Research, Society and Development, v. 9, n. 10, e7619109028, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9028

Essential oils control anthracnose in pepper seeds

Óleos essenciais controlam antracnose em sementes de pimentão

Los aceites esenciales controlan la "antracnosis" en las semillas de pimiento

Received: 10/04/2020 | Reviewed: 10/11/2020 | Accept: 10/15/2020 | Published: 10/18/2020

Daniele Maria do Nascimento
ORCID: https://orcid.org/0000-0001-7563-572X
São Paulo State University, Brazil
E-mail: daniele.nascimento@unesp.br
Paula Leite dos Santos
ORCID: https://orcid.org/0000-0002-0785-5031
São Paulo State University, Brazil
E-mail: santos.p.l@outlook.com
Marcos Roberto Ribeiro-Junior
ORCID: https://orcid.org/0000-0002-5024-1801
São Paulo State University, Brazil
E-mail: marcos.ribeiro@unesp.br
Maria Márcia Pereira Sartori
ORCID: https://orcid.org/0000-0003-4119-8642
São Paulo State University, Brazil
E-mail: maria.mp.sartori@unesp.br
Adriana Zanin Kronka
ORCID: https://orcid.org/0000-0002-9155-795X
São Paulo State University, Brazil
E-mail: adriana.kronka@unesp.br

Abstract
Pepper (*Capsicum annum*) is one of the main vegetables consumed in the Brazilian market, however, an important fungal disease, anthracnose, caused by *Colletotrichum gloeosporioides*, can decimate all its fruits in the field. Chemical control is the most commonly used measure of control, but, some active ingredients are proving ineffective, and consumers appreciate healthier options such as organic foods. Therefore, studies with natural substances that may help in the management of diseases have been encouraged. Because it is

transmitted via seed, seed treatment becomes an essential measure in the management and control of pepper anthracnose. The objective of this study was to evaluate the effect of basil (*Ocimum basilicum*), citronella (*Cymbopogon winterianus*), clove (*Syzygium aromaticum*), copaiba (*Copaifera langsdorfii*), eucalyptus (*Eucalyptus citriodora*), mint (*Mentha arvensis*), rosemary (*Rosmarinus officinalis*) and tea tree (*Melaleuca alternifolia*) essential oils on the development of *C. gloeosporioides*. Citronella, clove, eucalyptus, mint and basil essential oils totally inhibited the pathogen, regardless of the concentration used. These essential oils were then used to treat seeds infected with *C. gloeosporioides*, evaluating the physiological and health quality of these seeds. Clove essential oil reduced the incidence of fungi in seeds inoculated with the pathogen, without affecting their physiological quality.

Keywords: Anthracnose; *Capsicum annum*; Mycelial growth; Physiological quality.

Resumo

O pimentão (*Capsicum annum*) é uma das principais hortaliças consumidas no mercado brasileiro, contudo, uma importante doença fúngica, a antracnose, causada por *Colletotrichum gloeosporioides*, pode dizimar todos os seus frutos em campo. O controle químico é a medida mais empregada de controle e, atualmente, alguns princípios ativos vêm se mostrando ineficientes, além do mais, os consumidores prezam por opções mais saudáveis, como os alimentos orgânicos. Logo, estudos com substâncias naturais que possam auxiliar no manejo de doenças vêm sendo incentivadas. Por ser transmitido via semente, o tratamento de sementes torna-se uma medida essencial no manejo e controle da antracnose. Diante do exposto, objetivou-se avaliar o efeito de óleos essenciais de manjericão (*Ocimum basilicum*), citronela (*Cymbopogon winterianus*), cravo (*Syzygium aromaticum*), copaiba (*Copaifera langsdorfii*), eucalipto (*Eucalyptus citriodora*), hortelã (*Mentha arvensis*), alecrim (*Rosmarinus officinalis*) e tea tree (*Melaleuca alternifolia*) sobre o desenvolvimento de *C. gloeosporioides*, assim como na qualidade fisiológica de sementes de pimentão após tratamento. Os óleos essenciais de citronela, cravo, eucalipto, hortelã e manjericão inibiram totalmente o patógeno, independente da concentração utilizada. O óleo essencial de cravo reduziu a incidência de fungos nas sementes inoculadas com o patógeno, sem afetar a qualidade fisiológica destas.

Palavras-chave: Antracnose; *Capsicum annum*; Crescimento micelial; Qualidade fisiológica.

