EFFECT OF ESSENTIAL OILS ON *COLLETOTRICHUM GOSSYPII VAR. CEPHALOSPORIOIDES*

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Abstract

The identification and use of vegetable products with fungitoxic properties constituted in a research area that can contribute to minimize the production costs and to preserve the environment. With the objective of finding alternative forms of control to the causal agent of Cotton Ramulose evaluate in vitro antifungal effect of 10 essential oils (O.E.) on spore germination and mycelia growth of *Colletrotrichum gossypii* var. *cephalosporioides*. The following essential oils were tested: citronella (*Cymbopogon winterianus* Jowitt), cinnamon (*Cinnamomum zeylanicum* Blume), and eucalyptus globulus (*Eucalyptus globulus* Labill) clove (*Eugenia caryophyllus* (Spreng.) Bullock & S. G. Harrison), white thyme (*Thymus vulgaris* L.), peppermint (*Mentha piperita* L.), black pepper (*Piper nigrum* L.), origanum (*Origanum vulgare* L.), neem (*Azadirachta indicates* A. Juss) and white camphor (*Cinnamomum camphora* Ness). Were verified the growth micelial growth and the spores germination, respectively in PDA (potato-dextrose-agar) and spores suspension, added of the oils, at the concentrations of 0 (control), 0, 3 and 1% in relation to the total volume. The data obtained were calculated the mycelial growth inhibition percentage (MGIP) and the spore germination inhibition percentage (SGIP). Considering the effects on spore germination and mycelia growth, essentials oils *Cymbopogon winterianus, Origanum vulgare* and *Thymus vulgaris* have higher antifungal activities against *Colletotrichum gossypii* var. *cephalosporioides* and can become an option for the management of the cotton ramulose.

Introduction

Among several diseases that affect cotton (*Gossypium hirsutum* L.) in Brazil, the most important and provoke more damage is the common mosaic, the bacterial blight, Fusarium wilt and the ramulose (Silveira, 1965). Since 1999, ramulose is no more a leaf disease of minor importance and became the major cotton disease in the Brazilian cerrado region, could make unfeasible the cultivation, in case control measures are not taken (Chiavegato, 2005, Melo et al., 2008).

The ramulose or escobilla occurs only in Brazil and is caused by *Colletotrichum gossypii* South var. *cephalosporioides* A. S. Costa. Symptoms of the disease are spots leaves and holes, a shortening of internodes and excessive development of branches and leaves, causing a witches broom symptom. Diseased plants have dense mass of dark green foliage and few bolls (Hillocks, 2001; Cia & Salgado, 2005).

The fungus can survive in seeds and crop residues, with the spores spread by splashing rain or irrigation. The climatic conditions favor to the disease occurrence characterized by high relative humidity, generally above 80%, high rainfall, temperature between 25 ° C and 30 ° C and cloudy days (Chitarra, 2007). When susceptible varieties are grown in favorable climatic conditions, as observed in most regions of the Mato Grosso state, ramulose symptoms are striking in the early vegetative stage of culture, resulting in high severity and high loss in production (Iamamoto, 2007).

The identification and the use of plant products with fungitoxic properties constitute in a research area that can contribute to minimize the production costs and to preserve the environment. Several crude extracts and essential oils of plants have been tested on phytopathogenic fungi in various works (Takatsuka et al. 2003; Balbi-Peña et al., 2006, Pereira et al., 2006).

In order to seek alternative ways to control the causal agent of ramulose cotton, this study was carried out to evaluate in vitro the antifungal effect of 10 essential oils (EO) on spore germination and mycelial growth of *Colletrotrichum gossypii* var. *cephalosporioides.*

Materials and Methods

The study was carried out in Plant Disease Laboratory at the Faculty of Engineering, Ilha Solteira Campus, São Paulo State University "Júlio de Mesquita Filho" – UNESP, Ilha Solteira, and São Paulo State, Brazil.

We used the essential oils presented in Table 1, acquired of Ferquima Company Ind. and Com. (Vargem Grande Paulista, Sao Paulo, Brazil). They were evaluated at concentrations of 0 (control), 0,.3 and 1.0% over the volume. These concentrations were defined based on information from literature on the use of essential oils in other organisms, since no information was found on these essential oils and plant pathogen used in this work.

