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In vivo anti-mildew activity of essential oils against downy mildew of sunflower caused by *PLASMOPARA HALSTEDII*

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Abstract Commercial essential oils (EO) obtained from different parts of black cumin (*N. sativa*), mustard (*S. nigra*), St. John's wort (*H. perforatum*), garlic (*A. sativum*), grape (*V. vinifera*), and ginger (*Z. officinale*) plants were evaluated for in vivo anti-mildew activity against pathotypes '771' and '773' of *P.halstedii*. The EOs were examined at concentrations of 0.2%, 0.4%, and 0.6% in susceptible sunflower variety '08-TR-003' as seed, foliar, and seed + foliar treatments.It was concluded that seed treatments could be applicable and appropriate, due to the phytotoxic effects of foliar treatments at increasing concentrations. Moreover, it was observed that seed treatments with St. John's wort, mustard, grape, ginger, or garlic EOs at a concentration of 0.6% exhibited anti-mildew activity with a decrease in sporangium quantity above 80% for pathotypes '771' and '773'. Seed treatment with grape EO had the highest anti-mildew activity at a concentration of 0.6% and was found to be the most effective with a decrease in sporangium quantity above 90% for both pathotypes of the pathogen.

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Introduction

Sunflower has an important place among oilseed crops in terms of the cultivation area and production capacity. Total sunflower cultivation area reached 26 million hectares worldwide. Moreover, the vast majority of herbal crude oil production is supplied by sunflower, due to its high oil content.

Diseases of sunflower (*Helianthus annuus* L.) remain a major factor restricting capacity of crop yield of sunflower varieties in many parts of the world. The symptoms caused by *Plasmopara halstedii* (Farl.) Berl. & De Toni, which is a destructive pathogen of sunflower worldwide depend on the inoculum density, age of tissue, and environmental conditions. When environmental conditions are appropriate (100% relative humidity and temperature approximately 16–18 °C), it produces obvious white sporulation on the underside of cotyledons and causes dwarfing in sunflower plants (Ljubich and Gulya 1988; Spring 2001; Sakr 2010). So far, severe yield losses have been documented in regions with a temperate climate, due to sporangiospores released from sporangia in outbreak years (Viranyi and Spring 2011).

In recent years, chemical pesticides have been considered as the most effective management method to control plant diseases (Gulya *et al.* 1999; Sudisha et al. 2007, 2009, 2010). Although seed treatment of azoxystrobin fungicide has been informed to be an alternative for control of downy mildew, it is not adequate to suppress the disease development (Gulya 2001; Gulya 2002). These chemicals could be easily absorbed by soil and lead to ecosystem degradation, as well as contaminating of food crops (Ragsdale et al. 1993; Satapute et al. 2019). Also, target organisms could acquire resistance as a result of long-term and unconscious use of chemical pesticides. On the other hand, the majority of both oilseeds and confection type sunflower hybrids are commercially available, but they are susceptible to the common downy mildew races (Gulya 2007; Kleingartner 2005).

Recently, studies with plant-derived preparations have accelerated due to overuse of chemical pesticides. The antimicrobial effect of essential oils (EOs) on plant diseases has been an important topic of recent years. Nevertheless, only one publication about potential anti- mildew activity of *Bupleurum gibraltarium* EO against *P. halstedii* was recorded by Fernandez-Ocana et al. (2004). Also, there were a few publications in which the relevant EOs were used against other downy mildew pathogens, such as *Peronospora tabacina* in tobacco (Schiltz 1974), *Sclerospora graminicola* in pear millet (Deepak et al. 2005), and *Hyaloperonospora parasitica* in *B. juncea* (Bhatt et al. 2009).

EOs of Greek sage, black cumin, bay, mustard, St. John's wort, French lavender, garlic,

grape, and ginger have recently been tested at different concentrations for phytotoxic effects on sunflower plant (Er 2018). Also, anti-mildew activity measured as decrease of sporangium quantity of *P.halstedii* pathotypes (771 and 773) has been examined using the leaf disc assay (Er et al. 2020). It was found that the EOs of black cumin, mustard, St.John's wort, garlic, grape, and ginger would be appropriate and promising at concentrations of 0.2%, 0.4%, and 0.6% against the pathogen, due to their phytotoxic effects at increasing concentrations (Er 2018; Er et al. 2020).

