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Assessment of Some Grapevine Cultivars Against Powdery Mildew Disease and Enhancing Biochemical Resistance Using Some Compounds Under Field Conditions

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Abstract: Powdery mildew, caused by the obligate ascomycete *Erysiphae necator* Schw., remains a primary threat to viticulture worldwide. This study evaluated the susceptibility of several grapevine cultivars to powdery mildew and investigated the potential of various chemical compounds to enhance biochemical resistance under field conditions. Among the assessed cultivars, King Ruby exhibited the highest disease severity, followed by Thompson Seedless, whereas Prime and Starlight displayed the greatest resistance. The results demonstrated a significant reduction in total chlorophyll content in infected leaves compared to healthy ones, revealing a consistent inverse correlation between chlorophyll levels and disease intensity across all cultivars. Conversely, proline accumulation increased significantly following infection; resistant cultivars (Prime, Starlight, and Crimson) maintained the highest proline levels, while King Ruby and Thompson Seedless recorded the lowest. Regarding fruit quality, healthy berries from Prime and Starlight cultivars showed superior Total Soluble Solids (TSS%) compared to Thompson Seedless. Conversely, the highest Titratable Acidity (TA%) was observed in Thompson Seedless and King Ruby. Field treatments revealed that all tested chemicals significantly mitigated powdery mildew severity on the susceptible cv. King Ruby compared to the control. Calcium silicate, Potassium silicate, and Ataclawed demonstrated the highest efficacy, while micronized sulfur, calcium carbonate, and potassium bicarbonate showed moderate suppressive effects. Notably, all treatments enhanced the average fruit yield (kg/vine), with potassium bicarbonate and Ataclawed yielding the highest results. Furthermore, biochemical assays confirmed that all treatments stimulated the activities of defense-related enzymes, specifically peroxidase (PO) and polyphenol oxidase (PPO), alongside a marked accumulation of total phenolic content. These findings suggest that silicate-based compounds and Ataclawed offer promising, eco-friendly alternatives for integrated powdery mildew management in vineyards.

Key words: Potassium bicarbonate, Potassium silicate. TSS, PPO, PO

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the most widely cultivated fruit crops worldwide, with approximately 25 million feddans grown under temperate and tropical climatic conditions for fresh consumption, food processing, and medicinal purposes. It is considered among the most economically important and popular fruit crops globally. In Egypt, grapevine is extensively cultivated and includes both early- and late-ripening cultivars. Flame Seedless is regarded as the principal early-ripening seedless table grape cultivar. Grapes rank second among fruit crops in Egypt, with an estimated cultivated area of 205,497.88 feddans and a total production of 1,923,499.33 metric tons (**FAO, 2023**).

Powdery mildew, caused by the ascomycete *Erysiphe necator* Schw. [syn. *Uncinula necator* (Schw.) Burr., anamorph *Oidium tuckeri* Berk.], is one of the most destructive diseases affecting grapevine worldwide (**Gadoury *et al.,* 2012**). Because the pathogen is an obligate parasite and highly virulent on *Vitis vinifera*, repeated fungicide applications are generally required for effective disease management (**Pearson and Gadoury, 1992**). The disease is particularly severe in hilly and foothill vineyards characterized by high temperatures, wind, and moderate rainfall (**Essling *et al.,* 2021**).

According to **Gadoury *et al.* (2012)**, the pathogen overwinters as mycelium within dormant buds, producing infected shoots known as “flag shoots,” which are characterized by short internodes and leaves with rounded edges covered by whitish fungal colonies. Chasmothecia, which release ascospores capable of initiating new infections, also serve as important sources of inoculum. Since *E. necator* is a heterothallic ascomycete, chasmothecia are not present in all grape-growing regions. Therefore, management of overwintering inoculum and bud contamination should begin immediately after bud break (**Sawant *et al.,* 2015**).

Cultural practices such as green pruning to improve canopy aeration and agronomic techniques that reduce excessive vegetative growth are strongly recommended. Dense vegetation creates shaded and humid microclimates favorable for disease development. Under such conditions, both the frequency and severity of infections increase, while fungicide distribution becomes less effective, making disease management more difficult (**Austin *et al.,* 2011**).

Synthetic fungicides are commonly used to minimize the harmful effects of fungal diseases. However, according to **Tripathi and Dubey (2004)**, excessive use of these fungicides negatively affects human health and contributes to environmental pollution. Although sulfur-based compounds are not classified as highly hazardous pesticides, reducing their use is consistent with sustainable plant protection strategies. Due to its extensive application in viticulture, particularly in organic production systems, sulfur may also contribute to environmental contamination. Increased sulfate accumulation in soil and surface water adversely affects geological, chemical, and biological processes **(Hinckley *et al.,* 2020)**. Furthermore, sulfur has been reported to be toxic to natural antagonists of *E. necator*, thereby favoring the development of secondary pests **(Calvert and Huffaker, 1974). Magarey *et al.* (2000)** also indicated that sulfur applications under warm and humid conditions may induce phytotoxicity. Nevertheless, sulfur spraying at 600 g/100 L was reported to effectively control powdery mildew and improve grape yield compared with untreated plants (**Emmett *et al.,* 2003**). In addition, sulfur dioxide (SO_2) preservatives efficiently reduced infections without adversely affecting beneficial microorganisms (**Zhou *et al.,* 2019**).

Salts are widely used in the food industry because they are inexpensive, non-toxic, environmentally safe at appropriate concentrations, and generally accepted by consumers. Potassium, sodium, and calcium salts have been shown to inhibit the growth of *Botrytis cinerea* effectively (**Soltan *et al.,* 2006**). Moreover, different potassium and calcium salts applied before and after harvest reduced disease incidence in table grapes (**Soltan *et al.,* 2008**).

Silicon-based products are also considered environmentally friendly alternatives to synthetic fungicides. Numerous studies have investigated foliar applications of potassium silicate, sodium silicate, and silicic acid compounds (**Laane, 2018**). Application of potassium silicate (K_2SiO_3) to cucumber, muskmelon, zucchini squash, strawberry, wheat, and grapevine reduced powdery mildew severity while only slightly affecting plant growth and yield (**Guével *et al.,* 2007**). However, under severe disease pressure, potassium silicate treatments sometimes failed to provide adequate disease control in grapevines (**Reynolds *et al.*, 1996**). Foliar applications of silicic acid have also been reported to improve growth, yield, and fruit quality in several crops (**Soratto *et al.*, 2012**). These treatments significantly reduced diseases such as papaya smallpox, potato late blight, and tomato powdery mildew (**Realpe and Laane, 2008**).

