

## Chemical composition and biological activity of the essential oil of the fruits *Pimenta dioica* against *formae speciales* of fungus *Fusarium oxysporum*

Paulo Roberto Barros Gomes<sup>1\*</sup>, Francisco Reivilândio da Silva Barros Junior<sup>2</sup>, Jonas Batista Reis<sup>2</sup>, Gustavo Oliveira Everton<sup>2</sup>, Rayone Wesley Santos de Oliveira<sup>2</sup>, Hilton Costa Louzeiro<sup>3</sup>, Maria Alves Fontenele<sup>4</sup>, Adriana Crispim de Freitas<sup>4</sup>, Maria do Livramento de Paula<sup>5</sup>, Victor Elias Mouchrek Filho<sup>2</sup>

<sup>1</sup>Instituto Federal de Educação, Ciência e Tecnologia do Pará, 68440-000 Abaetetuba - PA, Brasil

<sup>2</sup>Universidade Federal do Maranhão, 65080-805, São Luís, MA, Brasil

<sup>3</sup>Universidade Federal do Maranhão, Campus Pinheiro, MA, Brasil.

<sup>4</sup>Coordenação de Engenharia de Alimentos, Universidade Federal do Maranhão, 65915-240, Imperatriz, MA.

<sup>5</sup>Universidade Federal do Maranhão, Departamento de Farmácia, 65080-805, São Luís, MA, Brasil.

\*Author for correspondence, E-mail: prbgomes@yahoo.com.br

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### SUMMARY

We determined the chemical composition and explored the hypothesis that the essential oil of the fruits of *Pimenta dioica* inhibits the mycelial development of fungi *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *passiflorae*, *F. subglutinans* f. sp. *ananas*, *F. oxysporum* f. sp. *vasinfectum* e *F. oxysporum* f. sp. *Cubense*. To do this, we extracted the oil by hydrodistillation, identified its components by gas chromatography coupled to mass spectrometry (GC-MS) and determined the fungal activity against five special forms of *Fusarium* species. The results showed that the oil had 76.88% of eugenol and inhibited the mycelial development of fungi up to 97.78% in an average of 7.2 days. Therefore, oil is a potential natural fungicide.

*Key words:* Eugenol, hydrodistillation, volatile compounds, *Pimenta dioica*, fungistatic activity.

## RESUMEN

### Composición química y actividad biológica del aceite esencial de los frutos *Pimenta dioica* contra *formae speciales* del hongo *Fusarium oxysporum*

Determinamos la composición química y exploramos la hipótesis de que el aceite esencial de los frutos de *Pimenta dioica* inhibe el desarrollo micelial de los hongos *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *passiflorae*, *F. subglutinans* f. sp. *ananas*, *F. oxysporum* f. sp. *vasinfectum* e *F. oxysporum* f. sp. *Cubense*. Para hacer esto, extrajimos el aceite por hidrodestilación, identificamos sus componentes por cromatografía de gases acoplada a espectrometría de masas (GC-EM) y determinamos la actividad fúngica contra cinco formas especiales de especies de *Fusarium*. Los resultados mostraron que el aceite tenía 76,88% de eugenol e inhibió el desarrollo micelial de hongos hasta 97,78% en un promedio de 7,2 días. Por lo tanto, el aceite es un potencial fungicida natural.

*Palabras clave:* Eugenol, hidrodistilación, compuestos volátiles, *Pimenta dioica*, actividad fungistática.

## INTRODUCTION

The intensification of agricultural production has led to an increase in the reduction of fungal diseases that attack fruits and vegetables. Among the genera of fungi that attack these crops, we have the *Fusarium*. This genus comprises filamentous fungi widely distributed in soils, plants, organic substrates and are responsible for the deterioration of food, productivity losses and diseases in humans and contaminated animals [1]. In addition, this genus has formations and special classifications according to the pathogenic criterion. Among this classification, we highlight the *formae speciales*: *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *passiflorae*, *F. subglutinans* f. sp. *ananas*, *F. oxysporum* f. sp. *vasinfectum* e *F. oxysporum* f. sp. *Cubense*. These special forms are responsible, respectively, for wilt on tomato, passion fruit, pineapple, okra and heliconias. In this case it is necessary to adopt measures that inhibit the development of these fungi and do not pose risks to consumers and the environment.

