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Essential oils of Tahiti lemon and cinnamon bark in control of storage fungi and the physiological and sanitary quality of beans

Óleos essenciais de limão Taiti e canela em casca no controle de fungos de armazenamento e na qualidade fisiológica e sanitária de feijão

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ABSTRACT: With the objective to evaluate the efficiency of essential oils of *Citrus latifolia* (Tahiti lemon) and *Cinnamomum zeylanicum* (cinnamon bark) in the control of plant pathogens *Penicillium* sp. and *Aspergillus* sp. and the quality of the bean seeds, two experiments were conducted. In the first one, the effect of essential oils of *C. latifolia and C. zeylanicum* was evaluated in vitro development of the fungi *Penicillium* sp. and *Aspergillus* sp. and, in the second one, the influence of essential oils on the physiological and sanitary quality of bean seeds. The variables mycelial growth, conidial germination and sporulation of *Penicillium* sp. and *Aspergillus* sp. were measured in the first experiment, while the seed germination test, first count of germination, germination speed index (GSI) and sanity test of bean seeds were measured in the second. The essential oil (EO) of *C. zeylanicum* was more efficient than *C. latifolia* in the control of *Aspergillus* sp. and *Penicillium* sp., but decreased the physiological quality of the beans seeds. The fungal diversity identified in the seed health test was composed by fungi of the genera *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium, Chaetomium* and *Rhizopus*. The results indicate the potential of the use of these EOs in the seeds treatment.

KEYWORDS: *Phaseolus vulgaris*; *Penicillium* sp.; *Aspergillus* sp.; *Cinnamomum zeylanicum*; *Citrus latifolia.*

RESUMO: Com o objetivo de avaliar a eficiência dos óleos essenciais de *Citrus latifolia* (limão taiti) e *Cinnamomum zeylanicum* (canela em casca) no controle dos fitopatógenos *Penicillium* sp. e *Aspergillus* sp. e na qualidade das sementes de feijão, foram conduzidos dois experimentos. No primeiro, avaliou-se o efeito dos óleos essenciais de *C. latifolia e C. zeylanicum* no desenvolvimento *in vitro* dos fungos *Penicillium* sp. e *Aspergillus* sp. e, no segundo, a influência dos óleos essenciais sobre a qualidade fisiológica e sanitária das sementes de feijão. As variáveis crescimento micelial, germinação de conídios e esporulação de *Penicillium* sp. e *Aspergillus* sp. foram aferidas no primeiro experimento, enquanto o teste de germinação de sementes, primeira contagem de germinação, índice de velocidade de germinação (IVG) e teste de sanidade de sementes de feijão foram aferidas no segundo. O óleo essencial (OE) de *C. zeylanicum* foi mais eficiente que *C. latifolia* no controle dos fungos *Aspergillus* sp. e *Penicillium* sp., mas diminuiu a qualidade fisiológica das sementes de feijão. A diversidade fúngica identificada no teste de sanidade de sementes foi composta por fungos dos gêneros *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium, Chaetomium* e *Rhizopus*. Os resultados indicam o potencial do uso desses óleos essenciais no tratamento de sementes.

PALAVRAS-CHAVE: *Phaseolus vulgaris*; *Penicillium* sp.; *Aspergillus* sp.; *Cinnamomum zeylanicum*; *Citrus latifolia.*

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INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) stands out among the agricultural crops in Brazil as a result of the extension of cultivated area, production value and importance as a source of protein for the population (TOLEDO et al., 2009). Bean is cultivated by approximately 2 million Brazilian farmers, being small farmers responsible for 64% of production in areas smaller than 5 hectares (STONE et al., 2013).

The contamination of the beans by fungi after harvesting can determine significant losses during the storage period, compromising the grains quality and the germination and vigor of the seeds (SILVA et al., 2013). In addition to direct damage to the grains and seeds, they may introduce pathogens into new areas or even introduce a race of a pathogen that may cause major damage to the culture (DALLA PRIA; SILVA, 2010; MENTEN, 1995). Thus, the treatment of the seeds constitutes an important method for preserving its original characteristics and for the prevention of contamination of areas free of pathogens spread by seeds.

Penicillium and *Aspergillus* genera are among the most important for stored bean grains and seeds (MARINO; MESQUITA, 2009). The use of fungicides applied in various formulations and dosages, has been the most usual and effective for the treatment of the seeds (VANZOLINI et al., 2000). However, the use of synthetic products causes adverse effects to the environment and human health (GHINI; KIMATI, 2000), arousing interest in the use of alternative methods with natural products for treatment of the seeds, in the form of extracts, powders and essential oils (EOs).

