Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2024v45n4p1109

Essential oil from orange peel in the control of *Botrytis cinerea* and in the postharvest conservation of 'Benitaka' table grape

Óleo essencial da casca da laranja no controle do *Botrytis cinerea* e na conservação pós-colheita da uva fina de mesa 'Benitaka'

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Highlights

Orange peel essential oil is efficient in controlling the gray mold.
The essential oil provides better postharvest conservation of 'Benitaka' table grapes.
The mycelial growth of *B. cinerea* was suppressed in the presence of the essential oil.

Abstract

The objective of this work was to evaluate the efficiency of essential oil from orange peel in the refrigerated conservation of the 'Benitaka' table grape, as well as to evaluate its *in vitro* effectiveness on *Botrytis cinerea,* the causal agent of gray mold. Grapes were harvested from a commercial field in the municipality of Cambira, Paraná, Brazil, during the 2022 and 2023 seasons. The experimental design was completely randomized, with four treatments and five replications of five bunches per plot. The treatments were: a) control; b) essential oil from orange peel at 4.0 mL of the commercial product (c.p.) L^{-1}; c) dual phase SO_2-generating pads containing 1 and 4 g of the active ingredient (a.i.) in the fast and slow phases, respectively; and d) essential oil from orange peel at 4.0 mL c.p. L^{-1} associated with the dual phase SO_2-generating pads containing 1 and 4 g of the a.i. in the fast and slow phases, respectively. The

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Received: Nov. 08, 2023 Approved: July 09, 2024

commercial product containing 61.14 g L^{-1} (6% w/v) of 4-isopropenyl-1-methylcyclohexane, the source of orange essential oil, was applied by spraying it directly onto the bunches. After drying, the grape bunches were stored in a cold chamber at 1.0±1°C and 95% relative humidity. The following variables were assessed 30 and 45 days after the beginning of cold storage: the incidence of gray mold on berries, loss of bunch mass, stem browning, shattered berries, and bleaching. The minimum inhibitory concentration for the development of *B. cinerea* was determined, and fungal mycelia were observed using scanning electron microscopy to evaluate the *in vitro* efficacy of orange essential oil. The data were subjected to analysis of variance, and the means were compared using Fisher's difference test at 5% probability. The effectiveness of orange essential oil in suppressing the development of *B. cinerea* was demonstrated both *in vivo* and *in vitro,* making it a safe alternative for the postharvest conservation of 'Benitaka' table grapes.
Key words: Gray mold. Essential oil. Fruit quality. Sulfur dioxide. *Vitis vinifera* L.

Resumo

O objetivo deste trabalho foi avaliar a eficiência do óleo essencial da casca da laranja na conservação refrigerada da uva de mesa 'Benitaka', bem como avaliar a sua eficácia *in vitro* sobre o fungo *Botrytis cinerea,* agente causal do mofo cinzento. As uvas foram obtidas de um campo de produção no município de Cambira, Paraná, Brazil nas safras de 2022 e 2023. O delineamento experimental foi inteiramente casualizado, com quatro tratamentos e cinco repetições, com cinco cachos por parcela, sendo avaliados os tratamentos: a) Controle; b) Óleo essencial da casca da laranja 4,0 mL p.c. L^{-1}; c) Folha geradora de SO_2 de liberação dupla fase contendo 1 e 4 g i.a. nas fases rápida e lenta, respectivamente; d) Óleo essencial da casca da laranja 4,0 mL p.c. L^{-1} associada à folha geradora de SO_2 de liberação dupla fase contendo 1 e 4 g i.a nas fases rápida e lenta, respectivamente. Como fonte de óleo essencial de laranja, empregou-se um produto comercial contendo 61,14 g L^{-1} (6% m/v) de 4-isopropenil-1-methilciclohexano, o qual foi aplicado por pulverização dirigida aos cachos. Após secos, os cachos de uva foram armazenados em câmara refrigerada a 1,0±1°C e 95% de umidade relativa do ar. Aos 30 e 45 dias após o início do armazenamento a frio foram avaliadas as variáveis: incidência de mofo cinzento nas bagas, perda de massa dos cachos, escurecimento da ráquis, degrana e branqueamento das bagas. Para a avaliação da eficácia *in vitro* do óleo essencial de laranja foi determinada a concentração inibitória mínima sobre o desenvolvimento de *B. cinerea,* além da observação dos micélios do fungo por meio de microscopia eletrônica de varredura. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste LSD de Fisher a 5% de probabilidade. A eficácia do óleo essencial de casca de laranja em suprimir o desenvolvimento de *B. cinerea* foi comprovada tanto *in vivo* como *in vitro,* sendo este biocomposto uma alternativa segura para a conservação pós-colheita de uvas de mesa 'Benitaka'.
Palavras-chave: Mofo cinzento. Dióxido de enxofre. Óleo essencial. Qualidade de frutos. *Vitis vinifera* L.

Introduction

The grapevine is one of the main fruit species cultivated worldwide. In 2021, the global grape production was 69,432,872 tons, grown in an area of 7,298,865 ha. Of the volume produced, approximately 43.3% is destined for consumption as fresh fruit (International Organisation of Vine and Wine [OIV], 2023). In addition to the nutritional value, the visual appearance of grapes is a characteristic of great relevance to the consumer market. Freshness, color, firmness, flavor, aroma, and tissue integrity are part of the set of attributes that define quality and, consequently, are determined as purchasing criteria for the consumer (Dantas et al., 2022).

