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Chemical composition and antibacterial activity of Moroccan *Artemisia mesatlantica* and *A. absinthium* essential oils against fire blight caused by *Erwinia amylovora*

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Abstract

The aim of this study was to find an alternative to chemical products used in the control of fire blight disease of rosaceous plants. In this work, essential oils of two Moroccan Artemisia species: *Artemisia mesatlantica* and *Artemisia absinthium* were screened for their antibacterial activity against *Erwinia amylovora* the causal agent of this disease. The EOs extraction was carried out by water distillation method in Clevenger-type apparatus and analyzed by gas Chromatography-Flame Ionization Detector (GC/FID) and by Gas Chromatography-Mass Spectrometry (GC/MS). The antibacterial activity of extracted EOs was tested *in vitro* based on the agar diffusion method and broth microdilution method and *in vivo* on detached immature pear fruits. The results revealed that the yields of extracted EOs were 1.25 and 0.58% respectively for *A. mesatlantica* and *A. absinthium*. The chemical composition analysis showed that investigated EOs were characterized with a high amount of β-Thujone (40.42% for *A. mesatlantica* and 36.25% for *A. absinthium)*. Both EOs have a similar MIC value (2.08%). *A. absinthium* EOs were found to be more effective than *A. mesatlantica* EOs since they show the lowest MBC. These results indicate that EOs of *A. absinthium* and *A. mesatlantica* contained compounds with antibacterial potential against *Erwinia amylovora*. In the bioassay test, the application of *A. absinthium* EOs on detached immature pear fruits infected by *E. amylovora* resulted in total suppression of disease severity. Such natural products may represent a sustainable alternative to the use of chemical pesticides.

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Keywords: antibacterial activity; *Artemisia absinthium*; *Artemisia mesatlantica*; bioassay test; *Erwinia amylovora*; *in vitro* test

Introduction

Fire blight caused by *E. amylovora* (Burrill), is the most serious bacterial disease affecting a wide variety of landscape plants in the rosaceous family, including pear, apple, quince etc., leading to enormous economic losses (Sterne, 1967; Gudžić *et al.*, 2002; Cakir *et al.*, 2004; Piqué *et al.*, 2015). *E. amylovora*, is a necrogenic, phytopathogenic gram-negative bacteria; it infects the host through openings natural in flowers (Droby *et al.*, 1989) or wounds, often following hailstorms. Indeed, the major economic impact of the fire blight disease has been reported in 40 countries (Sterne, 1967). The disease was first observed in 1780 in North America, in the Hudson Valley, and then spread rapidly to other regions (Sterne, 1967) around 1960. Since then, the disease has been widespread in Europe and countries of the Mediterranean region. The bacteria infect all parts of the plant, including blossoms, fruit, leaves, shoots, limbs, and trunks. Once the pathogen colonizes the parenchyma intercellular spaces of the bark consequently lead to the death of plant cells accompanied by a deformation of the cell walls associated with the formation of lysogenic cavities and outwardly the necrosis of infected tissues appeared. Subsequently, the necrosis extends downward from the apex accompanied by browning of the tissues (Vanneste, 2000).

Insusceptible hosts, bacteria can spread through the apoplast leading to systemic infection. The pathogen overwinters in cankers formed in dead woody tissue (Singh *et al.*, 1995).

Currently, control of fire blight disease relies mainly on the use of chemicals. However, the use of chemicals is subjected to registration and permission for use in various countries. Treatment of fire blight disease with streptomycin may achieve an effective control (Johnson and Stockwell, 1998). However, the use of this bactericide was prohibited because of the emergence of resistant strains in *E. amylovora* population as well as in non-target agents (Iacobellis *et al.*, 2005). Furthermore, due to the growing concern of consumers towards the use of antibiotics and synthetic products, the search for alternative control measures is necessary.

