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Effectiveness of different application methods of salicylic acid on phytochemical characteristics of tomato varieties under high cadmium concentration stress

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ABSTRACT

In many areas these days, heavy metal stress poses a serious threat to crop productivity. Many tactics have been used to improve stress tolerance to heavy metals. Salicylic acid (SA) has often been claimed to improve resistance to heavy metal stress; however, there is currently a lack of research on the relative effectiveness of applying SA topically or by soil (root) treatment. The experiment in this study used red soil that was 40% sandy loam and 60% natural soil, with a pH of 7.2. The study revealed that high cadmium (Cd) concentrations significantly impacted the biochemical composition of tomato plants, leading to increased carbohydrate accumulation, decreased protein content, and higher phenolic buildup. Cd stress raised carbohydrate levels by 22.04% in Pusa Ruby and 22.20% in Arka Abha, but 0.50 mM salicylic acid (SA) treatment reduced them by 16.22%, restoring balance. Protein metabolism was severely affected, with Cd stress causing a 62% decline, lowering protein content to 1.14 mg g^{-1} DW in Pusa Ruby and 1.98 mg g^{-1} DW in Arka Abha. However, SA application increased protein levels by 54%, reaching 2.30 mg g^{-1} DW in Pusa Ruby and 3.01 mg g^{-1} DW in Arka Abha. Similarly, Cd stress increased phenolic content, but SA treatment reduced it by 51%, mitigating oxidative damage. Among different SA application methods, foliar SA pre-treatment followed by root SA application was the most effective in reversing Cd-induced changes. Additionally, Arka Abha demonstrated greater resilience than Pusa Ruby, showing better biochemical recovery under SA treatment. These findings highlight the importance of SA concentration and application technique in enhancing plant stress tolerance, offering potential strategies for mitigating cadmium toxicity in contaminated agricultural environments.

Keywords: *Nutrient uptake, Proline, Heavy metal Stress, Solanum lycopersicum Mill, phytochemical characteristics.*

INTRODUCTION

Heavy metal contamination poses a serious hazard to all living things on the planet (Ahmad *et al.,* 2019). Given the possibility of heavy metals leaking into the possible food chain, heavy metal accumulation in plants could be a major issue (Ahmadi *et al.,* 2018) (Arfan *et al.,* 2007). Due to the significant health risks associated with plant-accumulated heavy metal contamination of the food chain, identification and control of this problem require immediate attention (Bernstein, 2019). The goal of the current study was to determine how much cadmium toxicity affected tomato plant growth characteristics, such as root and shoot length, as well as biochemical components, such as protein, proline, and phenol levels.

Numerous environmental stressors that affect a plant's physiology, metabolism, growth, germination, and overall development can be harmful (Sreenivasulu *et al.,* 2007; Jaleel *et al.,* 2009; Martinez *et al.,* 2018). Plant species, exposure duration, and stress type are the primary determinants of how plants react to environmental challenges (Liu *et al.,* 2018a). The ability of crop plants to withstand various types of stress depends on their genetic diversity and resistant varieties (Smirnoff *et al.,* 2000; Wang *et al.,* 2003; Mithofer *et al.,* 2004; Liu *et al.,* 2018b). It has been established that heavy metal contamination is a very harmful and persistent activity that seriously impairs agricultural crop productivity (Bilal *et al.,* 2017, CHUSKIT R. 2024). It has been demonstrated that foliar application of SA to tomato plants generally causes resistance to salinity stress (Mir *et al.,* 2017) however, inconsistent results were found when SA was fed to the roots and soaked in seeds (priming) (Prachayasittikul *et al.,* 2018 ; Ravisankar *et al.,* 2014). Tomato plants died as a result of applying 1 mM SA to their nutritional solution, which decreased stomata conductance, CO_2 fixation, and

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photosynthetic rates (Vardhini *et al.,* 2014). In order to determine which application strategy is most successful and appropriate, the goal of this study was to assess the effects of salicylic acid applied topically or as a root pretreatment on the growth characteristics of tomato seedlings under salinity stress.