The measures adopted to contain the action of these fungi are fungicides. However, we know that the prolonged use of synthetic fungicides has caused resistance in fungi and high toxicity to the environment and food. On the other hand, the incentives given in the development of natural fungicides are promising due to high biodegradability and

absence of waste in the environment. Thus, the use of natural fungicides ends up being an alternative in the fight against these phytopathogens [2-4].

A species of plant, whose essential oil has potential fungicidal action is *Pimenta dioica*. Studies have shown that essential oils extracted from fruits and leaves have an amount of eugenol of up to 79% [5, 6]. In the literature, the fungicidal activity of this component has been proven. However, because it is an oil there is the possibility of interaction with other components, thus interfering with bioactivity. However, the reported studies of the use of this essential oil are restricted only to the species *Fusarium oxysporum*, but none regarding the special forms.

Therefore, in this study we will determine the chemical composition and we will explore the hypothesis that the essential oil of the fruits of *Pimenta dioica* inhibits the mycelial development of fungi *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *passiflorae*, *F. subglutinans* f. sp. *ananas*, *F. oxysporum* f. sp. *vasinfectum* e *F. oxysporum* f. sp. *cubense*.

## MATERIAL AND METHODS

### Obtaining and extraction of essential oil

In this manner, we collected the fruits in the Cooperativa Agrícola Mista of the Onça LTDA Project, in the municipality of Taperoá-BA, Brazil, in August 2005, receiving the organic certificate from the Biodynamic Institute (IBD). After collection, they were dried under natural ventilation, crushed with the electric mill (Tecnal, model TE-340) and stored in polyethylene containers.

So, we extracted the oil by hydrodistillation and calculate the average yield from the measurements of density and gross material weight. For this, we weigh 30 grams of the samples and mix in 300 mL of distilled water. We then placed this mixture in a 1000 mL round bottom flask and coupled it to the Clevenger extractor under 100 °C heating in an electric blanket for four hours. After that time, the extracted oil was collected and dried by percolation in anhydrous solution of sodium sulfate. We performed these operations in triplicates and stored the samples in ampoules of amber glass under refrigeration to avoid possible losses of volatile constituents. We measured the density from a pycnometer.

### Chromatographic analysis GC/MS

The essential oil constituents were identified by gas chromatography coupled to mass spectrometry (GC-MS). We analyzed the volatile constituents in a Varian 2100 gas chromatograph coupled to an electron impact mass spectrometer and trap ion analyzer

using helium as drag gas with flow in the column of  $1\text{ mL}\cdot\text{min}^{-1}$ ; injector temperature:  $270\text{ }^{\circ}\text{C}$ , split 1:50; (15mx0.25mm) with stationary phase VF-1ms (100% methylsiloxane  $0.25\text{ }\mu\text{m}$ ) and furnace temperature programming from  $60$  to  $200\text{ }^{\circ}\text{C}$  with heating rate of  $8\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$  and  $200$  and  $290\text{ }^{\circ}\text{C}$ , with a heating rate of  $15\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ . In the Mass Spectrometer, the temperatures of the main fold ion trap and the transfer line were  $50$ ,  $190$  and  $200\text{ }^{\circ}\text{C}$ . We inject aliquots of  $1\text{ }\mu\text{L}$  (automatic injector CP-8410) of samples diluted in the proportion of  $20\text{ }\mu\text{L}$  in  $1.5\text{ mL}$  of hexane. We have identified and quantified the oil components by comparing them with the data obtained from authentic substances in reference libraries from the retention time.

### Obtaining fungi

In this way, we obtained *Fusarium* isolates from the Phytopathology Laboratory of the Nucleus of Agronomic Biotechnology of the State University of Maranhão (UEMA) (table 1), and we repeated them for Petri dish in PDA (Potato-Dextrose-Agar) medium, plastic film sealed and kept in ( $25 \pm 2\text{ }^{\circ}\text{C}$ ) for 7 days to evaluate the effect of the essential oil of *P. dioica* on the mycelial growth of phytopathogens.

