(DOI): 10.5777/PAeT.V5.N3.07

This article is presented in English with abstracts in Spanish and Portuguese.

Brazilian Journal of Applied Technology For Agricultural Science, Guarapuava-PR, v.5, n.3, p.61-66, 2012.

Scientific paper

Bioactivity of essential oils on the fungus *Didymella bryoniae* of the cucumber culture

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Abstract

The objective of this study was to evaluate the *in vitro* antifungal activity of the essential oil of five plant species in the inhibition of mycelial growth of the fungus *Didymella bryoniae* causer of the gummy stem blight of cucumber (*Cucumis sativa*). In the inhibition of the mycelial growth of the fungus *D. bryoniae* was used five concentrations (250, 500, 750, 1000 and 1250 ppm) of essential oil of citronella grass *(Cymbopogon nardus)*, lemon grass *(Cymbopogon citratus)*, peppermint *(Mentha piperita)*, lemongrass *(Lippia alba)* and eucalipto citriodora *(Eucalyptus citriodora)*, in five times of assessment (2, 4, 6, 8 and 10 days after transplanting). Another experiment was performed with lower concentrations of essential oil of citronella grass (150, 300, 450 and 600 ppm) in four times of assessment (3, 5, 7 and 9 days after transplanting). The essential oils were distributed on the surface of the PDA meium crop (potato-dextrose-agar) for assessment of mycelial growth in different concentrations. The experiments were conducted in a completely randomized design with four replications. The essential oil of lemongrass provided the highest effect of inhibition of mycelial growth of *D. bryoniae,* 100% of inhibition at all tested concentrations. The essential oil of eucalipto citriodora (*Eucalyptus citriodora)* provided the smaller effect of inhibition.

Keywords: Bioactivity; medicinal plants; essential oil.

Bioatividade de óleos essenciais sobre o fungo *Didymella bryoniae* da cultura do pepino

Resumo

O objetivo deste trabalho foi avaliar a atividade fungicida in vitro do óleo essencial de cinco espécies vegetais na inibição do crescimento micelial do fungo *Didymella bryoniae* causador crestamento gomoso da haste do pepino (*Cucumis sativus*). Na inibição do crescimento micelial do fungo *D. bryoniae* foram usadas cinco concentrações (250, 500, 750, 1000 e 1250 ppm) do óleo essencial de capim-citronela (*Cymbopogon nardus*), capim-limão (*Cymbopogon citratus*), hortelã-pimenta (*Mentha piperita*), erva-cidreira (*Lippia alba*) e eucalipto citriodora (*Eucalyptus citriodora*), em cinco épocas de avaliação (2, 4, 6, 8 e 10 dias após repicagem). Outro experimento foi realizado com concentrações menores do óleo essencial do capim citronela (150, 300, 450 e 600 ppm), em quatro épocas de avaliação (3, 5, 7 e 9 dias após repicagem). Os óleos essenciais foram distribuídos na superfície do meio de cultura BDA (batata-dextrose-ágar) para avaliação do crescimento micelial nas diferentes concentrações. Os experimentos foram instalados no delineamento inteiramente casualizado, com quatro repetições. O óleo essencial do capim-limão proporcionou o maior efeito de inibição do crescimento micelial do fungo *D. bryoniae*, 100 % de inibição em todas as concentrações utilizadas. O óleo essencial do eucalipto citriodora (*Eucalyptus citriodora*) proporcionou o menor efeito de inibição.

Palavras-chave: Bioatividade; plantas medicinais; óleo essencial

Bioactividad de aceites esenciales sobre hongos Didymella bryoniae en el cultivo de pepino

Resumen

El objetivo de este estudio fue evaluar la actividad antifúngica in vitro de aceite esencial de cinco especies de plantas en la inhibición del crecimiento micelial del hongo *Didymella bryoniae* causador del tizón gomoso del tallo del pepino (*Cucumis sativus*). En la inhibición del crecimiento del micelio del hongo *D. bryoniae* se utilizaron cinco concentraciones (250, 500, 750, 1000 y 1250 ppm) de aceite esencial de hierba citronela (*Cymbopogon nardus*), hierba limón (*Cymbopogon citratus*), menta