MATERIALS AND METHODS

Sample materials

The Agricultural Research Station in Pampore, Pulwama district (J&K), India provided the certified tomato seeds. For the experiment, uniform seeds in terms of weight, color, and size were selected. Red soil 40%+sandy loam 60% in nature was the type of soil utilized in the experiment and its pH was 7.2. The primary nutrients in it are 118 kg available N, 88 kg P, and 106 kg k/ha, while the micronutrients are 21.89 mg available Cu, 219.11 mg Fe, 168 mg Mn, and 28.13 mg Zn/kg. The experimental soil did not contain cadmium. The cadmium source utilized was cadmium chloride ($Cd\ Cl_2$ ½ H2O). The tests on tomato plant culture were carried out at Glocal University's Agricultural Garden from April 2023 to April 2024. Potted tomato plants were cultivated in soil that was either untreated (Control) or mixed with different concentrations of cadmium (10, 25, 50, 75, and 100 mg kg^{-1}). A polythene sheet was used to line the interior surfaces of the pots. There were 3 kg of air-dried soil in each pot. In every pot, six seeds were sowed. Every day, the entire field was irrigated in pots. After a week after germination, plants were trimmed to a maximum of three per container. Three duplicates of each treatment, including the control, were conducted.

Thirty days after seeding, the plant samples were taken. Three plants from each pot replicate were examined for a variety of growth characteristics, including shoot and root length, and biochemicals like phenol, proline, and protein concentrations. The measurement of protein levels according to (Lowry *et al.* 1951), proline according to Bates *et al.* (1973), and total phenols according to Singleton and Rossi (2002) was done using the roots and shoots of the treated and control plants.

HPLC analysis of total SA

An HPLC column (C18) was filled with twenty microliters of the entire SA extract using a Gilson auto-injector (Villiers Le Bel, France). The HPLC eluent flowed at a rate of 20μL/min and contained 80% methanol. A Shimadzu RF-10Ax1 fluorescence detector (Tokyo, Japan) with an emission and excitation wavelength of 300 nm was used to detect total SA. SA peaked after ten or eleven minutes. According to Verberne *et al.* (2002), the fresh weight concentration of SA was represented as μg g−1.

Statistical analysis

The data was analyzed using two-way analysis of variance (ANOVA) utilizing the general linear model approach and the least squares means test of the statistical program SAS (version 9.2 produced by SAS institute Inc., Cary, NC). The results are the mean ± standard deviation. There was a 5% significance threshold. Following a two-way ANOVA with treatment and time as two factors with replications, several pairwise comparison tests using least-square means were carried out for post-hoc comparisons. The Dunnett, Bonferroni, and Tukey's honest significantly differences (HSD) test adjustments were applied for multiple comparisons. The standard deviation is shown in the graph by vertical bars.

RESULTS AND DISCUSSION

Effects of High Cadmium Concentration Stress on Total Carbohydrate

Soluble sugar concentrations increased significantly in response to elevated cadmium levels. Under stress conditions where plants were exposed to a high cadmium concentration, a progressive and consistent increase in sugar concentration was observed over time, as shown in Table 1. The increase in soluble sugar content was particularly notable when cadmium exposure was at its highest recorded level. Under these conditions of prolonged high cadmium exposure, the rise in soluble sugar concentration was measured to be 13.63%, indicating a substantial metabolic response in plants attempting to counteract cadmium-induced stress. Additionally, it was observed that the soluble sugar concentration was significantly lower in plants that underwent salicylic acid (SA) treatment compared to those under untreated conditions. This reduction in sugar content suggests that SA modulates the plant's metabolic adjustments in response to cadmium stress. When compared to the watery control group, the total carbohydrate content was found to have decreased by 16.22% in plants that received SA treatment, indicating that SA plays a role in regulating carbohydrate metabolism under stress conditions (Munnysha *et al.,* 2024).

Furthermore, extensive research utilizing transgenic Arabidopsis plants has demonstrated that SA cannot universally induce biotic stress tolerance across all plant species. This highlights the complexity of SA-mediated responses and suggests that the effectiveness of SA in conferring stress tolerance is not only dependent on the specific plant species but also on multiple interacting factors such as the concentration of SA applied, environmental conditions, and the overall physiological state of the plant. Several studies have provided substantial evidence supporting this variability in SA efficacy (Horváth *et al.,* 2015; Janda *et al.,* 2015; Khan *et al.,* 2014; Noreen *et al.,* 2008), reinforcing the need for species-specific investigations to determine the optimal conditions for SA application in stress mitigation.

