J. Crop Prot. **2024, 13 (4): 385-397**

Research Article

Effect of the foliar fertilizers application on Grapevine trunk diseases

Omid Ali Amarloo[1*], **Safar Ali Mahdian**[1], **Mohammad Ali Tajick Ghanbari**[1] **and Hamid Mohammadi**[2]

1. Department of Plant Protection, Faculty of Agronomic Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.
2. Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.

Abstract: Grape trunk diseases are critical problems for grapes from the time of planting to the harvest stage. Grapes are susceptible to 29 fungal diseases due to their perennial woody trunk, so identifying and managing them is crucial. Among the methods of controlling these diseases, feeding with mineral fertilizers to reduce leaf symptoms resulting from the toxic secretions of fungi living in the woody part of the tree is of great importance. To manage these diseases, a mixture of fertilizers including calcium chloride $CaCl_2$, 466 g, magnesium nitrate $Mg(NO_3)_2$, 403 g, seaweed extract (75 ml) and sterile distilled water 466 ml per liter of foliar spraying on the selected treatments in Sohrabi's garden were used once every 20 days. Guaiacol peroxidase, catalase and leaf area index were measured in Sohrabi's garden during 2017 and 2018 and were compared with the control. In the treatments sprayed with the fertilizers, the average amount of guaiacol peroxidase enzyme was 12.72 µl compared to the control (10.6 µl) and the average amount of catalase enzyme was 83.68 µl compared to the control (31.85 µl). The average size of the leaf area in the foliar treatments in Sohrabi's garden was 11564 mm^2, compared to the control that was 4959 mm^2. The severity of the disease in the sprayed treatments (19.95 %) was lower than the control (56.6 %). These results are due to the increase in the leaf surface, which increases the amount of photosynthesis, and the increase in guaiacol peroxidase and catalase, which reduce the oxidative stress resulting from fungal secretions, which resulted in decreasing symptoms in foliar treatments.

Keywords: Grape decline, mineral fertilizers, oxidative stress, catalase, guaiacol peroxidase

Introduction

Grapevine trunk diseases (GTD) are currently considered one of the most important destructive challenges for grape growing, affecting the cuttings propagation to cultivation in the field and harvesting stages (Whiting, 2001; Bertsch *et al*., 2013). These destructive diseases have increased significantly in recent decades in all grape-producing countries (Baránek *et al*., 2018). Climatic changes are an additional pressure on grape cultivation. The

---

Handling Editor: Naser Safaie

[*] Corresponding authors: 21omid234100@gmail.com

Received: 27 December 2023, Accepted: 08 January 2025

stresses caused by these changes make the vines susceptible to GTD. Therefore, finding agricultural solutions and technologies that are economically and environmentally sustainable is an urgent need (Reis *et al*., 2019). At least 133 fungal species are associated with GTD worldwide involving four major trunk diseases: GTD, Esca, Eutypa dieback and Phomopsis dieback (Gramaje *et al*., 2018). Disease symptoms on infected grapevines can be variable. However, wood necrosis, brown streaking or cankers, discoloration, drying and stunting of foliage and dead spurs/cordons/vines are reported as the main GTD symptoms (Fontaine *et al*., 2016). Management of GTD is complicated and varies between geographical regions. Furthermore, scientific information on controlling GTD is limited and usually focuses on disease prevention and mitigation (Úrbez-Torres, 2011). With the prohibition of poisons for chemical control of this disease, such as sodium arsenite, prevention, including treatment of wounds, is the most effective management strategy in controlling GTD (Creaser and Wicks, 2004; Sosnowski *et al*., 2011b). However, these preventive measures and wound treatments can be costly (Kühn *et al*., 2017). As a result, recently it has been a priority for grape growers, processing industries and researchers to evaluate new active compounds and cultivation practices that can effectively reduce the contamination caused by GTD-causing pathogens (Úrbez-Torres, 2011). Integrated management strategies such as physical, chemical, biological, and nutritional that cause pruning care and wound coverage can enhance plant vigor and overall tree health and allow economically viable production in infected trees (Halleen and Fourie, 2016). A positive relationship exists between the expression of foliar symptoms and reduced quality and yield in diseased trees (Pacetti *et al*., 2021). The lack of quantitative and qualitative reduction in production in vines without leaf symptoms compared to healthy vines has led researchers to conduct studies to understand the mechanisms that reduce the expression of leaf symptoms and to try to improve the ability of infected plants to reduce the expression of symptoms (Guerin-Dubrana *et al*., 2013). Foliar application of some fertilizers effectively reduces the incidence and severity of foliar symptoms in GTD. In particular, a study tested the effect of foliar application with different nutrients to reduce symptoms in diseased trees. They evaluated quality parameters in branches without symptoms and vines with symptoms, which recorded higher calcium content in grape leaves without symptoms (Calzarano *et al*., 2009). These results led to further studies including the foliar application of a mixture of calcium, magnesium and seaweed during the growing season until the grape cluster is complete. The application of these minerals as fertilizers significantly reduced the symptoms of foliar disease. This reduction is probably due to the positive performance of each of these fertilizers in reducing the harmful effects of the substances secreted by the fungi living in the trunk of the grapevine that have been transferred to the leaves (Calzarano *et al*., 2014, 2018). The role of calcium, magnesium, and sodium has recently been investigated as different possible interaction mechanisms between these fertilizers and symptom reduction. These investigations were done by applying a mixture of fertilizers on diseased trees that were naturally asymptomatic and grapevines with disease symptoms. As a result, the trees with symptoms also became asymptomatic due to using fertilizers (Calzarano *et al*., 2021). The above results and the need to further investigate the nature of the mechanisms involved in the expression of foliar spraying symptoms and the effect of different mineral elements in the form of fertilizers on the occurrence of symptoms in the leaves and fruits of grapes infected with this disease encouraged us that in a study in 2017 and 2018, the effect calcium chloride and magnesium nitrate fertilizers and seaweed extract as a mixture should be checked on diseased vines without symptoms and with symptoms. In this research, we investigated the effect of using different mineral fertilizers in the form of foliar spraying during the grape growing season from BBCH 13 to BBCH 81 (Lorenz *et al*., 1995) on the size of the leaf surface index and the amount of two enzymes, catalase and guaiacol peroxidase. The reason for choosing these three criteria was that increasing the green area and reducing symptoms will increase photosynthesis. As a result, the grape yield will increase quantitatively and qualitatively. By participating in the antioxidant defense system and the detoxification cycle, the two mentioned enzymes can destroy the chloroplasts' hydrogen peroxide (H_2O_2) produced and help the plant remove and reduce the symptoms.

