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EFFECTS OF SALICYLIC ACID FOR ALLEVIATING BIOLOGICAL ACTIVITIES IN SORGHUM AGAINST TANNERY WASTEWATER

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Abstract

This study evaluated the protective effects of salicylic acid (SA) on *Sorghum bicolor* L. exposed to tannery wastewater containing heavy metals Cr, Pb, and Cd. Treatments included 30, 50, 70, and 100% tannery wastewater, 100 to 200 mg/l salicylic acid, and their combinations. Tannery wastewater (TWW) reduced root and shoot length, biomass, photosynthetic rate, and relative water content while increasing oxidative stress biomarkers such as malondialdehyde (MDA). Antioxidant enzymes (SOD, POD, CAT, NR, GR, and GPX) showed an initial increase under moderate tannery wastewater stress, followed by inhibition at higher levels. Application of 200 mg/l SA markedly improved growth by 30-41%, restored chlorophyll and carotenoids by 29-38%, and enhanced enzymatic antioxidants.

Introduction

Recent studies have emphasized that salicylic acid functions as a stress-induced indicating compound, acting as a potential plant growth regulator and an immunostimulant that activates plant defense mechanisms. It also plays a key role in various physiological and developmental processes, including morphogenesis (Kavulych *et al*. 2023). While Chen *et al*. (2023) highlighted its diverse effects on horticultural crops and the beneficial impacts of SA on several field crops under environmental stress. Exogenous application of SA has been shown to enhance plant tolerance to several abiotic stresses, including drought, salinity, and heavy metal toxicity. However, previous studies have also indicated that excessive SA concentrations may develop phytotoxic, resulting in reduced plant height and biomass (Shahrajabian and Sun 2024). Therefore, determining the optimal SA concentration that stimulates plant growth while minimizing toxicity is necessary. Kumar *et al*. (2024) established the optimal SA measure for improving the growth performance of *Sorghum bicolor* L. (Great Millet). Based on these findings, the present study re-evaluates these concentrations to assess their role in mitigating the adverse effects of tannery wastewater on the growth and physiological responses of *S. bicolor*.

Pakistan hosts over 800 tanneries, primarily located in Karachi, Multan, Kasur, and Sheikhupura. Although these industries contribute significantly to the national economy, most operate without adopting sustainable or eco-friendly waste management practices. Due to limited freshwater resources, wastewater irrigation has become common in many regions (Asif *et al*. 2025). However, tannery wastewater contains high concentrations of chromium, lead, sulfides, organic matter, and suspended solids, which pose severe environmental hazards. Heavy metals in wastewater exert both direct and indirect toxic effects on plants by disrupting enzymatic activities, generating oxidative stress, causing cellular damage, impairing photosynthesis, and hindering nutrient uptake (Rahman *et al.* 2024). The continuous discharge of untreated effluents into water bodies by leather industries not only deteriorates water quality but also pollutes agricultural soils, threatening food security, ecosystem stability, and public health (Naveed *et al*. 2025).

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Plants can absorb and accumulate heavy metals when exposed to irrigation with untreated or contaminated water sources, which demonstrates that irrigation with untreated wastewater significantly inhibits sorghum growth, whereas the use of treated wastewater enhances crop yield (Verma *et al*. 2022). Tannery effluents are among the primary contributors to heavy metal contamination, containing high levels of trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) chromium along with other toxic pollutants (Arshad *et al*. 2024). The remediation of heavy metals from wastewater remains a considerable challenge, as single plant species are often insufficient to fully eliminate metal toxicity from contaminated sites. Nevertheless, *Sorghum bicolor* has been identified as a promising phytoremediator capable of tolerating and accumulating chromium under stressful conditions (Shen *et al*. 2023). Previous studies have reported that *S. bicolor* mitigates Cr toxicity through bioaccumulation and biosorption mechanisms. It may also contribute to the conversion of highly toxic Cr^{6+} to the less toxic Cr^{3+} form within the rhizosphere, thereby reducing environmental toxicity and enhancing plant tolerance in tannery effluent-contaminated environments (Al-Huqail *et al*. 2024). Considering the above, the present study aims to investigate the potential of salicylic acid to alleviate the toxic impacts of tannery wastewater on *S. bicolor* by enhancing its physiological, biochemical, and enzymatic responses.