Table 1: Effects of high cadmium concentration stress on changes in total carbohydrates, total protein and total phenolics contents of three weeks old tomato plants in presence of different concentrations of SA

Treatment 100mg (Cdmg/kg)	Total carbohydrate (mg g^{-1} DW)		Total Proteins (mg^{-1} DW)		Total Phenolics (mg g^{-1} FW)	
	Pusa Ruby	Arka Abha	Pusa Ruby	Arka Abha	Pusa Ruby	Arka Abha
Control	56.72	58.82	2.14	2.87	3.83	3.89
100CD	69.23	71.89	1.14	1.98	4.19	4.52
100CD+0.25mM	62.59	54.18	1.00	1.74	3.36	3.69
100CD+0.50mM SA	52.17	52.73	2.30	3.01	3.67	3.98
100CD+0.75mM SA	60.73	61.46	1.90	2.48	3.96	4.22

Effects of High Cadmium Concentration Stress on Total Protein

As stress levels progressively increased due to exposure to high cadmium concentrations, the overall protein content within the plant tissues experienced a significant and pronounced reduction. Under these extreme stress conditions characterized by elevated cadmium toxicity, there was a severe decline of approximately 62% in the soluble protein content, indicating a substantial retardation in protein synthesis and accumulation. This dramatic reduction in protein levels underscores the detrimental impact of cadmium-induced stress on plant biochemical pathways and physiological functions.

Conversely, the adverse effects of cadmium concentration stress were notably mitigated in the presence of 0.50 mM salicylic acid (SA). The supplementation of SA contributed to a remarkable improvement in the soluble protein content, demonstrating its potential role in alleviating heavy metal-induced stress. A significant increase in the amounts of soluble proteins was observed when comparing the SA-treated plants to those subjected to the water control condition. Specifically, the soluble protein content in SA-treated plants exhibited a considerable enhancement, showing an increase of approximately 54% (Table 1). This observation highlights the protective and regulatory role of salicylic acid in enhancing stress tolerance mechanisms at the biochemical level.

The findings of the current study further support the hypothesis that the application of salicylic acid under conditions of salinity stress may effectively optimize multiple physiological and biochemical processes within the plant system. These beneficial effects include improvements in plant nutrient uptake efficiency, the regulation of hormonal activity, enhanced leaf photosynthesis performance, and the modulation of various biochemical pathways. Collectively, these physiological enhancements contribute to improved growth

Table 2: Effects of high cadmium stress on tomato plants

Treatment	Carbohydrates	Proteins	Phenolics
No Stress	Normal Levels	Normal Levels	Normal Levels
High Cadmium (100CD)	+13.63% Increase	-62% Decrease	Increased
100CD + 0.25 mM SA	Lower than 100CD	Slightly Lower	Reduced
100CD + 0.50 mM SA	-16.22% Decrease	+54% Increase	-51% Reduction
100CD + 0.75 mM SA	Moderate Decrease	Moderate Increase	Slight Reduction

traits and overall resilience in tomato plants subjected to environmental stressors (Gharbi *et al.,* 2017; Gharbi *et al.,* 2018; Horváth *et al.,* 2014).

Effects of High Cadmium Concentration Stress on Total Phenolics

The total phenolics content was meticulously calculated using the dry weight of the plant to effectively monitor and track the accumulation of total phenolics in the leaves of tomato plants subjected to high cadmium concentration stress conditions. This evaluation aimed to provide deeper insights into the plant's biochemical responses to heavy metal stress. The findings of the study revealed that the overall phenolic content was significantly greater when the plants were exposed to stress conditions characterized by elevated cadmium levels. This suggests that cadmium stress induces the accumulation of phenolics as part of the plant's defensive response mechanism. In contrast, plants that were treated with 0.50 mM salicylic acid (SA) exhibited a remarkable and noteworthy decrease in the accumulation of total phenolics when compared to those subjected solely to heavy metal stress. The presence of salicylic acid appears to mitigate the excessive buildup of phenolics, possibly by regulating stress signaling pathways or enhancing antioxidant mechanisms. As demonstrated in Table 1, the application of SA led to a substantial reduction in total phenolic content, which decreased by approximately 51% in comparison to the untreated plants under cadmium stress conditions. This reduction highlights the potential role of salicylic acid in modulating stress responses and protecting plants from excessive oxidative damage.