As a fruit with a high moisture content and delicate tissue, grapes are subject to several factors that can affect their quality and increase the risk of loss (Liu et al., 2015), such as injuries caused by handling, loss of water, and attack by pathogens, which damage the final quality of the product (Ahmed et al., 2018; Youssef et al., 2019). The main cause of postharvest losses in table grapes is the fungus *Botrytis cinerea,* the causal agent of gray mold disease (Martínez-Romero et al., 2007; Tessmann et al., 2019). This pathogen occurs in all producing regions of the world, causing losses of economic importance in the pre- and postharvest phases. It may remain latent in the field and only be expressed during the transport and refrigerated storage of grapes (Elad et al., 2015).

In order to prevent the deterioration of table grapes during storage, sulfur dioxide (SO_2) is used. Therefore, an SO_2-generating pad is placed inside a carton box above the bunches. SO_2 is used to control certain types of postharvest rot, including *B. cinerea* (Lichter et al., 2008). A plastic perforated liner associated with the SO_2-generating pad is used to facilitate the circulation of gas in the packaging, in addition to maintaining humidity and preventing loss of fruit mass (Zutahy et al., 2008).

However, excessive SO_2 accumulation caused by improper use can result in berry bleaching damage (Zutahy et al., 2008), unpleasant flavors, and residual SO_2, which can be dangerous to human health (Xiao et al., 2019; Yuan et al., 2022). Furthermore, chemical compounds can cause environmental problems (Garcia et al., 2019).

Alternative products have been sought to control diseases and limit the risks of ingestion and contamination of these chemical compounds (Arruda et al., 2011). Among these, essential oils, characterized as secondary metabolites of plants with low toxicity to mammals, have been studied as potent biofungicides and natural insecticides and have shown promise for practical use in the control of various phytopathogens (Silva & Bastos, 2007; Bonett et al., 2012; Simon et al., 2016; Garcia et al., 2019). Additionally, essential oils are listed as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA, 2024).

Orange essential oils can be extracted from fruit-processing residues (Ferronatto & Rossi, 2018). Since the annual Brazilian production of oranges corresponds to around 16.2 million tons (Instituto Brasileiro de Estatística e Geografia [IBGE], 2023), a consolidated source of the raw material is available. It is estimated that Brazil is responsible for 79% of the world's industrial production of juices from which orange

residues are obtained, a material of interest for the extraction of essential oils, producing approximately 3.5 million tons of waste every year (Associação Nacional dos Exportadores de Sucos Cítricos [Citrus BR], 2021). Brazil is among the four largest producers of citrus essential oils worldwide (Jacob, 2017).

Limonene is the main component of orange essential oil. Studies have reported that limonene has antioxidant action (Ferronatto & Rossi, 2018) and antimicrobial properties, even when diluted (Zhang et al., 2009). However, no studies have demonstrated the efficiency of orange peel essential oil in controlling *B. cinerea* or in the postharvest conservation of table grapes. Therefore, the objective of this study was to evaluate the efficiency of orange peel essential oil in preserving the 'Benitaka' table grape in cold storage conditions, as well as evaluating its *in vitro* effectiveness on the development of the fungus.

Material and Methods

Postharvest conservation of the 'Benitaka' table grape

Freshly bunches of 'Benitaka' table grape (*Vitis vinifera* L.) were obtained from a commercial field with a history of occurrence of *B. cinerea,* located in the municipality of Cambira, Paraná, Brazil coordinates 23°35'27" S 51°34' 14" W and 820 m elevation. The climate is classified as humid subtropical (Cfa) by Köppen, with an average winter temperature below 18 ºC, an average summer temperature above 22 °C and 1,600 mm of precipitation, which occurs mainly in the summer (Nitsche et al., 2019).

The experiment was conducted during two consecutive seasons in 2022 and 2023. Berries were harvested when they reached a soluble solid content of 14ºBrix. After harvesting, the boxes were transported to the Fruit Analysis Laboratory of the Agronomy Department at the State University of Londrina for further treatment and packaging of the bunches.

The experimental design was completely randomized, consisting of four treatments with five replicates, with each plot consisting of five bunches, stored individually in plastic clamshells with a capacity of 0.5 kg each and placed in carton boxes, each with a capacity of five clamshells. The following treatments were evaluated: a) control, b) essential oil from orange peel at 4.0 mL of the commercial product (c.p.) L^{-1}, c) dual phase SO_2-generating pads containing 1 and 4 g of the active ingredient (a.i.) in the fast and slow phases, respectively, and d) essential oil from orange peel at 4.0 mL c.p. L^{-1} associated with the dual phase SO_2-generating pads containing 1 and 4 g of the a.i. in the fast and slow phases, respectively. As a source of orange essential oil, the commercial product PREV-AM™ containing 61.14 g L^{-1} (6% w/v) of 4-isopropenyl-1-methylcyclohexane was used.

In the laboratory, the bunches were placed on a bench and selected. Next, the bunches were treated with orange essential oil by spraying the solution directly onto the branches. A pause time of 24 hours was given until the bunches was completely dried for subsequent packaging.