Received in: 22/03/2012 Accepted for publication: 28/10/2012

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piperita (*Mentha piperita*), hierba prontoalívio (*Lippia alba*) e eucalipto citriodora (*Eucalyptus citriodora*), en cinco épocas de evaluación (2, 4, 6, 8 y 10 días después del trasplante). Otro experimento se llevó a cabo con concentraciones más bajas del aceite esencial de hierba citronela (150, 300, 450 y ppm 600) en cuatro épocas de evaluación (días 3, 5, 7 y 9 después del trasplante). Los aceites esenciales se han distribuido sobre la superficie del medio de cultivo PDA (agar de dextrosa de patata) para evaluar el crecimiento del micelio en diferentes concentraciones. Los experimentos se llevaron a cabo en un diseño completamente al azar con cuatro repeticiones. El aceite esencial de hierba limón produjo el mayor efecto de inhibición del crecimiento micelial del hongo *D. bryoniae*, 100% de inhibición en todas las concentraciones utilizadas. El aceite esencial de eucalipto citriodora (Eucalyptus citriodora) fue el que causó el menor efecto de inhibición.

Palabras clave: Bioactividad, plantas medicinales, aceites esenciales

Introduction

The citronella grass (*Cymbopogon nardus*) plant originated in Ceylon and India, has medical use as a sedative and digestive. The genus *Cymbopogon* belongs to the family Poaceae, subfamily Panicoideae. This genus consists of eighty-five species. In Brazil the essential oil of *C. nardus* has traditionally been used as an insect repellent (CASTRO et al., 2010).

The essential oil extracted from *C. nardus* has high content of geraniol and citronellal. The geraniol has antiseptic activity, inhibiting the growth of fungi and bacteria. The citronellal is used as basic material for the synthesis of important chemical compounds called iononas and for synthesizing the vitamin A. This oil has insect repellent activity, and also a fungicide and bactericide (CASTRO et al., 2007).

The impacts of agricultural activity on the environment and in humans can be reduced through alternative control of plant diseases. The alternative control of plant diseases including biological control, the induction of resistance in plants and the use of alternative in the phytosanitary control, as extracts and vegetable oils, either directly by their fungitoxic action, or indirectly through the activation of defense mechanisms in treated crops (OLIVEIRA et al., 2011).

In the literature is possible to find several works that use the antimicrobial properties of secondary compounds of medicinal plants for the control of phytopathogenic agents (FIORI et al., 2000; FRANZENER et al., 2007; BONALDO et al., 2004; BALBI-PEÑA et al. 2006; MEDICI et al. 2007). According to Marques et al. (2004), the use of fungicides of vegetable origin could constitute an alternative and promising method for controlling plant diseases, as well as being easy to obtain and inexpensive, minimizing toxicity problems presented by synthetic chemical products.

Since the use of essential oils derived from medicinal plants has shown promising results in the control of plant pathogens, the aim of this study was to evaluate the antifungal activity of five kinds of essential oils on the inhibition of mycelial growth of fungus *Didymella bryoniae* causative of the blight gummy in the stem of the cucumber (*Cucumis sativus*).

Material and methods

The experiments were conducted at the Laboratory of Plant Pathology, University Campus of Gurupi, Universidade Federal do Tocantins - UFT. The geographical coordinates of landmarks are 11° 43′ S. latitude and 49° 04′ W. longitude and altitude of 300 m.

The isolated *D. bryoniae* (LAS-051) from the cucumber crop was obtained from the mycological collection of the Seed Pathology Laboratory, Department of Plant Pathology, Federal University of Lavras. In the procedure of transplanting of the fungus to Petri dishes containing mycelium of the fungus were incubated for eight days at a temperature of 25 ± 2 °C and 12 hours of photoperiod.

We tested five essential oils in inhibiting the micelial growth *in vitro* of the fungus *D. bryoniae*: citronella grass (*Cymbopogon nardus*), lemon grass (*Cymbopogon citratus*), lemon eucalyptus (*Eucalyptus citriodora*), peppermint (*Mentha piperita*) and lemon balm (*Lippia alba*). The concentrations of the oils tested were 250, 500, 750, 1000 and 1250 ppm in five *period*s of evaluation (2, 4, 6, 8 and 10 days after transplanting).

