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ORIGINAL ARTICLE

Eucalyptus staigeriana essential oil can control downy mildew in grapevine

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Abstract - Downy mildew (*Plasmopara viticola)* is the primary disease in viticulture worldwide, and your control requires synthetic fungicides applications to avoid quality and yield loss in the grapevines. However, alternatives to reduce synthetic fungicides are needed to ensure the consumer's health and the environment. Essential oils (EOs) are amongst the most promising natural plant protection alternatives because of their antifungal properties on several crop diseases. The present study objective was to determine the effect of *Eucalyptus staigeriana* EO *in vitro* and *in vivo* against *P. viticola*. The EO exhibited the highest activity *in vitro,* inhibiting 90% of the incidence and severity of disease caused by *P. viticola* in leaves of grapevines in the greenhouse. In the field (*in vivo*), treatment with EO could not control the disease; however, treatment with EO in consortium with conventional treatment reduced approximately 50% of the incidence and more than 90% of the severity of downy mildew disease in leaves, decreasing the application of synthetic fungicides by 50%.

Keywords: Alternative control. *Plasmopara viticola*. Eucalypt. Secondary metabolites. *Vitis*.

Óleo essencial de *Eucalyptus staigeriana* pode controlar o míldio em videiras

Resumo - O míldio (*Plasmopara viticola*) é a principal doença na viticultura mundial e seu controle requer a aplicação de fungicidas sintéticos para evitar perdas na qualidade e produtividade das videiras. No entanto, alternativas para reduzir o uso de fungicidas sintéticos são necessárias para garantir a saúde do consumidor e do meio ambiente. Os óleos essenciais (OE) estão entre as alternativas naturais de proteção de plantas mais promissoras devido às suas propriedades antifúngicas sobre várias doenças de plantas cultivadas. O objetivo do presente estudo foi determinar o efeito do OE de *Eucalyptus staigeriana in vitro* e *in vivo* contra *P. viticola*. O OE apresentou maior atividade *in vitro,* inibindo 90% da incidência e severidade da doença causada por *P. viticola* em folhas de videira em casa de vegetação. No campo (*in vivo*), o tratamento com OE não conseguiu controlar a doença; entretanto, o tratamento com OE em consórcio com o tratamento convencional reduziu em aproximadamente 50% a incidência e mais de 90% a severidade do míldio nas folhas, diminuindo a aplicação de fungicidas sintéticos em 50%.

Palavras-chave: Controle alternativo. *Plasmopara viticola*. Eucaliptus. Metabólitos secundários. *Vitis*.

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Introduction

Global food supply is highly dependent on agriculture, which would not be possible without pesticides against fungal diseases. In addition, there is growing consumer demand for sustainable food production, which requires developing alternative control for plant protection that reduces synthetic fungicides employment. Long-term fungicide applications have generated growing concerns over food safety because their use causes pathogen resistance and impacts consumers' health and environment (GISI; SIEROTZKI, 2008; KIM; KABIR; JAHAN, 2017).

The most devastating disease of viticulture worldwide is downy mildew, whose causal agent is *Plasmopara viticola* (Berk. & Curt.) Berl. & De Toni, belonging to Peronosporales order, is an obligate biotrophic oomycete pathogen of grapevine (FAWKE; DOUMANE; SCHORNACK, 2015; GESSLER; PERTOT; PERAZZOLLI, 2011; KASSEMEYER; GADOURYM; HILL, 2015). Downy mildew disease mainly infects leaves, where after infection, it produces in the adaxial leaf surface a typical oil spot lesions and a large number of spores on the abaxial leaf surface. Therefore, to guarantee grape production yield and quality, the control of *P. viticola* in the vineyard requires relatively large amounts of synthetic fungicides (CHEN *et al*., 2007; FRÖBEL; ZYPRIAN, 2019).

Essential oils (EOs) are natural products with fungicidal properties, low toxicity and environmental impact. Besides, they are well known for their biodegradable properties and do not leave any residual effect on food and, therefore they could be used as alternatives for synthetic fungicides in crops (PANDEY *et al*., 2017; ZAKER, 2016).

Trees of the *Eucalyptus* genus are widely grown in many parts of the world and comprise about 900 species. More than 300 species of this genus have been commercially used to produce EOs (BROOKER; KEING, 2006; DHAKAD *et al*., 2018). Several studies have shown the antifungal properties of *E. staigeriana* EO against grapevine phytopathogens *in vitro* and *in vivo*. Moreover, there is no report of phytotoxic effects of the EO on grapes or grapevines, demonstrating its potential use as an alternative control against phytopathogens (PEDROTTI *et al*., 2019; PEDROTTI *et al*., 2020). This study aimed to evaluate the EO of *E. staigeriana* in controlling *P. viticola* in *Vitis* spp. in the greenhouse and the field.

