

Essential oils in the control of *Colletotrichum gloeosporioides* f. Sp. *cepae* in onion seeds¹

Óleos essenciais no controle de *Colletotrichum gloeosporioides* f. sp. *cepae* em sementes de cebola

Maria Isabel Ordonez Lozada², Patricia Pereira da Silva³, Ricardo Borges Pereira³ and Warley Marcos Nascimento^{3*}

ABSTRACT - Anthracnose, one of the major diseases in the onion, is caused by the fungus *Colletotrichum gloeosporioides* f. sp. *cepae*. The main strategy for controlling this fungus is by the use of fungicide, with this technique being used on the majority of crops. Currently, methods of disease control are being studied that are less aggressive to the environment, with the use of essential oils proving to be an alternative. As such, the aim of this study was to evaluate the direct effect of the essential oils of basil (*Ocimum basilicum*), lemongrass (*Cymbopogon citratus* (DC) Stapf.), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*) and citronella (*Cymbopogon winterianus*) as an alternative in the control of the fungus *C. gloeosporioides* f. sp. *cepae*, in addition to determining its effect on the physiological quality of onion seeds. The essential oils of lemongrass and sage did not affect the physiological quality of the onion seeds, and all the essential oils used helped to inhibit spore germination in *C. gloeosporioides* f. sp. *cepae*. The essential oils of thyme, lemongrass and citronella at a concentration of 2000 ppm completely inhibited mycelial growth in the fungus. Treatment with the essential oil of lemongrass in seeds inoculated with the pathogen gave greater protection against fungal attack.

Key words: *Allium cepa*. Physiological quality. Conidial germination. Mycelial growth.

RESUMO - A antracnose causada pelo fungo *Colletotrichum gloeosporioides* f. sp. *cepae* é umas das principais doenças na cultura da cebola. A principal estratégia de controle deste fungo é o controle químico com fungicida sendo esta técnica utilizada na maior parte dos cultivos. Atualmente vem sendo estudados métodos de controle de doenças que sejam menos agressivos ao ambiente, mostrando-se como alternativa o uso de óleos essenciais. Diante do exposto, objetivou-se avaliar o efeito direto dos óleos essenciais de manjeriço (*Ocimum basilicum*), capim-limão (*Cymbopogon citratus* (DC) Stapf.), sálvia (*Salvia officinalis*), tomilho (*Thymus vulgaris*) e citronela (*Cymbopogon winterianus*) como alternativa no controle do fungo *C. gloeosporioides* f. sp. *cepae*, além de determinar seu efeito sobre a qualidade fisiológica de sementes de cebola. Os óleos essenciais de capim-limão e sálvia não afetaram a qualidade fisiológica das sementes de cebola e todos os óleos essenciais utilizados promoveram a inibição da germinação de esporos de *C. gloeosporioides* f. sp. *cepae*. Os óleos essenciais de tomilho, capim-limão e citronela, na concentração de 2.000 ppm, inibiram totalmente o crescimento micelial do fungo. O tratamento com o óleo essencial de capim-limão em sementes inoculadas com patógeno proporcionou maior proteção contra o ataque de fungo.

Palavras-chaves: *Allium cepa*. Qualidade fisiológica. Germinação de conídios. Crescimento micelial.

DOI: 10.5935/1806-6690.20190060

*Author for correspondence

Received for publication 21/02/2018; approved on 12/11/2018

¹Parte da Dissertação de Mestrado do primeiro autor apresentada à Universidade de Brasília, Brasília-DF

²Departamento de Agronomia, Universidade de Brasília/UnB, Campos Universitário Darci Ribeiro-Sul, Brasília-DF, Brasil, 70.297-400, misabell1223@gmail.com (ORCID ID 0000-0001-9387-3721)

³Embrapa Hortaliças, Caixa Postal 280, Brasília-DF, Brasil, 70.359-970, patybio55@yahoo.com.br (ORCID ID 0000-0002-6835-0053), ricardo-borges.pereira@embrapa.br (ORCID ID 0000-0003-0113-5049), warley.nascimento@embrapa.br (ORCID ID 0000-0002-6235-0917)

INTRODUCTION

Anthracnose is considered a disease of great importance in the onion (*Allium cepa* L.), the causative agent being the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. Et Sacc. f. sp. *cepae*, which survives in the soil, in crop remains and in seeds. Until now, anthracnose has been conventionally controlled with the use of different fungicides. Among these, the most important are those based on thiophanate-methyl, copper oxychloride, folpet, and futriafol (BRASIL, 2003), and which are registered in the Ministry of Agriculture, Livestock and Food Supply (MAPA). However, the indiscriminate use of these products can generate levels of resistance, with damage to the environment.

