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SHORT COMMUNICATION

Determination of anti-mildew activity of essential oils against downy mildew of sunflower caused by *Plasmopara halstedii*

Yesim Er[1] · Nuray Özer[2] · Yakup Zekai Katırcıoğlu[3]

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Abstract
To evaluate the anti-mildew activity of the commercial essential oils (EOs) against pathotypes '771' and '773' of *P. halstedii* in mineral salt medium, the EOs obtained from different parts of Greek sage, black cumin, bay, mustard, St. John's wort, French lavender, garlic, grape, and ginger plants were examined at concentrations of 0.2%, 0.4%, 0.6%, 0.8%, and 1% on leaf discs of susceptible sunflower variety '08-TR-003'. The ginger EO had the highest anti-mildew activity at a concentration of 1% and was found to be the most effective with a decrease in sporangium quantity above 90% for pathotypes '771' and '773'. The EOs of garlic, St. John's wort, and grape followed it, respectively, with a decrease in sporangium quantity above 80%.

Keywords Anti-mildew activity · Essential oil · Leaf disc · *Plasmopara halstedii*

Introduction

Diseases in sunflower (*Helianthus annuus* L.) are the most significant factor limiting the crop yield and genetic yield potential of sunflower varieties. The downy mildew caused by *Plasmopara halstedii* (Farl.) Berl. & De Toni is particularly one of the most important diseases of sunflower. When the relative humidity approaches 100% and air temperature is approximately 16–18 °C, it causes apparent white sporulation, firstly on the lower side of cotyledons, and dwarfing of the plants (Viranyi 1978; Sackston 1981; Ljubich and Gulya 1988; Spring 2001; Sakr 2010). So far, the disease has been reported on all continents and is more common in regions with a temperate climate than in regions with a subtropical climate, where sunflower is cultivated, causing significant yield losses due to sporangiospores released from sporangia in epidemic years (Viranyi and Spring 2011).

In recent years, the use of chemical pesticides against plant diseases is proposed as the most effective method (Gulya et al. 1999; Sudisha et al. 2007, 2009, 2010). The systemic chemical pesticides, which are used to control *P. halstedii,* can be easily absorbed by soil, and it causes the *degradation* of the ecosystem and pollution of our foods with these chemicals (Anonymous 1987; Ragsdale et al. 1993; Satapute et al. 2019). Besides, the resistance of target organism as a result of long-term use of systemic pesticides is another disadvantage causing the appearance of metalaxyl-resistant strains of *P. halstedii*. Although the seed treatment with azoxystrobin as an alternative pesticide has been registered for the control of downy mildew, it is not sufficient and satisfactory to assure 100% disease control (Gulya 2001, Gulya 2002). In addition to it, the vast majority of both oilseed and confection type sunflower hybrids, which are commercially available, are susceptible to the common downy mildew races (Gulya 2007).

Studies about the use of plant origin preparations for pest control have accelerated due to the unconscious and overuse of chemical pesticides. In this respect, the researches related to the antimicrobial effect of EOs on plant diseases have been the most crucial issue of recent years. However, only one documented study about possible anti-mildew activity of EOs on *P. halstedii* was reported by Fernandez-Ocana et al. (2004), although there were few studies which informed the anti-mildew activity of the alkaloids and extracts obtained from *Solanum* spp. and *Veratrum* spp. (Oros 2010), *Morinda citrifolia* (noni), *Zingiber officinale* (ginger), and *Tinospora*

✉ Yesim Er
y_err@hotmail.com

[1] Istanbul Directorate of Agricultural Quarantine, Istanbul, Turkey

[2] Department of Plant Protection, Faculty of Agriculture, Namık Kemal University, Tekirdağ, Turkey

[3] Department of Plant Protection, Faculty of Agriculture, Ankara University, Ankara, Turkey

cordifolia (Guduchi plant) (Girijamba et al. 2014) against the pathogen growth.

The aim of this study is to determine the anti-mildew activity of some essential oils, applied at different concentrations, and their effects on decrease in quantity of sporangium of *P. halstedii*, by using the leaf disc assay.