Material and Methods

Leaves of *Eucalyptus staigeriana* were collected in Caxias do Sul (RS, Brazil). After collection, the leaves were oven-dried at 30 ºC until a constant mass was obtained.

EO was extracted by steam distillation from dried plant leaves for one hour, according to PEDROTTI *et al*. (2020). The method described in PEDROTTI *et al*. (2020) was used for identifying and quantify compounds in the *E. staigeriana* EO. The EO was analyzed by GC/MS and GC-FID. GC-FID analysis for quantification of EO was carried out using a Hewlett Packard 6890 Series gas chromatograph, equipped with a HP-Chemstation data processing unit, using a HP-Innowax column (30 m x 320 µm i.d.) with 0.50 µm phase

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thickness. The temperature program was: 40 °C (8 min), rising to 180 °C (3 °C/min), then to 230 °C (20 °C/min), and held at 230 °C (10 min); injector and detector temperature were 250 °C; a split ratio of 1:50, hydrogen as carrier gas at 34 kPa. The sample volume injected was 1 µL, diluted in hexane (1:10). GC/MS analysis for identification of the compounds was performed on a mass spectrometer (HP 6890/MSD5973), equipped with an HP-Chemstation data processing unit (Wiley 275 library). Analysis was performed using a HP-Innowax cross-linked fused silica capillary column (30 m x 250 µm i.d.) with 0.50 µm phase thickness. The oven temperature program was the same used at GC-FID, the interface at 280 °C; split ratio 1:100; helium as carrier gas (56 kPa); flow ratio of 1.0 mL/min; ionization energy of 70 eV. The sample volume injected was 1 µL, diluted in hexane (1:10). The compounds of EO were identified by comparing the obtained spectra with those from the Wiley library (GC/MS) and by comparing the calculated linear retention indexes, relative to *n*-alkanes C8-C26 with literature data.

To evaluate the antifungal activity of EO in grapevine in the greenhouse (*in vitro*), *P. viticola* was collected from an unsprayed vineyard of *Vitis* spp. 'Isabella' (*Vitis labrusca* × *Vitis vinifera*) naturally infested, in Bento Gonçalves (RS, Brazil). Leaves with oil spot symptoms were collected and, sporangia were collected by washing the abaxial surfaces bearing freshly sporulating lesions with distilled water, and the inoculum concentration was adjusted to 10^5 sporangia mL^{-1} with a hemocytometer chamber. Branches of *Vitis* spp. 'Isabella' (*Vitis labrusca* × *Vitis vinifera*) were collected in the vineyard in Bento Gonçalves (RS, Brazil), with uniform morphological characteristics, derived from the former pruning material visually free of fungal and viral diseases. For sterilization, the branches were subjected to heat treatment in a hot water tank at 50 °C for 30 minutes. The branches were cut in 10-15 cm sections to obtain cuttings with one bud. The cuttings were rooted in pots (400 mL) with autoclaved plant substrate (Carolina Soil, Rio Grande do Sul, Brazil) and with the addition of five g/L of plant nutrition (Forth Cote, Osmocote, São Paulo, Brazil) and, maintained in a greenhouse at 25 ± 2 ºC, 60-80% relative humidity with a 16 hours photoperiod. The plants were watered twice a week without fertilization over the entire test period of three months. At the stage of four-six unfolded leaves, the tests were conducted. The antifungal activity of *E. staigeriana* EO was evaluated as curative and preventive treatments with EO emulsified with Tween 20 (1:1) at 1 µL mL^{-1} concentration. This concentration was defined according to PEDROTTI *et al*. (2019; 2020) due to its high efficiency against other pathogens of grapevines *in vitro* and *in vivo* (*Colletotrichum acutatum* and *Botrytis cinerea*) and no phytotoxic effect in grapevine leaves when applied in the field. In curative treatment, a sporangia suspension was inoculated (sprayed in leaves). After 24 hours, leaves were sprayed with EO. In preventive treatment, the same EO concentration was sprayed in leaves, and 24 hours later, inoculation was made as described above. For both experiments, grapevines were placed in small greenhouses (38 cm wide × 53 cm long × 40 cm high) and were incubated at 25 ± 2 °C / 100% relative humidity in the dark for 24 hours and, then returned to the initial condition (± 25 ºC, 60-80% relative humidity with a 16 hours photoperiod). After seven days, the grapevines were again kept at 25 ± 2 °C / 100 % relative humidity in the dark for 24 hours. After the

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incubation, disease incidence and severity were assessed. The incidence was evaluated for the presence or absence of symptoms of the disease. For severity, decayed area on the surface of leaves was visually evaluated using a scale described by BUFFARA *et al.* (2014). The experiment was also composed of a control treatment (received just water) and control with EO (1 µL mL^{-1} without inoculum application). Tests were conducted in triplicate with 12 grapevines for each treatment.