The EOs represent an important alternative to be exploited in the treatment of the seeds, because in addition to the fungicide effect they are normally biodegradable and have low toxicity to humans and animals (KRISCH et al., 2011; SIVAKUMAR; BAUTISTA-BAÑOS, 2014). Several EOs have been showing promising results for the treatment of the seeds, as the oils of *Allium sativum* L. and *Cinnamomum zeylanicum* in control of the fungus *Aspergillus flavus* (VIEGAS et al., 2005a), *Citrus latifolia* in the inhibition of conidial germination of *Penicillium digitatum* and *P. italicum* (FRENCH et al., 1978), *Moringa oleifera* on the mycelial growth of *Fusarium oxysporum, Cladosporim Sphaerospermum* and *Colletotrichum lindemuthianum* (SILVA et al., 2009).

In addition to efficiency in the microorganisms control, it is important that compounds present in the EO do not affect the seeds physiological quality. GOMES et al. (2016) found that clove oil in India at a concentration of 2 mL.L^{-1} reduced the physiological quality of lima bean seeds (*Phaseolus lunatus* L.). Similar results were observed by VIEGAS et al. (2005b) in which the oil of garlic (*Allium sativum* L.) and cinnamon (*Cinnamomun Zeylanicum* Breyn.) reduced the germination of peanut seeds (*Arachis hypogaea* L.).

Hypothetizing the potential use of EOs in the control of seed phytopathogenic fungi, the objective of this study was to evaluate the efficiency of EOs of *Citrus latifolia* and *Cinnamomum zeylanicum* in storage control of fungi *Penicillium* sp. and *Aspergillus* sp. in beans and their influence on the physiological quality of the seeds.

MATERIALS AND METHODS

The experiments were conducted in the Laboratories of Pathology and Germination and Plant Growth of the Universidade Federal da Fronteira Sul (UFFS), Laranjeiras do Sul, Paraná.

Obtainment of phytopathogenic fungi, essential oils and bean seeds

The fungi *Penicillium* sp. and *Aspergillus* sp. were isolated from common bean seeds variety Tuiuiú using the seed health test, incubation on paper substrate by the blotter test method (BRASIL, 2009). A single conidium was collected and cultivated in potato-dextrose-agar (PDA) and kept at 25 ± 2°C, being used with 10 days of growth. Whereas the EOs of *Citrus latifolia* and *Cinnamomum zeylanicum*, were purchased from Quinarí Casa das Essências, and the bean seed variety Tuiuiú purchased from rural producers in the municipality of Laranjeiras, Paraná.

Two experiments were conducted to achieve the work objectives. In the first experiment, the effect of EOs of *C. latifolia and C. zeylanicum* was evaluated in vitro development of the fungi *Penicillium* sp. and *Aspergillus* sp., and, in the second one, the influence of EOs on the physiological and sanitary quality of bean seeds.

Effect of essential oils in the development of *Penicillium* sp. and *Aspergillus* sp.

The experimental design was completely randomized with ten treatments and four replications. For each one of them was used EO of *Citrus lafifolia* and *Cinnamomum zeylanicum* concentrations of 0.1; 0.2; 0.4 and 0.8%, fungicide and control, for the fungi *Penicillium* sp. and *Aspergillus* sp. The culture medium in PDA was sterilized at a temperature of 121°C for 20 minutes. After reaching a temperature of approximately 40°C the aliquots of 0.1; 0.2; 0.4 and 0.8% of EOs of *C. latifolia* and *C. zeylanicum* were added.

The same procedure was performed for the addition of the aliquot in the proportion of 2.5% of the fungicide (150 g L^{-1} carbendazim + thiram 350 g L^{-1}) in PDA medium. The solutions PDA / EO and the PDA / fungicide were poured on Petri plates and it took 2 hours for the solidification of the medium. The growing medium for the control treatment counted only with PDA.

Portions of approximately 10 mm in diameter, containing the mycelium of fungi *Penicillium* sp. and *Aspergillus* sp., were deposited in the center of the Petri plates. The plates were sealed with plastic film and incubated at 25 ± 2°C for seven days. After this period, the following variables were measured: mycelial growth, conidial number formed and germinated.

The measurement of the diameters (cm) horizontal and vertical position of each colony was performed with the aid of a ruler by determining the average of the two values to infer the mycelial growth medium of the colony. Then, a count of the number of developed conidia was performed. Therefore, 10 mL of distilled water were added in the Petri dish, and the scraping of the colony with spatula was performed. The volume from adding water and scraping of the colony was filtered in two layers of gauze. After filtration, dilution of the suspension was performed, adding more 40 mL of distilled water, originating the suspension of 50 mL. In the suspension, the count of the number of conidia with a Neubauer chamber was carried out, with the aid of an optical microscope. A count of the number of conidia was held seven days after the beginning of the experiment.