Biological control using naturally occurring substances has emerged as an effective and safe strategy to control plant pathogens. EOs and plant extracts derived from medicinal and aromatic plants were usually used for flavouring, extending the shelf-life of food products, and in Moroccan traditional medicine (Wang *et al.*, 2010). These biological products present a promising alternative for the control of this threat due to their antimicrobial activities, non-phytotoxicity as well as their easy biodegradability in the environment (Fawcett and Spencer, 1970; Talibi *et al.*, 2012; Askarne *et al.*, 2013; Karim *et al.*, 2016). Rashidch *et al.* (2010) reported that the most antimicrobial properties of medicinal and aromatic plants are due at least in part to the presence of essential oils (EOs) produced by their secondary metabolism. EOs can be extracted from the whole plant or parts of the plant by several different methods, such as steam distillation (Dhifi *et al.*, 2016). Additionally, these oils have attracted the attention of researchers and the industry community due to their antimicrobial and antioxidant properties (Dung *et al.*, 2008).

The family of *Asteraceae*, includes 1500 genera and more than 26000 species that are distributed in all regions of the world, mainly in temperate areas (Barkley *et al.*, 2006). The genus *Artemisia* belonging to the family of *Asteraceae* is represented in Moroccan flora by 19 species, among them: *Artemesia. mesatlantica* and *Artemesia absinthium* (Zaim *et al.*, 2012). These plant species are a rich source of bioactive components (Ferreira et al. 2011) and were previously examined for their antimicrobial properties (Niño *et al.*, 2006).

Artemesia absinthium grows on uncultivated and arid terrain, on rocky slopes, along paths and fields, and it is extensively dispersed in Morocco (Iserin, 2001). The EOs of *A. absinthium* have shown antibacterial, antifungal, antitumoral, and insecticidal activities (Iserin, 2001). In contrast, *Artemesia mesatlantica,* which is

an endemic plant from Morocco, is widespread in the Middle Atlas, the High Atlas and the Anti-Atlas mountains regardless of the nature of the soil (Ouyahya, 1980). *Artemesia mesatlantica* exhibited antifungal and antibacterial activities (Bencheqroun *et al.*, 2012). Therefore, the aims of the present study were: i) to determine the chemical composition of *A. absinthium* and *A. mesatlantica EOs* grown in Morocco, and ii) to evaluate their effect to manage the fire blight disease of rosaceous plants caused by *E. amylovora in vitro* using aromatogram method on Petri dishes and under controlled laboratory conditions on detached immature pear fruits.

Materials and Methods

Plant material

Aerial parts (stems, leaves and fruits) of investigated Artemisia species were collected from their natural wild habitat, between March and May 2020, from two different locations of the Fez-Meknes region, Morocco: *A. mesatlantica* from Boulmane area and *A. absinthium* from the Meknes area. Voucher specimens were deposited in the herbarium of the bioactive molecules and environment, Moulay Ismail University, Faculty of Sciences, Meknes, Morocco. The collected plant samples were cleaned, shade-dried and stored in darkness at 4 °C until use.

Extraction of essential oils (EOs)

The essential oils (EOs) were obtained from dried aerial plant materials by steam training using a Clevenger type instrument for four hours, according to the following protocol: The biomass of each plant (500 g) was soaked in a flask containing sterile distilled water (SDW). The whole was boiled, and after the appearance of the first drop of distillate at the exit of the steam condensation tube, EOs was then driven by the steam and then condensed and recovered through a condenser.

Chemical characterization of EOs

The identification of the chemical components of each EOs was carried out by gas chromatography coupled to mass spectrometry (GC/MS); allowing a qualitative and quantitative determination of compounds. The Agilent 6890/5973 GC/MSD system was used for this purpose. The carrier gas was helium in let pressure 26 psi. The column was VF WAX (60 m × 0.25 mm, 0.5 µm) with temperature ranging from 60 to 250 °C at a heating rate of 5 °C min-1 for 10 min. Samples of EOs were first dissolved in hexane (10%). The injected volume was about 1 µl. The presence of the EOs compounds was calculated from the Peak area percent of each compound relative to the area percent of the entire GC/FID spectrum (100%), without correction factors. The components were identified by a combination of retention times (our own database) and mass spectra library NKS, 75 000 records.

Antibacterial activity

Bacterial culture

Erwinia amylovora, the causal agent of fire blight disease that affects rosaceous fruit trees, was obtained from the Laboratory of Phytopathology (ENA-Meknes, Morocco). The bacterium was isolated from infected pear trees and subcultured on Levan culture medium. The identity of the bacterium as *E. amylovora* was confirmed by PCR assays using the protocol described by (Llop *et al.*, 2000).

