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Research Article
Essential Oils with Inhibitory Capacities *Onpseudomonas syringae* pv. *actinidiae*, the Causal Agent of Kiwifruit Bacterial Canker

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Abstract

Background and Objective: Bacterial canker of kiwifruit caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) is one of the most severe bacterial disease of tree species and has been defined as a real pandemic. The Psa aggressiveness has made it very difficult to control with the use of single products, thus an integrated pest management seems to be key to successful control. The aim of this study was to evaluate the inhibitory capacity of 30 plant essential oils (EOs) against Psa. **Methodology:** The antimicrobial activity of EOs was performed by an *in vitro* assay based on an evaluation of the bacterial growth in a large volume of nutrient broth supplemented with EOs at different concentrations. The EOs that showed the strongest inhibitive capacities were further tested using a standardized broth microdilution method. Matching the results obtained with both tests, led to the selection of those EOs showing the strongest capacity to inhibit bacterial growth at the lowest concentrations. **Results:** This study shows that the most effective EOs against Psa were from clove bud (*Syzigium aromaticum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), cinnamon (*Cynnamon zeylanicum*) and to a lesser extent, garlic (*Allium sativum*). **Conclusion:** This large screening highlight the effectiveness of several EOs to be used for their antibacterial activity against Psa.

Key words: Antimicrobial activity, integrated control, *Actinidia chinensis*, *Actinidia deliciosa*, essential oils

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Kiwifruit bacterial canker is a devastating disease that affects *Actinida chinensis* and *Actinidia deliciosa* plantings, caused by the bacterium Psa[1]. Since 2008, Psa has caused severe damage first in Italy and subsequently in all the main kiwifruit cultivation areas in the world[2].

Due to the severely negative economic impact, the control of Psa has become of critical importance. Vine management-based practices such as the cutting and removal of infected vines and disinfection of pruning cuts are a key requisite. However, the disease can still spread from the vines in which these measures are applied and from those in which the bacterium is present as epiphytes or has not yet shown symptoms[3,4].

Breeding for resistance to Psa is on-going[5] and Psa-resistant germplasm has been reported in China[6]. Chemical treatments are best applied at an early stage of the disease[7]. In Asia and New Zealand, the use of antibiotics is legal for the control of plant pathogens, however in Italy and other European countries, it is banned and copper-based compounds are mostly used. The large use of these compounds has led to the insurgence of streptomycin-resistant Psa strains in Japan and Korea[8] and of copper-resistant Psa strains in Japan[9]. None of the strains isolated since 2008 in Italy have shown any resistance or tolerance to copper[10], however, the presence of the *P. syringae* pv. *syringae* copper-resistant strain in phyllosphere was recently recovered from Kiwifruit plants[11].

In Italy, several protocols based on the use of copper compounds together with sanitizers (benzoic acid, quaternary ammonium salts) and film forming products (chitosan, polyacrylic acids and amines) have been developed to protect Kiwifruit plants in the autumn-winter[12,13]. Due to the phytotoxicity of copper compounds on stalks and leaves[1,13,14] non phytotoxic compounds such as chitosan have been proposed as substitutes in the vegetative period[13]. Chitosan has an anti microbial power on a broad range of pathogens and is known to elicit the plant defence system and plant growth when used in the field[15].

Recently, acibenzolar-S-methyl (Bion® or Actigard® (Syngenta), an elicitor of host resistance was authorized in Italy with an emergency procedure for use on Kiwifruit for the control of bacterial canker (Ministry of Health, executive decree of 10 April 2017[16]). The efficacy of acibenzolar-S-methyl (ASM) has been reported[17] and the basis of the host response pathways are also under study[18,19]. It has also been reported that at high concentrations, ASM could decrease plant growth and cause phytotoxicity resulting in premature leave fall[14].

Overall these strategies have shown the potential to slow down disease progress, probably by decreasing the number of successful infection events and reducing the multiplication of the bacterium. Thus it is still important to find novel compounds, which when used in combination with other molecules, can contribute to the integrated control of bacterial canker. This is particularly urgent considering the recent legislation that specifies that several phytosanitary compounds such as copper should be replaced (Commission Implementing Regulation (EU) 2015/408 of 11 March 2015[20]).

Essential oils (EOs) could be safely used as effective pesticides with a low risk for human health and the environment and therefore represent a good alternative to conventional chemical pesticides[21].

The EOs are secondary metabolites known to have antimicrobial activities[22]. The action of EOs in the control of Gram-positive and Gram-negative plant pathogenic bacteria has been reported since 1963[23-26]. Generally, Gram-negative bacteria are more resistant to EOs than Gram-positive due to the differing structures of their cell walls[27]. Antimicrobial activity is due to their hydrophobic nature which facilitates the interaction with the lipids of cell membranes, thus affecting the cell permeability and structure which leads to cell death[28]. The *in vitro* inhibitive effect of two monoterpenes against Psa has been reported[10]. Vavala *et al.*[29] revealed that a mixture of EOs can kill Psa after one hour exposure and Minardi *et al.*[30] described the efficacy of the EO from *Monarda didyma* in the control of kiwifruit bacterial canker.