The essential oils of *C. nardus* and *C. citratus* had the extraction performed through hydrodistillation in Clevenger equipment with 1000 ml of distilled water and 100 g of dehydrated grass of the leaf citronella, for a period of 2 hours. At the end, yielded the essential oil of citronella grass (supernatant) with the auxiliary of a pipette, and thereafter stored in glass jars protected from light with aluminum foil wrapped. The other essential oils (*E. citriodora*, *L. alba* and *M. piperita*) were purchased in the market town of Gurupi-TO.

The aliquots of the essential oils were placed in the center of Petri dishes of 90 mm diameter

and containing BDA distributed over the surface in the center of the culture with the aid of the handle Drigalsky. Next, a disc of 6 mm in diameter containing mycelia of *D. bryoniae* with about 8 days was transplanted to the center of the plates which were sealed with plastic film and incubated at 25 ± 2 ° C B.O.D.

Later we performed another experiment to test lower concentrations of the essential oil of the citronella grass in inhibiting the micelial growth *in vitro* of the fungus *D. bryoniae*. The citronella grass was selected for the realization of this new experiment due to be a species that is well adapted to soil and climatic conditions of the south part of the Tocantins State and also for the commercial importance of the essential oil used in several commercial products. In this new experiment were tested four concentrations of the essential oil of the citronella grass (150, 300, 450 and 600 ppm) in four periods of evaluation (3, 5, 7 and 9 days after transplanting). The methodology used in this experiment was the same as in the previous experiment with higher concentrations of the essential oil.

The data were interpreted by analysis of variance and regression. In the factor oil concentrations, the averages were compared by Tukey test at 5% probability. In the Factor evaluation times, the equations were adjusted based on the "t" test of regression coefficients and the coefficient of determination. Statistical analysis was performed with SAEG (RIBEIRO JR. and MELO, 2009).

Results and discussion

It was observed in the essential oils of the lemongrass and peppermint the highest effects of in vitro inhibition of the mycelial growth of the fungus *D. bryoniae*. The essential oil of lemongrass (*C. citratus*) inhibited in 100% the mycelial growth of the fungus *D. bryoniae* at all concentrations tested (250, 500, 750, 1000 and 1250 ppm) and in the essential oil of peppermint (*M. piperita*) growth was observed at a concentration of 250 ppm at 10 DAT (days after transplanting).

SCAPIN et al. (2010) studied the bioactivity of medicinal plant extracts and concluded that extracts of rosemary and camphor were the ones that showed greater inhibition on mycelial growth of *Exserohilum turcicum* when compared with extracts of yarrow and lemon grass. With regard to the role of essential oil of *Mentha piperita*, PEREIRA et al. (2006) found that the essential oil of *Mentha piperita* inhibited mycelial growth of the fungi *Aspergillus niger* and *Aspergillus flavus* at concentrations of 1500 and 2000 mg Ml^{-1}, respectively.

The essential oil of lemon eucalyptus provided mycelial growth of fungus at all concentrations tested. In the control according to the regression equation adjusted was observed a radial growth rate of 7.78 mm day^{-1}, reaching 94.22 mm, in the last assessment time. The concentration of 1250 ppm showed mycelial growth of the fungus 4.73 mm day^{-1}, reaching 44.47 mm to 10 DAR (Table 1).

Table 1. Average values, regression equations and coefficient of determination (R^2) of the mycelial growth (mm) of the fungus *D. bryoniae*, at five different concentrations (C) (C_1 = 0 ppm, C_2 = 250 ppm C_3 = 500 ppm, C_4 = 750 ppm; C_5 = 1000 ppm and C_6 = 1250 ppm) of the essential oils *E. citriodora, L. alba* and *C. nardus* in five periods of evaluation.

