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Inducing resistance in cotton against *Colletotrichum gossypii* var. *cephalosporioides* with essential oils

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Abstract: This study aimed to evaluate the potential of essential oils of rosemary (*Rosmarinus officinalis*), baccharis (*Baccharis trimera*), lemon grass (*Cymbopogon citratus*), basil (*Ocimum basilicum*) and eucalyptus (*Corymbia citriodora*) in inducing resistance in cotton plants against *C. gossypii* var. *cephalosporioides*. The inductive effect of the essential oils was evaluated in plants growing in pots in the environment, which were treated with 1% essential oil at 47 days of age. 24 hours after elicitor treatment the plants were inoculated with a suspension of 1.5 x 10^5 conidia mL^{-1} of *C. gossypii* var. *cephalosporioides*. Five evaluations were performed disease and calculated the area under the disease progress curve. All essential oils showed potential for inducing resistance against cotton *C. gossypii* var. *cephalosporioides*.
Keywords: Medicinal plants, ramulosis, alternative control, *in vivo*.

Introduction

The cultivation of cotton in the north of Mato Grosso, has been conquering its space as an option to the growers, especially because weather in this area is favorable to the development of this cultivation. However, the cotton plant has a high rate of pest attacks, and diseases. Among the fungal diseases that attack the cotton cultivation, the ramulosis (*Colletotrichum gossypii* var. *cephalosporioides),* is one of the most important diseases of cotton plant in Brazil, and in high levels of severity it can occur losses in susceptible cultivars over 70% in production (Araújo et al., 2000).

The most used method by cotton growers to control pests and diseases is the chemical control, which are carried several applications of agrochemicals to control. Consequently, this control promotes a big environmental impact, contaminating rivers, soil, fauna, flora, and mainly resulting in harm to human health. Due those negative aspects mentioned, the search for substitutes for those agrochemicals, finds in plants an alternative of economic and ecological interest, quite promising (Souza et al., 2007). Furthermore, the diversity of active substances in medicinal plants motivates the development of researches evolving plant extracts and essential oils, in view of control *in vitro* of phytopathogens (Venturoso et al., 2010).

Several jobs demonstrate aqueous raw extracts and essential oils of medicinal plants and forest species present potential to control phytopathogens (Schwan-Estrada et al., 2000; Bonaldo et al., 2004; Rodrigues et al., 2006; Silva et al., 2009; Matiello & Bonaldo, 2013; Lima et al., 2014), by direct antifungal activity or induced resistance. The identification of vegetal species that has fungitoxic properties (Lima, 2007) or induced resistance is an alternative that can contribute to minimize the high production costs in agriculture, as well as environmental damage due to the use of agrochemicals.

The essential oil from rosemary (*Rosmarinus officinalis*) contains pinene, camphene, bornepol, cineole and also has tannins alkaloids, saponins, flavonoids and rosmarinic acid (Martins et al., 2003). Whereas baccharis have chemical constituents as tannins, lactones, saponins, essential oils, diterpenes, lignins, alpha and beta pinene, steroids, polyphenols, sesquiterpenes, ledol, sesquiterpene alcohols, nerotidol, spirulina, kaempferol, squalene, flavonoid glycosides, and many others (Couto, 2006). The principal essential oil of *Baccharis trimera* is the carquejol, which is also its main active constituent (Martins el al., 2003).

The chemical constituents present in the essential oil of lemon grass are: gerianiol, citral (anti-

spasmodic, antimicrobial, insecticidal and repellent), myrcene, cimbopogonol, dipentene, and others (Martins el al., 2003); the major chemical constituents present in basil are: thymol, estragole, methyl chavicol, linoal, camphor and tannins (Martins et. al., 2003). In the essential oil of eucalyptus (*C. citriodora***)** are found the chemical constituents: citronellol (approximately 85%), and geraniol, isopulegol, α and β pinene, cineol, guaiol, estragole, γ-element, nopineno, camphene, myrcene and β-cymene (Costa, 1986).

Therefore, this job aimed to evaluate the potential for essential oils of rosemary (*R. officinalis*), baccharis (*B. trimera*), lemon grass *(C. citratus),* basil *(O. basilicum),* and eucalyptus (*C. citriodora*) in order to induce resistance of cotton plants against *Colletotrichum gossypii* var. *cephalosporioides.*

Methods

For the development of this work were used essential oils of medicinal plants: rosemary (*R. officinalis*)*,* baccharis (*B. trimera*), basil (*O. basilicum)*, and lemon-grass (*C. citratus*) and eucalypt forest species (*C. citriodora*).