The bunches were then standardized, weighing approximately 0.5 kg, and placed in plastic clamshells packaged according to

their respective treatments. For packaging, the perforated plastic liner was initially placed in a corrugated cardboard box above the plastic liner. A unilaminar sheet of moisture-absorbing paper was placed in the bottom of the box, and five plastic clamshells containing the grape bunches were placed inside the box. When necessary (according to the treatment), a dual-phase SO_2-generating pad containing 1 g and 4 g of the active ingredient (a.i.) (fast and slow phases, respectively) was placed above the clamshells, and the liner was sealed with adhesive tape.

After completing the packaging process, the boxes with the grapes were stored in a cold chamber at 1.0 ± 1 ºC and relative air humidity greater than 90% for 45 days. The treatments were evaluated 30 and 45 days after the start of refrigerated storage, and the following variables were analyzed: incidence of gray mold on berries, stem browning, weight loss, and shattered berries. After 45 days, the incidence of bleaching, soluble solid content, and titratable acidity were evaluated.

The incidence of gray mold on berries was quantified using the following equation: incidence (%) = (number of infected berries/ total berries in the bunch) × 100 (Youssef & Roberto, 2014). Stem browning was assessed visually according to the methodology described by Ngcobo et al. (2011), assigning grades based on the browning level: 1, fresh and green; 2, light browning; 3, significant browning; and 4, severe browning. Mass loss was obtained by weighing the bunches at the initial moment of storage and at the time of each evaluation and was calculated using the following equation: mass loss (%) = (initial mass - final mass/initial mass) × 100 (Mattiuz et al., 2009). Shattered berries were assessed by counting the berries released from the bunch inside the bowl and expressed as percentages. The bleaching of berries was assessed according to the formula described by Henríquez and Pinochet (2016): bleaching of berries (%) = (number of berries with bleaching/total number of berries in the bunch) × 100.

For physicochemical analyses, samples consisting of 10 berries from each plot were collected after 45 days of storage. The berries were then crushed to extract juice. The soluble solid content was obtained using a digital refractometer (Atago Pal-1), and the results were expressed in °Brix. Titration using 10 mL of the extracted juice and a standardized 0.1 N NaOH solution in a semi-automatic titrator was carried out to determine the titratable acidity. The endpoint of the titration was pH = 8.2, and the result was expressed in percentage of tartaric acid, using the equation: % tartaric acid = (PM × M × V) / P, where: PM = molecular weight of tartaric acid; M = Molarity of the NaOH solution; V = Volume of NaOH used in the titration (mL); P = Sample volume (mL) (Instituto Adolfo Lutz [IAL], 2008).

In vitro efficacy of essential oil on the development of B. cinerea

Fungal isolates of *B. cinerea* were cultivated in potato dextrose agar (PDA) medium (Neogen Corporation, USA) at 25 °C, with a 12 hour/12 hour photoperiod, and stored in the same medium at 4 °C. All the isolates were deposited in the Microbial Culture Collection of the Phytopathology Laboratory of the Agronomy Department of the State University of Londrina.

Minimum Inhibitory Concentration (MIC)

MIC values for *B. cinerea* were determined using a Petri dish serial dilution method with PDA, in which 10 mL of BDA was mixed with respective concentrations of purified orange peel essential oil (0, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6%) in molten agar. Discs (8 mm in diameter) with B. cinerea mycelia were placed on the surface of the culture medium with the respective concentrations of the compound. The plates were incubated at 25°C with a 12h/12h photoperiod, and fungal growth was recorded after 10 days of incubation (total fungal growth on control plates). Each treatment was performed in six replicates, and the assay was repeated twice. The percentage of mycelial growth inhibition (MGI) was calculated according to the method described by Yahyazadeh et al. (2008):

$$MGI\ (\%) = \left(\frac{d_c - d_t}{d_c}\right) x\ 100$$

where dc (mm) is the average colony diameter in the control, and dt (mm) is the average colony diameter in each treatment. The effective doses of 50 and 80% (ED50 and ED80) were determined when growth was reduced by 50 or 80% compared with that of the control, respectively. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that completely inhibited fungal growth.

Scanning Electron Microscopy (SEM)

Fungal cultures grown for 4 days on PDA and treated with different concentrations of PCA (0 and ED80) were used for all SEM observations. Discs of 8 mm in diameter were cut from the cultures and placed in flasks containing 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4°C. The samples were kept in this solution for 4 hours for fixation and then washed with 0.1 M sodium cacodylate buffer (pH 7.2) for 10 min three times. Subsequently, the samples were dehydrated in a series of ethanol (70, 80, 90, and 100%) for 10 minutes, three times. The samples were dried to their critical point using CO_2 (BALTEC CPD 030 Critical Point Dryer), coated with gold (BALTEC SDC 050 Sputter Coater), and observed using an FEI Quanta 200 Scanning Electron Microscope operating at 30 kV (Simionato et al., 2017).

Statistical analysis

The percentage data were transformed into $\sqrt{x + 0{,}5}$ to vary normalization before ANOVA analysis. Subsequently, the data were subjected to analysis of variance, and the means of the treatments were compared using Fisher's least significant difference (LSD) test (p<0.05) in the R software.

