El-Sharkawy *et al.*
Egyptian Journal of Biological Pest Control (2023) 33:61
https://doi.org/10.1186/s41938-023-00707-x

Egyptian Journal of Biological Pest Control

RESEARCH

Open Access

Boosting biopesticide potential of *Trichoderma harzianum* for controlling the downy mildew and improving the growth and the productivity of King Ruby seedless grape

Hany H. A. El-Sharkawy[1*], Thoraya S. A. Abo-El-Wafa[2], Nada A. Mostafa[3] and Safaa A. M. Yousef[1]

Abstract

Background *Plasmopara viticola,* the causal agent of grape downy mildew, is one of the most serious grape diseases. Application of *Trichoderma harzianum* grown on different potato dextrose supplemented broth media using some chemical inducers (TSDCIS), i.e., thiamine (T2), a mixture of micronutrients (T3) and potassium tartrate (T4), compared to *Trichoderma* without amendment (T1), was conducted under field conditions to evaluate the potential of *T. harzianum* against grape downy mildew, improving the growth, as well as the yield quality, and quantity.

Results Foliar application of all TSDCIS significantly reduced the disease severity and increased the phenolic content, peroxidase, polyphenol oxidase enzyme activity, growth parameters, and yield parameters. *Trichoderma* growing on medium supplemented with potassium tartrate (T4) achieved the most significant reduction in the disease severity (78.9, 81.8%) than the control treatment in two growing seasons, respectively. In contrast, it decreased H_2O_2 content, lipid peroxidation, and cellular electrolyte leakage. Scanning electron microscopy observations revealed that the number of opened stomata, average stomatal area, and stomatal pore area decreased significantly in response to T4 treatment.

Conclusions It can be concluded that using *Trichoderma* growing on medium supplemented with potassium tartrate (T4) to biosafety control the downy mildew disease of grape and improve its growth, yield, and fruit quality is recommended.

Keywords *Plasmopara viticola*, Grape, *Trichoderma harzianum*, Biological control, Growth, Yield

Background

The grape (*Vitis vinifera* L.) crop, which belongs to the Vitaceae family, is one of the greatest widely grown commercial fruit crops in the world. The grapevine cultivar King Ruby seedless is one of the world's greatest widely cultivated species due to its high yield and coveted properties; however, its susceptibility to downy mildew acutely reduces yield.

Many diseases infect grape plants, especially downy mildew caused by *P. viticola* (Berk et Curt.) Berl. et de

*Correspondence:
Hany H. A. El-Sharkawy
drhanylasheen@arc.sci.eg
[1] Mycology Research and Plant Disease Survey Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt
[2] Viticulture Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt
[3] Agricultural Botany Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt

Tonia. The grape downy mildew: it is a heterotrophic like fungus that belongs to the Oomycetes and Peronosporaceae family and is the primary limiting factor in grape cultivation. It affects grape yield and wine quality by severely reducing growth and yield, resulting in up to 50% losses (Gessler et al. 2011). Chemical antioomycete fungicides are commonly used to control downy mildew disease, but their use has resulted in the emergence of pathogen resistance to many fungicides (Massi et al. 2021). Furthermore, the buildup of chemical fungicides in soil poses environmental risks to plants and non-target soil microbes (Bavaresco et al. 2019).

Because of the widespread public concern about human health and the safety of agricultural food products, as well as their impact on the environment, the use of biofungicides as safe alternatives to synthetic pesticides has increased rapidly. *Trichoderma* is highly marketable due to its ability to reduce several plant pathogens, i.e., *Puccinia graminis* and *P. viticola* (Kamble et al. 2021). *Trichoderma* spp. can directly affect the pathogen by competing for space and/or nutrients, secreting extracellular lytic enzymes, volatile and non-volatile poisonous metabolites, high proliferation antibiosis, mycoparasitism, and/or indirectly by inducing plant resistance (Kamble et al. 2021).

