Artigos Originais

ANTIFUNGAL ACTIVITY OF *Piper marginatum* L. (PIPERACEAE) ESSENTIAL OIL ON *IN VITRO Fusarium oxysporum* (SCHLECHT)

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ABSTRACT: *Piper marginatum* is used in popular medicine as a tonic, diuretic and for the treatment of snakes and insects bites. *Fusarium oxysporum* is a fungus which causes diseases in large agricultural areas in Brazil, such as in banana plantations. Current analysis evaluates the effects of the essential oil from leaves of *P. marginatum* on the *in vitro* growth of *F. oxysporum* colonies. Further, 5-mm diameter discs from isolated cultures of the fungus were placed in the center of 90-mm diameter petri dishes with potato dextrose agar. Four discs of filter paper were placed with 10 µL of essential oil, extracted by distillation, in the dishes' peripheral area. Controls consisted of discs without essential oil. A completely randomized design was used with four replications. Fungi growth was evaluated by measuring the diameter of the colonies every 24 hours, during 8 days. After 92 hours, the *F. oxysporum* colonies treated with essential oil had a 22.5-mm diameter, whereas controls developed mean 69.9-mm colonies. Results show that the essential oil from leaves of *P. marginatum* has an inhibitory effect on the *in vitro* growth of *F. oxysporum*.

KEYWORDS: Phytopathology; Biological Control; Natural Products.

ATIVIDADE FUNGICIDA DO ÓLEO ESSENCIAL DE *Piper marginatum L.* (PIPERACEAE) SOBRE *Fusarium oxysporum* (SCHLECHT) IN VITRO

RESUMO: *Piper marginatum* é usado na medicina popular como tônico, diurético e para tratar picadas de cobras e insetos. *Fusarium oxysporum* é um fungo que causa algumas doenças em grandes culturas vegetais no Brasil - como a banana. O objetivo deste trabalho foi avaliar o efeito do óleo essencial de folhas de *P. marginatum* no crescimento *in vitro* de colônias de *F. oxysporum*. Discos de 5 mm de diâmetro de isolados fúngicos foram colocados no centro de placas de Petri de 90 mm de diâmetro, contendo batata dextrose agar. Na área periférica das placas, foram colocados quatro discos de papel filtro contendo 10 µL de óleo essencial, extraído por um sistema de destilação. Como controle experimental, foram utilizados discos sem óleo essencial. Foi utilizado o delineamento inteiramente casualizado, com quatro repetições. A cada 24 horas, durante 8 dias, o crescimento fúngico foi avaliado, medindo-se o diâmetro das colônias. Após 92 horas, colônias de *F. oxysporum* com diâmetro médio de 22,5 mm foram observadas no tratamento com óleo essencial, enquanto no controle o diâmetro médio foi de 69,9 mm. Os resultados mostram que o óleo essencial de folhas de *P. marginatum* tem efeito inibitório sobre o crescimento *in vitro* de *F. oxysporum*.

PALAVRAS-CHAVE: Fitopatologia; Controle Biológico; Produtos Naturais.

INTRODUCTION

Problems regarding environmental pollution and toxicity to non-target organisms by synthetic products used in agriculture have stimulated the search for plant substances in the control of diseases (AMADIOHA, 2000). Other problems are residual effects, limited action spectrum and the pathogen resistance. Researches have been conducted to apply plant extracts and essential oils against phytopathogens in several cultures (FRANCO; BETTIOL, 2000; BENATO et al., 2002; CARRÉ et al., 2002; MOREIRA et al., 2002; SANTOS et al., 2007). Essential oils are among the most studied classes of vegetal compounds against insects, fungus and bacteria, and can already be found in pesticide formulations (ISMAN, 2000); are volatile, lipophilic and often fragant liquid substances; the use of plant substances, including essential oils, has already been used as a pesticide even before the advent of synthetic organic chemicals (FAZOLIN et al., 2007).

Piperaceae family has near three thousand species belonging to eight genus (DI STASI; HIRUMA-LIMA, 2002), with highlight to *Piper*, *Peperomia* and *Pothomorphe*. *Piper* has 700 species distributed in the tropics, among which 170 occur in Brazil (YUNCKER, 1972). These plants are a source of long-chain unsaturated isobutyl amides, which have proven insecticide and fungicide activities, such as piperine found in *Piper nigrum* L. (BASTOS, 1997).