The essential oils of rosemary, basil, baccharis, lemon-grass and eucalyptus were obtained in the company "Quinarí Casa das Essências".

The isolated of *C. gossypii* var. c*ephalosporioides* nº 107 was provided by EMBRAPA Algodão (Brazilian Company of Agricultural Research), and kept in Petri dishes with PDA (Potato Dextrose Agar) culture medium at 25 ° C in the dark.

Colonies with 6 days old were used to inoculum preparation, adding 10 mL of sterile water on the surface of Petri dish containing the pure colony of *C. gossypii* var. *cephalosporioides*. Then, the colony was scraped with a razorblade, the obtained suspension was filtered through cheesecloth, and 1 mL of the same was put in Neubauer Chamber to perform a spores counting.

Bioassay of protection for cotton against Colletotrichum gossypii var. cephalosporioides

Were used two varieties of cotton: LDCV 03 and LDCV 22, planted in black tapered vases with 22x21 cm dimension. As substrate, was used forest soil, and in each vase, were put 4 seeds. After the emergence, was performed the thinning, leaving only one plant per vase.

The essential oils were applied in a 1% concentration plus 1% Tween (polysorbate surfactant), in the cotton plants with 47 days old, with a spray to the runoff point. Sterile distillated water was used as a control and 24 hours later; of elicitor treatment, the plants were inoculated with conidia suspension of *Colletotrichum gossypii* var. *cephalosporioides* ($1{,}5x10^5$ conidia.mL^{-1}), and kept in humid chamber for 24 hours; After this period the plants were kept in environmental conditions.

The assessments of severity for ramulosis started after 13 days passed from inoculation, when was emergence of the first symptoms of disease, and were performed according to the scale of Costa et al. (1941) (Table 1).

Table 1. Rating scale for assessments of severity to ramulosis in cotton plants (Costa,1941).

Grade	Description of symptoms
1	No symptoms.
2	Few necrotic lesions on leaves.
3	Many necrotic lesions on leaves.
4	Plants with short upper internodes.
5	Plants with short upper internodes associated with overbudding.
6	Plants with short internodes with overbudding.
7	Plants with short internodes with overbudding without possibility of producing fruits (< 3 fruits / plant).
8	Overbudding accented with reduced size.
9	Excessive overbudding with reduced size and total absence of fruits.

After finish collecting data was made the area under the disease progress curve (AUDPC), using the equation proposed by Campebell & Madden (1990).

$$AACPD = \sum_{i=1}^{n} \left(\frac{(y_i + y_{i+1})}{2} \right) (t_{i+1} - t_i)$$

Where:

- N= is the number of assessments;
- yi and yi+1= are the values of severity observed in two consecutive assessments; and
- t(i+1)-ti, the interval between two evaluations.

Statistical analysis

The experimental design was completely randomized with five essential oils at 1% concentration. Five replications were used per treatment, wherein each replication consisted of one plant.

The obtained data was submitted to ANOVA, applying F-test at 5% level of significance, and the means were submitted to statistical analysis by Tukey test at 5% level of significance.

Results and discussion

In the cultivar LDCV 03 all the treatments measured presented a value for AUDPC statistically lower than the value of the control (water) (Figure 1), indicating was induced resistance in cotton plants, of this cultivar, against *Colletotrichum gossypii* var. *cephalosporioides.*

The oils of rosemary (AUDCP: 98,5), and baccharis (AUDCP: 99,1), had greater effect on the induction of resistance in cotton, reducing by 69,8% and 55,1% disease, respectively. The essential oils of lemon grass (AUDPC: 140,6), eucalyptus (AUDPC: 116,1), and basil (AUDPC: 108,6) indicated lower efficiency on induction of resistance against ramulosis in cotton.