**Table 1.** *Fusarium* isolates obtained from the UEMA Phytopathology Laboratory.

Species	Host	Origen (Country/ State)
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	Tomato ( <i>Lycopersicon esculentum</i> )	Pedrinhas (Brazil/Maranhão)
<i>F. oxysporum</i> f. sp. <i>passiflorae</i> ,	Passion fruit ( <i>Passiflora edulis</i> )	Cinturão verde (Brazil/Maranhão)
<i>F. subglutinans</i> f. sp. <i>ananas</i>	Pineapple ( <i>Ananas comosus</i> (L.) Merrill)	Turiaçu (Brazil/Maranhão)
<i>F. oxysporum</i> f. sp. <i>vasinfectum</i>	Okra ( <i>Abelmoschus esculentus</i> (L.) Moench)	Quebra-Pote (Brazil/Maranhão)
<i>F. oxysporum</i> f. sp. <i>Cubense</i>	Heliconia ( <i>Heliconia</i> sp)	Vassoral (Brazil/Maranhão)

### Fungistatic activity test

We added the essential oil of *P. dioica* in PDA flux medium in the presence of antibiotic at the concentration of  $1\mu\text{L}\cdot\text{mL}^{-1}$ . Subsequently, the medium was poured into Petri dishes, previously autoclaved. After solidification,  $6.0\text{ mm}$  diameter disc containing mycelium of *Fusarium oxysporum* isolates, 7 days old, were peeled to the center of each Petri dish, sealed with plastic film and kept at room temperature ( $25 \pm 2\text{ }^{\circ}\text{C}$ ). Plates containing PDA medium with phytopathogen without addition of essential oil served as controls. The evaluation of the mycelial growth was performed on the 10<sup>th</sup> day by measuring the radial growth of the colony on two orthogonal axes, and then averaged. Measurements were calculated by percentage inhibition of mycelial growth (PIC).

The expression that determines the calculation of PIC= 100. The design was completely randomized in a 2x5 factorial scheme (treatment x pathogens) with four replicates. The averages were compared by the Tuckey Test at the 5% probability level.

## RESULTS

### GC/MS chromatographic analysis

From the extraction, we obtain an average yield of 2.8% (m / m) and average density of 0.949 g mL<sup>-1</sup>. From the chromatographic analysis, we observed the presence of 17 peaks, indicating the presence of 17 compounds. We quantify these compounds from the retention time and compare these data with those obtained in the Nist 02 and Adams library [7]. In table 2, we show the 17 components of the essential oil with their respective retention times and percentage of the normalized area. Of these, the three largest are, respectively, eugenol,  $\beta$ -pinene, and 5-indanol.

**Table 2.** Identification of the compounds in the essential oil sample of the fruits of *P. dioica*.

Peak <sup>1</sup>	<sup>2</sup> RT (min)	Components	Percentage (%)
1	2.15	1-octen-3-ol	1.40
2	2.33	$\beta$ -Pinene	6.52
3	2.49	$\alpha$ - Pinene	0.28
4	2.66	o-Cymene	1.94
5	2.77	Limonene	4.09
6	3.56	Linalool	0.64
7	4.64	Sabinene	0.22
8	4.80	$\alpha$ - Terpeneol	0.13
9	5.76	5- Indanol	5.88
10	7.23	Eugenol	76.88
11	7.80	$\alpha$ - Cubebene	0.35
12	8.41	Caryophyllene	0.09
13	8.90	$\alpha$ - Caryophyllene	0.08
14	9.22	$\gamma$ - Muurolene	0.25
15	9.58	$\alpha$ - Candinene	0.19
16	9.74	$\alpha$ - Muurolene	0.22
17	9.88	$\delta$ - Candinene	0.76

**Note:** <sup>1</sup> Peak number in column elution order; <sup>2</sup> RT: Retention time of the compounds. Source: adapted from Monteiro *et al.* [8].

### Fungistatic activity

The essential oil of *P. dioica* fruits at  $1\mu\text{LmL}^{-1}$  concentration in PDA medium inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *passiflorae*, *F. subglutinans* f. sp. *ananas*, *F. oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *Cubense* both in a period of ten days of observation and in daily observations for the same period. That is, at ten days of observation, inhibition was 86.67 to 97.78% (table 3), while the daily analysis was 7.2 days (table 4).

