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APPLICATION OF HYDROGEN PEROXIDE IN THE PROTECTION OF GRAPEVINE (*VITIS VINIFERA* L.) AGAINST GRAPEVINE TRUNK DISEASES

MOGUĆNOST KORIŠTENJA VODIKOVOG PEROKSIDA U ZAŠTITI VINOVE LOZE (*VITIS VINIFERA* L.) OD BOLESTI DRVA

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ABSTRACT

Grapevine trunk diseases (GTDs) are major contributors to vineyard productivity decline, causing progressive vine deterioration and yield loss. Existing research highlights the need for effective alternative protection methods due to the limitations of conventional chemical treatments. Hydrogen peroxide (H_2O_2) has been identified as a potential treatment because of its antimicrobial properties; however, studies on its use in grapevine protection are still limited. This study aimed to evaluate the effectiveness of H_2O_2 application on symptomatic grapevines in reducing GTD symptoms and its impact on yield and qualitative must parameters. The experiment was conducted during the 2021 growing season on the grapevine cultivars Malvazija istarska and Cabernet Sauvignon. H_2O_2 (10%) at a dose 5 ml was injected into the vines, and treatment efficacy was assessed through a visual evaluation of vine health, as well as analyses of yield, sugar content, pH, and titratable acidity of grape must. Results showed that H_2O_2 application reduced GTD symptoms and promoted vine recovery, particularly when applied at early disease stages. While the treatment significantly improved yield in recovered vines of Malvazija istarska, no significant negative changes were observed in sugar content, pH, or titratable acidity of the must. The treatment has not affected differences in yield of Cabernet Sauvignon, sugar content, pH, or total acidity of the must. Further research is required to optimize treatment conditions and elucidate the mechanisms of H_2O_2 action in grapevine disease management.

Key words: hydrogen peroxide, grapevine trunk diseases, vineyard protection, Malvazija istarska, Cabernet Sauvignon

SAŽETAK

Bolesti drva vinove loze ubrajaju se među glavne čimbenike koji smanjuju produktivnost vinograda, uzrokujući progresivno propadanje trsa i gubitak prinosa. Dosadašnja istraživanja naglašavaju potrebu za učinkovitijim alternativnim metodama zaštite zbog ograničenja konvencionalnih kemijskih tretmana. Vodikov peroksid (H_2O_2) prepoznat je kao potencijalni tretman zbog svojih antimikrobnih svojstava no, istraživanja o njegovoj primjeni u zaštiti vinove loze još su ograničena. Cilj ovog istraživanja bio je procijeniti učinkovitost primjene vodikovog peroksida na simptomatičnim trsovima u smanjenju simptoma bolesti drva te njegov utjecaj na prinos i kvalitativne parametre mošta. Pokus je proveden tijekom vegetacijske sezone 2021. na sortama vinove loze 'Malvazija istarska' i 'Cabernet Sauvignon'. Vodikov peroksid (10 %) u količini od 5 ml injektiran je u trsove, a učinkovitost tretmana procijenjena je na temelju vizualne ocjene zdravstvenog stanja trsa, kao i analiza prinosa, sadržaja šećera, pH vrijednosti i ukupne kiselosti mošta. Rezultati pokazuju da primjena vodikovog peroksida smanjuje simptome bolesti drva i potiče oporavak trsa, osobito kada se primijenjuje u ranim fazama bolesti. Dok je tretman značajno poboljšao prinos kod oporavljenih trsova 'Malvazije istarske', nisu uočene značajne negativne promjene u sadržaju šećera, pH vrijednosti ili ukupnoj kiselosti mošta. Tretman kod Cabernet Sauvignona nije utjecao na razlike u prinosu, sadržaju šećera, pH vrijednosti niti ukupnoj kiselosti mošta.

Potrebna su daljnja istraživanja radi optimizacije uvjeta tretmana i razjašnjavanja mehanizma djelovanja vodikovog peroksida u upravljanju bolestima vinove loze.