In this study, thirty EOs were tested in an *in vitro* assay to verify their capacity to inhibit the growth of Psa. Among the EOs tested, eight were found to be particularly suitable and were thus further tested using the broth microdilution method and also by calculating the MIC90 using a regression according to Riccioni and Orzali[31]. The EOs from *Syzigium aromaticum*, *Thymus vulgaris*, *Origanum vulgare* and *Cinnamomum zeylanicum* showed the highest antimicrobial activity and thus highlighted their potential for successful use in kiwifruit protection strategies.

MATERIALS AND METHODS

Test organism and duration of the study: The Psa strain CRA-PAV 1625 used in this study was isolated from a Kiwifruit (*Actinidia chinensis*) plant showing typical bacterial canker symptoms and located in an orchard in the province of Latina (Southern Italy). Lyophilised stock culture was conserved in the CREA-DC collection and regenerated on nutrient agar 025%. D-glucose (NAG) for 48 h at 25±2°C. This study was developed in a time-range of two years (2014-2015).

Antibacterial activity of EOs: *In vitro* assay for the screening of EOs: all EOs were obtained from Sigma-Aldrich and are reported with their code number in Table 1. Each product was tested for its effect on bacterial growth and viability. The *in vitro* assay was performed following Loreti *et al.*[32] with modifications. A starter culture of Psa was prepared by selecting a single colony in 4-5 mL of nutrient broth supplemented with 5% sucrose (NSB) and incubated over night at 25±2°C and 170 rpm. The bacterial concentration was adjusted at 10^8 CFU mL^{-1} (colony forming units per mL). The turbidity readings were performed using a spectrophotometer at 660 nm. The cultures were prepared by diluting the starter culture in NSB broth until reaching a concentration of 10^8 CFU mL^{-1}. The EOs were added at different concentrations in a final volume of about 15 mL each. A culture of Psa CRA-PAV 1625 not supplemented with any oil was used as a control. Five replications were performed for each treatment. Negative controls consisted of sterile NSB broth and NSB broth supplemented with each oil. The bacterial cultures were incubated over night at 25°C and 170 rpm, then after 20-24 h of incubation, the optical density was measured at 660 nm. The bacterial growth was stopped at 1 OD turbidity, when the growth curve of Psa are ached the highest point in the exponential phase. The concentration series were as follow: 3, 30, 60, 150, 300, 600 and 1200 ppm. All oils were diluted in a final concentration of 0.5% (v/v) Tween-20 (Sigma-Aldrich) to enhance their solubility. Culturing Psa in 0.5% (v/v) Tween-20 was performed in order to prevent any bacterial growth inhibition by this diluent. The minimum inhibitory concentrations (MICs) were determined as the lowest oil concentrations to completely inhibit the bacterial growth after 20-24 h incubation in the conditions described above. Subcultures obtained from the bacterial inoculum grown under the MIC condition and the EOs at higher concentrations than the MIC (which ranged from 300-1200 ppm) were plated on NAG to check for any bactericidal effects. The complete absence of growth on the agar surface at the lowest EO concentration was considered to be the minimum bactericidal concentration (MBC). The effects of EOs from clove, cinnamon, oregano, thyme, basil, fennel, cumin and garlic were also evaluated with the MIC90 defined as the lowest oil concentration that led to a 90% growth inhibition compared with the oil-free control. This was determined by calculating the percentage inhibition of bacterial growth and using a regression equation in accordance with Riccioni and Orzali[31].

Standardized broth microdilution method: The eight most promising EOs selected by the *in vitro* assay (oregano, garlic, cinnamon, clove bud, cumin, basil, fennel and thyme oils) were tested using the standardized broth microdilution method in accordance with Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI M07-A9. 2012) to confirm their *in vitro* activity. Each oil was tested at the concentration series next to the previously obtained MIC value: 150, 300, 400, 600, 750, 900 and 1200 (ppm) for EOs from clove bud, cumin, cinnamon and garlic; 150, 250, 300, 400, 500, 600 and 1200 (ppm) for EOs from oregano, basil and fennel; 150, 195, 270, 300, 450, 600 and 1200 for EO from thyme. The dilution series were prepared as previously described in a 96-well microdilution plate and inoculated with the bacterial suspension at 10^6 CFU mL^{-1} in NSB. Wells containing sterile NSB alone and supplemented with the oils were used as negative controls. Wells containing Psa-inoculated NSB with and without Tween-20 were used as the positive oil-free controls. The plates were incubated at 25°C and the bacterial growth was measured after 48 h with a microplate photometer (Thermo Scientific™ Multiskan™ FC; λ = 620 nm). The experiment was repeated twice and each time was based on two replicate plates running in parallel with three replications within each plate. Thus each treatment relied on a total of 12 replications. To determine the MBC, 100 µL of bacterial suspension were taken aseptically from wells that did not present visible turbidity and plated on NAG. The MBC was considered as the lowest concentration of EOs that impeded the growth of visible colonies on NAG.