**Table 3.** Effect of *P. dioica* essential oil on mycelial growth of different *Fusarium* species in PDA medium for ten days of incubation at  $25 \pm 2$  °C.

Species	Diameter of colonies		PIC (%)
	With essential oil	Without essential oil	
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	1.20 aB	9.0 aA	86.67
<i>F. oxysporum</i> f. sp. <i>passiflorae</i> ,	0.2 cB	9.0 aA	97.78
<i>F. subglutinans</i> f. sp. <i>ananas</i>	0.35 bcB	9.0 aA	96.11
<i>F. oxysporum</i> f. sp. <i>vasinfectum</i>	0.89 abB	9.0 aA	90.11
<i>F. oxysporum</i> f. sp. <i>Cubense</i>	1.20 aB	9.0 aA	86.67

## DISCUSSION

*Fusarium* fungi are responsible for large losses in agricultural production. Thus, one of the alternatives adopted is the use of fungicides. However, the use of synthetic fungicides proved to be a poor choice, since they caused fungal resistance, low selectivity and generation of residues in the environment. Therefore, replacing this type of fungicide with a natural one ends up being a good alternative.

In this study, we extracted the essential oil from the fruits of *P. dioica*, identified the components and evaluated the inhibitory activity. From the extraction, we obtain a good yield. In the chromatographic analysis coupled to the mass spectrometer, we identified 7 teen compounds, in which the largest are, respectively, eugenol,  $\beta$ -pinene and 5-indanol. From the analysis of activity, we verified that the essential oil inhibited the mycelial development of the special forms of the species *Fusarium oxysporum* by up to 97.78% for an average period of 7.2 days. Therefore, the use of this essential oil shows itself as a potential fungicide.

The oil yield and the quantification of the major component that we obtain in this study are consistent with results obtained in the literature. The result of our income

**Table 4.** Effect of the essential oil of the *P. dioica* fruits on the mycelial growth of the *Fusarium* PDA species after ten days of incubation at 25 °C ± 2 °C.

Day	<i>F. oxysporum</i> f. sp. <i>lycopersici</i> <sup>a</sup>		<i>F. oxysporum</i> f. sp. <i>passiflorae</i> <sup>b</sup>		<i>F. subglutinans</i> f. sp. <i>anasas</i> <sup>c</sup>		<i>F. oxysporum</i> f. sp. <i>vasinfectum</i> <sup>d</sup>		<i>F. oxysporum</i> f. sp. <i>cubense</i> <sup>e</sup>	
	With oil	Without oil	With oil	Without oil	With oil	Without oil	With oil	Without oil	With oil	Without oil
1	0 bC	2.8 aG	0 bC	3.4 aG	0 bC	3.1 aG	0 bC	2.8 aH	0 bC	3.4 aG
2	0 bC	3.8 aF	0 bC	4.3 aF	0 bC	4.0 aF	0 bC	3.7 aG	0 bC	4.3 aF
3	0 bC	4.6 aE	0 bC	5.2 aE	0 bC	5.0 aE	0 bC	4.7 aF	0 bC	5.2 aE
4	0 bC	5.6 aD	0 bC	6.3 aD	0 bC	6.3 aD	0 bC	5.9 aE	0 bC	6.3 aD
5	0 bC	6.8 aC	0 bC	7.5 aC	0 bC	7.3 aC	0 bC	6.7 aD	0 bC	7.5 aC
6	0 bC	7.6 aB	0 bC	8.4 aB	0 bC	8.0 aB	0 bC	7.6 aC	0 bC	8.4 aB
7	0 bC	8.8 aA	0 bC	8.8 aA	0 bC	8.8 aA	0 bC	8.6 aB	0 bC	8.8 aA
8	0.5 bBC	9.0 aA	0 bC	9.0 aA	0.16 bB	9.0 aA	0.35 bA	9.0 aA	0 bC	9.0 aA
9	1.2 bAB	9.0 aA	0.15 bA	9.0 aA	0.54 bAB	9.0 aA	0.35 bA	9.0 aA	0.15 bA	9.0 aA
10	1.2 bAB	9.0 aA	0.2 bA	9.0 aA	0.9 bA	9.0 aA	0.35 bA	9.0 aA	0.2 bA	9.0 aA
average	0.3	6.71	0.042	7.11	0.16	6.95	0.1	6.7	0.042	7.11