There has recently been a growing search for alternative methods of disease control, such as the use of essential oils, due to their low environmental impact (XAVIER *et al.*, 2012). These have allelopathic characteristics, i.e. they have the capacity to interfere directly or indirectly in plants or in microorganisms through the release of chemical compounds (SIMONETO; CRUZ-SILVA, 2010) that are produced via secondary metabolism and act as allelochemicals. These compounds may also interfere with the physiological quality of the seeds and with normal plant development (SOYLU; KURT; SOYLU, 2010). For example, the essential oil of citronella (*Cymbopogon nardus* (L.) Rendle.) contains the compounds citronellal, geraniol and limonene, while the major component of lemongrass essential oil (*Cymbopogon citratus* Staph.) is citral, which is considered responsible for the allelopathic activity of the plant (GUIMARÃES *et al.*, 2008). The essential oil of basil (*Ocimum basilicum* L.) is composed of linalool, estragole, farnesene, eugenol and cineole, which have antifungal properties (MARTINEZ-VELAZQUEZ *et al.*, 2011).

Research shows that essential oils may have an allelopathic effect on the physiological quality of seeds. As such, Steffen, Antonioli and Steffen (2010) demonstrated that the essential oil of *Eucalyptus glandris* W. Hill ex Maiden favoured greater germination in eucalyptus seeds. According to Miranda *et al.* (2015), the essential oils of lemongrass (*Cymbopogon citratus* Staph.) and African basil (*O. gratissimum* L.) showed allelopathic potential on the germination and vigour of lettuce achenes that can be attributed to the content of the respective major constituents, citral and eugenol, whereas the allelopathic effect of the essential oil from basil is the joint result of all the components found in that oil.

Studies have been carried out to determine the effect of essential oils so as to develop an alternative technology for controlling pathogens. In this respect, Oliveira *et al.* (2017) demonstrated that lemongrass essential oil had a fungitoxic effect, inhibiting conidial germination in

Alternaria solani Sorauer and *Corynespora cassiicola* (Bert & Curt) Wei by 40%, results which indicate that essential oils can be used for inhibiting different types of fungi.

The aim of this study therefore, was to evaluate the effect of the essential oils of sage (*Salvia officinalis* Lineu), thyme (*Thymus vulgaris* Lineu), lemongrass (*Cymbopogon citratus* (DC) Stapf.), basil (*Ocimum basilicum* Lineu) and citronella (*Cymbopogon winterianus* Lineu) on the physiological quality of onion seeds, as well as on conidial germination and mycelial growth in *C. gloeosporioides* f. sp. *cepae*.

MATERIAL AND METHODS

The work was carried out in the Seed and Phytopathology Laboratory of Embrapa Hortaliças, in Brasília, from August 2014 to March 2016. Onion seeds of the 'Conquista' cultivar were used, produced in 2014 in the experimental area of Embrapa Hortaliças (15°56'00" S, 48°06'00" W, at an altitude of 997.62 m).

The medicinal plants, sage, thyme, lemongrass, basil and citronella, used to obtain the essential oils, were grown in the research area for organic vegetable production (APPOH) of Embrapa Hortaliças. Choice of the plants to be used in the study was based on the control potential shown by the essential oils in other pathosystems (BRITO *et al.*, 2010; PEREIRA *et al.*, 2008; SEIXAS *et al.*, 2011).

Fresh leaf material from each species was used to extract the essential oils. The plants were collected in the morning, at around 10:00, to find higher levels of essential oil (BLANK *et al.*, 2007), and then dried in an oven at 50 °C for 84 hours (RADÜNZ *et al.*, 2006). For each extraction, 200 g of dried leaf material and two litres of water were used, which underwent a process of hydrodistillation in a graduated Clevenger apparatus for two hours. The essential oils obtained were identified, protected from light and stored in a freezer at -20 °C until required (PEREIRA *et al.*, 2008).

The seeds were treated with a 1000 ppm and 2000 ppm dilution of each oil, as these concentrations had no effect on the physiological quality of the onion seeds in preliminary tests. Each batch of 50 seeds was placed in a plastic bag and 0.5 mL of oil was then added for 15 minutes. The seeds were then dried at room temperature for 24 hours. The control was treated with distilled water only.

To evaluate the effect of the different concentrations of essential oils on the physiological quality of the onion seeds, the following tests were carried out:

Germination - four replications of 50 seeds per treatment were sown on germination paper moistened with

distilled water at 2.5 times the weight of the dry paper, and placed in plastic boxes (Gerbox® - 11 x 11 x 3.5 cm). The seeds were then incubated in a BOD (Biochemical Oxygen Demand) vertical chamber germinator at 20 °C under constant light. The evaluations were carried out 6 and 12 days after setting up the test (BRASIL, 2009).

Germination speed index - during the 12 days of the germination test, daily counts were made of the germinated seeds; the totals were divided by the respective number of days elapsed from the start of the test, using the formula proposed by Maguire (1962): GSI: $G1 + G2 + \dots + Gn/N1 + N2 + \dots + Nn$, where: G1, G2 Gn is the number of germinated seeds; N1, N2 Nn is the number of days after seeding.

Accelerated aging - four replications of 50 seeds from each treatment were uniformly distributed over aluminium screens fitted into plastic boxes (Gerbox® - 11 x 11 x 3.5 cm) with 40 mL of saturated NaCl solution (40 g NaCl / 100 ml water) at the bottom. The boxes containing the seeds were closed and kept at 41 °C for 48 hours in an ageing chamber (COSTA; TRZECIAK; VILLELA, 2008). The seeds then underwent the germination test, where the number of germinated seeds was evaluated six days after the start of the test.

