



Antifungal Properties of *Pimenta racemosa* (Mill.) and *Mentha x piperita* (L.) Essential Oils against *Fusarium oxysporum* Causing Tomato Fruit Rot

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Fungal pathogens are responsible for the rot of tomatoes and cause health hazards to consumers as well as economic loss. The aims of this study were to evaluate the efficacy of essential oils from *Pimenta racemosa* (Mill.) and *Mentha x piperita* (L.) leaves as plant-based fungicide against *Fusarium oxysporum* strains associated with tomato rot. The chemical composition of the essential oils was determined by GC-MS analysis. Antifungal effects of essential oils against strain of *F. oxysporum* obtained from rotted tomato, were evaluated through the determination of Minimal Fungicide Concentration (MFC) and the Minimal Inhibitory Concentration (MIC). Results indicated that β -myrcene (19.48%), chavicol (15.39 %), and eugenol (46.65%) were the major components of the essential oil of *Pimenta racemosa* (Mill.). L-menthone (17.19 %), menthol (24.79%), and menthofuran (26.78 %) were the major components of the essential oil of *Mentha x piperita* (L.). Essential oil of *Pimenta racemosa* (Mill.) exhibited the highest antifungal activity against the growth of *Fusarium oxysporum* strain tested with a MIC and MFC of 0.75 and 1.25 μ l.ml⁻¹ respectively. These results provide an experimental basis for further research on the use of these plant extracts in the development of eco-friendly preservatives against fungi causing rots of tomato in post-harvest.

Keywords: *Pimenta racemosa* (Mill.); *Mentha x piperita* (L.); tomato; chemical composition; antifungal property; Benin.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important cultivated vegetable worldwide due to its nutritional and organoleptic properties [1-3]. Promoting the consumption of fresh vegetables has long been associated with a healthier lifestyle, which aiming to reduce the risk of micronutrient deficiencies (vitamins and minerals), as well as the occurrence of chronic diseases and cancer [4]. However, the availability of vegetables is affected by their high perishability, which is increasingly accentuated by various environmental factors such as climate change and pathogens throughout the production and storage chain [5]. According to Kang et al. [6], an unsuitable food storage can result in rapid water loss (wilting), pigments degradation (chlorophylls and carotenoids), as well as microbial spoilage.

According to Thirupathi et al. [7], post-harvest losses in fresh tomato fruit have been estimated at around 25.80%, because of the short post-harvest lifespan of tomatoes (2-3 weeks depending on the cultivar). During the post-harvest period, the quality of tomatoes continuously deteriorates, due to increased respiration and transpiration rates, as well as microbial-induced spoilage [8,9]. Various micro-

organisms are responsible for the post-harvest decay of tomatoes, with fungi or bacteria are the most destructive [10]. According to Chohan et al. [11], tomatoes are vulnerable to attack by different fungal pathogens, including soilborne, airborne and seedborne. Mycopathogens such as *Fusarium oxysporum* are one of the major causes of post-harvest tomato deterioration in humid regions. Indeed, *Fusarium oxysporum* is a species of anamorphic fungus that includes pathogenic and non-pathogenic strains. Phytopathogenic forms cause wilting of crops and are classified based on their host range. Some are subdivided into pathogenic races [12-14]. Recent findings have revealed that the use of chlorine-based disinfectants for decontaminating fresh food products is associated with the production of harmful and sometimes carcinogenic compounds [15]. Thus, more and more researchers are turning to natural products as disinfectants to reduce the incidence of chemical synthesized products in the food industries [16,17]. Among these natural products, essential oils (EO) extracted from medicinal and aromatic plants have attracted attention from scientific community, because of their interesting biological activities (including antioxidant, anti-inflammatory, antifungal and antibacterial properties) [18-21]. Various essential oils from aromatic plants have already been used as food

preservatives showing their bioefficacy [22,23]. Plants belonging to Myrtaceae family like *Pimenta racemosa* and Lamiaceae family like *Mentha x piperita*, have retained the attention of researchers, due to their widespread distribution worldwide and their traditional use in folk medicine to treat diseases [24,25]. In Benin, plant leaves are also used to preserve food by introducing them into grain barns to preserve stored products from insect and fungal damage. Thus, the present study aims to investigate the efficacy of essential oils extracted from the leaves of *Pimenta racemosa* and *Mentha x piperita* against fungi isolated from tomatoes.