Materials and Methods

A completely randomized block-designed pot experiment was conducted to examine the potential of salicylic acid for alleviating the biological functions of sorghum grown in irrigated tannery wastewater. Certified seeds of *Sorghum bicolor* were obtained from AGRIMAX SEEDS, Multan. Healthy seeds after disinfection were sown in the pots having a diameter of 12 x 8 inches, filled with loamy soil. The tannery wastewater was applied at 30, 50, 70, and 100% in irrigation form, while 100 and 200 mg/l of SA was applied through foliar spray. There were fifteen treatment combinations, keeping one as a control with three replicates each. After 60 days, the growth, biochemical, and enzymatic parameters collected from the samples were analyzed for the study. A scale was used to measure the lengths of the roots and shoots, while a precision digital balance was used to record the fresh biomass of root and shoot. The samples were oven-dried at 70°C for 48-72 hrs for the determination of dry weights of roots and shoots**.**

For the determination of photosynthetic and water retention responses a portable photosynthetic system (LI-6400; Li-Cor Inc., Lincoln, NE, USA) was used and relative water content (RWC) was calculated using the formula $RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100$. The activities of antioxidant enzymes were determined using standard spectrophotometric methods, superoxide dismutase (SOD) following Sambo *et al*. (2024), peroxidase (POD) according to Gahagan *et al*. (1968), catalase (CAT) following Aebi (1984), and glutathione reductase (GR) according to Foyer and Halliwell (1976). The accumulation of proline was quantified using the method of Bates *et al.* (1973), while nitrate reductase (NR) activity was measured by the method of Zhang *et al.* (2024). Similarly, chlorophyll, ascorbic acid contents, malondialdehyde (MDA), and proline were measured by Bates *et al*. (1973). Data was analyzed by conducting a two-way ANOVA in Statistix 8.1 software, while HSD Tukey's was applied to show the significant differences between the treatments at $p < 0.01$.

Results and Discussion

As shown in Fig. 1, all the growth parameters revealed a significant trend of decrease across various treatments of tannery wastewater. Salicylic acid, especially at 200 mg/l, impressively improved all features. At 30, 50, 70, and 100% TWW, root length decreased by 5, 10, 14 and 17%, respectively at $P<0.01$. SA alone caused a 6% at 100 mg/l and 14% at 200 mg/l, while at 200

mg/l, SA further increased by 9, 3, 2 and 4% under each stress level. Shoot length decreased 9 to 33% at the maximum concentration of TWW, while SA alleviated it to 12.05 and 24.92%, respectively. Similar trends were also noted in case of root and shoot fresh weights which decreased by 5 to 24% under stress but improved by 7-19% at the maximum level of SA. The shoot dry weight was also measured, decreased by 5.1-12.8%, and improved by 61.7% and 124.8% by using a minimum level of SA as compared to the control at $P < 0.01$. The maximum level of SA enhanced root dry weight by16.7, 15.4, 12.5, and 12% in comparison to the lower level.