Results and Discussion

Postharvest conservation of the 'Benitaka' table grape

There was a statistically significant difference in the incidence of gray mold on berries among treatments in both seasons during the two periods evaluated (Table 1). In the 2022 season, after 30 days of refrigerated storage, treatments in which dual-phase release SO_2-generating pads (DPGP) were used, with or without essential oil (EO), were

more efficient. However, when only EO was used, no differences were observed compared to the control group during this period. After 45 days of refrigerated storage, an increase in the incidence of gray mold on the berries was observed, and the efficiency of all the treatments used differed from that of the control; however, treatments that included DPGP were still the most efficient, presenting a very low incidence of the disease. The use of EO reduced the incidence of the disease by approximately 40% in relation to the control.

Table 1
Gray mold incidence of 'Benitaka' table grape (*Vitis vinifera* L.) at 30 and 45 days of cold storage (1.0 ± 1.0 ºC) individually packaged in plastic clamshells. Seasons of 2022 and 2023

Treatments	Gray mold incidence (%)			
	At 30 days of cold storage		At 45 days of cold storage	
	2022	2023	2022	2023
Control	5.17 a	2.83 a	7.22 a	4.58 a
EO	3.57 a	1.33 b	4.56 b	2.00 b
DPGP	0.91 b	0.00 d	1.14 c	0.00 d
EO + DPGP	0.53 b	0.42 c	1.60 c	0.58 c
F	26.79**	48.77**	38.59 **	62.66**
CV (%)	17.93	13.06	13.51	13.93

Means followed by the same letter are not different by Fisher´s test (p<0.05%). **: significant (p<0.01). CV: coefficient of variation. EO: essencial oil. DPGP: Dual phase SO_2-generating pad.

After 30 and 45 days of refrigerated storage in the 2023 season, the effect of the treatments tested against the control was again observed. The DPGP treatment was the most efficient, resulting in the complete absence of the disease even after 45 days of storage. This season, EO reduced the incidence of the disease by almost 60% in relation to the control after 45 days.

The DPGP treatment controlled the gray mold more efficiently than the other tested treatments. This is due to the fact that DPGP presents a double release of SO_2; that is, this sheet has different permeabilities, resulting in a rapid release of the gas in the first 48 hours of storage, which provides an eradication effect of spores from the skin of the berry, followed by a subsequent slow and continuous release of the gas for up to 60 days (Fernández-Trujillo et al., 2012; Champa, 2015). Therefore, this continuous release of gas was responsible for effectively controlling gray mold even after 45 days of storage.

Several studies have demonstrated the efficiency of SO_2-generating pads with a slow or double release phase in controlling gray mold in several table grape cultivars

(Ahmed et al., 2019; Chaves et al., 2019; Mühlbeier et al., 2021; Dantas et al., 2022; Aguiar et al., 2023). However, excessive SO2 accumulation can damage berries, resulting in bleaching damage (Zutahy et al., 2008; Sortino et al., 2017; Ahmed et al., 2018), cause environmental problems (Garcia et al., 2019), and sulfites are recognized as allergens by several international food safety regulatory bodies (Food and Agriculture Organization [FAO], 2005; Crisosto, 2008). Thus, orange peel EO could be an alternative to avoid sulfite residues on grape berries.

Little is known about the efficiency of EOs in controlling *B. cinerea* in postharvest table grapes, especially regarding the effect of EO from orange peel, which, to the best of our knowledge, has only been tested *in vitro* in a study carried out by Hernández et al. (2021). The authors studied the antifungal activity of orange peel polyphenolic extract (OPE) against three postharvest fungal pathogens: *Monilinia fructicola, Botrytis cinerea,* and *Alternaria alternata.* The OPE at the highest dose tested (1.5 g L^{-1}) completely inhibited mycelial growth and conidial germination of the three target fungi, indicating the potential of this essential oil.

In a study in which different EOs were evaluated in the control of gray mold in 'Rubi' grapes, including *Eugenia uniflora, Casearia sylvestris,* and *Melaleuca alternifolia* at a concentration of 100%, a reduction of 45.7; 43.1 and 21.2% of the disease was recorder, respectively, in relation to the control (Garcia et al., 2019). Considering that the commercial product used in this study had a concentration of 61.14 g L^{-1} orange peel essential oil, an advantage was observed compared to the essential oils evaluated by these authors, which were tested without dilution.

The potential of cinnamon and oregano EOs was evaluated for the control of gray mold on 'Taify' table grapes *in vitro* and *in vivo,* and it was verified that the essential oils at a concentration of 800 µL L^{-1} reduced the severity of gray mold by 58.9% and 42%, respectively, compared with the that of control (Almasaudi et al., 2022).

The antifungal effects of sage, lavender, mint, and tea tree oils were evaluated in the control of the fungus *B. cinerea* on table grapes. It was concluded that all four oils effectively inhibited pathogen growth and that the antifungal effects were dose-dependent *in vitro.* Furthermore, peppermint oil at 500 µL L^{-1} can significantly inhibit conidial germination and disease incidence *in vivo* (Xueuan et al., 2018).

The *in vitro* and *in vivo* effects of EOs from *Eucalyptus staigeriana* and *Eucalyptus globulus* were evaluated against *B. cinerea* and gray mold in wine grapes. It was recorded that the two EOs showed antifungal activity *in vitro* and that the EO from *E. staigeriana* at a concentration of 0.5 µL mL^{-1} was able to reduce the incidence and severity of gray mold caused by *B. cinerea* (Pedrotti et al., 2019).