Statistical analyses: One-way fully random analysis of variance (ANOVA) and the Tukey-Kramer test for multiple comparison were carried out to assess differences among experimental groups. With regard to the broth microdilution experiment, data were normalized to non-inoculated Tween-20 supplemented controls.

The results of pairwise comparisons were evaluated within a 99% confidence level with regard to the growth comparisons in NSB and a 95% confidence level for the broth micro-dilution assay. The CoStat-Statistics Software version 6 and 4 was used for the analyses.

RESULTS

Antibacterial activity of EOs

***In vitro* assay for the EO screening:** The MIC and the MBC obtained for each EO in the *in vitro* assay are reported in Table 1. About 17 EOs did not show any antibacterial effect on the Psa at any concentration tested. Six EOs-from garlic, cumin, eucalyptus, fennel, cinnamon and spearmint led to an inhibition in bacterial growth, while dill weed, coriander,

Table 1: Essential oils, their minimum inhibitory concentrations and minimum bactericidal concentrations against *Pseudomonas syringae* pv. *actinidiae* strain CRA-PAV 1625

Plant species	Common name	Code number	MICs (ppm)	MBCs (ppm)
Allium sativum	Garlic	cod. W250309	1200	NB
Anethum graveolens	Dill weed	cod. W238309	1200	1200
Apium graveolens	Celery seed	cod. W227102	NA	NB
Boswellia carterii	Frankincense or olibanum oil	cod. W281611	NA	NB
Cinnamomum camphora	Camphor tree	cod. W223115	NA	NB
Cinnamomum zeylanicum	Cinnamon, ceylon type	cod. W229202	1200	NB
Citrus limon	Lemon	cod. W262528	NA	NB
Coriandrum sativum	Coriander	cod. W233404	1200	1200
Cuminum cyminum	Cumin	cod. W234300	600	NB
Cymbopogon nardus	Citronella	cod. W230812	NA	NB
Elettaria cardamomum	Cardamom	cod. W224111	NA	NB
Eucalyptus polybractea	Eucalyptus	cod. W246603	1200	---
Foeniculum vulgare	Fennel	cod. W248207	600	NB
Lavandula angustifolia	Lavender	cod. 61718	NA	NB
Levisticum officinale	Lovage	cod. W265101	NA	NB
Liquidambar spp.	Styrax	cod. W303704	NA	NB
Melaleuca alternifolia	Tea tree	cod. W390208	1200	1200
Mentha piperita	Peppermint	cod. W284807	NA	NB
Mentha spicata	Spearmint	cod. 60987	1200	NB
Ocimum basilicum	Basil	cod. W211907	1200	1200
Origanum majorana	Marjoram	cod. W523208	NA	NB
Origanum vulgare	Oregano	cod. W282812	300	600
Piper nigrum	Black pepper	cod. W284505	NA	NB
Pogostemon patchouli	Patchouli	cod. W283800	NA	NB
Rosmarinus officinalis	Rosemary	cod. W299200	NA	NB
Salvia officinalis	Sage	cod. W300306	NA	NB
Santalum album	Sandalwood	cod. W300500	NA	NB
Syzygium aromaticum	Clove bud	cod. W232300	600	600
Thymus vulgaris	Thyme	cod. W306509	600	1200
Zingiber officinale	Ginger	cod. W252204	NA	NB

NA: Any antibacterial activity, NB: Any bactericidal activity, MIC: Minimal inhibition concentration, MBC: minimal bactericidal concentration. The code number of the Sigma product of each EOs was reported

Table 2: MIC90 values determined for each oil calculating the percentage inhibition of bacterial growth, this value is reported for all experiments performed: *In vitro* assay by blocking the Psa growth at 1 OD and CLSI standardized microdilution method

MIC90		
Oils	Psa growth at 1 OD*	Microdilution method**
Clove bud	551	875
Cinnamon	855	912
Oregano	287	830
Thyme	450	855
Cumin	520	>1200
Basil	440	>1200
Garlic	600-1200	>1200
Fennel	300-600	>1200

