Journal of
Plant Pathology & Microbiology
ISSN: 2157-7471

Erisléia-Meireles et al., J Plant Pathol Microbiol 2016, 7:2
DOI: 10.4172/2157-7471.1000333

Research Article Open Access

Phenylpropanoids Produced by *Piper divaricatum*, A Resistant Species to Infection by *Fusarium solani* f. sp. *piperis*, the Pathogenic Agent of Fusariosis in Black Pepper

Erisléia-Meireles N[1], Luciana-Xavier P[1], Alessandra-Ramos R[2], José-Guilherme MS[3,4] William-Setzer N[4] and Kelly-da-Silva JR[1]*

[1]*Graduate Program in Biotechnology, Federal University of Pará, Belém, Brazil*
[2]*Institute of Studies in Healthcare and Medicine, Federal University of South and Southeast of Pará, Maraba, Brazil*
[3]*Program of Graduate Studies in the Amazon Natural Resources, Federal University of Western Pará, Santarém, Brazil*
[4]*Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL, USA*

Abstract

In a previous study, the essential oil (EO) of *Piper divaricatum* showed strong antifungal activity *in vitro* against *Fusarium solani* F. sp. *piperis*. For this reason, the seedlings of *P. divaricatum* were inoculated with the pathogen to evaluate the *in-vivo* resistance. Evaluation of symptoms and analysis of secondary metabolite production from infected and non-infected plants occurred at 7, 21, 30 and 45 days post inoculation (dpi). Throughout the experiment, the infected seedlings did not display symptoms of infection or significant variation of total phenolic compounds. However, lipoxygenase (LOX) enzymatic activity, which is involved in the defense mechanism, had increased in the 21 and 45 dpi. In addition, the EOs were analyzed by GC-MS. The EOs from infected plants showed a substantial increase in the concentrations of phenylpropanoid compounds, particularly on days 21 and 45 dpi. The main constituent was methyl eugenol, which reached 95.3% in the infected sample at 45 dpi, followed by eugenol acetate with a variation of 6.1% to 10.7%, between 21 and 30 dpi. These results suggest an incompatible plant-pathogen interaction and involvement of various volatile compounds from *P. divaricatum* in the resistance mechanism.

Keywords: *Piper nigrum*; Plant-pathogen interaction; Volatile compounds; Methyl eugenol; Lipoxygenase activity; Phenolic content

Introduction

Piper divaricatum G. Mey. [syn: *Arthanthe adenophylla* Miq., *A. colubrina* (Link ex Kunth) Miq., *Peltobryon attenuatum* Klotzsch, *Piper apiculatum* C. DC, *P. belemense* C. DC, *P. colubrinum* Kunth, *P. crassifolium* (Miq.) C. DC, *P. crassum* Vell, *P. glabellum* (Miq.) C. DC, *P. glabrilimbum* C. DC, *P. insipiens* Trel. and Yunck., *P. nitidum* Vahl, *Schilleria colubrina* Link ex Kunth, among others] is distributed in northeastern and southeastern South America [1-3]. It is a shrub-like aromatic plant growing up to 3 m and is known in the Brazilian Amazon as "piper-pau-de-angola".

There have been several previous studies on the phytochemistry of *P. divaricatum*. The ethanolic extract of a specimen from Antioquia, Colombia, used locally as a medicinal and insecticide, showed 4,5-dioxyaporphine alkaloids, γ-asarone and 4-hydroxy-2,5-dimethoxy-allylbenzene, as the main constituents [4]. The seasonal and circadian studies of the essential oil of a specimen collected in Breves, Pará state, Brazil, showed methyl eugenol and eugenol as the principal volatile compounds [5]. Hierarchical cluster analysis of the leaf oils of eleven samples of *P. divaricatum*, collected in the Brazilian Amazon, resulted in the discovery of two different chemical types: a methyl eugenol + eugenol chemotype and an elemicin-rich chemotype [6]. The antioxidant and antifungal activities of the methyl eugenol + eugenol chemotype of *P. divaricatum* has also been reported [7]. Another specimen of *P. divaricatum*, harvested in Itabuna, Bahia state, Brazil, has provided oil from the leaves indicating a safrole-rich chemotype [8]. More recently, it was reported that the EO of the methyl eugenol + eugenol chemotype showed high *in-vitro* fungicidal potential against the phytopathogen *Fusarium solani* f. sp. *piperis*, which causes fusariosis in black pepper (*Piper nigrum L.*) [9].