Materials and Methods

Disease survey and sampling

During the spring and summer of 2017-2018, grapevine trunk diseases were investigated regarding the area under cultivation and the disease prevalence in northeast Iran, including North Khorasan provinces. Sampling was conducted from three vineyards in Badranlo, Qarjeh, Timurtash and Shirvan counties, the provinces' representative regions. Samples were collected from grapevines showing disease symptoms including yellowing, reduced growth, wilting and wood discoloration in cross-section. The predominant grape variety cultivated in these regions is Kolhadari which showed the highest percentage of GTD infection compared to other cultivated varieties. In addition to the symptoms of the disease that can be seen in the vines, the trunks infected with the disease are very brittle and slack due to the destruction of the inner wood, especially in the case of Esca disease. In each vineyard where sampling was done, the selected trees were marked to be clear for sampling in the next year, as well as other required operations such as foliar spraying. The collected samples were placed in separate plastic bags. Upon labeling the date, collection region, and the grape variety's name, they were transferred to the laboratory Neishabur Skills National University College of Agriculture for further studies and isolation of fungal agents.

Disease severity

Field studies were conducted in mid-September, just before the grape harvest (BBCH 89) (Lorenz *et al*. 1995), when some grapevines showed the highest leaf infection. Disease severity on leaves (as part of the grape crown) was recorded on a disease scoring scale from 0 to 5, where 0 = leaf without symptoms, 1 = 1-10%, 2 = 11-30%, 3 = 31-50%. 4 = 70-51%, 5 = 71-100%. The percentage of disease severity was calculated from the McKinney index: $\Sigma N \times 100 / (Y \times Z)$, where ΣN = total severity in each plant, Y = the number of grapes that were examined, Z = 5, which is the maximum score on the disease evaluation scale (Mc Kinney, 1923).

Fertilizing

A mixture containing 466 g of calcium chloride ($CaCl_2$), 403 g of magnesium nitrate $Mg(NO_3)_2$, 75 ml of seaweed extract, and 466 ml of distilled H_2O per liter (4 L/ha) was prepared. A 600 L of water per hectare mixtuer was used for foliar spraying with an electric atomizer in Sohrabi's garden. Foliar spraying in five selected times, from the stage of "open three leaves" (BBCH 13) to the stage of completion of grapes (BBCH 81) according to the plan of Lorenz (Lorenz *et al*. 1995), from early May to mid-August, at intervals of 20 Fasting was done (Calzarano *et al*., 2014).

leaf area index

Grid paper was used to measure the surface of the leaf. For this purpose, the leaf was first dried on the herbarium press and then placed on graph paper. Its surface was measured in mm^2, and then the total surface of the leaves of each treatment was averaged (Caldas *et al*., 1992). In the grid counting method, the leaf is first removed from the plant and placed on the grid paper, the leaf lines are made using a pencil on the grid paper, and finally, the leaf's surface is measured by counting the grids covered by the leaf.

Treatments

Based on the symptoms of leaves in Sohrabi's garden, the treatments were selected and divided into four groups: healthy, asymptomatic disease, symptomatic disease and control. In collecting leaves for each treatment, three trees were randomly selected in garden, and nine leaves from each tree, including three leaves from the middle part, three leaves from the right side and three leaves from the left side of the tree, were selected and separated from the petiole by a scalpel. Then, each part's area of three leaves was calculated and considered one sample. In this way, three leaf samples were prepared in each tree (an average of three leaves in each part), and 9 leaf samples were prepared for each treatment.

Catalase activity assay

Catalase activity assay was evaluated based on changes in hydrogen peroxide concentration at a wavelength of 240 nm. In this method, the catalase present in the plant extract reduces the absorption of this substance at the wavelength of 240 nm by breaking down the hydrogen peroxide present in this extract, and the activity of the enzyme was measured from the difference in absorption at the wavelength of 240 nm in the patient and control samples. The optical density (OD) of both cuvettes 158 was read using a spectrophotometer (Beers and Sizer, 1952; Aebi, 1984).

guaiacol peroxidase activity assay

To determine the amount of guaiacol peroxidase activity, the reaction mixture (2 ml) was prepared using 1 ml of 100 mM phosphate buffer (pH = 7), 250 µl of 0.1 mM EDTA, 1 ml of 5 mM guaiacol, 1 ml of 15 mM hydrogen peroxide, and 50 µl of the solution. The extracted enzyme was used. The reaction was started by adding the enzyme solution and the OD was measured at 470 nm for a minute. Enzyme activity was obtained based on the tetraguaiacol formed (Chance and Maehly,1955).

Statistical analysis

In this research, due to the same geographical conditions of both gardens and the sampling type, a completely random design (CRD) was used. SPSS software (version 24, 2017) and LSD test were used for statistical analysis of leaf surface, pathogenicity test and enzyme data.

Results

vineyard survey, sampling, and disease symptoms

In this study, leaf disease symptoms observed in vineyards were classified into two primary groups. The first group included leaves exhibiting chlorosis and interveinal necrosis, commonly referred to as "tiger skin symptoms" (Fig. 1A, B, C). The second group was characterized by a general decline in the health of vines. Symptoms in this group typically began as burning from the tips and edges of the leaves, gradually progressing inward until the entire leaf was affected and ultimately died (Fig. 1D, E).

