

Physiological and sanitary attributes of organic lettuce seeds treated with essential oils during storage

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Abstract

The use of quality seeds becomes crucial in the seed production system. In the production of organic seeds, there is a need for specific techniques. Therefore, the objective of this work was to evaluate the applications of clove, lemongrass, rosemary, eucalyptus, ginger and tea tree essential oils in concentrations of 0; 500; 1.000; 1.500 and 2.000 $\mu\text{L L}^{-1}$ plus Tween 80 (1%) in distilled water at lettuce seeds. Seed quality was determined by physical (water content), physiological (germination and vigor) and sanitary parameters immediately after seed treatment and at 60, 120 and 180 days after storage. The experimental design was a completely randomized design (DIA), in a $6 \times 4 + 1$ factorial scheme, with six essential oils and four concentrations + control treatment. The application of rosemary, ginger and tea essential oils to the lettuce seeds shortly after harvest did not interfere with germination. The other essential oils resulted in reduced seed germination and vigor. Increasing the concentration of essential oils, especially clove and lemongrass, reduced seed germination and vigor and the incidence of fungi. It was concluded that clove, lemongrass, eucalyptus, ginger, rosemary and tea tree essential oils reduced the quality of lettuce seeds and inhibited the development of *Alternaria* sp. Lettuce and from 60 days of storage the presence of *Cladosporium* sp. was not observed in lettuce seeds treated with lemongrass essential oil at a concentration of 1.000 $\mu\text{L L}^{-1}$, and in the treatment with clove essential oil (1.500 and 2.000 $\mu\text{L L}^{-1}$).

Keywords: *Lactuca sativa*, seed germination and vigor.

Introduction

Lettuce (*Lactuca sativa* L.) is a leafy vegetable from the Mediterranean region. The plant is autogamous and the inflorescence is chapter type, the fruit is an achene, whose seed is linked to the fruit by the region of the funicular developed from the ovary with a single egg, and the seeds are the main form of propagation (Sala & Nascimento, 2014).

The antifungal activities of plant products are known and used. Essential oils are recommended as one of the most promising antifungal agents (Kamazeri et al., 2012; Li et al., 2016; Nerilo et al., 2016). Essential oils are complex mixtures of volatile substances that include mainly terpenes, terpenoids, aromatic and aliphatic constituents, all characterized by low molecular weight (Bassolé & Juliani, 2012; Xavier et al., 2012).

However, it is essential that seed treatment products control or decrease pathogen infection, but

do not cause reduction in seed germination and vigor. Vokou et al. (2003) verified reduction of lettuce seed germination with treatment with tea tree essential oil. This oil contains more than 40% terpinen-4-ol, which is toxic depending on the amount used (Hammer et al., 2006). The objective of this work was to verify the interference of clove, lemongrass, rosemary, eucalyptus, ginger and tea tree essential oils at different concentrations on germination, vigor and sanity of organic lettuce seeds during storage.

Material and Methods

Organic lettuce seeds (*Elisa* variety) were supplied by the Mokiti Okada Foundation Research Center, produced in 2017. Products application to the seeds and the seeds analyzes were carried out at UEPG Seed Analysis Laboratory (State University of Ponta Grossa) in Ponta Grossa - PR.

Essential oils of clove [*Eugenia caryophyllus* (L.) Merrill & Perry], lemongrass [*Cymbopogon citratus*(DC) Stapf.], rosemary (*Rosmarinus officinalis* L.), eucalyptus (*Eucalyptus globulus* Labill.), ginger (*Zingiber officinale* Roscoe.) and tea tree (*Melaleuca alternifolia* Cheel.) were commercially acquired and were applied to the lettuce seed at concentrations of 0; 500; 1.000; 1.500 and 2.000 μ L L⁻¹ plus Tween 80 (1%) in distilled water (Brito et al., 2012).

The essential oils used for the research were analyzed for chemical composition in the chromatography laboratory of the Federal University of Minas Gerais (Table 1). The methodology used was High Resolution Gas

Chromatography with a gas chromatograph.

The application of the essential oils to the seeds was by immersion for a period of three minutes. For this, 6.6 grams of lettuce seeds were placed in 0.5; 1.0; 1.5; and 2.0 mL of each essential oil and added 1L of water, in addition to the control (Brito et al., 2012). Then the seeds were placed on two sheets of sterile blotting paper for drying in a natural environment until they reached 6% of water content. Subsequently, they were placed in aluminized bags for cold room storage at 10°C and 55% relative humidity. Seed quality evaluations were performed immediately after treatment and at 60, 120 and 180 days after storage.

Table 1. Gas chromatography analysis of the essential oils of clove (*Eugenia caryophyllus*), lemongrass (*Cymbopogon citratus*), rosemary (*Rosmarinus officinalis*), Eucalyptus (*Eucalyptus globulus*), ginger (*Zingiber officinale*) and tea tree (*Melaleuca alternifolia*).

Constituents (%)	Clove	Lemongrass	Rosemary	Eucalyptus	Ginger	Tea tree
1,8-cineole			39.6	89.9		1.4
Ar-curcumene					15.2	
Camphene		0.2	0.9		7.1	
Camphene						0.4
Camphor			27.7			
Carveol		0.1				
Cis-calameno						0.3
Citronellal		0.5				
Crisantenol		1.0				
Eugenol	87.3		0.6			
E- β -ocimene				0.6		
Geraniol	0.4	48.7				
Geranylacetate		0.9				
Germacrene d					3.0	
Sabinenehydrate					3.0	
Limonene		0.3	22.0	1.5	4.0	2.2
Linalool		0.2	0.8			
Myrcene		0.6	0.8	2.3		0.4
Neral		42.2				
Caryophyllene oxide	0.4	0.8				
p-cimene			1.6	0.2		2.8
Methylsalicylate	0.2					
Sesquifelandreno					12.6	
Terpinen-4-ol						42.5
Terpinolene				0.1		3.4
Trans verbenol		0.7				
Viridiflorino						0.4
Zingiberene					30.5	
Z- β -ocimene		0.1		0.1		
α -copaene	0.5					
α -farnesene					9.0	
α -phellandrene						1.0
α -humulene	1.8	0.6				
α -pinene			2.9	2.2	2.8	5.0
α -terpinene				1.4		10
α -terpineol						7.3
α -thujene						0.3
β -bisabolene					4.0	
β -caryophyllene	9.3	0.7	0.8			
β -gurjunene						0.2
β -pinene			1.5	1.6		0.4
γ -muurolene		0.2				
γ -terpinene		0.3				20.4
Others	0.4	1.5	0.8	0.3	8.7	1.7

Determination of water content

It was used the stove method adapted from Brazil (2009a). For this, 2.0 grams of seeds were placed in containers, which were kept open in a stove at 105°C ± 3 for 24 hours. Afterwards, the containers were capped and placed in a desiccator, until cooled for weighing. The result was expressed as a percentage of water.