Black pepper (*Piper nigrum L.*, Piperaceae) is one of the most famous and oldest spices in the world, with culinary and food preservative uses. The largest producers of black pepper are Brazil and India. The harvest in Brazil has been about 50 thousand tons per year, with about 90% produced in the Pará State [10]. The cultivation of black pepper has been threatened by infestations of *Phytophthora capsici* (in India) and *Fusarium solani* f. sp. *piperis* (in Brazil), two phytopathogenic fungi that cause severe diseases to the plant. Fusariosis leads to obstruction of the conducting vessels of the plant, root rot and death [11]. Control measures for the disease caused by *Fusarium* have been taken. However, variability and low homogeneity in the cultivation of *P. nigrum* has contributed to the spread of the fungus. Thus, strategies to increase black pepper resistance have been performed, including micrografting, micropropagation and *in-vitro* plant regeneration, development of molecular markers and identification of genes related to the infection process [12].

The resistance of different plants to pathogens may be related to the production of primary and secondary metabolites that act as natural antimicrobials [13]. In plant-pathogen interactions, several reactions occur simultaneously, and often the pathogen can overcome the structural and biochemical defenses of the plant in order to colonize its tissue [14]. Studies have suggested flavonoid and phenylpropanoid compounds to be responsible for the induction of fungal infection resistance, as observed in citrus fruits infected by *Penicillium digitatum* [15]. Some studies with native *Piper* species in the Amazon, such

***Corresponding author:** Kelly-da-Silva JR, Graduate Program in Biotechnology, Federal University of Para, Bethlehem 66075-110, Brazil, Tel: 55 94 3778 3253; E-mail: joycekellys@ufpa.br

Received February 09, 2016; **Accepted** February 11, 2016; **Published** February 25, 2016

Citation: Erisléia-Meireles N, Luciana-Xavier P, Alessandra-Ramos R, José-Guilherme MS, William-Setzer N, et al. (2016) Phenylpropanoids Produced by *Piper divaricatum*, A Resistant Species to Infection by *Fusarium solani* f. sp. *piperis*, the Pathogenic Agent of Fusariosis in Black Pepper. J Plant Pathol Microbiol 7: 333. doi:10.4172/2157-7471.1000333

as *P. aduncum* L., *P. colubrinum* Kunth (syn: *P. divaricatum*), *P. tuberculatum* Jacq., *P. hispidinervum* C. DC. and *P. hispidum* Sw. were carried out in search of specimens resistant to infection by *Fusarium*, aiming at development of interspecific hybrids [16]. *Piper colubrinum* showed strong resistance to infection in the soil by *F. solani* f. sp. *piperis* and, when used as a rootstock in *P. nigrum*, showed high tissue compatibility and fixation (98%). Unfortunately, the grafted seedlings have shown delayed compatibility issues, which decreased the life cycle in the field [17].

In this perspective, the aim of this study was to evaluate the resistance of *P. divaricatum* to *Fusarium* disease and analyze the production of metabolites after inoculation of the pathogen. In this context, it should be realized that metabolomics studies have assisted in understanding the biological system and have shown success in many plants, including, for example, *Arabidopsis thaliana* (L.) Heynh. and tobacco (*Nicotiana tabacum* L.) [18,19].

Materials and Methods

Plant and fungus material

Piper divaricatum was collected in the municipality of Breves, Pará state, Brazil, in July 2012. The cuttings containing two nodes were propagated in propylene bags with commercial fertilizer. The seedling growth was monitored for six months until root development. As a control group susceptible to *Fusarium* disease, some ten-month-old seedlings of *Piper nigrum*, which were obtained from the Bragantina cultivar, were used. *Fusarium solani* f. sp. *piperis* was isolated from an individual of *P. nigrum* with the symptoms of *Fusarium* disease, after twenty days of infection. Small pieces were cut near the site of infection, cleaned with ethanol, sterile water, and 10% sodium hypochlorite, for 1 min. After 10 days of contact with the medium (PDA, potato dextrose agar), at temperature of 27°C, the fungus was isolated and cultured in Petri dishes for use in the tests, which took place after 15 days of growth.