Trichoderma spp. can also boost plant growth and yield by directly stimulating plant hormones like indole-3-acetic acid, gibberellins, harzianolide, harzianopyridone, or harzianic acid (El-Sharkawy et al 2018b). These hormonal enhancements may increase chlorophyll formation, metabolism, cell division, and yield directly, or indirectly through pathogen resistance induction (El-Sharkawy et al. 2018b). Yousef et al. (2018) found out that adding various chemical inducers (micronutrient mixture, potassium tartrate, and thiamine) to a a potato dextrose broth (PDB) medium improved *T. harzianum* antagonism against *R. solani.* They also reported new anti-fungal compounds secreted by *Trichoderma* because of its growth on media supplemented by some chemical inducers such as 2,3-butanediol, decane, 2,4,6-trimethyl-, phenylethyl alcohol, acetic acid, 1H-benzocyclohepten-7-ol. The aim of this study was to upgrade the potential of *T. harzianum* a as a biofungicide against *P. viticola* using different potato dextrose media supplemented with some chemical inducers, i.e., thiamine, a mixture of micronutrients, and potassium tartrate, with the concentrate of spore suspension 10^8 spore ml^{-1} of *Trichoderma*, to evaluate the potential of *T. harzianum* against grapevine downy mildew, to improve the growth, as well as the yield quality, quantity, antioxidant activity, and stomatal opening.

Methods

Trichoderma, chemical materials, and cultivar

T. harzianum HL14 was isolated from the rhizosphere of a healthy bean in Ismailia governorate, Egypt, and was identified morphologically at the Department of Mycology and Plant Disease Survey, Plant Pathology Research Institute, Agricultural Research Center, Egypt. The grape used in the field experiments was the King Ruby seedless cultivar. All chemical materials used in the tests were obtained from Al-Gomhouria Co, Egypt.

Propagation of *T. harzianum*

Five-millimeter mycelial disks from a seven-day-old culture of *T. harzianum* HL14 were inoculated into 500 ml flasks containing 100 ml of potato dextrose broth media supplemented with different tested materials; potassium tartrate 30 mg, a mixture of micronutrients (mg) (Zn sulfate 50 mg, Mn sulfate 50 mg, boric acid 20 mg and selenium 0.01 mg), and thiamine 1 mg. The whole cultures of *Trichoderma* were amended to sterilized tap water, and the final concentration was adjusted to 10^8 spore ml^{-1} before suspensions were blended with 0.5% potassium soap (El-Sharkawy et al. 2018a, b).

Field experiment

The experiment was conducted during the growing seasons of 2020 and 2021 (April to August). The grape (King Ruby seedless cultivar) was grown in a special vineyard existing in the El-Khatatba region, Menoufia Governorate, Egypt. Ninety-six grapes**,** 8 years old, grown in sandy soil, and spaced (at 2 × 3 m) were chosen for this trial. The vines were trained to a quadrilateral cordon trellis using the Spanish baron shape system and were irrigated with a drip irrigation system. Physiochemical characteristics of the field soil were as follows: texture (sandy), organic content (1.3%), pH (8.21), electrical conductivity (5.11 ds/m, phosphorus content (24 ppm), potassium content (126 ppm), Cu content (21 ppm), Zn content (2 ppm), Mg content (1.9 ppm), and Fe content (2.56 ppm). The fertilizer doses were applied as recommended by the Egyptian Ministry of Agriculture and Land Reclamation. The experiment consisted of six treatments, each with four replicates, and each with four vines arranged in a completely randomized block design.

Spraying was applied at (1 l/vine) four times: the first at 50 days from buds burst, the second at 65 days after the buds burst, the third at 80 from the buds burst, and the fourth at the age of 95 from the buds burst. As a control, a set of vines was sprayed with water (+), and a chemical fungicide (dimethomorph + azoxystrobin, 80% WG) (Starchem Co, Egypt) was applied at 0.3 g l^{-1}. To spray the grapes, a 25 l agricultural knapsack motorized power sprayer with a solid cone nozzle (HT-767, Taizhou Tianyi

Agriculture & Forestry Machinery CO., Zhejiang, China) was used. The grape (surface leaves, branches, and clusters) was sprayed homogeneously until run-off happened. The experiment was carried out in the ambient weather conditions: mean temperature (24–36 °C), humidity (48–56 percent), rainfall (0–5 mm), and daily sunshine hours (9.5–11.8 h).

Disease assessment

In both growing seasons, the disease severity of the infected leaves grapes was evaluated two times, the first at the 65 days post the fruit set and the second at 95 post the fruit set. The disease severity was assessed depending on Townsend and Heuberger (1943). Fifteen randomly selected leaves from every grapevine were assessed for downy mildew severity using 6-grades-scale based on the symptoms of disease and leaf destruction according to Townsend and Heuberger (1943), where 0 = no symptoms, 1 = 1–10%, 2 = 10–25%, 3 = 25–50%, 4 = 50–75%, and 5 = 75–100% infection. The severity of the disease was determined using the following formula:

$$\text{DS (percentage)} = \frac{\Sigma ab}{AK}$$

where a represents the number of infected plants of the same severity grade, b represents the severity grade, A represents the total number of plants, and K represents the highest disease degree.