Effect of foliar fertilizers on disease severity

Applying foliar fertilizers over two consecutive years significantly reduced both the occurrence and severity of leaf symptoms in grapevines. In 2017, diseased vines treated with foliar spraying showed an average disease severity of 24.4%, compared to 57.7% in untreated control vines. Similarly, in 2018, disease severity dropped to 15.5% in treated vines, while control vines exhibited a severity of 55.5%. This marked reduction in symptoms during the second year was attributed to the continuous application of foliar fertilizers, which supplied the necessary minerals to the diseased vines.

Effect of foliar fertilizers on leaf area

Leaf area measurements were conducted in Sohrabi's vineyard (Table 1). Foliar spraying with a complete fertilizer mixture had a significant impact on the leaf area index ($F = 77.88$; $df_{t,e} = 3, 32$; $p < 0.0001$). No significant difference was observed between the healthy and asymptomatic treatments, but both had a substantially greater leaf area index compared to the symptomatic and control treatments. Notably, the symptomatic treatments also exhibited a significantly higher leaf area index compared to the control.

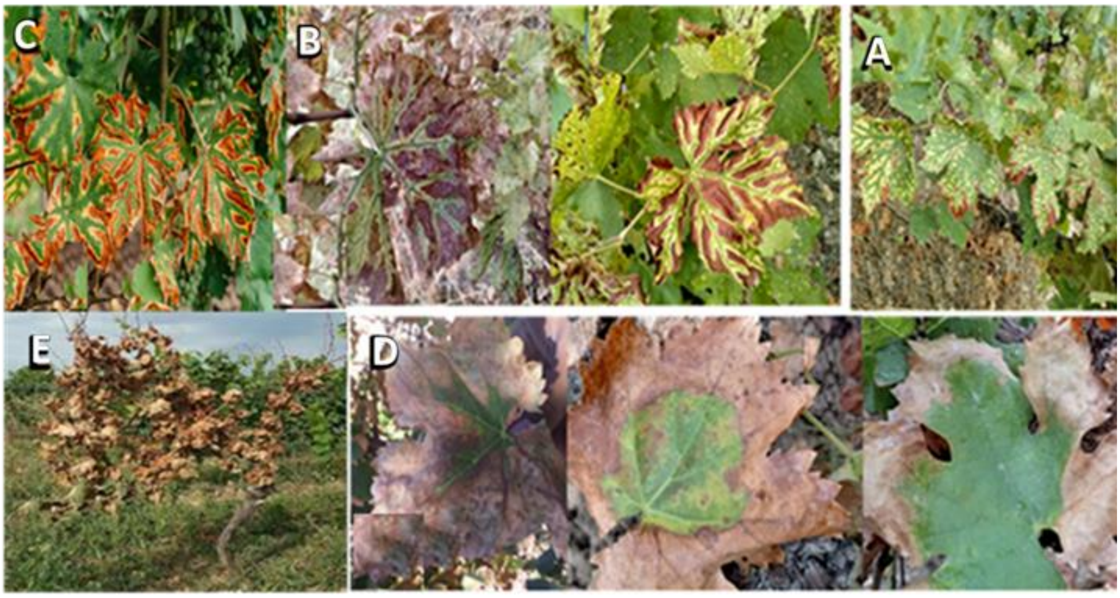

Figure 1 A- initial Esca symptoms on leaves; B- Typical Esca symptoms on leaves; C- tiger skin symptoms in vineyard; D- General decline symptoms on leaves; E- General decline damage in vineyard.

Foliar spraying and its effect on catalase and guaiacol peroxidase

Variance analysis results (Table 2) demonstrated significant differences at the 1% level due to sampling time, fertilizer application, and grapevine treatments. Additionally, the interaction between time and treatments showed significant effects. In five sampling periods, foliar spraying treatments were compared in terms of catalase and guaiacol peroxidase enzyme levels (Fig. 2). The study further explored the impact of the sampling time relative to foliar spraying (zero time, 48 hours post-spraying, and 72 hours post-spraying) on catalase activity.

Mean comparisons revealed that both catalase and guaiacol peroxidase levels increased over time following foliar spraying, with the highest concentrations observed 72 hours post-application (Fig. 3). This indicates that foliar spraying enhances the activity of these enzymes, contributing to improved plant defense mechanisms.