	Evaluation periods (days after transplanting)						
C	**2**	**4**	**6**	**8**	**10**	**Regression Equations**	**R^2**
				Essencial Oil of *E. citriodora*			
C1	23.42 a	53.54 a	72.14 a	76.18 a	90.00 a	Ŷ= 16.3252 + 7.7891EP**	0.89
C2	11.19 b	22.82 b	40.71 b	52.68 b	68.03 b	Ŷ=-3.9697 + 7.1763EP**	0.91
C3	9.85 b	20.02 bc	30.82 c	41.20 c	55.95 c	Ŷ=-2.4392 + 5.6686EP**	0.96
C4	10.03 b	19.67 bc	29.98 c	40.07 c	48.38 cd	Ŷ=0.4942 + 4.8558EP**	0.98
C5	7.85 b	16.73 bc	26.37 c	36.24 c	46.13 d	Ŷ=-2.1542 + 4.8036EP**	0.99
C6	6.99 b	15.13 c	25.98 c	35.34 c	44.22 d	Ŷ=-2.8635 + 4.7332EP**	0.99
				Essencial Oil of *L. alba*			
C1	23.42 a	53.54 a	72.14 a	76.18 a	90.00 a	Ŷ= 16.3252 + 7.7891EP**	0.89
C2	1.82 b	17.40 b	41.72 b	59.01 b	68.82 b	Ŷ=-14.9257 + 8.7803EP**	0.91
C3	0.00 b	0.00 c	9.76 c	25.58 c	50.41 c	Ŷ=-20.7710 + 6.3207EP**	0.78
				Essencial Oil of *C. nardus*			
C1	23.42 a	53.54 a	72.14 a	76.18 a	90.00 a	Ŷ= 16.3252 + 7.7891EP**	0.89
C2	0.00 b	0.00 b	5.17 b	15.81 b	28.69 b	Ŷ=-12.0220 + 3.6595EP**	0.75

*Averages followed by the same letter in the column do not differ by Tukey test (P> 0.05). ** Significant at 1% probability by "t" test.*

Regarding the essential oil of *L. alba* mycelial growth was observed only at concentrations of 250 and 500 ppm. The essential oil of lemon balm (*L. alba*) at a concentration of 500 ppm to 4, 6, 8 and 10 DAR presented significant difference with respect to concentration of 250 ppm. According to the regression equation adjusted was observed a rate of mycelial growth at a concentration of 500 ppm of essential oil of lemongrass of 6.32 mm day^{-1}, reaching 42.44 mm in the last evaluation period (Table 1).

The essential oil of citronella grass (*C. nardus*) gave 100% inhibition in mycelial growth of fungus at concentrations of 500, 750, 1000 and 1250 ppm. However, it was observed at a concentration of 250 ppm, a mycelial growth of fungus starting from 6 DAT. According to the regression equation adjusted was observed a rate of mycelial growth of fungus of 3.65 mm day^{-1}, reaching 24.57 mm at 10 DAT (Table 1).

Results similar to those obtained in this work, using medicinal plants to control plant diseases, have been reported by several researchers. VALARINI et al. (1994) working with essential oil of lemon grass (*Cymbopogon citratus*) observed complete inhibition of the mycelial growth of *Fusarium solani, Sclerotinia sclerotiorum* and *Rhizoctonia solani*. In another study, LIMA (2007) studied the essential oil of citronella (*Cymbopogon nardus*) was observed complete inhibition of the mycelial growth of *Colletotrichum gossypii*.

MEDICI et al. (2007) found that essential oils of lemon eucalyptus (*Eucalyptus citriodora*), citronella grass (*Cymbopogon nardus*), nin (*Azadirachta indica*) and thyme (*Thymus vulgaris*) at concentrations of 0.01%, 0.5%, 0, 3% and 1%, respectively, inhibit the germination of uredospores of *Phakopsora pachyrhizi*. Different results were found by BONALDO et al. (2004) who found the fungitoxicity of the *Eucalyptus citriodora* on the mycelial growth of *Rhizoctonia solani, Sclerotium rolfsii, Phytophthora sp, Alternaria alternata* and *Colletotrichum sublineolum*, being that this essential oil when distributed on the center of the surface of the culture did not promote germination inhibition of spores.

