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Evaluation of the Potential of Some Essential Oils in Biological Control against Phytopathogenic Agent *Pseudomonas syringae* pv. Tomato DC3000 Responsible for the Tomatoes Speck

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Abstract

To investigate biological control mains against phytopathogenic agent *Pseudomonas syringae pv.* tomato *DC3000*, responsible for bacterial speck, tests on the antibacterial activity of six essential oils were carried out. The essential oils, obtained by hydro distillation, was analyzed by gas chromatography (GC). The determination of the antibacterial activity of the essential oils carried out *in-vitro* using the well diffusion, micro atmosphere methods and the determination of MIC and CMB. Only essential oils of *Cedrus atlantica* had a negative activity against the bacterial strain. However, the overall results of this study suggested that *Mintha pulegium, Thym vulgaris, Eucalyptus globulus* essential oils had potential as a bio-pesticide for the control of bacterial speck disease of tomato.

Keywords: Biological control; *Pseudomonas syringae* pv. tomato *DC3000*; Bacterial speck; Essential oils; Antibacterial activity

Introduction

Bacterial speckle is a persistent and very common disease of field tomatoes, that induces a major phytosanitary problem, constituting a very destructive threat throughout the world [1]. It is manifested by the appearance on the foliage, the stems, or the fruit of small, greasy dark spots, rapidly becoming black and surround by wide and marked yellow aureole [2]. This pathology is caused by the gram-negative bacterium *P. syringae pv.* Tomato *DC3000*. Member of the gamma subgroup of the *Proteobacteria,* this bacterium can be present in the soil, in the plant rhizosphere, in the epiphyte and can be stored for 20 years in the seed without losing its pathogenicity [3]. Because of the richness of its metabolic pathway, it is often able to withstand many antiseptics or antibiotics. Indeed, a resistance to rifampicin has been established [4]. The pathogenicity of this strain has been a subject of several scientific studies and has become an important model of the organism in molecular pathology of plants, due to its genetic traceability and its genome that is fully sequenced. However, most of the studies carried out on *P. syringae pv.* tomato *DC 3000* have focused its molecular aspects, while the cellular and physiological aspects have not been yet developed [5].

Prophylactic measures were put in place to limit the progression of this disease through the management of hosts, soil or agricultural equipment, as well as antibiotics and copper salts. However, some emerging strains show strong resistance to all these products [6]. Thus, the populations are currently turning to medicinal plants as a natural alternative to synthetic antibiotics. They are known for their ability to produce a variety of compounds, especially essential oils, which can be used as a protection against pathogens and can therefore serve as antimicrobial substances [7]. Essential oils (EOs) are synthesized through secondary metabolic pathways of plants as communication and defense molecules. Generally, EOs play important roles in direct and indirect plant defenses against herbivores and pathogens [8].

Many studies are published every year indicating great prospects for EOs as active ingredients in the production of botanical pesticides. Nevertheless, only a very few commercial products based on EOs have been marketed and the number of newly introduced products remains minimal [9]. Here, the antibacterial activity of six essential oils was study against the phytopathogen agent *P. syringae* pv. tomato *DC3000* in order to develop a natural bactericide, in an attempt to prevent and/or eradicate the bacterial speck of the tomato.

Material and Method

Plant material

Six Eos were studied, they were obtained directly from leaves or aerial part of different vegetal species, as shown in Table 1. Plant material was harvested randomly, then washed and dried in a well-ventilated place at room temperature for ten days before their use. The samples were then isolated from each other's specimens and conserved for extraction.

Essential oil extraction

The six essential oils were obtained by 3.5 h hydrodistillation using the standard Clevenger apparatus. The oils were extracted from the distillate with hexane and dehydrated by passing through anhydrous sodium sulfate. After filtration, the solvent was removed by distillation under reduced pressure in a rotary evaporator at 35°C, and the pure oils stored in an amber vial kept under refrigeration (4°C), until their use.