Greenhouse emergence - four replications of 50 seeds per treatment were distributed over expanded-polystyrene (Styrofoam) trays of 200 cells containing Rohrbacher® commercial substrate made from composted pine bark. The trays were irrigated twice a day. The number of emerged seedlings was evaluated 20 days after seeding, and the results expressed as a percentage of seedling emergence.

The fungus used was *C. gloeosporioides* f. sp. *cepae* from the collection of the Plant Pathology Laboratory at Embrapa Hortaliças. The isolate was recovered by spreading onto Petri dishes (9 mm in diameter) containing a culture medium of 2% oatmeal; the dishes were placed in a BOD incubator at 25 °C, where they remained for 7 days under a photoperiod of 12 hours. The conidia which formed in the dishes were then detached from the surface of the plates with a soft-bristle brush and distilled water. The conidial suspension was later filtered through a double layer of gauze and the concentration adjusted to 3.7×10^5 conidia mL⁻¹ in a counting chamber.

To evaluate the direct *in-vitro* antifungal effect of the essential oils on conidial germination, five essential oils were tested: thyme, citronella, basil, sage and lemongrass, in concentrations of 500 ppm, 1000 ppm and 2000 ppm. *Tween* 20 was used as emulsifier at a concentration of 1000 ppm. Two controls were used: one treatment consisting of distilled water and the other consisting of *Tween* 20 at 1000 ppm only.

The culture medium used was water agar (1.0 L water, 20 g agar). This was prepared first, and after cooling

the essential oils were added to reach the final, pre-established concentrations, except for the controls. After homogenisation, around 25 ml of the culture medium was poured into Petri dishes (90 mm in diameter). The plates were scratched with a ballpoint pen, and divided into four quadrants. Five hundred µL of the 3.7×10^5 conidial suspension mL⁻¹ was continuously deposited onto each dish and spread over the surface of the dishes with a Drigalski loop. The dishes were placed in a BOD oven at 25 °C under a photoperiod of 12 hours. After 24 hours incubation, germination was stopped using a 0.2 mL solution of lactoglycerol, and the percentage of conidial germination determined, with 25 germinated conidia per quadrant being evaluated by trinocular stereoscopic microscope (Meiji) (PEREIRA *et al.*, 2008). The design was completely randomised, with two dishes per treatment, each divided into four quadrants, resulting in a total of eight replications.

To evaluate the effect of the essential oils on mycelial growth in the pathogen, concentrations of 1000 ppm and 2000 ppm of the oils from thyme, citronella, basil, sage and lemongrass were tested. *Tween* 20 was used as emulsifier at a concentration of 1000 ppm. Two controls were used, one for the treatment consisting of distilled water and the other for the treatment comprising *Tween* 20 at 1000 ppm.

The culture medium used was 2.0% oatmeal, prepared and autoclaved; after cooling to 45 °C to 50 °C (BRUNELLI *et al.*, 2006), the essential oils were added, except for the controls. The culture medium was then poured into Petri dishes (90 mm in diameter), and a mycelial disc of the pathogen (approximately 1 cm in diameter) was placed towards the centre of each Petri dish; the dishes were then sealed with plastic film and kept at 25 °C ± 2 °C under a photoperiod of 12 hours.

Evaluations were made daily, where the measurements corresponded to the mean value of two diametrically opposite measurements taken with the use of a graduated rule (mm). This was repeated until the control mycelium occupied the entire surface of the dish. The mycelial speed of growth index was calculated by adapting the formula proposed by Maguire (1962). The percentage inhibition of mycelial growth was then determined relative to the control. The experiment was conducted in a completely randomised design with four replications, each unit consisting of one Petri dish.

To evaluate the effect of the essential oils on onion seeds inoculated with *C. gloeosporioides*, three simultaneous experiments were carried out.

Onion seeds were inoculated by means of the water restriction method, using an osmotic potential of -1.0 MPa, determined in preliminary tests. First, Petri dishes were prepared with the BDA culture medium and the -1.0 MPa mannitol solution added after autoclaving. The culture

medium with the restrictor was poured into Petri dishes (90 mm in diameter) and an agar disk from the fungal colony, 1 cm in diameter, was placed at the centre of each dish. The dishes were then incubated in a BOD at 25 °C under a photoperiod of 12 hours for seven days. Fifty onion seeds were disinfested using 1% sodium hypochlorite for 1 min, rinsed in distilled water and dried at room temperature. The seeds were distributed in a single layer over each fungus colony. Each plate was then sealed and placed in a BOD oven at 25 °C under a photoperiod of 12 hours for five days, after which they were removed from the dishes (MACHADO *et al.*, 2007). They were then treated with the basil, citronella, lemongrass, thyme and sage oil at concentrations of 1000 ppm and 2000 ppm. Each batch of 50 seeds was treated with 0.5 mL oil for 15 minutes, dried at room temperature for 24 hours, and then subjected to health tests expressed as a percentage of infestation, germination and emergence in the greenhouse, as per the methodology described above. The experiment was carried out in a completely randomised design with 4 replications, each unit consisting of 25 seeds.