For counting the number of germinated conidia, microscopy slides with a layer of agar were placed in Petri plates lined with moistened filter paper. After the agar solidification, it was deposited on each end of the slide 30 µL of the conidial suspension of the fungi *Penicillium* sp. and *Aspergillus* sp., in the concentration of 2×10^5 conidia mL^{-1}. This concentration was determined by counting the conidia in a Neubauer chamber, so that 20 conidia were present in the central quadrant of the chamber. At the ends of the slide, it was also been added 30 µL of the respective treatments. In the control treatment, only the aliquot containing the conidia on the agar was added. The plates were incubated for 24 hours in the dark, being performed the paralysis of the conidia development by adding 10 µL of cotton blue on each point that contained the conidia on the slides after this period.

For the counting of germinated conidia, the slides were observed under an optical microscope. The count of 50 conidia was performed randomly in each plot for determination of the percentage of germinated conidia, being considered as germinated conidia the ones whose emitted germ tubes are greater than or equal to the smallest diameter of conidium.

The data were subjected to analysis of variance, being applied regression analysis or multiple comparison of means by Tukey test ($p < 0.05$), as appropriate.

Influence of treatments with essential oils of *C. latifolia* and *C. zeylanicum* on the physiological quality of beans seeds

For the germination tests and first count of germination, a completely randomized experimental design was used, in factorial scheme 2 × 9, being both fungi (*Penicillium* sp. and *Aspergillus* sp.) and nine treatments, being EO of *C. latifolia* at concentrations of 0.4 and 0.8% and *C. zeylanicum* at concentrations of 0.1; 0.2; 0.4 and 0.8%, control, positive control and negative control. For the GSI, the experimental design completely randomized was used, in factorial scheme 2 × 8, being both fungi (*Penicillium* sp. and *Aspergillus* sp.) and eight treatments, being EO of *C. latifolia* at concentrations of 0.4 and 0.8% and *C. zeylanicum* at concentrations of 0.1; 0.2; 0.4 and 0.8%, control, positive control and negative control. For the seed health test, it was used a completely randomized experimental design with eight treatments, being EO of *C. latifolia* at concentrations of 0.4 and 0.8% and *C. zeylanicum* at concentrations of 0.1; 0.2; 0.4 and 0.8%, positive control and negative control, for seeds inoculated with the fungi *Penicillium* sp. and *Aspergillus* sp. All tests were performed with four replicates per treatment.

The seeds were distributed in two plastic bags, and the inoculation of one of the portions was performed with the fungus *Penicillium* sp., and the other with the fungus *Aspergillus* sp. For the inoculation, 2 mL of suspension containing distilled water at a concentration of 2×10^5 conidia mL^{-1}, of the respective fungus in each plastic bag, and later the homogenization was performed by shaking the plastic bags. After being inoculated, the seeds remained exposed on the laboratory bench in the shade for approximately 4 hours, so that they were not wet.

After completing the inoculation, the seeds were treated with EOs of *C. latifolia* at concentrations of 0.4 and 0.8% and *C. zeylanicum* at concentrations of 0.1; 0.2; 0.4 and 0.8%. It was chosen to use only the highest concentrations of the EO of *C. latifolia,* because the two lowest concentrations did not differ from the control in the previous experiment. The positive control was treated with fungicide and the negative control was composed by seeds inoculated with the fungi *Penicillium* sp. and *Aspergillus* sp. and not treated with EOs. It was also used an additional control composed by non-inoculated and not treated seeds. In the case of treatment of positive control, the determination of carbendazim fungicide was carried out (150 g L^{-1} + thiram 350 g L^{-1}) at a dose of 300 mL.100 kg^{-1} of seed, plus the same volume in water. The seeds of each treatment were separated into individual plastic bags, and in each one of them, aliquots of the respective EOs and the same volume of water were added. For the negative control and additional control, it was used only the volume of water corresponding to twice the volume of fungicide used. After performing the treatments, the plastic bags were stirred for homogenization and the seeds moved to Kraft paper bags, being incubated for 14 days in the dark at approximately 20°C. The treatment of the seeds was performed 24 hours after inoculation of seeds with *Penicillium* sp. and *Aspergillus* sp.

Germination test and first count of germination

Four replications of 50 seeds for each treatment were used, arranged in rolls of paper moistened with distilled water in a proportion of 2.5 times the weight of the dry paper, which were kept in a germinator Mangelsdorf type, at a temperature of 25ºC with constant light. The evaluations were performed at 5th and 9th day counting the normal, abnormal, dead and dormant seedlings, and the results were expressed in percentage (BRASIL, 2009).