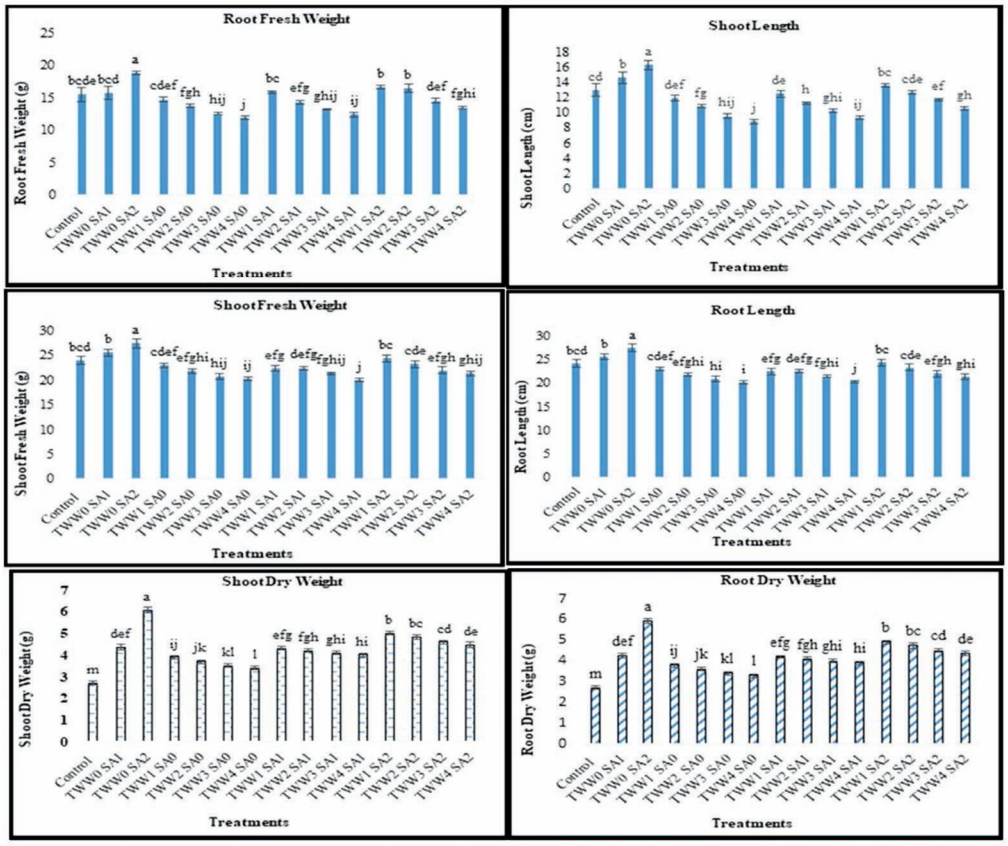

Fig. 1. Effects of tannery wastewater and salicylic acid on morphological parameters of sorghum crop conducted through Two-Way Anova. Different letters show the significant difference between the treatments analyzed through HSD Tukey's test at P<0.01.

As shown in Fig. 2, both the photosynthetic rate and relative water content showed same trend of variation across the different treatments as photosynthetic rate dropped by 17, 33, 50 and 58% when plants were solely exposed to tannery wastewater at 30, 50, 70 and 100%, respectively at P<0.01 while by adding 100 mg/l SA to the same wastewater levels increased photosynthesis by 21, 25, 39 and 40%, respectively, compared to the stress-only treatments. Improvements of 9, 5, 14 and 17% were seen when the SA dose was increased to 200 mg/l as compared to the control. A similar declining pattern of 13, 23, 35 and 46% was shown for relative water content, while after using the minimum level of SA, it increased by 5, 10, 16 and 29%, respectively at P<0.01. A

higher dose of 200 mg/l SA further increased relative water content by 3, 6, 9 and 10%, respectively.