Bacterial strains

The strain *P. syringae* pv. tomato *DC 3000* has been isolated, purified and identified in the laboratory of physiopathology, molecular

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Plant family	Scientific name	Local name	Collection site	Plant part
Lamiaceae	*Mentha pulegium*	fliyou	Fes	Leaves
	Rosmarinus officinalis	azir	Rabat	Aerial part
Lauraceae	*Thymus vulgaris*	Zaatar	Al-Hoceima	Aerial part
Myrtaceae	*Eucalyptus globulus*	kalitous	Rabat	Leaves
Pinaceae	*Cedrus atlantica*	larz	Rabat	Leaves
Rutaceae	*Citrus sinensis*	laymoun	Sidi Slimane	Bark

Table 1: Plant species used in the experiment for EOs extraction.

genetics and biotechnology (PGMB) [5]. This strain is characterized by the production of an endotoxin (pyoverdine) [10] and by its multiple resistance to antibiotics (rifampicin) [4]. The *P. syringae* pv. tomato cultures were grown on King's B and LB with rifampicin (50 g/ml) agar plates for strain verification.

Antibacterial activity

Agar diffusion: Agar well diffusion technique was carried out according to the method described by Mathabe et al. with some modifications [11]. Test agar plates were seeded with an overnight culture (equivalent to 10^7 to 10^8 CFU/mL). The seeded plates were left to dry for 3-5 min and a standard Pasteur pipette (5 mm) was used to cut uniform wells on the surface of the agar. The dilutions of EOs were prepared in agar 0.2% in a concentration range of (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512). The wells were then filled with 50, l of each concentrations of the respective essential oils. All the plates were incubated at 28°C for 24 h. Inhibition zone diameters (IZD) were measured after the incubation with the aid of a rule. The experiment was carried out in triplicate for each essential oil. Five EOs, which presented the highest IZDs, were selected for further investigation.

Micro atmosphere: Some of the volatile compounds (terpenes, sesquiterpenes) have poor water solubility and cannot be evaluated by agar diffusion method [12]. The volatile phase of essential oils exerts its inhibitory effect on the tested microorganisms [13]. To evaluate the antimicrobial activity of these volatile compounds against target bacteria, micro-atmosphere diffusion assay was performed using inverse Petri dish method [14]. Culture of *P. syringae* pv. tomato (10^8 CFU/mL) were inoculated on LB agar plates (20 ml). The EOs (10 l) were placed on a Whatman disc of 0.5 cm diameter in the middle of the cover. The Petri dishes were inverted and sealed with Petri film to prevent vapor transfer between samples as well the loss of volatile components of EOs. Samples were incubated for 24 h at 28°C.

Minimal inhibitory and minimal bactericidal concentration: Based on the previous screening five essential oils (*Citrus, Menthe, Romarin, Thym and Eucalyptus oils*) were identified to have potent antibacterial activity and their Minimum Inhibitory Concentrations (MIC) were determined. Different dilutions of each oil were prepared ranging from 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 to 1:512. All tubes were inoculated with an overnight broth culture of *P. syringae pv* tomato (10^8 CFU/mL) and incubated at 28°C for 24 h. The minimum concentration that completely inhibited macroscopic growth compared with the blank control was considered as the minimum inhibitory concentration (MIC) of the respective EOs. The minimal bactericidal concentration (MBC) was determined after determining the results for the MIC, by further sub culturing the last tube that showed visible growth and all the tubes in which there was no growth in solid LB medium. The plates were incubated at 28°C for 24 h. The MBC correspond to the lowest concentration that achieved a 99.9% decrease in viable bacteria [15].

Chemical composition of essential oils

The most potent oils, (*Citrus, Menthe, Romarin, Thym* and *Eucalyptus oils*) were analysed using a chromatographer in gas phase equipped with flame ionization detector (GC-FID, Trace GC ULTRA S/N 20062969, Thermo Fischer), equipped with a varian capillary chromatographic column (50 m length, 0.32 mm of diameter and film thickness 1.25 μm) of diphenyl dimethyl polysiloxane (5% diphenyl 95% dimethylsiloxane polysiloxane). The column temperature was programmed from 40 to 280°C for 5°C/min and finally held at that temperature for 10 min. The injector and the flam ionization detector (FID) temperature were 250°C and 260°C respectively. A volume of 1 μl of diluted oils in hexane solution (10%) were injected in the "split" mode at a ratio of 1:40. The debit of gas vector (azoth) was fixed to 1 ml/min. The percentage of each constituent in the oil was determined by area peaks.