For evaluation of the antifungal activity of EO in the field (*in vivo*), the test was carried out in a vineyard located in Bento Gonçalves (Southern Brazil) (-29.084463, -51.552576). The vineyard was implanted in 2017, with 2 m between lines and 1 m between plants and conducted in bi-lateral cordon training with spur pruning. The vineyard was composed of 'Isabella' (*Vitis labrusca* × *Vitis vinifera*) grafted on the rootstock 1103 Paulsen. Next to the experimental area, there are vineyards implanted for more than 80 years with an annual incidence of downy mildew. The experimental unit consisted of 12 plants formed by one line. Treatments consisted in: a control (non-treated plants), treatment with *E. staigeriana* EO emulsified with Tween 20 (1:1) at 1 µL mL^{-1} concentration, conventional treatment (was applied synthetic fungicide permitted for grapevine growing at the concentration indicated by the manufacture: Mancozeb 0.003 µg mL^{-1}), consortium treatment (*E. staigeriana* EO emulsified with Tween 20 (1:1) at 1 µL mL^{-1} concentration and Mancozeb 0.003 µg mL^{-1}, applied alternately every seven days). The plants were sprayed at intervals of seven days and, with reapplication of EO in the case of rain (Table 1).

Table 1. Days in which the different treatments were applied in 'Isabella' grapevines (*Vitis labrusca* × *Vitis vinifera*) in the field in Bento Gonçalves, RS, Brazil.

	Treatments			
Application day	Control	Treatment with essential oil	Conventional treatment	Consortium treatment
Sep 21, 2019		x	x	x*
Sep 28, 2019		x	x	x**
Out 06, 2019		x	x	x*
Out 12, 2019		x	x	x**
Out 19, 2019		x	x	x*
Out 22, 2019		x		x*
Out 26, 2019		x	x	x**
Out 29, 2019		x		
Nov 02, 2019		x	x	x*
Nov 07, 2019		x	x	x**
Nov 10, 2019		x		
Nov 17, 2019		x	x	x*
Nov 24, 2019		x	x	x**

* Treatment with *Eucalyptus staigeriana* essential oil (1 µL mL^{-1}); ** Treatment with Mancozeb 0.003 µg mL^{-1}

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The treatments started at the beginning of the bud burst (September 2019) and finished at the berry formation (pre-véraison) (November 2019). Spraying was done with two liters hand sprayer of pre-compression and was directed to leaves, preferably at dawn and to the pour point. The parameters evaluated were incidence and severity of disease of ten leaves collected (five leaves of each cordon, one leaf of each cane) every ten days, seven collections were performed (Table 2). The incidence and severity were evaluated as described above. The daily climatic data were obtained from an automatic meteorological station at Inmet (Instituto Nacional de Meteorologia), located at Embrapa Uva e Vinho (Brazil). Data were analyzed by ANOVA and the threshold for statistical significance was set at $p \leq 0.05$. In the case of statistical significance, Tukey's test was applied to separate the means. All statistical analysis was performed using SPSS 22.0 program.

Table 2. Date of collection of leaves of 'Isabella' grapevines (*Vitis labrusca* × *Vitis vinifera*) in the field in Bento Gonçalves, RS, Brazil.

Date	Collect
Out 02, 2019	1
Out 12, 2019	2
Out 22, 2019	3
Nov 01, 2019	4
Nov 11, 2019	5
Nov 21, 2019	6
Dec 01, 2019	7

Results and Discussion

E. staigeriana EO dried leaves yield 5.20% (mL $100g^{-1}$ of dried leaves). The analyses identified twenty-one compounds (Table 3) and, the major compounds found were citral 34.32% (21.83% geranial and 12.49% neral), limonene (20.60%), and 1.8-cineole (12.33%). Also, 88.08% of the compounds correspond to monoterpenes (30.36% hydrocarbons and 57.71% oxygenated) and 11.95% are esters compounds.

