Special Supplement: Pulses

Physiological and sanitary quality of lima bean seeds treated with essential oils[1]

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ABSTRACT

Pathogens in lima bean seeds grown by family farmers in the Northeast region of Brazil cause great losses and can make planting areas unviable, making it essential to search for control alternatives that do not harm the environment. This study aimed to evaluate the efficiency of lemongrass, citronella, fennel, eucalyptus and basil essential oils in controlling pathogens and the seed physiological quality of traditional lima bean varieties. Two hundred seeds of the Caramelo, Raio de Sol Amarela and Raio de Sol Vermelha varieties were used per treatment. The seed physiological quality was determined based on germination, emergence and vigor tests. The following fungal genera were identified: *Aspergillus*, *Penicillium* and *Fusarium*. The essential oils showed antifungal effects on the pathogens, in addition to increasing the germination, emergence, shoot and root length and dry mass, as well as the emergence speed index, without interfering with seed vigor.

KEYWORDS: *Phaseolus lunatus* (L.), *Aspergillus*, *Penicillium*, *Fusarium*, seed health.

RESUMO

Qualidade fisiológica e sanitária de sementes de feijão-fava tratadas com óleos essenciais

Patógenos em sementes de feijão-fava cultivado por agricultores familiares no Nordeste do Brasil causam grandes perdas e podem inviabilizar áreas de plantio, sendo fundamental a busca por alternativas de controle que não prejudiquem o meio ambiente. Objetivou-se avaliar a eficiência de óleos essenciais de capim-limão, citronela, erva-doce, eucalipto e manjericão no controle de patógenos e na qualidade fisiológica de sementes de variedades tradicionais de feijão-fava. Foram utilizadas 200 sementes por tratamento das variedades Caramelo, Raio de Sol Amarela e Raio de Sol Vermelha. A qualidade fisiológica das sementes foi determinada com base em testes de germinação, emergência e vigor. Os seguintes gêneros de fungos foram identificados: *Aspergillus*, *Penicillium* e *Fusarium*. Os óleos essenciais apresentaram ação antifúngica sobre os patógenos, além de incrementar a germinação, emergência, comprimento e massa seca da raiz e parte aérea, bem como o índice de velocidade de emergência, sem interferir no vigor das sementes.

PALAVRAS-CHAVE: *Phaseolus lunatus* (L.), *Aspergillus*, *Penicillium*, *Fusarium*, sanidade de sementes.

INTRODUCTION

Lima bean (*Phaseolus lunatus* L.) is a legume belonging to the Fabaceae family, which presents a high genetic diversity, hardiness and adaptability to the semiarid climate. Currently, it is considered the second most economically important species of the Phaseolus genus, surpassed only by the common bean (Jacinto Júnior et al. 2019, Soares et al. 2021). This crop is considered an alternative food source that contains essential compounds such as proteins and fibers that are fundamental for human nutrition (Soares et al. 2021, Lopes et al. 2024).

Traditional seeds are preserved and stored by family farmers, who are responsible for producing a wide variety of crops. However, diseases are among the obstacles to the production of traditional seeds, especially those transmitted by seeds, which lead to losses in plant production and productivity (Jacinto Júnior et al. 2019, Barreto et al. 2021). Thus, seed treatment ensures better results in controlling the spread of plant diseases (Mauri et al. 2019).

Pathogen control in seeds is carried out mainly through the use of synthetic fungicides, but these fungicides have undesirable effects, such as contamination of the environment, humans and

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animals. Thus, the use of essential oils has emerged as an alternative management method for the control of fungi in seeds, because the secondary metabolites present on them can have antimicrobial and antioxidant properties (Cutrim et al. 2019).

Essential oils do not affect the metabolism of seeds and improve their physiological quality and vigor, providing natural protection against attacks by microorganisms (Leite et al. 2018). The *Eucalyptus globulus* essential oil, for example, has fungicidal effects on some species such as *Portulaca oleracea*, *Lolium multiflorum*, *Echinochloa crusgalli* and *Nicotiana* (Ibáñez & Blázquez 2019).

Thus, the present study aimed to evaluate the efficiency of lemongrass, citronella, fennel, eucalyptus and basil essential oils in controlling pathogens and the seed physiological quality of traditional varieties of lima bean.

MATERIAL AND METHODS

The experiments were carried out at the Universidade Federal da Paraíba, in Areia, Paraíba state, Brazil, from April to September 2021.

Broad bean seeds of the Caramelo, Raio de Sol Amarela and Raio de Sol Vermelha varieties were obtained from the rural community of the Valdecy Santiago settlement, located in the city of Cajazeiras (38º53'11"S; 38º33"41"W), Paraíba state, from the 2021 harvest and homogenized and packaged in recyclable plastic bottles (5 ºC, for approximately 45 days).

The seeds were subjected to asepsis with sodium hypochlorite (1 %) for 3 min, washed twice in sterilized distilled water, and then dried on paper towels under laboratory conditions (25 ± 2 ºC).