MIC90: The lowest oil concentration that led to a 90% growth inhibition compared with the oil-free control, **In vitro* assay by blocking the Psa growth at 1 OD following Loreti *et al.*[32] with modification. **CLSI standardized microdilution method (CLSI M07-A9. 2012). The concentration is expressed in ppm

tea tree, basil, oregano, clove bud and thyme showed both an antibacterial and a bactericidal effects. The following EOs showed a MIC value of: 300 (oregano), 600 (cumin, fennel, clove bud, thyme) and 1200 ppm (garlic, dill weed, cinnamon, coriander, eucalyptus, tea tree, spearmint, basil). Oregano and clove bud oils had a MBC value of 600 ppm; dill weed, coriander, tea tree, basil and thyme oils had a MBC value of 1200 ppm. The EOs that led to at least a 40% bacterial growth inhibition with respect to the control already at 300 ppm, thus showing a relatively high bacteriostatic activity were considered as the most promising and selected for further analysis. The selected oils were: clove bud, cinnamon, oregano, thyme, cumin, fennel, basil and garlic oil (Fig. 1). Dill weed, coriander, eucalyptus, tea tree and spearmint oils did not meet this requirement (Fig. S1).

In order to acquire more accurate data on the antibacterial effects of the selected oils, MIC90 was calculated using a regression equation (Table 2). A good correlation between the growth inhibition and the oil concentrations with a regression coefficient of >0. 85 was found for the EOs from clove bud (MIC90: 551 ppm), cinnamon (MIC90: 855 ppm), oregano (MIC90: 287 ppm), thyme (MIC90: 450 ppm), cumin (MIC90: 520 ppm) and basil (MIC90: 440 ppm). Garlic and fennel oils did not show such correlation, thus value ranges at 600-1200 and 300-600 ppm, respectively were reported for MIC90.