Resumen

El pimiento (*Capsicum annum*) es una de las principales hortalizas consumidas en el mercado brasileño. Sin embargo, una importante enfermedad llamada "antracnosis", causada por el hongo Colletotrichum gloeosporioides ha llegado a causar perdidas significativas en el cultivo. Como manejo para esta enfermedad el control químico es la más utilizada. Sin embargo, el mercado se esta moviendo a consumir productos mas limpios y con menos uso de agroquímicos. En la actualidad, se ha demostrado que algunos ingredientes activos para el control de esta enfermedad son ineficientes. Por tanto, se fomentan los estudios con sustancias naturales que puedan ayudar en el manejo de enfermedades. Debido a que se transmite a través de la semilla, el tratamiento de la semilla se convierte en una medida esencial en el manejo y control de la antracnosis. Teniendo en cuenta la problemática presentada hemos establecido como objetivo el evaluar el efecto de los aceites esenciales de citronella (*Cymbopogon winterianus*), clavo (*Syzygium aromaticum*), eucalipto (*Eucalyptus citriodora*), albahaca (*Ocimum basilicum*), menta (*Rosmarinus officinalis*), copaiba (*Copaifera langsdorfii*), malaleuca (*Melaleuca alternifolia*), romero (Rosmarinus officinalis) sobre el desarrollo de *C. gloeosporioides* y como esta puede interactuar con la calidad fisiológica de las semillas de pimiento. Encontramos que el aceite esencial de clavo redujo la incidencia de hongo tanto en las semillas inoculadas como en la etapa in vitro. El resto de los aceites esenciales evaluados solo funcionaron con el control del patógeno en la etapa in vitro.

Palabras clave: Antracnosis; *Capsicum annum*; Crecimiento micelial; Calidad fisiológica.

1. Introduction

Anthracnose, caused by *Colletotrichum* species, infects over 3000 species of plants worldwide (O'Connell et al., 2012), including the Solanaceae family. The genus was ranked the eighth most important pathogenic fungal genus in the world (Dean et al., 2012). In Brazil, *Colletotrichum* causes major economic losses in fruits, especially pepper. Wet season and fields with a history of pathogen incidence favor the development of the disease, and 100% of the pepper fruits can be affected (Pavan et al., 2016)

Current management strategies for the anthracnose control are not efficient, because *Colletotrichum* species have acquired resistance to commonly used fungicides, such as azoxystrobion and thiabendazole (Kumar & Kudachikar, 2018; Torres-Calzada et al., 2013). Furthermore, this fungus can be transmitted via seeds (Pavan et al., 2016), which is an important source of inoculum, introduction and dissemination of the pathogen, thus making

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9028

the use of treated seeds an essential control measure.

Compounds derived from secondary metabolism of plants are alternatives for plant disease management (Donnarumma et al., 2015). The antimicrobial activity of natural plant products against plant pathogens has been extensively investigated worldwide and the results are promising (Bi et al., 2012; Deberdt et al., 2012; Paret et al., 2010; Pradhanang et al., 2003). Several previous studies have shown that essential oils can be used in the management of fungal disease (Dela Cueva & Balendres, 2018; Diánez et al., 2018; Üstüner et al., 2018). In greenhouse experiments, Bi et al. (2012) showed that oregano, red thyme and palmarosa essential oils reduced *Phytophthora capsici* population in soil and protected zucchini fruit against the pathogen. Chaijuckam and Davis (2010) found that cinnamon oil has potential to control *Rhizoctonia oryzae-sativa*, the cause of aggregate sheath spot disease in rice. Dagostin et al. (2010) demonstrated that *Salvia officinalis* extract can protect grapevine against *Plasmopara viticola* in both greenhouse and field experiments.

Alves et al. (2015) and Dela Cueva and Balendres (2018) reported the control of anthracnose in pepper and chilli fruits by natural plants products, suggesting that they play an important role in the disease management. In vitro studies indicate that the essential oils of *Zingiber officinale, Cymbopogon citratus, Piper nigrum, Pelargonium hortororum, Syzygium aromaticum, Melaleuca alternifolia, Corymbia citriodora* and *Mentha arvensis* are efficient in inhibiting *C. gloeosporioides* spores germination (Nascimento et al., 2019). However, to date, no studies have reported on the antifungal properties of essential oils applied in pepper seed treatment against *C. gloeosporioides*.

Thus, the aims of this study were to (i) evaluate the effect of eight essential oils on mycelial growth and sporulation of *C. gloeosporioides* in vitro, (ii) develop an efficient pepper seed treatment in order to reduce the incidence of anthracnose, and (iii) to investigate the effects of the selected essential oils on the germination and growth of pepper plants.

2. Methodology

Laboratory tests and greenhouse assays were conducted at the Department of Plant Protection, São Paulo, State University, Botucatu/SP, Brazil. The laboratory test aimed to select the essential oils with the most effective antimicrobial activity against *C. gloeosporioides* and to evaluate the effect of pepper seed treatments infected by *C. gloeosporioide*s treated with the selected essential oils, in the vivo experiments.

Test pathogen

Colletotrichum gloeosporioides was isolated from infected pepper seeds. The pathogen identity was confirmed by PCR and gene sequencing, the isolate was deposited in the fungal collection belonging to the Laboratory of Seed Pathology of São Paulo State University.