Table 1: Information about the plants that were obtained the essential oils used.

Common name	**Scientific name**	**Botanic family**	**Part of the plant used**
Black pepper	*Piper nigrum L.*	Piperaceae	Fruits
Cinnamon	*Cinnamomum zeylanicum* Blume	Lauraceae	Leaves
Citronella	*Cymbopogon winterianus* Jowitt	Poaceae	Leaves
Clove	*Eugenia caryophyllus* (Spreng.) Bullock & S.G. Harrison	Myrtaceae	Leaves
Eucalyptus globulus	*Eucalyptus globulus* Labill	Myrtaceae	Leaves
Neem	*Azadirachta indica* A. Juss	Meliaceae	Seeds
Origanum	*Origanum vulgae* L.	Lamiaceae	Leaves
Peppermint	*Mentha piperita* L.	Lamiaceae	Leaves
White camphor	*Cinnamomum camphora* Ness	Lauraceae	Wood
White thyme	*Thymus vulgaris* L.	Lamiacea	Flowers/Leaves

Fonte: Ferquima (2011)

We used an isolate of *Colletrotrichum gossypii* var. *cephalosporioides*, identified as MMBF 246, obtained from the fungal collection "Mario Barreto Figueiredo," the Biological Institute, São Paulo, which was growing in a test tube in PDA medium.

Effect of essential oils on the mycelial growth: This determination was performed in Petri dishes containing PDA + polysorbate + essential oil or PDA + polysorbate (control). In Petri dishes containing PDA was peaked culture of *Colletotrichum gossypii* var. *cephalosporioides* which was preserved in PDA. These plates were incubated at 25 °C incubator for seven days. After this period, discs of 7 mm in diameter were cut of the edges of the culture, which were transferred to Petri dishes containing the culture medium to be evaluated. The addition of essential oils, the PDA culture medium was made at a temperature between 40 and 45 °C. To the mixture of oils and PDA stay well mixed, the dispersant was added polysorbate (Tween 80) to 0.5% under manual shaking for thirty seconds. After homogenization, 15 mL of enriched PDA was poured per Petri dish 90 mm in diameter. We used concentrations of 0.3 and 1.0 and 0% (control) of oils in the culture medium of PDA. After solidification of the medium in the Petri dish was transferred over a disk PDA colony Cgc para the center of each plate. Then the plates were sealed and kept in incubator at 25 °C in the dark. The evaluations were conducted 14 days, by measuring the diameter of mycelial growth in two perpendicular positions per plate, then calculating the average.

Effect of essential oils on spore germination: For the production of spores was performed similar procedure to that used in obtaining the fungal colony. Then were added 10 mL of sterile distilled water in each petri dish, releasing the spores to water with a brush. The spore suspension was filtered through cheesecloth and the concentration of double spore suspension was calibrated in the 2 x 10^4 spores / mL. Then suspensions were prepared containing concentrations of the oils to be analyzed and the spore suspension. To the mixture of oils and sterile distilled water to stay well mixed, the dispersant was added polysorbate (Tween 80) to 0.5% under manual shaking for thirty seconds. These suspensions were pipetted into 60 mL aliquots and placed in excavated slides; these were placed in petri dishes and incubated at 25 °C in the dark for 5 hours. At the end of this period, we proceeded to interrupt the germination process using a drop of lactoglicerol on each slide. As a witness, we used sterile distilled water and polysorbate + spore suspension. In optical microscope held the count of 100 spores in each cell, determining the number of spores germinated and not germinated. As spore germinated was considered spores that had a germ tube equal to or greater than its width.

The experimental design was completely randomized with 21 treatments (ten essential oils, two concentrations and control) and four replications. Each plot was excavated by a blade or a petri dish. Of data was calculated the percentage of spores germinated and the percentage of mycelial growth for each treatment. It was then determined the percentage of inhibition of spore germination (SGA) and the percentage inhibition of mycelial growth (PIC) for each treatment with the control. These data were statistically analyzed and means were compared by Scott-Knott test at 5% probability.