The results obtained by testing *E. staigeriana* EO in leaves of grapevines in the greenhouse are reported in Table 4. The inoculum treatment presented incidence of downy mildew in more than 60% of grapevines, with a severity of 7.71%. Curative treatments with EO showed a significant reduction in the development of *P. viticola* compared with the control with inoculum, reducing more than 90% the incidence and severity of disease, demonstrating its effectiveness. However, there was no significant difference in disease incidence and severity between treatment with EO and control with inoculum in preventive treatment. EO did not cause any phytotoxic effect on the leaves. After seven days of fungus inoculation, leaves with the disease presented oil spot lesions on the adaxial leaf surface accompanied by massive sporulation on the abaxial surface. No disease symptom was observed in control and control with EO treatment.

In the field, during the evaluation period, climatic conditions showed slight variations of temperature

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(between 16.35 and 20.97 ºC; an average of 18.89 ºC) and humidity (between 64.80 and 84.75 %; an average of 75.02%), but significant variations of precipitation, mainly in the evaluations comprised between 12-Oct to 11-Nov that showed the highest precipitation indexes (between 5.42 and 10.12 mm; an average of 8.59 mm) (Figure 1 A and B).

Table 3. Chemical composition of essential oil from *Eucalyptus staigeriana*.

Compounds	**RI** [1]	**RA**[2]
Monoterpenes Hydrocarbons		**30.36**
α-pinene	13.942	1.10
α-phelandrene	21.895	0.27
Myrcene	21.995	0.64
Limonene	23.861	20.60
γ-terpinene	26.309	0.62
Cis-β-ocimene	26.704	0.36
ρ-cymene	27.677	0.73
δ-terpinene	28.285	6.04
Oxygenated Monoterpenes		**57.71**
1,8-cineole	24.268	12.33
Linalool	40.518	0.62
Terpinen-4-ol	42.987	1.05
Neral	46.356	12.49
Geranial	48.349	21.83
Citronellol	49.295	1.31
Nerol	50.664	3.01
Geraniol	52.347	5.07
Esters		**11.75**
Citronellyl acetate	45.338	0.60
Terpinyl acetate	46.787	6.64
Neryl acetate	47.931	2.43
Geranyl acetate	49.038	2.08
Other		**0.20**
Geranic acid	63.270	0.20

[1] RI, retention index determined relative to n-alkanes (C8–C20). [2] RA, Relative amounts of the compounds identified based on the area of each peak in the total chromatogram area.

The climatic conditions, such as high precipitation associated with high humidity and temperature, are directly related to the high disease incidence rates and severity since these conditions are favorable to the development of *P. viticola*. As observed in control, the high precipitation rates observed after the third evaluation (22-Oct) increased the disease incidence rates, which reached more than 90% of the assessed leaves (Figure 1 A). Furthermore, it also presented a significant increase in disease severity during the same period (22-Oct), which affected more than 60% of the leaf area (Figure 1 B).

Treatment with *E. staigeriana* EO in vineyard reduced the incidence of downy mildew in leaves (*P.*

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viticola) compared to control in evaluations carried 02-Oct to 12-Oct (reduction of 39 and 40 % respectively compared to control), and the severity in the evaluation carried in 02-Oct (reduction of 60 % compared to control). In the other evaluations, EO could not reduce the incidence and severity of the disease (Figure 1 A and B). During the evaluations, the leaves did not show any symptoms of phytotoxicity.

The conventional treatment and consortium treatment significantly reduced the development of *P. viticola* in the leaves. Approximately 50% less incidence and more than 90% reduction in severity of downy mildew disease was observed compared to control in all evaluations (Figure 1 A and B). In addition, the antifungal efficiency of both treatments (conventional and consortium) was comparable and not significantly different.

Table 4. Effect of *Eucalyptus staigeriana* essential oil applied in grapevines 'Isabella' (*Vitis labrusca* × *Vitis vinifera*) in greenhouse on incidence and severity of downy mildew caused by *Plasmopara viticola.*

Treatments	Incidence (%)	Severity (%)
Control	00.00 ± 0.00 c	00.00 ± 0.00 b
Control with essential oil	00.00 ± 0.00 c	00.00 ± 0.00 b
Control with inoculum	60.85 ± 3.17 a	07.71 ± 1.98 a
Preventive treatment with essential oil 1 µL mL^{-1}	51.58 ± 2.05 a	07.99 ± 1.78 a
Curative treatment with essential oil 1 µL mL^{-1}	04.15 ± 1.47 b	00.15 ± 0.55 b

Values are the average of ten replicates per treatment ± SD. Letters indicate the comparison among the different treatments. Means followed by same letter do not differ by Tukey test ($p \leq 0.05$).