Discussion

Some vines affected by grape trunk diseases (GTD) exhibit variable disease symptoms across different years, making them indistinguishable from healthy plants at times. The complete or partial disappearance of foliage symptoms in certain diseased vines indicates that other physiological or environmental factors may contribute to the expression of these symptoms. In years when symptoms are absent, diseased trees demonstrate yields-both quantitative and qualitative- that are comparable to those of healthy plants (Calzarano and Di Marco, 2007, 2008; Bertsch *et al*., 2013). Leaf area plays a critical role in various physiological studies related to plant growth, photosynthetic efficiency, evapotranspiration, and responses to fertilizers and irrigation. Therefore, both the health status and leaf area significantly influence overall growth and production (Blanco and Folegatti, 2005). It has been established that toxic metabolites produced by GTD fungi within infected grape tissues can be transported to the crown via sap flow. This transport contributes to foliar symptoms associated with grape trunk diseases (Sparapano *et al*., 1998; Mugnai *et al*., 1999; Surico, 2009). The predominant hypothesis regarding symptom development involves phytotoxic substances generated by these pathogens being moved into the leaves through the host's vascular system (Sparapano *et al*., 1998; Evidente *et al*., 2000). The entry of these phytotoxic substances secreted by fungi residing in woody tissues into leaf tissue acts as a stress factor for plants. These toxic metabolites can trigger plant responses such as necrotic lesions on leaves-a hypersensitivity reaction characterized by the formation of antimicrobial compounds including enzymes and stilbene derivatives. (Heath, 2000). Typically observed is an increase in foliage symptoms during specific developmental stages of grapes-from flowering through cluster formation until harvest time (Calzarano *et al*., 2016), which includes distinct chlorosis-necrosis patterns between leaf veins known as "tiger skin" in Esca-infected plants (Mugnai *et al*., 1999). Stress-induced oxidative reactions lead to free radical production within plant cells-comprising superoxide anions (O^{2-}) hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), among others-which can damage carbohydrates, fats and nucleic acids structures damaging cellular integrity further exacerbated under stress conditions caused excessive reactive oxygen species production relative antioxidant capacity leads oxidative stress (Bian and Jiang, 2009; Sharma *et al*., 2012). Since there are no pathogens present on foliage specifically involved in this disease it becomes evident that only those toxic secretions from pathogens induce stress response subsequent metabolic activity generates symptomatic damage revealed tissue impact. To mitigate against environmental stresses plants utilize various enzymatic non-enzymatic antioxidant systems control excess reactive oxygen species levels resulting from such conditions (Mittler, 2002). Non-enzymatic defense comprises low-molecular-weight antioxidants beta-carotene ascorbic acid reduced glutathione while enzymatic counterparts include superoxide dismutase catalase guaiacol peroxidases glutathione reductase respectively. While non-stressed scenarios maintain equilibrium between ROS generation scavenging capacity antioxidant defenses any tipping point triggers oxidative distress thus necessitating robust antioxidative mechanisms (Yang *et al*., 2017). Key enzymes like guaiacol peroxidases dismutase play frontline roles combating ROS yet their resultant product hydrogen peroxide remains potentially harmful requiring removal facilitated catalases recycling pathways involving catalase or guaiacol peroxidase ascorbate, ascorbate glutathione cycle (Asada, 1992; Laspina *et al*., 2005). A significant management strategy lies supplying essential mineral elements throughout growing seasons aiming preemptively address tension prevent onset oxidative demands (Sparapano *et al*., 1998; Mugnai *et al*., 1999; Surico, 2009). Calcium depletion in diseased grape leaves with symptoms were treated with NPK fertilizer mixed with calcium chloride. Therefore, unlike what was observed in untreated grapes, the level of symptoms in these grapes was reduced and the reduction in symptoms in diseased grapes sprayed with NPK fertilizers mixed with calcium chloride could be due to the reduction in the metabolic activity of the toxic secretions of the fungi as a result of this foliar spray. Similar results were observed in the present study in garden where nitrogen fertilizer or fresh animal manure was used at the beginning of the season. The reduction of calcium content can weaken the role of calcium in reducing the intensity of the plant's oxidative response and the activity of superoxide dismutase, guaiacol peroxidase, catalase and ascorbate guaiacol peroxidase increased (Lecourieux *et al*., 2006; Lima *et al*., 2012).

Table 1 Mean leaf area index of grapvine in foliar treatments (Healthy, asymptomatic, and symptomatic) sprayed with complete fertilizer and control in 2017 and 2018.

Year	Treatments	Mean leaf area (mm^2)
2017	Healthy	13326a
	Asymptomatic	12766a
	Symptomatic	8358b
	Control	4866b
2018	Healthy	13589a
	Asymptomatic	12868a
	Symptomatic	8476b
	Control	5053c

Means followed by the same letters are not significantly different (LSD test, $P < 0.05$).

Table 2 Mean squares (MS) of different periods of foliar spraying on catalase and guaiacol peroxidase activity of grapevine in foliar-sprayed and control treatments in 2017 and 2018.

Period	SOV	df	MS. Cat	F	MS. Gua	F
First period	Treatment (Tr)	3	0.1067	7.674**	8.2463	0.895ns
	Time (T)	2	0.0630	4.532*	0.0004	4.055*
	Tr × T	6	0.0399	2.870*	0.0002	2.349ns
	Error	24	0.0139		9.2083	
Second period	Treatment (Tr)	3	0.0821	7.334**	5.8074	0.734ns
	Time (T)	2	0.0691	6.171**	0.0005	6.046**
	Tr × T	6	0.0429	3.831**	8.4518	1.068ns
	Error	24	0.0112		7.9083	
Third period	Treatment (Tr)	3	0.2745	16.55**	0.0004	5.050**
	Time (T)	2	0.1298	7.828**	0.0007	8.901**
	Tr × T	6	0.1151	6.944	0.0003	3.582*
	Error	24	0.0165		8.2361	
Fourth period	Treatment (Tr)	3	0.2675	17.121**	960.02	4.680*
	Time (T)	2	0.1225	7.838**	1135.1	5.534*
	Tr × T	6	0.1182	7.568**	610.43	2.976*
	Error	24	0.0156		205.12	
Fifth period	Treatment (Tr)	3	0.2772	16.65**	4.7055	0.570ns
	Time (T)	2	0.1363	8.188**	0.0004	4.952*
	Tr × T	6	0.1135	6.814**	0.0001	1.610ns
	Error	24	0.0166		8.2611	

Cat: Catalase, Gua. Guiacol guaiacol peroxidase, **Significant at $P < 0.01$, *Significant at $P < 0.05$, ns: Non-significant.