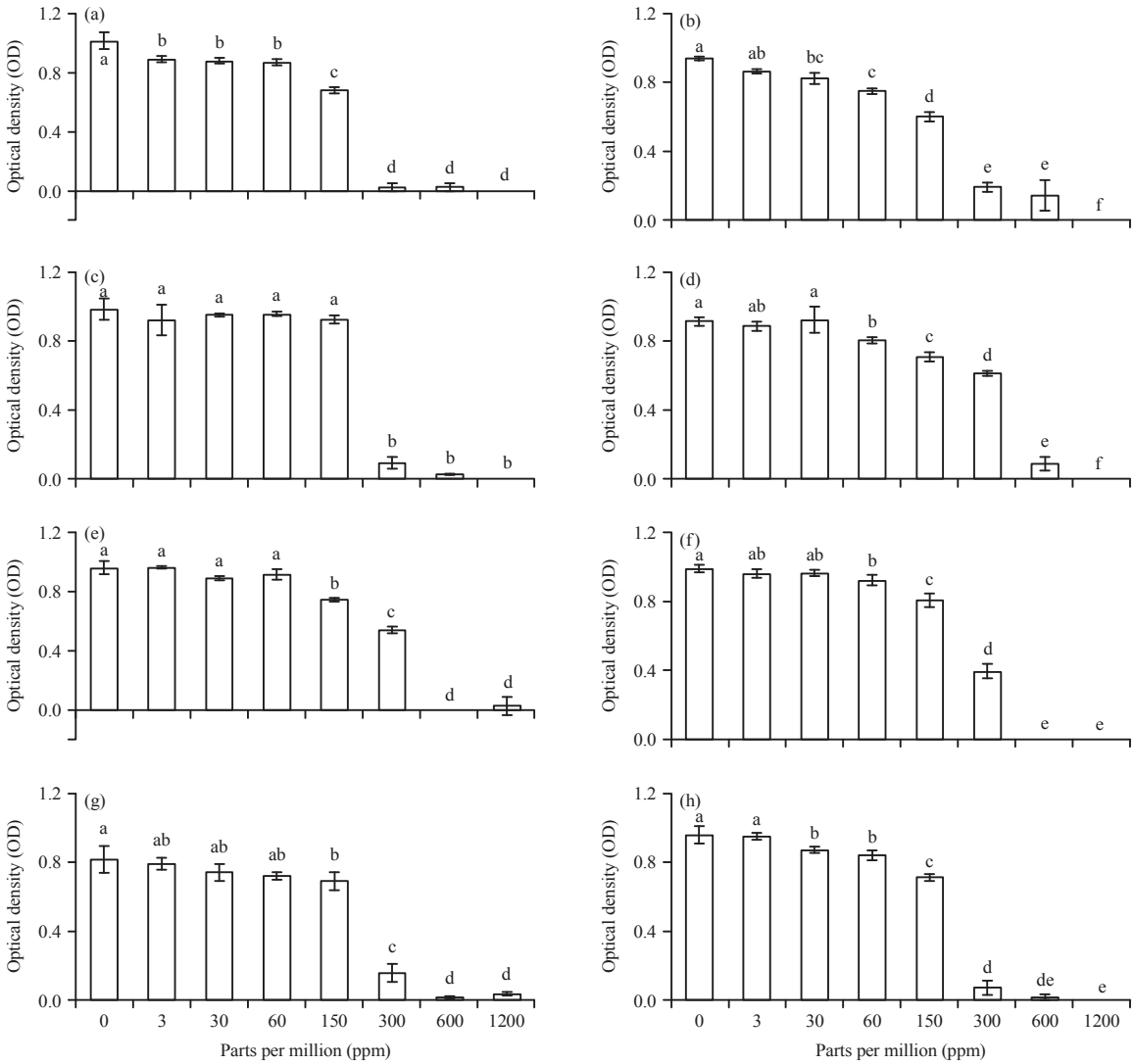

Fig. 1(a-h): Antibacterial activity of (a) Oregano, (b) Garlic, (c) Basil, (d) Cinnamon, (e) Clove bud, (f) Cumin, (g) Fennel and (h) Thyme essential oils against *Pseudomonas syringae* pv. *actinidiae* by *in vitro* assay: Effects of different concentrations of essential oils on bacterial growth measured as optical density (OD) (λ = 660 nm)

Data reported are the means of the experiment replications. The error bar shows the Standard Deviation. Significance differences ($p \leq 0.01$) found using the Tukey-Kramer test for multiple comparisons are indicated as letters a-f: Same letter within the same graph means no statistical significance among the treatments

Standardized broth microdilution method: The broth microdilution method was applied to the EOs from clove bud, cinnamon, oregano, thyme, cumin, garlic, basil and fennel, which were selected following the results of bacterial growth in the first *in vitro* assay. The effects of the different EOs on bacterial growth in the broth microdilution method are shown in Fig. 2. A bacteriostatic action was not confirmed for fennel: in fact the bacterial growth with the oil did not differ significantly from the control at any concentration tested. A significant bacterial growth inhibition was observed for basil only at 500 and 1200 ppm, thus showing the low antibacterial effectiveness of the oil. Cumin oil also showed a poor, though significant inhibition compared to the control. Interestingly the inhibition was not associated with a dose-related response. Conversely, a significant bacteriostatic action was revealed for oregano oil starting from 400 ppm, thyme oil starting from 300 ppm and for clove, cinnamon and garlic oils from 150 ppm. In general EOs from oregano, garlic, cinnamon, clove and thyme were the most effective in terms of inhibitive capacity.