Estrela and contributors (2005) studied the activity of amides analogs to piperine, with the groups *N*-hexyl, *N*-isopropil and *N*-isopenthyl linked to isopenthyl (3,4-metilenodioxiphenyl)-amide, and observed high toxicity to the *Spodoptera frugiperda* caterpillar. The essential oil of some species of *Piper*, such as *P. hispidinervum* and *P. aduncum* are rich sources of the phenylpropanoids safrole, dillapiol and sarisan (LIMA et al., 2009). These species are largely found in the Amazon and are highlighted by their antimicrobial and insecticide properties (BERGO; MENDONÇA; SILVA, 2005; SILVA; BASTOS, 2007).

Studies have demonstrated the efficiency of the essential oil from *Piper* on the control of fungal plant diseases. Bastos (1997) showed the fungicidal effect of *P. aduncum* essential oil on the germination of basidiospores and mycelial growth of *Crinipellis perniciosa* (Stahel) Singer and other phytopathogens. Salgado and contributors, (2003) showed the fungicide effect of eucalyptus essential oils extracted from leaves of *Eucalyptus urophylla, E. citriodora* and *E. camaldulensis* on *Fusarium oxysporum, Botrytis cinerea and Bipolaris sorokiniana*. Silva e Bastos (2007) used successfully essential oils from *P. dilatatum*, *P. callosum* and *P. marginatum* var. *anisatum* leaves against *Crinipellis perniciosa*.

Piper marginatum is used in the popular medicine in Brazil as a soothing, tonic, for diuretic purpose and to treat snakes and insects bites (CHAVES; OLIVEIRA; SANTOS, 2006; ARAÚJO JÚNIOR et al., 1999).

Fusarium oxysporum is a fungus which causes some diseases in great vegetal cultures in Brazil - like banana, beans and soybeans. This fungus is found in the soil, causing root rot and wilt, and it is important due to the economic damage caused in the agricultural sector and its cosmopolitan distribution (GHINI; NAKAMURA, 2001).

The objective of this work was to evaluate the effect of the essential oil from leaves of *P. marginatum* on the *in vitro* growth of *F. oxysporum* colonies.

2 MATERIAL AND METHODS

Leaves of *P. marginatum* were collected at the Embrapa Rondônia experimental area, in Porto Velho, Rondônia, Brazil. The essential oil was extracted by a distillation system, according to the methodology described by Craveiro, Fernandes and Andrade (1981). The oil yield was of 5 $mL.kg^{-1}$ of leaves. Discs of 5 mm diameter from isolated cultures of the fungus were placed in the center of 90 mm diameter Petri dishes with potato dextrose agar. In the peripheral area of the dishes four discs of filter paper were placed with 10 µL of essential oil. As for control discs without essential oil were used. The dishes were kept at 25°C during 8 days (192 hours). A completely ramdomized design was used with four replications (of four dishes). Every 24 hours the growth of the fungi was evaluated by measuring the diameter of the colonies. Statistical analyses were performed by Tukey test, at a 5% probability level.

3 RESULTS AND DISCUSSION

After 192 hours, the essential oil of *P. marginatum* demonstrated intense inhibitory effect on the *F. oxysporum* growth. The oil treatment showed average diameter of 22.5 mm (Figure 1), compared with the control where the colonies cover the area of 69.9 mm diameter (Figure 2).

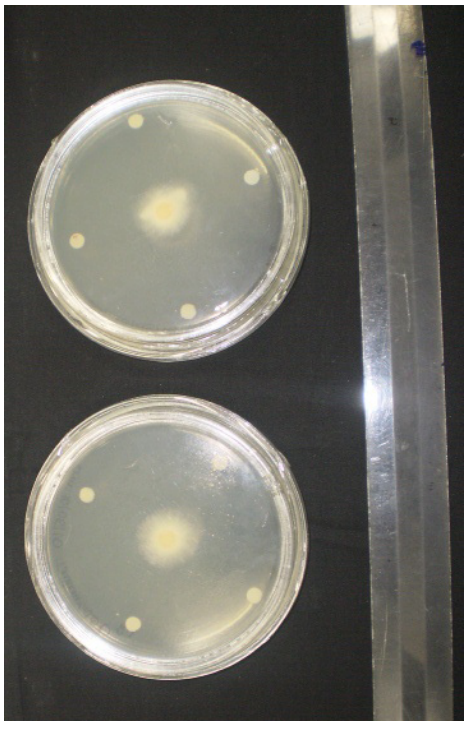

Figure 1 Treatment using oil essential the P. marginatum after 192 hours.