Antibacterial screening

The agar disc diffusion method was employed to test the antibacterial activity of the essential oils as previously described by (Serban *et al.*, 2011; Yakoubi *et al.*, 2014; Doukkali *et al.*, 2018). Briefly, 15 ml of Levan medium was poured into Petri dishes, and an aliquot (100 µl) of the bacterial suspension adjusted to 1×10^7

CFU/ml was spread over the surface of the medium and left dried under aseptic conditions. Sterilized paper Whatman discs (6 mm in diameter and 3 disc/plate), loaded with increasing volumes of EOs (concentration, 2, 6 and 10 µl/disc), were deposited on the agar surface medium inoculated beforehand with the bacterial suspension. The antibiotic streptomycin (2, 6 and 10 µl/disc) was used as a positive control. To allow the diffusion of EOs into the culture medium, Petri dishes were kept at 4 °C for 2 hours. Petri plates free of EOs were served as negative controls. The antibacterial activity was determined by measuring the diameter of the inhibition zone (mm) with a digital caliper 72-h post-incubation period at 28 °C. The results were expressed as mean ± SD. All tests were performed in triplicates, and each experiment was repeated twice over time. Sensitivity to EOs was rated based on the diameter of the inhibition zone according to (Ponce et al. 2003): non-sensitive (-) for diameters less than 8 mm; sensitive (+) for diameters from 8 to 14 mm; very sensitive (+ +) for diameters from 15 to 19 mm and extremely sensitive (+ + +) for diameters over 20 mm.

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) according to the NCCLS (1999) guidelines with small modifications. All experiments were performed in Levan medium using a 96-well microtiter plate. Fresh overnight bacterial cultures of *E. amylovora* (in lag phase) were used to prepare the cell suspension at a concentration of 1×10^6 CFU/mL. The EOs were first diluted in DMSO; the highest concentration to be tested was 4.16%. Afterward, 170 µl of Levan medium was dispensed in each well of the culture plate, and an aliquot (10 µl) of each EOs was added into the first well. Serial two-fold dilution was made at a concentration range from 4.16 to 0.016% (v/v). Finally, each well of the culture plate was inoculated with 20 µl of the bacterial suspension (1×10^6 CFU/ml). Two controls were prepared (Levan + *E. amylovora*) and (Levan + *E. amylovora* +DMSO). The contents of the wells were mixed, and the microplate was incubated at 28 °C for 24 h.

MIC, defined as the lowest concentration of the EOs that inhibits the growth of the tested bacterium, was determined by quantitative tetrazolium based colorimetric method. Furthermore, 50 µl of iodonitrotetrazolium chloride (INT) was added to each well; the plates were incubated at 28 °C for 4 to 5 minutes. Wells with pink colour were indicative of bacterial growth. The lowest concentration at which no visible colour change was observed was considered as the MIC. MBC was determined by sub-culturing aliquots from wells yielding a negative microbial growth in the broth microdilution test on the surface of free Levan medium for 24 h at 28 °C.

Bioassay on immature pear fruit

Bioassays were conducted under controlled laboratory conditions on detached immature pear fruits to evaluate the effect of *A. mesatlantica* and *A. absinthi* EOs on fire blight development in artificially injured and inoculated immature pear fruits. These latter were surface disinfected by immersion in a sodium hypochlorite solution (1.5%) for 30 min, and washed three times with SDW (Benada et al. 2021). Each pear fruit was wounded to 5 mm of every well and injected with 50 µl of suspension of the pathogen (1×10^8 CFU/ml). After two hours at room temperature, the inoculated fruits were treated with EOs of each plant (*A. mesatlantica* and *A. absinthi*) with 30 µl per wound at concentrations of 4.16% and 2.08%. Inoculated fruits were treated with streptomycin and Uninoculated fruits (50 µl of SDW Instead of the pathogen) and Uninoculated fruits (with 30 µl of EOs for phytotoxicity test) were used as controls. All fruits were incubated for 7 days in plastic bags at 28 °C in sterile desiccators in a controlled environment chamber at 25 ± 1 °C with a relative humidity of 95% (RH) (Sharifazizi *et al.*, 2017; El Khetabi *et al.*, 2020). Positive results were recorded when bacterial necrosis was detected in the well. For each concentration, four replicates were conducted in two independent experiments over time. The following formulas were used to calculate the disease's severity: Disease severity (%) = [(average lesion diameter of treatment/average lesion diameter of control)] × 100.