Growth parameters assessment

At 30 days after the fruit set, the vines vegetative growth parameters in non-fruiting shoots were assessed in response to the applied treatments. The parameters assessed included the number of leaves, leaf area (cm^2), shoot length (cm), and pruning/vine weight (g). The seventh leaf from the tip was chosen to measure the leaf area per vine (Montero et al. 2000). A weight of pruning was performed during winter pruning (g).

Yield and its components parameters

Harvesting occurred 80 days after the fruit set, when the soluble solid content (SSC Brix) of berries reached 16–17% in berry juice. The total yield per vine (kg) and the weight of 100 berries (g) were estimated. The average cluster weight was determined by weighting six clusters per grape (g). The average yields per (kg) grape and the number of clusters per grape were measured. The weight of 100 berries (g), cluster length (cm), cluster width (cm), berry length (cm), and width (cm) were all determined.

Chemical properties of berries

The chemical properties of berries were assessed at 80 days after fruit set, when the soluble solid content (SSC, °Brix) of berries reached 16–17% in berry juice. SSC (TSS, °Brix) was determined using a Master T hand refractometer (ATAGO Co., Ltd., Japan). Total acidity (g tartaric acid/100 ml juice) was determined using AOAC (2000), and the (SSC/acidity) ratio was calculated.

Biochemical analyses

The activities of peroxidase and polphenol oxidase enzymes were measured according to Galeazzi et al. (1981) and Maxwell and Bateman (1967), respectively, in the leaves. According to Malick and Singh (1980), the total phenols content was also determined. According to Shao et al. (2005), lipid peroxidation in grapevine leaves was estimated. According to the method described by Jana and Choudhuri (1981), hydrogen peroxide was estimated. The proline content was calculated using the methods described by Arbona et al (2003). Ascorbic acid was determined using the method described by Law et al (1983).

Scanning electron microscope

After the second spray, samples were collected for SEM examination. 1 cm^2 samples from control untreated and T4 treatment were dehydrated with a series of dilutions of ethanol (10–100), every stage for 10 min, dried with a critical point dryer (TEC-030), gold-coated using a sputter coater and examined using SEM (JEOL 100 CX-II ASID-4D, Tokyo, Japan).

Statistical analysis

All the data were analyzed using the statistical analysis system CoStat (CoHort Software, USA) version 6.4 (CoStat 2005). Significant treatment differences were evaluated by using Duncan's multiple range test (Duncan 1995) at $P \leq 0.05$.

Results

Impact on the disease severity

The data offered in Table 1 showed that all tested treatments significantly reduced disease severity than the untreated plants (C). The highest reduction was obtained by treatment T4, recording 78.9 and 81.8% in both experimental seasons, respectively. In contrast, the lowest reduction was obtained by treatment T1, which recorded 63.2 and 69.7% in each season.

Impact on the vegetative growth parameters, and weight of pruning

In grape plants, infection with *P. viticola* resulted in a dramatic decrease in the tested growth parameters; however, treatment with supplemented *Trichoderma* reduced the effect of this disease on growth parameters. As shown in (Table 2), all *Trichoderma* supplemented treatments

Table 1 Effect of TSDCIS on the disease severity of downy mildew King Ruby seedless grape during 2020 and 2021 growing seasons at 30 days post the fruit set

Treatment	Season 2020		Season 2021	
	Disease severity%	Reduction%	Disease severity%	Reduction%
C	17.1a	–	16.5a	–
F	2.8 f	83.6	2.4f	85.5
T1	6.3b	63.2	5.0b	69.7
T2	5.3c	69.0	4.0c	75.7
T3	4.2d	75.4	3.7d	77.6
T4	3.6e	78.9	3.0e	81.8

According to the Duncan multiple range test ($P \leq 0.05$), columns denoted by a same letter are not significantly different

C, control untreated; F, fungicide; T1, whole culture of *Trichoderma* control; T4, whole culture of *Trichoderma* growing on medium supplemented with K; T3, whole culture of *Trichoderma* growing on medium supplemented with Mn; T2, whole culture of *Trichoderma* growing on medium supplemented with thiamine

increased leaves number, shoot length, leaf area, and pruning weight than the untreated control. In both seasons, spraying the plants with T4 gave the best effects on the leaf number, plant length, leaf area, and pruning weight.