Germination speed index

The test was conducted in conjunction with the germination test, performing the daily evaluation of seedlings from the moment in which the first normal seedling appeared, which were computed and withdrawn from the substrate each day. This evaluation was extended to the 9th day after the assembly of the experiment, allowing to determine the GSI (MAGUIRE, 1962) (Equation 1).

$$GSI = N1/D1 + N2/D2 + ... + Nn/Dn \quad (1)$$

Being GSI the germination speed index, Nn the number of seedlings germinated on the nth day after sowing, and Dn the nth day after implanting the test.

Seed health testing

Four replications of 25 seeds were used for each treatment, and the seeds placed in gearbox type boxes, disinfested with alcohol 70% and lined with two sheets of filter paper moistened with 2.5 times the volume of their weight in water. Then, the boxes were sealed with plastic film and placed in BOD at 25°C and without photoperiod, for 24 hours. Afterwards, they were placed at a temperature of -20°C for 24 hours, and then returned to BOD at 25°C for five days, when the pathogens present in the seeds were identified. The seeds used in the sanity test, received the same treatments as those used in the germination test. In light of this, it is supposed that the presence of the fungi *Aspergillus* sp. and *Penicillium* sp. in seeds inoculated with the fungus *Aspergillus* sp. and *Penicillium* sp., respectively, are supposed to be from the same inoculation, and not from the natural flora present in the seeds.

RESULTS AND DISCUSSION

The EOs of *C. latifolia* and *C. zeylanicum* influenced the mycelial growth of both *Penicillium* sp. and *Aspergillus* sp. The mycelial growth of *Penicillium* sp. was reduced with the increase in the concentration of *C. latifolia*, differing 0.4% from the control (Table 1), whereas the EO of *C. zeylanicum* completely inhibited the mycelial growth in all the concentrations used. These results show the potential fungicidal activity of EO of *C. zeylanicum,* which was as efficient as the fungicide in the control of the mycelial growth of the fungus *Penicillium* sp.

The use of EO of *C. latifolia* caused reduction in the sporulation of *Penicillium* sp. in all the concentrations used, except for the concentration of 0.2%, which did not differ from the control; whereas the EO of *C. zeylanicum,* promoted the complete inhibition of sporulation of *Penicillium* sp. in all the concentrations. The EOs have different secondary metabolites, presenting biological activities that can be elicitor or antimicrobial (CUNHA et al., 2015; OOTANI et al., 2013; SILVA et al., 2014). OOTANI et al. (2016) also observed

Table 1. Mycelial growth, sporulation and conidial germination of *Penicillium* sp. in potato-dextrose-agar culture medium with different concentrations of essential oils of *Citrus latifolia* and *Cinnamomum zeylanicum*.

Treatments	Mycelial[1] growth (cm)	Sporulation[2] (number of conidia/colony 10^7)*	Germination (%)*
Control	2.63 d	17.50 d	38.00 d
Fungicide	0.00 a	0.00 a	14.00 c
EO *C. latifolia* 0,1%	2.61 cd	8.43 c	51.00 d
EO *C. latifolia* 0,2%	2.65 d	18.75 d	50 d
EO *C. latifolia* 0,4%	2.17 c	9.37 c	44.50 d
EO *C. latifolia* 0,8%	1.66 b	3.95 b	40.65 d
EO *C. zeylanicum* 0,1%	0.00 a	0.00 a	4.50 b
EO *C. zeylanicum* 0,2%	0.00 a	0.00 a	1.50 ab
EO *C. zeylanicum* 0,4%	0.00 a	0.00 a	0.50 ab
EO *C. zeylanicum* 0,8%	0.00 a	0.00 a	0.00 a

[1]OE C.latifolia $y = -2.8244 - 1.4652x$ $R^2 = 96.49$; [2]OE C.latifolia $y = Y = 10.3463 + 18.7255x - 34.0683x^2$ $R^2 = 50.52$; Fungicide: carbendazim 150 g L^{-1} + thiram 350 g L^{-1}; means followed by the same letter in column do not differ statistically among themselves by Tukey test ($p < 0.05$); *for the purpose of analysis, the data were transformed to square root of Y + 1.0 - Sqrt (Y + 1.0); EO: essential oils.

total inhibition of fungal development in seeds of cowpea (*Vigna unguiculata* (L.) Walp.) stored when treated with EO of *Cymbopogon nardus* L., being indicated as a potential alternative in the seed treatment in the concentration starting from 10%.

The EO of *C. latifolia* was not effective in the reduction of the conidial germination of *Penicillium* sp., however, the EO of *C. zeylanicum* decreased this parameter in proportion to the concentration of oil until it reached 100% control at a concentration of 0.8% (Table 1). CHAGAS et al. (2014), in a study on the influence of fungicides, EOs and biological agents to control *Amphobotrys ricini* in castor bean plant, also verified total control in the mycelial growth with application of EO of *C. zeylanicum* at a concentration of 0.3%.