Statistical analysis

All datasets were subjected to statistical analysis of variance (ANOVA 2) using SPSS statistical software (version 20, IBM SPSS Statistics 20). When the effect was revealed to be significant, the SNK test was used to segregate treatments at $P < 0.05$.

Results

Chemical composition

The yields of EOs obtained from dried aerial parts of tested plants were 1.25% and 0.58% on a dry weight basis (v/w) for *A. mesatlantica* and *A. absinthium*, respectively. The chemical composition of both EOs was performed using GC/FID and GC/MS techniques (Figure 1). Fourteen compounds were identified in the case of *A. mesatlantica* EOs, representing 80.95% of total compounds (Table 1). *A. mesatlantica* EOs were characterized by a large amount of monoterpens (75.56%), including oxygenated monoterpenes as major compounds (74.44%). Sesquiterpenes represented only 5.39% of total compounds. Oxygenated sesquiterpenes (4.03%) represented the main classes of volatile compounds. The predominant components are β-Thujone (40.42%), camphor (12.14%), α-Thujone (6, 98%), 1,8-cineole (4.62%), Borneol (4.07%), Terpinene-4-Ol (3.27%), and Sabinol (2.94%) (Table 1).

Table 1. Chemical composition of essential oils of *A. mesatlantica*

Peaks	RT (min)	Compound	%
1	9.3	Sabinene	1.12
2	12.0	**1-8 Cineole**	**4.62**
3	18.8	**α-Thujone**	**6.98**
4	19.5	**β-Thujone**	**40.42**
5	21.7	**Camphor**	**12.14**
6	23.8	**Terpinene-4-Ol**	**3.27**
7	26.3	Sabinol	2.94
8	26.4	**Borneol**	**4.07**
9	33.5	Oxyde De Caryophyllene	1.36
10	35.3	Spirojatamol	1.49
11	36.4	Spirojatamol Isomere	0.81
12	36.4	Santalol Isomere	0.82
13	37.9	α-Bisabolol	0.56
14	42.0	Oxyde A D'α-Bisabolol	0.35
Total			**80.95**
Monoterpenes			**1.12**
Oxygenated Monoterpenes			**74.44**
Sesquiterpenes			**1.36**
Oxygenated Sesquiterpenes			**4.03**

For *A. absinthium* essential oil, 17 compounds were identified, representing 90.52% of total compounds (Table 2). *A. absinthium* EOs were also characterized by a large amount of monoterpens (87.63%). Oxygenated monoterpenes (70.86%) and monoterpenes (16.77%) represent the main classes of volatile compounds. The most predominant components were β-Thujone (36,25%), Camphor (24,42%), Terpinen-4-ol (8,16%), β-Myrcène (5.45%), Sabinene (3.18%), y-terpinene (2.09%), Camphene (1.80%), P-Cymene (1.63%), α-Pinène (1.60%), α-Thujone (1.22%), and Germacrene (1.22%) (Table 2).