As shown, several EOs can potentially control post-harvest diseases and thus can be used as an alternative strategy for controlling fungal diseases. Essential oils are known for their antimicrobial and biodegradable properties and their lack of residual effects on fresh produce (Bakkali et al., 2008). EOs suppress fungal growth, often prevent hyphal growth and sporulation, disrupt nutritional absorption and metabolism, disrupt the plasma membrane, damage mitochondrial structure, and interfere with enzymatic and respiratory processes (Patel & Jasrai, 2011).

Regarding the weight loss of bunches, no statistical differences were observed between the treatments used in any of the seasons or evaluation periods (Table 2). Weight loss is one of the key factors that determine the excellence and quality of table grapes; that is, the greater the amount of water lost by the product, the more it develops quality loss problems (Ahmed et al., 2019). As a result of weight loss, the wrinkling of the berries stands out, which occurs when the loss reaches approximately 4 to 5%, thus affecting the appearance and firmness ideal for consumption (Gorgatti et al., 1993). However, the mass loss of the 'Benitaka' grape bunches in this study was very low and did not affect the characteristics of the bunches.

Table 2
Weight loss of 'Benitaka' table grape (*Vitis vinifera* L.) at 30 and 45 days of cold storage (1.0 ± 1.0 ºC) individually packaged in plastic clamshells. Seasons of 2022 and 2023

Treatments	Weight loss (%)			
	At 30 days of cold storage		At 45 days of cold storage	
	2022	2023	2022	2023
Control	2.33 a	1.18 a	3.13 a	1.81 a
EO	1.62 a	1.38 a	2.08 a	1.89 a
DPGP	2.35 a	1.24 a	3.07 a	1.67 a
EO + DPGP	1.61 a	1.37 a	2.26 a	2.03 a
F	1.94 ^{ns}	0.70 ^{ns}	2.30 ^{ns}	1.30 ^{ns}
CV (%)	13.00	6.97	12.25	6.27

Means followed by the same letter are not different by Fisher´s test (p<0.05%). ^{ns}: not significant. CV: coefficient of variation. EO: essencial oil. DPGP: Dual phase SO_2-generating pad.

It is important to highlight that fresh vegetables have a high-water content and are subject to dehydration (shriveling). Furthermore, the longer the refrigerated storage period, the greater the weight loss due to water loss from the bunches (Colombo et al., 2018), as shown in Table 2. Furthermore, water loss is directly proportional to the vapor pressure difference (VPD) between the product and its environment, and the VPD is inversely related to the relative humidity of the air around the product (Kader, 2013). Therefore, the use of perforated plastic liners is important, as it helps maintain humidity, prevents the loss of fruit mass, and preserves the freshness of the rachis (Antoniolli & Lima, 2008).

The grapes subjected to the various treatments evaluated in this study maintained the freshness of the stems in both seasons (Table 3). However, in the 2022 season, after 30 and 45 days of cold storage, both treatments differed from the control, showing better preservation of the freshness

of the stems; at 45 days of storage, the EO + DPGP treatment was the most efficient. In the 2023 season, no difference was observed between the treatments after 30 days of storage; however, after 45 days, the stems of the control bunches were darker than those of the other treatments, showing reduced quality conservation of the stems.

Table 3
Stem browning scores of 'Benitaka' table grape (*Vitis vinifera* L.) at 30 and 45 days of cold storage (1.0 ± 1.0 ºC) individually packaged in plastic clamshells. Seasons of 2022 and 2023

Treatments	Stem browning [a]			
	At 30 days of cold storage		At 45 days of cold storage	
	2022	2023	2022	2023
Control	1.32 a	1.04 a	1.88 a	1.24 a
EO	1.08 b	1.00 a	1.60 b	1.04 b
DPGP	1.12 b	1.00 a	1.40 b	1.04 b
EO + DPGP	1.00 b	1.00 a	1.04 c	1.04 b
F	6.62 **	1.00 [ns]	17.83 **	3.84 *
CV (%)	10.47	4.43	12.64	10.46

[a] Stem browning scores: (1) fresh and green, (2) light browning, (3) significant browning, and (4) severe browning (Ngcobo et al., 2011). Means followed by the same letter are not different by Fisher´s test (p<0.05%). **: significant (p<0.01). *: significant (p<0.05). [ns]: not significant. CV: coefficient of variation. EO: essencial oil. DPGP: Dual phase SO_2-generating pad.

The 'Benitaka' table grape bunches from all treatments were suitable for commercialization based on the stem browning scores. According to Nelson (1983), the quality of the grape is only affected when the stems of the bunches are subjected to severe browning.

Both EO and DPGP positively affected stem browning maintenance, which may be due to the antioxidant capacity of both treatments. SO_2 has an antioxidant effect, which affects the catalytic mechanism of some enzymes (Gorgatti et al., 1993), and EOs also have antioxidant capabilities, which decrease the diffusion of oxygen and reduce the rate of respiration (Shehata et al., 2020). Therefore, both worked to maintain the freshness of the bunch rachis, keeping it fresh and green even after 45 days of storage.

