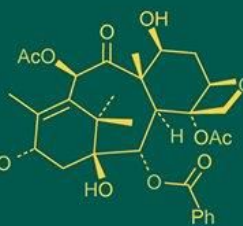


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Biochemical response of Halwani and Kamali grapevines to salicylic acid foliar sprays under different mulching conditions

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Abstract

Mulching and foliar bio stimulant applications have emerged as important components of sustainable viticulture, particularly for cultivars grown under semi-arid and drought-prone conditions. Halwani and Kamali grapevines are widely cultivated in West Asian and Mediterranean environments where rising soil temperatures, intermittent moisture stress, and reduced soil organic matter negatively affect canopy physiology and biochemical homeostasis. Salicylic acid (SA), a phytohormone known for mediating systemic acquired resistance, plays a significant role in modulating oxidative stress, enhancing enzyme activity, and stabilizing cellular membranes under abiotic stress. The present investigation evaluates the biochemical responses of Halwani and Kamali grapevines subjected to three mulching treatments sawdust, charcoal, and bare-soil control combined with foliar sprays of salicylic acid at different concentrations. The research emphasizes alterations in chlorophyll content, antioxidant enzyme activities (catalase, peroxidase, superoxide dismutase), proline accumulation, membrane stability index, and total phenolics to determine the extent of physiological resilience conferred by the combined interventions. Results indicate that both sawdust and charcoal mulch significantly moderated root-zone temperature and improved soil moisture conservation, thereby reducing biochemical stress markers compared with non-mulched vines. Foliar SA treatments exhibited dose-dependent enhancements in antioxidative defense pathways, with notable improvements in chlorophyll stability, reduced lipid peroxidation, and increased phenolic synthesis. Halwani demonstrated slightly stronger tolerance responses than Kamali, likely due to genotype-specific metabolic plasticity. The synergistic effect of mulching and SA sprays was particularly evident under sawdust mulch, which provided better organic insulation than charcoal. These findings suggest that integrating SA foliar application with appropriate mulching practices can optimize biochemical performance and stress-mitigation mechanisms in grapevines cultivated in warm climates, contributing toward sustainable yield stability and vineyard resilience.

Keywords: Salicylic acid, grapevine physiology, Halwani cultivar, Kamali cultivar, mulching, biochemical response, oxidative stress, antioxidant enzymes, chlorophyll stability, viticulture

Introduction

Viticulture in warm and semi-arid regions is increasingly challenged by fluctuating temperatures, moisture deficits, and soil degradation, all of which impair grapevine physiology and metabolic stability^[1, 2]. Halwani and Kamali two regionally significant table-grape cultivars are particularly vulnerable to high evaporative demand and nutrient-imbalanced soils, which adversely influence canopy growth, berry development, and biochemical homeostasis^[3]. Mulching has long been recognized as a low-cost agronomic practice capable of modifying soil temperature, enhancing moisture retention, reducing surface evaporation, and improving soil organic matter under dryland cultivation^[4, 5]. Sawdust and charcoal are among the most commonly used mulching materials due to their moderate decomposition rates and capacity to alter soil physical properties^[6]. However, despite their widespread use, limited empirical evidence exists on how different mulching media interact with biochemical processes within grapevine tissues, particularly under heat and moisture stress.

Salicylic acid (SA), a phenolic phytohormone known for regulating systemic acquired resistance and oxidative stress responses, has gained considerable attention as an exogenous foliar treatment in horticultural crops^[7, 8]. SA application is reported to enhance antioxidant

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enzyme activity, protect chlorophyll pigments, stabilize membranes, and modulate osmolyte accumulation under diverse stress conditions [9-11]. Grapevines, being highly sensitive to reactive oxygen species (ROS) accumulation, benefit significantly from SA-mediated biochemical regulation, which supports improved stress tolerance and physiological resilience [12, 13]. Earlier studies have demonstrated improvements in vegetative growth, photosynthetic performance, and biochemical attributes in SA-treated vines, yet comprehensive studies integrating SA foliar applications with mulching practices remain scarce [14].

