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EFFECT OF ESSENTIAL OILS ON THE “IN VITRO” MICELIAL GROWTH OF THE FUNGUS *LASIODIPLODIA THEOBROMAE*

EFEITO DE ÓLEOS ESSENCIAIS SOBRE O CRESCIMENTO MICELIAL DO FUNGO *LASIODIPLODIA THEOBROMAE* “IN VITRO”

*[1]Talita Lopes Viana; [1]Walmor Moya Peres; [1]Grace Queiroz David; [1]Dilânia Lopes de Matos; [1]Ostenildo Ribeiro Campos; [1]Oscar Mitsuo Yamashita; [1]Marco Antonio Camillo de Carvalho; [2]Paulo Cezar Ceresini
[1]Universidade do Estado de Mato Grosso/UNEMAT; [2]Universidade Estadual Paulista/UNESP

*Autor correspondente: e-mail: yama@unemat.br

ABSTRACT

Studies have shown that plant essential oils have the potential for alternative control of several economically important crop diseases. The objective of this work was to evaluate the effect of garlic (*Allium sativum* L.), coconut (*Cocos nucifera* L.), copaiba (*Copaifera landesdorff*), castor bean (*Ricinus communis* L.) and melaleuca (*Melaleuca alternifolia*) essential oils on mycelial growth. fungus *Lasiodiplodia theobromae* at non-toxic concentrations to plants. The experiment was carried out at the UNEMAT Microbiology and Phytopathology Laboratory. The experimental design was completely randomized with five treatments and one control, using five replications. From the growth averages, the Mycelial Growth Speed Index (IVCM) and the Growth Inhibition Percentage (GIP) were obtained. Data were subjected to analysis of variance, followed by Tukey test at 5% significance level. All treatments had good results in controlling the mycelial growth of the fungus L. theobromae, and the treatments with melaleuca and coconut essential oil showed the best results and did not differ statistically.
Keywords: Antifungal activity, Phytopathogen, Alternative control.

RESUMO

Estudos têm revelado que óleos essenciais de plantas apresentam potencial para o controle alternativo de diversas doenças. O objetivo deste trabalho foi avaliar o efeito de óleos essenciais de alho (*Allium sativum* L.), coco (*Cocos nucifera* L.), copaíba (*Copaifera langsdorffi*), mamona (*Ricinus communis* L.) e melaleuca (*Melaleuca alternifolia*) no crescimento micelial do fungo *Lasiodiplodia theobromae* em concentrações não tóxicas para as plantas. O experimento foi realizado no Laboratório de Microbiologia e Fitopatologia da UNEMAT, Alta Floresta, MT, Brasil. O delineamento experimental utilizado foi o inteiramente casualizado com cinco tratamentos e uma testemunha, utilizando cinco repetições. A partir das médias de crescimento foi obtido o Índice de Velocidade de Crescimento Micelial (IVCM) e o Percentual de Inibição de Crescimento (PIC). Os dados foram submetidos a análise de variância, seguido de teste Tukey ao nível de significância de 5%. Todos os tratamentos tiveram bom resultado no controle do crescimento micelial do fungo *L. theobromae*, sendo que os tratamentos com óleo essencial de melaleuca e coco foram os que apresentaram os melhores resultados não diferindo entre si estatisticamente.
Palavras-chave: Atividade antifúngica, Fitopatógeno, Controle alternativo.

1 INTRODUCTION

The fungus *Lasiodiplodia theobromae*, belonging to the Deuteromycetes class, is a pathogen responsible for diseases in several economically important crops, which induces the symptoms of fruit rot, cancer and drought [1].

The pathogen is characteristic of tropical and subtropical regions, where it occurs in about 500 plant species. Previously *L. theobromae* was considered an opportunistic fungus, but over the years it has been a serious problem for producers in various agroecosystems, among

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the most damaged species are avocado, citrus, coconut, Argentine eucalyptus, jackfruit, cassava, melon, fig, hose, guava, papaya and grapevine [2].

According to [3], Brazil ranks third in the world fruit production ranking and is responsible for 5.7% of the harvested volume, with a production of 41.5 million tons. It is clear that the incidence of plant fungi in cultivation areas can be a limiting factor in the amount of production and also in the quality of what is produced.

It is noteworthy that the control method most used by producers is still chemical control. Despite the significant contribution of these products to agricultural production, the continuous and indiscriminate use of pesticides causes environmental problems such as the emergence of resistant pathogens and the interruption of natural biological control, causing disease outbreaks and favoring the emergence of secondary pests [4].

According to [5], most of the chemical contaminants present in ground and surface waters are related to agricultural sources, and pesticides assume a prominent place, due to the intensity of their consumption in Brazil.