Impact on the yield and its components

Table 3 shows that spraying with TSDCIS and fungicide significantly increased all yield parameters: yield per vine (kg), number of clusters per vine, cluster width (cm), cluster length (cm), cluster weight (g), and 100 berries weight (g), than the untreated control. In both seasons, the T4 treatment produced the highest values in terms of yield per vine, number of clusters per vine, cluster width, cluster length, cluster weight, and the weight of 100 berries. T1, or a chemical anti-immersed fungicide, on the other hand, yielded the lowest values in this regard.

Impact on the antioxidant enzyme activity, and total phenols

TSDCIS significantly increased the activity of POD and PPO enzymes, as well as the total phenol content in grape plants, as shown in Table 4. T4 had the highest enzyme activity for peroxidase and polyphenol oxidase, as well as the highest total phenol content and ascorbic acid.

Impact on the proline, H_2O_2, lipid peroxidation, and cell membrane stability index

Data in Table 5 showed the effects of the studied treatments on proline, H_2O_2, lipid peroxidation, and cell membrane stability index. The results showed that TSDCIS significantly reduced proline, H_2O_2, and lipid peroxidation, while increasing the cell membrane stability index. T4 achieved the best results than the untreated control.

Electron microscopy observations

Compared to untreated grapes (Fig. 1), SEM analysis revealed that T4 resulted in a significant decrease in the number of opened stomata as well as in the average of the stomatal area and pore area on the abaxial leaf surface.

Impact on the berry chemical properties

Table 6 shows the effects of the TSDCIS studied on soluble solids content (SSC), SSC/acidity ratio, and acidity. TSDCIS improved the chemical properties than the untreated grapevines. Grapes sprayed with T4, on the other hand, demonstrated chemical properties superior to grapes. T4 applied gave the best value in terms of soluble solids content (SSC), SSC/acidity ratio, and reduced the total acidity when compared to untreated control grapes.

Table 2 Effect of TSDCIS on vegetative growth, and weight of pruning of King Ruby seedless grapevines during 2020 and 2021 growing seasons at 30 days post the fruit set

Treatment	Number of leaves		Leaf area $(cm)^2$		Shoot length(cm)		Weight of pruning/vine(g)	
	Season 2020	Season 2021	Season 2020	Season 2021	Season 2020	Season 2021	Season 2020	Season 2021
C	16.3f	17.0e	126.6f	128.0f	196.7e	220.0e	200.0e	2166.7e
F	20.7e	21.0d	135.2e	140.2c	280.0d	288.3d	3100.0d	3200.0d
T1	24.0c	24.7bc	136.8d	137.2d	303.3bc	313.3c	3266.7cd	3466.7cd
T2	22.3d	23.3c	138.7c	135.5e	296.7c	306.7c	3466.7c	366.7bc
T3	25.3b	26.3b	141.0b	141.0b	316.7b	330.0b	3800.0b	3900.0b
T4	27.3a	28.3a	143.1a	144.7a	343.3a	253.3a	4500.0a	4600a

According to the Duncan multiple range test ($P \leq 0.05$), columns denoted by a same letter are not significantly different

C, control untreated; F, fungicide; T1, whole culture of *Trichoderma* control; T4, whole culture of *Trichoderma* growing on medium supplemented with K; T3, whole culture of *Trichoderma* growing on medium supplemented with Mn; T2, whole culture of *Trichoderma* growing on medium supplemented with thiamine

Table 3 Effect of TSDCIS on the yield and its components parameters of King Ruby seedless grapevines during 2020 and 2021 growing seasons at 80 days post the fruit set

Treatment	Yield/vine(kg)		Cluster width (cm)		Cluster length (cm)		Cluster weight (g)		100 berries weight (g)		Number of clusters/Vine	
	Season 2020	Season 2021	Season 2020	Season 2021	Season 2020	Season 2021	Season 2020	Season 2021	Season 2020	Season 2021	Season 2020	Season 2021
C	7.9f	9.5f	18e	19e	21e	21.3e	473.3e	493.3f	300.0f	306.0e	15.0e	16.0d
F	12.4e	14.3e	20d	22d	25d	26d	666.7d	689.1e	347.0e	363.7d	18.0d	19.0c
T1	13.4d	14.3e	23c	25c	30c	31c	720.0c	730.0d	359.3d	394.0c	19.0 cd	20.0c
T2	17.3c	22.3c	25b	26bc	33b	34b	730.0c	773.3c	390.0c	400.0bc	20.0c	23.0b
T3	19.8b	22.8b	25b	27ab	31c	33b	850b	870.0b	400.0b	403.3b	23.0b	24.3b
T4	21.8a	24.7a	27a	28a	35a	36a	900.0a	911.8a	405.0a	411.0a	25.0a	27.0a