The major compounds identified in *E. staigeriana* EO were: citral, 1.8-cineole, and limonene, as was previously described in the literature for this specie (MACEDO *et al*., 2010; PEDROTTI *et al*., 2019; TOMAZONI *et al*., 2017), indicating that the EO composition is highly specie stable, with a low influence of environmental factors.

OEs have great potential for use as biofungicides, as they have demonstrated effectiveness in controlling many plants fungal pathogens *in vitro* and *in vivo* (FALASCA *et al*., 2016; PEDROTTI *et al*., 2019; PEDROTTI *et al*., 2020; ROMANAZZI *et al*., 2012; TOMAZONI *et al*., 2017; TOMAZONI *et al*., 2018). Furthermore, as a natural antifungal product, EOs are assumed to be more acceptable and less harmful to the environment than synthetic products and could be used as an alternative for controlling phytopathogens.

Treatments in a greenhouse with *E. staigeriana* EO in leaves reduced more than 90% of the incidence and severity of disease caused by *P. viticola* in curative treatment, demonstrating its effectiveness. Another study carried out by RIENTH *et al*. (2019) with grapevine has confirmed that EOs could be an efficient alternative treatment against *P. viticola* in the greenhouse. They used cuttings of grapevine (*Vitis vinifera*) 'Chasselas' infected with *P. viticola* to expose to *Origanum vulgaris* and *Thymus vulgaris* EOs continuous

fumigation at different concentrations, during two application periods (24 hours and ten days). Both EO were highly efficient in inhibiting *P. viticola* development on leaves and reduced disease severity by up to 98%.

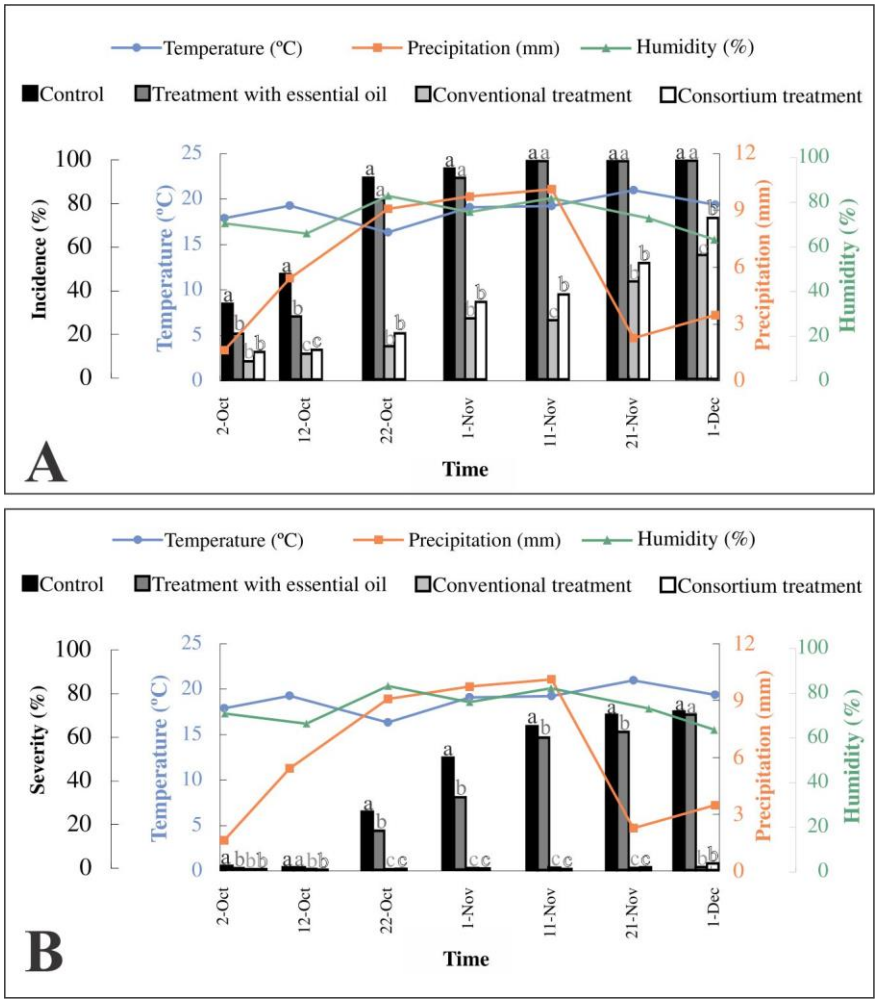

Figure 1. Effect of different treatments *in vivo* applied in vineyard of *Vitis* spp. (*Vitis vinifera* × *Vitis labrusca)* 'Isabella' on incidence (A) and severity (B) of diseases caused by *Plasmopara viticola.* Values are the average of twelve replicates per treatment ± SE. Letters indicate the comparison among the different treatments. Means followed by same letter do not differ by Tukey test ($p<0.05$).