Another concern regarding the development of new alternative control methods is regarding the organic producers, whose market has grown significantly, with an average annual rate of 20 to 30% in 2016 and may move around US$1.0 billion [6]. This concern is also reported by [7], emphasizing that in production systems where the use of chemical control is not allowed, such as in organic cultivation, there is a need for alternative methods, with proven efficiency, to control pests and diseases.

Due to these factors, the demand for alternative products that serve as pesticides and cause less damage to the environment, whether chemical, biological, organic or natural, has been growing. This category may include various biofertilizers, syrups, biocontrol agents and essential oils [8]. The use of essential oils from medicinal plants has shown promising results in the control of plant pathogens [9].

Plant fungicides have been used for centuries. Research involving the demand for plant-derived fungicides has been increasing in recent years. Essential oils are generally a very complex mixture of hydrocarbons, alcohols and aromatics found in all living plant tissues, usually concentrated in bark, flowers, leaves, rhizomes and seeds [10].

Importantly, despite the great potential of the use of essential oils in sustainable agriculture, these plant compounds can impair plant development and germination if used indiscriminately [11]. Thus, it is important to develop research that demonstrates that, in the

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correct form and concentrations, these natural products can be important tools for phytosanitary management in various situations.

The objective of this study was to evaluate the fungistatic effect of essential oils on the in vitro alternative control of *L. theobromae*.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Microbiology and Phytopathology of UNEMAT - State University of Mato Grosso, Campus Alta Floresta, Brazil.

The essential oils of coconut, garlic, castor bean, copaiba and melaleuca, obtained in local trade, in health food stores were tested. The culture medium used was Potato-Dextrose-Agar (BDA).

The fungus was obtained from the Microbiology Laboratory Mycotheca where it was stored in the form of BDA discs with fungal mycelium in glasses containing sterile distilled water. The pathogen was grown in Petri dishes in sterile BDA culture medium. Prior to obtaining the pure colonies of the fungus, they were evaluated daily for macromorphology through the pigmentation, texture, consistency and shape of the back and reverse colonies and their growth velocity, obtaining the final diameter equal to from the petri dish.

The colonies were then stored in BOD-type growth chambers at 25 ºC and 12 hours photoperiod for 7 days. On this occasion, immature (spherical shaped unicellular hyaline color) and mature (dark brown, multicolored oval shaped) conidia were observed.

In the experiment, only the concentration of essential oils at 1% was used for all treatments, ie 2.5 mL of product for each 250 mL of BDA. The same ratio of Nutrifix® adhesive spreader (2.5 mL of product/250 mL of BDA) was also added for a uniform dilution of the oil to the aqueous medium and 250 mg of amoxicillin antibiotic for each 250 mL of BDA to avoid contamination. from the middle by bacteria.

The experimental design was completely randomized, and each treatment consisted of 5 repetitions, and each repetition consisted of 4 Petri dishes. Thus, a total of 6 treatments were evaluated, 5 essential oils and 1 control. For the preparation of the culture media in the Petri dishes, 10 ml of BDA was poured into them. After solidification of the medium, a fungal mycelium disc (9 mm in diameter) was placed in the central region of each plate. For the evaluation of the mycelial growth of the fungus *L. theobromae*, an axis was traced on the back of the plate to perform the measurements.

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The plates were placed in a germination chamber (BOD) at 25 °C with a photoperiod of 12 hours for 2 days; time needed for the control fungus to grow by fully occupying the Petri dish. The evaluation of mycelial growth consisted of daily measurement of the diameter of the colonies in two perpendicular directions, with the aid of a millimeter ruler, obtaining the average values of mycelial growth.

From the mycelial growth averages the mycelial growth rate index (IVCM) was obtained, as proposed by [12]:

$$IVCM = \frac{\Sigma(D - Da)}{N}$$

On what:

D = current average diameter of the colony;

Da = average diameter of the previous day colony;

N = number of days after inoculation;

The growth inhibition percentage (GIP) of the treatments relative to the control was determined according to the formula cited by [13]:

$$GIP = \frac{(Dt - Do)}{Dt} x100$$

On what:

Dt = witness diameter

Do = treatment diameter

The obtained data were submitted to analysis of variance, followed by the test of comparison of means (Tukey) at the significance level of 5%, with the aid of the statistical software Sisvar® [14].

RESULTS AND DISCUSSIONS

Significant effect was found in all essential oil treatments on *Lasodiplodia theobromae* mycelial development. On the first day of evaluation, as shown in (Table 1), we observed that the growth rate of coconut, melaleuca, copaíba and castor oil treatments did not differ, showing that garlic essential oil had the highest growth compared to the other treatments, but showed a significant difference in relation to the control.