Analysis of the results

According to the width of the inhibition zone diameter expressed in mm, results were appreciated following the criteria of Fertout-Mouri et al. [16]. Thus, the IZDs were sorted out as follows: for diameters equal to or below 8.0 mm, the bacteria were classified as insensitive (-) to the action of EOs; for diameters between 8.0 to 14.0 mm, as moderately sensitive (+); for diameters between 14.0 to 20.0 mm, as sensitive (++); and for diameters longer than 20.0 mm, as extremely sensitive bacteria. Also, data analyses were performed using Principal Component Analysis (PCA) [17].

PCA provides the data for diagrams in which both objects (EOs) and variables (EOs classes components and antibacterial inhibition) obtained from the experimental screening. This method aims at reducing the multivariate space which objects (EOs) are distributed but are complementary in their ability to present results [18]. PCA was carried out using function

'PCA' from the statistical PASS software. The variables have been selected using function from the statistical software.

Results

Antimicrobial effects of EOs against *P. syringae* pv. tomato DC 3000 in agar diffusion assay

The antibacterial effects of six EOs against *P. syringae* pv. tomato DC 3000 using the agar diffusion method are summarized in Table 2. The results revealed that all of the EOs were effective against the bacterial strain with varying magnitudes, except the *C. atlantica*, which showed no antibacterial activity. Generally, the strain was very sensitive to many of the tested pure EOs. *Eucalyptus* oil and thym oil showed maximum activity against this bacterial specie tested. These oils retain their antimicrobial capacity despite being highly diluted (IZD=9 mm at dilution 1/64) and (IZD=10 mm at dilution 1/64) respectively. *Mentha* oil has the greatest antibacterial effect at the highest concentration (IZD =35 mm at dilution 1/2, IZD=35 mm at dilution ¼); however, this effect decreased until it no longer exists as it is diluted. Moderate effects were seen in *Citrus* oil and *Rosmarinus* oil. There was no inhibition of growth with the vehicle control (0.2% agar).

Essential oil	Diameter of inhibition zone (mm)							
	Pure	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Eucalyptus globulus	30 (+++)	25 (+++)	21 (+++)	16 (++)	13 (+)	12 (+)	9 (+)	6 (-)
Mentha pulegium	45 (+++)	35 (+++)	30 (+++)	9 (+)	6 (-)	00 (-)	00 (-)	00 (-)
Citrus sinensis	31 (+++)	22 (+++)	19 (++)	14 (++)	12 (+)	6 (-)	00 (-)	00 (-)
Thym vulgaris	33 (+++)	26 (+++)	25 (+++)	20 (++)	16 (++)	11 (+)	10 (+)	9 (-)
Rosmarinus officinalis	21 (+++)	19 (++)	14 (++)	11 (+)	9 (+)	8 (-)	6 (-)	5 (-)
Cedrus atlantica	00 (-)	00 (-)	00 (-)	00 (-)	00 (-)	00 (-)	00 (-)	00 (-)

The inhibitory activity is represented by the mean diameter of the growth inhibition zone (including the well, 0.5 cm). Essential oils were classified as (+++) extremely sensitive, (++) sensitive, (+) moderately sensitive, (-) not sensitive. No inhibitory effect was found with the 0.2% agar solution used as a control.

Table 2: Determination of the mean inhibition zone diameter (mm) of the six tested EOs against *Pseudomonas syringae* pv. *tomato* DC 3000.

Essential oils						
Diameter of inhibition zone (mm)						
Test organism						
Pseudomonas syringae	*Eucalyptus globulus*	*Mentha pulegium*	*Citrus sinensis*	*Thym vulgaris*	*Rosmarinus officinalis*	*Cedrus atlantica*
pv tomato	40 (+++)	36 (+++)	38 (+++)	28 (+++)	22 (++)	5 (-)

The inhibitory activity is represented by the mean diameter of the growth inhibition zone (including the well, 0.5 cm). Essential Oils were classified as (+++) extremely sensitive, (++) sensitive, (-) not sensitive.