Silicon applications have been associated with increased mechanical resistance in plant tissues (**Epstein, 2001**). This enhanced resistance is mainly attributed to the reinforcement of structural barriers such as cell walls and cuticles, which limit pathogen penetration in many pathosystems (**Van Bockhaven, 2013**). Such reinforcement restricts both mechanical penetration and enzymatic degradation by pathogens (**Sousa *et al.*, 2013**). In addition to these physical effects, silicon-mediated disease resistance is associated with increased accumulation of phenolic compounds, flavonoid phytoalexins, and enhanced activities of defense-related enzymes and antimicrobial compounds, including chitinases, peroxidases, and polyphenol oxidases (**Fortunato *et al.*, 2012**). Silicon also activates plant defense signaling pathways involving ethylene, salicylic acid, and jasmonic acid (**Ghareeb *et al.*, 2011**). Furthermore, silicon-induced resistance is linked to the regulation of defense-related genes and proteins (**Fauteux *et al.*, 2005**).

Defense-related enzymes play essential roles in enhancing plant resistance against pathogens (**Takuo *et al.*, 1993**). Oxidative enzymes such as peroxidase (PO) and polyphenol oxidase (PPO) catalyze the formation of oxidative phenolic compounds, including lignin, which strengthen plant cell walls and contribute to defense responses (**Avdiushko *et al.*, 1993**). Polyphenol oxidase is particularly important in defense against fungal and bacterial pathogens through the oxidation of phenolic compounds into quinones (**Hassan and Abo-Elyousr, 2013**). PPO-generated reactive quinones are also associated with lignification processes that protect wounded plant tissues from infection (**Morishita *et al.*, 2003**). Peroxidase activity has similarly been linked to hypersensitive responses, papilla formation, and lignin polymerization from monolignols (**Sorokan and Maksimov, 2013**).

Therefore, the present study was conducted under Egyptian field conditions to evaluate the resistance of certain grapevine cultivars to powdery mildew disease and to compare the effects of selected salt applications and chemical treatments on disease severity, vine yield, and fruit quality.

2. Materials and Methods

1. Disease Severity Assessment of Grapevine Powdery Mildew

The present study was conducted during 2025 and 2026 growing seasons in Giza Governorate, Egypt. A field survey of grapevine powdery mildew was carried out on 8–10-year-old vines of the following cultivars: 'Crimson', 'King Ruby', 'Thompson Seedless', 'Superior Seedless', 'Flame Seedless', 'Prime', and 'Starlight'. For each cultivar, fifteen vines were randomly selected and divided into three replicates to evaluate the disease under conditions of natural infection. Disease severity, defined as the percentage of leaf area or clusters covered by sporulating fungal colonies, was estimated according to the methodology described by **El-Morsi *et al.* (2012).** Infection levels were classified into five categories using a 0–5 rating scale: 0 = no visual infection; 1 = 1–5%; 2 = 6–25%; 3 = 26–50%; 4 = 51–75%; and 5 = more than 75% of the leaf area or cluster surface covered by fungal colonies.

Disease severity (%) = $(\Sigma (n \times v) / 5 N) \times 100$.

Whereas:

n = number of infected leaves or clusters in each category, v = numerical values of each category and N = total number of the infected leaves or clusters.

2. Physiological and biochemical measurements

2.1. Chlorophyll content and reduction percentage

Chlorophyll content was determined using a portable chlorophyll meter (SPAD-502, Minolta, Japan). SPAD readings were converted to chlorophyll content (mg mL^{-1}) according to the method described by **Monje and Bugbee (1992)**.

The percentage reduction in chlorophyll content was calculated using the following equation:

Reduction (%) = {(Chlorophyll content in healthy leaves−Chlorophyll content in infected leaves)/ Chlorophyll content in healthy leaves} ×100

2.2. Proline content and accumulation

Proline content in grapevine leaves was determined according to the method of **Bates *et al.* (1973)**. Fresh leaf samples (100 mg) were homogenized in 10 mL of 3% sulfosalicylic acid using a mortar and pestle. The homogenate was centrifuged at 5000 rpm for 10 min, and the supernatant was collected. The acid ninhydrin reagent was prepared by dissolving 1.25 g ninhydrin in 30 mL glacial acetic acid, followed by the addition of 20 mL of 6 M phosphoric acid. For the assay, 2 mL of plant extract was mixed with 2 mL of acid ninhydrin reagent and 2 mL of glacial acetic acid in a test tube. The mixture was heated in a boiling water bath (100°C) for 1 h, then immediately cooled in an ice bath to terminate the reaction. Subsequently, 4 mL of toluene was added, and the mixture was vigorously shaken for several seconds. The chromophore-containing toluene layer was separated, and its absorbance was measured at 520 nm using toluene as a blank. Proline concentration was expressed as mg g^{-1} fresh weight.

The percentage increase in proline content was calculated as follows:

Increase (%) = {(Proline content in infected leaves−Proline content in healthy leaves) /Proline content in infected leaves} ×100

3. Fruit quality attributes

3.1. Total soluble solids (TSS)

Total soluble solids (TSS %) were determined according to **Mehaisen (1999)**. Fresh grape juice samples were prepared and measured using a hand refractometer (Model BX-1, Brix 0–32%).

3.2. Titratable Acidity (TA%)

Total acidity of grape berries was determined by titrating 10 mL of grape juice against 0.1 N NaOH using phenolphthalein as an indicator. Acidity percentage was calculated as tartaric acid according to **Soltan (2016)**.

4. Field Experiment and treatment application

This investigation was conducted over two consecutive seasons (2025 and 2026) in a private vineyard located in Giza Governorate, Egypt, to evaluate powdery mildew severity on the leaves and clusters of cv. King Ruby. The selected grapevines were eight years old, planted in sandy soil at a spacing of 2 × 2.75 meters, and supported by a drip irrigation system. The vines were cane-pruned and trellised using the Spanish Paron system. Pruning was carried out during the second week of January in both seasons, maintaining a consistent crop load of 60 buds per vine. Each treatment was applied to 15 vines, organized into three replicates. The tested chemical compounds (Table 1) were administered as foliar sprays four times at 15-day intervals. The initial application commenced upon the first of March of 2025 and 2026. Control vines were sprayed with water under the same conditions.