There was no statistical difference between the treatments used (Table 4) regarding shattered berries (% of loose berries) for either season. Furthermore, the averages were low, varying from 0.17 to 3.27% after 45 days of storage, thus meeting the minimum identity and quality requirements for table grapes in accordance with the Normative Instruction of the Ministério da Agricultura, Pecuária e Abastecimento (nº 69/2018) (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2018).

Table 4
Shattered berries of 'Benitaka' table grape (*Vitis vinifera* L.) at 30 and 45 days of cold storage (1.0 ± 1.0 ºC) individually packaged in plastic clamshells. Seasons of 2022 and 2023

Treatments	Shattered berries (%)			
	At 30 days of cold storage		At 45 days of cold storage	
	2022	2023	2022	2023
Control	1.52 a	0.08 a	2.54 a	0.25 a
EO	1.82 a	0.08 a	3.11 a	0.25 a
DPGP	1.75 a	0.17 a	2.60 a	0.25 a
EO + DPGP	1.60 a	0.08 a	3.27 a	0.17 a
F	0.10 ^{ns}	0.22 ^{ns}	0.31 ^{ns}	0.13 ^{ns}
CV (%)	25.83	15.43	24.27	4.5

Means followed by the same letter are not different by Fisher´s test (p<0.05%). ^{ns}: not significant. CV: coefficient of variation. EO: essencial oil. DPGP: Dual phase SO_2-generating pad.

It is important to highlight that several factors may be related to shattered berries, such as dehydration, dryness of the rachis, the incidence of gray mold, and tissue sensitivity to excess SO_2 (Neves et al., 2008). Furthermore, depending on the intensity or position of occurrence, loose berries can often go unnoticed by consumers when using plastic clamshells; this, in addition to providing a combination of practicality, protection, and marketing for table grapes, also prevents loose berries from becoming a problem in markets, as they will remain inside the packaging (Saito & Xiao, 2017; Mühlbeier et al., 2021).

Excessive exposure of grapes to SO_2 can result in damage from bleaching (Lurie et al., 2006; Zutahy et al., 2008). Bunches treated with SO_2-generating pads with or without essential oil resulted in bleached berries in both seasons. In the summer season of 2022, damage due to bleaching of the berries varied from 6.0 to 11.4% for the DPGP and EO + DPGP treatments, respectively, after 45 days of storage, and in the early season of 2023, it varied from 2.9 to 5.4% for the EO + DPGP and DPGP treatments, respectively. In both seasons, there was a statistically significant difference between the treatments and control.

The generation of the acids H_2SO_3 and H_2SO_4 by SO_2 after contact with the water vapor inside the packaging is responsible for inducing bleaching or discoloration of berries (Crisosto & Mitchell, 2002). Initially, only the color of the berries was affected, and bleaching was classified as a mild defect caused by SO_2 damage, with a maximum limit of 12%. As this defect progresses, the berry area may become soft, and cracks may eventually appear, resulting in juice leakage. In this case, bleaching is classified as a serious defect caused by SO_2 damage, with a maximum limit of 4% as accepted by the United States Department of Agriculture [USDA] (1971).

Some bunches of 'Benitaka' grapes subjected to treatments with DPGP, after 45 days, showed levels higher than the acceptable limit of bleaching. In this case, it would be necessary to take appropriate measures to reduce the occurrence of this damage, such as reducing the active ingredient content present in the SO_2 generating sheet or even using a plastic liner with a larger ventilation area, thus allowing better gas dissipation.

The chemical characteristics of the fruits were not affected by any of the treatments. Furthermore, both characteristics were well-preserved during the refrigerated storage period. At the beginning of the storage period, the soluble solid content (°Brix) was 14.44 and 13.23 ºBrix in the 2022 and 2023 seasons, respectively, and the titratable acidity (% tartaric acid) was 0.61 and 0.87% in the 2022 and 2023 seasons, respectively (Table 5).

Table 5
Soluble solids (SS) and titratable acidity (TA) of 'Benitaka' table grape (*Vitis vinifera* L.) at 45 days of cold storage (1.0 ± 1.0 ºC) individually packaged in plastic clamshells. Seasons of 2022 and 2023

Treatments	Soluble solids - SS (°Brix)		Titratable acidity - TA (% tartaric acid)	
	At 45 days of cold storage			
	2022	2023	2022	2023
Control	14.56 a	13.08 a	0.60 a	0.75 a
EO	14.58 a	12.96 a	0.60 a	0.84 a
DPGP	14.44 a	13.22 a	0.59 a	0.78 a
EO + DPGP	14.32 a	12.94 a	0.59 a	0.80 a
F	0.07 ^{ns}	0.81 ^{ns}	0.07 ^{ns}	3.17 ^{ns}
CV (%)	1.32	3.01	3.64	6.05

Means followed by the same letter are not different by Fisher´s test (p<0.05%). ^{ns}: not significant. CV: coefficient of variation. EO: essencial oil. DPGP: Dual phase SO_2-generatin.

In vitro efficacy of essential oil on the development of B. cinerea

All tested orange peel EO concentrations inhibited the mycelial growth of *B. cinerea.* It was also observed that the higher the concentration of the product, the greater its toxic activity on the fungus, causing a greater reduction in the development of the mycelial halo (Figure 1). The minimum inhibitory concentration (MIC) was obtained at the highest evaluated concentration of 1.6%, at which a small growth of the mycelial halo was observed; however, it was not able to grow in the agar with the essential oil, and the fungus developed on the disc only.