Tested essential oils

The essential oils used in this study were: rosemary (*Rosmarinus officinalis* L. - containing 23,5% of β-mirceno; 22% of 1,8 cineol and 20% of α-pineno), citronella (*Cymbopogon winterianus* Jowitt - containing 41% of geraniol; 29% of citronelal and 13% of citronelol), clove (*Syzygium aromaticum* L. - containing 81% of eugenol), copaiba (*Copaifera langsdorfii* Desf. - containing 45% of β-cariofileno), eucalyptus (*Eucalyptus citriodora* Hook - containing 75% of citronelal), mint (*Mentha arvensis* L. - containing 52,50% of mentol), basil (*Ocimum basilicum* L. - containing 74,15% of metil chavicol and 17,60% of linalol) and tea tree (*Melaleuca alternifolia* Cheel - containing 32,60% of terpinen-4-ol; 19,80% of Y-terpineno and 3,55% of terpinoleno). All essential oils were purchased from Destilaria Bauru (Bauru, São Paulo, Brazil).

Effects of essential oils on radial growth and sporulation

The essential oils were added to molten culture medium potato-dextrose-agar (PDA) to achieve final concentrations of 0.25%, 0.50% and 0.75%. Control was constituted of PDA without the addition of essential oils. Petri dishes containing the culture medium were inoculated in the center with a 5 mm of mycelial disk removed from peripheral regions of *C. gloeosporioides*, which actively grew on culture medium (de Billerbeck et al., 2001) The Petri dishes were incubated at 25 °C under a 12 h photoperiod. Radial growth was determined when at least one of the treatments reached the border of the petri dish, measuring two mutually perpendicular diameters on each Petri dish and taking the average value, having subtracted the diameter of the mycelial disk. The experiment was conducted twice, and three replicates were considered for each concentration.

Based on the last evaluation data, the percentage of colony inhibition (PI) was calculated using the equation (de Billerbeck et al., 2001): PI = (control diameter - treatment diameter/control diameter) x 100.

For the evaluation of sporulation, three mycelial disks were removed from each plate and transferred to tubes containing 5 ml of distilled water with Tween 80 (one drop in 100 ml of water). These tubes were shaken for three minutes, obtaining a suspension of conidia for sporulation evaluation. The concentration of the conidia was determined in a Neubauer chamber.

Seed inoculation

Mycelial disks of *C. gloeosporioides* were transferred to petri dishes containing PDA with mannitol (33.10 g/L). After the fungus colonized the whole plate, pepper seeds previously disinfested with sodium hypochlorite 2% for one minute, were deposited in a single layer, remaining in contact with the colonies for 24 h (Machado et al., 2017). After this, the seeds were disinfested again and dried. The uninoculated seeds (control) were submitted to the same process, but without the presence of the inoculum in the culture medium.

Seed treatment

Seeds were treated with 0.25% of essential oil diluted in distilled water with Tween 80 in a beaker and shaken for 5 min. This essential oil concentration (0.25%) was selected based on the in vitro assay, where the essential oils selected for this stage inhibited the pathogen in the lowest concentration. After treatment, seeds were allowed to air-dry under room temperature on paper towel for 24 h.

Seed treatment effect on sanitary quality of seeds

Sixteen replications of 25 seeds from each treatment were distributed in plastic Petri dishes, containing three sheets of blotting paper moistened with sterile distilled water. The plates containing the seeds were incubated in BOD at 20 °C ± 2° with 12 hours light, for seven days (Brasil, 2009). The sanitary examination was performed evaluating each seed individually with stereomicroscope, observing the occurrence of fungal structures and mycelium. The results were expressed as percentage of seeds infected by *C. gloeosporioides*.

Effect on seed germination, aerial part and roots

Seed germination tests were conducted using samples of 400 pepper seeds per treatment. Eight replications of 50 seeds were plated uniformly into two layers of moist

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9028

blotter paper, being covered with a third paper and rolled up. The rolls were incubated at 25 °C and the seedlings were counted at seven (first germination count) and 14 days, according to the Rules for Seed Analysis (Brasil, 2009). The results were expressed as percentage of dead seeds and normal seedlings. Ten normal seedlings were selected from this test for aerial part and root measurement. The results were expressed in centimeters.

Seedling emergence and emergence speed index

Four replications of 50 seeds were distributed on plastic trays containing sand. Trays were kept in a greenhouse at room temperature and irrigated daily. Percent seedling emergence was determined 14 days after sowing by counting plants with shoot lengths of at least 1 cm. The number of emerged seedlings was recorded daily to determine the speed of emergence index according to Maguire (1962).