The Sisvar v.5.6 statistical software was used (FERREIRA, 2008) for statistical analysis of the data. The qualitative mean values were grouped by the Scott-Knott test ($p < 0.05$), and regression graphs were generated for the quantitative mean values using the Excel software.

RESULTS AND DISCUSSION

The untreated seeds had a first count of 87%, with a significant statistical difference in this count (Table 1). Seeds treated with the essential oils of thyme at the two concentrations

(1000 ppm and 2000 ppm), and citronella and basil at 2000 ppm, showed 82%, 71%, 78% and 78% of germinated seeds respectively at the first reading, with no statistical difference, but did show a difference in relation to the control. These results differed from those found by Araújo Neto and Araújo (2012) in fennel (*Foeniculum vulgare* Mill), where treatment with a 2.5% concentration of citronella essential oil increased the percentage of germinated seeds at the first count.

The untreated seeds presented a germination of 96%. The essential oil of thyme at concentrations of 1000 ppm and 2000 ppm, and citronella at 2000 ppm, resulted in germination of 93%, 90% and 90% respectively (Table 1). It was found that citronella oil showed a decrease in germination as its concentration increased. This may be due to the presence of compounds such as citronellal and geraniol, high concentrations of which may inhibit germination (XAVIER *et al.*, 2012).

For the germination speed index, no significant differences were found between the treatments and the control. With regard to the accelerated ageing test, the treatments did not differ from one another or in relation to the control. This test allows the vigour and storage potential of the seeds to be evaluated, and shows that germination in seeds treated with the essential oils was not affected by extreme conditions and that the seeds could be considered vigorous.

For the greenhouse emergence test, the untreated seeds had an emergence of 93% (Table 1). In this test, the basil oil at a concentration of 2000 ppm resulted in a statistical difference with the control, showing a lower number of emerged seeds (65%). The other treatments do not differ statistically from one other or from the control.

Table 1 - Mean values for first count (FC), germination (G) and greenhouse emergence (EM) in onion seeds

Treatment	Concentration (ppm)	FC (%)	G (%)	EM (%)
Sage	1000	92 a	96 a	89 a
Sage	2000	87 a	94 a	88 a
Thyme	1000	82 b	93 b	88 a
Thyme	2000	71 b	90 b	81 a
Lemongrass	1000	92 a	95 a	87 a
Lemongrass	2000	90 a	97 a	94 a
Basil	1000	84 a	94 a	84 a
Basil	2000	78 b	95 a	65 b
Citronella	1000	92 a	96 a	85 a
Citronella	2000	78 b	90 b	79 a
Control	-	87 a	96 a	93 a
CV (%)	6.86	2.90	4.34	

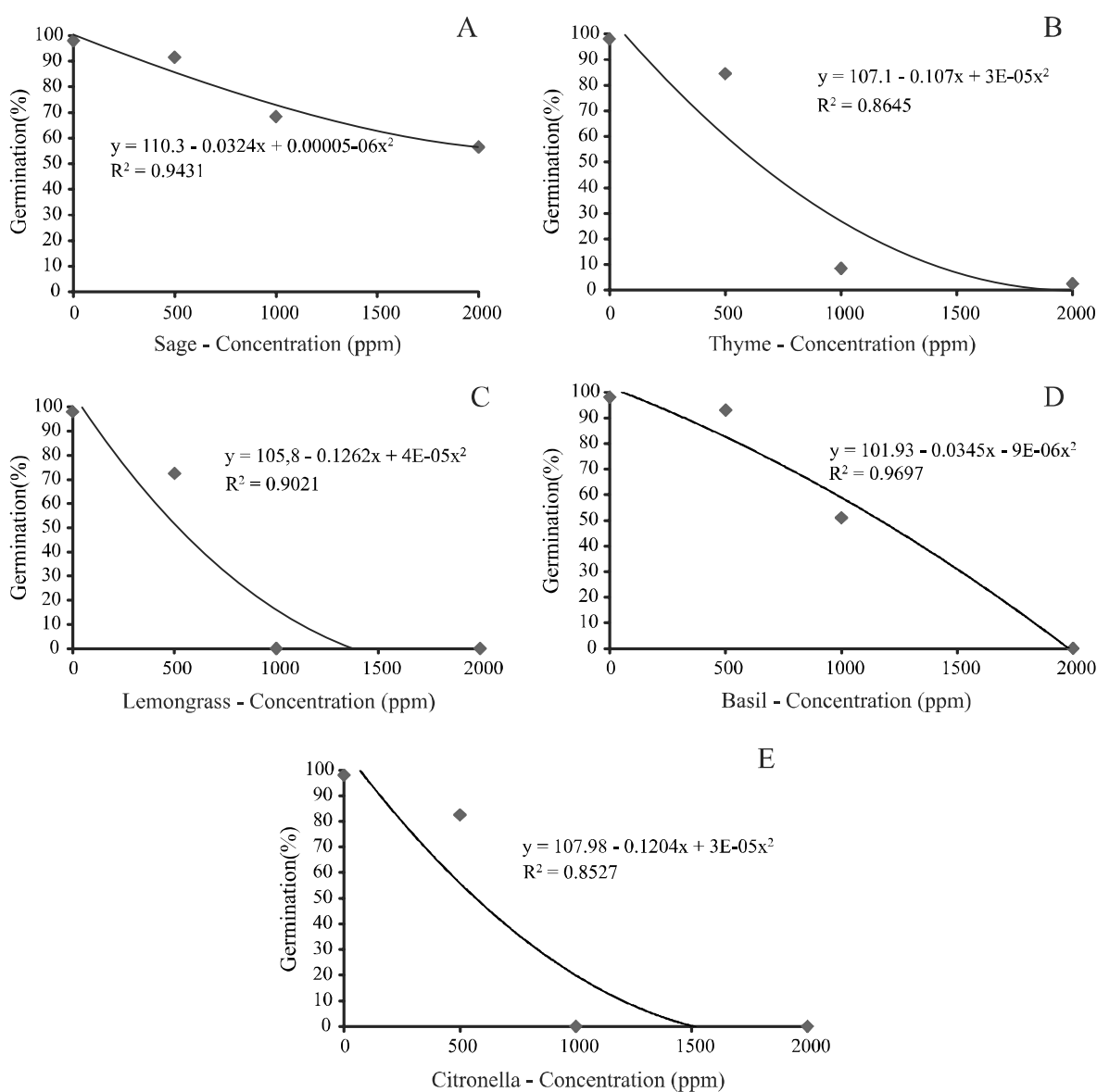
Mean values followed by the same letter do not differ by Scott-Knott test at a level of 5% probability