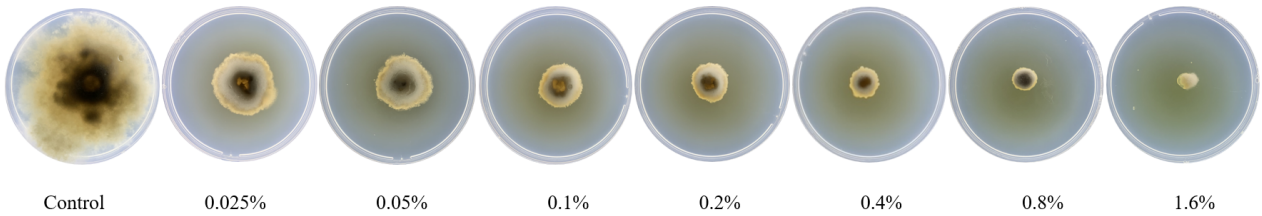

Figure 1. Growth of *B. cinerea* after 10 days of incubation with different concentration of orange peel essential oil. The Minimum inhibitory concentration (MIC) can be observed in the test containing 1.6% of the essential oil.

These results corroborate those of Hernández et al. (2021), who evaluated the ability of phenolic extracts from orange peels to inhibit the conidial germination and radial growth of *M. fructicola, B. cinerea,* and *A. alternata.* The authors observed that at the highest concentration tested, 1.5 g L^{-1}, the germination and growth of the conidia of the three fungal pathogens were completely inhibited. In a study evaluating EOs from *Aloysia citriodora, Cymbopogon winterianus,* and *Ocimum americanu,* increased doses of EOs reduced the mycelial growth of *Colletotrichum* sp., *B. cinerea,* and *Monilinia fructicola* (Fontana et al., 2021). In another study, it was recorded that the EOs of *Cinnamomum verum* and *Origanum vulgare* showed significant activity against the germination of *B. cinerea* spores compared to the control and decreased the germination of spores in approximately 65.2% at the highest doses (800 µL L^{-1}) (Almasaudi et al., 2022). The immediate mechanism of EOs is linked to the lipophilic property of the oil molecules, which bind firmly to the membranes, thus altering selectivity and aiding penetration through the membranes, resulting in a loss of energy from the microbial cell and suppressing the development of the fungus (Knaak & Fiuza, 2010).

The effective concentrations of 50 and 80% (ED50 and ED80) were 0.05 and 0.4%, respectively, with the lowest dose evaluated being sufficient to inhibit more than 50% of mycelial growth (Figure 2). ED80 was reached at the concentration recommended by the manufacturer of the commercial product, which corroborates the results obtained in the in vivo test, in which the essential oil was not high enough to inhibit the development of the fungus completely but was able to significantly reduce the percentage of infected grape berries compared to that in the control.

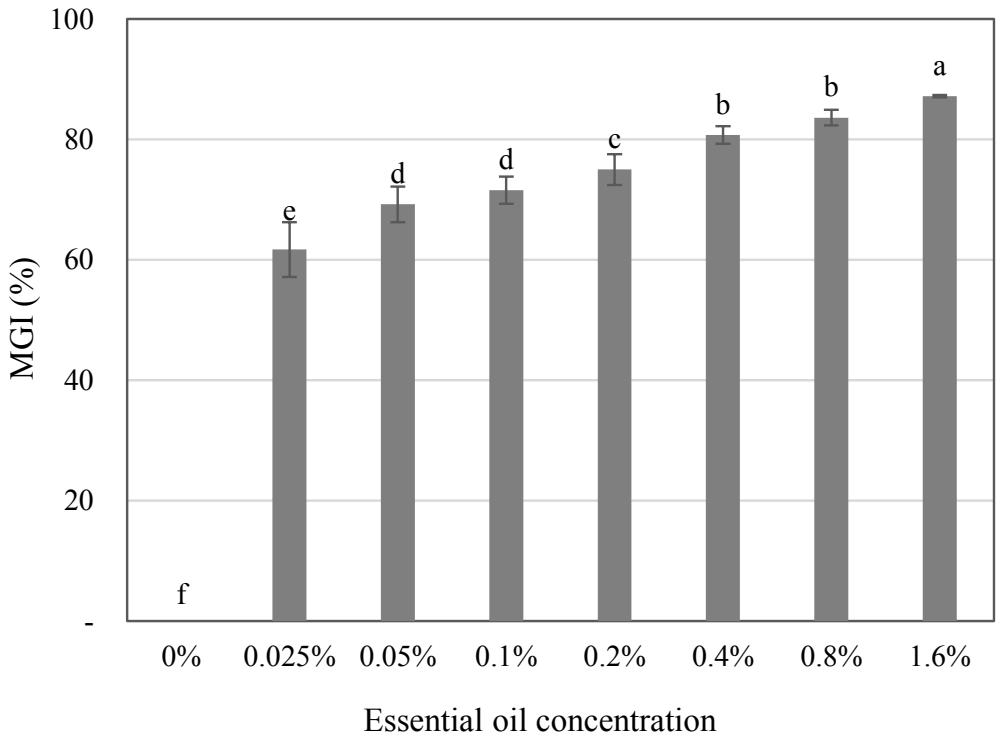

Figure 2. Mycelial growth inhibition percentage (MGI%) of *B. cinerea* as a function of different concentrations of orange essential oil. Means followed by the same letter are not different by Fisher´s test (p<0.05%).