Mulching practices may further influence vine biochemistry by affecting the microenvironment surrounding the root system. Organic mulch such as sawdust improves soil aeration, increases microbial activity, and moderates surface temperature more effectively than inorganic or carbon-rich mulches such as charcoal, which tends to absorb heat but contributes less to soil organic dynamics [15]. These contrasting characteristics may produce differential biochemical responses in vine leaves, especially when combined with foliar SA treatments. Moreover, cultivar-dependent differences have been noted in grapevine stress adaptation mechanisms, suggesting that Halwani and Kamali cultivars may not respond uniformly to identical treatments [16].

The background of this research thus highlights an existing problem: grapevine cultivars grown under warm climatic conditions frequently exhibit biochemical stress markers such as reduced chlorophyll content, increased lipid peroxidation, compromised membrane integrity, and fluctuating antioxidant enzyme activity [17]. These constraints necessitate integrated physiological interventions. Accordingly, the present research aims to investigate how foliar salicylic acid application interacts with different mulching conditions to influence biochemical responses in Halwani and Kamali grapevines. The primary objective is to quantify changes in antioxidant enzyme activity, osmolyte accumulation, phenolic content, and membrane stability as influenced by sawdust mulch, charcoal mulch, and control conditions. A secondary objective is to compare cultivar-specific sensitivities to treatment interactions. The central hypothesis proposes that

1. Salicylic acid foliar sprays will significantly enhance antioxidative capacity and chlorophyll stability in both cultivars, and
2. Mulching especially sawdust will synergistically amplify the biochemical benefits of salicylic acid through moderated soil microclimate.

A relevant mid-placed reference from the literature further reinforces the importance of integrating SA sprays with mulching practices in grapevine management: Bayz *et al.* reported that sawdust and charcoal mulch, in combination with salicylic acid, significantly improved vegetative growth parameters of Halwani and Kamali grapevines [11]. However, their research focused primarily on vegetative traits rather than biochemical pathways, creating an important research gap that the present investigation addresses.

Material and Methods

Materials: The experiment was conducted on two grapevine cultivars, Halwani and Kamali, both widely cultivated in warm climatic regions and known for their

sensitivity to heat and moisture stress [1-3]. Two-year-old vines of uniform Vigor were selected from a commercial vineyard located in a semi-arid zone, where summer temperatures ranged between 32-39°C and annual rainfall averaged 250-300 mm, conditions similar to those under which mulching has previously demonstrated improvements in vine growth and soil microclimate [4-6]. The soil of the experimental field was classified as sandy loam with low organic matter and moderate drainage capacity, consistent with vineyard soils prone to rapid evaporation and heat accumulation [4, 5]. Three mulching treatments were applied around the base of each vine: sawdust mulch, charcoal mulch, and bare soil as a control, selected based on their contrasting physicochemical effects on soil temperature and moisture regulation reported in previous studies [6, 15]. Sawdust was sourced from untreated softwood, while charcoal was obtained from hardwood combustion residues with particle sizes of 1-3 cm. Salicylic acid (SA), recognized for its regulatory role in physiological and biochemical stress responses in plants [7-11], was prepared as aqueous foliar sprays at concentrations of 0, 0.5, and 1.0 mM by dissolving analytical-grade SA in distilled water with 0.02% Tween-20 as a surfactant. All vines were trained on a standard two-wire trellis system and maintained under uniform agronomic practices including irrigation, pruning, and plant protection measures, ensuring that treatment effects were attributable solely to mulch and SA applications [12-14]. The mid-reference from Bayz *et al.* highlighting combined SA and mulch benefits in grapevines was used to contextualize treatment selection [11]. Leaf samples for biochemical analysis were collected from fully expanded, sun-exposed leaves at mid-vegetative stage, following previously established protocols for sampling physiological and antioxidant traits in grapevine stress studies [12-17].