Table 3: Antimicrobial activity of six essential oils against using micro atmosphere method *Pseudomonas syringae* pv. *Tomato DC 3000.*

Essential oils	CMI µl/ml	CMB µl/ml	CMB/CMI ratio	Effect
Thym vulgaris	125	250	2	Bactericide
Mentha pulegium	500	500	1	Bactericide
Eucalyptus globulus	3125	62,5	2	Bactericide
Citrus sinensis	62,5	250	4	Bacteriostatic
Rosmarinus officinalis	31,25	250	8	Bacteriostatic

Table 4: The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the essential oils selected against *Pseudomonas syringae pv tomato DC3000.*

Antimicrobial effects of EOs against *Pseudomonas syringae pv* tomato DC 3000 in micro atmosphere assay

The results of antibacterial activity of six EOs against *P. syringae* pv. tomato DC 3000 using the micro atmosphere assay are presented in Table 3. *Eucalyptus* and *Citrus* EOs caused the highest inhibition diameter of approximately 40 mm against *P. syringae*. Mentha pulegium was very active against *P. syringae* causing 36 mm of inhibition (Table 3). *Rosmarinus officinalis* and *T. vulgaris* EOs inhibited the growth diameter of *P. syringae* by 28 and 22 mm, respectively, while *C. atlantica* caused no inhibition.

Minimal inhibitory and minimal bactericidal concentration of the EOs

The minimum inhibitory (MIC) and bactericidal (MBC) concentrations, two parameters that respectively quantify the bacteriostatic and bactericidal potential of bioactive compounds, were determined for the five EOs with substantial antibacterial activity against *P. syringae* p. tomato *DC3000.* As is shown in Table 4, the obtained values were in the ranges of 31.25 to 500 l/ml for MIC and 62.5 to 500 l/ml for MBC. This study revealed that *Mentha* oil showed an absolute bactericidal effect with MIC/MBC ratio of one against the tested organism, followed by *Eucalyptus* and *Thym* oil with a bactericidal effect (MIC/MBC ratio=2). Whereas remaining oils showed a MIC/MBC ratio greater than two, which mean they exhibit a bacteriostatic effect. Overall, these results are in agreement with the inhibitory activities shown above.

Chemical composition of essential oils

GC analysis detected the major components in the selected EOs. Different compounds such as aldehydes (e.g., citral), phenol compounds (e.g., eugenol, carvacrol), alcohols (e.g., terpineol, linalool), and monoterpenes were detected. All components are summarized in Table 5. Given the high number of analyzed compounds (98), and the individuals analyzed (five essential oils), a data technique was used to summarize them. To facilitate synthesis, the compounds were transformed into mathematical variables. This transformation of qualitative into quantitative allowed the approach to summarizing technique namely the analysis in Principal Components (ACP) (Figure 1). The high number of data (490=98 compounds × 5 individuals) which may have redundancy (some variables are correlated with other variables) ordered this choice. Because of this redundancy, it is possible to reduce the observed variables to a reduced number of synthetic variables. Thus, the analysis in Principal Components is a method of reducing variables that can be used to achieve the goal of summarizing a high number of compound variables. The five natural components (EOs selected) were summarized in two artificial components. These two first artificial axes explain 92% of the variance of the data. The majority of compounds hold a paltry contribution in the formation of the two axes. However, the 1-8 cineole and the pinene contribute positively in axis 1. Limonene, carvacrol and caryophyllene, contribute negatively in axis 1. Therefore, axis 1 is the main component that summarizes the major components of essential oils. Axis 2 represents the MICs (Figure 1). The matrix effect allows the following conclusion: Essential oils act by their chemical nature and their concentrations majority components.