Germination Test

Eight repetitions of 25 seeds distributed on two sheets of paper moistened 2.5 times the weight of the dry paper were placed in plastic boxes previously disinfected with sodium hypochlorite. The plastic boxes were placed in a germination chamber with constant temperature of 20°C ± 2, with the evaluations on the 4th day after sowing (first count of the germination test - FCG) and 7th day (Brasil, 2009a). Results were expressed as percentage of normal seedlings.

Seedling emergence

Four replicates of 50 seeds were sown in 200-cell plastic trays. The seeds were sown to a depth of 1 cm and one seed per cell was placed. Substrate composed of peat, concealers, vermiculite, charcoal and pine bark was used. The substrate was moistened daily with water equivalent to 60% of its holding capacity, with the trays in the greenhouse at 25°C and 70% relative humidity. The evaluations, considering the developed structures of seedlings, were carried out from the 1st day until 21st days after sowing and the results were expressed as percentage of emerged seedlings (Nakagawa, 1994).

Seedling emergence speed index

It was calculated from the data obtained from the daily seedling emergence assessments. The index was represented by the number of seedlings emerged daily until the 21st day after sowing, according to Maguire's adapted formula (1962).

Sanitary Parameter Evaluation

To evaluate the sanitary parameter, 200 seeds (8 replicates of 25 seeds) were placed in plastic boxes previously disinfected with sodium hypochlorite on two sheets of filter paper moistened 2.5 times the weight of dry paper. The seeds remained for seven days in a BOD incubation chamber at 20 °C with a 12 hour photoperiod. The evaluation was performed at seven days after sowing, using stereoscopic microscope and light microscope, classifying the fungi by gender. The results were expressed as percentage of occurrence of the observed fungi (Brasil, 2009b).

Statistical analysis

The experimental design was a completely randomized design (DIA), in a 6x4 + 1 factorial scheme, with six essential oils and four concentrations + control treatment, with four replications for the germination and vigor analysis (seedling emergence, seedling emergence speed index and germination test first count) and eight replications for the analysis sanitary. Data were analyzed for variance analysis to verify the significance of the interaction of the essential oil and concentration factors. Essential oil concentration data, when significant, were subjected to polynomial regression analysis, up to the third degree, and when significant for essential oil, the means were compared by Tukey test (5%). When necessary, the data were transformed into $\text{arc sen } \sqrt{(x + 0.5) / 100}$ and analyzes were performed using the program R Studio.

Results and Discussion

The amount of water in lettuce seeds ranged from 6.5 to 7.0% from the beginning of storage until 180 DOS (days of storage). There was a significant interaction for the essential oil and concentration factors for all analyzed variables, except for the incidence of *Alternaria* sp. at 180 days of storage.

For the germination test first count, in lettuce seeds treated with eucalyptus, ginger and tea tree essential oils, germination was superior in relation to the results of treatments with rosemary, clove and lemongrass essential oils. The results of lemongrass essential oil use indicated that there was a significant reduction in lettuce seed germination. With application of this oil at a concentration of 1.000µl L⁻¹ there was no lettuce seed germination (Table 2).

At 60 DOS for treatments with eucalyptus, ginger and tea tree essential oils at concentrations of 500, 1.000 and 2.000µl L⁻¹, lettuce seed germination at 4 days was higher than treatments with clove, lemongrass and rosemary essential oils. These results were also observed at 120 DOS for the same oils at 1.000, 1.500 and 2.000µl L⁻¹ concentrations and at 180 DOS at 500, 1.500 and 2.000µl L⁻¹ concentrations (Table 2).

The results related to lettuce seed germination at 7days indicate that post-harvest treatments with eucalyptus, ginger and tea tree essential oils showed higher germination than treatments with clove, lemongrass and rosemary essential oils, regardless of the used concentration. This variation in results remained until 180 DOS. After 60 days of storage, these results were also observed in the treatment with rosemary essential oil (Table 2).

Shokouhian et al. (2016) verified for lettuce seeds

(*Lactuca sativa*) that the application of the essential oils of rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*) and anise (*Pimpinella anisum*) in concentrations of 25 and 50% reduced germination significantly compared to the control treatment. Using the essential oil of *Eucalyptus globulus* L. (0.15%, for 120 minutes) to evaluate lettuce seed (*Lactuca sativa* L.) germination and growth of seedlings. Garbim et al. (2014) indicated that there was a significant reduction germination of the seed with the use of this essential oil.

For storage up to 60 days, the application of tea tree essential oil at a concentration of up to 1.000µl L⁻¹ kept the lettuce seeds with 74% germination, and with the

500 µl L⁻¹ ginger essential oil the seed germination was 70% (Table 2), values according to the minimum value of the standard established in the legislation. In the post-harvest, there was a linear reduction in lettuce seed germination results, with increase of clove and rosemary essential oils concentration.

Miranda et al. (2015) found that the use of lemongrass essential oil and its main component, the citral monoterpene (isomeric mixture of neral and geranial), inhibited the germination of lettuce seeds. In the present study, lemongrass essential oil had 48.7% geranial (Table 1), which is likely to cause a reduction in lettuce seed germination soon after harvest.

Table 2. Germination test count (%) of lettuce seeds at 4 and 7 days after sowing, submitted to the application of essential oils at different concentrations in post-harvest, 60, 120 and 180 days of storage.