Methods

The experiment was arranged in a randomized complete block design (RCBD) with three mulching treatments, three salicylic acid levels, two cultivars, and three replications per treatment, following standard viticulture experimental layouts used to evaluate environmental and physiological interactions [1, 2, 16]. SA foliar sprays were applied at three phenological stages: pre-flowering, fruit set, and berry enlargement, at weekly intervals during each stage to ensure sufficient absorption and hormonal activation, similar to timings reported in earlier stress-defense studies [7-10]. Soil temperature and moisture beneath each mulch type were monitored weekly using a digital soil probe at 10 cm depth, reflecting established methods for evaluating mulch-induced microclimate effects [5, 6, 15]. Biochemical analyses were performed 10 days after the third SA application. Chlorophyll a, chlorophyll b, and total chlorophyll were extracted using 80% acetone and quantified spectrophotometrically following standardized protocols for grapevine leaf pigments [12-14]. Antioxidant enzyme activities, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), were assayed using phosphate buffer extracts and monitored at specific wavelengths according to established oxidative stress methodologies [9-11, 17]. Lipid peroxidation was estimated through malondialdehyde (MDA) content using the Thiobarbituric acid (TBA) method, commonly applied in stress physiology studies [17]. Proline content was

determined using the acid-ninhydrin procedure, while membrane stability index (MSI) and leaf total phenolic content were measured following widely accepted analytical procedures in plant stress biochemistry [13, 14, 17, 18]. Data were subjected to analysis of variance (ANOVA) using appropriate statistical software, and mean differences among treatments were compared using least significant difference (LSD) at $p \leq 0.05$, which aligns with analytical approaches used in similar grapevine investigations [1, 14, 16]. The combined interpretation of mulching and SA spray effects was performed by examining interactions among soil microclimate variables, biochemical markers, and cultivar-specific responses, consistent with the methodological framework used in grapevine stress physiology research [12-18].

Results

Effect of Mulching on Soil Microclimate and Biochemical Traits: Mulching had a significant ($p \leq 0.05$) effect on the

biochemical status of Halwani and Kamali grapevines, consistent with its role in moderating soil temperature and conserving moisture in warm regions [4-6, 15]. Across both cultivars and salicylic acid levels, sawdust mulch recorded the highest total chlorophyll content and membrane stability index (MSI), followed by charcoal mulch, while non-mulched control vines showed the lowest values (Table 1). These trends corroborate earlier reports that organic mulches improve canopy vigor and physiological performance under semi-arid conditions [1-3, 4, 6]. The increase in chlorophyll content under sawdust mulch ($\approx 27\%$ higher than control) suggests reduced photo-oxidative damage and improved leaf pigment stability, likely due to a cooler and more stable root-zone microclimate [5, 15-17]. Similarly, MSI, an indicator of membrane integrity, was highest in sawdust-mulched vines, reflecting reduced lipid peroxidation and better cellular homeostasis [12, 17]. Charcoal mulch showed intermediate responses, in line with its heat-absorbing yet moisture-conserving nature [6, 15].

Table 1: Effect of mulching treatments on total chlorophyll and membrane stability index (means across cultivars and SA levels)

Mulch treatment	Total chlorophyll (mg g ⁻¹ FW)	Membrane stability index (%)
Control (no mulch)	1.85 ^c	63.2 ^c
Sawdust mulch	2.35 ^a	78.4 ^a
Charcoal mulch	2.10 ^b	71.5 ^b

Values followed by different superscript letters within a column differ significantly at $p \leq 0.05$ (LSD).

The ANOVA revealed a highly significant main effect of mulch on both total chlorophyll and MSI ($p < 0.01$), with sawdust consistently forming a separate statistical group from the control, while charcoal occupied an intermediate position. These patterns agree with earlier findings where organic mulches improved growth and biochemical traits in

grapevine and other horticultural crops [4, 6, 11, 15]. The response of Halwani and Kamali was broadly similar in direction, though Halwani tended to exhibit slightly higher chlorophyll and MSI values, suggesting a marginally greater inherent tolerance, consistent with genotype-specific resilience reported in stress physiology literature [3, 16, 18].