Discussion

EOs play important roles in direct and indirect plant defenses against herbivores and pathogens. Indeed, their active substances, show good biological activity and provide insecticidal, nematicidal, ovicidal, fungicidal, and bactericidal effects against pathogens and pests that are important factors in agricultural yield [19]. Every year, many studies

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Compounds	*E. globulus*	*M. pulegium*	*C. sinensis*	*R. officinalis*	*T. vulgaris*
Tricyclene	0	0	0	0,34	0
3-Methylbutanal	0,11	0	0	0	1,1
α-Pinene	17,35	4,3	0,53	24,5	0
α-Thujene	0	0	0	0	0,61
α-Fenchene	0,02	0	0	0	0
Camphene	0,06	0,8	0	7,8	0,79
β-Pinene	0,53	4,46	0,03	2,96	0,22
Sabinene	0	1,61	0,47	0,01	0
Verbenene	0,3	0	0	0	0
3-Carene	0	0	0,07	0,19	0,09
Myrcene	0,33	2,1	1,84	1,71	1,4
α-Phellandrene	0,24	0	0	0,01	0,09
α-Terpinene	0,04	0	0	0,01	1,25
Limonene	4,65	5,75	94,39	4,7	0,56
β-Phellandrene	0,13	0	0,24	0	0,2
1,8-Cineole	61,76	1,11	0	22,6	0,32
(z)-β-Ocimene	0,06	0,04	0	0	0
γ-Terpinene	0,59	0,04	0	0,01	3,64
(E)-β-Ocimene	0,07	0,07	0,03	0	0
3-Octanone	0	0	0	0,67	0
p-Cymene	2,47	0,07	0,01	2,62	22,3
Terpinolene	0,09	0,07	0,01	0,01	0,13
(E)-Sabinene	0	0	0	0	0,59
Octanal	0	0	0,3	0	0
Nonanal	0	0	0,05	0	0
cis-Limonen-1,2-epoxide	0	0	0,07	0	0
trans-Limonen-1,2-epoxide	0	0	0,04	0	0
Citronellal	0	0	0,04	0	0
α-Copaene	0	0	0,02	0	0
Decanal	0	0	0,29	0	0
β-Cubene	0	0	0,02	0	0
Isoamyl isobutyrate	0,07	0	0	0	0
p-Cymenene	0,07	0	0	0	0
δ-Elemene	0,05	0	0	0	0
α-Copaene	0,03	0	0	0	0
Camphor	0,27	0	0	22,8	0,32
α-Gurjuine	0,26	0	0	0	0
Octanol-3	0	2,42	0	0	0
menthone	0	16,31	0	0	0
Menthofuran	0	0,64	0	0	0
Isomenthone	0	12,1	0	0	0
β-Bourbonene	0	0,2	0	0	0
Linalool	0,07	0,16	0,4	0,47	5,48
Octanol	0	0	0,1	0	0
Menthyl acetate	0	1,8	0	0	0
Isopulegol	0	1,42	0	0	0
Neomenthol	0	4,08	0	0	0
Pinocarvone	0,18	0	0	0	0
β-Guiene	0,14	0	0	0	0
β-Caryophyllene	0,07	0,59	0,02	0	1,07
Linalyl acetate	0	0	0	0,02	0
Bornyl acetate	0	0	0	1,34	0,1
Isobornyl acetate	0	0	0	0,04	0
Methylcarvacrol	0	0	0	0	0,54
Terpinen-4-ol	0,48	0,59	0,02	1,01	1,1
Neral	0	0	0,06	0	0
Iso-isopulegol	0	1,17	0	0	0
Neo-isomenthol	0	0,8	0	0	0
Menthol	0	33,66	0	0	0
Isomenthol	0	0,41	0	0	0