5. Disease assessment and treatment efficacy

Before each spraying time, disease severity % was evaluated as mentioned before and the efficacy (%) was calculated using the following scale according to **Mousa *et al*. (2006).**

Efficacy (%) = ((a-b)/a) ×100

Where:

a = disease severity (powdery mildew %) in the control.

b = disease severity (powdery mildew %) in treated trees.

6. Tested Chemical Compounds and Application Rates (Table 1).

Product	Chemical formula	Conc./100 L water
Micronic sulfur 80%WP	Sulphur	250g
Calcium silicate	$CaSiO_3$	100g
Calcium carbonate	$Ca(HCO_3)_2$	100g
Potassium bicarbonate	$KHCO_3$	100g
Potassium silicate	K_2SiO_3	100g
Ataclawed 38%WG	Boscalid+ Pyraclostrobin	50g

7. Defense-related enzyme activities and total phenolic content

To evaluate the effect of the tested commercial products at the concentrations presented in Table (1) on defense-related enzyme activities and total phenolic content in grapevine cv. King Ruby, 0.5 g of fresh leaf tissue was homogenized at 0–4°C in 3 mL of 50 mM Tris-HCl buffer (pH 7.8) containing 1 mM EDTA-Na_2 and 7.5% polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 12,000 rpm for 20 min at 4°C, and the resulting supernatants were used for enzyme assays.

Enzyme activities were determined spectrophotometrically using a UV-160A spectrophotometer, and all measurements were conducted at 25°C. Peroxidase (PO) activity was assayed according to **Hammerschmidt *et al*. (1982)**, while polyphenol oxidase (PPO) activity was determined following the method of **Malik and Singh (1980)**.

Total phenolic content (TPC) was estimated according to **Meighani *et al.* (2014)** and expressed as mg gallic acid equivalent (GAE) per 100 mL of grapevine leaf extract. The activities of oxidative enzymes (PO and PPO) and total phenolic content were determined two days after the final spray application.

8. Yield assessment (kg vine^{-1})

At harvest, when berry total soluble solids (TSS) in the control treatment reached approximately 15–16%, five clusters per vine were randomly collected and weighed. The average cluster weight was multiplied by the total number of clusters per vine to estimate the average yield per vine according to **Ahmed (2018)**.

The percentage increase in yield was calculated using the following equation:

Increase in yield (%) = ((Treatment yield − Control yield)/ Treatment yield) ×100

9. Statistical analysis

All obtained data from both experimental seasons were subjected to analysis of variance (ANOVA) according to **Snedecor and Cochran (1990)**. Mean comparisons were performed using Duncan's Multiple Range Test at the 5% probability level (**Duncan, 1955**).

3. Results

1. Evaluation of cultivar susceptibility to powdery mildew

1.1. Disease severity (DS)

Field evaluation of the seven grapevine cultivars during 2025 and 2026 growing seasons revealed significant differences ($P < 0.05$) in their susceptibility to powdery mildew caused by *Erysiphae necator* (Table 2). Disease severity (DS) varied markedly among cultivars, indicating substantial genetic variability in host response under natural field conditions. Among the tested cultivars, King Ruby consistently exhibited the highest susceptibility, recording the greatest disease severity on both leaves and clusters throughout the assessment period. The maximum DS reached 14.66% during May in both seasons, confirming the high vulnerability of this cultivar to powdery mildew infection. Thompson

Seedless also showed high susceptibility, particularly during the late stages of the growing season, where disease development increased markedly on grape clusters.

In contrast, Prime and Starlight were identified as the most resistant cultivars. Both cultivars remained free from visible symptoms during the early evaluation period (March), with only slight disease development detected toward the end of the season. Their consistently low DS values across both years indicate a stable and durable level of resistance under field conditions. Crimson, Superior Seedless, and Flame Seedless exhibited intermediate reactions, characterized by moderate disease severity values that increased gradually over time. This intermediate response suggests partial resistance or tolerance mechanisms that may limit, but not completely suppress, pathogen development.

A clear temporal trend was observed across all cultivars, with disease severity progressively increasing from March to May. This pattern likely reflects the increasing suitability of environmental conditions during late spring and early summer for pathogen establishment, sporulation, and secondary spread. The relatively similar disease responses recorded during the two consecutive seasons further suggest that cultivar reaction was predominantly determined by genetic background rather than seasonal fluctuations in environmental conditions. Generally, the obtained results clearly distinguish King Ruby as a highly susceptible cultivar suitable for subsequent disease management and chemical induction studies, whereas Prime and Starlight may represent promising genetic sources of resistance for grapevine breeding programs aimed at improving powdery mildew resistance.

Table (2). Evaluation of cultivar susceptibility to powdery mildew

Cultivars	2025						2026					
	March		April		May		March		April		May	
	[a)]DS		DS		DS		DS		DS		DS	
	[b)]C	[ca)]L	C	L	C	L	C	L	C	L	C	L
Crimson	5.33c [d)]	5.33b	5.33c	6.66c	5.33c	6.66c	1.66d	2.66d	1.66e	2.66c	1.66e	2.66d
King Roby	11.66a	9.33a	12.00a	14.60a	12.00a	14.66a	11.33a	6.66b	11.33a	9.33b	12.00a	14.66a
Thompson seedless	8.33b	5.33b	8.33b	13.33b	8.33b	13.33b	4.66b	10.66a	8.00b	13.66a	11.00b	13.66b
Superior seedless	1.66e	5.33b	1.66e	6.66c	1.66e	6.66c	1.66d	5.33c	3.33d	9.33b	3.33d	9.33c
Flame seedless	3.33d	5.33b	3.33d	6.66c	3.33d	6.66c	3.33c	5.33c	6.66c	9.33b	6.66c	9.33c
Prime	0.00f	0.00c	0.00f	5.33d	0.00f	5.33d	0.00e	0.00e	0.00f	0.00d	0.00f	0.00e
Starlight	0.00f	0.00c	0.00f	3.33e	0.00f	3.33e	0.00e	0.00e	0.00f	0.00d	0.00f	0.00e

[a)]DS= Disease severity [b)]C =Clusters [c)]L= leaves
[d)] The treatments with the same letter or letters in the same column did not differ significantly at P≤0.05.