Essential oil	4thDAY					7thDAY				
	Post-harvest									
	Concentration (µl L ⁻¹)									
	0	500	1.000	1.500	2.000	0	500	1.000	1.500	2.000
Clove	32 ^{ns}	16 c*	10 bc	2 c	1 b	96 ^{ns}	82 b	63 b	38 c	33 c
Lemongrass	32	1 d	0 d	0 c	0 b	96	38 c	21 c	14 d	0 d
Rosemary	32	10 c	4 cd	4 c	3 b	96	76 b	70 b	68 b	53 b
Eucalyptus	32	27 a	22 a	14 ab	14 a	96	95 a	93 a	93 a	87 a
Ginger	32	24 ab	21 a	18 a	14 a	96	92 a	91 a	90 a	88 a
Tea tree	32	25 b	14 ab	7 bc	7 ab	96	95 a	94 a	85 a	83 a
60 DOS										
Clove	31 ^{ns}	12 b	6 b	1 c	1 b	70 ^{ns}	17 c	7 d	5 d	3 c
Lemongrass	31	1 c	0 b	0 c	0 b	70	6 c	0 d	0 d	0 c
Rosemary	31	10 b	4 b	3 c	3 b	70	55 b	42 c	42 c	41 b
Eucalyptus	31	24 a	20 a	14 ab	13 a	70	61 b	54 c	51 bc	49 b
Ginger	31	24 a	21 a	18 a	14 a	70	70 b	67 b	66 a	64 a
Tea tree	31	20 a	20 a	8 bc	7 ab	70	73 a	74 a	59 ab	54 ab
120 DOS										
Clove	29 ^{ns}	10 bc	6 b	1 b	0 b	66 ^{ns}	18 b	8 d	2 c	1 c
Lemongrass	29	1 c	0 b	0 b	0 b	66	6 c	0 d	0 c	0 c
Rosemary	29	9 bc	4 b	3 b	3 b	66	55 a	41 c	41 c	39 b
Eucalyptus	29	24 a	19 a	13 a	14 a	66	58 a	55 b	49 b	48 b
Ginger	29	20 ab	19 a	15 a	11 a	66	64 a	62 ab	61 a	61 a
Tea tree	29	21 ab	20 a	20 a	12 a	66	65 a	66 a	60 a	44 b
180 DOS										
Clove	18 ^{ns}	11 b	6 c	0 b	0 b	58 ^{ns}	15 c	7 d	1 c	1c
Lemongrass	18	1 b	0 c	0 b	0 b	58	4 d	0 d	0 c	0 c
Rosemary	18	7 b	3 c	3 b	3 b	58	49 b	40 c	42 b	35 b
Eucalyptus	18	18 a	19 a	14 a	14 a	58	54 b	54 b	50 ab	50 a
Ginger	18	17 a	15 b	14 a	14 a	58	55 b	54 b	52 a	51 a
Tea tree	18	17 a	16 b	17 a	13 a	58	67 a	65 a	58 a	45 a

*Means followed by the same letter in the column, within each storage period, do not differ statistically by Tukey test at 5% significance; ns = not significant.

Until 120 days of storage (DOS), the results of lettuce seed germination at 4 days after sowing (DAS) decreased linearly with increasing concentrations of eucalyptus, ginger and tea tree essential oils (Figure 1). At 180 DOS, the germination percentage decreased linearly with clove essential oil (Figure 1-D).

According to regulation 457 of December 18, 1986, lettuce seeds must have a minimum germination of 70% for commercialization (Brasil, 1986). In the present

work, seeds treated with Clove essential oil from (500 µl L⁻¹) and Rosemary oil at concentration (1.000µl L⁻¹) after harvest, or with eucalyptus essential oils, Ginger and tea tree at all concentrations after harvest remained with germination above 70%.

In treatments with lemongrass and tea tree essential oils the results analysis followed the quadratic model. At 60 DOS the application of eucalyptus essential oil linearly reduced the germination of lettuce seeds as

a function of the concentrations used, and the results regarding the use of other essential oils was more

adequate with the quadratic model (Figure 2).

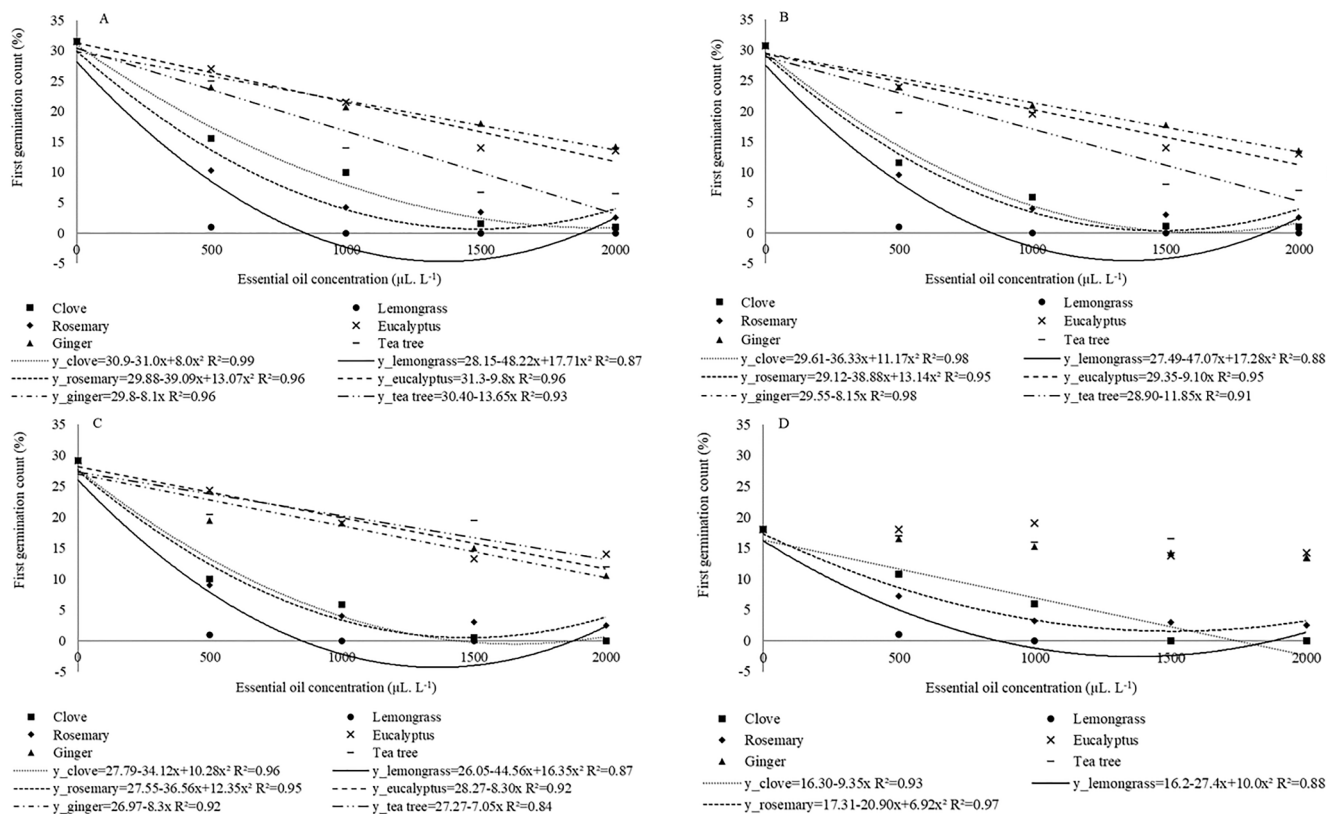


Figure 1. Regression analysis of the first germination count (%) of lettuce seeds submitted to the application of essential oils in different concentrations at post-harvest (A), 60 (B), 120 (C) and 180 (D) days of storage.