Studies conducted by Blank *et al.* (2004) demonstrated that the essential oil of basil has great agronomic potential for linalool extraction, both due to its oil content and its being a short-cycle plant. This chemical compound can affect the germination potential of seeds treated with this oil (BRITO *et al.*, 2010), agreeing with the results obtained in this work. Other studies were carried out by Magalhães *et al.* (2014), who found that basil oil affected the seedling emergence pattern of maize (*Zea mays* L.) as the concentration increased (0 $\mu\text{L L}^{-1}$, 5 $\mu\text{L L}^{-1}$, 10 $\mu\text{L L}^{-1}$, 15 $\mu\text{L L}^{-1}$ and 20 $\mu\text{L L}^{-1}$).

The treatments with the essential oils of sage and lemongrass did not differ statistically from the control in the various tests, showing no effect on the physiological quality of the onion seeds. These results agree with those of Simonetto and Cruz-Silva (2010) who verified that different concentrations (0%, 7.5%, 15%, 22.5% and 30%) of the aqueous extract of sage oil showed no statistical interaction with the germination of maize (*Zea mays* L.) or tomato (*Solanum lycopersicum* L.) seeds compared to the control.

In the *in-vitro* evaluation of essential oils on conidial germination and mycelial growth in *C. gloeosporioides* f. sp.

Figure 1 - Spore germination in *Colletotrichum gloeosporioides* f. sp. *cepae* subjected to different concentrations (0, 500, 1000 and 2000 ppm) of the essential oils of sage (*Salvia officinalis*) (A), thyme (*Thymus vulgaris*) (B), lemongrass (*Cymbopogon citratus* (DC) Stapf.) (C), basil (*Ocimum basilicum*) (D) and citronella (*Cymbopogon winterianus*) (E) in a culture medium of 2.0% oatmeal treated with the respective oils



cepa, it was found that all the essential oils caused a quadratic reduction in conidial germination as the concentration increased (Figure 1). From the concentration of 1000 ppm, the essential oils of lemongrass and citronella reduced conidial germination completely. On the other hand, the essential oils of thyme and basil caused the 100% inhibition of conidial germination from the concentration of 2000 ppm, whereas sage oil reduced conidial germination by 56% at the highest concentration (2000 ppm). The treatment consisting of *Tween* at 1000 ppm showed no statistical differences between the doses used and the control.

The results obtained in this experiment agree with those obtained by other authors, where the use of essential oils reduces conidial germination in different pathogens. As such, Cruz *et al.* (1997) found that lemongrass oil was efficient in controlling *Fusarium* spp., inhibiting mycelial growth and spore germination. Silva *et al.* (2009) found that lemongrass oil controlled *C. gloeosporioides*, causing the 100% inhibition of conidial germination in the pathogen. Furthermore, Guimarães *et al.* (2008) found pathogen inhibition with lemongrass due to the citral component which exerts an antifungal effect on the conidia.

All the treatments with the essential oils inhibited mycelial growth in *C. gloeosporioides* in relation to the control, which showed no inhibition of the pathogen (Table 2). At a concentration of 2000 ppm, the essential oils of thyme, lemongrass and citronella completely inhibited mycelial growth in *C. gloeosporioides*. Sage oil at 2000 ppm inhibited growth of the fungus by 64.39% relative

to the control, followed by the essential oil of thyme at 1000 ppm, with 49.01% inhibition. The essential oil of basil at the concentration of 1000 ppm inhibited fungus growth by 32.41%. The essential oils of sage, lemongrass and citronella at the concentration of 1000 ppm, and of basil at 2000 ppm, inhibited growth of the pathogen by 18.99%, 18.62%, 18.01% and 17.21% respectively, not differing from one other, but differing from the control. The standard treatment with *Tween* at 1000 ppm did not significantly inhibit mycelial growth in the pathogen.