Table 1. Mycelial growth (mm) and *Lasiodiplodia theobromae* mycelial growth rate index (IVCM) under the effect of vegetable oils.

Treatments / Vegetable Oils	Mycelial Growth (mm) (mm)	IVCM

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	1st. evaluation	2nd. evaluation	
T_1- Coconut	0.93 a	1.98 a	0.53 a
T_2- Melaleuca	1.08 a	1.78 a	0.35 a
T_3- Copaiba	1.63 a	2.88 ab	0.63 a
T_4- Castor bean	2.30 a	3.85 b	0.63 a
T_5- Garlic	7.95 b	9.20 c	0.78 a
T_6- Witness	21.45 c	40.00 d	9.28 b
CV%	17.96	8.71	25.26

* Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability.

On the second day of evaluation it was possible to observe that the treatments with coconut, melaleuca and copaíba essential oil did not differ statistically, and the castor oil treatment did not differ significantly from the copaíba oil treatment (Figure 1). The treatment with garlic oil obtained the highest values, showing a lower efficiency compared to the other treatments, but it still differed significantly from the control. Melaleuca oil showed better results in inhibiting mycelial growth of the fungus *Lasiodiplodia theobromae*, as shown in the table above. Melaleuca essential oil also significantly reduced mycelial growth of the fungi *Macrophomina phaseolina*, *Sclerotinia sclerotiorum* and *Alternaria alternata* at all levels. Concentration (0.2, 0.4, 0.6 and 0.8%), suggesting that the oil may be useful in the treatment of fungal infections in plants [15].

Too [16] analyzing the effects of different concentrations of essential oils (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) on the growth of the fungus *Colletotrichum gloeosporioides*, in pepper, showed that the andiroba, coconut and eucalyptus oils showed ability to inhibit the growth of the fungus as its concentration was increased, differing from the control from the 1.0% concentration. Similar result to that obtained in the present work, considering that the treatment with tea tree essential oil was the most efficient, followed by coconut essential oil.

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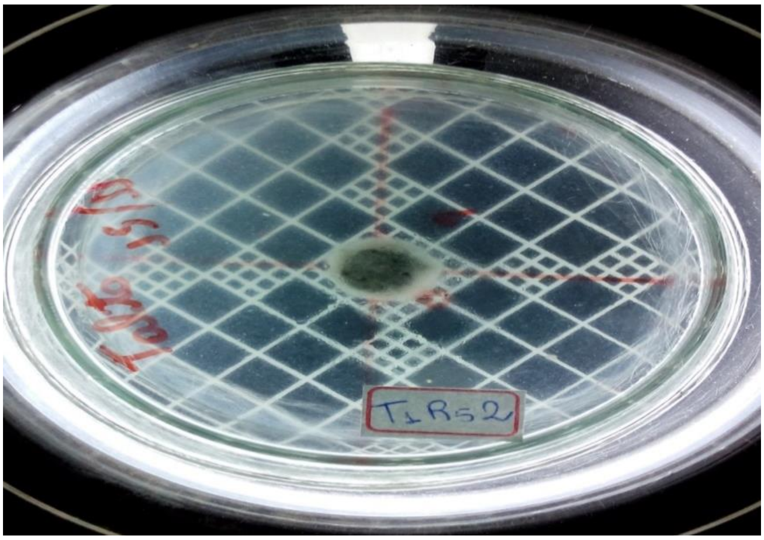

Figure 1. *Lasiodiplodia theobromae* mycelial growth with coconut oil treatment on the second assessment day.

And [17] testing essential oils of different copaiba species extracted from different parts of the plant, verified their potential to inhibit the mycelial growth of *Fusarium solani*, causal agent of foot and root rot in black pepper plants.

Research indicates satisfactory control of the fungi *Rhizoctonia solani*, *Sclerotium rolfsi*, *Macrophomia phaseolina*, *Bipolar sorokiniana* and *Colletotrichum gloeosporioides*, showing a broad antifungal activity linked to compounds present in copaiba oil [18].

Castor oil compared to coconut and melaleuca had a less satisfactory result in inhibiting mycelial growth of the fungus *L. theobromae*. Similar results were obtained by [19] when evaluating the fungitoxic effect of alcoholic extracts of castor bean leaves tested on *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium expansum*, *Fusarium poae* and *Fusarium moniliform* fungi.

According to Table 1 the least efficient treatment was with garlic oil, but compared to control the treatment still obtained a good result. Further studies would be needed to prove the full effectiveness of the oil in controlling *L. theobromae*, with higher dosages, for example, since garlic is known to have an active ingredient with fungicidal action as found by [20], evaluating the toxicity of garlic extract on *Cylindrocladium clavatum*, *Fusarium moniliform*, and *Rhizoctonia solani*.