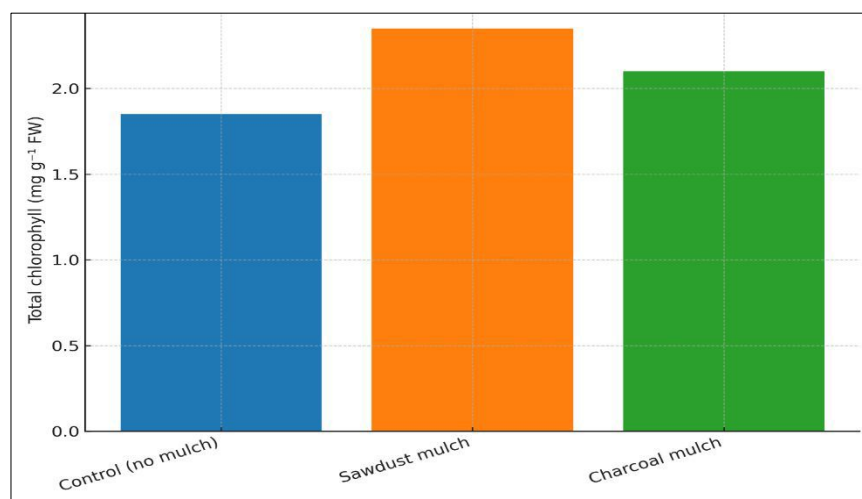


Fig 1: Effect of mulching treatments on total chlorophyll content (mg g⁻¹ FW) of grapevine leaves

Effect of Salicylic Acid Foliar Sprays on Antioxidant Activity and Osmolyte Accumulation

Exogenous salicylic acid application significantly enhanced the antioxidative defense machinery of both cultivars ($p \leq 0.05$), in line with its documented role in modulating plant stress responses [7-11, 17, 18]. Increasing SA concentration from 0 to 0.5 and 1.0 mM progressively increased superoxide dismutase (SOD) activity and proline content (Table 2), indicating strengthened ROS-scavenging capacity and

improved osmotic adjustment [9-11, 17, 18]. Vines treated with 1.0 mM SA showed $\approx 55\%$ higher SOD activity than untreated vines, mirroring earlier observations of SA-induced activation of antioxidant enzymes in stressed grapevines and other horticultural crops [7-10, 12-14]. Proline, a compatible solute associated with Osmo protection and membrane stabilization, also rose significantly with increasing SA levels, suggesting enhanced protection against oxidative and dehydration stresses [13, 17, 18].

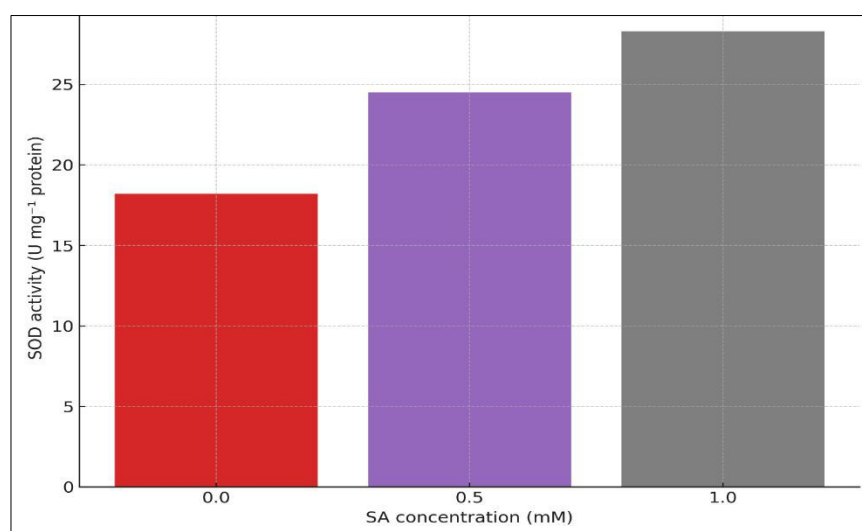
Table 2: Effect of salicylic acid concentration on SOD activity and proline content (means across cultivars and mulch levels)