Materials and methods

The essential oils of Greek sage (*Salvia triloba* L.), black cumin *(Nigella sativa* L.), bay (*Laurus nobilis* L.), mustard (*Sinapis nigra* L.), St. John's wort (*Hypericum perforatum* L.), French lavender (*Lavandula stoechas* L.), garlic (*Allium sativum* L.), grape (*Vitis vinifera* L.), and ginger (*Zingiber officinale* L.), obtained from different parts of plants, were purchased as 100% pure and reliable commercial preparations. The susceptible sunflower variety '08-TR-003' was used for the study and obtained 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. The mineral salt (MS) medium (180 mg D-glucose, 1.3 mg asparagine, 5.3 mg $(NH_4)\ 2SO_4$, 2.4 mg $MgSO_4{\cdot}7H_2O$, 1.1 mg $CaCl_2$, 3.1 mg K_2HPO_4, 1.6 mg KH_2PO_4, 1.7 µg thiamine HCl, for 1 l) (Nelson and Hsu 1994) was used to produce sporangia of *P. halstedii* for the leaf disc assay conducted on watch glasses in a diameter of 80 mm. The first true leaves of 20 days old of sunflower plants were collected and rinsed with *SDW*. The true leaves were cut into discs of 1 cm diameter following the drying process. Essential oils containing Tween 20 (0.5% v/v) were applied to the lower surface of leaf discs at different concentrations with a dose-adjusted spray (Fernandez-Ocana et al. 2004). The leaf discs were allowed to absorb the essential oils for 20 min and then placed in sterile watch glasses in a diameter of 80 mm (one disc per glass), containing 500 µl of MS medium at a concentration of 10^5 sporangia/ml. The watch glasses were placed on sterile petri dishes, spraying SDW on top of them and taken into a climate room (at 16 °C with a photoperiod of 14 h) for 7 days. The experiment was replicated five times with the discs of five leaves. The EO-free MS medium, containing only SDW and Tween 20 (0.5% v/v), was 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 of 7 days, the physiological saline solution was prepared by adding 9 g NaCl to 1 L of SDW and transferred to each leaf disc placed into Eppendorf tubes to pass sporangia into the solution (Sakr et al. 2007). Consequently, the quantity of sporangium of each leaf disc was counted by Thoma slide.

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 sporangium of the pathogen after EO treatments. The decrease in sporangium quantity after EO treatments was calculated using the following formula (Deans and Svoboda 1990).

$$\%\ \text{Decrease}\ (D) = (A - T/A) \times 100$$

A Average quantity of sporangium per leaf disc in control treatment

T Average quantity of sporangium per leaf disc in essential oil treatment

The essential oils were analysed by GC/MS (gas chromatography/mass spectrometry) technique. Component analysis of the EOs was performed with Shimadzu QP2010-Ultra model GC/MS. Components of the EOs were separated based on holding time of the fused silica capillary, and the evaluation procedures were carried out by the GC/MS instrument library. The total program time was 59.67 min. The identification of EO components was made by computerized library matching (NIST27, NIST107, NIST147, and WILEY7).

Results

The EOs of black cumin and mustard were evaluated for three concentrations (0.2%, 0.4%, and 0.6%) due to their *phytotoxic effects* at higher concentrations (Er 2018). For pathotype 771, sporangium quantity after treatment of the EOs at all concentrations, except some concentrations of the EOs of Greek sage (0.2, 0.8, and 1.0%) and bay (0.2 and 0.4%), was significantly lower than the control treatment. However, Greek sage EO showed an increase in quantity of sporangium at concentrations of 0.2% and 1%, although it did not statistically differ from the control treatment. The ginger EO at concentrations of 0.8% and 1%, the St. John's wort EO at a concentration of 1%, and the garlic EO at a concentration of 0.8%, exhibited a decrease in sporangium quantity above 90% (Table 1).

For pathotype 773, sporangium quantity at most of the applied concentrations of Greek sage EO was statistically similar to the control treatment. The sporangium quantity in leaf discs treated with other EOs, except some concentrations of French lavender (0.2%) and bay (0.4%) EOs, was significantly lower than the control treatment, occurring a decrease in sporangium quantity above 50%. Particularly, the ginger EO at concentrations of 0.8% and 1%, the EOs of St. John's wort, garlic, and grape at a concentration of 1% exhibited a decrease in sporangium quantity above 90% (Table 1).