Fungal infection

To prepare the solution of inoculum, 10 mL of sterile water was added to the Petri dishes and the conidia were removed with a spatula. The concentration of spores in the suspension was 2.1×10^6 mL^{-1}, measured in a Neubauer chamber. For inoculation, 18 seedlings of *P. divaricatum* and 18 seedlings of *P. nigrum* were removed from the plastic bags and their roots washed under running water to remove excess soil. Micro wounds were made in the roots of the seedlings to facilitate the penetration of spores, followed by immersion for 5 min in the conidial suspension. For the control group, only distilled water was used. The seedlings were replanted and maintained in a greenhouse for 45 days with water irrigation twice per day. The monitoring of secondary metabolites in the plant and observation of disease symptoms were made at intervals of 7, 21, 30 and 45 days post-inoculation (dpi). After 65 days, the pathogen was again isolated from the seedlings of *P. divaricatum*. Figure 1 illustrates the experimental design.

Folin-Ciocalteu total phenolics determination

The leaves of infected and uninfected plants were collected at random in steps 7, 21, 30 and 45 dpi. Fresh plant material (1 g) was cut and extracted with 20 ml of ethanol/water (7: 3), at 60°C for 30 min. The extracts were obtained in triplicate and stored in amber vials under refrigeration at 4°C. The total phenolics content was determined by the Folin-Ciocalteu method [20]. An aliquot of 500 µL was used to react with 250 µL of reagent (1N) and 1250 µL of sodium carbonate (75 g L^{-1}). After 30 min incubation in the dark, the absorbance of the mixture was read at 760 nm using a UV-Visible spectrophotometer. The experimental calibration curve was prepared using gallic acid at concentrations of 0.5 and 10.0 mg L^{-1} and the content of total phenolics was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry leaves (mg GAE g^{-1}).

Lipoxygenase (LOX) activity

To prepare the substrate, 78 µL of linoleic acid (Sigma-Aldrich, USA) and 90 µL Tween 20 (Sigma-Aldrich) were mixed with 10 mL of boiling water and a few drops of sodium hydroxide (0.5 N). The final volume was adjusted to 25 mL, resulting in a sodium linoleate solution (10 mM), which was stored at -20°C. The LOX activity determination was carried out with 5 µL of crude leaf extract and 50 µL of sodium linoleate (10 mM), mixed with 1950 µL of sodium phosphate buffer (50 mM) at pH 6.5. The absorbance at 234 nm for the reaction was monitored for 150 s, at continuous intervals of 30 s, using a UV-Visible spectrophotometer. The increase in absorbance at 234 nm indicated the presence of a conjugated double bonds system in the formed hydroperoxide [21].

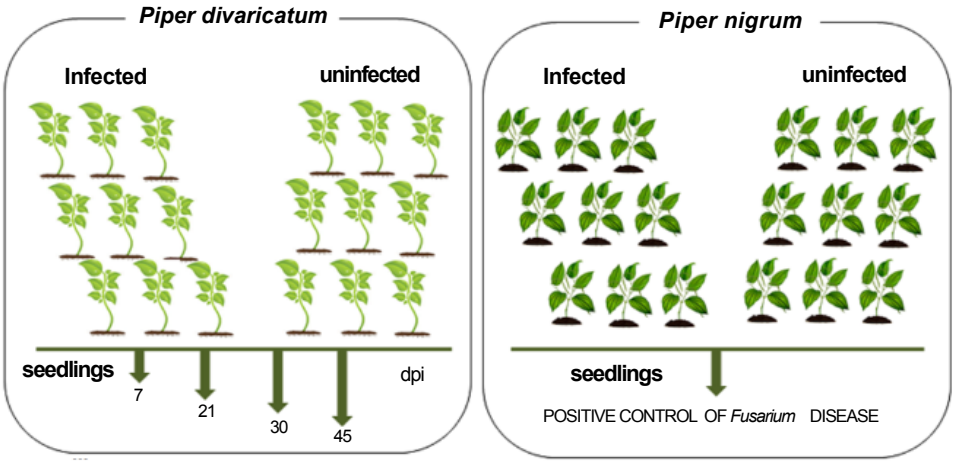

Figure 1: Schematic drawing of the treatments to evaluate the resistance of *Piper divaricatum* seedlings after inoculation with *Fusarium solani* f. sp. *piperis*. Seedlings of *Piper nigrum* cv. Bragantina were used as positive control of the disease. dpi = days post inoculation.