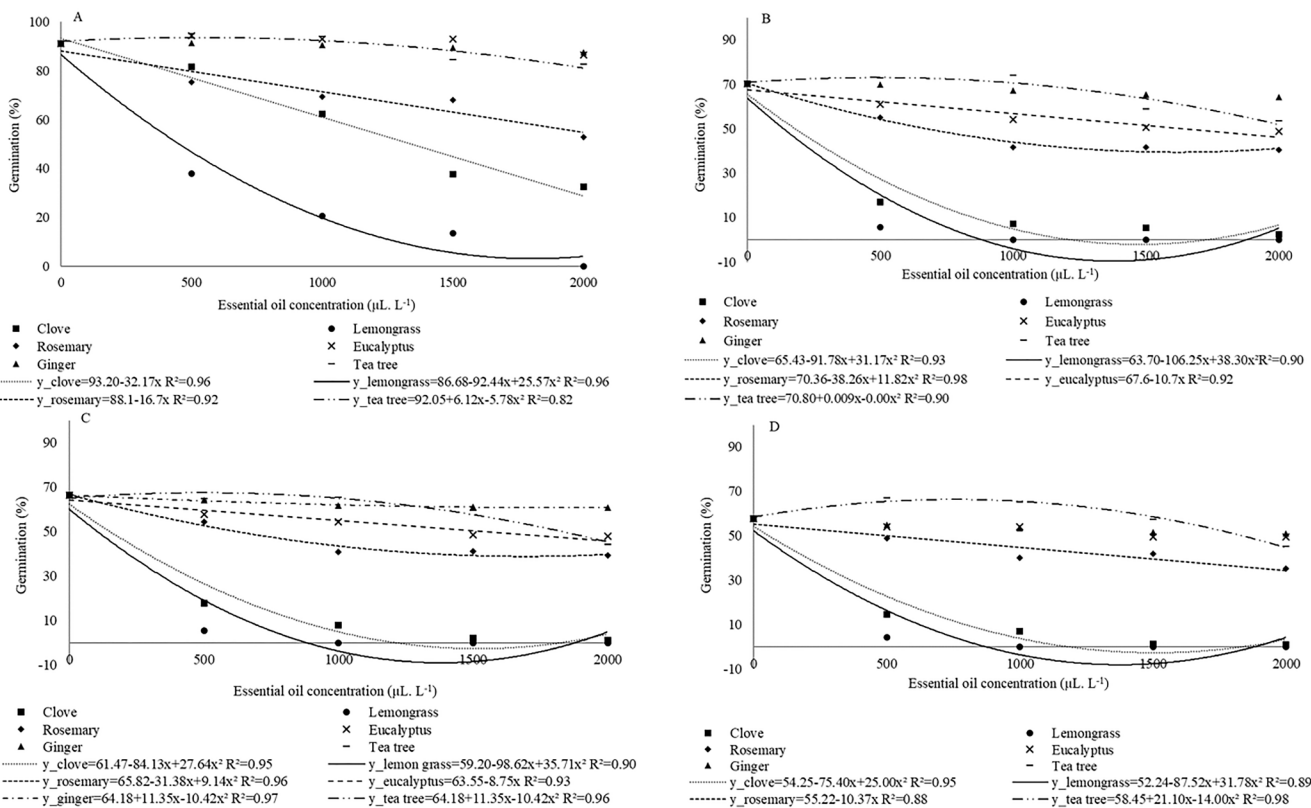


Figure 2. Regression analysis of lettuce seed germination submitted to the application of essential oils in different concentrations at post-harvest (A), 60 (B), 120 (C) and 180 (D) days after storage.

Lettuce seedling emergence was also influenced by the application of essential oils. There was a significant interaction between the concentration and essential oil factors in post-harvest seed and storage (Figure 3).

There was a reduction in the percentage of normal seedlings evaluated in the emergence speed index (Figure 3).

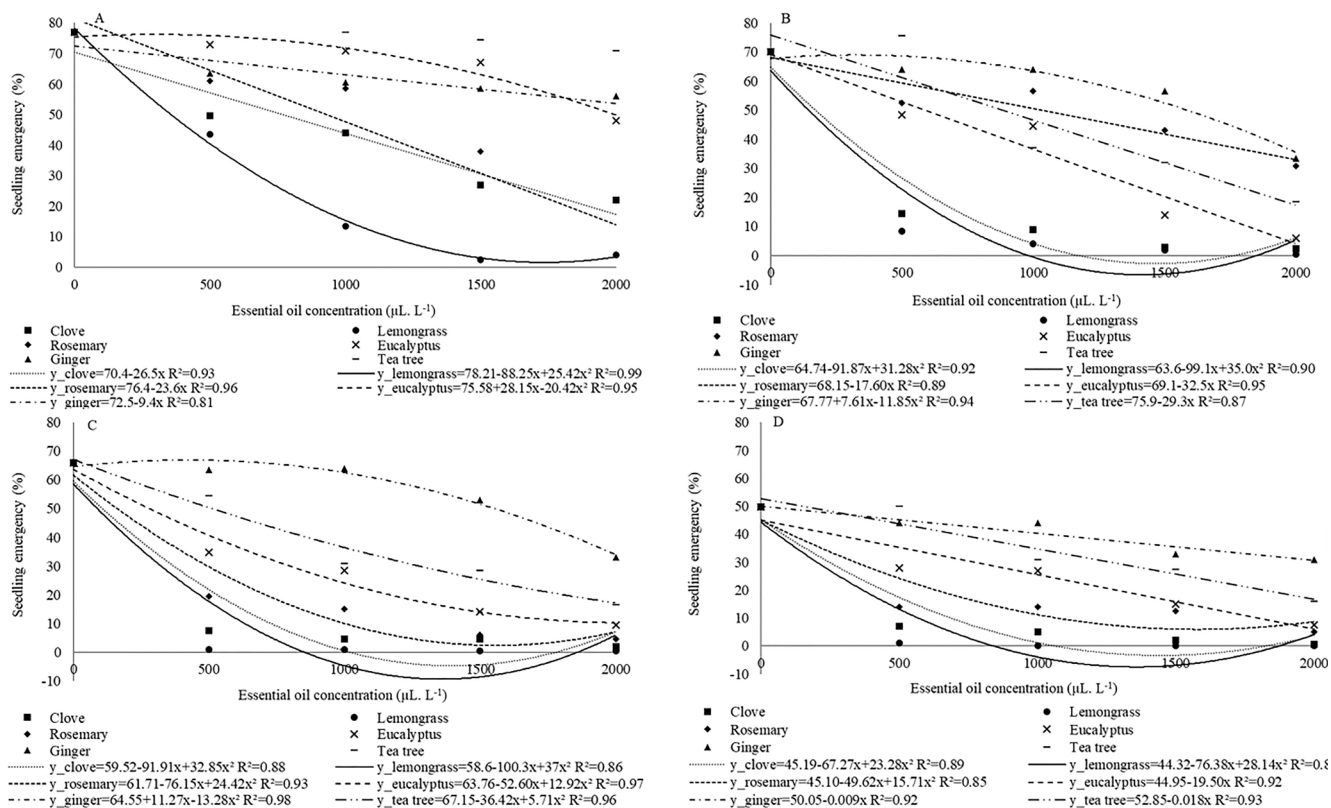


Figure 3. Regression analysis of lettuce seedling emergence (%) submitted to the application of essential oils in different concentrations at post-harvest (A), 60 (B), 120 (C) and 180 (D) days of storage.