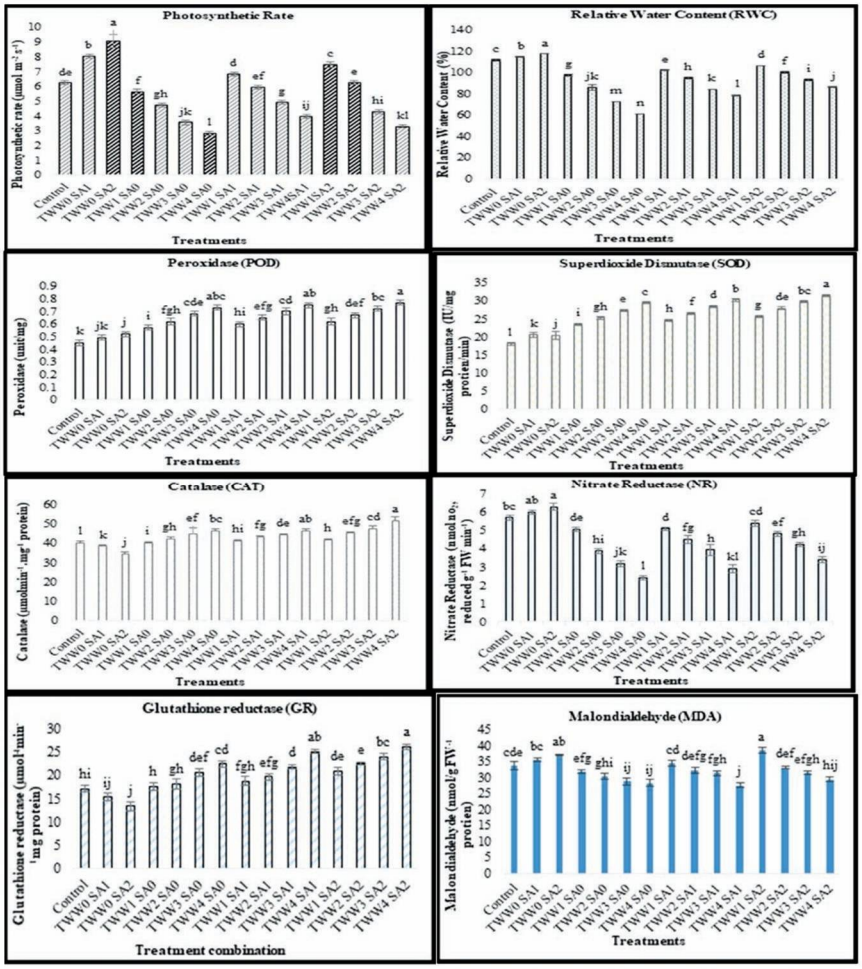

Fig. 2. Effects of tannery wastewater and salicylic acid on Physiological parameters of sorghum crop conducted through Two-Way Anova. Different letters show the significant difference between the treatments analyzed through HSD Tukey's test at P<0.01.

As shown in Fig. 2, TWW alone increased peroxidase activity by 26.6, 37.7, 51.1 and 62.2%, respectively at P<0.01, but SA alone increased POD by 8.88 and 15.55%, respectively, at its varying levels. In the same manner, SOD increased to 24, 40, 62 and 63% when exposed to tannery wastewater alone, whereas it decreased to13 and 15% with SA alone. The oxidative stress, the increased level of CAT was also noted under various levels of TWW, but it was observed to be mitigated by 6 and 15% by using varying levels of SA. Nitrate reductase decreased under different treatments of TWW, ranging from 5.05 to 2.4; however, SA alone alleviated it by 5 and 10%, respectively. In combined form, the SA restored it from 5.1 to 5.55 at 30%, 4.5 to 5.1% at 50%, 3.93 to 4.3 at 70%, while 2.9 to 3.2 at 100% as compared to lonely treatments of TWW also at

P<0.01. Strong dose-dependent enhancement of the glutathione-based antioxidant system was confirmed by the fact that GR activity increased with TWW from 17.5% (30%) to 22.38% (100%), which slightly decreased under SA alone by 15.21 and 13.38%, respectively at P<0.01. An obvious increase in MDA content was observed with rising concentrations of tannery wastewater. At 30% TWW treatment, MDA increased by 15-20% while at 60% TWW**,** it rose by nearly 35 to 40%. The 100% of TWW treatment showed the highest accumulation, approximately 55 to 60% at P<0.01.

As shown in Fig. 3, Proline increased by 8.6-34% under TWW alone, while this effect was found to be mitigated 80, 87, 98 and 108% at 30-100% of TWW combined with the maximum level of SA at P<0.01. Salicylic acid at 200 mg/l increased chlorophyll content by 7.53, 9.08, 10.18 and 3.13% at 30-100% TWW, while similar protective trends were seen in ascorbic acid, which decreased with TWW alone but steadily improved with salicylic acid. At 30-100% TWW, 200 mg/l salicylic acid improved ascorbic acid to 11.08, 15.14, 8.96 and 15.36%. The trend of increase was seen in GPX concentration with the increase of TWW and found maximum at the