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Aromaderdrene	2,63	0	0	0	0,2
Alloaromadendrene	0,51	0	0	0	0
trans-Pinacarvol	1	0	0	0	0
α-Terpinyl acetate	1,36	0	0	0	0
α-Terpineol	1,04	0,32	0,15	0	0
Dodecanal	0	0	0,05	0	0
Germcrene D	0	0,46	0	0	0
Isoborneol	0	0	0	0,06	0
α-Humulene	0	0	0	0,02	0
α-terpineol	0	0	0	1,93	0,27
Borneol	0,09	0	0	1,77	1,72
δ-Cadinene	0	0	0	0	0,16
Geranyl acetate	0,19	0	0	0	0
trans-p-Menth-1 (7),8-dien-2-ol	0,17	0	0	0	0
trans-Carveol	0,05	0	0	0	0
Valencene	0	0	0,06	0	0
Geranial	0	0	0,09	0	0
Geraniol/*p*-cymen-8-ol	0,18	0	0,02	0	0,29
cis-p-Menth-1 (7),8-dien-2-ol	0,1	0	0	0	0
$C_{15}H_{26}O$	0,12	0	0	0	0
Globulol	0,36	0	0	0	0
Epi-Globulol	0,09	0	0	0	0
β-Eudesmol	0,05	0	0	0	0
Piperitone	0	0,58	0	0	0
Carvone	0	0,67	0,08	0	0
Geranyl acetate	0	0	0,01	0	0
δ-Cardinene	0	0	0,01	0	0
Citronellol	0	0	0,02	0	0
Nerol	0	0	0,02	0	0
Geraniol	0	0	0,02	0	0
β-Sinensal	0	0	0,04	0	0
α-Sinensal	0	0	0,02	0	0
Nootkatone	0	0	0,02	0	0
Verbenone	0	0	0	0	0
Caryophyllene epoxide	0	0	0	0	0,16
Thymol	0	0	0	0	50
Carvacrol	0	0	0	0	2,64

Table 5: Chemical composition of five selected essential oils.

are published highlighting great prospects for EOs as active production of botanical pesticides and bactericides [20].

Nevertheless, to the best of our knowledge, no studies have been carried out on the effect of essential oils against the phytopathogenic agent *P. syringae* pv. tomato DC 3000 and thus identify the possibility of developing a bio-bactericide. The essential oils selected in this study have antimicrobial, antiseptic, and disinfectant actions, given by their contents in terpenes, aromatic aldehydes, terpenic aldehydes and phenolic compounds, among other components (Table 5) which are evaluated by different authors previously [21,22].

This work showed that the essential oils inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium, or weak [23]**.** In our study, *Mentha puligium*, *T. vulgaris* and *E. globulus* oils exhibited strong activity against the selected bacterial strain. Several studies [24-26] have shown that mentha, thym and *eucalypus* had strong and consistent inhibitory effects against various pathogens. The antibacterial activity has been attributed to the presence of some active constituents in the oils. Regarding the chemical composition of *M. pulegium* essential oil, 18 volatile compounds accounting for 98.77% were detected (Table 5), the majority compounds are pulegone (64.5%) and menthol (33.66%). Nonetheless, there is a great variability in the chemical composition of *M. pulegium* essential oil among the studies performed so far [27]. Such variability may be related with different plant's vegetative phases and with environmental conditions (seasonal and geographical variations, soil composition) [28]. This EO is considered of high importance in medicine and it has been traditionally used in medicine for treatment of disorders, colds, gastronomy (culinary herb), aromatherapy and cosmetics [29]. It is also known for its antispasmodic, carminative, antiseptic, anti-inflammatory [30], antioxidant [31], and antimicrobial properties [32]. However, volatiles of *T. vulgaris* essential oil revealed 20 different compounds accounting for 96.17% of its composition whose majority compounds are phenols (49.3%), thymol (49.3%) and carvacrol (47.1%), responsible for the antimicrobial effect Thyme essential oil [33]. A study carried out on three species of Moroccan thyme revealed that the three species of thyme have thymol among the majority constituents whose rate varies from 37.5% to 55.9% depending on the species. High antibacterial activity of these essential oils to *Erwinia chrysanthemi* and *Bacillus subtilis* was detected. This activity was related to the presence of phenols in the chemical composition [34]. Fifty-four compounds were identified in the leaf essential oil of *E. globulus*, the major constituents were: 1.8 cineole (22.35%), other

