# Antifungal Properties of *Pimentaracemosa* (Mill.) and *Mentha x piperita* (L.) Essential Oils against *Fusariumoxysporum* Causing Tomato Fruit Rot

**Abstract**

Fungal pathogens are responsible for the rot of tomatoesandcausehealthhazardstoconsumersaswellas economic loss. The aims of this study were to evaluate the efficacy of essential oils from *Pimentaracemosa*(Mill.) and *Mentha x piperita* (L.) leaves as plant-based fungicide against *Fusariumoxysporum* 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 *Pimentaracemosa* (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 *Pimentaracemosa* (Mill.) exhibited the highest antifungal activity against the growth of *Fusariumoxysporum* strain tested with a MIC and MFC of 0.75 and 1.25 $\mu l.ml^{-1}$ 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***Pimentaracemosa*(Mill.),*Menthaxpiperita* (L.), tomato,chemicalcomposition, antifungalproperty, Benin.



## Introduction

Tomato (*Solanumlycopersicum*L.) is an important cultivated vegetable worldwide due to its nutritional and organoleptic properties (Ayari et al. 2015; El-Garhy et al. 2020; Akotowanou et al. 2022). Promoting the consumption of fresh vegetables has long been associated with a healthier lifestyle,whichaimingtoreducetheriskof micronutrient deficiencies (vitamins and minerals), as well as the occurrence of chronic diseases and cancer (Jahan et al. 2020).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 (Cortbaouiand Ngadi 2015). According to Kang et al. (2002), an unsuitable food storage can resultin rapid water loss (wilting), pigments degradation (chlorophylls and carotenoids), as well as microbial spoilage. According to Thirupathi et al. (2006), 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,thequalityoftomatoescontinuously deteriorates,dueto increased respiration and transpiration rates, as well as microbial-inducedspoilage(Zapata et al. 2008; Tzortzakis et al. 2019).Variousmicro-organisms are responsible for the post-harvest decay of tomatoes, with fungiorbacteriaarethemostdestructive(Obetta et al. 2011).Accordingto Chohan et al. (2016), tomatoes are vulnerable to attack by different fungal pathogens, including soilborne, airborneand seedborne. Mycopathogens such as *Fusariumoxysporum*are one of the major causes of post-harvest tomato deterioration inhumid regions. Inded, *Fusariumoxysporum* 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(Nasir et al. 2015; Sajad et al. 2017; Shakya and Aryal 2021). Recent findings have revealedthat the use of chlorine-based disinfectants for decontaminating fresh food products is associated with the production of harmful and sometimes carcinogenic compounds (Imaizumi et al. 2018). Thus, more and more researchers are turning to natural products as disinfectants to reduce the incidence of chemical synthesized products in the food industries (Kawhena et al. 2020; Panahirad et al. 2020). 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 (Burt 2004; Falleh et al. 2020;Xylia et al. 2021;Adjou et al. 2022). Various essential oils from aromatic plants have already been used as food preservatives showing their bioefficacy(Adjou et al. 2012; Soumanou and Adjou 2016). Plants belonging to Myrtaceae family like *Pimentaracemosa* and Lamiaceae family like *Mentha x piperita*, have retained the attention of researchers, due to their widespread distribution worldwideand their traditionaluse in folk medicine to treat diseases(Adjou et al.2017; Dolghi et al. 2022). 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 *Pimentaracemosa*and*Menthaxpiperita* againstfungiisolatedfrom tomatoes.

## MaterialandMethods

### *Collectionofplant leaves*

Plant materials used for essential oil (EO) extraction consisted of

leavesfrom*Pimentaracemosa*(Figure 1)and*Menthaxpiperita*(Figure 2).Leaves of *Pimentaracemosa* and *Menthapiperita* 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.

***Essentialoil extraction***

"The collected plant materials were stored in the laboratory between 19 °C and 20 °C in the shade of the sunlight throughout the period of extraction. 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 in tightly sealed amber glass vials for further analyses" (de Billerbeck et al. 2001).

***Gaschromatography–massspectrometryanalysis***

"Thevolatilecompoundsof*Pimentaracemosa*and*Menthax piperita*oilsweredeterminedbyusinggas-chromatography/mass-spectrometry(GC/MS).The analysis conditions were as described in our previouslypublished study" (Tanoh et al. 2020).