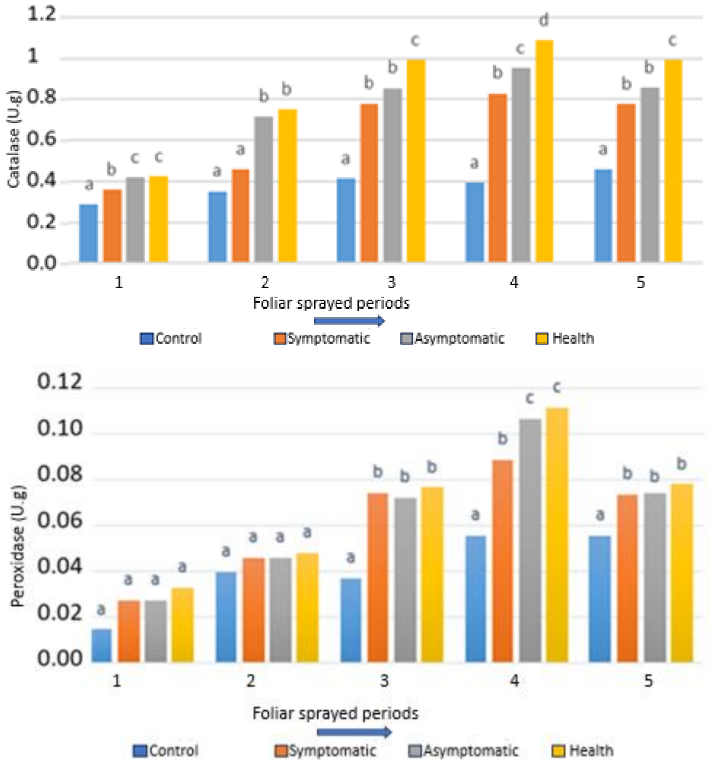

Figure 2 Comparison of the average levels of catalase and guaiacol peroxidase activity of grapevine in sprayed and control fertilizer treatments separately in five different periods (early May to mid-August).

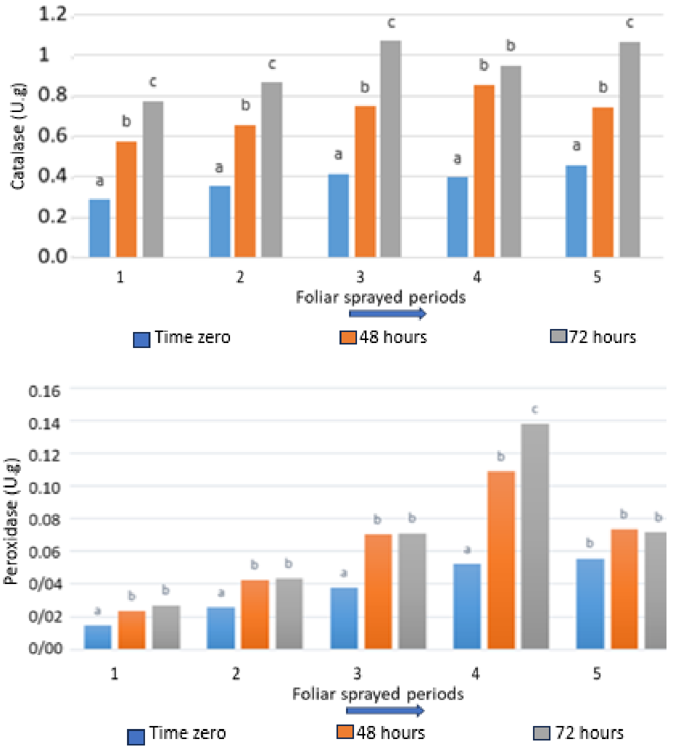

Figure 3 The effect of sampling time on catalase and guaiacol peroxidase activity of grapevine (zero, 48 and 72 hours after foliar fertilizer spraying).

Magnesium deficiency causes symptoms of interveinal chlorosis in leaves, which are similar to those that lead to tiger skin in Esca-infected grapes (Shaul, 2002; Marschner, 2012). In addition, magnesium can play a role in the detoxification of phytotoxins, as shown in Eutypa dieback, where eutypine is converted to the non-toxic compound eutypineol via Mn^{2+} and Mg^{2+} (Colrat *et al*., 1999). Application of fertilizers in the form of foliar spraying in each period (once every 20 days) increased the amount of catalase and guaiacol peroxidase regularly. These results are consistent with previous findings (Colrat *et al*., 1999; Mittler, 2002; Calzarano *et al*., 2017a,b; Yang *et al*., 2017). In the present study we applied complete fertilizers incorporating $CaCl_2$ and $Mg(NO_3)_2$ occurred five designated intervals spanning phenological stages open three-leaf (BBCH 13) until full fruiting (BBCH 81) sequentially administered every twenty days commencing (Lorenz *et al*. 1995), from early May to mid-August (Calzarano *et al*., 2014). The amount of catalase in the second period was not significantly different in the asymptomatic treatment from the healthy treatment, but the healthy treatment and the asymptomatic treatment were significantly different from the symptomatic treatment, while the symptomatic treatment also had a significant difference from the control. In the third period, the expected result was seen, the symptomatic treatment did not have a significant difference from the asymptomatic treatment. This result means that foliar spraying with fertilizers has been able to increase the catalase level in the symptomatic treatment and reduce symptoms. These effects increase the final product in this treatment to the same extent as the asymptomatic treatment. However, the symptomatic treatment had a significant difference from the control, and this difference was constantly increasing in different periods. The effect of foliar spraying on the guaiacol peroxidase activity was also increasing in all periods, but in the third period, the amount of this increase in the symptomatic treatment was equal to the healthy treatment, which is a very interesting and noteworthy result because the healthy trees had green leaves and no symptoms. In the fourth period, in the symptomatic treatment the enzyme activity increased and were significantly higher from the control, and this difference was much greater than in the first period. In the fifth period, in all treatments, the enzyme activity decreased compared to the fourth period, which is due to the movement of minerals from the leaves to the fruits. This indicates that the application of ferilizer in disease treatments, especially in the symptomatic treatment, has been able to increase the amount of catalase and guaiacol peroxidase, thereby increasing the plant's response to oxidative responses and reducing the number of symptoms. Comparing the average results for both enzymes showed that after the fourth period of foliar spraying (80 days after the first foliar spraying), the highest catalase and guaiacol peroxidase activity was observed. Foliar spraying in successive periods compensates for the lack of these effective mineral elements in chlorophyll and leaf parenchyma and prevents symptoms. The lowest value was observed in the first period for control grapes. Collection of leaves from foliar treatments was done at three different times, including leaf collection immediately before foliar application as time zero, 48 and 72 hours after foliar application. Comparison of the data obtained from spraying solution in each period and with the passage of time showed that after spraying the catalase and guaiacol peroxidase activities increased. So that 72 hours after spraying showed the highest increase compared to the previous times. Subsequently analyzing expressions related each component activity collected samples revealing synergistic trends catalytic exertions distinctly elevated following initial post-treatment respective intervals ensuring adequate compensation elemental deficiencies manifested during transitional phases evolving maturation climactic changes (Lecourieux *et al*., 2006; Calzarano *et al*., 2018, 2021).