It is noted from Figure 2 that all treatments significantly interfered with fungal growth, reaching approximately 100% inhibition. The treatments with essential oil of melaleuca 96% and coconut 95% had the highest inhibition percentage and did not differ statistically, followed by copaíba 93% that did not differ from melaleuca and coconut, and castor bean with 90%, the

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garlic oil treatment obtained the most moderate percentages with 77%, a satisfactory result compared to the control that obtained 0% inhibition.

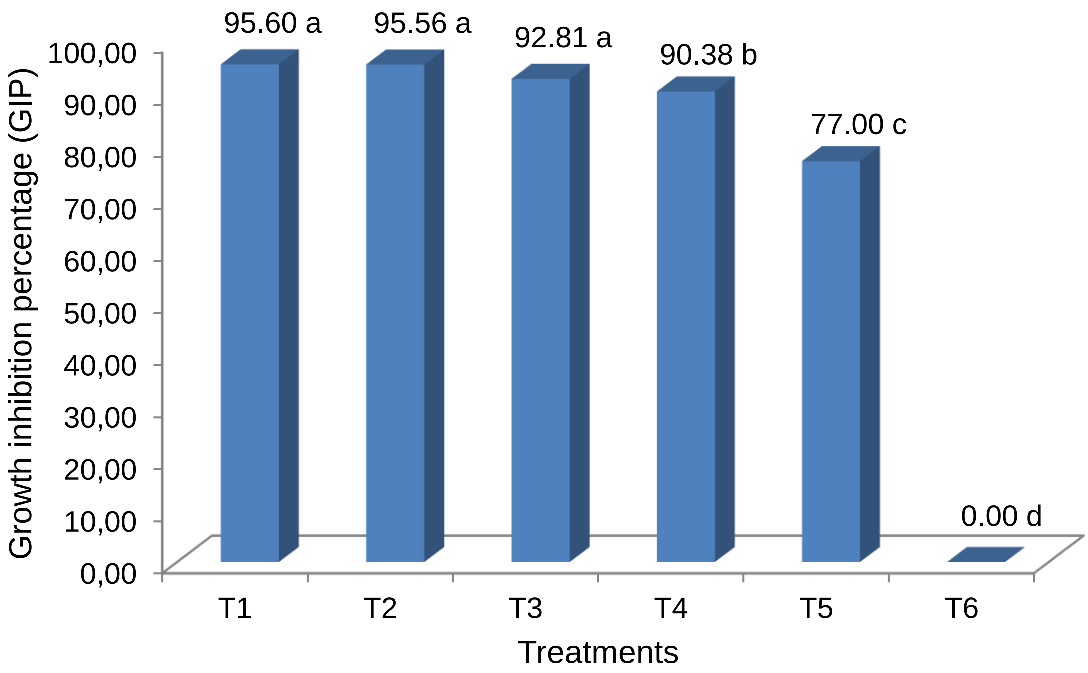

Figure 2. Growth Inhibition Percentage (GIP) of *Lasiodiplodia theobromae* on vegetable oil effect.

There are studies that prove that the presence of substances capable of inhibiting fungal diseases in plant extracts and oils, and it is a reality that deserves more careful studies, mainly because all treatments obtained good results, emphasizing the tea tree and coconut oil. Which in turn has been little studied for alternative control for plant fungi, and even at the 1% concentration controlled the fungus *L. theobromae* famous for its severity.

[21] showed in studies that complete inhibition of mycelial growth of the fungus L. theobromae was obtained with the essential oil of rosemary (*Lippia sidoides*), which exhibited a toxic effect on the fungus at all concentrations used (3, 6, 9, 12 and 15 mL).

Studies with *Rhizoctonia solani* evaluated with copaiba oil-resin showed in general that, as concentration was increased, inhibition of mycelial growth in vitro was more efficient, with concentrations (0.1; 0.2; 0.5 and 0.75%) GIP ranged from 66.44 to 76.44% [18].

The antifungal activity of essential oils in general according to [22], is related to their hydrophobic property, which when in contact with fungus interacts with cell membrane wall lipids and mitochondria, altering their permeability. causing structural disturbances, which can

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promote exposure of cellular content, including exposure of the cell nucleus. In addition, oil components can bind to ions and molecules (hormones) in other cells.

CONCLUSION

There is a potential effect of the essential oils of melaleuca, coconut, copaiba, castor and garlic resulting in the difficulty of mycelial development of the fungus *Lasodiplodia theobromae*.

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