1.2. Total Chlorophyll Content

The results presented in Table 3 demonstrated that powdery mildew infection significantly affected ($P \leq 0.05$) total chlorophyll content in grapevine leaves across all evaluated cultivars during 2025 and 2026 growing seasons. In general, infected leaves exhibited a pronounced decline in chlorophyll concentration compared with healthy leaves, indicating the detrimental effect of *Erysiphe necator* infection on the photosynthetic machinery of grapevine plants. The magnitude of chlorophyll reduction varied considerably among cultivars, reflecting differences in their susceptibility to powdery mildew. King Ruby, identified previously as the most susceptible cultivar, exhibited the greatest decline in chlorophyll content, with reduction percentages reaching 33.64% in 2025 and 20.00% in 2026. Similarly, Superior Seedless and Flame Seedless showed substantial decreases in chlorophyll levels, with reductions exceeding 20% under severe infection conditions.

In contrast, the resistant cultivars Prime and Starlight maintained comparatively higher chlorophyll stability under infection stress. These cultivars recorded the lowest reduction percentages, reaching only 8.85% in 2025 and approximately 9.5–10.7% during 2026, indicating a greater ability to preserve photosynthetic efficiency despite pathogen challenge. Although Thompson Seedless exhibited the highest absolute chlorophyll content in both healthy and infected leaves, its relative reduction following infection was greater than that observed in the resistant cultivars, suggesting that high baseline chlorophyll content alone does not necessarily confer enhanced disease tolerance. Overall, the obtained results reveal a strong inverse relationship between powdery mildew severity and chlorophyll retention.

Table (3). Impact of powdery mildew infection on leaf total chlorophyll content during 2025 and 2026 seasons

Cultivars	Total chlorophyll on leaves (mg/ ml^{-1})					
	Season 2025			Season 2026		
	Healthy	Infected	Reduction (%)	Healthy	Infected	Reduction (%)
Crimson	15.66d	13.61b	13.09	16.80e	14.00b	16.64
King Roby	17.33b	11.50e	33.64	17.50 c	14.00b	20.00
Thompson seedless	19.33a	16.45a	14.89	20.22a	18.30a	9.49
Superior seedless	16.50c	12.55d	23.93	17.20d	13.30d	22.67
Flame seedless	16.50c	13.50c	18.18	18.50b	13.50c	27.02
Prime	13.77e	12.55d	8.85	14.7g	13.30d	9.52
Starlight	13.77e	12.55d	8.85	14.9f	13.30d	10.73

The treatments with the same letter or letters in the same column did not differ significantly at $P \leq 0.05$.

1.3. Proline Accumulation

The results presented in Table 4 demonstrated a significant increase ($P \leq 0.05$) in leaf proline content in all evaluated grapevine cultivars following powdery mildew infection during 2025 and 2026 growing seasons. The observed accumulation of proline indicates its important role as a physiological and biochemical marker associated with plant responses to biotic stress caused by *Erysiphe necator*. Marked differences among cultivars were observed in their capacity to accumulate proline under infection stress. The resistant cultivars, Starlight and Prime, exhibited the greatest increases in proline content, with accumulation percentages exceeding 60% during 2025 season. Among all cultivars, Starlight recorded the highest proline concentration, reaching 0.84 mg g^{-1} fresh weight in the second season.

In contrast, the highly susceptible cultivar King Ruby showed the lowest capacity for proline accumulation, with increases ranging only between 20.9% and 21.6% compared with healthy leaves. Moderate responses were recorded in Crimson, Superior Seedless, and Flame Seedless, where proline increases ranged approximately from 33% to 43%, reflecting an intermediate level of stress adaptation. The obtained results suggest a strong positive association between proline accumulation and cultivar resistance to powdery mildew. The enhanced proline content observed in resistant cultivars may contribute to improved osmotic adjustment, protection of cellular membranes, stabilization of proteins and enzymatic systems, and scavenging of reactive oxygen species generated during pathogen attack. Consequently, the ability to accumulate proline efficiently may represent an important biochemical component of the defense mechanism involved in grapevine resistance to powdery mildew infection.

Table (4). Accumulation of leaf proline content in response to powdery mildew infection in the seven grapevine cultivars during 2025 and 2026 **seasons**

Cultivars	Proline (mg/g^{-1} fresh wt.)					
	Season 2025			Season 2026		
	Healthy	Infected	Increasing (%)	Healthy	Infected	Increasing (%)
Crimson	0.45b	0.77 c	41.55	0.45b	0.79c	43.03
King Roby	0.47a	0.60g	21.66	0.49a	0.62f	20.96
Thompson seedless	0.39e	0.61f	36.06	0.39d	0.62f	37.09
Superior seedless	0.40d	0.65e	38.46	0.44c	0.68d	35.29
Flame seedless	0.44c	0.66d	33.33	0.44c	0.67e	34.32
Prime	0.32g	0.79b	59.49	0.32e	0.82b	60.97
Starlight	0.33f	0.81a	59.25	0.33e	0.84a	60.17

The treatments with the same letter or letters in the same column did not differ significantly at P≤0.05.

1.4. Total Soluble Solids (TSS)

The results presented in Table 5 revealed that powdery mildew infection significantly affected (P ≤ 0.05) the total soluble solids (TSS%) of grape berries in all evaluated cultivars during 2025 and 2026 growing seasons. In general, infected berries exhibited lower TSS values than healthy berries, indicating that *Erysiphae necator* infection adversely influenced sugar accumulation and fruit maturation processes. Considerable variation among cultivars was observed in both the absolute TSS values and the degree of reduction caused by disease infection. Across both seasons, Starlight consistently maintained the highest TSS content under both healthy and infected conditions, reaching up to 21.30% in healthy berries and 18.00% in infected berries. In contrast, Thompson Seedless recorded the lowest overall TSS values, reflecting a greater negative impact of powdery mildew on berry quality.

Table (5). Effect of Powdery Mildew Infection on Total Soluble Solids (TSS %) during 2025 and 2026 seasons.