The EO of *C. zeylanicum* showed similar results to those observed with the fungicide in three parameters, being thus a potential natural fungicide, completely inhibiting the mycelial growth and sporulation of the fungus *Penicillium* sp.

According to MARJANLO et al. (2009), among the several alternatives, EOs have called the attention of researchers, for their potential in protection of plants. Among the EOs, *C. zeylanicum* has been explored by presenting biological activities, such as antifungal effect (BITU et al., 2016) and antibacterial (AL-BAYATI; MOHAMMED, 2009).

As observed for the fungus *Penicillium* sp. (Table 1), the EO of *C. latifolia* promoted reduction in the mycelial growth of *Aspergillus* sp. To the extent of increasing the concentration of the latter, given that only the 0.1% concentration did not differ from the control. The EO of *C. zeylanicum* also promoted total inhibition in the mycelial growth of *Aspergillus* sp. in all the concentrations used (Table 2).

No concentration of the EO of *C. latifolia* was efficient to reduce the sporulation of *Aspergillus* sp. (Table 2). In contrast, the EO of *C. zeylanicum* proved to be efficient, completely inhibiting the mycelial growth and sporulation of *Aspergillus* sp., in all concentrations. The conidial germination of *Aspergillus* sp. was reduced in all concentrations of *C. zeylanicum* and at a concentration of 0.8% of *C. latifolia* (Table 2). It should be emphasized that, in low concentrations, 0.2 and 0.4% of *C. zeylanicum* was capable of promoting complete inhibition on germination of conidia of *Aspergillus* sp.

The EOs represent an important alternative for treatment of seeds, because in addition to the fungicide effect (KRISCH et al., 2011) they are normally biodegradable and have low toxicity to humans and animals (KRISCH et al., 2011, SIVAKUMAR; BAUTISTA-BAÑOS, 2014). Checking the efficiency of EOs in sanitary and physiological quality in lima beans seeds (*Phaseolus lunatus* L.), the EO of basil was also able to promote the reduction in the development of *Aspergillus* sp. at a concentration of 0.1% (GOMES et al., 2016).

Regarding the treatment with fungicide (Table 2), it was noted a reduction in mycelial growth, sporulation and also on conidia germination of *Aspergillus* sp., however, there was no full control as observed in the mycelial growth and sporulation of *Penicillium* sp. (Table 1).

In view of the potential that the seeds have in the spread of diseases and chemical treatments that are performed for the control of these pathogens, the use of EOs of *C. latifolia* and *C. zeylanicum* may represent an alternative method for controlling phytopathogenic fungi in stored seeds. The results presented in this study demonstrate the greatest potential of fungicidal activity of EO of *C. zeylanicum*, which was able to completely inhibit the mycelial growth and sporulation of *Penicillium* sp. and *Aspergillus* sp.

The inoculation of the common bean seeds with the fungi *Aspergillus* sp. and *Penicillium* sp., drastically reduced the seeds germination in 31 and 41.5%, respectively, compared to the uninoculated and not treated control (Table 3).

Table 2. Mycelial growth, sporulation and conidial germination of *Aspergillus* sp. in potato-dextrose-agar culture medium with different concentrations of essential oils of *Citrus latifolia* and *Cinnamomum zeylanicum*.

b1	mycelial (cm) Growth[1]	Sporulation[2] (number of conidia/colony 10^7)*	Germination[3] (%)*
Control	7.20 f	2.50 cd	90.00 c
Fungicide	2.12 b	0.64 b	3.33 a
EO *C. latifolia* 0,1%	6.78 ef	2.50 cd	69.50 bc
EO *C. latifolia* 0,2%	6.48 e	2.81 d	62.00 bc
EO *C. latifolia* 0,4%	5.37 d	2.39 cd	49.50 bc
EO *C. latifolia* 0,8%	4.70 c	1.01 bc	45.00 b
EO *C. zeylanicum* 0,1%	0.00 a	0.00 a	12.50 a
EO *C. zeylanicum* 0,2%	0.00 a	0.00 a	0.00 a
EO *C. zeylanicum* 0,4%	0.00 a	0.00 a	0.00 a
EO *C. zeylanicum* 0,8%	0.00 a	0.00 a	1.00 a

$^{1OE\ C.latifolia}$ $y = 7.5166 - 6.7665x + 4.0389\ x^2$ $R^2 = 98.52$; $^{2OE\ C.latifolia}$ $y = 3.0784 - 2.3936x$ $R^2 = 86.49$; $^{3OE\ C.latifolia}$ $y = 69.0217 - 33.3913x$ $R^2 = 84.25$; Fungicide: carbendazim 150 g L^{-1} + thiram 350 g L^{-1}; means followed by the same letter in column do not differ statistically among themselves by Tukey test ($p < 0.05$); *for the purpose of analysis, the data were transformed to square root of Y + 1.0 - Sqrt (Y + 1.0); EO: essential oils.