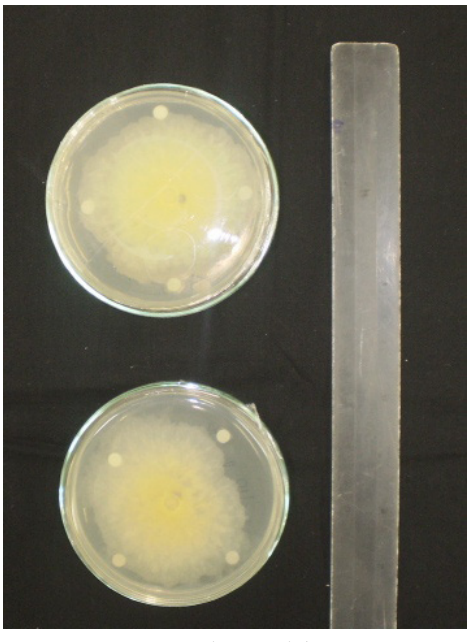

Figure 2 Treatment not using oil essential the P. marginatum after 192 hours.

Other species have been studied as alternative for controlling the agricultural fungi proliferation, most of all regarding plants reported as medicinal (VANDERLINDE; ONOFRE, 2010). Pereira and contributors (2006), studied the essential oil of *Rosmarinus officinalis* L., *Allium cepa* L., *Ocimum basilicun* L., *Mentha piperita* L. and *Origanum vulgari* L., at 500; 1,000; 1,500 and 2,000 mg.mL^{-1} on *Fusarium sp*., *Aspergillus ochraceus* Wilhelm., *Alternaria flaves* Link and *A. niger* Van. growth and found that the *O. vulgari* oil inhibited the fungi growth in all the used concentrations, except for *A. niger*, which had the mycelial growth inhibited by concentrations up to 1,000 mg.mL^{-1}. Krauze-Baranowska (2002), evaluated essential oils from *Pinus ponderosa*, *P. resinosa* and *P. strobus* on three *Fusarium* species. The most relevant result was the inhibition effect caused by *P. ponderosa* oil, which inhibited 100% of growth of all the tested fungi at 2 and 5% of dilution. Silva (2005) tested the extract of *Pterodon emarginatus* Vog. fruits on the mycelial growth of the fungi *Alternaria brassicae*, *F. oxysporum*, *Rhizoctonia solani* and *Ceratocystis fimbriata* during six days, and reported its effectiveness, with inhibitory effect of 62; 70; 74 and 82%, respectively. The authors argue that this can be an ecologically and economically viable alternative, since the fruits are obtained without the prejudice of the trees. Pereira (2005) tested essential oils of *Matriocaria chamomilla* L., *Rosmarinus officinalis* L. and *Mentha piperita* L. medicinal plants for the control of *in vitro* culture contaminants at 5 and 10% dilutions. The first two were efficient against *Aspergillus* sp. and *Penicillium* sp. at 5% and *M. piperita* was efficient at 10% dilution.

Austran and contributors (2009) identified 40 chemical compounds in essential oil of *P. marginatum* leaves (Table 1).

Table 1 Volatile compounds identified in the essential oil of Piper marginatum L.