2. MATERIALS AND METHODS

2.1 Collection of Plant Leaves

Plant materials used for essential oil (EO) extraction consisted of leaves from *Pimenta racemosa* (Fig. 1) and *Mentha x piperita* (Fig. 2). Leaves of *Pimenta racemosa* and *Mentha piperita* were collected respectively in one of the large market gardening sites located in Abomey-Calavi and Cadjèhoun (South Benin), and identified at the Benin national herbarium, where voucher specimens are deposited.

2.2 Essential Oil Extraction

Plant materials collected were stored in the laboratory between 19 °C and 20 °C. Essential oils were obtained by hydrodistillation of the leaves (450 g) for 5 hours using a Clevenger-type apparatus. The extracted essential oils were dried over anhydrous sodium sulfate (Na_2SO_4) and stored at +4 °C [26].

2.3 Gas Chromatography–mass Spectrometry Analysis

Chemical composition of essential oils from *Pimenta racemosa* and *Mentha x piperita* leaves, were determined by using gas-chromatography/mass-spectrometry (GC/MS). The analysis conditions were as described in our previously published study [27].

2.4 Collection of Tomato Fruits

Different infected tomato fruits were collected from seven different markets located in the municipalities of Klouekanmey, one of the major tomato production localities in southern Benin.

Samples were collected in sterilized polyethylene bag.

2.5 Fungal Strain

Subcultures of *Fusarium oxysporum* strains obtained from rotted tomato [28], were used in the present study. Strains are repeatedly sub-cultured on Sabouraud medium before their use.

2.6 Antifungal Assay

The antifungal assay was performed as described by Billerbeck et al. [26], using de Sabouraud medium with different concentrations of essential oil (0.75, 1.25, 2.5 or 5.0 $\mu\text{l}\cdot\text{ml}^{-1}$) and Tween 20. About 20 ml of the medium was poured into glass Petri dishes (9 cm). *Fusarium oxysporum* grown on Sabouraud medium, were transplanted (subcultured), using a 6 mm diameter disc carrying spores from the anamorph mold, on the surface of a Petri dish containing the aforementioned Sabouraud medium and essential oil at different concentrations. Control plates were also inoculated following the same procedure. Plates were incubated at 25 °C, and the mycelial growth was evaluated as described by Yehouenou et al. [29]. The percentage inhibition (PI) of fungal growth was evaluated as described by Kumar et al. [30] using the following equation: $\text{PI} = [1 - (d/dc)] \times 100$ (where d and dc represent the diameters of the growth zone in the test plate, and in the control plate, respectively).

2.7 Determination of the Fungistatic or Fungicidal Activity

The fungistatic or fungicidal activity was determined by reintroducing the disc from the lowest experimental concentrations where no mycelial growth was observed, into a new culture medium without essential oil [29]. If the mycelial growth is consistently inhibited, the plant extract is fungicidal at this concentration, allowing the determination of the Minimal Fungicide Concentration (MFC). In the contrary case, the plant extract has a fungistatic activity, which is related to the Minimal Inhibitory Concentration (MIC).

2.8 Statistical Analysis

Experiments were performed in triplicate, and data analyzed are represented as means \pm SD subjected to one-way ANOVA. Tukey's multiple range test were used to separate means when Anova was significant ($P < 0.05$) (SPSS 10.0; Chicago, IL, USA).



Fig. 1. *Pimenta racemosa* (Mill.) J.W. Moore



Fig. 2. *Mentha x piperita* (L.)