According to the Duncan multiple range test ($P \leq 0.05$), columns denoted by a same letter are not significantly different

C, control untreated; F, fungicide; T1, whole culture of *Trichoderma* control; T4, whole culture of *Trichoderma* growing on medium supplemented with K; T3, whole culture of *Trichoderma* growing on medium supplemented with Mn; T2, whole culture of *Trichoderma* growing on medium supplemented with thiamine

Table 4 Effect of TSDCIS on antioxidant enzyme activity, total phenols content at 30 days post the fruit set during 2020 season

Treatment	POD activity (Unit/g FW)	PPO activity (Unit/g FW)	Total phenols (mg g FW)	Ascorbic acid (mg g FW)
Control	3.4d	3.6e	594.0f	1.74e
Fungicide	3.1d	3.03d	521.9e	1.38f
T1	6.4c	5.1c	882.5d	2.11d
T2	7.2b	5.7b	1171.2c	2.43c
T3	7.5ab	6.0b	1192.7b	2.72b
T4	8.0a	6.6a	1459.1a	3.05a

According to the Duncan multiple range test ($P \leq 0.05$), columns denoted by a same letter are not significantly different

C, control untreated; F, fungicide; T1, whole culture of *Trichoderma* control; T4, whole culture of *Trichoderma* growing on medium supplemented with K; T3, whole culture of *Trichoderma* growing on medium supplemented with Mn; T2, whole culture of *Trichoderma* growing on medium supplemented with thiamine

Table 5 Effect of TSCIS on proline, H_2O_2, lipid peroxidation, cell membrane stability% of King Ruby seedless grapevines leaf during 2020 season at 30 days post the fruit set

Treatment	Proline µg g^{-1} FW	H_2O_2 µg g^{-1} FW	Lipid peroxidation MDA	Cell membrane stability index %
C	28.3a	58.4a	8.12a	85.3f
F	23.9b	56.3b	7.4b	86.69e
T1	20.0c	54.7c	6.7c	88.0d
T2	16.5d	54.03d	6.02d	89.1c
T3	13.2e	49.6e	5.3e	90.3b
T4	10.1f	47.4f	4.6f	91.5a

According to the Duncan multiple range test ($P \leq 0.05$), columns denoted by a same letter are not significantly different

C, control untreated; F, fungicide; T1, whole culture of *Trichoderma* control; T4, whole culture of *Trichoderma* growing on medium supplemented with K; T3, whole culture of *Trichoderma* growing on medium supplemented with Mn; T2, whole culture of *Trichoderma* growing on medium supplemented with thiamine

Discussion

Because of its high productivity and desirable characteristics, the King ruby seedless cultivar is one of the most common and preferred grape cultivars. However, its susceptibility to downy mildew severely limits its output. The present study showed that all tested the TSDCIS under field conditions led to upgrade the potential of *T. harzianum* against grape downy mildew, improved the growth, as well as the yield quality and quantity. Obtained data were consistent with the results of Yousef et al. (2018), who found that adding some chemical inducers (potassium tartrate, micronutrient mixture, and thiamine) to media of *Trichoderma* increased the potential antagonism activities of *T. harzianum* against *R. solani*. Also, Kamble et al. (2021) found that *T. longibrachiatum* elicited defense responses in against downy mildew grapevines.

Treatments with *Trichoderma* triggered a significant defense against *P. viticola* via numerous functions, including (1) triggering the resistance via a plant transcriptome and proteome composite reprogramming (Perazzolli et al. 2012); (2) triggering various defense enzymes, like cellulase, chitinases, 1,3-glucosidases, and ligninase, which can minimize the growth of oomycete like-fungi by decay of the cell walls (Dourou and La Porta 2023); (3) fabrication of various anti-oomycete antibiotics such as, pyrones, viridian, koninginins, gliovirin, which are poisonous to the oomycete pathogen (Kamble et al. 2021); (4) pilling up of numerous phenolic components, which showed a major role against *P. viticola* because of their anti-oomycete toxic nature (Olowe et al. 2022); (5) elicitation of several elicitors that boost various signaling agents such as salicylic and jasmonic acid (Kamble et al. 2021); (6) triggering of PPO and POD, and ascorbic acid; (7) reduction H_2O_2 content, lipid peroxidation, and cellular electrolyte leakage and (8) decreased significantly, the average stomatal area, stomatal pore areas, the number of closed stomata.