Ključne riječi: vodikov peroksid, bolesti drva vinove loze, zaštita vinograda, Malvazija istarska, Cabernet Sauvignon

1. INTRODUCTION

Grapevine Trunk Diseases (GTDs) represent a major and increasing threat to vineyard longevity and productivity worldwide, with incidence rising markedly since the late 20th century (Kenfaoui et al., 2022). These diseases are caused by complexes of fungi belonging to genera such as *Eutypa*, *Phaeomoniella*, *Phaeoacremonium*, *Fomitiporia*, and members of the family Botryosphaeriaceae, as well as *Diaporthe*, *Cytospora*, *Cadophora*, and

Fusarium species (Bertsch et al., 2013; DeKrey et al., 2022; Li et al., 2023). Other important fungi associated with GTDs are from genera like *Kalmusia*, *Alternaria*, and *Cladosporium* (Bekris et al., 2021). The fungal communities vary by region and cultivar but commonly they colonize perennial woody tissues, leading to internal necrosis, dieback, and eventual vine death (Perez-Gonzalez et al., 2022). Management is complicated by inconsistent symptoms, long latent periods, and strong modulation by environmental and biological stresses (Muntean et al., 2022).

Effective curative options for GTDs remain largely unavailable (Fontaine et al., 2016; Mondello et al., 2018). The ban on sodium arsenite, formerly the only effective treatment against Esca, has left viticulture reliant on preventive practices such as pruning wound protection and sanitation, which are ineffective once internal infections are established (Gramaje et al., 2018; Eichmeier et al., 2018; Grozić et al., 2019). This limitation underscores the need for therapeutic strategies capable of acting directly within woody tissues.

Hydrogen peroxide (H_2O_2) has emerged as a candidate compound due to its dual role as a signalling molecule and antimicrobial agent in plant defence (Fourie & Halleen, 2006; Smirnoff & Arnaud, 2019). As a relatively stable reactive oxygen species, H_2O_2 regulates defence gene activation, restricts pathogen spread, and can induce localized programmed cell death at infection sites (Waszczak et al., 2018; Mittler et al., 2022). However, conventional applications of H_2O_2 in viticulture have produced inconsistent results, largely due to limited penetration into perennial tissues (Mondello et al., 2018).

Trunk injection (endotherapy) has therefore gained attention as an alternative delivery method, enabling direct introduction of active compounds into the transpiration stream and contact with vascular pathogens (Aćimović et al., 2014). Previous studies have shown that endotherapy can reduce Esca symptom expression and stimulate vegetative recovery (Calzarano et al., 2004; Dula et al., 2007).

Given the scarcity of empirical data on H_2O_2 endotherapy, particularly regarding cultivar-specific responses, this study evaluates the curative potential of trunk injection with a 10% H_2O_2 solution in ‘Malvazija istarska’ and ‘Cabernet Sauvignon’ grown in the Istria region, with emphasis on symptom mitigation and grape must quality.

2. MATERIALS AND METHODS

The study was conducted during the 2021 growing season in two commercial vineyards owned by the Rossi family in Vižinada (Istria County, Croatia), established on red soil (terra rossa) and managed under sustainable practices. The experiment involved two cultivars grown in separate vineyards: 'Malvazija istarska' (clone VCR4, Kober rootstock), planted in 2004 at the Brzomila site (0.6 ha, 2400 vines, 45°20'15.8"N 13°43'46.8"E), and 'Cabernet Sauvignon' (clone VCR11, SO4 rootstock), planted in 2007 at the Babušea site (0.5 ha, 2,000 vines, 45°20'22.0"N 13°42'57.1"E). Vine spacing in both vineyards was 2.5 × 1 m.

Curative treatment was carried out during the 2021 growing season at the time of symptom observation (4 August 2021), and assessments were conducted over a two-month period until harvest. The treatment consisted of intramedullary trunk injection of 5 mL of a 10% hydrogen peroxide (H_2O_2) solution using a medical syringe. Two holes (8 mm diameter) were drilled into each trunk (at the graft union and at the head of the vine), and the solution was evenly distributed between them; injection sites were subsequently sealed with grafting wax. The study evaluated vines treated by the grower in summer 2020 as well as symptomatic vines treated during summer 2021.