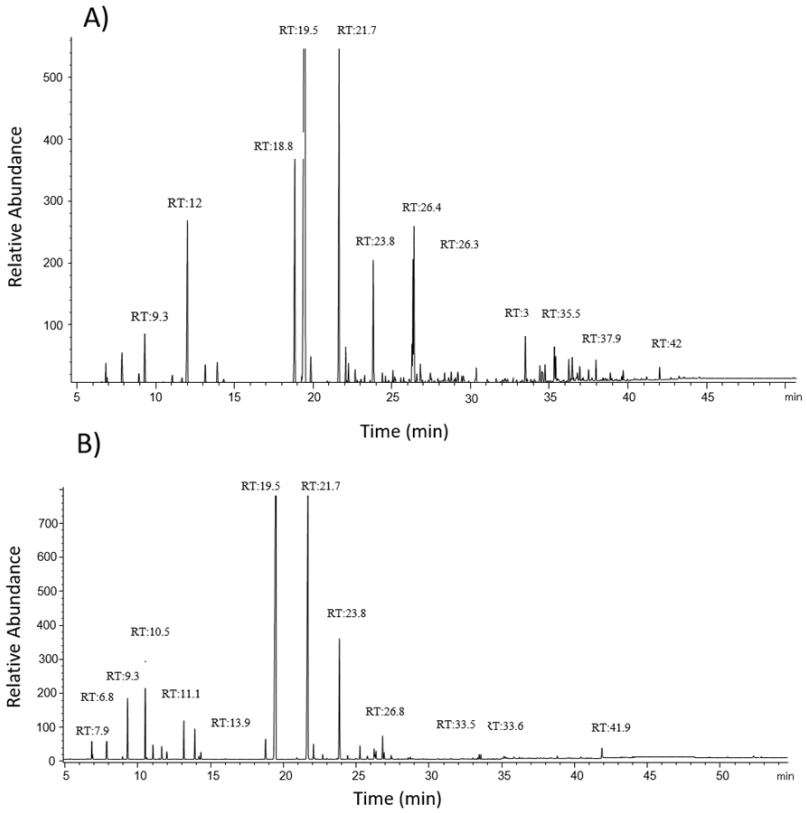

Figure 1. *Artemisia mesatlantica* (A) and *A. absinthium* (B) essential oils chromatogram (GC/FID)

Table 2. Chemical composition of essential oils of *A. absinthium*

Peaks	RT (min)	Compound	%
1	6.8	α-Pinène	1.60
2	7.9	Camphene	1.80
3	9.3	**Sabinene**	**3.18**
4	10.5	**β-myrcène**	**5.45**
5	11.1	**α-terpinene**	0.77
6	13.1	y -Terpinene	2.09
7	13.9	P-Cymene	1.63
8	18.8	**α-thujone**	1.22
9	19.5	**β-thujone**	**36.25**
10	21.7	**Camphor**	**24.42**
11	22.1	Linalol	0.81
12	23.8	**Terpinen-4-ol**	**8.16**
13	25.3	Alcool thuyylique	0.73
14	26.8	Germacrene	1.22
15	33.5	Oxyde de caryophyllene	0.28
16	33.6	E-methyleugenol	0.25
17	41.9	Chamazulene	0.66
Total			**90.52**
Monoterpenes			**16.77**
Oxygenated Monoterpenes			**70.86**
Sesquiterpenes			**2.16**
Alcools			**0.73**

Antibacterial activity

The essential oils from *A. mesatlantica* and *A. absinthium* were screened for their abilities to inhibit the in vitro growth of *E. amylovora*, the causal agent of fire blight diseases of rosaceous plants. As shown in Table 3, both investigated EOs exhibited a potent antibacterial activity against tested bacterium when 6 µl of EOs was used per disc. Moreover, both EOs inhibited the *E. amylovora* growth with an inhibition zone diameter of 15 ± 0.5 mm for *A. mesatlantica* and 12.8 ± 34 mm for *A. absinthium*. The inhibition zone diameter increased significantly with increasing the amount of essential oil per disc (Figure 2). The inhibition zone diameters of the positive control were significantly higher than that of investigated EOs even at the lower dose (2 µl/disc).