The aim of this study is to determine the anti-mildew activity of these essential oils at certain concentrations and their effects on decrease of *P.halstedii* sporangium quantity under in vivo conditions.

Material and methods

Essential oils and plant material

The essential oils of grape (*V. vinifera*), black cumin *(N. sativa*), garlic (*A. sativum*), mustard (*S. nigra*), ginger (*Z. officinale*), and St. John's wort (*H. perforatum*) were purchased from medicinal herb stores as 100% reliable commercial preparations. The susceptible sunflower variety '08-TR-003' was tested in the study and supplied from Agricultural Research Institute of Trakya. *Plasmopara halstedii* pathotypes '773' and '771', known to be the most aggressive in Marmara and Thrace regions, were obtained from May Seed Company. Sunflowers were planted in 22 × 15 cm pots with a mixture of 1/3 perlite +2/3 sterile peat for in vivo experiments.

Production of pathotypes of sunflower downy mildew

Sunflower seeds were first disinfected with 1% sodium hypochlorite (NaOCl) for 5 min and rinsed 2 times with sterile distilled water (SDW). The seeds were then germinated at room temperature in the dark for 3–4 days. Young plants were separately inoculated with sporangia suspensions (10^5 sporangia/ml) of both pathotypes at 16 °C for 24 h in the dark and transferred to pots (22 × 15 cm) containing 1/3 perlite+2/3 sterile peat mixture (Viranyi 1977 and Gulya et al. 1991, 1998). The experiment was carried out at 20 °C with a photoperiod of 16 h in a climate room. When the first true leaves of infected plants reached 2–3 mm wide, they were covered with a moistened transparent nylon bag and incubated for 48 h at 18 °C (Fernandez-Ocana et al. 2004 and Evci et al. 2011).

Determination of in vivo anti-mildew activity of essential oils

The EOs were examined at concentrations of 0.2% 0.4% and 0.6% as seed, foliar, and seed+foliar treatment. To determine the anti-mildew activity of the seed treatments against downy mildew caused by *P. halstedii*, the essential oils (EOs) containing Tween 20 (0.5% *v*/v) were applied to sunflower seeds homogenously at different concentrations with a dose-adjusted spray (Fernandez-Ocana et al. 2004). After germination of the seeds, they were inoculated as mentioned in production of pathotype sections.

For foliar treatment, the EOs were sprayed separately at concentrations of 0.2% to 0.6% to upper and lower surfaces of cotyledons with a dose-adjusted spray for 2 times, when cotyledons were observed to emerge and before the seedlings were covered with a moistened transparent nylon bag. In addition, seed+foliar treatment of EOs was implemented according to the same procedure as seed and foliar treatments**.** In the present study, sunflower seedlings were incubated at 18 °C with a photoperiod of 16 h under moist conditions and the sporangium quantity was evaluated after 48 h. The experiment was attempted with an average of 5 independent replicates; EO-free pots, containing SDW and a spore suspension of the pathogen (1×10^5 sporangia/ml), were used as a control (Spring et al. 1997; Spring et al. 1998; Mounira et al. 2011; La Torre et al. 2014). After an incubation period, a physiological saline solution was prepared by adding 9 g NaCl to 1 l of SDW and added to each cotyledon placed into eppendorf tubes to pass sporangia into the solution (Sakr et al. 2007). The quantity of sporangia for each cotyledon was counted by Thoma slide.

The decrease in quantity of sporangia after essential oil treatments was calculated using the following formula (Deans and Svoboda 1990).

% Decrease (D) = [(C-T/C)] × 100.

C: Average quantity of sporangia per cotyledon in control treatment.

T: Average quantity of sporangia per cotyledon in essential oil treatment.

Identification of essential oil components by gas chromatography/mass spectrometry (GC/MS)

The essential oils were analyzed by GC/MS (Gas Chromatography/Mass Spectrometry) using a Shimadzu QP2010-Ultra model GC/MS. Components of the EOs were separated based on holding time of the fused silica capillary and evaluation procedures were carried out by the GC/MS instrument library. The oven program started with an initial temperature of 60 °C, kept for 5 min, was heated at 4 °C/min to 260 °C and reached 300 °C with an increase every 15 min, held for 2 min. The injection volume was 1 μL and the injection port temperature was 250 °C. Helium gas (40:1 split ratio and 1 ml/min) was supplied as a carrier gas. For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. The total program time was 59.67 min. Identification of EO components was made by computerized library matching (NIST27, NIST107, NIST147, and WILEY7).