Dry matter mass of the seedling aerial part and root

Ten normal seedlings per repetition of the previous test were used. The aerial part and root system were oven dried at 60 ° C for 48 hours, and then weighed in analytical balance for mass determination. The result was expressed in grams.

3. Results and Discussion

Effects of essential oils on radial growth and sporulation

The results of mycelial growth and percentage of colony inhibition (PI) of both assays are reported in Table 1. Except for the essential oils of rosemary and copaiba, at 0.25%, in the first assay, all other treatments differed from the control by Dunnet test, promoting a decrease in mycelial growth. The essential oils of clove, citronella, eucalyptus, mint and basil completely inhibited fungal growth at all concentrations, being considered effective in the in vitro control of this pathogen. Concentrations higher than 0.25% of tea tree essential oil inhibited the radial growth of *C. gloeosporioides* completely. The same was observed for rosemary essential oil at 0.75% in both assays. The correlations between inhibition percentage and concentration were observed for the copaiba essential oil, showing that the inhibition percentage was proportional to concentration.

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9028

Table 1. Mycelial growth and percentage of colony inhibition (PI) of *Colletotrichum gloeosporioides*, isolated from pepper, cultivated in PDA and PDA culture medium with essential oils at different concentrations.

Essential oils		Assay 1		Assay 2	
		Mycelial growth (cm)	PI (%)	Mycelial growth (cm)	PI (%)
Rosemary	0.25%	8.3 a A	2.12	4.3 a A*	49.14
	0.50%	2.6 b B*	70.00	0.0 b B*	100.00
	0.75%	0.0 b C*	100.00	0.0 b B*	100.00
Clove	0.25%	0.0 c A*	100.00	0.0 c A*	100.00
	0.50%	0.0 b A*	100.00	0.0 b A*	100.00
	0.75%	0.0 b A*	100.00	0.0 b A*	100.00
Copaiba	0.25%	6.0 ab A	29.88	4.9 a A*	42.24
	0.50%	5.9 a A*	30.20	4.7 a AB*	45.14
	0.75%	5.2 a A*	39.7	4.3 a B*	49.14
Citronella	0.25%	0.0 c A*	100.00	0.0 c A*	100.00
	0.50%	0.0 b A*	100.00	0.0 b A*	100.00
	0.75%	0.0 b A*	100.00	0.0 b A*	100.00
Eucalyptus	0.25%	0.0 c A*	100.00	0.0 c A*	100.00
	0.50%	0.0 b A*	100.00	0.0 b A*	100.00
	0.75%	0.0 b A*	100.00	0.0 b A*	100.00
Mint	0.25%	0.0 c A*	100.00	0.0 c A*	100.00
	0.50%	0.0 b A*	100.00	0.0 b A*	100.00
	0.75%	0.0 b A*	100.00	0.0 b A*	100.00
Basil	0.25%	0.0 c A*	100.00	0.0 c A*	100.00
	0.50%	0.0 b A*	100.00	0.0 b A*	100.00
	0.75%	0.0 b A*	100.00	0.0 b A*	100.00

	0.25%	4.5 b A*	47.41	2.5 b A*	70.16
Tea tree	0.50%	0.0 b B*	100.00	0.0 b B*	100.00
	0.75%	0.0 b B*	100.00	0.0 b B*	100.00
PDA		8.5	-	8.5	-

For mycelial growth, in each test:
a, b, c - at each concentration level, mean values of essential oils followed by the same lowercase letter do not differ by Tukey's test (P> 0.05).
A, B - at each level of essential oil, averages of concentrations followed by the same capital letter do not differ by Tukey test (P> 0.05).
* Each treatment differed from the control (BDA) by Dunnett's test (P <0.01).
Source: Authors.

Results related to sporulation are presented in Table 2. There was no sporulation for oils that totally inhibited fungal development. For the copaiba essential oil, there was no significant difference in sporulation at all three concentrations in the two trials. At the concentration of 0.25%, sporulation of the treatment containing tea tree essential oil did not differ significantly from that of copaiba, and both differed from rosemary, which presented the lowest sporulation.

Table 2. Sporulation of *Colletotrichum gloeosporioides*, isolated from pepper, cultivated in PDA and PDA culture medium with essential oils at different concentrations.