Cultivars	Total soluble solids (%)					
	Season 2025			Season 2026		
	Healthy	Infected	Reduction (%)	Healthy	Infected	Reduction (%)
Crimson	18.00d	16.70c	7.22	18.60 d	16.40b	11.82
King Roby	17.90e	15.00e	16.20	17.60e	15.30d	13.06
Thompson seedless	15.60g	13.00f	16.66	15.90g	13.00f	18.23
Superior seedless	18.90c	16.90b	10.58	18.90c	16.00c	15.34
Flame seedless	16.80f	15.00e	10.70	16.50f	15.00e	9.09
Prime	19.60b	16.00d	18.36	19.20b	16.00c	16.66
Starlight	21.30a	18.00a	15.49	21.00a	18.00a	14.28

The treatments with the same letter or letters in the same column did not differ significantly at P≤0.05.

With respect to disease-induced reduction, Thompson Seedless exhibited the greatest decline in TSS, reaching 18.23% during the 2026 season. Similarly, Prime showed a pronounced reduction of 18.36% in 2025. Although King Ruby was previously identified as one of the most susceptible cultivars to powdery mildew infection, the reduction in its TSS values ranged between 13.06% and 16.20%, suggesting that susceptibility to disease severity does not always correspond proportionally with fruit quality deterioration. Conversely, Crimson and Flame Seedless displayed comparatively lower reductions in TSS in some seasonal evaluations, indicating a relatively greater capacity to preserve berry quality under infection stress. These differences among cultivars may reflect variations in physiological tolerance, carbohydrate metabolism, and the efficiency of assimilate translocation during pathogen challenge. Generally, the obtained results demonstrate that powdery mildew infection not only affects vegetative growth but also significantly impairs fruit quality by reducing soluble sugar accumulation in grape berries.

1.5. Titratable Acidity (TA)

The results presented in Table 6 demonstrated that powdery mildew infection significantly affected ($P \leq 0.05$) titratable acidity (TA) in grape berries across all evaluated cultivars during 2025 and 2026 growing seasons. In general, infected berries exhibited lower acidity values than healthy berries, indicating that *Erysiphe necator* infection interfered with normal organic acid metabolism during berry development and ripening. Under healthy conditions, Thompson Seedless and King Ruby consistently recorded the highest TA values, reaching up to 0.55%, whereas Starlight exhibited the lowest acidity levels, ranging from 0.33% to 0.36% across both seasons.

The magnitude of TA reduction following infection varied considerably among cultivars and between seasons. During 2025 season, the greatest reduction in acidity was observed in Crimson and Prime, both recording a decrease of 15.38%. In contrast, King Ruby exhibited the highest degree of stability, with only a 3.84% reduction in TA. However, during the 2026 season, the impact of infection became more pronounced in Superior Seedless and Flame Seedless, which exhibited TA reductions of 23.07% and 20.40%, respectively. Despite the decline caused by infection, King Ruby and Thompson Seedless maintained comparatively higher absolute TA values under infected conditions than the remaining cultivars.

Table (6). Total acidity (TA) in seven grapevine cultivars grown under natural infection by powdery mildew during 2025 and 2026 seasons

Cultivars	TA = Total acidity					
	Season 2025			Season 2026		
	Healthy	Infected	Reduction (%)	Healthy	Infected	Reduction (%)
Crimson	0.39e	0.33c	15.38	0.37e	0.32d	13.51
King Roby	0.52c	0.50a	3.84	0.55a	0.50a	9.09
Thompson seedless	0.55a	0.50a	9.09	0.55a	0.30e	9.09
Superior seedless	0.53b	0.50a	5.66	0.39d	0.30e	23.07
Flame seedless	0.47d	0.43b	8.51	0.49b	0.39b	20.40
Prime	0.39e	0.33c	15.38	0.40c	0.33c	17.50
Starlight	0.33f	0.30d	9.09	0.36f	0.30e	16.66

The treatments with the same letter or letters in the same column did not differ significantly at $P \leq 0.05$.

2. Evaluation of Chemical Treatments Against Powdery Mildew

2.1. Disease Suppression Efficiency

The results presented in Tables 7 and 8 demonstrated that all tested chemical treatments significantly reduced powdery mildew severity ($P \leq 0.05$) on both leaves and clusters of the highly susceptible grapevine cultivar King Ruby during the 2025 and 2026 growing seasons, compared with the untreated control. The obtained data clearly indicated substantial differences among the evaluated compounds in their ability to suppress *Erysiphe necator* under field conditions.

During the early stage of both seasons (March), all treatments provided complete protection against powdery mildew infection, achieving 100% efficacy and maintaining vines free from visible disease symptoms on leaves and clusters. However, as the season progressed and environmental conditions became increasingly favorable for pathogen development during April and May, distinct variations in treatment performance became evident. Among the tested compounds, calcium silicate consistently exhibited the highest efficacy throughout both seasons, maintaining complete disease suppression with 0.00% disease severity on both leaves and clusters until the end of the experimental period. Similarly, potassium silicate and Boscalid + Pyraclostrobin (Ataclawed) demonstrated highly stable and prolonged protective effects, achieving complete disease control during the late stages of the season and effectively preventing disease establishment under high infection pressure.

Table (7). Efficacy of some chemical treatments against powdery mildew on cv. King Roby during season 2025

Treatment	March				April				May			
	*DS		*Eff		*DS		*Eff		*DS		*Eff	
	*C	*L	*C	*L	*C	*L	*C	*L	*C	*L	*C	*L
Micronic sulfur	0.00b	0.00b	100	100	1.66b	5.33b	86.16	61.39	3.33b	5.33b	72.25	63.64
Calcium silicate	0.00b	0.00b	100	100	0.00c	0.00c	100	100	0.00d	0.00c	100	100
Calcium carbonate	0.00b	0.00b	100	100	1.66b	5.33b	86.16	61.39	3.33b	5.33b	72.25	63.64
Potassium bicarbonate	0.00b	0.00b	100	100	1.66b	5.33b	86.16	61.39	1.66c	5.33b	86.16	63.64
Potassium silicate	0.00b	0.00b	100	100	0.00c	0.00c	100	100	0.00d	0.00c	100	100
Ataclawed	0.00b	0.00b	100	100	0.00c	0.00c	100	100	0.00d	0.00c	100	100
Control	11.66a	9.33a	--	--	12.00a	14.66a	--	--	12.00a	14.66a	--	--

[a)]DS= Disease severity [b)]C =Clusters [c)]L= leaves
[d)] The treatments with the same letter or letters in the same column did not differ significantly at $P \leq 0.05$.