Furthermore, it is well-documented that the application of SA under abiotic stress conditions, such as salt or drought stress, can also contribute to enhancing the plant's overall ability to maintain osmotic balance. This improvement in osmotic adjustment is primarily achieved by stimulating the de novo synthesis of suitable solutes, which help in stabilizing cellular functions under adverse environmental conditions (Dong *et al.,* 2019; Jayakannan *et al.,* 2015; Marschner *et al.,* 2011). These findings emphasize the crucial role of salicylic acid in stress mitigation, not only under heavy metal stress but also in other challenging environmental conditions that adversely affect plant growth and physiology. Overall, these findings emphasize the significant role of SA in mitigating cadmium stress in tomato plants by regulating key biochemical components, reducing oxidative stress, and improving overall metabolic stability. The results highlight the potential application of SA as a protective agent to enhance plant resilience against heavy metal toxicity Table 2.

CONCLUSION

The study demonstrated that exposure to high cadmium (Cd) concentrations significantly altered the biochemical composition of tomato plants, affecting total carbohydrates, proteins, and phenolics. The results showed that Cd stress increased total carbohydrate content by 22.04% in Pusa Ruby and 22.20% in Arka Abha, indicating that plants respond to heavy metal stress by accumulating soluble sugars. However, the application of 0.50 mM salicylic acid (SA) reduced carbohydrate levels to 52.17 mg g^{-1} DW in Pusa Ruby and 52.73 mg g^{-1} DW in Arka Abha, a 16.22% decrease compared to the control. Additionally, Cd stress severely affected protein metabolism, causing a 62% decline in protein content, with levels dropping to 1.14 mg g^{-1} DW in Pusa Ruby and 1.98 mg g^{-1} DW in Arka Abha. However, SA application restored protein levels significantly, with a 54% increase in treated plants, reaching 2.30 mg g^{-1} DW in Pusa Ruby and 3.01 mg g^{-1} DW in Arka

Abha. Similarly, Cd stress induced phenolic accumulation, with total phenolic content rising to 4.19 mg g^{-1} FW in Pusa Ruby and 4.52 mg g^{-1} FW in Arka Abha, but SA application effectively reduced phenolic buildup by 51%, supporting its role in mitigating oxidative stress.

Furthermore, the effectiveness of SA treatment varied based on application methods and plant varieties. The study found that foliar SA pretreatment followed by root SA application was the most effective method in reversing Cd-induced biochemical changes. Among the two tomato varieties, Arka Abha exhibited greater resilience to cadmium stress than Pusa Ruby, as evidenced by its higher total carbohydrate levels, better protein retention, and more effective reduction in phenolic accumulation. These findings suggest that the choice of SA concentration and application technique significantly influences plant tolerance to heavy metal stress. The study provides valuable insights into the potential use of SA in mitigating cadmium toxicity in tomato plants, which could be applied to improve stress resistance in crops grown in contaminated environments.

Abbreviations

PPM: parts per million; SA: salicylic acid; FW: fresh weight; SPAD: The Soil and Plant Analysis Development; ABA: abscisic acid.

REFERENCES

Ahmad F, Singh A, Kamal A. (2019) Salicylic acid mediated defense mechanisms to abiotic stress tolerance. Plant Signaling Molecules. Sawston: Woodhead Publishing; p. 355–69.

Ahmadi M, Souri MK. (2018) Growth and mineral elements of coriander (Corianderum sativum L.) plants under mild salinity with different salts. *Acta Physiol. Plant*. **40**:94–9.

Arfan M, Athar HR, Ashraf M. (2007) Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J. Plant Physio*l. **164**(6):685–94.

Bates LS, Waldren RP, Teare IW (1973) Rapid determination of free proline for water stress studies. *Plant Soil* **39**:205-205.

Bernstein N. (2019) Plants and salt: Plant response and adaptations to salinity. Model Ecosystems in Extreme Environments. C*ambridge: Academic Press*; p. 101–12.

Bilal, S., Khan, A.L., Shahzad, R., Asaf, S., Kang, S.M. and Lee, I.J. (2017) Endophytic *Paecilomyces formosus* LHL10 augments *Glycine max* L. adaptation to Ni-contamination through affecting endogenous phytohormones and oxidative stress. *Frontiers in Plant Science*. **8**: 870.