Volatiles

The volatile concentrate of leaves (3 g) of *P. divaricatum* was obtained by hydrodistillation with continuous extraction with *n*-pentane using a Likens-Nickerson apparatus for 2 h. After extraction, an aliquot of 1 µL was analyzed by gas chromatography. Qualitative analysis was carried out on a THERMO DSQ II GC–MS instrument, under the following conditions: DB-5 ms (30 m 0.25 mm; 0.25 mm film thickness) fused-silica capillary column; programmed temperature: 60-240°C (3°C min^{-1}); injector temperature: 250°C; carrier gas: helium, adjusted to a linear velocity of 32 cm s^{-1} (measured at 100°C); injection type: 1 µL of solution, splitless mode, split flow was adjusted to yield a 20:1 ratio; septum sweep was a constant 10 mL min^{-1}; EIMS: electron energy, 70 eV; temperature of ion source and connection parts: 200°C. Quantitative data regarding the volatile constituents were obtained by peak-area normalization using a FOCUS GC/FID operated under GC-MS similar conditions, except for the carrier gas, which was nitrogen. The retention indices were calculated for all volatile constituents using an *n*-alkane (C8–C40, Sigma–Aldrich) homologous series. Individual components were identified by comparison of both mass spectrum and GC retention data with authentic compounds, which were previously analyzed and stored in private library, as well as with the aid of commercial libraries containing retention indices and mass spectra of volatile compounds commonly found in essential oils [22].

Statistical analysis

The extracts and essential oils were obtained of leafs collected from five individuals. The chemical composition of volatile compounds is represented as mean. In the Total phenolic content and LOX activity assays, the samples were assayed in triplicate, and the results are shown as means ± standard deviation. Analysis of variance was conducted, and the differences between variables were tested for significance by Tukey test, using GraphPad Prism 5.0 software. Differences at $p < 0.05$ were considered statistically significant.

Results

The resistance of *P. divaricatum* seedlings were compared to the resistance of *P. nigrum* seedlings to the infection and symptoms of *Fusarium* disease for 45 days (Figure 2). Throughout the duration of the experiment, no wilting symptoms or drying of the branches of *P. divaricatum* were observed. The plants were healthy and some of the individuals started their normal reproductive period with flowering and fruiting. In the experiment with *P. nigrum*, however, the first signs of yellowing leaves began on 21 dpi. By 30 dpi there was obvious necrosis of some leaves, with the advancement of these effects until the 45th day. The progression of infection in *P. nigrum*, with the total drying of the branches and death of most plants, occurred by 65 dpi (Figure 2).

During the experiment, the total phenolic content ranged from 10 to 18 mg GAE g^{-1} in the fresh leaf matter. No statistically significant differences were observed between infected and non-infected plants. On the other hand, the lipoxygenase activity was higher at days 21 and 45 dpi. At day 21, the lipoxygenase activity in infected plants was 2.2 ± 0.4 × 10^{-7} M s^{-1}, which corresponded to an increase of about 50% over the uninfected plants (1.5 ± 0.3 × 10^{-7} M s^{-1}). On 45 dpi, the lipoxygenase activity of the infected plants was 1.6 ± 0.2 × 10^{-7} M s^{-1}, which corresponded to twice the rate observed for the uninfected plants (0.8 ± 0.1 × 10^{-7} M s^{-1}) (Figure 3).

The essential oils of *P. divaricatum* were analyzed by GC and GC-MS. These concentrates were obtained from infected and non-infected seedlings, between 7 and 45 dpi. Eighteen constituents were identified in the essential oils and phenylpropanoids were the main components, comprising about 94% of the total composition. Additionally, there was a substantial increase in phenylpropanoid concentrations in the leaf oils of infected plants compared to non-infected plants, particularly in the samples collected at 21 and 45 dpi (Table 1).

At 7 dpi was possible to visualize qualitative and quantitative differences in the essential oils of *P. divaricatum*, depending on the infection. α-Pinene, myrcene and (*Z*)-β-ocimene were identified only in infected seedlings, but there was an increase in production of methyl eugenol (88.6 to 91.0%) and (*E*)-β-ocimene (0.3 to 1.1%), and a decrease in the production of β-caryophyllene and germacrene D. At 21 dpi, in addition to the presence of linalool, there was an increase in the production of methyl eugenol (81.5 to 88.3%) and (*E*)-β-ocimene (1.3 to 2.0%), a decrease in eugenol acetate, β-elemene and β-caryophyllene, and the absence of β-copaene, α-humulene, bicyclogermacrene and germacrene A. At 30 dpi, in addition to production of eugenol, there was a decrease in the production of methyl eugenol (93.3 to 85.7%),