<sup>a</sup> The averages followed by the same letter do not differ statistically from each other. We applied the Tukey test at the 5% probability level. CV% = 10.8; DMS = 0.43 (columns) represented by capital letters DMS = 0.7 (lines), represented by lower case letters. \* BC.V% = 4.0; DMS = 0.16 (columns) represented by upper case letters DMS = 0.27 (lines), represented by lower case letters. \* C The Tukey test was applied at a 5% probability level. DMS = 0.38 (columns) represented by upper case letters DMS = 0.61 (lines), represented by lower case letters. \* D CV% = 6.2; DMS = 0.24 (columns) represented by capital letters DMS = 0.4 (lines), represented by lower case letters. \* and CV% = 5.39; DMS = 0.22 (columns) represented by capital letters DMS = 0.36 (lines), represented by lowercase letters.

was 2.8% (m/m). This value is within the stipulated range when the essential oil is extracted from the fruits of *P. dioica*, which is 1.5 to 4.5%. Generally, the major components of this oil are eugenol, and the amounts thereof range from 60 to 79% [5, 6, 9]. Thus the results of our study, whose value was 76.88% for the eugenol component, are in agreement with those found in the literature [10-13].

We know that the essential oil extracted from *P. dioica* has antioxidant and cytotoxic activity against some cancer strains [13], molluscicide against *Biomphalaria glabrata* [14], acaricide [12], antibacterial in foods [15, 16], larvicide and adulticide against *Aedes aegypti* [17], antiradical [18] and antifungal against only *Fusarium oxysporum* and *F. verticillioides* [19]. As we see, until now there is no study of the use of this essential oil against special forms *F. oxysporum* f. sp. *passiflorae*, *F. subglutinans* f. sp. *ananas*, *F. oxysporum* f. sp. *vasinfectum* e *F. oxysporum* f. sp. *Cubense*.

Despite the lack of studies for these special forms using the essential oil of *P. dioica*, we found that some components present in the oil have inhibitory activity. For example, the studies performed by [20, 21] showed, respectively, that  $\alpha$ -Pinene has low inhibition against *F. subglutinans* and high against *F. oxysporum* f. sp. *vasinfectum*. In our study, this component was present, but its action with other components increased the inhibition rate against *F. subglutinans* to values of 96.11%, whereas activity was still maximal for *F. oxysporum* f. sp. *vasinfectum*. The study by Zabka *et al.* [19] showed that the eugenol obtained from the essential oil of *P. dioica* inhibited in 100% the growth of fungi *F. oxysporum* and *F. verticillioides*.

Although the study of Zabka *et al.* [19] was not applied to the special forms of *F. oxysporum*, we observed that there were differences between our results and theirs. To explain this difference, we resort to the influence of seasonality. As this variable influences the oil yield, it will also influence the biological activity of an essential oil. In our study, the collection and extraction of the oil occurred in the month of August, whose season is winter. In a study of the larvicidal activity of essential oils of the leaves of *Laurus nobilis* and *Tetradenia riparia* showed higher activity in the spring season and low in the other seasons [22, 23]. Therefore, several factors may influence the bioactivity of essential oil.

Although our study was limited only to verify mycelial inhibition, its merit lies in the potentiality and use of this essential oil as a natural fungicide. However, studies determining minimum inhibitory concentration, in loco and toxicity are required to ensure the safety and proper use of this material.



## CONCLUSION

We analyzed the essential oil obtained from the fruits of *Pimenta dioica* and determined the fungistatic activity. From the chemical analysis, we identified 7 teen compounds, in which the three largest are, respectively, eugenol,  $\beta$ -pinene and 5-indanol. From the biological activity, we verified that the essential oil inhibited the fungi of the special forms of the species *Fusarium oxysporum* in an average period of 7 days. Therefore, oil is a potential fungicidal agent.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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