TSDCIS enhanced all the tested growth and yield parameters, outperformed the untreated control. *Trichoderma* secretes numerous secondary metabolites with various biological roles that enhance plant growth, i.e., (1) phytohormones and growth promoters i.e., harzianolide, indole acetic acid, harzianopyridone, or harzianic acid; (2) increase the nutrient uptake (Tan et al. 2022); (3) reducing the damage caused by *P. viticola* (Kamble et al. 2021); (4) growth promoter the volatile compound 2,3-butanediol (Yousef et al. 2018); (5) additionally, *Trichoderma* application can improve photosynthesis by increasing chloroplast size and grana number (Tan et al. 2022).

One of the greatest crucial defensive mechanisms documented in this study via the SEMexamination was the triggering effect of T4 on the stomatal pore area, stomatal closure and stomatal area. The stomata pore is the only gate for the entry of the fungus *P. viticola* in grape leaves, so controlling its lock plays a significant role in managing *P. viticola.* Their zoospores may be attracted near the stomata via chemotactic attraction in order to penetrate the grapevine leaves (Allègre et al. 2007). From the results obtained by SEM observation, that T4 elicited the stomatal closure, stomatal pore area, and stomatal area by 2,3-butanediol. This was in similar to that obtained by Wu et al. (2018) who found that *Bacillus amyloliquefaciens* FZB42 secreted 2, 3-butanediol caused stomatal closure in *Arabidopsis thaliana*. This study emphasized the triggering of the ROS-scavenging system in grapevines treated with TSCIS for enzymatic and

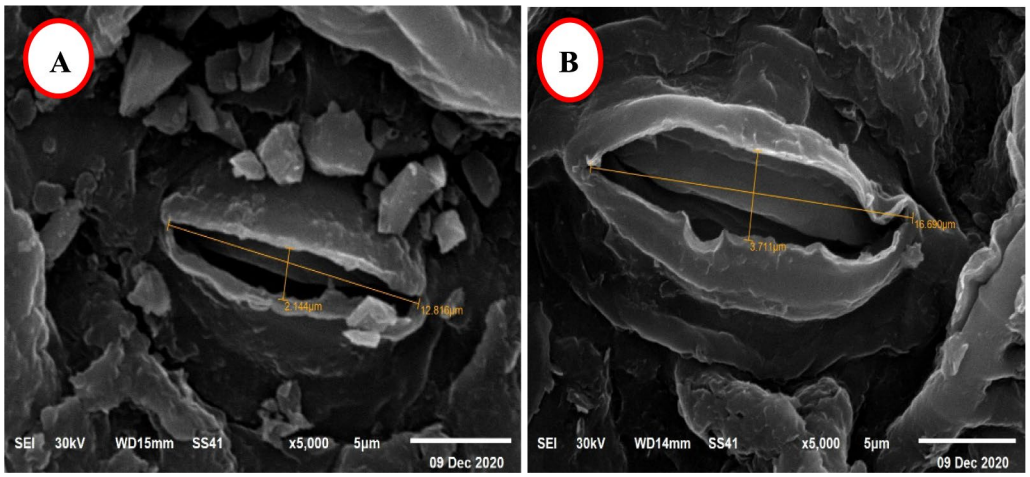

Fig. 1 Scanning electron micrographs of a grapevine leaves infected with *Plasmopara viticola* showing **A** closed stomata in leaf sprayed with T4 treatment, **B** opened stomata in a leaf from the untreated control grapevine (bar = 5)

Table 6 Effect of TSCIS on the chemical properties of berries of King Ruby seedless grapevines during 2020 and 2021 growing seasons at 80 days post the fruit set

Treatment	Soluble solids content		Total acidity (g tartaric acid/100 ml juice)		Soluble solids content/acidity	
	Season 2020	Season 2021	Season 2020	Season 2021	Season 2020	Season 2021
C	16f	16.4e	0.80a	0.70a	20.0d	23.43c
F	18e	18.5d	0.70b	0.56b	25.71d	32.83b
T1	20.0d	21c	0.50c	0.53b	42.19c	39.67b
T2	20.7c	22bc	0.40c	0.43c	50.0b	55.00a
T3	22b	23ab	0.40c	0.40c	55.0ab	57.50a
T4	23a	24a	0.40c	0.40c	57.44a	56.50a

According to the Duncan multiple range test ($P \leq 0.05$), columns denoted by a same letter are not significantly different