***Collectionoftomatofruits***

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.

***Fungal strain***

Subcultures of *Fusariumoxysporum* strainsobtained from rotted tomato (Akotowanou et al. 2023), were used in the present study. Strains are repeatedly sub-cultured on Sabouraud medium before their use.

***AntifungalAssay***

The antifungal assay was performed as described by Billerbecketal.(2001), using de Sabouraudmediumwithdifferent concentrationsofessentialoil(0.75,1.25,2.5or5.0µl.ml$^{-1}$) and Tween 20. About20mlofthemediumwaspouredintoglassPetri dishes(9cm).*Fusariumoxysporum*grownonSabouraudmedium,were transplanted (subcultured), using a 6 mm diameter disc

carryingsporesfromtheanamorphmold,onthesurfaceofaPetridishcontainingtheaforementionedSabouraudmediumandessentialoilatdifferentconcentrations. Controlplateswerealsoinoculated followingthesameprocedure.Plateswereincubatedat 25 °C, and the mycelial growth was evaluatedas described by Yehouenoue t a l. (2012). The percentageinhibition(PI)offungalgrowthwasevaluated asdescribedbyKumaretal. (2007)usingthefollowing equation: PI = [1- (d/dc)] ×100 (where d and dc represent the diameters of the growthzoneinthetestplate,andinthecontrolplate,respectively).

***Determinationofthe FungistaticorFungicidalactivity***

"Experimental concentrations where no mycelial growth was observed, were used for the determination of fungistatic or fungicidal activity],by reintroducing the mycelial disc that did not germinate at the end of the incubation, into anew culture medium(the former one) withoutessentialoil". (Yehouenoue t a l. 2012).Ifthemycelialgrowthisconsistentlyinhibited, the plant extract is fungicidal at this concentration, allowingthedeterminationoftheMinimalFungicide Concentration(MFC).Inthecontrarycase,itbecamesfungiostaticactivitywhichisrelatedtotheMinimal Inhibitory Concentration (MIC).

***StatisticalAnalysis***

Experimentswereperformedintriplicate,anddataanalyzed are represented as means ±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).

**Resultsanddiscussion**

By hydrodistillation, the leaves of *Pimentaracemosa*(Mill.) and *Mentha x piperita* (L.) yielded 4.3% and 1.76% (w/w) respectively, of essential oils. Chemical analysis of the componentsoftheessentialoilof*Pimentaracemosa*(Mill.) v i a GC/MSledto t h e identificationof26components, representing 99.98 % of the essential oil of *Pimentaracemosa*(Table 1). *Pimentaracemosa*oil exhibitedchemical 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*Menthaxpiperita*(L.)allowedtheidentificationof 22 components, representing 99.9% of the essential oil (Table 2). The chemical composition was characterized by L-menthone(17.19%),menthofuran(26.78%)andmenthol (24.79%). Results from the evaluation

of the antifungal potential of the essential oils against *Fusariumoxysporum*(Table 3), indicated that oils exhibited pronounced antifungal activity against the growth of *Fusariumoxysporum*.Specifically, the MICand MFC of essential oil of *Pimentaracemosa*, was found to be respectively 0.75µl.ml$^{-1}$and 1.25µl.ml$^{-1}$ against the fungal tested. However, the MICand MFCof the essential oil of *Mentha x piperita*, was found to be 3.75µl.ml$^{-1}$and 5 µl.ml$^{-1}$µl.ml$^{-1}$respectively. Results of mycelial percentage growth inhibition(PI),calculated asdescribedbyKumaretal. (2007), are presentedinTable4. These results indicatedthatthe 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 (Voda et al. 2003). This study investigates the antifungal potential of essential oils from fresh leaves of *Pimentaracemosa*and *Mentha x piperita* as promising plant-based antimicrobials against strain of *Fusariumoxysporum*associated with tomato rots in post-harvest. The results indicated thatboth essential oils were found to be effective. However, the antifungal activity of essentialoilof *Pimentaracemosa*was found to be highlypronounced against the strain of *Fusariumoxysporum*, with the lowest MFC. The antifungal potential of *Pimentaracemosa*essential oil, may be attributedto some highly fungitoxic components in the oil. Indeed. GC/MS analysis indicated that the essential oil of *Pimentaracemosa*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 includingantimicrobial, anti-inflammatory, analgesic, antioxidant and anticancer properties. It is also widely used in food industry to protect food against spoilage (Pavesi et al. 2018; Ayou et al. 2022). Several other studies have confirmed the antibacterial potentialof eugenol against pathogens (Moharram et al. 2018; Youssef et al. 2021; SuhrandNielsen 2003). Itisbelieved thatthisantibacterialactivitycouldbeduetothe inhibitionofextracellularenzymes 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 (Salehi et al., 2019).In the present study, GC/MS data on the chemical composition of the oils depicted remarkable variation with the earlier reports on the oils