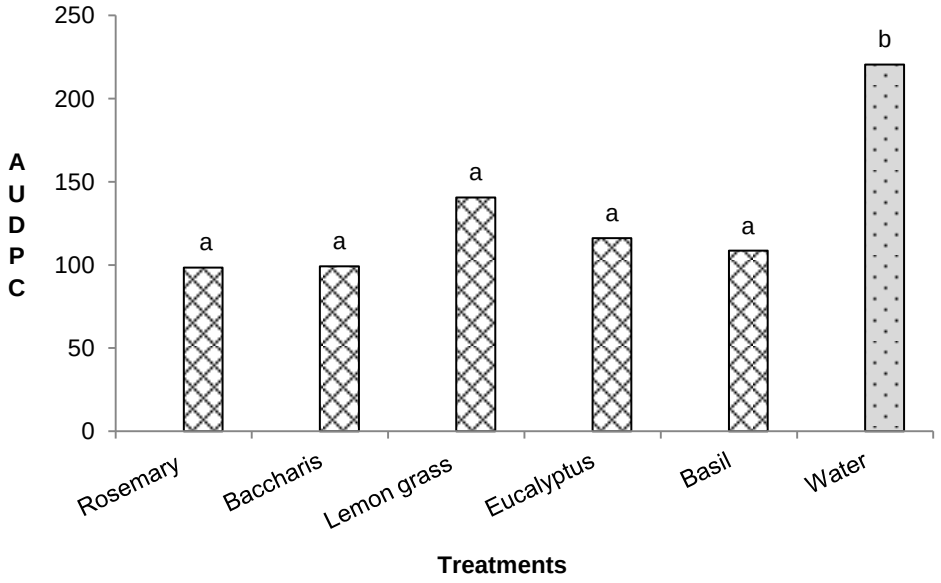

Figure 1. Area under the disease progress curve (AUDPC) caused by *Colletotrichum gossypii* var. *cephalosporioides*, in plants of cotton cultivar LDCV 03, treated with essential oils of medicinal plants and forest specie. Means followed as the same letters does not differ by Tukey test at 5% level of significance.

Pereira et al. (2006) studied the effect of essential oils of condiments in antifungal activity of *Fusarium* sp.; *Aspergillus ochraceus; Aspergillus flavus e Aspergillus niger,* and noted that the oils of rosemary, mint, onion and basil presented inhibitory effect of fungal growth from the concentration of 1500 $mg.mL^{-1}$.

Arieira et al. (2010), has measured the efficiency, *in vitro* and *in vivo*, the oils of eucalyptus (*Eucalyptus citriodora*) and neem (*Azadirachta indica*) to control *Colletotrichum acutatum* in strawberry. Observed that in *in vitro* test, all the treatments (concentration of 0; 0,25; 0,5; 1,0 and 1,5%) presented significant reduction mycelial of fungus when compared to the control. Afield, only the oil of neem, in 0,5% concentration, presented a significant effect, reducing floral abortion and occurrence of diseased fruits. Other studies report the effectiveness of eucalyptus essential oil, as well as other species, in control of fungus, nevertheless those studies measured the mycelial growth in controlled conditions and, often, the efficiency is related with high doses of essential oils. Stangarlin et al. (1999) observed that the reduction 14-34% in appressorium formation of *Colletotrichum graminicola* was obtained when the eucalyptus extract had been applied in concentrations superior to 10%. In a study carried out by Lee et al. (2007), the eucalyptus oil inhibited the growth of the *Botrytis cinerea*, *Rhizoctonia solani, Fusarium oxysporum* and *Pythim ultimum*, in 91, 87, 57 and 50%, respectively, although did not inhibit mycelial growth of *C. gloeosporioides*.

In the Figure 2, it presents the results of AUDPC of ramulosis in the cultivar LDCV 22 plants of cotton, when treated different essential oils of medicinal plants. There was significant differences between treatments, and all the essential oils studied differed from the control (water).

The essential oil of basil presented lower AUDPC (82,75), being the most efficient in induction of resistance in cotton plant against *C. gossypii* var. *cephalosporioides*. The induction of resistance was also observed by Felipe et al. (2004), barley sprayed with the extract of basil, presenting 92-96 % of protection against *Bipolaris sorokiniana***.**

The treatments with essential oil of rosemary (AUDPC: 106,92), lemon grass (AUDPC: 113,25), and eucalyptus (AUDPC: 17,50) do not differ, reducing in 31,9%, 27,6% and 24,9%, respectively. Therefore, those essential oils are potential tools for alternative control of *C. gossypii* var. *cephalosporioides.*