This antimicrobial or antifungal activity of EOs might mainly be due to the properties of their terpenes/terpenoids. These compounds can disrupt the cell membrane (alteration and inhibition of cell wall formation, dysfunction of the fungal mitochondria, inhibition of efflux pumps, and/or reactive oxygen species production), causing cell death or inhibiting fungi's sporulation and germination (NAZZARO *et al*., 2017; TANG *et al*., 2018; ZHENG *et al*., 2015).

However, in this study, preventive treatment with *E. staigeriana* EO could not control the incidence and

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severity of the disease, this effect is probably due to the rapid volatilization of the EO (TUREK; STINTZING, 2013) that was applied 24 h before the inoculation of *P. viticola;* this strongly suggests that the antifungal effect of EOs acts during the early stages of the infection cycle. Thus, there is still no way to say whether the inhibitory effect is due to its direct effect on zoospores (before infection) or by inhibiting the hyphae development within the leaf (after infection). An alternative hypothesis is that EO stimulates innate plant immunity by activating pattern-triggered immunity or effector-triggered immunity, which would inhibit de infection or limit hyphae growth inside the leaf (RIENTH *et al*., 2019).

P. viticola undergoes numerous infection cycles during a vegetative grapevine season, where the infection levels are directly related to climatic conditions (CHEN *et al*., 2020; MASSI *et al*., 2021). Therefore, in our study, we related the high incidence and severity of downy mildew on leaves according to the climatic conditions, such as high precipitation associated with high humidity and temperature.

E. staigeriana EO in vineyard reduced the incidence and severity of downy mildew in leaves (*P. viticola)* compared to control in the first and second evaluations (02-Oct to 11-Nov). After, the high temperature, humidity, and mainly the increase in precipitation from the second evaluation caused the appearance of high incidence and severity of downy mildew in leaves in control and treatment with EO, demonstrating that the OE was not able to control the disease. However, consortium treatment and conventional treatment reduced approximately 50% the incidence and more than 90% of the severity of downy mildew disease in leaves, compared to control and treatment with EO in all evaluations. Demonstrating that EO can be applied in consortium with synthetic fungicides, reducing the application of synthetic fungicides by 50%.

Similarly, LA TORRE *et al*. (2014) used a commercial formulation containing 23.8% (w/w) of tea-tree essential oil (BM-608) in the field on grapevines ‘Malvasia di Candia’ against downy mildew and showed that the formulation was able to control the development of downy mildew. However, they were not as effective as the reference product (copper).

According to DAGOSTIN *et al*. (2011) and TUREK; STINTZING (2013), under field conditions, the efficacy of natural products as the EO is often limited by their sensitivity to environmental factors such as humidity, pH, heat, and UV radiations and their inherent physicochemical characteristics, such as rain fastness, application time, permeability, and volatility.

The alternative control failure can also be due to the pathogen nature and the conditions in which infection occurs. According to PERTOT *et al*. (2017), without treatment with a highly effective preventive substance, *P. viticola* penetrates the leaves tissues rapidly and, only treatment with systemic products can partially stop the downy mildew after infection. Moreover, secondary infections may occur almost daily in periods of frequent rain, when the alternative controls may be inefficient to control the downy mildew because they are quickly deteriorated by environmental factors, in contrast to systemic treatment with synthetics products (PERTOT *et al*., 2017).

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Therefore, though it is unexpected that EO application completely inhibits infections, they could lower the disease pressure and reduce synthetic fungicides. Unfortunately, there are few studies with EOs in the field for the control of downy mildew, and chemical control to date is still the most effective way to extensively reduce the impact of disease caused by *P. viticola* in the vineyard, but the application in the field of fungicides synthetics is not always desirable. Moreover, regulations on pesticide use may become more restrictive in the future, forcing grape growers to reduce the fungicide applications. Thus, from this study, we can see that it is possible to reduce the use of chemical products using *E. staigeriana* EO in consortium with synthetic fungicide, handling hazards, concern about pesticide residues on food, and the threat to human health and the environment.