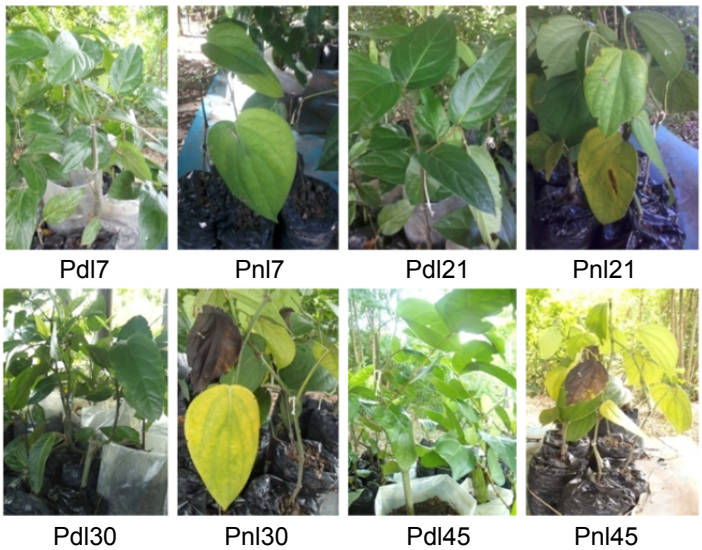

Figure 2: Symptoms of the *Fusarium* disease. Comparison of seedlings of *Piper divaricatum* (Pd) and *Piper nigrum* (Pn), in the intervals of 7 to 45 days.

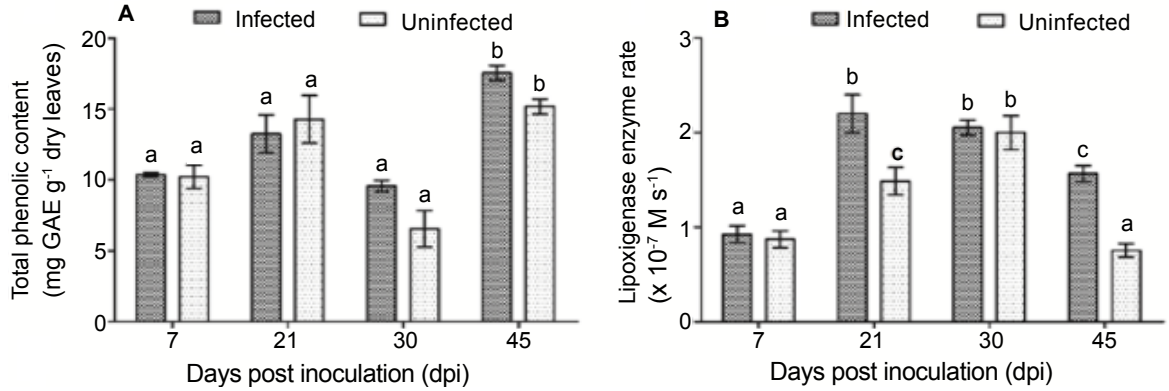

Figure 3: (A) Content of phenolic compounds in extracts of *Piper divaricatum*. (B) The speed of lipoxygenase enzyme. [a,b,c]Different letters represent a statistically significant difference ($p < 0.05$).