Statistical analysis

Statistical analysis data were subjected to ANOVA (one-way analysis of variance). Significant differences ($p < 0.05$) were tested by the General Linear Model (GLM) procedure using the Duncan's Multiple Range Test (DMRT) for quantity of pathogen sporangia after EO treatments.

Results

Seed, foliar, and seed+foliar treatments of the EOs at increasing concentrations against both pathotypes of *P.halstedii* were found to have remarkable anti-mildew activity compared to control treatments.

For pathotype 771, seed treatment with black cumin EO at a concentration of 0.2% was determined to have the lowest anti-mildew activity with a decrease of 46.3% in sporangium quantity (Table 1). The seed+foliar treatment with EOs of ginger, mustard, garlic, grape, and St. John's wort at a concentration of 0.6% exhibited a decrease in sporangium quantity above 98%.

For pathotype 773, seed treatment with black cumin EO at a concentration of 0.2% was determined to have the lowest anti-mildew activity with a decrease of 38% in sporangium quantity (Table 2). The seed+ foliar treatment with EOs of ginger, St John's wort, and garlic at a concentration of 0.6% exhibited a decrease in sporangium quantity of approximately 100%.

The essential oils exhibiting significant anti-mildew activity against both pathotypes of *P.halstedii* were evaluated by GCMS analysis (Table 3).

Discussion

Although there are a limited number of studies examining the anti-mildew activity of EOs against *P. halstedii*, the present study is the first report indicating that EOs of mustard, grape,St. John's wort, ginger, and garlic

Table 1 The percentage and quantity of sporangia of pathotype 771 of *P. halstedii* after essential oil treatments at different concentrations

		Seed Treatment		Foliar Treatment		Seed+Foliar Treatment	
Essential oils	Concentration (%)	Sporangium quantity (×10^4) / cotyledon	Decrease in sporangium quantity (%)	Sporangium quantity (×10^4) / cotyledon	Decrease in sporangium quantity (%)	Sporangium quantity (×10^4) / cotyledon	Decrease in sporangium quantity (%)
Black cumin	0.2	35.54±7.10 b*	46.3	19.335±5.22cde*	70.8	9.775±3.19d-j*	85.2
	0.4	21.835±5.45 c	67.0	18.05±5.02 c-g	72.7	8.34±2.70 d-j	87.4
	0.6	19.78±4.97 cd	70.1	10.705±3.15 c-j	83.8	5.715±1.90 hıj	91.3
Mustard	0.2	11.29±3.50 c-j	82.9	18.58±4.98 c-f	71.9	4.43±1.84 hıj	93.3
	0.4	10.395±3.32c-j	84.3	15.275±4.37c-h	76.9	2.25±1.16 ıj	96.6
	0.6	8.36±3.29 d-j	87.3	12.84±3.94 c-j	80.6	0.675±0.35 j	98.9
St.John's wort	0.2	8.495±3.12 d-j	87.1	10.085±3.53 d-j	84.7	4.785±1.98 hıj	92.7
	0.4	7.67±2.81 e-j	88.4	9.335±3.25 d-j	85.9	2.63±1.20 ıj	96.0
	0.6	6.28±2.72 g-j	90.5	6.765±2.81 f-j	89.7	0.825±0.41 j	98.7
Garlic	0.2	13.35±3.78 c-ı	79.8	11.995±3.91 c-j	81.8	3.015±1.47 ıj	95.4
	0.4	11.98±3.39 c-j	81.9	8.025±2.96 d-j	87.8	2.41±1.11 ıj	96.3
	0.6	6.605±2.72 f-j	90.0	6.805±2.41 f-j	89.7	0.72±0.39 j	98.9
Grape	0.2	7.01±2.90 f-j	89.4	13.62±4.45 c-ı	79.4	3.825±1.78 hıj	94.2
	0.4	6.035±2.65 g-j	90.8	13.39±4.20 c-ı	79.7	2.755±1.32 ıj	95.8
	0.6	5.805±2.59 hıj	91.2	12.55±4.17 c-j	81.0	0.665±0.33 j	98.9
Ginger	0.2	7.865±3.25 d-j	88.1	7.745±3.18 e-j	88.3	2.3±1.0970ıj	96.5
	0.4	7.12±2.90 f-j	89.2	7.395±2.97 e-j	88.8	1.325±0.60 ıj	98.0
	0.6	6.46±2.74 f-j	90.2	7.245±2.87 f-j	89.0	0.57±0.27 j	99.1
Control		66.27±9.34 a	–	66.27±9.34 a	–	66.27±9.34 a	–