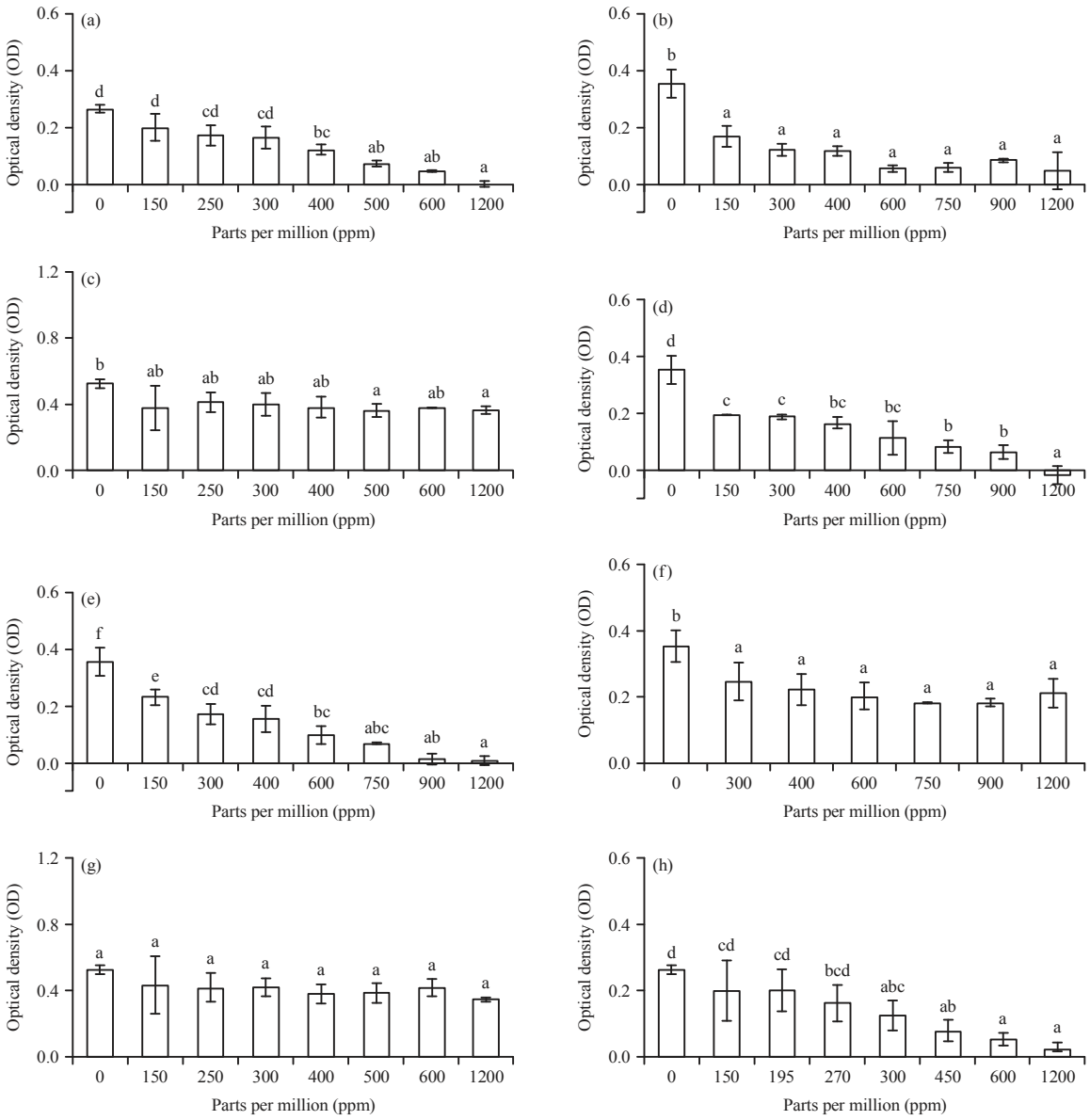

Fig. 2(a-h): Antibacterial activity of (a) Oregano, (b) Garlic, (c) Basil, (d) Cinnamon, (e) Clove bud, (f) Cumin, (g) Fennel and (h) Thyme essential oils against *Pseudomonas syringae* pv. *actinidiae* by standardized CLSI microdilution method: effects of different concentrations on bacterial growth measured as optical density (OD) ($\lambda = 620$ nm)

The concentrations are expressed in ppm. Data reported are the means of the experiment replications. The error bar shows the Standard Deviation. Significance differences ($p \leq 0.01$) found using the Tukey-Kramer test for multiple comparisons are indicated as letters a-f: Same letter within the same graph means no statistical significance among the treatments

Values of MIC90, calculated using a regression equation, are reported in Table 2. A good correlation between the growth inhibition and the oil concentrations with a regression coefficient of >0.85 was shown for clove bud (MIC90: 875 ppm), cinnamon (MIC90: 912 ppm), oregano (MIC90: 830 ppm) and thyme (MIC90: 855ppm) oils. At 1200 ppm garlic oil showed a slightly lower inhibition percentage than 90% (= 86%). For cumin, basil and fennel up to a concentration of 1200 ppm, no MIC90 value was found. Clove bud and cinnamon oils were the only EOs that showed a bactericidal effect on Psa (at 1200 ppm).