Disease expression was assessed visually on treated and untreated vines, and symptom severity was classified according to the percentage of affected canopy (Disease Severity Index (DSI) (Bock et al., 2022)): 0% (asymptomatic), 25%, 50%, 75%, and 100% (dead vine). For enological analyses, grape samples were collected at harvest on 24 September 2021 for 'Malvazija istarska' and 4 October 2021 for 'Cabernet Sauvignon'. Four experimental variants were included, each with four replications: untreated asymptomatic (A–UT), untreated symptomatic (S–UT), treated symptomatic recovered (S–TR), and treated symptomatic not recovered (S–TNR).

Yield per vine (16; 4 vines for each variant in 4 repetitions) was determined using a precision scale (RADWAG PS 6000.R2). Sugar content was measured in crushed grape samples using a digital refractometer (HI 96814) and expressed as °Brix. Must pH was measured using a pH meter (inoLab pH 720), and total acidity was determined by titration of 10 mL of must with 0.75 M NaOH using bromothymol blue as an indicator.

Statistical analyses were performed using R software (version 4.4.2, R Core Team, 2024). Results are presented as mean ± standard deviation. Homogeneity of variance was verified using Levene's test, and treatment effects were evaluated by one-way ANOVA. When significant differences were detected ($p < 0.05$), means were separated using Tukey's HSD test.

3. RESULTS

Visual assessment after hydrogen peroxide (H_2O_2) treatment showed a cultivar-specific response and reduced symptom severity in treated vines (Figure 1). In 'Malvazija istarska', 27% of treated vines fully recovered and became asymptomatic (0%), while 13% and 9% exhibited mild (25%) and moderate (50%) symptoms, respectively. Treatment efficacy declined sharply in advanced disease stages, as 47% of vines were fully desiccated, indicating irreversible vascular damage.

In 'Cabernet Sauvignon', complete recovery was lower, with only 12% of vines classified as asymptomatic. Most surviving symptomatic vines exhibited severe symptoms (75%; 22%), and 52% of vines were dead. These results identify a clear efficacy threshold, with H_2O_2 showing markedly reduced effectiveness in vines exhibiting more than 50% symptom severity, irrespective of cultivar.

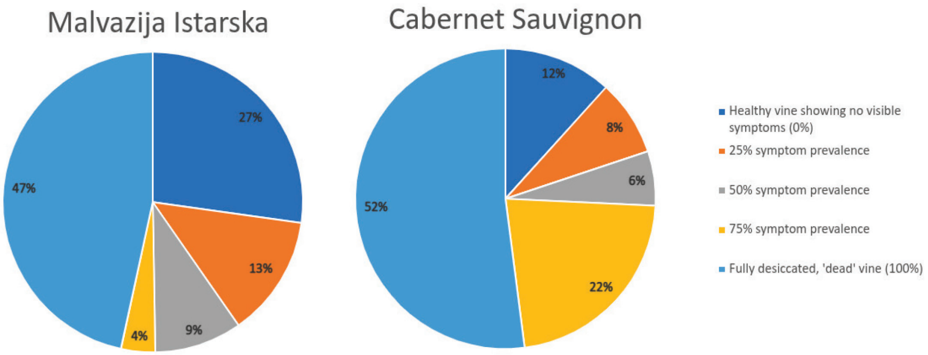

Figure 1 Percentage of grapevine vines classified according to symptom severity in the cultivars 'Malvazija istarska' and 'Cabernet Sauvignon' after curative treatment.

Slika 1. Postotak trsova vinove loze razvrstanih prema jačini simptoma kod sorata 'Malvazija istarska' i 'Cabernet Sauvignon' nakon kurativnog tretmana.

The analysis of yield per vine (Figure 2 A) demonstrated that the curative treatment contributed to the recovery of production capacity, particularly in vines that showed visual recovery (S-TR variant). In 'Malvazija istarska', the treated, visually recovered vines (S-TR) achieved the highest average yield of 4706 g/vine, significantly higher than the untreated symptomatic control (S-UT) at 2470 g/vine. Notably, the recovered vines also outperformed the untreated healthy control (A-UT) group, indicating a vigorous vegetative response to the treatment. Vines that were treated but remained symptomatic (S-TNR) yielded 2750 g/vine.