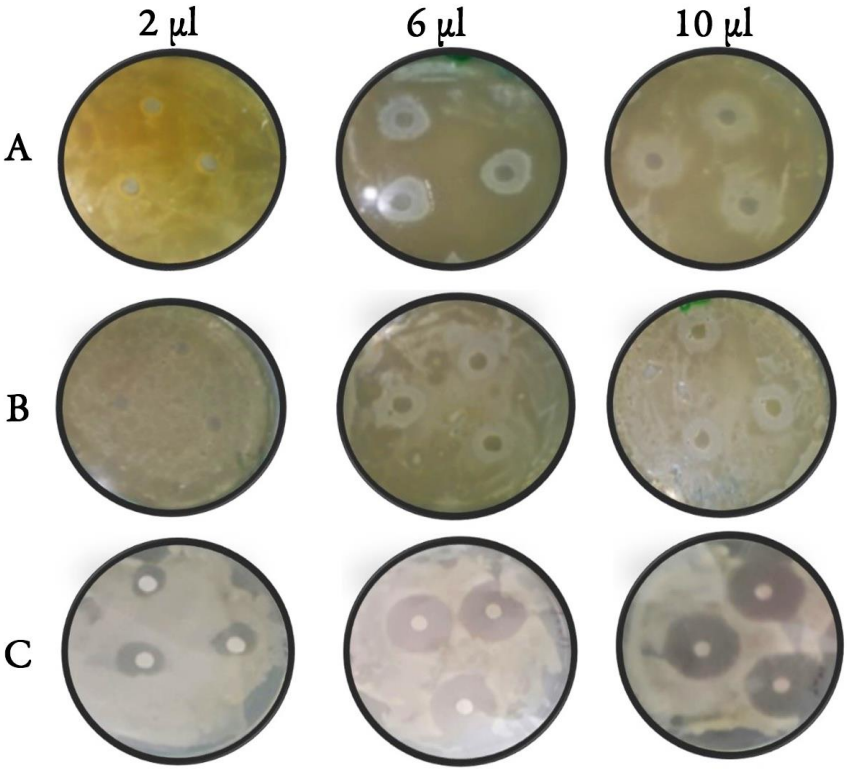

Figure 2. Antibacterial activity (zones of inhibition) of *Artemisia mesatlantica* (A), *Artemisia absinthium* (B) EOs and streptomycin (C) against *Erwinia amylovora,* the causal agent of fire blight

Table 3. The inhibitory effect (mm) of *A. mesatlantica* and *A. absinthium* (mm) EOs on the growth of E. *amylovora* 72 hours post-incubation

Treatments	Negative control	2 µl	6 µl	10 µl
A. mesatlantica	0.0	7^{b}± 0.5 (-)	15^{ab}± 0.5 (+)	17^{a}± 0.5 (++)
A. absinthium	0.0	0^{a} ± 0 (-)	12.8^{a} ± 0.34 (+)	24.8^{b} ± 0.85 (+++)
Streptomycin	0.0	20^{c}± 1.05 (+++)	27^{b}± 0.78 (+++)	29^{c}± 0.72 (+++)

- Values are means (± SE) standard error for bioassay conducted twice over time in triplicates.
- Inhibition percentage followed by the same letters was not significantly different according to the SNK test ($P < 0.05$). (-): inactive; (+): active; (++): very active ;(+ + +): extremely active

Determination of MIC and MBC

The antibacterial activity of *A. mesatlantica* and *A. absinthium* EOs was studied by determining their MIC and MBC values using broth microdilution method. According to the results shown in Table 4,

investigated EOs exhibited significant antibacterial activity against *E. amylovora* with a MIC value of 2.08% for both EOs.

A. absinthium EOs were found to be more effective than *A. mesatlantica* EOs, showing an MBC value of 2.08%, while for *A. mesatlantica* EOs MBC value was 4.16%. Moreover, the ratios MIC/MBC as shown in Table 4 was of 1 for *A. absinthium* EOs confirming its bactericidal activity, while *A. mesatlantica* EOs exhibited a bacteriostatic rather than a bactericidal activity (MIC/MBC) >1).

Table 4. MIC and MBC of EOs of *A. mesatlantia* and *A. absinthium* against *E. amylovora*

	MIC	MBC	MBC/MIC
A. mesatlantia	2.08%	4.16%	2
A. absinthium	2.08%	2.08%	1

Bioassay on immature pear fruit

Immature pear fruits inoculated with *E. amylovora* and previously treated with EOs were compared with untreated control. The results showed that EOs of *A. absinthium* at concentrations of 2.08% and 4.18% suppressed totally the disease severity of fire blight caused by *E. amylovora* under controlled laboratory conditions in artificially wounded and inoculated fruits (100% symptoms inhibition; 0% disease severity) (Table 5). The EOs of *A. mesatlantica* at concentrations of 2.08% and 4.18% reduced the disease severity by 94.01% and 67.42%, respectively. The EOs of *A. absinthium* plant exhibited a highly significant reduction in disease severity compared to both EOs of *A. mesatlantica* and the streptomycin (200 µg/ml) (P ≤0.05) (Table 5).