Both the seeds inoculated with *Aspergillus* sp. and *Penicillium* sp. presented higher germination percentages in relation to the increase of the concentration of the EO of *C. latifolia*, which highlights its potential fungicidal activity for both fungi. When the EO of *C. zeylanicum* was used, the bean seeds showed a reduction in the germination to the extent of increasing the oil concentration. Thus, in spite of being more efficient in the fungal inhibition (Tables 1 and 2) the EO of *C. zeylanicum* showed phytotoxic effect to the seeds (Table 3).

When the seeds inoculated with *Penicillium* sp. and *Aspergillus* sp. were compared, no difference was observed among the treatments, except for seeds treated with fungicide (positive control) and treated with *C. zeylanicum* 0.2% (Table 3), which showed lower germination of seeds inoculated with *Aspergillus* sp.

In the first count of germination and GSI (Tables 4 and 5) it was observed the same behavior of the germination test, in which both the seeds inoculated with *Aspergillus* sp. and the ones inoculated with *Penicillium* sp. presented a greater force in proportion to the increase in the concentration of the EO of *C. latifolia*. On the other hand, when the EO of *C. zeylanicum* was used, the bean seeds reduced the force to the extent of increasing the oil concentration, demonstrating once more, the fungicidal potential presented by the EO of *C. latifolia*, and the phytotoxic effect of EO of *C. zeylanicum* to the bean seeds. In a study on the allelopathic potential of the EO of medicinal plants on the germination of pepper seeds, MOURA et al. (2013) also observed lower germination capacity in seeds treated with EO of *C. zeylanicum*, which was capable of completely inhibiting the germination of the pepper seeds at a concentration of 1%.

In general, when seeds inoculated with *Penicillium* sp. and *Aspergillus* sp. were compared, it was noted greater negative effect on the vigor of the seeds inoculated with *Penicillium* sp. (Tables 4 and 5). *Penicillium* sp. is one of the main fungi responsible for the deterioration of stored seeds, being capable of reaching the tissues and cause a reduction in the force of the seeds and on their physiological quality (SOUZA et al., 2017). When the chemical treatment of the seeds was performed, there was a reduction in the germination, first count of germination and GSI in comparison to the negative control and the treatments with

Table 3. Germination of seeds inoculated with *Aspergillus* sp. and *Penicillium* sp. and treated with different concentrations of essential oils of *Citrus latifolia* and *Cinnamomum zeylanicum*.

Treatments	Germination (%)	Germination (%)
	***Aspergillus* spp.**	***Penicillium* spp.**
Control	93.50 Aa	93.50 Aa
Negative Control	62.50 Acd	52.00 Acd
Positive control	31.50 Ae	13.50 Bf
EO *C. latifolia* 0,4%	52.00 Ad	60.00 Abc
EO *C. latifolia* 0,8%	77.50 Ab	69.50 Ab
EO *C. zeylanicum* 0,1%	77.00 Abc	68.00 Ab
EO *C. zeylanicum* 0,2%	54.75 Ad	39.25 Bde
EO *C. zeylanicum* 0,4%	33.00 Ae	24.75 Aef
EO *C. zeylanicum* 0.8%	12.50 Af	10.00 Af

Control non-inoculated and not treated seeds; negative control: inoculated and not treated seeds; positive control: seeds treated with the fungicide carbendazim 150 g L^{-1} + thiram 350 g L^{-1}; means followed by the same uppercase letter in the lines do not differ statistically among themselves by Tukey test ($p < 0.05$); EO: essential oils.

Table 4. First count of germination of seeds inoculated with *Aspergillus* sp. and *Penicillium* sp. and treated with different concentrations of essential oils of *Citrus latifolia* and *Cinnamomum zeylanicum*.

Treatments	First count of germination (%)	First count of germination (%)
	***Aspergillus* spp.**	***Penicillium* spp.**
Control	31.50 Ab	31.50 Aab
Negative Control	24.50 Abc	6.00 Ac
Positive control	8.00 Acd	4.00 Ac
EO *C. latifolia* 0,4%	25.00 Abc	17.50 Abc
EO *C. latifolia* 0,8%	60.50 Aa	39.00 Ba
EO *C. zeylanicum* 0,1%	59.00 Aa	18.00 Bbc
EO *C. zeylanicum* 0,2%	36.00 Ab	12.5 Bbc
EO *C. zeylanicum* 0,4%	30.00 Ab	12.00 Bbc
EO *C. zeylanicum* 0.8%	3.00 Ad	5.50 Ac

Control non-inoculated and not treated seeds; negative control: inoculated and not treated seeds; positive control: seeds treated with the fungicide carbendazim 150 g L^{-1} + thiram 350 g L^{-1}; means followed by the same uppercase letter in the lines do not differ statistically among themselves by Tukey test ($p < 0.05$); EO: essential oils.