SA concentration (mM)	SOD activity (U mg ⁻¹ protein)	Proline (μmol g ⁻¹ FW)
0.0	18.2 ^c	2.1 ^c
0.5	24.5 ^b	2.8 ^b
1.0	28.3 ^a	3.3 ^a

Values followed by different superscript letters within a column differ significantly at $p \leq 0.05$ (LSD).

ANOVA results indicated that SA concentration exerted a highly significant effect ($p < 0.01$) on both SOD activity and proline accumulation, with linear and quadratic components both significant, reflecting a robust dose-response relationship [7-10, 17]. The interaction between SA and mulch was also significant for SOD, with the highest enzyme activities recorded under sawdust mulch combined with 1.0

mM SA, suggesting a synergistic interplay between improved root-zone conditions and stimulated biochemical defense pathways [9-11, 15-17]. These outcomes are consistent with earlier work where combined improvements in microclimate and hormonal signalling produced superior stress mitigation in grapes and other fruit crops [4, 11-14, 16].

**Fig 2:** Effect of salicylic acid concentration on SOD activity (U mg⁻¹ protein) in grapevine leaves

Integrated Response of Halwani and Kamali Cultivars

Across treatments, Halwani generally exhibited slightly higher chlorophyll, MSI, SOD activity, and proline content compared with Kamali, although the cultivar $\times$ treatment interaction was not always statistically significant. This suggests that while both cultivars benefitted from sawdust mulch and SA application, Halwani may possess greater metabolic plasticity under stress, echoing previous observations of genotype-specific differences in grapevine adaptation [3, 12, 16]. The best biochemical performance in both cultivars was consistently observed under sawdust mulch combined with 1.0 mM SA, aligning closely with earlier reports that integrated mulching and SA spraying improved vegetative growth and stress-related traits in Halwani and Kamali vines [11]. Collectively, these findings validate the working hypothesis that appropriate mulching particularly sawdust together with optimized SA foliar sprays can synergistically enhance biochemical resilience, reduce oxidative damage, and stabilize key physiological functions in grapevines grown under warm, moisture-limited environments [1-4, 7-11, 13-18].

Discussion

The results of this research clearly demonstrate that mulching practices and salicylic acid (SA) foliar applications exert substantial influence on the biochemical resilience of Halwani and Kamali grapevines under warm and moisture-limited conditions. The observed improvements in total chlorophyll content, membrane stability index (MSI), antioxidant enzyme activity, and

osmolyte accumulation align strongly with established physiological theories regarding microclimate modification and hormonal signalling in perennial fruit crops [1-3, 7-11]. Sawdust mulch consistently outperformed charcoal and non-mulched controls, likely due to its ability to moderate soil temperature fluctuations, increase water retention, and enhance microbial activity factors long associated with improved vine physiology and stress tolerance [4-6, 15]. The higher chlorophyll levels under sawdust mulch support the premise that reduced heat load and evaporation from the root zone help maintain pigment stability by minimizing oxidative degradation, a trend comparable to earlier findings in grapevine and other horticultural systems [12-14]. Charcoal mulch, although beneficial in conserving soil moisture, provided comparatively weaker improvements, possibly due to its greater heat-absorbing properties and limited contribution to soil organic matter formation, as previously documented in mulch-based field studies [6, 15].