Table 5. Effect of EOs of *A. absinthium* and *A. mesatlantica* at concentrations of 2.08% and 4.18% against *E. amylovora* infection (expressed as disease severity) on detached immature pear fruits after 7 days incubation at 28 °C. The pathogen was used at 1×10^8 CFU/ml

Treatment	Disease severity (%)	
	Essential oils concentration	
	2.08%	4.16%
A. mesatlantica	$94{,}01 \pm 0.02^{d}$	67.42 ± 0.01^{c}
A. absinthium	0 ± 0^{a}	0 ± 0^{a}
Streptomycin	31.62 ± 0.017^{b}	31.62 ± 0.017^{b}

Values are means of four replicates. Inhibition percentage followed by the same letters were significantly different according to Tukey's test (P <0.05).

Discussion

Fire blight is one of the most devastating diseases of the *Rosaceae* family worldwide. The disease is caused by the bacterium *Erwinia amylovora* (Burrill, 1883), which can destroy the entire orchard and consequently cause enormous economic damage. Several control methods were used to manage this disease. However, most of these methods are not efficient and have serious effects on the environment, human and animal health. For this reason, the global trend is oriented to the search for new ecofriendly alternatives that respect human health and the environment as well. In this context, the current study was conducted.

The current study shows that β-Thujone represents the major component in both investigated essential oils. Thus, *A. mesatlantica* and *A. absinthium* plant species can be classified as β-Thujone chemotypes. Obtained results indicated that the chemical composition of investigated EOs was qualitatively and quantitatively different and the oxygenated monoterpenes fraction was the most abundant with 74.44 and 70.86% for *A.*

mesatlantica and *A. absinthium* respectively. These results are in agreement with those of (Kliks, 1985; Cruz *et al.*, 2007) who reported that despite the variability in chemical composition, the EOs mainly consisted of mono and sesquiterpenes.

Previous studies demonstrated that the geographical origin and existence of chemotypes within plant species are factors that can directly influence the chemical composition of EOs (Chekem *et al.*, 2010). The differences observed in the chemical composition of EOs can also be explained by drying, harvesting period, extraction technique, and the developmental stage of the plant (Aberchane *et al.*, 2001; Bourkhiss *et al.*, 2009).

Furthermore, several studies pointed out the chemical composition of Moroccan chemotypes of *A. mesatlantica* EOs. Holeman *et al.* (1991) in the region near Ifrane (Morocco), showed that *A. mesatlantica* EOs were rich in β-thujone (60%). In the same region, Ouyahya *et al.* (1990) reported that the major constituents of *A. mesatlantica* EOs were β-thujone (34%) and camphor (32%), which is in line with the results of this study. In the region of Ifrane, β- thujone in *A. mesatlantica* EOs represent 33.9% of total compounds (Boumhara *et al.*, 2014). Bencheqroun et *al.* (Bencheqroun *et al.*, 2012) reported that β-thujone (56.33%), camphene (7.48%), camphor (4.17%), 1,8-cineol (2.63%), α-thujone (1.38%) were the main components of *A. mesatlantica* EOs. The chemical composition of *A. absinthium* EOs has also been studied in different locations in Morocco and β-thujone (39.69%), sabinyl acetate (10.96%) and α-thujone (7.25%) were determined as the major constituents of *A. absinthium* EOs from the region of Guigou and Errachidia (Derwich *et al.*, 2009). *A. absinthium* EOs from the region of Taounate were characterized by β- thujone (35.6%), chamazulene (3%) (Fouad *et al.*, 2015). While, *A. absinthium* EOs from western Canada were characterized by high amounts of myrcene (10.8%), trans-thujone (10.1%) and trans-sabinyl acetate (26.4%) (Lopes-Lutz *et al.*, 2008). Sabinene (17.56%) was the main constituent in *A. absinthium* EOs from Turkey (Erel *et al.*, 2012).