In contrast, Micronic sulfur, calcium carbonate, and potassium bicarbonate showed a progressive decline in efficacy as disease pressure increased. During May of both seasons, these treatments recorded higher disease severity values on leaves, accompanied by noticeable reductions in efficacy. In the 2025season, their efficacy against leaf infection declined to approximately 63.64%, whereas during the 2026 season efficacy decreased further to nearly 50.00%. Despite this reduction, potassium bicarbonate maintained relatively better protection of grape clusters than Micronic sulfur and calcium carbonate, with cluster efficacy remaining above 71.44% under severe infection conditions. The untreated control vines consistently exhibited the highest disease severity levels throughout both seasons, confirming the high susceptibility of King Ruby to powdery mildew infection and emphasizing the importance of effective disease management strategies under Giza field conditions. Overall, the obtained results highlight the superior and consistent performance of silicate-based compounds and Ataclawed compared with conventional sulfur- and bicarbonate-based treatments.

Table (8). Efficacy of some chemical treatments against powdery mildew on cv. King Roby during season 2026

Treatment	March				April				May			
	*DS		*Eff		*DS		*Eff		*DS		*Eff	
	*C	*L	*C	*L	*C	*L	*C	*L	*C	*L	*C	*L
Micronic sulfur	1.00b	0.00b	87.5	100	1.20 c	5.33b	85.00	49.71	3.33b	5.33b	71.44	50.00
Calcium silicate	0.00c	0.00b	100	100	0.00d	0.00c	100	100	0.00d	0.00c	100	100
Calcium carbonate	0.00c	0.00b	100	100	1.66b	5.33b	80.00	49.71	1.66c	5.33b	85.76	50.00
Potassium bicarbonate	1.00b	0.00b	87.5	100	1.66b	5.33b	80.00	49.71	1.66c	5.33b	85.76	50.00
Potassium silicate	1.00b	0.00b	87.5	100	0.00d	0.00c	0.00	100	0.00d	0.00c	100	100
Ataclawed	0.00c	0.00b		100	0.00d	0.00c	0.00	100	0.00d	0.00c	100	100
Control	8.00 a	10.66 a	--	--	8.00a	10.66a	--	--	11.66a	10.66 a	--	--

[a)]DS= Disease severity [b)]C =Clusters [c)]L= leaves
[d)] The treatments with the same letter or letters in the same column did not differ significantly at P≤0.05.

2.2. Effect on Yield Parameters

The results presented in Table 9 demonstrated that all tested chemical treatments significantly increased ($P \leq 0.05$) the average fruit yield (kg vine^{-1}) of the highly susceptible grapevine cultivar King Ruby compared with the untreated control during both the 2025 and 2026 growing seasons. The observed yield enhancement reflected the effectiveness of these treatments in reducing powdery mildew severity and improving vine productivity under field conditions.

Among the evaluated treatments, potassium bicarbonate consistently produced the highest yield values, recording 27.00 kg vine^{-1} in 2025 and 27.50 kg vine^{-1} in 2026. These increases corresponded to yield improvements of approximately 40.74% and 39.27%, respectively, relative to the untreated control. Ataclawed (Boscalid + Pyraclostrobin) ranked second, producing significantly high yields of 25.00 and 26.60 kg vine^{-1} during the two successive seasons. Potassium silicate and calcium silicate/carbonate treatments also resulted in considerable yield enhancement, with increases ranging from 27.39% to 30.43% over the control treatment. These findings further support the positive role of silicate-based compounds in maintaining vine health and productivity under powdery mildew infection pressure.

Table (9). Impact of some chemical treatments on the yield of grapevine during 2025 and 2026

Treatments	2025		2026	
	Yield (kg/vine)	Increases %	Yield (kg/vine)	Increases %
Micronic sulfur	19.50e	17.94	19.80e	15.65
Calcium silicate	22.50d	28.88	23.00d	27.39
Calcium carbonate	22.50d	28.88	23.00d	27.39
Potassium bicarbonate	27.00a	40.74	27.50a	39.27
Potassium silicate	23.00c	30.43	24.00c	30.41
Ataclawed	25.00b	36.00	26.60b	37.21
Control	16.00f	--	16.70f	--

The treatments with the same letter or letters in the same column did not differ significantly at P≤0.05.

In contrast, Micronic sulfur produced the lowest yield values among the treated vines, ranging from 19.50 to 19.80 kg vine^{-1} across the two seasons. Nevertheless, despite its comparatively lower performance, Micronic sulfur still significantly outperformed the untreated control, which consistently recorded the lowest yields (16.00 and 16.70 kg vine^{-1} in 2025 and 2026, respectively). At general the obtained results indicate that effective suppression of powdery mildew was directly associated with improved grapevine productivity. Although silicate-based compounds and Ataclawed (Boscalid + Pyraclostrobin) exhibited superior disease control efficiency, potassium bicarbonate and Ataclawed (Boscalid + Pyraclostrobin) appeared to provide additional beneficial effects on vine vigor and fruit productivity.

2.3. Effect on Defense Enzymes and Phenolic Compounds

2.3.1. In leaves of grapevine

The biochemical analysis presented in Table 10 demonstrated that all tested treatments significantly enhanced the defense response of grapevine plants through the activation of defense-related enzymes and the accumulation of total phenolic compounds compared with the untreated control. These findings indicate that the evaluated compounds not only suppress powdery mildew development directly but also stimulate host biochemical defense mechanisms associated with resistance to *Erysiphe necator*. Regarding oxidative enzyme activity, calcium silicate and potassium silicate exhibited the strongest stimulatory effects, recording the highest peroxidase (PO) activities of 0.45 and 0.44 units, respectively. The same treatments also induced the greatest increase in polyphenol oxidase (PPO) activity, with both compounds reaching 1.30 units. In contrast, the untreated control exhibited the lowest enzyme activities, recording only 0.36 units for PO and 0.90 units for PPO. Potassium bicarbonate and calcium carbonate also enhanced the activities of both enzymes, although to a lesser extent than the silicate-based treatments.