The need for sustainable alternatives to replace or reduce the number of treatments with synthetic molecules is vital from environmental and public health perspectives. The present study showed that *E. staigeriana* EO exhibited the highest antifungal activity *in vitro*, inhibiting 90% of the incidence and severity of disease caused by *P. viticola* in the curative treatment of grapevines in the greenhouse. In the vineyard on the field (*in vivo)*, the treatment with EO only, could not control the disease; however, consortium treatment (treatment with EO with conventional treatment) reduced approximately 50% the incidence and more than 90% of the severity of disease caused by *P. viticola* in leaves of grapevines, decreasing the application of synthetic fungicides. These results provide essential information regarding *E. staigeriana* EO's effect in the control of downy mildew and could potentially represent a natural strategy to reduce the use of synthetic fungicides. So, new alternatives to developing innovative plant protection strategies using volatile organic compounds from plant EOs are possible.

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Conflict of interests

The authors declare that the research was conducted in the absence of any potential conflicts of interest.

Ethical statements

The authors confirm that the ethical guidelines adopted by the journal were followed by this work, and all authors agree with the submission, content and transfer of the publication rights of the article to the journal. They also declare that the work has not been previously published nor is it being considered for publication in another journal.

The authors assume full responsibility for the originality of the article, and may incur on them, any charges arising from claims, by third parties, in relation to the authorship of the article.

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References

BROOKER, M. I. H.; KEING, D. A. **Field guide to Eucalyptus. Third edition**. Melbourne: Bloomings Book, 2006.

BUFFARA, C. R. S. *et al.* Elaboration and validation of a diagrammatic scale to assess downy mildew severity in grapevine. **Ciência Rural**, v. 44, n.8, 2014. DOI: https://doi.org/10.1590/0103-8478cr20131548.

CHEN, M. *et al.* Forecasting severe grape downy mildew attacks using machine learning. **Plos one**, v. 15, n. 3, 2020. DOI: https://doi.org/10.1371/journal.pone.0230254.

CHEN, W. J. *et al.* At least two origins of fungicide resistance in grapevine downy mildew populations. **Applied and environmental microbiology**, v.73, p. 5162–5172, 2007. DOI: https://doi.org/10.1128/AEM.00507-07.

DAGOSTIN, S. *et al.* Are there alternatives to copper for controlling grapevine downy mildew in organic viticulture? **Crop protection**, v. 30, n. 7, p. 776–788, 2011. DOI: https://doi.org/10.1016/j.cropro.2011.02.031.

DHAKAD, A. K. *et al.* Biological, medicinal and toxicological significance of *Eucalyptus* leaf essential oil: a review. **Journal of the Science of Food and Agriculture**, v. 98, p. 833-848, 2018. DOI: https://doi.org/10.1002/jsfa.8600.

FALASCA, A. *et al*. GC-MS analysis of the essential oils of *Juniperus communis* L. berries growing wild in the Molise region: Seasonal variability and *in vitro* antifungal activity. **Biochemical Systematics and Ecology**, v. 69, p. 166-175, 2016. DOI: https://doi.org/10.1016/j.bse.2016.07.026.

FAWKE, S.; DOUMANE, M.; SCHORNACK, S. Oomycete interactions with plants: infection strategies and resistance principles. **Microbiology and Molecular Biology Reviews**, v. 79, p. 263-280, 2015. DOI: https://doi.org/10.1128/MMBR.00010-15.

FRÖBEL, S.; ZYPRIAN, E. Colonization of different grapevine tissues by *Plasmopara viticola* - A histological study. **Frontiers in Plant Science**, v. 10, p. 951, 2019. DOI: https://doi.org/10.3389/fpls.2019.00951.

GESSLER, C.; PERTOT, I.; PERAZZOLLI, M. *Plasmopara viticola*: a review of knowledge on downy mildew of grapevine and effective disease management. **Phytopathologia Mediterranea**, v. 50, n. 1, 2011.

GISI, U.; SIEROTZKI, H. Fungicide modes of action and resistance in downy mildews. **European Journal of Plant Pathology**, v. 122, n. 1, p.157–67, 2008. DOI: https://doi.org/10.1007/s10658-008-9290-5.

KASSEMEYER, H-H.; GADOURYM, D. M.; HILL, G. Downy Mildew. In Compendium of Grape Diseases, Disorders, and Pests. 2nd Edn., eds W. Wilcox, W. D. Gubler, and K. J. Uyemoto (St. Paul: APS Press), p. 46–52, 2015.

KIM, K-H.; KABIR, E.; JAHAN, S. A. Exposure to pesticides and the associated human health effects. **Science of the total Environment**, v. 575, p. 525–535, 2017. DOI: https://doi.org/10.1016/j.scitotenv.2016.09.009.

LA TORRE, A. *et al*. Evaluation of essential plant oils for the control of *Plasmopara viticola*. **Journal of essential oil research**, v. 26, n. 4, p. 282–91, 2014. DOI: https://doi.org/10.1080/10412905.2014.889049.