Until 1.500 µL L⁻¹ in the post-harvest of teatree and eucalyptus essential oils the results of seedling emergence were higher than those treated with clove, lemongrass, rosemary and ginger essential oil at the same concentrations (Table 3).

At 60 DOS, the emergence of lettuce seedlings reduced linearly as a function of rosemary, eucalyptus and tea tree essential oil concentrations (Figure 4-B). At 120 DOS, the emergence response of lettuce seedlings was quadratic for all essential oils (Figure 4-C). In addition, at 180 DOS the reduction was linear for treatments with eucalyptus and tea tree oils (Figure 4-D).

Table 3. Lettuce seedling emergence (%) and lettuce emergence speed index (ESI) submitted to the application of essential oils at different concentrations at post-harvest, 60, 120 and 180 days of storage

There was a reduction in ESI with increasing concentrations of essential oils, except for the treatment with tea tree essential oil after harvest. In the post-harvest treatment with clove, rosemary and ginger essential oils this reduction was linear, while in treatments with lemongrass and eucalyptus the response was quadratic

(Figure 4).

According to the analyzes performed to determine the physiological attribute of lettuce seeds (germination test, seedling emergence and ESI), treatments with clove and lemongrass essential oils caused greater losses in seed germination and vigor in relation to seeds treated with ginger and tea tree essential oils. Flávio et al. (2014) found a reduction in the results related to the germination test first count and the germination in sorghum seeds treated with cinnamon essential oil at a concentration of 10%.

The main fungi observed in lettuce seeds were *Cladosporium* sp. and *Alternaria* sp. In treatments with eucalyptus, tea tree essential oils (up to 1.000µL L⁻¹) and ginger there was a higher incidence of *Cladosporium* sp. compared to clove, lemongrass and rosemary essential oil treatments. From 60 DOS, the presence of *Cladosporium* sp. was not observed in lettuce seeds treated with lemongrass essential oil at a concentration of 1.000µL L⁻¹, and in the treatment with clove essential oil (1.500 and 2.000µL L⁻¹) (Table 4).

Gomes et al. (2016) found a reduction in the incidence of *Cladosporium* sp. from the concentration of 1.53 mL L⁻¹ of eucalyptus essential oil.

Table 3. Lettuce seedling emergence (%) and lettuce emergence speed index (ESI) submitted to the application of essential oils at different concentrations at post-harvest, 60, 120 and 180 days of storage.

Essential oil	Seedling emergence (%)					ESI				
	Post-harvest					Post-harvest				
	Concentration ($\mu\text{L L}^{-1}$)					Concentration ($\mu\text{L L}^{-1}$)				
	0	500	1.000	1.500	2.000	0	500	1.000	1.500	2.000
Clove	77.0 ^{ns}	50.0 bc*	44.0 c	27.0 c	22.0 c	14.0 ^{ns}	9.0 bc	8.0 c	4.9 c	4.0 c
Lemongrass	77.0	44.0 c	14.0 d	3.0 d	4.0 d	14.0	7.9 c	2.5 d	0.5 d	0.7 d
Rosemary	77.0	61.0 b	59.0 b	38.0 c	30.0 c	14.0	11.1 b	10.6 b	6.9 c	5.4 c
Eucalyptus	77.0	73.0 a	71.0 a	67.0 a	48.0 b	14.0	13.0 a	13.0 a	13.0 a	8.7 b
Ginger	77.0	64.0 b	61.0 b	59.0 b	56.0 b	14.0	11.5 b	11.0 b	10.6 b	10.2 b
Tea tree	77.0	81.0 a	77.0 a	75.0 a	71.0 a	14.0	14.6 a	14.0 a	13.5 a	12.9 a
60 DOS										
Clove	70.0 ^{ns}	15.0 c	9.0 d	3.0 c	3.0 b	12.7 ^{ns}	2.6 c	1.6 d	0.5 c	0.5 b
Lemongrass	70.0	9.0 c	4.0 d	2.0 c	1.0 b	12.7	1.5 c	0.7 d	0.4 c	0.1 b
Rosemary	70.0	53.0 b	57.0 ab	43.0 ab	31.0 a	12.7	9.5 b	10.3 ab	7.8 ab	5.6 a
Eucalyptus	70.0	49.0 b	45.0 bc	14.0 c	6.0 b	12.7	8.8 b	8.1 bc	2.5 c	1.1 b
Ginger	70.0	64.0 ab	64.0 a	57.0 a	34.0 a	12.7	11.6 ab	11.6 a	10.3 a	6.1 a
Tea tree	70.0	76.0 a	37.0 c	32.0 b	19.0 b	12.7	13.7 a	6.7 c	5.8 b	3.4 ab
120 DOS										
Clove	66.0 ^{ns}	8.0 e	5.0 d	5.0 d	2.0 d	12.0 ^{ns}	1.4 e	0.8 d	0.8 d	0.4 d
Lemongrass	66.0	1.0 f	1.0 d	1.0 d	1.0 d	12.0	0.2 f	0.2 d	0.1 d	0.1 d
Rosemary	66.0	20.0 d	15.0 c	6.0 d	5.0 cd	12.0	3.5 d	2.7 c	1.1 d	0.8 cd
Eucalyptus	66.0	35.0 c	29.0 b	14.0 c	10.0 c	12.0	6.3 c	5.2 b	2.5 c	1.7 c
Ginger	66.0	64.0 a	64.0 a	53.0 a	33.0 a	12.0	11.5 a	11.6 a	9.6 a	6.0 a
Tea tree	66.0	55.0 b	31.0 b	29.0 b	17.0 b	12.0	9.9 b	5.6 b	5.2 b	3.0 b
180 DOS										
Clove	50.0 ^{ns}	7.0 cd	5.0 cd	2.0 d	1.0 c	9.0 ^{ns}	1.3 cd	0.9 cd	0.4 d	0.1 c
Lemongrass	50.0	1.0 d	0.0 d	0.0 d	0.0 c	9.0	0.2 d	0.0 cd	0.0 d	0.0 c
Rosemary	50.0	14.0 c	14.0 c	13.0 c	5.0 c	9.0	2.5 c	2.5 c	2.3 c	0.9 c
Eucalyptus	50.0	28.0 b	27.0 b	15.0 c	8.0 bc	9.0	5.1 b	4.9 b	2.7 c	1.4 bc
Ginger	50.0	44.0 a	44.0 a	43.0 a	31.0 a	9.0	8.6 a	8.5 a	8.5 a	5.6 a
Tea tree	50.0	50.0 a	31.0 b	28.0 b	16.0 b	9.0	8.8 a	5.6 b	5.0 b	2.9 b