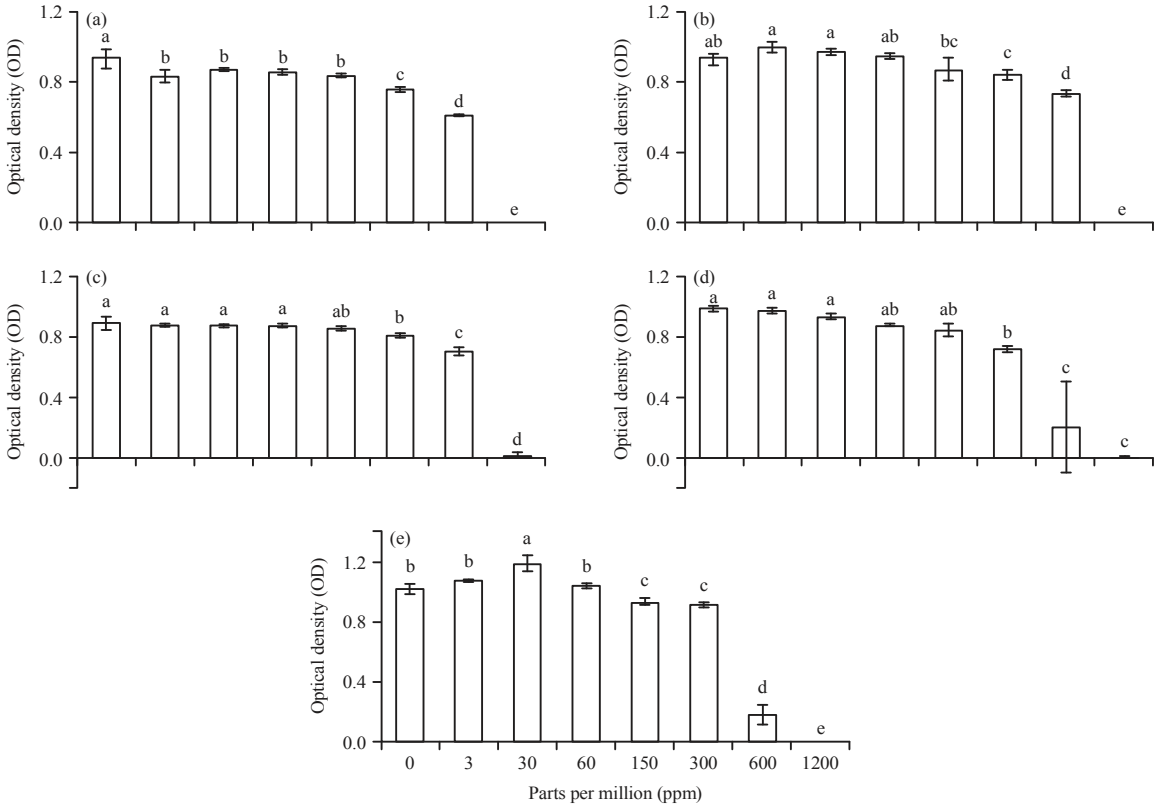

Fig. S1(a-e): Antibacterial activity of (a) Tea tree, (b) Dill weed, (c) Eucalyptus, (d) Spearmint and (e) Coriander oils against *Pseudomonas syringae* pv. *actinidiae* by *in vitro* assay: Effects of different concentrations of essential oils on bacterial growth measured as optical density (OD) ($\lambda = 660$ nm)

The concentrations are expressed in ppm. Data reported are the means of the experiment replications. The error bar shows the Standard Deviation. Significance differences ($p \leq 0.01$) found using the Tukey-Kramer test for multiple comparisons are indicated as letters a-f: Same letter within the same graph means no statistical significance among the treatments

DISCUSSION

In this study was performed for the first time a large screening of EOs to verify their possible antibacterial activity against Psa. In particular, among thirty tested EOs, the following resulted promising: clove bud, thyme, oregano, cinnamon and to a lesser extent, garlic. This micro-organism is the causal agent of the kiwifruit bacterial canker, one of the main pandemic diseases of recent years. Controlling the bacterial canker is difficult due to the limited availability of antimicrobial substances. Antibiotic and copper compounds are still the common chemicals used to contain Psa[33], however, their use is hampered by their potential negative effects on the environment and animal health[34]. Antibiotics are already forbidden in Italy and copper have been included among the compounds that will be replaced when the recently approved legislation becomes enforceable (Commission Implementing Regulation (EU) 2015/408 of 11 March 2015[20]).

The search for new compounds that can promote a bacteriostatic or a bactericidal effect against Psa, thus remains urgent. Natural products obtained from aromatic plants represent a potential source of molecules with biological activities and with a low or negligible environmental impact. EOs are of great interest due to their potential to prevent or control bacterial growth in the field due to their antimicrobial capacities. There are several studies on the inhibition effects of EOs on micro-organisms dangerous for human health, for example those contaminating food and also on plant pathogenic bacteria[35,26]. Due to their anti-microbial activity, EOs are increasingly adopted as pesticides in agriculture[21].

In current study, it was used a multi-phase approach for the final selection of the most interesting EOs tested. This choice derived by the fact that the inhibitory activities may vary using different approaches[22,36]. The following factors may influence the results: microbial growth, exposure of the micro-organism to plant oil, oil solubility, use and quantity of the emulsifier.

As a first approach was apply an *in vitro* assay, based on growing the bacteria until the highest point of its exponential curve, in order to include only the EOs that led to at least a 40% bacterial growth inhibition with respect to the control (already at 300 ppm), thus showing a relatively high bacteriostatic activity. The selected EOs, clove bud, cinnamon, oregano, thyme, garlic, cumin, basil and fennel, were then tested with the standardized broth microdilution method. Combining the results of the two different approaches and taking into account both MIC90 and MBC values enabled us to identify the most effective EOs against Psa: Clove bud, oregano, thyme, cinnamon and to a lesser extent, garlic. This efficacy was revealed by their high bacteriostatic efficacy and their MIC90 expressed at low concentrations, whereas, they did not show evident bactericidal activity (because this was not confirmed by the standardized microdilution method).

The antibacterial activities of clove oil against seven different genera of plant pathogenic bacteria were investigated by Huang and Lakshman[37]. Among the tested bacteria, the most sensitive to clove oil was *Ralstonia solanacearum* and its use as an alternative control measure to control tomato and geranium bacterial wilt has been proposed by the authors. The efficacy of this EO has been known for many years. In Maruzzella *et al*.[23] tested *in vitro* activities of 123 essential oils against four phytopathogenic bacteria: *Erwinia carotovora*, *Corynebacterium michiganense*, *Pseudomonas striafaciens* and *P. glycines* and found clove EO to be highly inhibitory against all tested bacteria[21]. Clove oil also inhibited the growth of *Xanthomonas vesicatoria* and led to a loss of integrity of their cell wall *in vitro*[38].