Conclusion

In summary, our study highlights the complex relationship between grape trunk diseases (GTD), environmental factors, and plant physiological responses. The variable expression of symptoms in diseased vines highlights the need for a nuanced understanding of GTD dynamics and their impact on vine health and productivity. Our findings suggest that management strategies incorporating mineral supplementation- such as calcium and magnesium- can significantly mitigate disease symptoms while enhancing leaf area and overall vine vigor. These results align with previous research, reinforcing the critical need for effective nutrient management as part of integrated disease management practices in viticulture. Future research should continue to explore these relationships further, examining additional factors influencing grapevine resilience to trunk diseases. Ultimately, adopting proactive nutritional strategies will be essential for sustainable viticulture practices aimed at safeguarding vineyard health against evolving challenges posed by grapevine pathogens.

Reference

Aebi, H. 1984. Catalase in vitro. Methods in Enzymology, 105: 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Asada, K. 1992. Ascorbate guaiacol peroxidase hydrogen peroxide scavenging enzyme in plants. Physiologica Plantarum, 85: 235-241.

Baránek, M., Armengol, J., Holleinová, V., Pe˘cenka, J., Calzarano, F., Pe˘názová, E., Vachun, M. and Eichmeier, A. 2018. Incidence of symptoms and fungal pathogens associated with grapevine trunk diseases in Czech vineyards: First example from a north-eastern European grape-growing region. Phytopathologia Mediterranea, 57: 449-458. DOI: 10.14601/Phytopathol_Mediterr-22460.

Beers, R. F. and Seizer, I. W. 1952. Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide by Catalase. Journal of Biological Chemistry, 195: 133-140. https://doi.org/10.1016/S0021-9258(19)50881-X.

Bertsch, C. M., Ramírez-Suero, M., Magnin-Robert, P., Larignon, J., Chong, E., Abou-Mansour, A., Spagnolo, C. and Clèment Fontaine, F. 2013. Grapevine trunk diseases: complex & still poorly understood. Plant Pathology, 62: 243-265. https://doi.org/10.1111/j.1365-3059.2012.02674.x.

Bian, S. and Jiang, Y. 2009. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of kentucky bluegrass in response to drought stress and recovery. Science Horticulturae, 120: 264-270. https://doi.org/10.1016/j.scienta.2008.10.014.

Blanco, F. F. and Folegatti, M. V. 2005. Estimation of leaf area for greenhouse cucumber by linear measurements under salinity and grafting. Scientia Agricola, 62: 305-309. DOI:10.1590/S0103-90162005000400001.

Caldas, LS., Bravo, C., Piccolo, H. and Faria, C. 1992. Measurement of leaf area with a hand-scanner linked to a microcomputer. Revista Brasileira de Fisiologia Vegetal. 4: 17- 20.

Calzarano, F. and Di Marco, S. 2007. Wood discoloration and decay in grapevines with Esca proper and their relationship with foliar symptoms. Phytopathologia Mediterranea, 46: 96-101. DOI: https://doi.org/10.36253/phyto-5208.

Calzarano, F., Amalfitano, C., Seghetti, L. and Cozzolino, V. 2009. Nutritional status of vines affected with Esca proper. Phytopathologia Mediterranea, 48: 20-31. DOI: https://doi.org/10.14601/Phytopathol_Mediterr-2872.

Calzarano, F., Cichelli, A. and Odoardi, M. 2001. Preliminary evaluation of variations in composition induced by Esca on cv. Trebbiano d'Abruzzo grapes and wines. Phytopathologia Mediterranea, 40: 443-448. DOI:10.14601/Phytopathol_Mediterr-1633

Calzarano, F., D,'Agostino, V., Pepe, A., Osti, F., Della Pelle, F., de Rosso, M., Flamini, R. and Di Marco, S. 2016. Patterns of phytoalexins in the grapevine leaf stripe disease (Esca complex)/grapevine pathosystem. Phytopathologia Mediterranea, 55: 410-426. DOI: https://doi.org/10.14601/. Phytopathologia Mediterranea, -18681.

Calzarano, F., Di Marco, S., D'Agostino, V., Schiff S.and Mugnai, L. 2014. Grapevine leaf stripe disease symptoms (Esca complex) are reduced by a nutrients and seaweed mixture. Phytopathologia Mediterranea, 53: 543-558.

Calzarano, F., Di Marco, S. and Cesari, A. 2004. Benefit of fungicide treatment after trunk renewal of vines with different types of Esca necrosis. Phytopathologia Mediterranea, 43: 116-124. https://www.jstor.org/stable/26456695.

Calzarano, F., Osti, F., Baránek, M. and Di Marco, S. 2018. Rainfall and temperature influence expression of foliar symptoms of grapevine leaf stripe disease (Esca complex) in vineyards. Phytopathologia Mediterranea, 57: 488-505. DOI: https://doi.org/10.14601/Phytopathol_Mediterr-24454.

Calzarano, F., Osti, F., D'Agostino, V., Pepe, A. and Di Marco, S. 2017a. Mixture of calcium, magnesium and seaweed affects leaf phytoalexin contents and grape ripening on vines with grapevine leaf stripe disease. Phytopathologia Mediterranea, 56: 394-401. DOI: https://doi.org/10.14601/Phytopathol_Mediterr-22023.