Essential oils		**Assay 1**		**Assay 2**	
		Sporulation		**Sporulation**	
		log (x+1)¹	**Spores number/mL²**	**log (x+1)¹**	**Spores number/mL²**
Rosemary	0.25%	1.32 bc A*	0.08×10^4	1.39 b A*	0.50×10^4
	0.50%	1.23 b A*	0.17×10^4	0.00 b B*	0
	0.75%	0.00 b A*	0	0.00 b B*	0
Clove	0.25%	0.00 c A*	0	0.00 b A*	0
	0.50%	0.00 b A*	0	0.00 b A*	0
	0.75%	0.00 b A*	0	0.00 b A*	0

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9028

Copaiba	0.25%	3.60 a A	0.50 $x10^4$	4.94 a A*	37.25 $x10^4$
	0.50%	3.70 a A	0.50 $x10^4$	4.23 a A	2.25 $x10^4$
	0.75%	4.65 a A	48.91 $x10^4$	4.59 a A*	36.25 $x10^4$
Citronella	0.25%	0.00 c A*	0	0.00 b A*	0
	0.50%	0.00 b A*	0	0.00 b A*	0
	0.75%	0.00 b A*	0	0.00 b A*	0
Eucalyptus	0.25%	0.00 c A*	0	0.00 b A*	0
	0.50%	0.00 b A*	0	0.00 b A*	0
	0.75%	0.00 b A*	0	0.00 b A*	0
Mint	0.25%	0.00 c A*	0	0.00 b A*	0
	0.50%	0.00 b A*	0	0.00 b A*	0
	0.75%	0.00 b A*	0	0.00 b A*	0
Basil	0.25%	0.00 c A*	0	0.00 b A*	0
	0.50%	0.00 b A*	0	0.00 b A*	0
	0.75%	0.00 b A*	0	0.00 b A*	0
Tea tree	0.25%	3.10 ab A*	9.17 $x10^4$	4.11 a A*	1.75 $x10^4$
	0.50%	0.00 b A*	0	0.00 b B*	0
	0.75%	0.00 b A*	0	0.00 b B*	0
PDA		5.93	87.91 $x10^4$	5.92	91.00 $x10^4$

For mycelial growth, in each test:
a, b, c - at each concentration level, mean values of essential oils followed by the same lowercase letter do not differ by Tukey's test (P> 0.05).
A, B - at each level of essential oil, averages of concentrations followed by the same capital letter do not differ by Tukey test (P> 0.05).
* Each treatment differed from the control (BDA) by Dunnett's test (P <0.01).
Source: Authors.

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.9028

Effect of essential oil in seed treatment

The untreated uninoculated control had a low incidence of fungus (1.3%), whereas in the untreated inoculated control the incidence was 75.8% (Table 3). The treatments composed of essential oils of basil and eucalyptus did not differ significantly from the inoculated control in relation to the incidence of *C. gloeosporioides*. The other treatments with essential oils differed, especially the clove and citronella essential oils, with 7.5% and 24%, respectively, of pathogen incidence in seeds.

Table 3. Incidence of *Colletotrichum gloeosporioides* on pepper seeds, submitted to treatment with essential oils.

Essential oils	*C. gloeosporioides* incidence	
	arcsen √(x/100)	(%)[1]
Clove 0.25%	15.01 d	7.5
Citronella 0.25%	29.00 c	24.0
Eucalyptus 0.25%	54.93 a	65.0
Mint 0.25%	41.89 b	44.8
Basil 0.25%	59.68 a	72.0
Untreated inoculated seeds	62.09 a	75.8
Non-inoculated untreated seeds	3.19 e	1.3
F	87.26**	
dms	10.47	
CV (%)	25.94	

a, b, c ... - in the column, averages followed by the same letter do not differ among themselves by the Tukey test at the 5% probability level.
Source: Authors.

There were no significant differences among treatments at the first germination count at five days (Table 4); however, at the final count, at seven days, the highest percentage of germinated normal seedlings was verified in the treatment with clove essential oil (95.25%), not differing from the untreated control (94%), but differing from untreated inoculated control (90%) and citronella essential oil, which resulted in low germination of treated seeds (87.50%).

Table 4. First count of pepper seed germination submitted to treatment with essential oils.

Essential oils	First count		Germination		Lengh (cm)		
	arcsen √(x/100)	(%)[1]	arcsen √(x/100)	(%)[1]	Primary root	Hypocotyl	Total
Clove	60.33 a	74.00	77.80 a	95.25	7.7 a	2.9 a	10.6 a
Citronella	63.83 a	80.50	69.39 c	87.50	6.8 a	2.7 a	9.5 a
Eucalyptus	65.42 a	82.25	7563 ab	93.75	6.1 a	2.9 a	9.0 a
Mint	69.69 a	87.75	73.88 abc	92.25	6.5 a	2.9 a	9.4 a
Basil	68.54 a	86.50	74.11 abc	92.50	7.2 a	3.0 a	10.2 a
Untreated inoculated seeds	64.21 a	81.00	71.78 bc	90.00	6.7 a	2.5 a	9.2 a
Non-inoculated untreated seeds	69.54 a	87.75	75.89 ab	94.00	7.8 a	3.1 a	10.9 a
F	1,79^{NS}		5.17**		2.39 ^{NS}	1.14 ^{NS}	2.84 ^{NS}
Dms	11,98		5.65		1.88	0.89	2.04
CV (%)	7,91		3.32		11.78	13.53	9.04

Source: Authors.