Constituents	RI[a]	RI[b]	Pd7i	Pd7ni	Pd21i	Pd21ni	Pd30i	Pd30ni	Pd45i	Pd45ni
α-Pinene	934	932	0.1*			0.1				0.9
Myrcene	990	988	0.1*			0.1				0.1
Limonene	1026	1024								0.3
(*Z*)-β-ocimene	1035	1032	0.1*		0.2	0.2	0.1-	0.2		
(*E*)-β-ocimene	1045	1044	1.1+	0.3	2.0+	1.3			0.5	0.8
Linalool	1095	1095			0.1*					
Eugenol	1357	1356	**2.1**	**2.2**	**0.8**	**0.7**	**0.6***		**0.1-**	**1.2**
β-Elemene	1390	1389	2.9	2.7	1.4-	3.3	1.5+	1.0	1.4-	2.5
Methyl eugenol	1403	1403	**91.0+**	**88.6**	**88.3+**	**81.5**	**85.7-**	**93.3**	**95.3+**	**80.6**
β-Caryophyllene	1416	1417	1.0-	1.9	0.4-	1.5	0.6	0.8	0.7-	1.3
β-Copaene	1430	1430		0.3		0.2		0.1	0.1*	
α-Humulene	1455	1452		0.2		0.1	0.4+	0.1	0.1	0.1
Germacrene D	1484	1484	0.7-	2.2	0.4-	0.7		1.5	0.7-	1.7
Asaricin	1495	1495		**0.3**						
Bicyclogermacrene	1500	1500	0.1-	0.2		0.1		0.2	0.1-	0.2
Germacrene A	1508	1508	0.2	0.3		0.1		0.1		
Eugenol acetate	1522	1521	**0.4-**	**0.7**	**6.1-**	**9.4**	**10.7+**	**2.3**	**1.0-**	**9.1**
Elemicin	1555	1555	**0.1-**	**0.2**	**0.2**	**0.2**	**0.2-**	**0.3**	**0.1**	**0.1**
Hydrocarbon monoterpenes			1.4+	0.3	2.2	1.7	0.1-	0.2	0.5-	2.1
Monoterpene oxygenated					0.1					
Hydrocarbon sesquiterpenes			4.9-	7.8	2.2-	6.0	2.5-	3.8	3.1-	5.8
Phenylpropanoids			93.6	92.0	95.4+	91.8	97.2	95.9	96.5+	91.0
Total			98.5	99.8	97.7	97.8	99.8	99.4	99.6	96.8

RI[a] = Calculated retention index (DB-5ms capillary column, C_8-C_{30} *n*-alkanes); RI[b] = Literature retention index (Adams, 2007); *Constituent emission only in infected plants; +Constituents with increased content in infected plants; -Constituents with reduced content in infected plants; bold letters/numbers = phenylpropanoids

Table 1: Variation in the essential oil compositions of *Piper divaricatum* infected and non-infected by *Fusarium solani* f. sp. *piperis*, over a period of 45 days.

increased production of eugenol acetate (2.3 to 10.7%) and an absence of germacrene D, bicyclogermacrene and germacrene A. Finally at 45 dpi, there was a significant increase in methyl eugenol content (80.6 to 95.3%), decreased production of eugenol acetate (9.1 to 1.0%), and lack of α-pinene, myrcene and limonene.

Discussion

Incompatibility can explain the absence of symptoms of *Fusarium* infection in *P. divaricatum* in plant-pathogen response. In the compatible reactions, if the pathogen is virulent and the host susceptible, the disease occurs. In incompatible reactions if the pathogen is virulent and the host resistant, the disease is not installed [23]. For *Piper nigrum*, the positive control group of *Fusarium* disease, the wilting of aerial parts after 21 dpi was observed, and after that, necrosis of the root tissue.

Pathogens that cause wilting in plants, such as *F. solani* f. sp. *piperis*, have a hemi-biotrophic life cycle. Initially, it is installed in living tissue and then in the dead tissue [24]. After colonization of the root tissue, distribution in the vascular system occurs and, with the death of the plant, the fungus in the infected tissue survives as a saprophyte [25]. During the development stage, toxins are released in the host tissue, which in turn induces the production of defense molecules in an attempt to inhibit invasion. This process occurs within the vascular system, causing the obstruction of vessels that carry nutrients and water [26].

The plant response to pathogen attack is a complex mechanism, involving several defense strategies and different biochemical pathways [27]. The chain of events begins with the recognition of elicitors (molecules responsible for activating the integrated signaling network) by specific receptors of the host, and ends with the activation of

resistance genes [28]. This induced-defense mechanism is represented by two steps: a hypersensitivity reaction that induces the rapid elimination of cells located at the site of infection and the systemic response acquired, more lasting and effective, which spreads in all plant tissues [29].

The interaction with pathogens directly affects the metabolism of the host plant, which reorganizes its primary metabolism to create defense mechanisms [30]. The production of total phenolic compounds in the leaves of *P. divaricatum* did not vary as a result of the infection (Figure 2). However, in the infected seedlings, the lipoxygenase activity had increased in 21 and 45 dpi, as well as the concentration of the phenylpropanoids in the leaf essential oils (Table 1).