Calzarano, F., Osti, F., D'Agostino, V., Pepe, A., Della Pelle, F., De Rosso, M., Flamini, R. and Di Marco, S. 2017b. Levels of phytoalexins in vine leaves with different degrees of grapevine leaf stripe disease symptoms (Esca complex of diseases). Phytopathologia Mediterranea, 56: 494-501. https://doi.org/10.14601/Phytopathol_Mediterr-22055.

Calzarano, F., Pagnani, G., Pisante, M., Bellocci, M., Cillo, G., Metruccio, E.G. and Di Marco, S. 2021. Factors Involved on Tiger-Stripe Foliar Symptom Expression of Esca of Grapevine. Plants. 10, 1041; https://doi.org/10.3390/plants10061041.

Chance, B. and Maehly, A. C. 1955. Assay of catalase and guaiacol peroxidase. Methods in Enzymology, 2: 764-775. http://dx.doi.org/10.1016/S0076-6879(55)02300-8.

Colrat, S., Deswarte, C., Latché, A., Klaébe, K., Bouzayen, M., Fallot, J. and Roustan, J. P. 1999. Enzymatic detoxification of eutypine, a toxin from Eutypa lata, by Vitis vinifera cells: Partial purification of an NADPH-dependent aldehyde reductase. Planta, 207: 544-550.

Creaser, M. L. and Wicks T. J. 2004. Short-term effects of remedial surgery to restore productivity to *Eutypa lata* infected vines. Phytopathologia Mediterranea, 43: 105-107. DOI: https://doi.org/10.14601/Phytopathol_Mediterr-1737.

Evidente, A., Sparapano, L., Andolfi, A. and Bruno, G. 2000. Two naphthalenone pentaketides from liquid cultures of *Phaeoacremonium aleophilum*, a fungus associated with Esca of grapevine. Phytopathologia Mediterranea, 39: 162-168. https://doi.org/10.14601/Phytopathol_Mediterr-1559.

Gramaje D., Urbez-Torres, J. R. and Sosnowski, M. R. 2018. Managing Grapevine Trunk Diseases With Respect to Etiology and Epidemiology: Current Strategies and Future Prospects. Plant Disease. 2018 Jan;102:12-39. doi: 10.1094/PDIS-04-17-0512-FE. Epub 2017 Dec 7.

Guerin-Dubrana, L., Labenne, A., Labrousse, J. C., Bastien, S., Rey, P. and Gégout-Petit, A. 2013. Statistical analysis of grapevine mortality associated with Esca or Eutypa dieback foliar expression. Phytopathologia Mediterranea, 52: 276-288. https://doi.org/10.14601/Phytopathol_Mediterr-11602.

Halleen, F. and Fourie P. H. 2016. An integrated strategy for the proactive management of grapevine trunk disease pathogen infections in grapevine nurseries. Enology and Viticulture . 37:104-114. DOI:10.21548/37-2-825.

Heath, M. C. 2000. Hypersensitive response-related death. Plant Molecular Biology, 44: 321-334. DOI: 10.1023/a:1026592509060.

Kühn, A., Zappata, A., Gold, R. E., Zito, R. and Kortekamp, A. 2017. Susceptibility of grape pruning wounds to grapevine trunk diseases and effectiveness of a new BASF wound protectant. Phytopathologia Mediterranea, 56: 48-50.

Laspina, N. V., Groppa, M. D., Tomaro, M. L. and Benavides, M. P. 2005. Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. Plant Science, 169: 323-330.

Lecourieux, D., Ranjeva, R. and Pugin, A. 2006. Calcium in plant defence-signalling pathways. New Phytologist. 171: 249-69. doi: 10.1111/j.1469-8137.2006.01777.x.

Lima, M. R. M., Ferreres, F. and Dias, A. C. P. 2012. Response of *Vitis vinifera* cell cultures to *Phaeomoniella chlamydospora*: Changes in phenolic production, oxidative state and expression of defence-related genes. European Journal of Plant Pathology. 132: 133-146. DOI:10.1007/s10658-011-9857-4

Lizaso, J. I., Batchelor, W. D. and Westgate, M. E. 2003. A leaf area model to simulate cultivar-specific expansion and senescence of maize leaves. Field Crops Research, 80: 1-17.

Lorenz, D. H., Eichhorn, K. W., Bleiholder, H., Close, R., Meier, U. and Weber, E. 1995. Phenological growth stages of the grapevine (*Vitis vinifera* L). Encoding and description of the phenological stages of the grapevine according to the extended BBCH scheme. 100 Growth stages of the grapevine Australian Journal of Grape and Wine Research. 1: 100-103. DOI:10.1111/j.1755-0238.1995.tb00085.x.

Marschner, P. 2012. Marschner’s mineral nutrition of higher plants. Book. Third Edition. 2012. Academic Press; London, UK. pp. 178-189.

Mc Kinney, H. H. 1923. Influence of soil, temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Journal Agricultural Research, 26: 195-217. http://om.ciheam.org/article.php?IDPDF=800231.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Science. 7: 405-410. https://doi.org/10.1016/S1360-1385(02)02312-9.

Mugnai, L., Graniti, A. and Surico, G. 1999. Esca (black measles) and brown wood streaking: Two old and elusive diseases of grapevines. Plant Disease, 83: 404-418. https://doi.org/10.1094/PDIS.1999.83.5.404.

Pacetti, A., Moretti, S., Pinto, C., Compant, S., Farine, S., Bertsch, C. and Mugnai, L. 2021.

Trunk Surgery as a Tool to Reduce Foliar Symptoms in Diseases of the Esca Complex and Its Influence on VineWood Microbiota. Journal of Fungi. 2021 Jun 29: 521- 548. doi: 10.3390/jof7070521.