Table 1 The percentage and quantity of sporangium of *P. halstedii* pathotypes after essential oil treatments at different concentrations

		Pathotype 771		Pathotype 773	
Essential oil	Concentration (%)	Sporangium quantity ($\times 10^4$)/leaf disc	Decrease in quantity of sporangium (%)	Sporangium quantity ($\times 10^4$)/leaf disc	Decrease in quantity of sporangium (%)
Greek sage	0.2	144.8 ± 12.25 ab*	0.0	164.2 ± 16.1 4 ab*	8.0
	0.4	93.4 ± 11.54 cd	32.3	142.6 ± 15.26 bc	20.1
	0.6	104.6 ± 11.50 cd	24.1	147.2 ± 15.72 ab	17.5
	0.8	123.2 ± 18.24 abc	10.6	171.4 ± 16.38 ab	4.0
	1.0	149.8 ± 17.41 a	0.0	178.0 ± 10.18 a	0.3
Black cumin	0.2	98.8 ± 15.11 cd	28.4	45.8 ± 9.11 f–k	74.4
	0.4	57.0 ± 14.69 e–g	48.7	26.8 ± 4.49 g–k	85.0
	0.6	24.6 ± 4.73 g–k	82.8	34.0 ± 6.47 g–k	81.0
Bay	0.2	122.6 ± 18.48 abc	11.0	73.2 ± 20.41 d–f	59.0
	0.4	118.0 ± 22.63 bcd	14.4	114.6 ± 18.56 c	35.8
	0.6	97.6 ± 16.05 cd	29.2	70.8 ± 21.07 d–f	60.4
	0.8	61.2 ± 8.35 ef	55.6	49.6 ± 11.36 d–ı	72.2
	1.0	23.2 ± 3.30 g–k	83.2	48.4 ± 13.52 e–j	72.9
Mustard	0.2	87.4 ± 11.91 de	36.6	81.4 ± 16.96 de	54.4
	0.4	48 ± 5.30 f–j	65.2	52.0 ± 6.81 d–h	70.9
	0.6	56.2 ± 5.06 e–g	59.3	62.2 ± 5.05 d–g	65.2
St. John's wort	0.2	57.6 ± 8.26 e–g	58.3	44.4 ± 10.63 f–k	75.2
	0.4	24.4 ± 5.01 g–k	82.3	41.6 ± 10.41 f–k	76.7
	0.6	15.8 ± 3.24 ı–k	88.6	34.6 ± 6.24 g–k	80.7
	0.8	18.2 ± 3.72 ı–k	86.8	20.2 ± 2.47 h–k	88.7
	1.0	13.8 ± 3.63 j–k	90.0	12.4 ± 1.91 jk	93.1
French lavender	0.2	86.0 ± 9.40 de	37.6	82.8 ± 19.38 d	43.6
	0.4	56.2 ± 11.96 e–g	59.3	49.0 ± 4.84 d–ı	72.6
	0.6	31.8 ± 6.0116 f–k	77.0	29 ± 4.19 g–k	83.3
	0.8	40.0 ± 7.85 f–k	71.0	21.6 ± 5.76 h–k	88.0
	1.0	49.6 ± 11.05 f–ı	64.1	19.6 ± 4.41 h–k	89.0
Garlic	0.2	24.2 ± 6.44 g–k	82.5	40.6 ± 9.00 f–k	77.3
	0.4	24 ± 5.80 g–k	82.6	30.0 ± 5.62 g–k	83.2
	0.6	23.2 ± 4.43 g–k	83.2	27.6 ± 8.18 g–k	84.6
	0.8	11.8 ± 2.17 k	91.5	26.2 ± 3.12 g–k	85.4
	1.0	16.4 ± 4.88 ı–k	88.1	13.2 ± 1.24 ı–k	92.7
Grape	0.2	53.4 ± 8.33 f–h	61.3	60.8 ± 3.62 d–g	66.0
	0.4	29.6 ± 4.14 f–k	78.6	32.6 ± 4.00 g–k	81.8
	0.6	35.2 ± 9.59 f–k	74.5	28 ± 6.23 g–k	84.4
	0.8	33.8 ± 4.18 f–k	75.5	19.6 ± 1.60 h–k	89.1
	1.0	21.4 ± 4.14 h–k	84.5	14.2 ± 1.28 ı–k	92.1
Ginger	0.2	38.8 ± 6.52 f–k	71.9	43.6 ± 6.63 f–k	75.6
	0.4	37.4 ± 4.43 f–k	72.9	29.4 ± 5.31 g–k	83.6
	0.6	29.4 ± 4.54 f–k	78.7	27.4 ± 7.01 g–k	84.7
	0.8	2.4 ± 1.60 k	91.1	16.6 ± 1.50 h–k	90.7
	1.0	7.0 ± 1.92 k	95.0	11.2 ± 1.31 k	93.8
Control		137.8 ± 16.41 ab	–	178.4 ± 16.54 a	–