(Adjou et al. 2017; Dolghiet al. 2022; Ayoubet al. 2022). Youssef et al. (2021) and Suhr and Nielsen (2003)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 ashas been done in thisstudy.Based on the results from this investigation, essential oil of *Pimentaracemosa*, with its high antifungal potentiality, provides an opportunity to avoid synthetic chemical preservatives and offers novel approach to the management of tomato storage fungi.

**Conclusion**

The present study provides evidence that the essential oil of *Pimentaracemosa*, containing β-myrcene, chavicol, and eugenol as major components, exhibited potent antifungal activity against strain of *Fusariumoxysporum*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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**Table1.**Componentsidentifiedasconstituentsofessentialoilof*Pimentaracemosa*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-Allylphenylacetate | 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 |

**Table2.**Componentsidentifiedasconstituentsofessentialoilof*Menthax 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-sabinenehydrate | 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 |
| Menthylacetate | 17.764 | 0.32 |
| Menthofurolactone | 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 |

T**able3.***Fusariumoxysporum*colonydiametersrecorded(mm)withessentialoilsof*Pimentaracemosa*and *Menthaxpiperita*

| **Days** | **Control (without oils)** | **Essential oils concentrations** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **0.75µl.ml$^{-1}$** | | **1.25µl.ml$^{-1}$** | | **2.5µl.ml$^{-1}$** | | **3.75µl.ml$^{-1}$** | | **5.0µl.ml$^{-1}$** | |
| | | ***P.racemose*** | ***M. piperita*** | ***P. racemosa*** | ***M. piperita*** | ***P.racemosa*** | ***M. piperita*** | ***P.racemosa*** | ***M. piperita*** | ***P.racemosa*** | ***M. piperita*** |
| 1 | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a |
| 2 | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a |
| 3 | 26.0±0.0b | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a |
| 4 | 32.0±0.3b | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a |
| 5 | 37.0±0.1b | 6.0±0.0a | 32.0±0.4b | 6.0±0.0a | 24.0±0.1b | 6.0±0.0a | 22.0±0.5b | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a |
| 6 | 44.0±0.3b | 6.0±0.0a | 41.0±0.3b | 6.0±0.0a | 34.0±0.4b | 6.0±0.0a | 30.0±0.1b | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a |
| 7 | 48.5±0.2b | 6.0±0.0a | 44.5±0.3b | 6.0±0.0a | 42.0±0.7b | 6.0±0.0a | 41.0±0.4b | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a | 6.0±0.0a |

*Diameterof 6 mm is theinoculation point.Values (mm) are mean (n=3)±SD. The means followed bysame letterin the same column arenotsignificantlydifferent according toANOVAand Tukey's multiple comparison tests*

**Table4.**Percentageofmycelialgrowthinhibition(PI)

| **Concentrationsofessentialoils** | **Percentageofinhibition(%)** | |
|---|---|---|
| | ***Pimentaracemosa*** | ***Mentha x piperita*** |
| **0.75µl.ml$^{-1}$** | 100±0.0a | 8.24±0.30a |
| **1.25µl.ml$^{-1}$** | 100±0.0a | 13.40±0.20b |
| **2.5µl.ml$^{-1}$** | 100±0.0a | 15.46±0.40b |
| **3.75µl.ml$^{-1}$** | 100±0.0a | 100±0.0c |
| **5.0µl.ml$^{-1}$** | 100±0.0a | 100±0.0c |

*Values (mm) are mean (n=3)±SD. The means followed bysame letterin the same column arenotsignificantlydifferent according toANOVAand Tukey's multiple comparison tests*

**Figure 1.***Pimentaracemosa* (Mill.) J.W. Moore

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