MACEDO, I. T. F. *et al.* Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes. **Veterinary Parasitology**, v. 173, p. 93-98, 2010. DOI: https://doi.org/10.1016/j.vetpar.2010.06.004.

MASSI, F. *et al.* Fungicide resistance evolution and detection in plant pathogens: *Plasmopara viticola* as a case study. **Microorganisms**, v. 9, n. 1, p. 119, 2021. DOI: https://doi.org/10.3390/microorganisms9010119.

ISSN: 0104-907. ISSN ONLINE: 2595-7686.
Received in 06 Jul. 2021. Accepted in 18 Nov. 2021.

NAZZARO, F. *et al.* Essential oils and antifungal activity. **Pharmaceuticals**, v. 10, n. 4, p. 86, 2017. DOI: https://doi.org/10.3390/ph10040086.

PANDEY, A. K. *et al.* Essential oils: sources of antimicrobials and food preservatives. **Frontiers in Microbiology**, v. 7, n. 2161, 2017. DOI: https://doi.org/10.3389/fmicb.2016.02161.

PEDROTTI, C. *et al.* Alternative control of grape rots by essential oils of two Eucalyptus species. **Journal of Science of Food and Agriculture**, v. 99, n. 14, p. 6552-6561, 2019. DOI: https://doi.org/10.1002/jsfa.9936.

PEDROTTI, C. *et al.* Essential oil as sustainable alternative for diseases management of grapes in postharvest and in vineyard and its influence on wine. **Journal of Environmental Science and Health, Part B**, v. 56, n. 1, p. 73-81, 2020. DOI: https://doi.org/10.1080/03601234.2020.1838827.

PERTOT, I. *et al.* A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. **Crop Protection**, v. 97, p. 70–84, 2017. DOI: https://doi.org/10.1016/j.cropro.2016.11.025.

RIENTH, M. *et al.* Oregano essential oil vapour prevents *Plasmopara viticola* infection in grapevine (*Vitis vinifera*) and primes plant immunity mechanisms. **Plos one**, v. 14, n. 9, 2019. DOI: https://doi.org/10.1371/journal.pone.0222854.

ROMANAZZI, G. *et al.* Recent advances on the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes. **Postharvest Biology and Technology**, v. 63, p. 141–147, 2012. DOI: https://doi.org/10.1016/j.postharvbio.2011.06.013.

TANG, X. *et al.* Antifungal activity of essential oil compounds (geraniol and citral) and inhibitory mechanisms on grain pathogens (*Aspergillus flavus* and *Aspergillus ochraceus*). **Molecules**, v. 23, p. 2108, 2018. DOI: https://doi.org/10.3390/molecules23092108.

TOMAZONI, E. Z. *et al.* Screening for inhibitory activity of essential oils on fungal tomato pathogen *Stemphylium solani* Weber. **Biocatalysis and Agricultural Biotechnology**, v. 16, p. 364-372, 2018. DOI: https://doi.org/10.1016/j.bcab.2018.08.012.

TOMAZONI, E. Z. *et al. In vitro* and *in vivo* activity of essential oils extracted from *Eucalyptus staigeriana*, *Eucalyptus globulus* and *Cinnamomum camphora* against *Alternaria solani* Sorauer causing early blight in tomato. **Scientia Horticulturae**, v. 223, p. 72–77, 2017. DOI: https://doi.org/10.1016/j.scienta.2017.04.033.

ISSN: 0104-907. ISSN ONLINE: 2595-7686.
Received in 06 Jul. 2021. Accepted in 18 Nov. 2021.

TUREK, C.; STINTZING, F.C. Stability of essential oils: a review. **Comprehensive Reviews in Food Science and Food Safety**, v. 12, n. 1, p. 40–53, 2013. DOI: https://doi.org/10.1111/1541-4337.12006.

ZAKER, M. Natural plant products as eco-friendly fungicides for plant diseases control- a review. **The Agriculturists**, v. 14, n. 1, p. 134-141, 2016. DOI: https://doi.org/10.3329/agric.v14i1.29111.

ZHENG, S. *et al.* Citral exerts its antifungal activity against *Penicillium digitatum* by affecting the mitochondrial morphology and function. **Food Chemistry**, v. 178, p. 76–81, 2015. DOI: https://doi.org/10.1016/j.foodchem.2015.01.077.

ISSN: 0104-907. ISSN ONLINE: 2595-7686.
Received in 06 Jul. 2021. Accepted in 18 Nov. 2021.