*Means followed by the same letter in the column, within each storage period, do not differ statistically by Tukey test at 5% significance; ns = not significant.

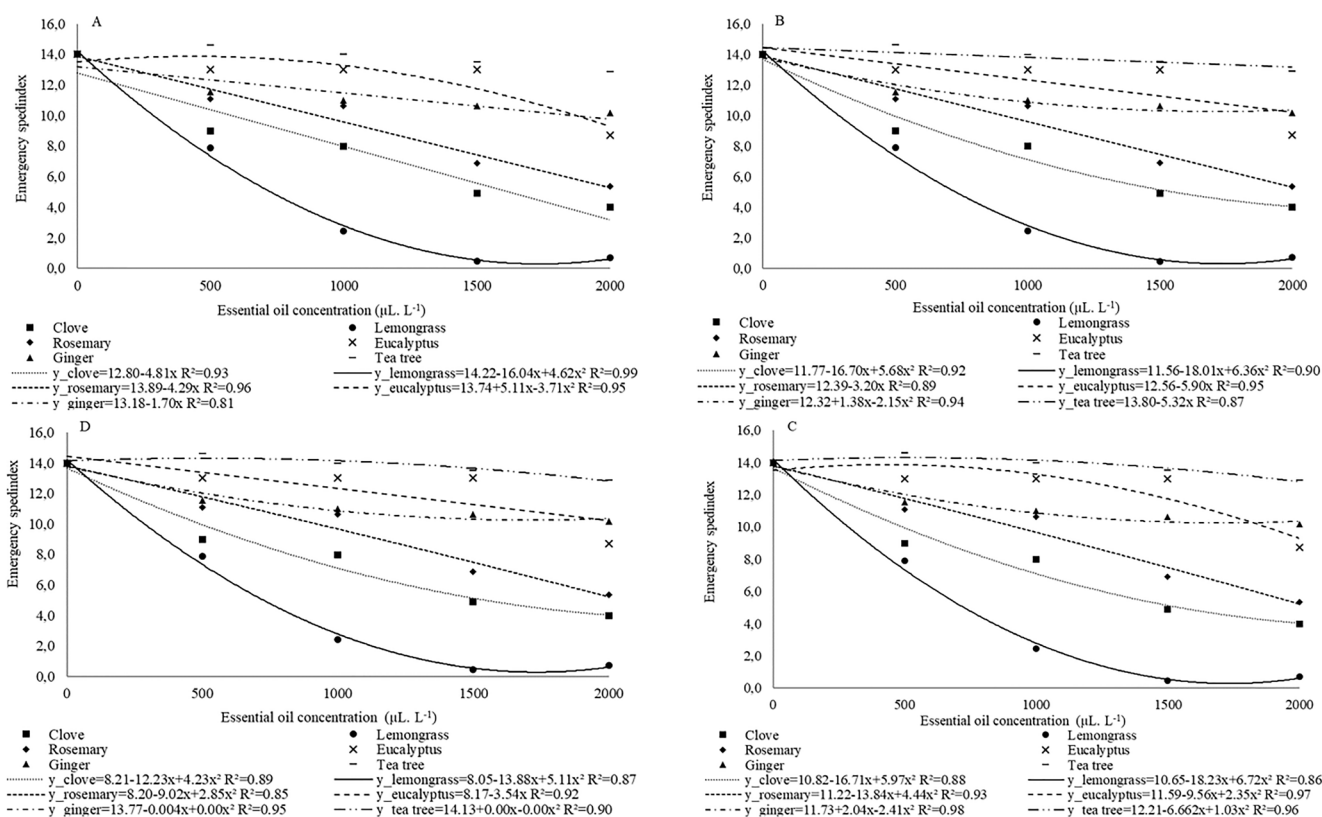


Figure 4. Regression analysis of lettuce emergence speed index (ESI, %) submitted to the application of essential oils in different concentrations at post-harvest (A), 60 (B), 120 (C) and 180 (D) days of storage.

Table 4. Incidence of *Cladosporium* sp. (%) in lettuce seeds submitted to the application of essential oils in different concentrations at post-harvest, 60, 120 and 180 days of storage.

Essential oil	<i>Cladosporium</i> sp. (%) - Post-harvest					
	Concentration (μL^{-1})					
	0	500	1.000	1.500	2.000	
Clove	100.0 ^{ns}	14.0 c*	10.0 c	5.5 d	5.0 d	d
Lemongrass	100.0	2.0 c	2.0 c	8.5 d	2.5 d	d
Rosemary	100.0	67.0 b	65.3 b	61.5 b	34.5 c	c
Eucalyptus	100.0	90.0 a	86.5 a	70.8 b	70.0 b	b
Ginger	100.0	99.5 a	99.0 a	98.5 a	88.5 a	a
Tea tree	100.0	100.0 a	89.5 a	36.5 c	35.0 c	c
60 DOS						
Clove	97.8 ^{ns}	13.0 c	6.8 c	0.0 e	0.0 c	c
Lemongrass	97.8	0.0 d	2.0 c	4.5 e	1.5 c	c
Rosemary	97.8	89.4 b	85.3 b	80.0 c	33.0 b	b
Eucalyptus	97.8	91.5 b	91.3 b	91.3 b	88.3 a	a
Ginger	97.8	99.3 a	98.0 a	98.0 a	88.5 a	a
Tea tree	97.8	93.0 b	90.0 b	36.0 d	34.8 b	b
120 DOS						
Clove	96.9 ^{ns}	13.0 c	7.0 c	0.0 c	0.0 e	e
Lemongrass	96.9	0.00 d	1.50 c	2.0 c	1.5 e	e
Rosemary	96.9	89.0 a	87.5 a	92.0 a	38.8 c	c
Eucalyptus	96.9	93.8 a	94.0 a	90.5 a	88.5 a	a
Ginger	96.9	94.0 a	94.3 a	91.5 a	74.8 b	b
Tea tree	96.9	76.0 b	76.0 b	28.5 b	23.0 d	d
180 DOS						
Clove	95.8 ^{ns}	12.5 c	7.5 b	0.0 d	0.0 d	d
Lemongrass	95.8	0.0 d	1.0 c	7.0 c	1.5 d	d
Rosemary	95.8	96.0 a	94.5 a	92.5 a	44.0 b	b
Eucalyptus	95.8	95.3 ab	95.5 a	95.0 a	94.5 a	a
Ginger	95.8	93.5 ab	95.0 a	90.5 a	74.5 a	a
Tea tree	95.8	90.5 b	92.5 a	28.0 b	16.5 c	c

*Means followed by the same letter in the column, within each storage period, do not differ statistically by Tukey test at 5% significance; ns = not significant.