A pronounced increase in total phenolic content (TPC) was also observed in response to all tested treatments. Potassium silicate induced the highest accumulation of phenolic compounds (0.69), followed by calcium silicate (0.66) and Ataclawed (0.64). These values represented more than a twofold increase compared with the untreated control, which recorded the lowest phenolic content (0.23). Although Micronic sulfur exhibited comparatively lower efficacy than the silicate-based compounds, it still significantly increased TPC levels, reaching 0.50.

The enhanced activities of PO and PPO enzymes, together with the accumulation of phenolic compounds, suggest that these treatments stimulated important biochemical defense pathways involved in grapevine resistance. Peroxidase and polyphenol oxidase are known to contribute to lignification, oxidation of phenolic substrates, strengthening of cell walls, and inhibition of pathogen development, whereas phenolic compounds possess antimicrobial and antioxidant properties that limit fungal colonization. The obtained results indicate that, the superior performance of calcium silicate, potassium silicate, and Ataclawed in controlling powdery mildew is closely associated with their ability to induce systemic resistance mechanisms through the activation of antioxidant enzymes and the enhancement of fungitoxic phenolic metabolism. Consequently, these compounds may provide a dual mode of action involving both direct disease suppression and stimulation of host defense responses.

Table (10). Effect of some chemical treatments on defense-related enzyme activities and total phenolic content in leaves of grapevine cv. King Ruby

Enzyme & phenol	Control	Effect of the tested products on enzyme activities and phenolic content.					
		Micronic sulphur	Calcium silicate	Calcium carbonate	Potassium bicarbonate	Potassium silicate	Ataclawed
Peroxidase (PO)	0.36b	0.36c	0.45c	0.36c	0.41c	0.44c	0.36c
Polyphenol oxidase (PPO)	0.90a	0.90a	1.30a	0.90a	0.99a	1.30a	0.90a
Total phenolic content (TPC)	0.23c	0.50b	0.66b	0.55b	0.60b	0.69b	0.64b

The treatments with the same letter or letters in the same column did not differ significantly at P≤0.05.

2.3.2. In berries of grapevine

The biochemical response of grape berries (cv. King Ruby) to the tested chemical treatments is presented in Table 11. Similar to the responses observed in leaf tissues, all evaluated compounds significantly enhanced the activities of defense-related enzymes and stimulated the accumulation of phenolic compounds in berry tissues compared with the untreated control ($P \leq 0.05$). These findings indicate that the induced resistance response extended beyond vegetative tissues to include reproductive organs, thereby contributing to enhanced berry protection against powdery mildew infection. Regarding oxidative enzyme activities, calcium silicate and potassium bicarbonate induced the highest peroxidase (PO) activities, recording 0.77 and 0.79 units, respectively, compared with only 0.48 units in the untreated control. Likewise, substantial increases in polyphenol oxidase (PPO) activity were detected in response to all treatments. Calcium silicate produced the highest PPO activity (1.66 units), followed by potassium bicarbonate (1.45 units) and potassium silicate (1.30 units), whereas the control treatment recorded the lowest PPO value (0.99 units).

A significant increase in total phenolic content (TPC) was also observed in berry tissues following treatment application. The highest phenolic accumulation was recorded in berries treated with calcium silicate, potassium silicate, and Ataclawed (Boscalid + Pyraclostrobin), with values ranging from 0.86 to 0.89, compared with only 0.56 in the untreated control berries. These increases clearly demonstrate the ability of the tested compounds to stimulate secondary metabolite production associated with plant defense responses.

The enhanced activities of PO and PPO enzymes, together with the elevated phenolic content in berry tissues, suggest that these treatments effectively activated systemic biochemical defense mechanisms throughout the grapevine plant. Oxidative enzymes may contribute to reinforcement of cell walls, oxidation of phenolic substrates, and restriction of pathogen colonization, whereas phenolic compounds are known to possess antimicrobial and antioxidant properties that inhibit fungal development. At general the present results confirm that silicate-based compounds, potassium bicarbonate, and Ataclawed (Boscalid + Pyraclostrobin) effectively induce systemic resistance not only in leaves but also in grape berries, thereby enhancing their biochemical defense capacity against *Erysiphae necator*.

Table (11). Effect of some chemical treatments on defense-related enzyme activities and total phenolic content in berries of grapevine cv. King Ruby

Enzyme & phenol	Control	Effect of the tested products on enzyme activities and phenolic content.					
		Micronic sulphur	Calcium silicate	Calcium carbonate	Potassium bicarbonate	Potassium silicate	Ataclawed
Peroxidase (PO)	0.48c	0.58c	0.77c	0.58c	0.79c	0.78c	0.48c
Polyphenol oxidase (PPO)	0.99a	0.99a	1.66a	0.99a	1.45a	1.30a	1.00a
Total phenolic content TPC	0.56b	0.80b	0.89b	0.88b	0.86b	0.89b	0.86b

The treatments with the same letter or letters in the same column did not differ significantly at $P \leq 0.05$.

4. Discussion

Grapevines, being deciduous perennial fruit crops, are cultivated worldwide and represent a significant component of the global agricultural economy. The history of grape cultivation dates back approximately 13,000 years in Egypt, where grapes were not only discovered but also depicted on the walls of ancient temples and tombs, including that of Queen Nefertari (**Kassem *et al.*, 2011**). Due to their high nutritional value, distinctive flavor, wide range of uses, and strong economic returns, grapes (*Vitis vinifera* L.) are considered among the most important fruit crops for both domestic and international markets. They are processed into various products such as jams, beverages, juices, raisins, and other derivatives. In addition, grapes possess several medicinal properties and are highly digestible (**Kareem *et al.* 2022**). Globally, grapevine production reaches approximately 77 million tons, ranking fifth among the most produced fruits after bananas, watermelons, apples, and oranges (**FAOSTAT, 2021**).

Powdery mildew is one of the most destructive diseases affecting grapevines. It is caused by *Erysiphe necator*, an obligate ectoparasite of *Vitis* species, particularly *V. vinifera*. Most cultivated varieties are highly susceptible, as the pathogen can infect all green tissues of the vine. Under favorable environmental conditions—moderate temperatures and high relative humidity without rainfall—the pathogen produces multiple infection cycles and abundant conidia, leading to severe yield and economic losses (**Gadoury *et al.*, 2012**). Management of powdery mildew relies heavily on repeated fungicide applications from flowering through version and sometimes until harvest. However, excessive chemical use has negative ecological impacts and contributes to the development of fungicide resistance, thereby reducing long-term efficacy and contradicting sustainability goals (**Dufour *et al.*, 2011**). Therefore, alternative strategies that enhance plant defense mechanisms have gained increasing attention (**Filippi *et al.*, 2019**). In this context, breeding programs have focused on developing resistant cultivars, and several wild species and interspecific hybrids have been identified as potential sources of resistance (**Atak, 2017**).