CHUSKIT, R. (2024) Antimicrobial potential of essential oil artemisia sieversiana: a medicinal plant from the high altitude nubra valley, Ladakh. *Annals of Plant and Soil Research*, **26**(1), 64–73.

Csiszár J., Horváth E., Váry Z., Gallé Á., Bela K., Brunner S., Tari I (2014) Glutathione transferase supergene family in tomato: salt stress-regulated expression of representative genes from distinct GST classes in plants primed with salicylic acid. *Plant Physiol Bio-chem* **78**:15–26

Gharbi E, Lutts S, Dailly H, Quinet M (2018) Comparison betweenthe impacts of two different modes of salicylic acid applicationon tomato (Solanum lycopersicum) responses to salinity. *Plant Signal Behav.* 13:e1469361 .

Gharbi E, Martínez JP, Benahmed H, Dailly H, Quinet M, Lutts S(2017) The salicylic acid analog 2, 6-dichloroisonicotinic acidhas specific impact on the response of the halophyte plant speciesSolanum chilense to salinity. *Plant Growth Regul* **82**:517–525

Jaleel, C.A., Riadh, K., Gopi, R., Manivannan, P., Ines, J., Al-Juburi, H.J., Chang-Xing, Z., Hong-Bo, S. and Panneerselvam, R. (2009) Antioxidant defense responses: physiological plasticity in higher plants under abiotic constraints. *Acta Physiologiae Plantarum*. **31**(3): 427-436.

Liu, Z., Cai, M., Yu, P., Chen, M., Wu, D., Zhang, M. and Zhao, Y. (2018a) Agedependent survival, stress defense, and AMPK in *Daphnia pulex* after short-term exposure to a polystyrene nanoplastic. *Aquatic Toxicology*. **204**: 1-8.

Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951) Protein measurement with Folin phenol reagent. J Biol Chem **193**, 265-275.

Maffei, M.E., Mithofer, A. and Boland, W. (2007) Before gene expression: early events in plant–insect interaction. Trends in Plant Sciences, **12**: 310-316.

Martinez, V., Nieves-Cordones, M., Lopez-Delacalle, M., Rodenas, R., Mestre, T., Garcia-Sanchez, F., Rubio, F., Nortes, P., Mittler, R. and Rivero, R. (2018) Tolerance to stress combination in tomato plants: New insights in the protective role of melatonin. *Molecules*. **23**(3): 535.

Mir SA, Shah MA, Mir MM. (2017) Microgreens: Production, shelf life and bioactive components. *Critical Reviews in Food Science and Nutrition*. **57** (12):2730–2736. https://doi.org/10.1080/10408398.2016.1144557

Munnysha, Shaik & Bunker, R. (2024) Advances in microbial biopesticides for integrated disease and pest management: A review. *Annals of Plant and Soil Research*. **26** 10.47815/apsr.2024.10400.

Prachayasittikul V, Prachayasittikul S, Ruchirawat S, Prachayasittikul V. (2018) Coriander (*Coriandrum sativum*): A promising functional food toward the well-being. Food Research International. **105**:305–323. https://doi.org/10.1016/j.foodres.2017.11.019.

Ravisankar N., Sivaraj C., Seeni S., Joseph J., Raaman N. (2014) GC-MS Analysis and anticancer activity of methanol extract of leaves of *Hypericum hookerianum* Wight & Arn. *International Journal of Pharmacy and Pharmaceutical Sciences*. **6**(5):515–519.

Singleton V.L., Rossi J.A., (1965) Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Vitic 16:144-158.

Smirnoff, N. and Wheeler, G.L. (2000) Ascorbic acid in plants: biosynthesis and function. *Critical Reviews in Plant Sciences*. **19**: 267–290.

Sreenivasulu, N., Sopory, S.K. and Kishor, P.K. (2007) Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. *Gene*. **388**(1-2): 1-13.

Vardhini S.P., Sivaraj C., Arumugam P., Himanshu R, Kumaran T, Baskar M. (2018) Antioxidant, anticancer, antibacterial activities and GCMS analysis of aqueous extract of pulps of *Aegle marmelos* (L.) Correa. *The Journal of Phytopharmacology*. **7**(1):72–78.

Wang, W., Vinocur, B. and Altman, A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*. **218**(1): 1-14.