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

Table 2 Results of GC/MS analysis of essential oils

Major components and peak areas (%) of the EOs							
Ginger EO	(%)	Garlic EO	(%)	John's wort EO	(%)	Grape EO	(%)
Benzyl alcohol	43.07	Linoleic acid	61.89	Camphor	20.67	Furaneol	27.73
Bornyl acetate	31.33	Palmitic acid	12.17	L-Fenchone	14.36	Triacetin	21.99
						2-Butanone,4-(4 hydroxyphenyl)	8.52
				Linalool	11.65	Beta-ionone	6.26
ar-Curcumene	3.14	Ethyl oleate	9.09	Cis-Ocimene	10.23	Vanillin	4.48
				1,8-Cineole	8.48	Tetrapentacontane	3.64
Zingiberene	2.90	Linoleic acid, butyl ester	3.35	Endobornyl acetate	2.39	Maltol	3.50
						Tetratriacontane	3.30
ß-Bisabolene	2.45					Hexacontane	2.29
ß-Sesquiphellandrene	1.88	Ethyl palmitate	3.11	Borneol	2.26	n-Hexatriacontane	1.58
Oleic acid	1.39	Methyl oleate	1.97	Ledene	2.02	Heptadecanoic acid, methyl ester	1.48
Camphene	1.35	Diallyl disulphide	1.77	Camphene	1.80	Alpha-ionone	1.18

The essential oils, exhibiting a significant anti-mildew activity against both pathotypes of *P. halstedii*, were evaluated by GC/MS analysis (Table 2).

Discussion

The EOs of St. John's wort, garlic, grape, and ginger, starting from concentrations of 0.4% or 0.6% in the current study, significantly decreased the sporangium quantity of *P. halstedii*. Particularly, the ginger EO exhibited maximum anti-mildew activity at concentrations of 0.8% and 1% by inhibiting the release of sporangiospores of both pathotypes of *P. halstedii.* The findings were in agreement with data obtained by a previous study, which indicated that *Bupleurum gibraltarium* EO at a concentration of 0.5% was particularly found to be effective by reducing percentage of sporangia of *P. halstedii* on young sunflower plants (Fernandez-Ocana et al. 2004). There was an agreement with Oros (2010) and Girijamba et al. (2014), who reported that the alkaloids obtained from *Solanum* spp. and *Veratrum* spp., and the methanolic extracts obtained from *Morinda citrifolia*, *Zingiber officinale*, and *Tinospora cordifolia* inhibited the motion and release of sporangiospores of *P. halstedii*. In addition, it was reported that tobacco leaves treated with a monoterpene 'β-ionone', which is found in composition of the grape EO, markedly reduced the lesion development and sporulation of *Peronospora tabacina* (the agent of tobacco downy mildew) (Schiltz 1974; Salt et al. 1986). Accordingly, the anti-mildew activity of EOs was most probably due to the presence of various bioactive compounds (terpenes, alcohols, ketones) inhibiting the release of sporangiospores from sporangia in the present study.

When taking into account that the EOs exhibiting a high anti-mildew activity, the EOs of ginger, garlic, St. John's wort, and grape showed a significant decrease in sporangium quantity, respectively, and were identified as the most effective EOs against both pathotypes of the pathogen. The authors of this study suggested the presence of EO components with a synergistic anti-mildew activity. 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 was concluded that 'the monoterpenes (benzyl alcohol, bornyl acetate)' and 'sesquiterpenes (ar-curcumene, zingiberene, ß-bisabolene, ß-sesquiphellandrene, camphene)' in ginger EO composition; 'the monoterpenes (camphor, 1,8-cineole, cis-ocimene, linalool, fenchone)' in St. John's wort EO composition; 'the fatty acids' particularly 'linoleic acid' in garlic EO composition; 'the monoterpenes (maltol, β-ionone)' in grape EO composition, detected at high ratios, interacted with and penetrated into the leaf discs. Accordingly, these compounds of the EOs played an important role to prevent the release of sporangiospores attacking to the plant tissue, by disrupting the basic enzymes and membrane integrity and resulting in consequently metabolic disruption of the pathogen cells.

We hope that plant-derived preparations containing the EOs of ginger, garlic, grape, and St. John's wort will be significantly promising and applicable for the control of downy mildew of sunflower. We consider that plant origin pesticides will be used more economically and reliable to provide a better alternative for eco-friendly control of plant pathogens than chemical pesticides, because these plants can be cultivated by farmers.

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Compliance with ethical standards

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

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