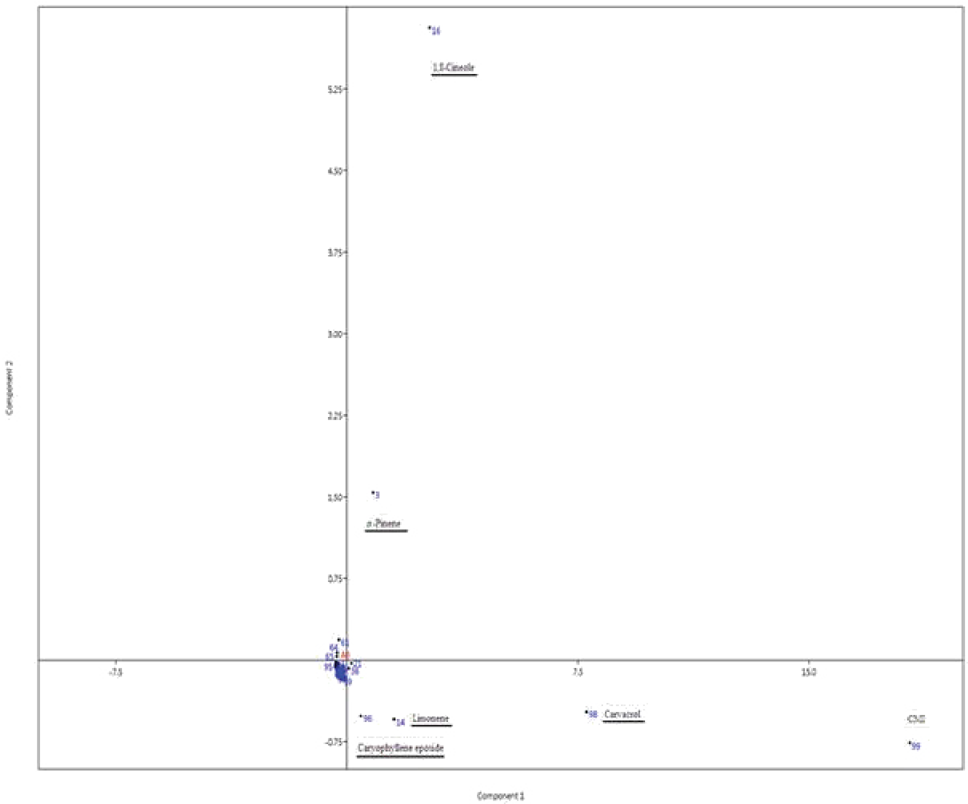

Figure 1: Principal Component Analysis (PCA) of five essential oils based on their antibacterial activity against Principal Components Analysis shows the five EOs (natural components) summarized in two artificial components. Axis 1 represents the major components of essential oils. Axis 2 represents the MICs. 1-8 cineole and the pinene contribute positively in axis 1. On the other side, Limonene, carvacrol and caryophyllene, contribute negatively in axis 1. The majority of compounds hold a derisive contribution in the formation of the two axes.

components present in appreciable contents were limonene, (7.01%), solanol (6.05%), β-pinene (5.20%), trans-verbenol (4.02%), terpinen-4-ol (3.10%), aristolene (2.35%), terpinyl acetate (2.10%), isosativene (1.85%), sabinène (1.49%), α-myrcène (1.15%) and α-terpinéol (1.10%). The antimicrobial activity of *Eucalyptus* is attributed to eucalyptol, or 1.8-cineole which is a monoterpene belonging to the class of ethers [35]. It has antibacterial and antioxidant properties [36]. Several studies have already demonstrated the antimicrobial activity of *Eucalyptus* compounds in different domains of life sciences [37,38]. Indeed, *E. globulus* is an antiseptic of the respiratory tract, expectorant, analgesic, decongestant, hypoglycemic, a detoxifying action of diphtheria toxins and Tetanus, anti-inflammatory, improves respiratory function tests, mucolytic, bronchial antispasmodic, febrifuge, very pronounced bronchopulmonary tropism, drying in high proportion [26,39]. Also, the 1.8-cineola contained in Eucalyptus has been shown to be effective in reducing the dose of corticosteroids used by asthma sufferers [40] and to combat the common cold [41,42].