Agreeing with the results of this study, Silva *et al.* (2009) evaluated the effect of lemongrass essential oil on mycelial growth in *C. gloeosporioides* at concentrations of 1 $\mu\text{L mL}^{-1}$, 3 $\mu\text{L mL}^{-1}$, 5 $\mu\text{L mL}^{-1}$ and 10 $\mu\text{L mL}^{-1}$ in the passion fruit (*Passiflora edulis* Sims), demonstrating that from the concentration of 1 $\mu\text{L mL}^{-1}$, the essential oil inhibited growth of the pathogen by 100%. According to Itako (2009), lemongrass oil resulted in a fungitoxic effect on germination and mycelial growth in *Cladosporium fulvum* Cooke, with a reduction of 96.30% in spore germination at a concentration of 26.46% in relation the control. On the other hand, the oil at a concentration of 40.67% showed 20.03% inhibition of mycelial growth in the pathogen.

Pereira *et al.* (2011), evaluated the effect of citronella, thyme and lemongrass oil at concentrations of 1000 $\mu\text{L L}^{-1}$, 1500 $\mu\text{L L}^{-1}$ and 2000 $\mu\text{L L}^{-1}$ on the control of *Cercospora coffeicola*, and found that the oils totally inhibit conidial germination in *Cercospora coffeicola*, besides reducing mycelial growth in the pathogen. On the other hand, studies

Table 2 - Inhibition of mycelial growth in *Colletotrichum gloeosporioides* f. sp. *cepa* subjected to the essential oils of sage (*Salvia officinalis*) (A), thyme (*Thymus vulgaris*) (B), lemongrass (*Cymbopogon citratus* (DC) Stapf.) (C), basil (*Ocimum basilicum*) (D) and citronella (*Cymbopogon winterianus*) (E) at concentrations of 1000 ppm and 2000 ppm, *Tween* 20 and the control

Treatment	Concentration (ppm)	Inhibition (%)
Sage	1000	18.99 b
Sage	2000	64.39 e
Thyme	1000	49.01 d
Thyme	2000	100 f
Lemongrass	1000	18.62 b
Lemongrass	2000	100 f
Basil	1000	32.41 c
Basil	2000	17.21 b
Citronella	1000	18.01 b
Citronella	2000	100 f
<i>Tween</i> 20	1000	2.02 a
Control	--	0.00 a
CV (%)	7,64	

Mean values followed by the same letter do not differ by Scott-Knott test, $p < 0.05$

by Nascimento, Nery and Rodrigues (2008) on the control of *Colletotrichum gloeosporioides* from papaya fruit with basil-leaf extract showed no statistical difference in mycelial growth for eight days compared to the control.

For the seeds inoculated with *C. gloeosporioides* f. sp. *cepae*, each treatment differed from the control, which showed 65% germination (Table 3), except for the treatment with basil oil at 2000 ppm which resulted in 72% of germinated seeds. Seeds treated with the essential oils of lemongrass and basil at 1000 ppm, and the essential oil of citronella at both concentrations (1000 ppm and 2000 ppm), showed 88%, 84%, 85% and 88% germination respectively. These oils differed from the control and the other treatments, having the highest values for germination. The essential oils of sage and thyme at the two concentrations, and of lemongrass at 2000 ppm, had 80%, 79%, 79%, 78% and 81% germination respectively, without differing statistically from one another, showing a greater number of germinated seeds compared to the control.

In the health test, each treatment differed from the control, which showed more infected seeds with a rate of 73%, proving that essential oils have an effect in controlling the pathogen, as they had a lower percentage infection (Table 3). The treatments with the essential oils of lemongrass at the two concentrations (1000 ppm and 2000 ppm), and of basil and citronella at 2000 ppm, did not differ statistically from one other, but differed from the control, having the lowest number of seeds with symptoms of the pathogen, respectively 5%, 2%, 11%, and 10%; the treatment with sage oil at both concentrations presented 54% and 45% respectively in relation to the control. These

two treatments had the highest number of infected seeds in comparison with the other essential-oil treatments, and were considered the treatments that exerted the least control over the pathogen, followed by the essential oils of citronella and basil at the concentration of 1000 ppm, and thyme at both concentrations (1000 ppm and 2000 ppm), with a percentage infection of 22%, 19%, 31% and 20% respectively.

From the results of the test for emergence (Table 3), all the treatments with essential oils showed a statistical difference in relation to the control, which had a percentage emergence of 12%. The treatment with lemongrass oil at 1000 ppm, showed the highest percentage emergence of 71% compared to the control, with the treatment with sage oil at 1000 ppm being the least effective of the oil treatments, reflected in its percentage seedling emergence of 26%. The remaining treatments showed no statistical differences between one another.