Lipoxygenases catalyze the stereospecific addition of molecular oxygen in the *cis,cis*-1,4-pentadiene system of polyunsaturated fatty acids to form hydroperoxides, which are metabolized enzymatically to produce jasmonic acid and methyl jasmonate [31]. The stress caused by pathogen attack raises the levels of jasmonic acid and its methyl derivative which, in turn, upregulate the biosynthesis of phenolic and terpene compounds through the accumulation of phenylalanine ammonia-lyase and terpene-synthase enzymes, respectively [32-34].

The variation of volatile constituents in *P. divaricatum* was highly significant, when caused by *Fusarium* infection, given that some compounds were produced or changed, presumably for self-protection of the plant, in response to damage caused by the pathogen. In this regard, it is to highlight the increase in the content of phenylpropanoid compounds and the emission of monoterpene compounds, throughout the experiment [35,36]. Phenylpropanoids have numerous functions in plants, ranging from structural constituents, growth, and reproductive biochemistry and physiology to chemoecological interactions with microbes, animals (particularly insects), and neighboring plants [37]. Except for the 30th day, the emission (increase) of methyl eugenol was significant in the infected seedlings. Several studies of the inhibitory properties of methyl eugenol against plant pathogens have been described [38]. Both eugenol and eugenol acetate have shown biological activity against various pathogens.

In summary, *Piper divaricatum* showed no *Fusarium* disease symptoms during inoculation with *F. solani* f. sp. *piperis*. Also, there was increased lipoxygenase activity and variation in the production of volatile compounds such as monoterpene and sesquiterpene hydrocarbons. The disease resistance is likely related to the presence and increased production of phenylpropanoids.

Acknowledgements

We are grateful for CNPQ, CAPES and FAPESPA/PA for their financial support.