Although only one application of the product was carried out in the post-harvest treatment, very promising results were achieved. Orange peel EO can be applied to bunches in the field as an alternative or complementary form to synthetic fungicides to eliminate or at least reduce their environmental impacts. The addition of synthetic fungicides is currently the main strategy for controlling postharvest diseases. However, their use has been increasingly limited by the emergence of resistant fungal strains associated with their excessive application (Feliziani et al., 2013). Treatment with bioinputs can protect plants against pathogens by inducing local and systemic resistance (Abo-Elyousr et al., 2021) and is considered safe for both the environment and human health (Feliziani et al., 2013; Palou et al., 2016).

Observations made using scanning electron microscopy showed deformation of the hyphae of *B. cinerea* after exposure to orange peel essential oil on agar, where the hyphae appeared to be withered. In Figure 3, it is possible to identify the normal growth of hyphae in the control treatment, as well as deformed hyphae when exposed to orange peel essential oil.

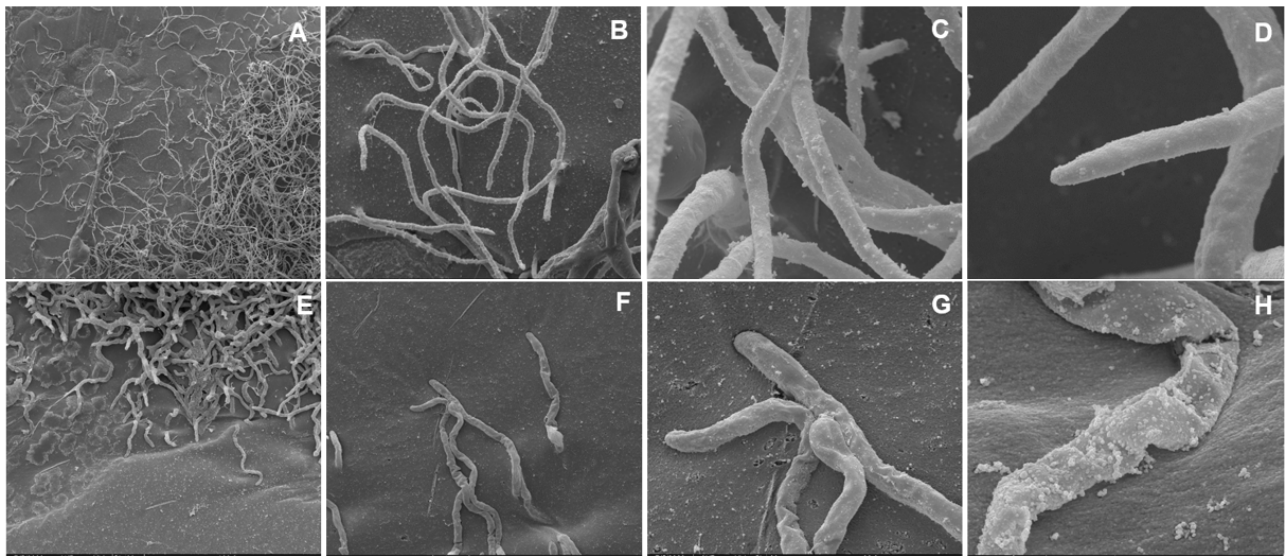

Figure 3. Scanning electron microscopy on the antifungal effect of orange peel essential on mycelial growth of *B. cinerea* after 10 days of incubation.
(A-D) Control, not treated with essential oil; (E-H) Treated with essential oil in concentration 0.4%. In the magnitudes of 200× (A: bar 500 µm), 800× (E: bar 100 µm), 1.600× (B,F: bar 50 µm), 6.000x (C,G: bar 20 µm) and 12.000x (D,H: bar 10 µm).

The mycelia obtained from the edge of the *B. cinerea* colony in the control treatment showed hyphae with a typical "network" structure and smooth surface. On the other hand, in the presence of orange peel EO at a concentration of 0.4% (concentration recommended by the manufacturer), the hyphae appear abnormal, lose their turgidity, and form superficial protuberances, indicating that the orange essential oil presents toxic and compromises the growth of *B. cinerea,* which results in deformation of the structure of the hyphae. Furthermore, EO caused a significant decrease in the size of the hyphal network, resulting in marked shrinkage and wrinkling of the hyphae in addition to inhibiting fungal growth. Similar results were obtained by Xueuan et al. (2018), who evaluated the effect of *Mentha piperita* essential oil on *B. cinerea,* and by Wang et al. (2019), who evaluated the effect of *Litsea cubeba* essential oil on *B. cinerea.* The authors reported the same observations regarding the structure of the hyphae by scanning electron microscopy.

Conclusions

The effectiveness of essential oil from orange peel in suppressing the development of *B. cinerea* has been demonstrated both *in vivo* and *in vitro,* making this biocompound a safe alternative for the postharvest conservation of 'Benitaka' table grapes.

Acknowledgments

The authors offer their heartfelt thanks to ORO AGRI BRAZIL for providing Essential oil from orange peel (PREV-AM™).

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