For the parameter's seedling length, emergence speed index, final emergence and dry matter (Table 5), there were no significant differences among treatments.

Table 5. Index of emergence speed (IES), final emergence and dry matter of pepper plants, from seeds submitted to treatment with essential oils.

Essential oils	IES	Final emergence		Dry matter (g)
		arcsen √(x/100)	(%)[1]	
Clove 0.25%	11.0 a	61.5 a	75.0	0.493 a
Citronella 0.25%	9.1 a	60.3 a	74.5	0.462 a
Eucalyptus 0.25%	12.0 a	72.8 a	90.5	0.473 a
Mint 0.25%	12.2 a	68.4 a	86.0	0.467 a
Basil 0.25%	11.4 a	69.8 a	88.0	0.477 a
Untreated inoculated seeds	12.2 a	73.3 a	91.5	0.486 a
Non-inoculated untreated seeds	11.0 a	64.7 a	81.0	0.472 a
F	1.51^{NS}	1.59^{NS}		1.01^{NS}
dms	4.02	19.94		0.062
CV (%)	15.14	11.41		5.59

a - in each column, averages followed by the same letter do not differ among themselves by the Tukey test at the 5% probability level.
1original date
Source: Authors.

Pepper anthracnose management is currently extremely difficult due to the fact that this fungus is a seed-borne transmitted and the chemical treatments are not efficient. Our study is the first to examine the effect of essential oils on the *C. gloeosporioides* isolated from pepper fruits, and the possibilities of these oils to be used as a seed treatment to control the incidence of anthracnose. We assessed the in vitro antifungal activities of eight essential oils against *C. gloeosporioides* and evaluated the mycelial growth and sporulation on PDA medium with different concentrations of the emulsified essential oils.

Our results demonstrated that clove, citronella and mint essential oils can suppress *C. gloeosporioides* development in vitro and that, at 0.25%, these essential oils can either protect pepper seeds from increased incidence of anthracnose in greenhouse conditions.

The in vitro results obtained from rosemary and tea tree essential oils showed that they are able to delay the growth of the fungi within a short period of time but cannot completely inhibit it. Essential oils are volatile compounds that are easily degraded when exposed to factors such as temperature, oxygen and light (Beyki et al., 2014). The atmosphere in Petri dishes (in vitro studies) and in field is not favorable to these compounds. Therefore, in order to maintain the properties of the essential oil, technologies need to be developed, such as the encapsulation of oils with chitosan and mesoporous silica nanoparticles (MSNPs) (Villegas-Villegas-Rascón et al., 2018; Cadena et al., 2018).

Our findings concerning *C. gloeosporioides* control by essential oils are in agreement with previous reports. Yilmaz, Ermis, and Boyraz (2016) showed that the application of rosemary essential oil resulted in slight inhibition of *C. gloeosporioides* mycelial growth, and Andrade and Vieira (2016) demonstrated the fungicidal effect of the same essential oil, as well as mint and tea tree essential oils on mycelial growth and sporulation of *C. gloeosporioides isolated* from papaya.

The potential of citronella essential oil for the management of chilli anthracnose was investigated by Dela Cueva and Balendres (2018). In the in vitro test the oil inhibited mycelial growth, germ tube elongation and reduced conidial germination of *C. acutatum*. Citronella and basil essential oil were also evaluated on the mycelial growth of *C. lindemunthianum* (Jyoti et al. 2014). More recently, clove, rosemary, basil and tea tree essential oils showed antagonistic activity against *C. gloeosporioides* on a Petri plate assay (Rabari et al., 2018).

In the present study, no phytotoxic effects were observed on pepper plants when the seeds were treated with essential oils. This is not the first observation of positive effects of essential oils on plant growth, but it is the first with pepper seeds besides the suppressive effect of clove, citronella and mint essential oils in the incidence of seed-borne *Colletotrichum*.

The analysis of the commercial clove essential oil used in this study revealed its chemical composition and a large amount of eugenol was found. We propose that the antimicrobial mode of action of clove essential oil could be linked to eugenol, which is reported in the literature to be the major component of this essential oil. A recent study by Deberdt et al. (2018) demonstrated that *Pimenta racemosa* var. *racemosa* essential oil, in which eugenol is the major component, influenced tomato resistance to bacterial wilt. Wang and Fan (2014) showed eugenol is an effective activator of plant systemic acquired resistance by triggering the salicylic acid biosynthesis pathway. These authors demonstrate the induction of systemic resistance in tomato against tomato leaf curl virus.

The major limitations regarding the application of essential oils in seeds happen due to the volatilization of these oils, not providing an appropriate period of residual effect. In order to be effective in seed treatment, essential oils must be able to promote seed protection for extended periods. Technologies development for the application of essential oils to seeds should enable their use in seed treatment.