Even though earlier studies have reported better antimicrobial activity for Citrus oil [43,44] our study showed least inhibitory activity of citrus in addition to rosmarinus and cedrus oils. The EOs of rosemary and citrus have medium antibacterial activity (Table 2) against *Pseudomonas syrinage* pv. tomato *DC 3000* compared to mentha, thyme and eucalyptus. Camphor (22.8%), and 1.8 cineole (22.7%) are main constituents of rosmary oil (Table 5). The use of this EO since ancient times in traditional medicine is justified by its anti-inflammatory [45], anti-rheumatic [46], antiseptic [47], antimicrobial [48], and antioxidant properties [49].

The main compound of citrus is limonene 94.39% (Table 5), and this compound is characterized by amphiphilic properties, which allows the interaction with the cytoplasmic membrane, membrane fluids, proteins, lipids, and other molecules vital to microbial cells [50]. However, citrus oil demonstrated a more significant antibacterial activity in micro atmosphere assay (Table 3). It has been established that the effectiveness of EOs in vapor phase could be completely different from direct contact in solid and liquid phase. The hydrophilic components of EOs are more critical in direct contact than volatile substances, whereas in vapor phase the volatile components could be both hydrophilic and hydrophobic [51]. This may occur due to high number of monoterpenes in vapor of EOs, they can attack the bacteria easily compare to liquid phase [52]. Thus, citrus as well as menthe and eucalyptus oils can be used as air decontaminants in fields and they can be good candidates to be used in agriculture as bio bactericide. Also, due to their volatility, they will not change organoleptic properties of tomatoes.

Cedrus oil had no antimicrobial activity on *Pseudomonas syrinage pv* tomato *DC* 3000 (Table 2), or the property was very low (Table 3). This behavior may be due to a problem of oil solubility because it is known that the different susceptibility of the bacteria to the substances may be due to variations in the cell wall structure, lipid, and protein composition of the cytoplasmic membrane as well as in specific physiological processes [53]. The GC-MS analysis of essential oil extracted from *C. atlantica* revealed the presence of twenty-six compounds, representing 88.59% of the total composition. The main compounds identified are as follows:

α-(E)-atlantone (19.3%), β-himachalene (15.1%), 8-Cedren-13-ol, (13.1%), α-himachalene (5.1%), cedroxyde (4.6%) and deodarone (4.6%) [54]. Unlike our results, *C. atlantica* showed very effective bactericidal activity with the strongest inhibition zone: 12 at 25 mm and 6 at 22 mm for Gram-negative (*Escherichia coli, Pseudomonas aeroginosa and Klebsiella pneumonia*) and Gram-positive (*Staphylococcus aureus, Enterococcus faecalis, Bacillus sphericus* and *Staphylococcus intermedius*) bacteria, respectively [55]. The essential oil of *C. atlantica* has been already studied and shown to possess antimicrobial [55-57], antifungal [57], anti-inflammatory [58], and molluscicidal [59] activities. Identification and quantification of potent bioactive compounds from these plants were also performed.

Conclusion

The present study allowed to observe the antimicrobial activity of *Mentha pulegium*, *C. atlantica*, *Citrus sinensis*, *Rosmary officinalis*, *E. globulus*, *T. vulgaris* against the phytopathogenic bacterium *P. syringae* pv. tomato *DC3000*. To our knowledge, it was the first time that the effectiveness of these essential oils was determined against this strain. Only essential oils of *C. atlantica* had a negative activity against the bacterial strain. From the six essential oils studied, three (*mentha, thym and eucalyptus*) were highly effective against the bacterium tested. The phytochemical analysis, in conjunction with the *in vitro* microbiological test, allowed us to obtain EO composition and antimicrobial activity correlations. All these findings are an interesting outcome on complex matrices of natural origin, such as EOs, in an attempt to prevent and/or eradicate the bacterial speck of the tomato. As pesticides, plant essential oils should continue to make inroads into the market place, especially as the arsenal of conventional pesticide products becomes increasingly constrained, and consumers become ever more discerning about pesticide residues in the food supply, the workplace, and the outdoor environment.

Conflicts of Interest

The authors declare no conflicts of interest related to this work.

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