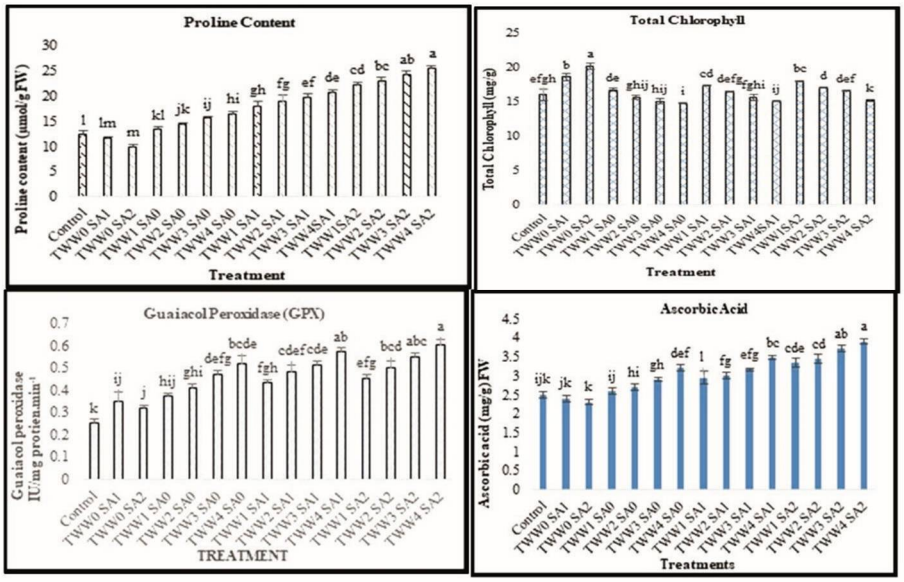

Fig. 3. Effect of tannery wastewater and salicylic acid on biochemical parameters of sorghum crop conducted through Two-Way Anova. Different letters show the significant difference between the treatments analyzed through HSD Tukey's test at P<0.01.

highest concentration, but a minimal effect was seen when SA was added with its maximum dose. All of the non-enzymatic antioxidants together showed that oxidative defense was strongly impacted by both increasing effluent stress and higher salicylic acid dosage. Salicylic acid, especially at 200 mg/l, greatly improved tolerance by preserving redox balance, membrane integrity, and pigment stability under heavy metal-induced stress. The previous results verified that both the growth and physiology of *S. bicolor* are strongly inhibited by chromium-rich tannery wastewater (Benamara *et al.* 2025). Similar trends were shown in the present study, where increased TWW levels (300-100%) decreased biomass by 30-35% and plant height by 20-25%, suggesting metabolic inhibition and growth suppression brought on by heavy metal accumulation. As shown in Fig. 4, all growth parameters and others, including photosynthetic rate, relative water

content, and nitrate reductase, showed highly positive correlation (r = 0.926-1.000) while all antioxidant enzyme activities and ascorbic acid showed moderate correlation (r=0.825–0.995) at P<0.01. On the other hand, significant negative correlations (r = –0.783 to–0.855) were observed between the root, shoot, and catalase, while a positive correlation was observed between shoot fresh weight to root length (r = 0.999) and shoot dry weight to Root dry weight (r = 1.000). The strong inverse correlations were seen between catalase and shoot fresh weight (r =–0.807) and between catalase and photosynthetic rate (r = –0.845) at P<0.01. The principal component analysis