Table 5. Germination speed index of seeds inoculated with *Aspergillus* sp. and *Penicillium* sp. and treated with different concentrations of essential oils of *Citrus latifolia* and *Cinnamomum zeylanicum*.

Treatments	GSI	GSI
	***Aspergillus* spp.**	***Penicillium* spp.**
Negative Control	5.49 Ab	4.25 Abc
Positive control	2.42 Ade	1.06 Be
EO *C. latifolia* 0,4%	4.58 Abc	4.97 Aab
EO *C. latifolia* 0,8%	7.95 Aa	6.24 Ba
EO *C. zeylanicum* 0,1%	8.15 Aa	5.91 Ba
EO *C. zeylanicum* 0,2%	5.24 Ab	3.23 Bcd
EO *C. zeylanicum* 0,4%	3.39 Acd	2.26 Bde
EO *C. zeylanicum* 0.8%	0.94 Ae	0.89 Ae

Negative control: seeds inoculated and not treated; positive control: seeds treated with the fungicide carbendazim 150 g L^{-1} + thiram 350 g L^{-1}; means followed by the same uppercase letter in the lines and the same lowercase letter in the columns do not differ statistically among themselves by Tukey test ($p < 0.05$); GSI: germination speed index; EO: essential oils.

EOs (Tables 3, 4 and 5). These results indicate that the fungicide causes higher phytotoxicity to bean seeds among the evaluated treatments. As CONCEIÇÃO (2013) points out, when some chemicals are used directly in seeds, a decrease in germination and survival of seedlings may be caused by phytotoxicity problems. The level of phytotoxicity will depend on the cultivar used, plant protection product, applied dose and the stage of development in which the product was applied (TAKANO et al., 2015).

Regarding the results obtained in the seed health test, it was observed that, for the bean seeds inoculated with the fungus *Aspergillus* sp. (Table 6) there was no significant action of the EO of *C. latifolia* in control of the genus of the mentioned fungus, whereas the EO of *C. zeylanicum* promoted increasing reductions in the incidence of the fungus *Aspergillus* spp. with the increase in its concentration. For the fungus *Penicillium* spp., the EO of *C. zeylanicum* promoted total inhibition on incidence of the fungus.

The decrease in fungal development starting from the increase in the concentration of EOs was also verified by AQUINO et al. (2014), who observed a reduction in the germination of conidia of the fungus *Colletotrichum gloeosporioides* with increasing concentration of pepper-rosmarin, lemon grass and basil-harpsichord.

Still on the sanity test, in the case of the fungus *Cladosporium* spp., its incidence also decreased with the increase in concentrations of EOs of *C. latifolia* and *C. zeylanicum,* whereas the occurrence of fungus *Rhizopus* spp. influenced by the different treatments did not happen. This last result may indicate greater resistance of the genus *Rhizopus* to alternative seeds treatment with EOs, being necessary the study of other control methods for this genre. *Fusarium* spp. and *Chaetomium* spp. had low occurrence in the seeds (Table 6).

For the seeds inoculated with the fungus *Penicillium* sp. (Table 7), there was no difference among the treatments for the incidence of the genus *Aspergillus*. Regarding the occurrence of the fungus *Penicillium* spp., the EO of *C. zeylanicum* reduced the incidence regardless of the concentration used and also the EO of *C. latifolia* in 0.4% concentration. The fungus *Rhizopus* spp. was totally controlled by the EO of

Table 6. The presence of the fungi *Aspergillus* spp. (Asp.), *Penicillium* spp. (Pen), *Cladosporium* spp. (CLA), *Fusarium* spp. (FUS), *Chaetomium* spp. (CHA) and *Rhizopus* spp. (Rhi) in seeds inoculated with the fungus *Aspergillus* sp. and treated with different concentrations of essential oils of *Citrus latifolia* and *Cinnamomum zeylanicum*.