ROZWALKA et al. (2008) studied the essential oil of lemon grass (*Cymbopogon citratus*) which allowed mycelial growth inhibition of 100% of the fungus *Colletotrichum gloeosporioides* using 10 uL aliquot of on the plate surface. FIORI et al. (2000) evaluated the essential oils of lemon grass (*Cymbopogon citratus*), mentrasto (*Ageratum conyzoides*) and lemon eucalyptus (*Eucalyptus citriodora*) and found 100% inhibition of the mycelial growth and spore germination of *D. bryoniae* with a rate of 20 uL. BONALDO et al. (2007) observed that the essential oil of lemon eucalyptus (*Eucalyptus citriodora*) at all rates tested (5, 10, 20, 40 and 60 uL), inhibited mycelial growth and conidial germination of *Alternaria alternata* and *Colletotrichum sublineolum* and *sclerotia* of *Sclerotium rolfsii*.

In the experiment using lower concentrations of the essential oil of the citronella grass was observed at concentrations of 300, 450 and 600 ppm at 3 DAT, 100% inhibition of the mycelial growth of the pathogen. A concentration of 600 ppm showed 100% inhibition at all times (Table 2).

There was a decrease in the mycelial growth when increased the concentration of the essential oil of the citronella grass. According to the regression adjusted we observed a mycelial growth rate in the concentration of 450 ppm of 1.50 mm day $^{-1}$, reaching 11.15 mm in the last evaluation period. The control showed mycelial growth rate of 9.80 mm day^{-1}, reaching in the last evaluation period mycelial growth of 88.35 mm.

Table 2. Average values, regression equations and coefficient of determination (R2) of the mycelial growth (mm) of the fungus *D. bryoniae* in four concentrations (C) (C1 = 0, C2 = 150 ppm C3 = 300 ppm, C4 = 450 ppm; C5 = 600 ppm) of the essential oil of the citronella grass, into four evaluation periods.

	Evaluation periods (days after transplanting)					
C	**3**	**5**	**7**	**9**	**Regression Equation**	**R^2**
C1	46.34 a	69.85 a	79.06 a	90.00 a	$\hat{Y}$= 9.6936 + 9.804EP**	0.91
C2	6.59 b	17.05 b	31.52 b	58.30 b	$\hat{Y}$= -12.6900 + 7.0770EP**	0.88
C3	0.00 c	6.78 c	12.32 c	29.23 c	$\hat{Y}$= -8.0286 + 3.5391EP**	0.83
C4	0.00 c	0.00 d	5.87 c	12.15 d	$\hat{Y}$= -3.9408 + 1.5093EP**	0.74
C5	0.00	0.00	0.00	0.00	$\hat{Y}$= 0	

*Averages followed by the same letter in the column do not differ by Tukey test (P> 0.05). ** Significant at 1% probability by "t" test.*

PERINI (2009) evaluated the essential oil of eucalyptus (*Eucalyptus citriodora*) on mycelial growth of the fungus *Pyricularia grisea* with the concentration of 90 µL and found inhibitory effect of 100% of mycelial growth of fungus. In this same manner, the essential oil of the citronella grass on the rates of 30, 60, 90, 120 and 150 µL gave 100% inhibition of the mycelial growth of the fungus *Pyricularia grise.*

The union of various chemical compounds in the composition of essential oils can act in synergistic harmony and present a broad fungicidal or fungistatic activity (SILVA 2003). However, it should be considered that the toxicity of the essential oils is influenced by soil and climaric factors and genetics of plants, which can interfere with the chemical composition of plants and their biological activity.

Conclusions

The essential oils of the lemongrass and peppermint had the greatest effect of *in vitro* inhibition of the mycelial growth of the fungus *D. bryoniae* of cucumber. The essential oil of the lemongrass inhibited in 100% the mycelial growth of the fungus at all concentrations. The essential oil of the citronella grass also showed high inhibitory effect in vitro of the fungus *D. bryoniae*, effect of inhibition greater than the essential oils of the lemon eucalyptus and lemongrass.

The results showed that essential oils studied had significant fungitoxic action against the pathogens tested. This therefore shows good prospects for experimental use of these oils in the control of plant pathogens in conditions of green house and field. The possibility of using natural products which exhibit low toxicity is translated into an advantage for being a procedure less aggressive to the environment.

Financial Organs

This work was supported by the National Council for Scientific and Technological Development (CNPq) and the Coordination of Improvement of Personnel of Superior Level (CAPES).

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