*Each value is the mean (±standard error) of 5 replications. Means in sporangium quantity column followed by different letter differ significantly according to Duncan's multiple range test at the $p<0.05$ level.

exhibit a high anti-mildew activity and are promising treatments against *P.halstedii* on sunflower plant. Particularly, seed treatment with grape EO demonstrated maximum anti-mildew activity with a decrease in sporangium quantity above 90% at a concentration of 0.6%. Nevertheless, anti-mildew mechanisms of the EOs are complicated; anti-mildew activity is likely not dependent on only one mechanism, as the pathogen cell could be affected by different chemical groups found in the composition of EOs.

The EOs of mustard, grape, garlic, St. John's wort, and ginger remarkably decreased the sporangium quantity of *P. halstedii*, in particular at a concentration of 0.6%. The results were in agreement with data from a previous study, which pointed out that *Bupleurum gibraltarium* EO at a concentration of 0.5% (5.0 ml/L) was effective on sunflower plants to reduce percentage of sporangia of *P. halstedii* (Fernandez-Ocana et al. 2004).

Deepak et al. (2005) reported that a ginger-derived preparation reduced sporulation of downy mildew (*Sclerospora graminicola*) in pearl millet. The similar results were obtained from another obligate plant pathogen, *'Blumeria graminis* f. sp. *tritici'* in wheat seeds (Vechet et al. 2005, 2009). Another study showed that a ginger-derived preparation at a concentration of 0.2% (2000 ppm) significantly controlled *Erysiphe pisi* in pea seed (Singh et al. 1991). Seed treatment of a ginger-derived preparation at a concentration of 2.5% remarkably suppressed powdery mildew of mungbean caused by *Oidium sp*. (Ayub et al. 1997). These previous studies were in agreement with the present study, which remarked that seed treatment with ginger EO had anti-mildew activity with a decrease in sporangium quantity above 83% at all concentrations against both pathotypes of the pathogen. It was also concluded that seed treatment with ginger EO was more applicable and reliable, since it demonstrated an anti- mildew activity even at low concentrations and no phytotoxic effect compared to foliar treatments (Er 2018).

The anti-mildew activity of seed and seed+foliar treatments of garlic EO at a concentration of 0.6%

Table 2 The percentage and quantity of sporangia of pathotype 773 of *P. halstedii* after essential oil treatments at different concentrations

		Seed Treatment		**Foliar Treatment**		**Seed+Foliar Treatment**	
Essential oils	Concentration (%)	Sporangium quantity (×10^4) / cotyledon	Decrease in sporangium quantity (%)	Sporangium quantity (×10^4) / cotyledon	Decrease in sporangium quantity (%)	Sporangium quantity (×10^4) / cotyledon	Decrease in sporangium quantity (%)
Black cumin	0.2	46.2±8.86 b*	38.0	18.98±5.40 cde*	74.5	16.255±4.86 c-g*	78.2
	0.4	36.16±7.38 b	51.5	17.08±5.01 c-f	77.1	14.71±4.64 c-ı	80.2
	0.6	21.885±5.78 c	70.6	15.74±4.22 c-h	78.8	12.145±4.57 c-ı	83.7
Mustard	0.2	19.09±5.34 cde	74.4	15.715±4.37 c-h	78.9	9.51±3.05 c-ı	87.2
	0.4	11.285±4.13 c-ı	84.8	14.67±4.91 c-ı	80.3	4.68±1.81 d-ı	93.7
	0.6	7.89±3.23 c-ı	89.4	11.39±4.44 c-ı	84.7	0.935±0.49 hı	98.7
St.John's wort	0.2	16.395±4.99 c-g	78.0	20.815±5.72 c	72.0	4.53±2.04 d-ı	93.9
	0.4	12.44±4.16 c-ı	83.3	14.59±4.75 c-ı	80.4	2.975±1.36 f-ı	96.0
	0.6	10.825±3.71 c-ı	85.4	7.995±3.23 c-ı	89.2	0.65±0.33 ı	99.1
Garlic	0.2	19.375±5.17 cd	74.0	16.095±4.41 c-g	78.4	4.23±1.93 e-ı	94.3
	0.4	15.695±4.84 c-h	78.9	14.6±4.17 c-ı	80.4	2.98±1.44 f-ı	96.0
	0.6	12.105±4.59 c-ı	83.7	7.195±2.88c-ı	90.3	0.69±0.39 ı	99.0
Grape	0.2	13.34±4.16 c-ı	82.1	13.55±4.43c-ı	81.8	8.21±2.71 c-ı	88.9
	0.4	10.31±3.53 c-ı	86.1	12.455±4.01 c-ı	83.3	4.235±1.79 e-ı	94.3
	0.6	5.695±2.50 d-ı	92.3	9.2±3.45 c-ı	87.6	0.97±0.48 hı	98.6
Ginger	0.2	12.29±4.14 c-ı	83.5	7.595±3.05 c-ı	89.8	3.34±1.58 f-ı	95.5
	0.4	11.21±3.75 c-ı	84.9	5.67±2.40 d-ı	92.3	1.625±0.96ghı	97.8
	0.6	9.725±3.56 c-ı	86.9	5.625±2.51 d-ı	92.4	0.57±0.30 ı	99.2
Control		74.595±9.87 a	-	74.595±9.87 a	-	74.595±9.87 a	-