In the 'Cabernet Sauvignon' vineyard, the highest yield was recorded in the healthy untreated control (A-UT) at 3367 g/vine. However, the treated vines that exhibited visual recovery (S-TR) produced 2893 g/vine, representing a substantial improvement over the untreated symptomatic control (S-UT), which yielded 2397 g/vine. The treated vines that did not recover visually (S-TNR) produced the lowest yield at 2169 g/vine. These results confirm that while GTDs severely impact yield, H_2O_2 treatment can partially restore productivity in vines that respond vegetatively to the injection.

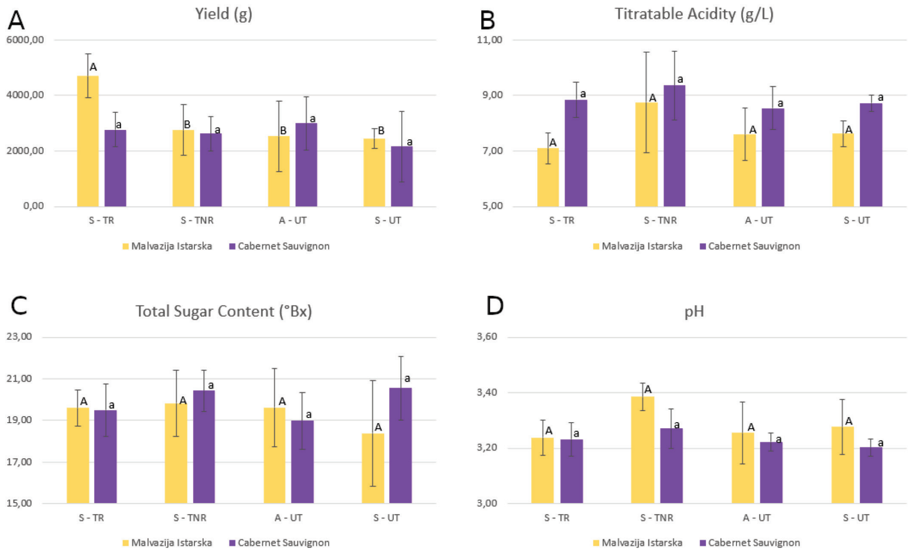

Figure 2 Impact of hydrogen peroxide trunk injection on grape yield and must quality parameters (titratable acidity, total sugar content, and pH) for 'Malvazija istarska' and 'Cabernet Sauvignon' across different treatment variants.
Slika 2. Utjecaj ubrizgavanja vodikovog peroksida u deblo na prinos grožđa i parametre kvalitete mošta (titracijska kiselost, ukupni sadržaj šećera i pH) za 'Malvaziju istarsku' i 'Cabernet Sauvignon' u različitim varijantama tretmana.

Different uppercase letters indicate statistically significant differences between variants for 'Malvazija istarska', while different lowercase letters indicate significant differences for 'Cabernet Sauvignon' ($p<0.05$). Error bars represent standard deviation. Abbreviations: S–TR (Symptomatic-Treated/Recovered), S–TNR (Symptomatic-Treated/Not-Recovered), A–UT (Asymptomatic-Untreated), S–UT (Symptomatic-Untreated).

Velika slova označavaju statistički značajne razlike između varijanti za 'Malvaziju istarsku', mala slova označavaju značajne razlike za 'Cabernet Sauvignon' ($p<0,05$). Stupci pogreške prikazuju standardnu devijaciju. Kratice: S–TR (Simptomatičan-Tretiran/Oporavljen), S–TNR (Simptomatičan-Tretiran/Nije oporavljen), A–UT (Asimptomatičan-Netretiran), S–UT (Simptomatičan-Netretiran).

Chemical analysis of the grape must showed that H_2O_2 injection did not cause statistically significant changes in the main oenological parameters, confirming that grape quality was not adversely affected. Sugar content (Figure 2C) ranged from 18.38 °Brix (S–TR) to 19.83 °Brix (S–TNR) in 'Malvazija istarska' and from 18.98 to 20.55 °Brix in 'Cabernet Sauvignon', with no significant differences between treated and control variants.