3. RESULTS AND DISCUSSION

By hydrodistillation, the leaves of *Pimenta racemosa* (Mill.) and *Mentha x piperita* (L.) yielded 4.3% and 1.76% (w/w) respectively, of essential oils. Chemical analysis of the components of the essential oil of *Pimenta racemosa* (Mill.) via GC/MS led to the identification of 26 components, representing 99.98 % of the essential oil of *Pimenta racemosa* (Table 1). *Pimenta racemosa* oil exhibited chemical compositions characterized by β -myrcene (19.48%), eugenol (46.65%) and chavicol (15.39 %), as the major components. Similarly, the chemical analysis by GC/MS of essential oil of *Mentha x piperita* (L.) allowed the identification of 22 components, representing 99.9% of the essential oil (Table 2). The chemical composition was characterized by L-menthone (17.19 %), menthofuran (26.78 %) and menthol (24.79%). Results from the evaluation of the antifungal potential of the essential oils against *Fusarium oxysporum* (Table 3), indicated that oils exhibited pronounced antifungal activity against the growth of *Fusarium oxysporum*. Specifically, the MIC and MFC of essential oil of *Pimenta racemosa*, was found to be respectively $0.75 \mu\text{l.ml}^{-1}$ and $1.25 \mu\text{l.ml}^{-1}$ against the fungal tested. However, the MIC and MFC of the essential oil of *Mentha x piperita*, was found to be $3.75 \mu\text{l.ml}^{-1}$ and $5 \mu\text{l.ml}^{-1}$ respectively. Results of mycelial percentage growth inhibition (PI), calculated as described by Kumar et al. [30], are presented in Table 4. These results indicated that the radial growth of strains was inhibited by the essential oils tested, with a percentage of growth inhibition (PI) significantly influenced by essential oil concentration and the chemical composition of the essential oil ($P < 0.05$). Indeed, essential oils are natural mixtures containing organic substances from plants, which have a long history of application as antimicrobial agents [31]. This study investigates the antifungal potential of essential oils from fresh leaves of *Pimenta racemosa* and *Mentha x piperita* as promising plant-based antimicrobials against strain of *Fusarium oxysporum* associated with tomato rots in post-harvest. The results indicated that both essential oils were found to be effective. However, the antifungal activity of essential oil of *Pimenta racemosa* was found to

be highly pronounced against the strain of *Fusarium oxysporum*, with the lowest MFC. The antifungal potential of *Pimenta racemosa* essential oil, may be attributed to some highly fungitoxic components in the oil. Indeed, GC/MS analysis indicated that the essential oil of *Pimenta racemosa* has main components with high antimicrobial activity, such as eugenol and Pinene. Several studies have reported that eugenol is incorporated as a functional ingredient in numerous industrial products due to its vast range activities including antimicrobial, anti-inflammatory, analgesic, antioxidant and anticancer properties. It is also widely used in food industry to protect food against spoilage [32,33]. Several other studies have confirmed the antibacterial potential of eugenol against pathogens [34-36]. It is believed that this antibacterial activity could be due to the inhibition of extracellular enzymes synthesis and disruption of the cell wall structure. However, it is also important to underline that, despite the high proportion of eugenol in this essential oil, its high antimicrobial could be due to the synergic action of all components presents in the oil. Indeed, α -pinene also present in *P. racemosa* essential oil as a minor compound, has a wide range of pharmacological activities, including antibiotic resistance modulation, anticoagulant, antitumor, antimicrobial, antimalarial, antioxidant, anti-inflammatory, anti-Leishmania, and analgesic effects [37]. In the present study, GC/MS data on the chemical composition of the oils depicted remarkable variation with the earlier reports on the oils [24,25,33]. Youssef et al. [35] and Suhr and Nielsen [36] reported that the chemical profile of essential oil is influenced by the harvest period, and the climatic, seasonal and geographical conditions, which could affect the amount and composition of active constituents in the oil. Therefore, it should be important to qualitatively and quantitatively investigate the chemical composition of essential oil before their recommendation for practical exploitation as has been done in this study. Based on the results from this investigation, essential oil of *Pimenta racemosa*, with its high antifungal potentiality, provides an opportunity to avoid synthetic chemical preservatives and offers novel approach to the management of tomato storage fungi.

Table 1. Components identified as constituents of essential oil of *Pimenta racemosa* used

Constituents	Kovats Retention Index (KI)	Percentage
Thujene	8.2	0.10
Alpha-pinene	8.366	0.66
1-octen-3-ol	9.585	1.60
α-myrcene	9.954	19.48
Phellandrene	10.238	1.24
Alpha-terpinene	10.553	0.25
Cymene	10.762	0.61
Limonene	10.885	5.51
Eucalyptol	10.944	1.49
β-ocimene	11.372	0.72
Gamma-terpinene	11.65	0.23
α-terpinolene	12.415	0.31
Linalool	12.704	2.13
4-terpineol	14.667	0.71
Menth-1-en-8-ol	14.993	0.57
Decanal	15.308	0.32
Chavicol	16.528	15.39
Neral	16.929	0.11
4-Allylphenyl acetate	18.657	0.64
Eugenol	19.127	46.65
Caryophyllene	20.283	0.40
Isoeugenol	20.86	0.15
α-humulene	20,994	0,16
α-farnesene	21,989	0,14
Aceteugenol	22,385	0,28
Camphorene	30,033	0,16