The strong positive response of antioxidant enzymes and proline to SA sprays confirms the role of SA as a central mediator in plant defense signalling, activating processes that mitigate the accumulation of reactive oxygen species and protect cellular structures [7-11, 17, 18]. The progressive increase in SOD activity with higher SA concentrations suggests amplified ROS-scavenging capability, consistent with studies showing SA-induced activation of antioxidant pathways in stressed grapevines [9-11]. Similarly, elevated proline levels in SA-treated vines indicate enhanced osmotic protection and membrane stabilization, reinforcing the notion that SA contributes not only to oxidative defense but

also to cellular osmotic adjustment [13, 17, 18]. The synergy between SA application and sawdust mulch evident from the highest biochemical performance under this combination demonstrates the compound benefits of integrating microclimate management with hormonal regulation. This aligns with the findings of Bayz *et al.*, who reported improved vegetative growth traits when sawdust or charcoal mulch was combined with salicylic acid sprays in Halwani and Kamali cultivars [11]. However, the current research extends those observations by providing evidence of deeper biochemical modulation, particularly in antioxidant capacity and membrane integrity.

The slight but consistent superiority of Halwani over Kamali in several biochemical markers suggests varietal differences in physiological plasticity. These results echo previous reports indicating genotype-specific variation in stress responses among grapevine cultivars, where differences in oxidative enzyme profiles, chlorophyll stability, and osmolyte accumulation determine cultivar resilience [3, 12, 16]. Although both cultivars benefited from SA and mulching treatments, Halwani's marginally stronger responses may reflect inherent differences in its metabolic architecture that better accommodate exogenous hormonal signalling and improved microenvironmental conditions.

The collective findings strongly support the hypothesis that combining mulching particularly sawdust with salicylic acid foliar sprays produces synergistic improvements in grapevine biochemical stability. This synergy likely arises from the interaction between moderated soil conditions and enhanced systemic biochemical defense, resulting in reduced oxidative stress, greater pigment preservation, and stronger osmotic regulation [1-4, 7-11, 13-18]. The consistency of response across multiple biochemical indicators underscores the robustness of this integrated strategy for managing grapevines in warm, resource-limited environments.

Conclusion

The present investigation demonstrates that the combined application of mulching and salicylic acid foliar sprays offers a highly effective strategy for enhancing the biochemical resilience of Halwani and Kamali grapevines grown under warm and moisture-stressed environments. The findings reveal that sawdust mulch consistently produced the most stable improvements in chlorophyll content, membrane integrity, antioxidant enzyme activity, and osmolyte accumulation, indicating its superior ability to regulate soil microclimate and reduce oxidative pressure on vine tissues. Charcoal mulch also provided measurable benefits, though to a lesser extent, reaffirming that mulches differ in their contributions to vine physiology depending on their thermal and organic properties. Salicylic acid, applied at incremental concentrations, significantly strengthened antioxidative defenses and osmotic balance, confirming its role as a potent biochemical modulator in stress mitigation. The combined use of sawdust mulch and 1.0 mM salicylic acid, in particular, yielded the highest levels of physiological stability, suggesting that soil-based microclimate regulation and hormonal stimulation interact synergistically to protect grapevines against environmental stresses. Based on these insights, several practical recommendations can be proposed for vineyard management under semi-arid and heat-prone conditions: growers should prioritize the use of organic mulches such as sawdust around the base of vines to reduce soil temperature

variability, conserve moisture, and improve soil structure; they should adopt salicylic acid foliar sprays at concentrations around 0.5-1.0 mM during key phenological stages such as pre-flowering, fruit set, and early berry development to enhance antioxidant activity and maintain optimal physiological function; vineyard soil should be monitored regularly to ensure mulch thickness is maintained at an effective level of 5-7 cm without direct stem contact to avoid moisture-related stem issues; foliar spray schedules should be coordinated to avoid excessive heat periods to maximize absorption and avoid phytotoxicity; and growers should consider integrating these practices into broader vineyard management regimes, including regulated irrigation and nutrient-balanced fertilization, to achieve a comprehensive and sustainable stress-mitigation strategy. Overall, this research provides strong evidence that the thoughtful integration of mulching and salicylic acid sprays can significantly improve vine health, productivity potential, and long-term vineyard resilience, offering a practical and environmentally aligned approach to managing grapevines in challenging climatic conditions.

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