Reis, P., Pierron, R., Larignon, P., Lecomte, P., Abou-Mansour, E., Farine, S., Bertsch, C., Jacques, A., Trotel-Aziz, P., and Rego, C. 2019. Vitis Methods to understand and develop strategies for diagnosis and sustainable control of grapevine trunk diseases. Phytopathology, 109: 916-931. https://doi.org/10.1094/PHYTO-09-18-0349-RVW.

Sharma, D., Rawat, I. and Goel, H. G. 2012. Antioxidant and prebiotial of some cucurbits. Research Journal of Medicinal Plant. 6: 500-510.

Shaul, O. 2002. Magnesium transport and function in plants: The tip of the iceberg. BioMetals, 15: 307-321. DOI: 10.1023/A:1016091118585.

Shen, W. B., Huang L. Q. and Xu, L. L. 1997. Ascorbate guaiacol peroxidase in plants. Journal of Zhejiang University-SCIENCE B.14: 1110-1120. doi: 10.1631/jzus. B1300105.

Sosnowski, M. R., Wicks, T. W. and Scott E. S. 2011b. Control of Eutypa dieback in grapevines using remedial surgery. Phytopathologia Mediterranea, 50: S277-S284. DOI: https://doi.org/10.14601/Phytopathol_Mediterr-8919.

Sparapano, L., Bruno, G. and Graniti A. 1998. Esopolisaccaridi fitotossici sono prodotti in coltura da due specie di *Phaeoacremonium associate* al complesso del “mal dell’Esca” della vite. Phytopathologia Mediterranea 39: 16-20. DOI:10.14601/Phytopathol_Mediterr-1539.

Surico, G. 2009. Towards a redefinition of the diseases within the Esca complex of grapevine. Phytopathologia Mediterranea, 48: 5-10.

Úrbez-Torres, J. R. and Gubler, W. D. 2011. Susceptibility of grapevine pruning wounds to infection by *Lasiodiplodia theobromae* and *Neofusicoccum parvum*. Plant Pathology 60: 261-270. DOI:10.1111/j.1365-3059.2010.02381.x.

Whiting, E. C., Khan, A. and Guber, W. D. 2001. Effect of temperature and water potential on survival and mycelial growth of *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. Phytopathologia Mediterranea. 51: 37-50. DOI:10.14601/Phytopathol_Mediterr-9499.

Yang, T., Groenewald J. Z., Cheewangkoon, R., Jami, F., Abdollahzadeh, J., Lombard, L. and Crous, P. W. 2017. Families, genera and species of Botryosphaeriales. Fungal Biology.121: 322-346. doi: 10.1016/j.funbio.2016.11.001. Epub 2016 Nov 21.

تأثیر کاربرد کودهای محلول‌پاشی بر بیماری‌های تنه انگور

امیدعلی عمارلو[۱*]، صفرعلی مهدیان[۱]، محمدعلی تاجیک قنبری[۱] و حمید محمدی[۲]

۱- گروه گیاه‌پزشکی، دانشکده علوم زراعی، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران.
۲- گروه گیاه‌پزشکی ، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان، ایران.
پست الکترونیکی نویسنده مسئول مکاتبه: 21omid234100@gmail.com
دریافت: ۶ دی ۱۴۰۲؛ پذیرش: ۱۸ دی ۱۴۰۳

چکیده: بیماری‌های تنه انگور از زمان کاشت تا مرحله برداشت از مشکلات حیاتی انگور است. انگور به‌دلیل داشتن تنه چوبی چندساله، مستعد ابتلا به ۲۹ بیماری قارچی است، بنابراین شناسایی و مدیریت آن‌ها بسیار مهم است. ازجمله روش‌های کنترل این بیماری‌ها، تغذیه با کودهای معدنی برای کاهش علائم برگ ناشی از ترشحات سمّی قارچ‌های ساکن در قسمت چوبی درخت از اهمیت بالایی برخوردار است. برای کنترل این بیماری‌ها، مخلوطی از کودهای حاوی کلریدکلسیم $CaCl_2$، ۴۶۶ گرم، نیترات منیزیم $Mg(NO_3)_2$، ۴۰۳ گرم، عصاره جلبک دریایی (۷۵ میلی‌لیتر) و آب مقطر استریل ۴۶۶ میلی‌لیتر در لیتر محلول‌پاشی، روی تیمارهای انتخابی در باغ سهرابی هر ۲۰ روز یک‌بار مورد استفاده قرار گرفت. پراکسیداز، کاتالاز و شاخص سطح برگ در باغ سهرابی طی سال‌های ۱۳۹۶ و ۱۳۹۷ اندازه‌گیری و با شاهد مقایسه شدند. در تیمارهای سمپاشی شده با کودها، میانگین آنزیم پراکسیداز ۱۲/۷۲ میکرولیتر نسبت به شاهد (۱۰/۶ میکرولیتر) و میانگین آنزیم کاتالاز ۸۳/۶۸ میکرولیتر نسبت به شاهد (۳۱/۸۵ میکرولیتر) بود. متوسط اندازه سطح برگ در تیمارهای محلول‌پاشی شده در باغ سهرابی ۱۱۵۶۴ در مقایسه با شاهد (۴۹۵۹) میلی‌متر مربع بود. شدت بیماری در تیمارهای اسپری شده (۱۹/۹۵ درصد) کم‌تر از گروه شاهد (۵۶/۶ درصد) بود. این نتایج به‌دلیل افزایش سطح برگ است که باعث افزایش میزان فتوسنتز می‌شود و افزایش پراکسیداز و کاتالاز که استرس اکسیداتیو ناشی از ترشح قارچ را کاهش می‌دهد که منجر به کاهش علائم در تیمارهای محلول‌پاشی می‌شود.

واژگان کلیدی: کاهش انگور، کودهای معدنی، استرس اکسیداتیو، کاتالاز، پراکسیداز