Table 2. Components identified as constituents of essential oil of *Mentha x piperita*

Constituents	Kovats Retention Index (KI)	Percentage
Alpha-pinene	8.366	0.87
Sabinene	9.414	0.52
β-pinene	9.489	1.19
β-myrcene	9.88	0.22
Limonene	10.869	2.11
Eucalyptol	10.939	3.81
Gamma-terpinene	11.65	0.22
Cis-sabinene hydrate	11.869	0.93
L-menthone	14.164	17.19
Menthofuran	14.431	26.78
Menthol	14.677	24.79
Isomenthol	14.859	0.47
Pulegone	16.202	5.73
Piperitone	16.527	0.22
3-menthene	16.983	0.86
Carane	17.448	9.66
Menthyl acetate	17.764	0.32
Menthofuro lactone	18.651	0.30
Caryophyllene	20.272	1.92
β-farnesene	20.93	0.40
Germacrene	21.55	1.12
Aromadendrene	23.786	0.36

Table 3. *Fusarium oxysporum* colony diameters recorded (mm) with essential oils of *Pimenta racemosa* and *Mentha x piperita*

Days	Control (without oils)	Essential oils concentrations									
		0.75 $\mu\text{l.ml}^{-1}$		1.25 $\mu\text{l.ml}^{-1}$		2.5 $\mu\text{l.ml}^{-1}$		3.75 $\mu\text{l.ml}^{-1}$		5.0 $\mu\text{l.ml}^{-1}$	
		<i>P. racemosa</i>	<i>M. piperita</i>	<i>P. racemosa</i>	<i>M. piperita</i>	<i>P. racemosa</i>	<i>M. piperita</i>	<i>P. racemosa</i>	<i>M. piperita</i>	<i>P. racemosa</i>	<i>M. piperita</i>
1	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a
2	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a
3	26.0 \pm 0.0 b	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a
4	32.0 \pm 0.3 b	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a
5	37.0 \pm 0.1b	6.0 \pm 0.0 a	32.0 \pm 0.4b	6.0 \pm 0.0 a	24.0 \pm 0.1b	6.0 \pm 0.0 a	22.0 \pm 0.5b	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a
6	44.0 \pm 0.3 b	6.0 \pm 0.0 a	41.0 \pm 0.3b	6.0 \pm 0.0 a	34.0 \pm 0.4b	6.0 \pm 0.0 a	30.0 \pm 0.1b	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a
7	48.5 \pm 0.2b	6.0 \pm 0.0 a	44.5 \pm 0.3b	6.0 \pm 0.0 a	42.0 \pm 0.7b	6.0 \pm 0.0 a	41.0 \pm 0.4b	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a	6.0 \pm 0.0 a

Diameter of 6 mm is the inoculation point. Values (mm) are mean (n=3) \pm SD. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests

Table 4. Percentage of mycelial growth inhibition (PI)

Concentrations of essential oils	Percentage of inhibition (%)	
	<i>Pimenta racemosa</i>	<i>Mentha x piperita</i>
0.75 $\mu\text{l.ml}^{-1}$	100 \pm 0.0 a	8.24 \pm 0.30a
1.25 $\mu\text{l.ml}^{-1}$	100 \pm 0.0 a	13.40 \pm 0.20b
2.5 $\mu\text{l.ml}^{-1}$	100 \pm 0.0 a	15.46 \pm 0.40b
3.75 $\mu\text{l.ml}^{-1}$	100 \pm 0.0 a	100 \pm 0.0 c
5.0 $\mu\text{l.ml}^{-1}$	100 \pm 0.0 a	100 \pm 0.0 c

Values (mm) are mean (n=3) \pm SD. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests

4. CONCLUSION

The present study provides evidence that the essential oil of *Pimenta racemosa*, containing β -myrcene, chavicol, and eugenol as major components, exhibited potent antifungal activity against strain of *Fusarium oxysporum* causing tomato rots in post-harvest. Nevertheless, further investigations on the molecular identification of fungi tested are required, as well as the synergistic effects of essential oils could be exploited so as to maximize the antifungal activity of oils and to minimize the concentrations required to achieve a particular antifungal effect.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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