Results and Discussion

It was found that essential oils evaluated at concentrations of 0.3 and 1%, provided the percentages of inhibition of mycelial growth and spore germination of *Colletotrichum gossypii* var. *cephalosporioides* from 14 to 100% and from 11 to 100% (Table 2), respectively. The greatest inhibition of mycelial growth were found for the essential oils of *Cinnamomum zeylanicum, Cymbopogon winterianus*, *Eugenia caryophyllus*, *Thymus vulgaris*, in two concentrations, and *Mentha piperita* in 1% concentration, which inhibited 100% of mycelial growth. Inhibition of mycelial growth intermediate, between 50 and 95% were obtained for the essential oils of *Mentha piperita*, at a concentration of 0.3%, and *Origanum vulgare*, in both concentrations. The lower inhibition of mycelial growth of *Colletotrichum gossypii* var. *cephalosporioides* below 40% was found for the essential oils of *Cinnamomum camphora*, *Eucalyptus globulus*, *Azadirachta indica* and *Piper nigrum.*

On the germination of spores of *Colletotrichum gossypii* var. *cephalosporioides* (Table 2), 100% inhibition was observed in the essential oils of *Cymbopogon winterianus*, *Mentha piperita*, *Origanum vulgare*, *Thymus vulgaris*, in two concentrations. For the essential oils of *Cinnamomum camphora*, *Cinnamomum zeylanicum*, *Eugenia caryophyllus*, *Eucalyptus globulus*, *Azadirachta indica* and *Piper nigrum* were obtained percentages of inhibition of spores below 65%.

There are several studies evaluating the effect of essential oils on pathogens, but few about *Colletotrichum gossypii* var. *cephalosporioides*. Zanandrea et al. (2004) found the inhibitory effect of essential oil of *Origanum vulgare* on the mycelial growth *of Alternaria* sp., *Bipolaris oryzae*, *Curvularia* sp., *Gerlachia oryzae*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* isolated from rice. Abreu (2006) observed the effect of essential oils of *Cinnamomum zeylanicum, Cymbopogon citratus, Syzygium aromaticum, Eucalyptus citriodora, Melaleuca alternifolia* and *Mentha piperita* in control of *Alternaria solani* in vitro and under field conditions. Pereira (2006) found fungitoxicity of the essential oil of *Thymus vulgaris* on conidia of *Cercospora coffeicola*, at concentrations of 500, 1000 and 2000 ppm, with a reduction in germination of 27, 30 and 45% respectively. Medice et al. (2007) observed that the essential oils of *Thymus vulgaris* and *Cymbopogon nardus,* at concentrations of 0.5, 1.0 and 0.3%, 100% inhibited germination of the spores *Phakospsora pachyrhizi*. Melo et al. (2008) reported inhibition of mycelial growth of *Colletotrichum gossypii* var. *cephalosporioides* the essential oil of *Cymbopogon winterianus*.

Considering the inhibition of spore germination and mycelial growth of *Colletotrichum gossypii* var. *cephalosporioides*, essential oils of *Cymbopogon winterianus, Origanum vulgare, Thymus vulgaris* presented as oils with higher antifungal activities. Other studies should be conducted to assess the efficiency and viability of these essential oils in ramulose control of cotton in the field of culture.

Table 2. Mycelial growth inhibition percentage (MGIP) and spore germination inhibition percentage of (SGIP) of *Colletotrichum gossypii* var. *cephalosporioides* by 10 essential oils. Ilha Solteira, SP, Brazil. 2011.

Essential oil	MGIP – Concentration		SGIP – Concentration	
	0.3%	1.0%	0.3%	1.0%
Cinnamomum camphora	29 c[1]	40 d	19 b	58 c
Cinnamomum zeylanicum	100 f	100 f	52 e	63 d
Cymbopogon winterianus	100 f	100 f	100 g	100 e
Eugenia caryophyllus	100 f	100 f	62 f	64 d
Eucalyptus globulus	27 b	36 c	11 a	40 a
Mentha piperita	69 d	100 f	100 g	100 e
Azadirachta indica	14 a	18 a	46 d	59 c
Origanum vulgare	89 e	91 e	100 g	100 e
Piper nigrum	25 b	30 b	38 c	46 b
Thymus vulgaris	100 f	100 f	100 g	100 e

[1]Means followed by different letters in each column are significantly different by to Scott-Knott's test (5%).