Studies have shown the effectiveness of essential-oil treatment on infected seeds. For example, Hillen *et al.* (2012) demonstrated that the essential oils of rosemary (*Rosmarinus officinalis* L.), candeia (*Eremanthus erythropappus* (DC) MacLeish) and palmarosa (*Cymbopogon martinii* (Roxb.) Wats. Var. *Motia* Burk) had different allelopathic effects on seed germination, i.e. rosemary oil gave the lowest percentage of germinated asymptomatic seeds in the bean (*Phaseolus vulgaris* L.) (56.25%) and maize (*Zea mays* L.) (53.50%), while the lowest percentage of germinated asymptomatic soybean seeds (*Glycine max* L.) (0.00%) was obtained with the use of palmarosa essential oil.

Table 3 - Mean values for percentage germination, seed infection and emergence in onion seeds (*Allium cepa*) inoculated with *Colletotrichum gloeosporioides* f. sp. *cepae* and treated with essential oils

Treatment	Concentration (ppm)	Germination (%)	Infection (%)	Emergence (%)
Sage	1000	80 b	54 b	26 c
Sage	2000	79 b	45 b	45 b
Thyme	1000	79 b	31 c	53 b
Thyme	2000	78 b	20 c	46 b
Lemongrass	1000	88 a	5 d	71 a
Lemongrass	2000	81 b	2 d	58 b
Basil	1000	84 a	19 c	44 b
Basil	2000	72 c	11 d	47 b
Citronella	1000	85 a	22 c	49 b
Citronella	2000	88 a	10 d	51 b
Control	-	65 c	73 a	12 d
CV (%)	7.03	19.42	13.15	

Mean values followed by the same letter do not differ by Scott-Knott test, $p < 0.05$

According to Gomes *et al.* (2016), who evaluated the effect of essential oils on health and physiological quality in seeds of the broad bean (*Phaseolus lunatus* L.) and found a negative effect on first count and speed of emergence index with the application of a 2 mL.L⁻¹ concentration of the essential oil of clove (*Caryophyllus aromaticus* L.), whereas the essential oils of *Copaifera langsdorffi* Desf and basil considerably reduced the percentage incidence of the fungi *Aspergillus* spp., *Rhizopus* sp. and *Aspergillus niger* associated with seeds of the broad bean.

CONCLUSIONS

1. The essential oils of thyme, lemongrass, citronella, basil and sage reduce mycelial growth and conidial germination in *Colletotrichum gloeosporioides* f. sp. *cepae*;
2. The essential oils of thyme, lemongrass, citronella, basil and sage at concentrations of 1000 ppm and 2000 ppm reduce infection caused by *C. gloeosporioides* f. sp. *cepae*;
3. The essential oil of lemongrass at 1000 ppm is an alternative for the treatment of onion seeds in the control of *Colletotrichum gloeosporioides* f. sp. *cepae* by providing greater protection against the fungus.