Must pH remained stable across all treatments (Figure 2D), ranging from 3.23 to 3.39 in 'Malvazija istarska' and from 3.20 to 3.27 in 'Cabernet Sauvignon'. While no negative effects on sugar content or pH were detected, a slight increase in total acidity was observed in treated vines (Figure 2B), with the highest values recorded in treated recovered 'Malvazija istarska' (8.75 g/L) and treated symptomatic 'Cabernet Sauvignon' (9.36 g/L). Overall, these results indicate that the treatment preserves the oenological potential of the grapes without compromising technological maturity.

4. DISCUSSION

The results demonstrate that trunk injection with a 10% hydrogen peroxide (H_2O_2) solution is a viable curative method for reducing symptom severity of Grapevine Trunk Diseases (GTDs), particularly Esca, in 'Malvazija istarska' and 'Cabernet Sauvignon' grown in the Istria region. The reduction in foliar symptoms and stimulation of vegetative recovery, especially in 'Malvazija istarska', indicate that directly introducing oxidative compounds into the xylem can partially compensate for vascular damage caused by pathogenic fungi.

By targeting pathogens within the woody tissues, endotherapy overcomes key limitations of conventional preventive measures, which are largely ineffective once infections are established (Darrieutort & Lecomte, 2007; Mondello et al., 2018).

The effectiveness of this approach aligns with previous studies highlighting the advantages of delivering active substances directly into the transpiration stream (Calzarano et al., 2004; Mondello et al., 2018), ensuring more efficient distribution within perennial organs compared to surface applications (Aćimović et al., 2014). The observed recovery likely reflects both the microbicidal activity of H_2O_2 and its role as a signaling molecule that primes host defences. In the xylem, H_2O_2 may act as an elicitor of defence-related gene cascades and lignification processes, thereby restricting fungal spread and contributing to the restoration of physiological function (Calzarano et al., 2004; Yadeta & Thomma, 2013; Lima et al., 2017).

The agronomic relevance of this physiological recovery is supported by the marked restoration of yield, particularly in visually recovered 'Malvazija istarska' vines, which nearly doubled production compared to untreated symptomatic controls. This response is notable given that Esca infections are associated with yield losses exceeding 50% in untreated vineyards (Fontaine et al., 2016). Although recovery in 'Cabernet Sauvignon' was less pronounced, treated recovered vines consistently outperformed symptomatic controls, indicating partial restoration of productivity.

Differences in recovery rates between cultivars highlight the importance of cultivar-specific anatomical and defence traits. Variations in vessel architecture and the formation of tyloses are known to influence tolerance to GTDs (Pouzoulet et al., 2017). The sharp decline in efficacy above 50% symptom expression suggests a threshold beyond which xylem occlusion by tyloses and gels limits solution translocation and treatment effectiveness (Pouzoulet et al., 2017).

Importantly, the absence of negative effects on must quality parameters confirms that 10% H_2O_2 endotherapy preserves the oenological potential of the crop. This finding is consistent with studies on trunk injection of ozonated water, which also reported no detrimental impacts on grape and wine quality (Campayo et al., 2020; Campayo et al., 2022), and provides new evidence supporting the oenological safety of H_2O_2 trunk injection.

5. CONCLUSION

This study confirms that trunk injection with a 10% hydrogen peroxide (H_2O_2) solution is an effective curative approach for Grapevine Trunk Diseases (GTDs), particularly Esca. The treatment reduced symptom expression and promoted vegetative recovery in 'Malvazija istarska' and 'Cabernet Sauvignon', with the highest efficacy in vines showing less than 50% symptom severity.

Yield recovery, especially in 'Malvazija istarska', highlights the agronomic relevance of this approach, while cultivar-specific responses indicate the influence of anatomical and physiological traits. Importantly, H_2O_2 endotherapy did not affect must quality, confirming its oenological safety.

This research was conducted over a single growing season and included a limited number of vineyards, therefore the results should be interpreted within these experimental limitations. Further research conducted over multiple years and locations would enable optimization of treatments and improved understanding of the mechanisms by which H_2O_2 acts in GTDs management.

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