4. Final Considerations

Overall, the results of this study indicate that clove essential oil inhibits the in vitro growth of *C. gloeosporioides*, reduces anthracnose impact on pepper seeds, and is not phytotoxic at the concentration of 0.25% to pepper seeds and later, for the development of the seedlings. This essential oil has potential as component of an integrated disease management program for pepper antrachnose.

Further studies should be conducted evaluating the effect of its major compound (eugenol) on the pathogen and on the seed. The technology for applying this essential oil to the seed must also be improved, e.g. through encapsulation, prolonging its effect on the pathogen.

References

Alves, K. F., Laranjeira, D., Câmara, M. P. S., Câmara, C. A. G., & Michereff, S. J. (2015). Efficacy of plant extracts for anthracnose control in bell pepper fruits under controlled conditions. *Horticultura Brasileira*, 33(3), 332–338.

Andrade, W. P., & Vieira, G. H. C. (2016). Efeito dos óleos essenciais sobre a antracnose *in vitro* e em frutos de mamoeiro. *Revista Brasileira de Plantas Medicinais*, 18(1), 367–372. https://doi.org/10.1590/1983-084X/15_089

Beyki, M., Zhaveh, S., Khalili, S. T., Rahmani-Cherati, T., Abollahi, A., Bayat, M., Tabatabaei, M., & Mohsenifar, A. (2014). Encapsulation of *Mentha piperita* essential oils in chitosan-cinnamic acid nanogel with enhanced antimicrobial activity against *Aspergillus flavus*. *Industrial Crops and Products*, 54, 310–319. https://doi.org/10.1016/j.indcrop.2014.01.033

Bi, Y., Jiang, H., Hausbeck, M. K., & Hao, J. J. (2012). Inhibitory effects of essential oils for controlling *Phytophthora capsici*. *Plant Disease,* 96(6), 797–803. https://doi.org/10.1094/PDIS-11-11-0933

de Billerbeck, V. G., Roques, C. G., Bessière, J. M., Fonvieille, J. L., & Dargent, R. (2001). Effects of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. *Canadian Journal of Microbiology*, 47(1), 9-17.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. (2009). *Regras para análise de sementes*.

Cadena, M. B., Preston, G. M., Van der Hoorn, R. A., Flanagan, N. A., Townley, H. E., & Thompson, I. P. (2018). Enhancing cinnamon essential oil activity by nanoparticle encapsulation to control seed pathogens. *Industrial Crops and Products*, 124, 755-764.

Chaijuckam, P., & Davis, R. M. (2010). Efficacy of natural plant products on the control of aggregate sheath spot of rice. *Plant Disease,* 94(8), 986–992. https://doi.org/10.1094/PDIS-94-8-0986

Dagostin, S., Formolo, T., Giovannini, O., & Pertot, I. (2010). *Salvia officinalis* extract can protect grapevine against *Plasmopara viticola*. *Plant Disease,* 94(36), 575–580. https://doi.org/Doi 10.1094/Pdis-94-5-0575

Dean, R., Van Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, 13(4), 414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

Deberdt, P., Davezies, I., Coranson-Beaudu, R., & Jestin, A. (2018). Efficacy of leaf oil from *Pimenta racemosa* var. *racemosa* in controlling bacterial wilt of tomato. *Plant Disease*, 102(1), 124–131. https://doi.org/10.1094/PDIS-04-17-0593-RE

Deberdt, P., Perrin, B., Coranson-Beaudu, R., Duyck, P. F., & Wicker, E. (2012). Effect of *Allium fistulosum* extract on *Ralstonia solanacearum* populations and tomato bacterial wilt. *Plant Disease*, 96(5), 687-692.

Dela Cueva, F., & Balendres, M. A. (2018). Efficacy of citronella essential oil for the management of chilli anthracnose. *European Journal of Plant Pathology*, 1–8. https://doi.org/10.1007/s10658-018-1491-y

Diánez, F., Santos, M., Parra, C., Navarro, M. J., Blanco, R., & Gea, F. J. (2018). Screening of antifungal activity of twelve essential oils against eight pathogenic fungi of vegetables and mushroom. *Letters in Applied Microbiology*. https://doi.org/10.1111/lam.13053

Donnarumma, L., Milano, F., Trotta, S., & Annesi, T. (2015). Use of essential oils in control strategies against zucchini powdery ildew. *Journal of Phytopathology*, 163(11–12), 877–885. https://doi.org/10.1111/jph.12387

Jyoti, B., Mk, S., Jameel, A., & Dinesh, C. (2014). Antifungal efficacy of essential oils against common bean anthracnose caused by *Colletotrichum lindemuthianum*. 3(4), 22–29.