The antimicrobial activity of EO from *Cinnamomum zeylanicum* against a wide variety of bacteria and its potential even at low concentrations is also well known[39]. The antimicrobial action of this EO is considered to arise mainly from its hydrophobicity which can disrupt the bacterial cell membrane leading to ion leakage[40]. In line with this, Joshi *et al*.[41] proved that eugenol and carvacrol inhibit quorum sensing in pectobacteria and reduce their virulence.

Similarly, thyme and oregano have also been shown to be inhibitive against phytopathogenic bacteria, more effectively than antibiotics[42,34]. There is a lot of information on the chemical composition, antimicrobial and antioxidant activities of EOs obtained from various *Origanum* species and their commercial applications[43]. The antibacterial activity of oregano oil is attributed to major terpene components, such as thymol and carvacrol, which are known to inhibit pathogenic bacteria[44].

The EO composition can be affected by the experimental conditions and the chemotype[44]. For example there are several species and varieties of thyme with different chemotypes, i.e., geraniol, thymol, carvacrol, linalool[45].

The antimicrobial effect of the terpenes geraniol and citronellol on Psa was studied by Ferrante and Scortichini[10], who had already showed their inhibition action on the *in vitro* growth of *Erwinia amylovora*[24].

Cumin has been shown to be highly effective against several genera of phytopathogenic bacteria, but to a lesser extent against the genus *Pseudomonas*[26]. In current study the anti-microbial effect of cumin was not confirmed by the two different approaches and thus was not considered as one of the most promising EOs.

The antibacterial activity of garlic is also well known[46]. The antimicrobial activities of garlic extracts and other plant alliums are primarily based on allicin, which has been found to have inhibitory and bactericidal activities against the *Burkholderia cepacia* Complex [47].

The efficacy in controlling Psa through the use of the EO of *Monarda didyma* has also been reported[30] and Vavala *et al*.[29] showed the ability of a mixture of EOs from round leaved mint (*Mentha suaveolens*), rosemary and tea tree oils to kill Psa after 1 h of exposure. They observed that the EOs in the mixture were able to kill Psa at a concentration sixteen times lower than the corresponding MIC value of each EO used alone. In our study rosemary did not show a bactericidal effect against Psa, probably because was used lower concentrations than those used by Vavala *et al*.[29].

It was found that at higher concentrations, the majority of the texted EOs showed an inhibitory effect against Psa, however, they were also phytotoxic towards kiwifruit causing local desiccation on the treated leaves. The phytotoxic potential of essential oils have also been studied and strategically used in weed management for the inhibition and delaying of seed germination and seedling growth of many weeds[48]. However, this potential could be dangerous if the phytotoxic effect is expressed in crop plants, thus compromising the crop production. For this reason the antibacterial effects at relatively low concentrations of the EOs selected in this study are thus of particular interest. Lucas *et al*.[38] reported that lower EO concentrations than used for inhibition of bacterial growth *in vitro* are still able to reduce the severity of bacterial spot caused by *Xanthomonas vesicatoria*, indicating the existence of additional factors conditioning the action of EOs *in vivo*.

Further in depth studies are being planned to evaluate their potential toxicity towards Kiwifruit plants and their efficacy in reducing disease symptoms, in order to suggest effective formulations for use in crop protection. The main aim of this study was to provide a large screening of EOs against Psa to create directly comparable, quantitative, antimicrobial data. Clove bud, thyme, oregano, cinnamon and garlic oils were revealed to be the best performing EOs against Psa. Thus their use is suggested in an integrated control of Psa in which their combination with other molecules can led to new pesticides in order to contribute to the management of a diseased orchard. This aspect is particularly urgent in consideration of the recent legislation that specifies that copper, widely used for Psa control should be replaced (Commission Implementing Regulation (EU) 2015/408 of 11 March, 2015[20]).

CONCLUSION

The multiphasic approach of the *in vitro* screening of 30 EOs showed that clove bud (*Syzigium aromaticum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), cinnamon (*Cinnamomum zeylanicum*) and to a lesser extent, garlic (*Allium sativum)* shows an antibacterial activity against Psa.

SIGNIFICANCE STATEMENTS

This study highlights the possibility to use the most promising EOs discovered in this study (i.e., clove bud, thyme, oregano, cinnamon and to a lesser extent garlic) as antibacterial agent against Psa. These evidences highlight the possibility to develop appropriate formulation based on these EOs to support the development of eco-friendly disease management of bacterial canker affected Kiwifruit orchard.

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