Treatments	Asp. (%)	Pen. (%)	Cla. (%)	Fus. (%)	Cha. (%)	Rhi. (%)
Negative Control	23.00 bc	13.00 bc	3.00 ab	2.00 a	0.00 a	65.25 a
Positive control	18.00 b	2.00 abc	0.00 a	0.00 a	0.00 a	96.00 a
EO *C. latifolia* 0,4%	15.00 b	10.00 abc	6.00 b	4.00 a	1.00 a	34.00 a
EO *C. latifolia* 0,8%	14.00 b	2.00 ab	1.00 a	0.00 a	0.00 a	100.00 a
EO *C. zeylanicum* 0,1%	38.75 c	10.00 abc	1.00 a	0.00 a	0.00 a	37.00 a
EO *C. zeylanicum* 0,2%	23.00 bc	20.00 c	1.00 a	0.00 a	0.00 a	36.00 a
EO *C. zeylanicum* 0,4%	14.75 b	1.00 ab	0.00 a	0.00 a	6.00 a	42.00 a
EO *C. zeylanicum* 0,8%	2.00 a	0.00 a	0.00 a	0.00 a	1.00 a	65.00 a

Negative control: inoculated and not treated seeds; positive control: seeds treated with the fungicide carbendazim 150 g L^{-1} + thiram 350 g L^{-1}; means followed by the same letter in column do not differ statistically among themselves by Tukey test ($p < 0.05$); *for the purpose of analysis, the data were transformed to square root of Y + 1.0 - SQRT (Y + 1.0); EO: essential oils.

Table 7. The presence of the fungi *Aspergillus* spp. (Asp.), *Penicillium* spp. (Pen), *Cladosporium* spp. (CLA), *Fusarium* spp. (FUS), *Chaetomium* spp. (CHA) and *Rhizopus* spp. (Rhi) in seeds inoculated with the fungus *Penicillium* sp. and treated with different concentrations of essential oils of *Citrus latifolia* and *Cinnamomum zeylanicum*.

Treatments	Asp. (%)	Pen. (%)	Cla. (%)	Fus. (%)	Cha. (%)	Rhi. (%)
Negative control	11.00 a	61.25 c	4.00 a	0.00 a	0.00 a	18.00 ab
Positive control	3.00 a	18.00 b	8.00 a	0.00 a	0.00 a	12.00 ab
EO *C. latifolia* 0,4%	2.00 a	28.00 b	0.00 a	0.00 a	0.00 a	25.00 ab
EO *C. latifolia* 0,8%	3.00 a	65.00 c	2.00 a	0.00 a	0.00 a	54.75 ab
EO *C. zeylanicum* 0,1%	6.00 a	1.00 a	2.00 a	0.00 a	0.00 a	88.00 b
EO *C. zeylanicum* 0,2%	7.00 a	2.00 a	0.00 a	0.00 a	2.00 a	39.00 ab
EO C. zeylanicum 0.4%	3.00 a	0.00 a	0.00 a	0.00 a	0.00 a	23.00 ab
EO *C. zeylanicum* 0,8%	1.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a

Negative control: seeds inoculated and not treated; positive control: seeds treated with the fungicide carbendazim 150 g L^{-1} + thiram 350 g L^{-1}; Asp.: *Aspergillus* spp.; Pen: *Penicillium* spp.; CLA: *Cladosporium* spp.; FUS: *Fusarium* spp.; CHA: *Chaetomium* spp.; Rhi: *Rhizopus* spp.; means followed by the same letter in column do not differ statistically among themselves by Tukey test ($p < 0.05$); *for the purpose of analysis, the data were transformed to square root of Y + 1.0 - SQRT (Y + 1.0) ; EO: essential oils.

C. zeylanicum in 0.8% concentration. The fungi, *Cladosporium* spp., *Fusarium* spp. and *Chaetomium* spp. had low incidence.

By the results presented in Tables 6 and 7, it is observed that the control exercised by the EOs, on the different genera of fungi in bean seeds, was similar to the results obtained with conventional treatment using a fungicide, which proves the efficiency of waste oils as an alternative in the pathogenic fungi control. It is also observed that the genera of fungi of greater occurrence in bean seeds were *Aspergillus*, *Penicillium* and *Rhizopus*. The results show the potential of these EOs in the treatment of common bean seeds and the importance of advancing in studies to facilitate the practical use in the field.

CONCLUSIONS

The EO of *Cinnamomum zeylanicum* was more efficient in the control of the fungi *Aspergillus* sp. and *Penicillium* sp., but affects the physiological quality of the beans seeds as the fungicide.

The EO of *Citrus latifolia* was less efficient in the control of the fungi *Aspergillus* sp. and *Penicillium* sp., however it affects in lower degree the physiological quality of the bean seeds.

The fungal diversity identified in seed health testing is composed by Aspergillus spp., *Penicillium* spp., *Cladosporium* spp., *Fusarium* spp., *Chaetomium* spp. and *Rhizopus* spp., with greater frequency for *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp.

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