Kumar, A., & Kudachikar, V. B. (2018). Antifungal properties of essential oils against anthracnose disease: a critical appraisal. *Journal of Plant Diseases and Protection*, 125(2), 133–144. https://doi.org/10.1007/s41348-017-0128-2

Machado, A. Q., Machado, J. D. C., Vieira, M. D., Cassetari Neto, D., & Souza, M. V. (2007). Potencial do uso da restrição hídrica em testes de sanidade de sementes de algodoeiro. *Fitopatologia Brasileira*, 32(5), 408-414.

Maguire, J. D. (1962). Speed of Germination—Aid in selection and evaluation for seedling emergence and vigor 1. *Crop Science*, 2(2), 176–177.

Nascimento, D. M. D., Santos, P. L. D., & Kronka, A. Z. (2019). Essential oils inhibit *Colletotrichum gloeosporioides* spore germination. *Summa Phytopathologica*, 45(4), 432-433.

O'Connell, R. J., Thon, M. R., Hacquard, S., Amyotte, S. G., Kleemann, J., Torres, M. F., Damm, U., Buiate, E. A., Epstein, L., Alkan, N., Altmüller, J., Alvarado-Balderrama, L., Bauser, C. A., Becker, C., Birren, B. W., Chen, Z., Choi, J., Crouch, J. A., Duvick, J. P., … Vaillancourt, L. J. (2012). Lifestyle transitions in plant pathogenic *Colletotrichum* fungi deciphered by genome and transcriptome analyses. *Nature Genetics*, 44(9), 1060–1065. https://doi.org/10.1038/ng.2372

Paret, M. L., Cabos, R., Kratky, B. A., & Alvarez, A. M. (2010). Effect of plant essential oils on *Ralstonia solanacearum* race 4 and bacterial wilt of edible ginger. *Plant Disease*, 94(5), 521–527. https://doi.org/10.1094/PDIS-94-5-0521

Pavan, M. A., Krause-Sakate, R., Moura, M. F., & Kurozawa, C. (2016). *Doenças das plantas cultivadas*. In L. Amorim, J. A. M. Rezende, A. Bergamin Filho, & L. E. A. Camargo (Eds.), Manual de Fitopatologia (pp. 677–686). Agrônomica Ceres.

Pradhanang, P. M., Momol, M. T., Olson, S. M., & Jones, J. B. (2003). Population density and bacterial wilt incidence in tomato. *Plant Disease*, 87(4), 423–427. https://doi.org/10.1094/PDIS.2003.87.4.423

Rabari, V. P., Chudashama, K. S., & Thaker, V. S. (2018). In vitro screening of 75 essential oils against *Colletotrichum gloeosporioides*: A causal agent of anthracnose disease of mango. *International Journal of Fruit Science*, 18(1), 1–13. https://doi.org/10.1080/15538362.2017.1377666

Torres-Calzada, C., Tapia-Tussell, R., Higuera-Ciapara, I., & Perez-Brito, D. (2013). Morphological, pathological and genetic diversity of *Colletotrichum* species responsible for anthracnose in papaya (*Carica papaya* L). *European Journal of Plant Pathology*, 135(1), 67–79. https://doi.org/10.1007/s10658-012-0065-7

Üstüner, T., Kordali, S., & Usanmaz Bozhüyük, A. (2018). Herbicidal and fungicidal effects of *Cuminum cyminum*, *Mentha longifolia* and *Allium sativum* essential oils on some weeds and fungi. *Records of Natural Products*, 12(6), 619–629. https://doi.org/10.25135/rnp.80.18.05.106

Villegas-Rascón, R. E., López-Meneses, A. K., Plascencia-Jatomea, M., Cota-Arriola, O., Moreno-Ibarra, G. M., Castillón-Campaña, L. G., Sánchez-Mariñez, R. I., & Cortez-Rocha, M. O. (2018). Control of mycotoxigenic fungi with microcapsules of essential oils encapsulated in chitosan. *Food Science and Technology*, 38(2), 335–340. https://doi.org/10.1590/1678-457X.04817

Wang, C., & Fan, Y. (2014). Eugenol enhances the resistance of tomato against tomato yellow leaf curl virus. *Journal of the Science of Food and Agriculture*, 94(4), 677–682. https://doi.org/10.1002/jsfa.6304

Yilmaz, A., Ermis, E., & Boyraz, N. (2016). Investigation of in vitro and in vivo anti - fungal activities of different plant essential oils against postharvest apple rot diseases *Colletotrichum gleosporioides*, *Botrytis cinerea* and *Penicillium expansum*. *Journal of Food Safety and Food Quality*, 67(5), 113–148. https://doi.org/10.2376/0003-925X-67-122

Percentage of contribution of each author in the manuscript

Daniele Maria do Nascimento – 30%

Paula Leite dos Santos – 30%

Marcos Roberto Ribeiro Junior – 7,5%

Maria Márcia Pereira Sartori – 7,5%

Adriana Zanin Kronka – 25%