There was a linear reduction in the incidence of *Cladosporium* sp. due to the increase of clove, rosemary and tea tree oils concentrations in post-harvest (Figure 5).

For the incidence of *Alternaria* sp. in lettuce seeds there was interaction of oil and concentration factors up to 120 DOS. Post-harvest incidence of *Alternaria* sp. was lower in seeds with lemongrass essential oil treatment (500 μL^{-1}) in relation to the other essential oils. At the concentrations of 1.000, 1.500 and 2.000 μL^{-1} of clove and lemongrass essential oils, the results were similar. Until 60 DOS, the essential oils of ginger, tea tree and eucalyptus were not efficient for the control of *Alternaria* sp. on lettuce seeds (Table 5).

There was no interaction between essential oils and different concentrations at 180 DOS. However, it can be observed that the results related to clove and lemongrass essential oils had a statistically significant reduction in the incidence of *Alternaria* sp. in relation to other treatments (Table 5).

This result was similar to that obtained by Pereira et al. (2016) who verified that the essential oil of *Eucalyptus globulus* was not efficient to control the pathogens present in seeds of *Schinus molle* L. (aroeira).

Table 5. Incidence of *Alternaria* sp. (%) in lettuce seeds submitted to the application of essential oils in different concentrations at post-harvest, 60 and 120 days of storage

The use of ginger (post-harvest and 60 DOS) and eucalyptus (post-harvest) essential oils were not efficient in reducing *Alternaria* sp. in relation to the control treatment (Figure 6 A and B). At 180 days of storage, even though the interaction between the factors did not show significant variation, there was a linear reduction in the incidence of *Alternaria* sp. The use of essential oils compared to the control result was valid, according to the proposed model, with more marked reductions to initial concentrations, tending to stabilize from the higher concentrations (Figure 6 D).

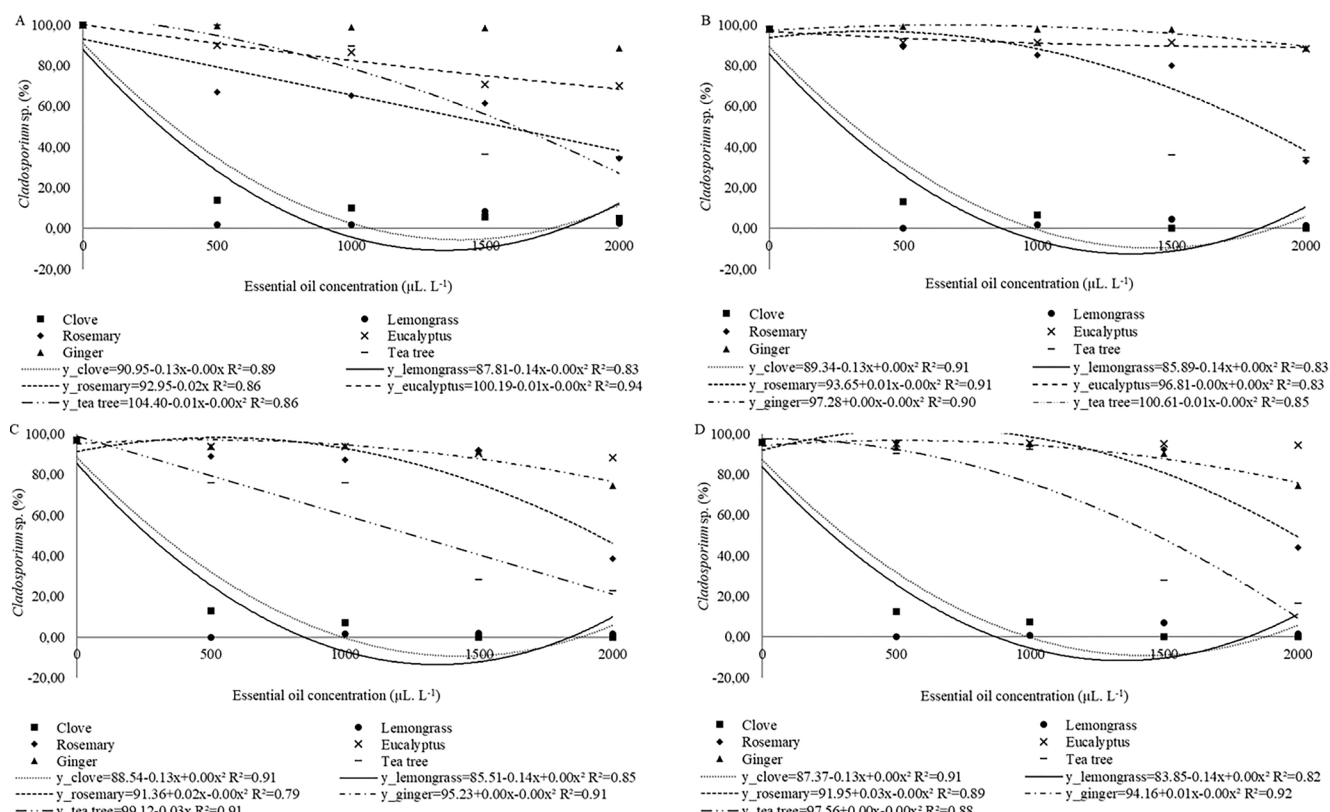


Figure 5. Regression analysis of the incidence of *Cladosporium* sp. (%) in lettuce seeds submitted to the application of essential oils in different concentrations at post-harvest (A), 60 (B), 120 (C) and 180 (D) days of storage.

Table 5. Incidence of *Alternaria* sp. (%) in lettuce seeds submitted to the application of essential oils in different concentrations at post-harvest, 60 and 120 days of storage.