REFERENCES

- ARAÚJO NETO, A. C.; ARAÚJO, P. C. Atividade antifúngica do óleo essencial de citronela em sementes de Erva-doce (*Foeniculum vulgare* Mill.). **Revista Verde de Agroecologia e Desenvolvimento Sustentável**, v. 7, n. 1, p. 189-195, 2012.
- BLANK, A. F. *et al.* Caracterização morfológica e agronômica de acessos de manjerição e alfavaca. **Horticultura Brasileira**, v. 22, n. 1, p. 113-116, 2004.
- BLANK, A. F. *et al.* Influence of season, harvest time and drying on Java citronella (*Cymbopogon winterianus* Jowitt) volatile oil. **Revista Brasileira de Farmacognosia**, v. 17, n. 4, p. 557-564, 2007.
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **AGROFIT**: sistema de agrotóxicos fitossanitários. Brasília, DF, 2003. Disponível em: <http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Acesso em: 2 jul. 2018.
- BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. **Regras para análise de sementes**. Brasília, DF, 2009. 399 p.
- BRITO, N. M. *et al.* Efeitos de óleos essenciais na germinação de sementes de *Cereus jamacaru*. **Revista Ciência Agronômica**, v. 5, n. 2, p. 207-211, 2010.
- BRUNELLI, K. R. *et al.* Effect of culture media and light exposure on the sporulation of *Cercospora zea-maydis*. **Summa Phytopathologica**, v. 32, n. 1, p. 92-94, 2006.
- COSTA, C. J.; TRZECIAK, M. B.; VILLELA, F. A. Potencial fisiológico de sementes de brássicas com ênfase no teste de envelhecimento acelerado. **Horticultura Brasileira**, v. 26, n. 2, p. 144-148, 2008.
- CRUZ, M. E. S. *et al.* Efeito do óleo essencial de *Cymbopogon citratus* (capim-limão) no crescimento micelial de fungos fitopatogênicos. **Summa Phytopathologica**, v. 23, n. 1, p. 63-65, 1997.
- FERREIRA, D. F. Sisvar: um programa para análises e ensino de estatística. **Revista Symposium**, v. 6, n. 1, p. 36-41, 2008.
- GOMES, R. S. S. *et al.* Eficiência de óleos essenciais na qualidade sanitária e fisiológica em sementes de feijão-fava (*Phaseolus lunatus* L.). **Revista Brasileira de Plantas Mediciniais**, v. 18, n. 1, p. 279-287, 2016.
- GUIMARÃES, L. G. L. *et al.* Influência da luz e da temperatura sobre a oxidação do óleo essencial de capim-limão (*Cymbopogon citratus* (DC.) stapf.). **Química Nova**, v. 31, n. 6, p. 1476-1480, 2008.
- HILLEN, T. *et al.* Atividade antimicrobiana de óleos essenciais no controle de alguns fitopatógenos fúngicos *in vitro* e no tratamento de sementes. **Revista Brasileira de Plantas Mediciniais**, v. 14, n. 3, p. 439-445, 2012.
- ITAKO, A. T. *et al.* Controle de *Cladosporium fulvum* em tomateiro por extratos de plantas medicinais. **Revista Arquivos do Instituto Biológico**, v. 76, n. 1, p. 75-83, 2009.
- MACHADO, A. Q. *et al.* Potencial do uso da restrição hídrica em testes de sanidade de sementes de algodoeiro. **Fitopatologia Brasileira**, v. 32, n. 5, p. 408-414, 2007.
- MAGALHÃES, C. R. I. *et al.* Óleos essenciais na emergência de grãos de milho (*Zea mays* L.). **Enciclopédia Biosfera**, v. 10, n. 19, p. 228-349, 2014.
- MAGUIRE, J. D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. **Crop Science**, v. 2, p. 176-177, 1962.
- MARTINEZ-VELAZQUEZ, G. A. *et al.* Acaricidal effect and chemical composition of essential oils extracted from *Cuminum cyminum*, Pimenta dioica and *Ocimum basilicum* against the cattle tick *Rhipicephalus* (Boophilus) *microplus* (Acari: Ixodidae). **Parasitology Research**, v. 108, n. 2, p. 481-487, 2011.
- MIRANDA, C. A. S. F. *et al.* Atividade alelopática de óleos essenciais de plantas medicinais na germinação e vigor de aquênios de alfaca. **Semina: Ciências Agrárias**, v. 36, n. 3, p. 1783-1798, 2015. Suplemento 1.
- NASCIMENTO, L. C.; NERY, A. P.; RODRIGUES, L. N. Controle de *Colletotrichum gloeosporioides* em mamoeiro, utilizando extratos vegetais, indutores de resistência e fungicida. **Acta Scientiarum. Agronomy**, v. 30, n. 3, p. 313-319, 2008.
- OLIVEIRA, J. B. S. *et al.* Homeopatas de óleos essenciais sobre a germinação de esporos e indução de fitoalexinas. **Revista Ciência Agronômica**, v. 48, n. 1, p. 208-215, 2017.
- PEREIRA, R. B. *et al.* Extrato de casca de café, óleo essencial de tomilho e acibenzolar-S-metil no manejo da cercosporiose-

do-cafeeiro. **Pesquisa Agropecuária Brasileira**, v. 43, n. 10, p. 1287-1296, 2008.

PEREIRA, R. B. *et al.* Potential of essential oils for the control of Brown eye spot in coffee plants. **Ciência e Agrotecnologia**, v. 35, n. 1, p. 115-23, 2011.

RADÜNZ, L. L. *et al.* Influência da temperatura do ar de secagem no rendimento do óleo essencial de hortelã-comum (*mentha x villosa* Huds). **Engenharia na Agricultura**, v. 14, n. 4, p. 250-257, 2006.

SEIXAS, P. L. *et al.* Controle fitopatológico do *Fusarium subglutinans* pelo óleo essencial do capim citronela (*Cymbopogon nardus* L.) e do composto citronelal. **Revista Brasileira de Plantas Mediciniais**, v. 13, p. 523-526. 2011.

SILVA, A. C. *et al.* Efeito in vitro de compostos de plantas sobre o fungo *Colletotrichum gloeosporioides* Penz. isolado do maracujazeiro. **Ciência e Agrotecnologia**, v. 33, p. 1853-1860, 2009.

SIMONETO, E. L.; CRUZ-SILVA, C. T. Alelopatia de sálvia sobre a germinação e o desenvolvimento do milho, tomate e girassol. **Revista Cultivando o Saber**, v. 3, n. 3, p. 48-56, 2010.

SOYLU, E. M.; KURT, S.; SOYLU, S. In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. **International Journal of Food Microbiology**, v. 143, n. 3, p. 183-189, 2010.

STEFFEN, R. B.; ANTONIOLLI, Z. I.; STEFFEN, G. P. K. Efeito estimulante do óleo essencial de eucalipto na germinação e no crescimento inicial de mudas de *Eucalyptus grandis*. **Pesquisa Florestal Brasileira**, v. 30, n. 63, p. 100-206, 2010.

XAVIER, M. V. A. *et al.* Viabilidade de sementes de feijão caupi após o tratamento com óleo essencial de citronela (*Cymbopogon winterianus* Jowitt). **Revista Brasileira de Plantas Mediciniais**, v. 14, p. 250-254, 2012.



This is an open-access article distributed under the terms of the Creative Commons Attribution License