The positive results obtained in this study indicated that EOs contained active compounds that are responsible at least in part for the antibacterial activity. These results corroborated those of (Kokoskova and Pavela, 2007) who reported that EOs of *A. absintium* from USA exerted a significant inhibitory effect against *E. amylovora* with an inhibition zone diameter of 12.5 ± 1.5mm. In another study, Elazzouzi *et al.* showed that *Artemisia ifranensis* EOs from Timahdite region in Morocco presented an inhibitory effect against *E. amylovora* with inhibition zone diameter of 13.5±0.08 mm. Furthermore, both investigated EOs were rich in oxygenated monoterpenes and monoterpenes, which are well known for their antimicrobial activities (Gudžić *et al.*, 2002; Cakir *et al.*, 2004). Several studies reported that the antibacterial activity of EOs can be attributed to the antibacterial properties of some compounds such as: β-Thujone, Camphor (Tantaoui-Elaraki *et al.*, 1993; Senatore *et al.*, 2005), α-Thujone, 1,8-cineole) (Jalsenjak *et al.*, 1987; Prudent *et al.*, 1993; Sivropoulou *et al.*, 1997), Terpinen-4-ol (Filipowicz *et al.*, 2003). In addition, other minor components such as Borneol have been also reported to have antimicrobial potential (Knobloch *et al.*, 1989). Furthermore, a previous study on *Lavandula mairei* collected in the southwest of Morocco, demonstrated that oxygenated monoterpenes and monoterpenes were the major components of *Lavandula mairei's* EOs and these EOs exhibited a most efficient antibacterial power against some human bacterial pathogens (El Hamdaoui *et al.*, 2018). However, many studies have concluded that there was a synergistic effect between all compounds of EOs and the greatest antimicrobial activity was recorded when the major compounds were used in combination with minor compounds (Dorman and Deans, 2000; Jirovetz *et al.*, 2006).

Previous study reported that *Artemisia ifranensis* EOs have a strong antibacterial power against *E. amylovora* (MIC= 1/250 v/v; MBC= 1/50 v/v) (Elazzouzi *et al.*, 2018). In other works, the MIC values of EOs of *Artemisia judiaca* and *Artemisia monosperma* collected from Egypt and tested against *Erwinia carotovora* and *Agrobacterium tumefaciens* were in the range of 525 to 675 $mg.l^{-1}$ (Badawy and Abdelgaleil, 2014). Plant extracts have also been tested against *Erwinia* sp. In fact, the chloroform extract of *Artemisia nilagirica*

exhibited the highest inhibitory effect against *Erwinia sp.* with a MIC value of 32µg.ml^{-1} (Ahameethunisa and Hopper, 2010).

In vivo bioassay on inoculated immature pear fruits infected by *E. amylovora* and treated with *A. absinthium* EOs resulted in a total absence of disease severity (fruits without symptoms) suggesting a protective capability of these EOs. Several studies have reported the efficacy of EOs use against *E. amylovora* on immature pear fruits. Indeed, Hasanzadeh (Hasanzadeh, 2005) reported that *Satureia hortensis* EOs reduced the symptoms of fire blight disease caused by *E. amylovora* in immature pear fruit over a period of 6-day. Similarly, the study of Akhlaghi et *al.* (2020) indicated that the use of EOs of *Apium graveolens* and *Curcuma longa* showed a very high reduction in *E. amylovora* progression on immature pear fruits.

Conclusions

In this work, the chemical composition of EOs from two *Artemisia* species and their subsequent antibacterial activities against *E. amylovora* were investigated. Qualitative and quantitative analyses of both EOs revealed 14 and 17 compounds for *A. mesatlantica* and *A. absinthium* respectively. Both species are considered β-Thujone chemotypes.

The results of the biological antibacterial assay showed *in vitro and in vivo* efficiency of *A. mesatlantica* and *A. absinthium* in suppressing *E. amylovora* with higher antimicrobial activity for *A. absinthium.* Therefore, the results presented in this study might contribute to the good knowledge of antibacterial potential of these species and the possibility of using them further in an integrated control strategy against this devastating bacterial disease. Moreover, other studies involving the use of plant extracts of these species in controlling this disease are needed in order to compare their efficiency to that obtained with their EOs.

Authors' Contributions

Conceptualization: LD, AT, FG, BT and RL; Performed research: LD and SE; Supervision: AT, FG, BT and RL; Writing - original draft: LD, LA and SE; Writing - review and editing: LD, LA and RL. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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