*Each value is the mean (±standard error) of 5 replications. Means in sporangium quantity column followed by different letter differ significantly according to Duncan's multiple range test at the $p<0.05$ level.

against both pathotypes of the pathogen was similar to a previous study, which exhibited a significant suppression of *Hyaloperonospora parasitica* in *B. juncea* treated with a garlic-derived preparation at a concentration of 1% (Bhatt et al. 2009). In the present study, the seed+foliar treatment even at a concentration of 0.6% was sufficient to assure nearly 100% control of downy mildew caused by *P. halstedii*.

St. John's wort-derived preparations have been reported to be effective against the vast majority of plant pathogens such as *Penicillium canescens*, *Fusarium oxysporum*, *A. alternata, Sclerotinia sclerotiorum, Rhizoctonia solani,* and *Fusarium graminearum* under in vitro conditions (Gudzic et al. 1997; Couladis et al. 2003; Kordali et al. 2007; Maskovic et al. 2011); in present study, St. John's wort EO was found to have a high anti-mildew activity above 70% at all concentrations with seed, foliar, and seed+foliar treatments against both pathotypes of the pathogen.

When taking into account that foliar treatment with grape seed oil provided approximately 99% control of powdery mildew (*Sphaerotheca pannosa* var. *rosae*) in rose plant (Wojdylo 2015) and powdery mildew (*Podosphaera leucotricha*) in apple (Northover et al. 1993), the foliar treatment with grape EO at a concentration of 0.6% showed an anti-mildew activity with a decrease in sporangium quantity above 80% against both pathotypes of *P. halstedii.* However, seed treatment with grape EO at a concentration of 0.6% would be more efficient and reliable, since it provides adequate anti-mildew activity against both pathotypes of the pathogen when considering phytotoxic effect of the foliar treatment at increasing concentrations (Er 2018).

Seed treatment with mustard EO at a concentration of 0.6% showed an anti-mildew activity above 85% against both pathotypes of *P. halstedii* and was sufficient to control the disease significantly. These findings were in agreement with other researchers (Borgen and Lars 2001), who reported that seed treatment of wheat and rye with mustard-derived preparations against *Tilletia tritici* and *Urocystis occulta* led to a reduction in disease rates above 90%. Therefore, it was concluded