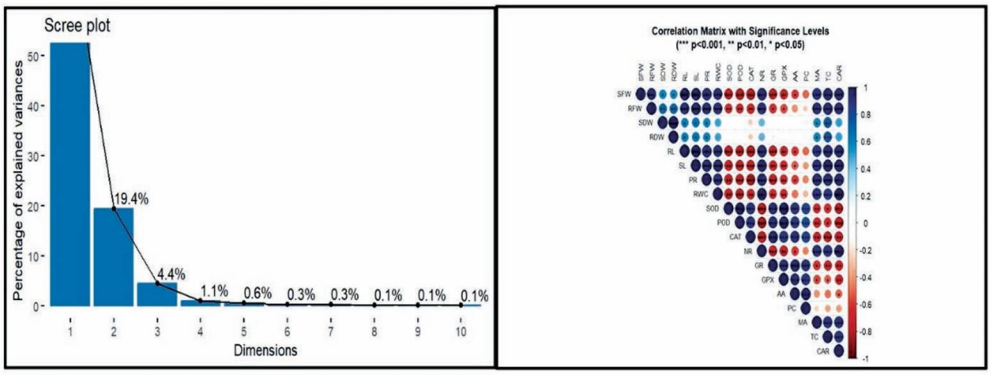

Fig. 4. Scree plot showing the dimension-based variations among all parameters of the plant under the effect of TWW and SA at P<0.01.

showed that the highest variation was shown in Dim1 (73.4%), while Dim2 showed the variability of 19.4% and progressively to Dim10 (0.1) at P<0.01. In the existing study, SOD and CAT activity were increased by moderate TWW concentrations, indicating an early antioxidative response; however, these enzymes decreased at higher stress levels. Salicylic acid's stimulatory impact was demonstrated, especially at 200 mg L^{-1}, when enzymatic activity increased by 35-40%, indicating SA's function in preventing oxidative damage caused by metals. Cevik *et al.* (2025) showed similar reactions, under pesticide stress after SA administration. However, differences in the kind and severity of pollutants may account for the variability in enzyme magnitude between experiments. In line with results by Ali *et al.* (2021), who found POD amplification after heavy metal exposure, peroxidase activity likewise steadily rose under TWW stress. However, at higher $HgCl_2$ concentrations, their experiment revealed a rapid drop in POD, suggesting enzyme inhibition at extreme toxicity. On the other hand, POD activity in the current investigation increased even at 100% TWW and reached a 71.11% increase above control when SA was applied. This discrepancy probably results from SA's capacity to stabilize enzymes under complicated effluent stress, as opposed to the previous study's lack of any protective agent. Increased MDA level indicates oxidative damage caused by tannery wastewater, which is comparable to the patterns reported by Ghosh *et al.* (2022) under waterlogging stress. The current experiment showed a definite dose-dependent increase in proline, even though the study did not quantify it. The highest increase (108%) was seen when 200 mg L^{-1} SA was paired with 100% TWW. In non-stressed plants, SA alone marginally decreased proline, indicating a regulatory rather than stimulatory function under ideal circumstances. These results corroborate SA's cooperative function in enhancing osmotic adjustment and stress tolerance. TWW significantly reduced photosynthetic rate (10-15% at 30% TWW and 40-45% at 100%), demonstrating how sensitive CO_2 absorption is to heavy metal poisoning. The current study demonstrated that SA was

more successful than citric acid in maintaining chlorophyll under Cr stress, increasing total chlorophyll by 15% at 100 mg/l and 30% at 200 mg/l, despite Aslanzadeh *et al.* (2024) emphasizing pigment loss as a major indicator of photosynthetic impairment. This implies that SA increases pigment stability through enhanced antioxidant defense while simultaneously reducing metal toxicity (Wang *et al.* 2025). Under TWW stress, relative water content dramatically decreased, which is in line with previous research showing that leachate-based irrigation reduced RWC in alfalfa and basil by 28-37% (Bourdoug *et al.* 2025). Nevertheless, SA treatment significantly reduced these effects, increasing RWC by 10% at 200 mg/l and 29% at 100 mg/l. These findings show that SA successfully improves stomatal control, osmotic balance, and water retention, allowing plants to stay more hydrated under osmotic stress brought on by salt and metals.

The present study confirmed that salicylic acid can efficiently mitigate the toxic effects of tannery effluent on Sorghum, by inducing biological changes such as the activation of antioxidant enzymes and other growth-promoting strategies. The effect of tannery wastewater was found to be significant as its concentrations increased from the lowest to the highest, but as salicylic acid was applied, the effect of wastewater was found to be alleviated to a great extent. This shows that it has great potential in altering the biological functions of plants, thus proving a sustainable solution for good agricultural practices.

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