Number	Compound[a]	RI[b]	RI[literature]	Leaf (%)
1	β-Pinene	984	980	0.3
2	δ-3-Carene	1016	1011	0.4
3	Sylvestrene	1028	1027	0.2
4	β-(Z)-Ocimene	1044	1040	0.5
5	Linalool	1094	1098	0.6
6	Isopentyl isovalerate	1103	1103	0.2
7	α-Terpineol	1192	1189	0.1
8	δ-Elemene	1341	1339	3.4
9	α-Cubebene	1352	1351	-
10	Isoledene	1376	1373	0.4
11	α-Copaene	1379	1376	0.6
12	β-Elemene	1394	1391	1.1
13	Ethyl hydroquinone	1416	1412	-
14	(E)-Caryophyllene	1423	1418	7.5
15	α-Guaiene	1443	1439	0.5
16	α-Humulene	1456	1454	0.5
17	Seychellene	1465	1460	-
18	α-Acoradiene	1467	1463	5.1
19	β-Acoradiene	1470	1466	1.3
20	γ-Gurjunene	1476	1473	-
21	γ-Himachalene	1480	1499	1.3
22	Germacrene D	1482	1480	-
23	β-Selinene	1487	1485	-
24	cis-β-Guaiene	1494	1490	0.4
25	Valencene	1495	1491	-
26	Bicyclogermacrene	1497	1494	9.4
27	(E)-Methyl isoeugenol	1500	1495	0.8
28	trans-β-Guaiene	1504	1500	0.2
29	γ-Cadinene	1516	1513	0.2
30	(Z)-Nerolidol	1543	1534	-
31	γ-Cadinene	1545	1538	1.4
32	Elemol	1552	1549	9.7
33	Elemicin	1556	1554	0.3
34	Ledol	1567	1565	0.5
35	Caryophyllene oxide	1585	1581	-
36	Dihydro-AR-turmerone	1596	1591	-
37	cis-Isolongifolanone	1610	1606	-
38	(Z)-Asarone	1624	1622	30.4
39	Patchouli alcohol	1662	1659	16.0
40	(E)-Asarone	1682	1679	6.4
	Total			**99.6**

[a]Constituents listed in order of elution on a non-polar DB-5 column
[b]Retention indices (RI) calculated from retention times in relation to those of a series of n-alkanes on a 30 m DB-5 capillary column. SOURCE: AUSTRAN et al., (2009)

Piper plants have been largely studied due to their biological activities. Silva and Bastos (2007) evaluated the activity of several oils from *Piper* plant leaves on the *Crinipellis perniciosa* basidiospores and mycelia growth. The most efficient species and dilutions of the oils were: *P. marginatum* var. *anisatum* (0.5 µL.mL^{-1}), *P. dilatatum* (0.4 µL.mL^{-1}) and *P. callosum* (0.5 µL.mL^{-1}), causing total inhibition of the growth. The data showed also that the basidiospores are more sensitive than the mycelia to the studied oils. Morandim and contributors (2006), reported the high antifungal activity of the *P. aduncum* and *P. tuberculatum* fruit oil against the fungi *Cladosporium cladosporioides* and *C. sphaerospermum*, with minimum inhibitory concentration (MIC) of 10 µg.mL^{-1}. These plants also contain safrole, which suggests its antimicrobial property. Zacaroni and contributors (2009) observed the toxic effect of essential oil from *P. hispidinervum* leaves on *Bipolaris sorokiniana*, *F. oxysporum* and *C. gloeosporioides*. The total inhibition occurs at concentrations of 200 µg.mL^{-1} in *B. sorokiniana* and 1000 µg.mL^{-1} in *F. oxysporum* and *C. gloeosporioides*. Bastos and

Albuquerque (2004) evaluated the *P. aduncum* essential oil in the control of *F. oxysporum*, observing total inhibition of mycelia growth and conidia germination using 100 $\mu g.mL^{-1}$ of oil. On the *in vivo* conditions only 1% of the oil was able to reduce rot in banana fruits. Hanada, Gasparotto and Pereira (2004) explain the inhibitory effect of the *P. hispidinervum* essential oil on *Mycosphaerella fijiensis* Morelet conidia germination to the presence of safrole in the essential oil. *Parmar* and contributors *(1997) found safrole, myristicin, eugenol, dillapiol and apiol as the main compounds of the essential oils of the Piperaceae family. All these compounds have antimicrobial and antioxidant properties.*

4 CONCLUDING REMARKS

The results reported here show the *in vitro* efficiency of *P. marginatum* essential oil against *F. oxysporum*, which suggests the potential of its use in agriculture, mainly regarding banana, beans and soybeans crops. However, field experimentations and toxicological tests must be done to determine the applicability and effectiveness of it in *ex vitro* conditions.

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