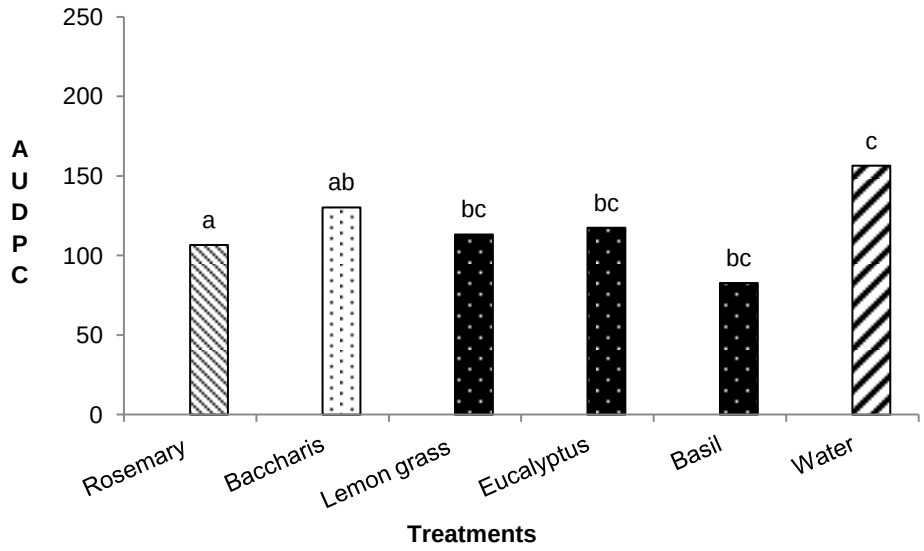

Figure 2. Area under the ramulosis (*Colletotrichum gossypii* var. *cephalosporioides*) progress curve, in cotton plants – cultivar LDCV 22, treated with essential oils of medicinal plants and forest specie. Means followed as the same letters does not differ by Tukey test at 5% level of significance.

Bonett et al. 2010 obtained satisfactory results when they studied the inhibitory potential of rosemary essential oil, on the mycelial growth of *Colletotrichum gloeosporioides,* evidencing that this plant has antifungal compound.

Camatti-Sartori et al. (2011) studied the potential of acetic and ethanol extracts of rosemary, horsetail, ginger, garlic, chamomile, bay leaves, basil, mint and eucalyptus to control *Fusarium* sp. e *Botrytis* sp. of flowers, and showed a higher percentage of inhibition to mycelial growth by acetic extract of rosemary for both fungi.

According to study by Rozwalka et al. (2008), assessing the antifungal effect of extracts, decoctions and essential oils of medicinal and aromatic plants, there was an inhibitory effect of extracts of rosemary and ginger on the mycelial growth of *Glomerella cingulate* and *C. gloeosporioides* indicating potential for anthracnose control in guava fruit.

Carnelossi et al. (2009) studied the effect of lemon grass essential oil, eucalyptus and peppermint and *Artemisia dracunculus* in post-crop control of *Colletotrichum gloeosporioides* using *in vivo* test in papaya fruits, and observed a lower AUDPC in treatment with lemon grass essential oil and eucalyptus. Júnior et al. (2009) also observed complete inhibition of spore germinat ion of *C. gloeosporioides,* under treatment with lemon grass essential oil. Guimarães et al. (2011) observed high fungitoxicity in lemon grass essential oil on pathogenic fungi *Alternaria alternata*, *C. gloeosporioides, Fusarium oxysporum cubense* and *Bipolaris* sp.

In the present study the baccharis essential oil (AUDPC: 130,25) in the cultivar LDCV 22, provided less control of the disease by inducing resistance.

Importantly, the essential oils studied at a concentration of 1% did not cause phytotoxicity on cotton plants. Based on the present study, it was observed that the essential oils showed great potential in ramulosis control in field, independent of cultivar. Future studies must be realized to enable the use of this technology by cotton farmers. However, to small producers, the cost of this technology can be high, in this case, the use of aqueous raw extracts (has low cost of obtaining), can be a promising alternative. In this sense, the future studies should be conducted aiming to determine the time and the proper concentration of the extracts for applications on plants.

Conclusions

Essential oils of rosemary (*R. officinalis*), coot (*B. trimera*), lemongrass (*C. citratus*), basil (*O. basilicum*) and eucalyptus (*C. citriodora*) induced resistance in cotton against *Colletotrichum gossypii* var. *cephalosporioides.*

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