Summary

Studies on essential oils on pathogens have been conducted and these can become an option for control of plant diseases. In this study it was found that the essential oils of *Cymbopogon winterianus*, *Origanum vulgare*, *and Thymus vulgaris* have antifungal activities against *Colletotrichum gossypii* var. *cephalosporioides* in vitro. Other studies should be conducted to assess the efficiency and viability of these essential oils in ramulose control of cotton in the field of culture conditions.

References

Abreu, C. L. M. 2006. Controle de *Alternaria solani* em tomateiro (*Lycopersicon esculentum*) com óleos essenciais, 2006, 71f., Tese (Doutorado em Agronomia – Horticultura) - UNESP – FCA Faculdade Ciências Agronômicas. Botucatu.

Balbi-Penã, M. I.; Becker, A.; Stangarlin, J. R.; Franzener, G.; Lopes, M. C.; Schwan-Estrada, K. R. F. 2006. Controle de *Alternaria solani* em tomateiro por extratos de Cúrcuma longa e curcumina – I. Avaliação in vitro. Fitopatologia Brasileira, 1: 10-14.

Chiavegato, E. J. 2005. O problema crescente do uso de fungicidas na cultura do algodoeiro: manejo e propostas para a minimização do problema. In: CONGRESSO BRASILEIRO DE ALGODÃO, 5. 2005. Salvador. Algodão, uma fibra natural: Anais... Salvador: ABAPA; Campina Grande: Embrapa Algodão. 220p

Chitarra, L. G. 2007. Identificação e controle das principais doenças do algodoeiro. Campina Grande: Embrapa Algodão. 65 p. (Embrapa Algodão. Cartilha, 2)

Hillocks, R.J. 2001. Anthracnose. Compendium of cotton diseases. Kirpatrick, T.L. & Rothrock, C.S. (ed.). 2nd. Ed. St. Paul: APS Press. p. 34.

Iamamoto, M. M. 2007. Doenças do algodoeiro: interação patógeno-hospedeiro. Jaboticabal: Funep. 62 p

Medice, R.; Alves, E.; Assis, R.T.; Junior, R.G.M.; Lopes, E.A.G.L. 2007. Óleos essenciais no controle da ferrugem asiática da soja *Phakopsora pachyrhizi* Syd. & P. Syd. **Ciência Agrotecnica**, Lavras, v.31, n.1, p.83-90.

Melo, R. M. C .A.; Melo Filho, P. A.; Câmara, M. P. S.; Câmara, A. G. C.; Santos, R. C. 2008. Prospecção de óleos vegetais para controle da Ramulose do algodoeiro. In: JORNADA DE ENSINO, PESQUISA E EXTENSÃO DA UFRPE, 8., 2008, Recife. Anais... Recife: UFRPE. 1 CD- ROM.

Pereira, M.C.; Vilela, G.R.; Costa, L.M.A.S. 2006. Inibição do desenvolvimento fúngico através da utilização de óleos essenciais de condimentos. Ciência Agrotecnológica, v.30, n.4, p.731-733.

Silveira, A.P. 1965. Moléstias. Fungos e bactérias. In: Neves, O.S.;Cavaleri, P.A.; Verdade, F.C.; Junqueira, A.A.B.;Gridi-Papp, I.L.; Ortolani, A.A.; Silva, N.M.; Righi, N.R.; Ferraz, C.A.M.; Corrêa, D.M.; Calcagnolo, G.;Silveira, A.P.; Costa, A.S.; Carvalho, A.M.B.; Mendes, H.C.; Fuzatto, M.G.; Corrêa, F.; Berzaghi, M.N. Cultura e adubação do algodoeiro. São Paulo, Instituto Brasileiro de Potassa. p.417-33

Takatsuka, F. S.; Silva, I. D.; Oliveira, M. F.; Czepak, C.; Oliveira, C. M. A.; Cunha, M. G. 2003. Efeito do óleo essencial de açafrão (*Curcuma longa*) sobre o desenvolvimento micelial de fungos. 36° Congresso Brasileiro de Fitopatologia, Uberlândia, Brasil, v.28, p.361

Zanandrea, I.; Juliano, D.S.; Andréa, B.M.; Juliane, L.; Veridiana, K.B. 2004. Atividade do óleo essencial de orégano contra fungos patogênicos do arroz: crescimento micelial em placas. Revista Brasileira de Farmacognosia, v.14, supl. 01, p. 14-16.