Table 3 Results of GC/MS analysis of essential oils

Essential oil	Compounds	Peak area (%)
Ginger	Camphene	1.35
	Bornyl acetate	31.33
	Benzyl Alcohol	43.07
	ar-Curcumene	3.14
	Zingiberen	2.90
	ß-Bisabolene	2.45
	ß-Sesquiphellandrene	1.88
	Oleic acid	1.39
Garlic	Diallyl disulphide	1.77
	Palmitic acid	12.17
	Ethyl palmitate	3.11
	Methyl oleate	1.97
	Linoleic acid	61.89
	Linoleic acid, butyl ester	3.35
	Ethyl oleate	9.09
John's wort	Cis-Ocimene	10.23
	Camphene	1.80
	1,8-Cineole	8.48
	L-Fenchone	14.36
	Linalool	11.65
	Camphor	20.67
	Borneol	2.26
	Endobornyl acetate	2.39
	Ledene	2.02
Grape	Heptanoic acid methyl ester	1.48
	Maltol	3.50
	1,2,3-Propanetriol monoacetate	2.11
	Triacetin	21.99
	α-Ionone	1.18
	ß-Ionone	6.26
	2-Butanone, 4-(4-hydroxyphenyl-)	8.52
	Methyl2-[2,4a-epidioxy-5,6,7,8-tetrahydro-5,5,8a-trimethyl-2H-]	1.37
	Tetratriacontane	3.30
	Vanillin	4.48
	Propanedioyldihydrazide	1.39
	4-Hydroxy-2,5-dimethylfuran-3(2H)-one (furaneol)	1.58
	Hexacontane	2.29
	Tetrapentacontane	3.64
Mustard	D-Limonene	1.89
	l-(+)-Ascorbic acid 2,6-dihexadecanoate	9.57
	Linoleic acid ethyl ester	2.84
	9-Octadecenoic acid (Z)-, methyl ester	1.66
	1-Triacontanol	1.45
	Octadec-9-enoic acid	70.58
	Octadecanoic acid	9.13
Black Cumin	D-Limonene	31.62
	d-Carvone	44.82
	2,4-Decadienal	1.74
	Cyclohexanol, 2-(1-methylpropyl)	2.57
	Tetrapentacosan	6.01
	Tetrapentacontane, 1,54-dibromo	1.61

that mustard-derived preparations could be used effectively as a seed treatment against *P.halstedii*.

The EOs of ginger, grape, St.John's wort, garlic, and mustard exhibited a remarkable decrease in sporangium quantity and were determined as the most effective EOs against both pathotypes of the pathogen. It was suggested that there was a possible synergistic anti-mildew activity depending on the presence and amount of EO components.

Based on previous studies (Cunningham and Pickard 1985; Naganawa et al.1996; Parveen et al. 2004; Tiwari and Kakkar 2009; Kim et al. 2012; Aala et al. 2014; Del Olmo et al. 2017), it is probable that the following compounds in the content of tested EOs, detected at certain ratios, interacted with and penetrated into the sunflower plants: monoterpenes (benzyl alchocol,bornyl acetate,fenchone,1,8-cineole, camphor, linalool, Cis-Ocimene, β-ionone, maltol), fatty acids (linoleic acid, oleic acid), and sesquiterpenes (ar-Curcumene, Zingiberen, ß-Bisabolene, camphene, ß-Sesquiphellandrene). These components of the EOs.

probably took an important part in directly inhibiting the germination of sporangia and/or release of sporangiospores, or acting as an elicitor to activate plant defense responses by triggering a variety of bioactive compounds such as phenolic compounds; these factors caused to disrupt fungal cell membrane integrity and affect the activity of basic enzymes, and consequently death of the pathogen cells (Pinto et al. 2006; Park et al. 2009; Ben-Jabeur et al. 2015).

We hope that plant-derived preparations containing the EOs of ginger, St. John's wort, grape, mustard, and garlic will be significantly applicable and promising to control the downy mildew of sunflower.

Conclusion

Most of the EO treatments tested on sunflower plants were found to exhibit a significant anti-mildew activity against *Plasmopara halstedii,* which is an important pathogen of sunflower worldwide. It was concluded that anti-mildew activity of the EOs was predominantly due to monoterpenes, sesquiterpenes, and fatty acids found at certain ratios in the composition of the EOs. Although the results are satisfactory and promising, the EOs with high anti-mildew potency and the interactions of EO compounds with each other should be reserached for formulations that are effective, non-toxic and non-polluting. Hopefully, the present study will accelerate the use of plant origin pesticides as an alternative tool that can be used instead of chemical pesticides and other low-efficiency or harmful methods for the control of downy mildew caused by *P.halstedii*.

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Declarations

Human and/or animals rights We confirm there was no research on any humans or animals.

Informed consent All authors have reviewed the manuscript and consented to submission of the study to the European Journal of Plant Pathology.

Conflict of interest The authors declare that they have no conflict of interest.

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