C, control untreated; F, fungicide; T1, whole culture of *Trichoderma* control; T4, whole culture of *Trichoderma* growing on medium supplemented with K; T3, whole culture of *Trichoderma* growing on medium supplemented with Mn; T2, whole culture of *Trichoderma* growing on medium supplemented with thiamine

non-enzymatic mechanisms, resulting in a significant minimization in lipid peroxidation and H_2O_2 content. The observed boost in POD and PPO enzyme activity suggested that the enzymatic antioxidant mechanism has been activated. On the other hand, the observed boost in total phenolic and ascorbic acid contents prop up the induction of the non-enzymatic antioxidant function. Another line of evidence for the role of TSCIS in disease resistance is the accumulation of secondary metabolites. Our findings showed that TSCIS cause hyper-piling up of phenolic compounds in grapevine leaves. These accumulations could be attributed to the activation of the phenylpropanoid pathway via increased phenyl amino lyase (PAL) gene expression and phenolic compound synthesis (Sood et al. 2013). This is not only to the mechanical function of phenolic compounds function in cell walls but also to their antifungal and antioxidant features (Kamble et al. 2021). *P. viticola* led to a decrease in the photosynthetic pigments and CO_2 diffusion within the mesophyll tissue, as well as misshaping of the chloroplast ultrastructure, which led to photosynthesis decay (Nogueira Júnior et al. 2020).

Conclusion

The results of the present investigation confirmed the possibility of upgrading the potential of biopesticide from *T. harzianum* for controlling the downy mildew of King Ruby seedless grapevine, improving the growth and yield by adding some supplemented materials to the medium of growth, where the best treatment that can suffices for this purpose was when plants were treated with whole culture of *Trichoderma* growing on medium supplemented with potassium tartrate (T4) treatment. Overall, it can be concluded that using T4 to control the downy mildew disease of grapevines and improve their growth, yield, and fruit quality was effective and environmentally safe.

Abbreviations

TSDCIS	*Trichoderma harzianum* grown on different potato dextrose broth supplemented media using some chemical inducers
SEM	Scanning electron microscopy
PAL	Phenyl amino layase
PPO	Polyphenol oxidase
POD	Peroxidase
C	Control untreated
F	Fungicides
T1	Whole culture of *Trichoderma* control
T4	Whole culture of *Trichoderma* growing on medium supplemented with potassium tartrate
T3	Whole culture of *Trichoderma* growing on medium supplemented with micronutrients
T2	Whole culture of *Trichoderma* growing on medium supplemented with thiamine
SSC	Soluble solid content

Acknowledgements
Not applicable.

Author contributions
HE contributed to the idea and design of the work, disease assessment, biochemical analysis, statistical analyses, and manuscript and photographs editing. TA contributed to the field experiment, growth and yield evaluation, analysis of the berries chemical properties, and NM contributed to the biochemical analysis, SEM, TEM investigation. SY contributed to the idea and the Trichoderma media preparations. All authors revised and approved the final manuscript.

Funding
Not applicable.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 25 March 2023 Accepted: 27 May 2023
Published online: 02 June 2023