The cultivar King Ruby, evaluated in this study, is considered a highly valuable table grape due to its attractive fruit quality, late ripening, crisp texture, and high nutritional value (**Al-Obeed *et al.*, 2010; Asmaa and Aboryia, 2020**). However, its susceptibility to powdery mildew necessitates effective disease management strategies. Previous studies have demonstrated the role of silicon in enhancing plant resistance to powdery mildew. For instance, silicon application in cucumber was shown to improve resistance against *Sphaerotheca fuliginea* by enhancing structural and biochemical defenses (**Menzies *et al.*, 1991b; Samuels *et al.*, 1991b**). Both silicon and phenolic compounds were observed to accumulate at infection sites, suggesting a localized defense response (**Menzies *et al*., 1991b**; **Samuels *et al*., 1991a**). Even foliar-applied silicon may be absorbed and temporarily enhance resistance, as evidenced by reduced pathogen colonization after silicon removal prior to inoculation. This suggests that silicon-induced resistance may be rapid but transient in nature (**Samuels *et al.*, 1991b**). Similarly, potassium silicate has been reported to improve postharvest disease resistance and fruit quality, as demonstrated in apple fruits against *Penicillium expansum* (**Tarabih *et al.*, 2014**). These findings support the concept that silicon-based compounds can enhance both pre- and postharvest disease resistance.

Induced resistance has been widely recognized as a sustainable alternative to synthetic fungicides, offering broad-spectrum protection against pathogens (**Yildirim *et al.*, 2002**). However, increasing reports of fungicide resistance in powdery mildew populations have raised concerns about long-term chemical control effectiveness (**Staudt, 1997; Alimad *et al.*, 2017; Singh *et al.*, 2017**). Consequently, integrated disease management strategies involving multiple compounds and repeated seasonal applications are required (**Rhouma *et al.*, 2016; Innerebner *et al.*, 2020; Matrood and Rhouma, 2021a, b**).

Calcium-based treatments have also been shown to enhance fruit quality and disease resistance by strengthening cell wall structure and reducing physiological deterioration. Postharvest calcium applications have been reported to reduce fungal incidence and maintain fruit firmness in crops such as strawberries, peaches, nectarines, and apples (**Dunn and Able, 2006**). In addition, calcium treatments can reduce respiration rate, delay ripening, and improve overall fruit quality (**Raese and Drake, 2006**). Similar effects were reported in papaya, where calcium improved titratable acidity and soluble solids while reducing weight loss and maintaining ascorbic acid content (**Mahmud *et al.*, 2008**).

In organic viticulture, sulfur remains the primary control measure for powdery mildew; however, its repeated application leads to excessive spraying, environmental concerns, and negative effects on beneficial organisms (**Dent, 1995; Gubler, 1996**). Moreover, sulfur residues may negatively affect fruit and wine quality and contribute to environmental pollution (**Calvert and Huffaker, 1974**). Therefore, precise timing of application is essential, as inorganic compounds act preventively rather than curatively (**Rutto *et al*, 2021**). Disease forecasting models can help optimize fungicide application timing and reduce unnecessary treatments (**Chen *et al.*, 2020**). Several studies have demonstrated the effectiveness of fungicide combinations and rotation strategies in controlling powdery mildew. For example, wettable sulfur and mixtures of penconazole, azoxystrobin, and trifloxystrobin have shown high efficacy when applied during critical growth stages (**Margal *et al.*, 1998**). However, limiting strobilurin applications is necessary to delay resistance development (**Wicks and Hitch, 2002**). Similarly, potassium bicarbonate combined with hexaconazole has shown superior control compared to individual fungicides (**Adsule *et al.*, 2008**), while penconazole has also been reported to achieve up to 88.48% disease control (**Thind e*t al.*, 2010**).

At the physiological level, proline accumulation plays a key role in plant stress responses by maintaining osmotic balance and cellular stability (**Kisor *et al*., 2005**). Variations in titratable acidity among grape cultivars have been reported in several studies, reflecting genetic and environmental influences (**El-Morsy *et al.*, 2017; Mohamed and Khairy, 2017; Sharma *et al.*, 2017**). Polyphenol oxidase activity is strongly influenced by cultivar, developmental stage, and environmental conditions (**Sapis *et al.* 1983**). Grapes are rich in polyphenols, with seeds containing the highest proportion (60–70%), followed by skins (28–35%) and pulp (around 10%) (**Sochorova *et al.*, 2020**). Defense-related enzymes such as peroxidase and polyphenol oxidase are closely associated with induced resistance mechanisms. Enhanced activities of these enzymes have been reported in several crops following treatment with elicitors or fungicides, indicating their role in strengthening plant defense responses (**Monir *et al.*, 2021; Martinez *et al.*, 2004; Hassan *et al.*, 2024**).

5. Conclusions

This study revealed clear differences in powdery mildew susceptibility among grapevine cultivars, where cv. King Ruby and Thompson Seedless were the most susceptible, while cv. Prime and cv. Starlight showed the highest levels of resistance. Powdery mildew infection significantly reduced chlorophyll content, while increasing proline accumulation in infected leaves. Fruit quality parameters also varied among cultivars, with higher TSS% in Prime and Starlight, and higher TA% in Thompson Seedless. All tested chemical treatments significantly reduced disease severity in cv. King Ruby, with calcium silicate, potassium silicate, and Ataclawed showing the highest efficacy. Treatments also improved yield compared with the control, with potassium bicarbonate and Ataclawed producing the highest fruit yield. In addition, all treatments enhanced defense-related enzyme activities (PO and PPO) and increased total phenolic content, indicating activation of plant defense mechanisms. Overall, the results confirm that integrating resistant cultivars with effective chemical elicitors improves disease control, physiological performance, and productivity of grapevines under field conditions.

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Conflict of interests

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