References

1. Yuncker TG (1972) The Piperaceae of Brazil. I Piper- Group 1,2,3,4. Hoehnea 2: 19-366.
2. Tebbs MC (1990) Revision of Piper (Piperaceae) in the new world 2. The taxonomy of *Piper* section Churumayu. Bulletin of the British Museum. Natural History Botany 20: 193-236.
3. Missouri Botanical Garden.
4. Avella E, Díaz PP, de Díaz AM (1994) Constituents from Piper divaricatum. Planta Med 60: 195.
5. Andrade EHA, Da Silva JKR, Ribeiro AF, Guimarães EF, Maia JGS (2009) Chemotaxonomy, seasonality and biological activity of Piper divaricatum, based on the analysis of the essential oil. Belém, Brazil: MPEG pp: 359-368.
6. Andrade EHA, Guimarães EF, Maia JGS (2009) Variability in chemical essential oils of Piper species of Belém Amazon, Brazil: Faculty of Chemical Engineering, Federal University of Pará.
7. da Silva JK, Andrade EH, Guimarães EF, Maia JG (2010) Essential oil composition, antioxidant capacity and antifungal activity of Piper divaricatum. Nat Prod Commun 5: 477-480.
8. Barbosa QPS, Da Câmara CAG, Ramos CS, Nascimento DCO, Lima-Filho JV, et al. (2012) Chemical composition, circadian rhythm and antibacterial activity of essential oils of Piper divaricatum: a new source of safrole. Quím Nova 35: 1806-1808.
9. Da Silva JK, Silva JR, Nascimento SB, da Luz SF, Meireles EN, et al. (2014) Antifungal activity and computational study of constituents from Piper divaricatum essential oil against Fusarium infection in black pepper. Molecules 19: 17926-17942.
10. Lemos TLG, Matos FJA, Alencar JW, Craveiro AA, Clark AM, et al. (1990) Antimicrobial activity of essential oils of Brazilian plants. Phytother Res 4: 82-84.
11. Duarte MLR, Albuquerque FC (1999) Doenças da cultura da pimenta-do-reino. Belém, Brazil: Embrapa Amazônia Oriental pp: 159-208.
12. Lemos OF, Poltronieri MC, Rodrigues SM, Menezes IC, Mondin M (2011) Conservation and Breeding of Pimenteira - the - kingdom (*Piper nigrum* L.) Associated with the techniques of biotechnology. Belém, Brazil: Embrapa Amazônia
13. Zhang Y, Lubberstedt T, Xu M (2013) The genetic and molecular basis of plant resistance to pathogens. J Genet Genomics 40: 23-35.
14. Ballester A, Lafuente MT, González-Candelas L (2013) Citrus phenylpropanoids and defense against pathogens. Part II: gene expression and metabolite accumulation in the response of fruits to Penicillium digitatum infection. Food Chem 136: 285-291.
15. Poltronieri MC, Lemos OF, Albuquerque FC (1999) Pepper, United (*Piper nigrum* L.) Brazilian Agricultural Research Corporation.
16. Albuquerque FC, Duarte MLR, Benchimol RL, Endo T (2001) Resistance native piperaceae the Amazon to infection caused by Nectria haematococca f. sp. piperis. Acta Amaz 31: 341-348.
17. Kim JK, Bamba T, Harada K, Fukusaki E, Kobayashi A (2007) Time-course metabolic profiling in Arabidopsis thaliana cell cultures after salt stress treatment. J Exp Bot 58: 415-424.
18. Blount JW, Masoud S, Sumner LW, Huhman D, Dixon RA (2002) Over-expression of cinnamate 4-hydroxylase leads to increased accumulation of acetosyringone in elicited tobacco cell-suspension cultures. Planta 214: 902-910.
19. Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Method Enzymol 299: 152-178.
20. Axelrod B, Cheesbrough TM, Laasko S (1981) Lipoxygenases from soybeans. Method Enzymol 71: 441-451.
21. Adams R (2007) Identification of Essential Oil Components by Gas Chromatography Mass Spectrometry, Carol Stream, Illinois, USA: Allured Publishing Corporation.
22. Resende MLV, Salgado SML, Chaves ZM (2003) Active oxygen species in plant defense response to pathogens. Fitopatol Bras 28: 123-130.
23. Cavalcanti LS, Coelho RSB, Perez JO (2002) Use two methods inoculation in cultivar resistance evaluation and bean lines to *Fusarium oxysporum* f sp. phaseoli. Cienc Rural 32: 1-5.
24. Michereff J, Domingos T, Andrade M (2005) Ecology and management of pathogens root in tropical soils. Pernambuco, Brazil.
25. Wheeler T, Rush M (2001) Soil inhabitant. Wiley 935-947.
26. Dixon RA, Harrison MJ (1990) Activation, structure, and organization of genes involved in microbial defense in plants. Adv Genet 28: 165-234.
27. Veronese P, Ruiz MT, Coca MA, Hernandez-Lopez A, Lee H, et al. (2003) In defense against pathogens. Both plant sentinels and foot soldiers need to know the enemy. Plant Physiol 131: 1580-1590.
28. Hammond-Kosack K, Jones J (2000) Responses to plant pathogens. Biochem Mol Biol Plant 21: 1102-1156.
29. Pérez-Bueno ML, Pineda M, Diaz-Casado E, Barón M (2014) Spatial and temporal dynamics of primary and secondary metabolism in Phaseolus vulgaris challenged by Pseudomonas syringae. Physiol Plantarum 153: 161-174.

30. Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. Plant Cell 14 Suppl: S153-164.

31. Dudareva N, Pichersky E, Gershenzon J (2004) Biochemistry of plant volatiles. Plant Physiol 135: 1893-1902.

32. Fonseca S, Chico JM, Solano R (2009) The jasmonate pathway: the ligand, the receptor and the core signalling module. Curr Opin Plant Biol 12: 539-547.

33. Santino A, Taurino M, De Domenico S, Bonsegna S, Poltronieri P, et al. (2013) Jasmonate signaling in plant development and defense response to multiple (a) biotic stresses. Plant Cell Rep 32: 1085-1098.

34. Holopainen JK (2004) Multiple functions of inducible plant volatiles. Trends Plant Sci 9: 529-533.

35. Snoeren TA, Kappers IF, Broekgaarden C, Mumm R, Dicke M, et al. (2010) Natural variation in herbivore-induced volatiles in Arabidopsis thaliana. J Exp Bot 61: 3041-3056.

36. Tan KH, Nishida R (2012) Methyl eugenol: its occurrence, distribution, and role in nature, especially in relation to insect behavior and pollination. J Insect Sci 12: 56.

37. Yang NW, Li AL, Wan FH, Liu WX, Johnson D (2010) Effects of plant essential oils on immature and adult sweet potato white fly, Bemisia tabaci biotype. B Crop Prot 29: 1200-1207.

38. Menniti AM, Gregori R, Neri F (2010) Activity of natural compounds on Fusarium verticillioides and fumonisin production in stored maize kernels. Inter J Food Microbiol 136: 304-309.