References

Allègre M, Daire X, Héloir MC, Trouvelot S, Mercier L, Adrian M et al (2007) Stomatal deregulation in *Plasmopara viticola*-infected grapevine leaves. New Phytol 173:832–840. https://doi.org/10.1111/j.1469-8137.2006.01959.x
AOAC (2000) Official methods of analysis, 18th edn. Association of Official Analytical Chemists Inc, Gaithersburg
Arbona V, Flors V, Jacas J, Garcia-Agustin P, Gomez-Cadwenas A (2003) Enzymatic and non-enzymatic antioxidant responses of *Carrizo citrange* a salt-sensitive citrus rootstock, to different levels of salinity. Plant Cell Phys 44:388–394
Bavaresco L, Squeri C, Vercesi A (2019) Field evaluation of new plant protection products against *Plasmopara viticola*. BIO Web Conf 12:01007. https://doi.org/10.1051/bioconf/20191201007
CoStat 2005 Cohort Software, 798 Lighthouse Ave. PMB 320 Monterey, USA
Dourou M, La Porta CAM (2023) A Pipeline to investigate fungal–fungal interactions: trichoderma isolates against plant-associated fungi. J Fungi 9:461. https://doi.org/10.3390/jof9040461
Duncan DB (1995) Multiple ranges and multiple F test. Biometrics 11:1–42
El-Sharkawy HHA, Abo-El-Wafa TSA, Ibrahim SA (2018a) Biological control agents improve the productivity and induce the resistance against downy mildew of grapevine. J Plant Pathol 100:33–42
El-Sharkawy HHA, Rashad YM, Ibrahim SA (2018b) Biocontrol of stem rust disease of wheat using arbuscular mycorrhizal fungi and *Trichoderma* spp. Physiol Mol Plant Pathol 103:84–91
Galeazzi MAM, Sgarbieri VC, Constantinides SM (1981) Isolation, purification and physicochemical characterization of polyphenoloxidases (PPO) from a Dwarf variety of banana (*Musa cavendishii*, L). J Food Sci 46:150–155. https://doi.org/10.1111/j.1365-2621.1981.tb14551.x
Gessler C, Pertot I, Perazzolli M (2011) *Plasmopara Viticola*: a review of knowledge on downy mildew of grapevine and effective disease management. Phytopathol Mediterr 50:3–44
Jana S, Choudhuri MA (1981) Glycolate metabolism of three submerged aquatic angiosperms during aging. Aquatic Bot 12:345–354
Kamble MV, Joshi SM, Hadimani S, Jogaiah S (2021) Biopriming with rhizosphere *Trichoderma harzianum* elicit protection against grapevine downy mildew disease by triggering histopathological and biochemical defense responses. Rhizosphere. https://doi.org/10.1016/j.rhisph.2021.100398
Law MY, Charles SA, Halliwell B (1983) Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. The effect of hydrogen peroxide and of Paraquat. Biochem J 210:899–903. https://doi.org/10.1042/bj2100899
Malick CP, Singh MB (1980) Estimation of total phenols, plant enzymology and histo enzymology. Kalyani Publishers, New Delhi, p 286
Massi F, Torriani SFF, Borghi L, Toffolatti SL (2021) Fungicide resistance evolution and detection in plant pathogens: *Plasmopara viticola* as a case study. Microorganisms. https://doi.org/10.3390/microorganisms9010119
Maxwell DP, Bateman DF (1967) Changes in the activities of some oxidases in extracts of Rhizoctonia-infected bean hypocotyls in relation to lesion maturation. Phytopathology 57:132–136
Montero FJ, De Juan JA, Cuesta A, Brasa A (2000) Nondestructive methods to estimate leaf area in *Vitis vinifera* L. HortScience 35:696–698. https://doi.org/10.21273/hortsci.35.4.696
Nogueira Júnior AF, Tränkner M, Ribeiro RV, von Tiedemann A, Amorim L (2020) Photosynthetic cost associated with induced defense to *Plasmopara viticola* in grapevine. Front Plant Sci 11:235. https://doi.org/10.3389/fpls.2020.00235
Olowe OM, Nicola L, Asemoloye MD, Akanmu AO (2022) Babalola OO 2022 *Trichoderma*: potential bio-resource for the management of tomato root rot diseases in Africa. Microbiol Res 257:126978
Perazzolli M, Moretto M, Fontana P, Ferrarini A, Velasco R, Moser C, Delledonne M, Pertot I (2012) Downy mildew resistance induced by *Trichoderma harzianum* T39 in susceptible grapevines partially mimics transcriptional changes of resistant genotypes. BMC Genom 13:660–113
Shao HB, Liang ZS, Shao MA, Wang BC (2005) Changes of some physiological and biochemical indices for soil water deficits among 10 wheat genotypes at seedlings stage. Colloids Surf B 42:107–113
Sood N, Sohal BS, Lore JS (2013) Foliar application of benzothiadiazole and salicylic acid to combat sheath blight disease of rice. Rice Sci 20:5. https://doi.org/10.1016/s1672-6308(13)60155-9
Tan B, Li Y, Deng D, Pan H, Zeng Y, Tan X, Zhuang W, Li Z (2022) Rhizosphere inoculation of *Nicotiana benthamiana* with *Trichoderma harzianum* TRA1-16 in controlled environment agriculture: effects of varying light intensities on the mutualism-parasitism interaction. Front Plant Sci 13:989155. https://doi.org/10.3389/fpls.2022.989155
Townsend GR, Heuberger JW (1943) Methods for estimating losses caused by diseases in fungicide experiments. Plant Dis Rep 27:340–343
Wu L, Li X, Ma L, Borriss R, Wu Z, Gao X (2018) Acetoin and 2,3-butanediol from *Bacillus amyloliquefaciens* induce stomatal closure in *Arabidopsis thaliana* and *Nicotiana benthamiana*. J Exp Bot 69(22):5625–5635. https://doi.org/10.1093/jxb/ery326
Yousef SAM, Salem SH, El-Sharkawy HHA (2018) Effect of some chemical inducers on antagonistic potential of *Trichoderma harzianum* against *Rhizoctonia solani* and its metabolites production. J Plant Prot Path Mansoura Univ 9(8):497–506

Publisher's Note