Essential oil	<i>Alternaria</i> sp. (%) - Post-harvest				
	Concentration ($\mu\text{L L}^{-1}$)				
	0	500	1.000	1.500	2.000
Clove	100.0 ^{ns}	30.0 b*	7.0 c	6.0 d	6.5 c
Lemongrass	100.0	15.0 c	14.5 c	8.5 d	10.0 c
Rosemary	100.0	92.5 a	70.8 b	68.5 b	48.5 b
Eucalyptus	100.0	100.0 a	98.0 a	93.0 a	90.5 a
Ginger	100.0	100.0 a	100.0 a	100.0 a	100.0 a
Tea tree	100.0	100.0 a	97.5 a	45.5 c	44.0 b
60 DOS					
Clove	100.0 ^{ns}	17.5 c	8.3 c	4.5 d	6.6 d
Lemongrass	100.0	6.0 c	6.0 c	5.5 d	2.3 d
Rosemary	100.0	91.5 b	71.8 b	68.3 b	48.5 c
Eucalyptus	100.0	98.5 a	98.0 a	98.0 a	87.5 b
Ginger	100.0	100.0 a	100.0 a	99.5 a	99.5 a
Tea tree	100.0	100.0 a	96.3 a	49.0 c	43.8 c
120 DOS					
Clove	29.3 ^{ns}	9.5 bc	9.0 abc	0.0 c	1.5 b
Lemongrass	29.3	4.5 c	4.5 c	4.0 bc	1.0 b
Rosemary	29.3	14.8 ab	14.0 ab	14.0 a	11.0 a
Eucalyptus	29.3	18.3 a	16.0 a	15.0 a	12.5 a
Ginger	29.3	16.0 ab	16.0 a	9.5 ab	9.3 a
Tea tree	29.3	7.0 c	7.0 bc	10.5 ab	11.0 a
<i>Alternaria</i> sp. (%)					
Clove		5.40			c*
Lemongrass		3.18			c
Rosemary		11.50			a
Eucalyptus		9.70			ab
Ginger		9.45			ab
Tea tree		8.20			b

*Means followed by the same letter in the column, within each storage period, do not differ statistically by Tukey test at 5% significance; ns = not significant.

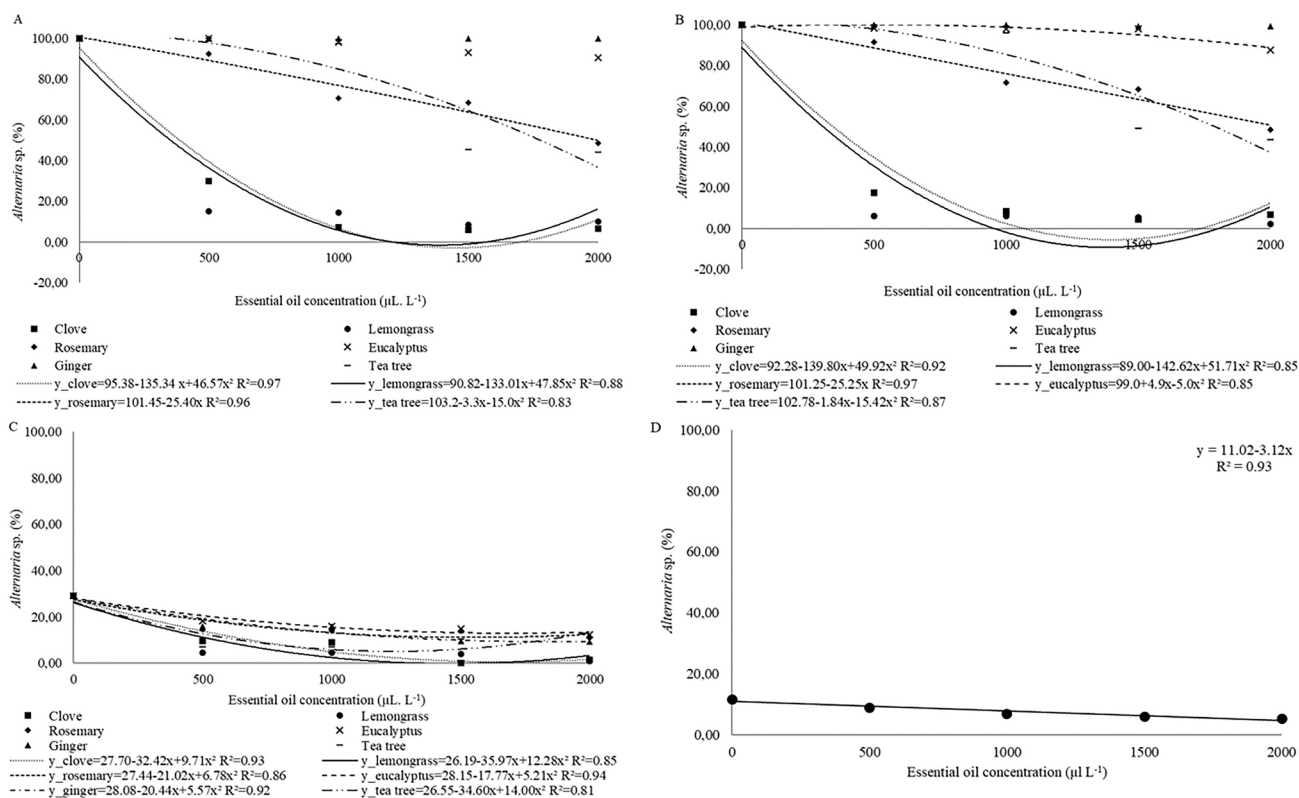


Figure 6. Incidence of *Alternaria* sp. (%) in lettuce seeds submitted to the application of essential oils in different concentrations at post-harvest (A), 60 (B), 120 (C) and 180 (D) days of storage.

Lemongrass and clove essential oils were the most efficient for reducing the incidence of *Alternaria* sp., although they were also the ones that caused a statistically significant reduction in lettuce seed germination and vigor. According to Miranda et al. (2015) the effects of an essential oil on seeds and seedlings is often attributed to its chemical composition, which affects seed germination and plant growth by causing morphological and physiological changes in plants.

Conclusions

The application in lettuce seeds of essential oils of clove, lemongrass, eucalyptus, ginger, rosemary and tea tree caused a reduction in lettuce seed germination and vigor. Application in lettuce seeds of clove and lemongrass essential oils at concentrations above 1.000μL L⁻¹ and rosemary and tea tree at concentrations above 1.500μL L⁻¹ are efficient for reducing the incidence of *Alternaria* spp. It is not technically feasible to apply clove, lemongrass, eucalyptus, ginger and tea tree essential oils post-harvest and to store lettuce seeds, due to the reduction of the physiological parameter with concentrations above 500 μL L⁻¹.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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