The determination of the seed moisture content was carried out via the oven method at 105 ± 3 ºC, for 24 h. Four samples of 100 g were weighed on an analytical scale with accuracy of 0.0001 g. The average weight was expressed in grams and the moisture content as percentage (Brasil 2009), the latter being calculated as 100 * (P - p)/(P - t), where P is the wet weight, p the dry weight and t the container weight.

The seeds were immersed in the different treatments for 3 min, in a beaker, as follows: control - seeds immersed in sterilized distilled water; Captana fungicide (240 g 100 kg^{-1} of seeds); lemongrass essential oil (*Cymbopogon citratus*); citronella essential oil (*Cymbopogon nardus*); fennel essential oil (*Pimpinella anisum* L.); basil essential oil (*Ocimum basilicum* L.); and eucalyptus essential oil (*Eucalyptus globulus*), at a concentration of 1 %, diluted in sterilized distilled water with the addition of 3 drops of Tween 20 to break the tension of the oils and allow homogenization.

For the health test, the seeds were subjected to the aforementioned treatments. One hundred seeds were used per treatment and divided into 10 replicates of 10 seeds each. The seeds were incubated in 9-mm Petri dishes on a double layer of sterilized filter paper moistened with sterilized distilled water. The dishes were incubated for 7 days at a temperature of 25 ± 2 ºC, with a photoperiod of 12 h. The fungi detection and identification were performed via slides and lactophenol, with the aid of a stereoscopic microscope, and the results compared with descriptions in the literature (Seifert et al. 2011). The obtained results were expressed as percentage of infected seeds.

To evaluate the effects of the essential oils on the physiological quality of seeds, they were subjected to the treatments previously described. For the germination analysis, 100 seeds were used, distributed in four replicates of 25 seeds, placed in a triple layer of Germitest® paper arranged in a roll and moistened with sterilized distilled water with 2.5 times the weight of the dry paper and distributed in a biological oxygen demand (BOD) germination chamber at 25 ± 2 ºC, with a 12-h photoperiod.

From the moment the radicle emerged (observed on the 4th day), the evaluation was carried out until the 9th day of germination. Normal seedlings, abnormal seedlings with primary infection, dead seeds, hard seeds and germinated seeds were counted (Brasil 2009). The germination speed index was calculated together with the germination test, in which the number of germinated seeds was recorded daily and determined according to Maguire (1962).

The shoot and root lengths were assessed from a cut at the base of the seedling neck, with the aid of a ruler graduated in centimeters. To determine the mass, a cross-sectional cut was made in the seedling, using a sterile scalpel. The seedlings' shoots and roots were placed in Kraft paper bags separately and placed in an oven with forced air circulation at 65 ºC, for 48 h, until a constant weight was reached. For the emergence test, 100 seeds per treatment (four replicates of 25 seeds) were used, which were sown in trays containing sterilized and moistened sand, in a greenhouse with daily watering. The germinated seeds were evaluated on the 4th and 9th days after sowing (Brasil 2009). The shoots and roots were separated and placed in Kraft paper bags and in an oven with forced air circulation at 65 ºC, for 48 h, until they reached a constant weight. After this period, the seedlings' dry mass was weighed using an analytical scale.

The chemical compositions of the essential oils of citronella, lemongrass, fennel, eucalyptus and basil were determined using gas chromatography-mass spectrometry. The analysis was performed with a Shimadzu GC-210 chromatograph equipped with a QP2010 Plus mass selective detector. The equipment operation was calibrated with a fused silica capillary column RTX-5MS (30 m × 0.25 mm × 0.25 µm film thickness), with a column temperature of 60-240 ºC (3 ºC min^{-1}); injector temperature of 220 ºC; carrier gas helium; and splitless injection with an injected volume of 1 µL of a 1:1,000 hexane solution.

For the mass spectrometer, the impact energy was 70 volts, and the ion source and interface temperature were 200 ºC. A homologous series of n-alkanes (C_9H_{20}.......$C_{26}H_{54}$) was injected under the same conditions as those used for the samples. The obtained spectra were compared with the Nist and Wiley library database 229, and the retention index calculated for each constituent was compared with that tabulated according to Adams (2007).

The seeds were subjected to health, emergence and germination tests, in a completely randomized experimental design, with treatments arranged in a 7 × 3 factorial scheme, being seven treatments and three lima bean cultivars.

The obtained data were subjected to analysis of variance (Anova) via the F test, and the means compared by the Tukey test ($p \leq 0.05$) using the R® statistical software (R Core Team 2020). To analyze the incidence of fungi, the data were previously transformed to $\sqrt{x} + 1$.

RESULTS AND DISCUSSION

The average values for seed moisture content were 9.61 % for Caramelo, 9.33 % for Raio de Sol Amarela and 9.76 % for Raio de Sol Vermelha. Five fungal genera associated with lima bean seeds, such as *Aspergillus* sp., *Fusarium* sp., *Cladosporiuim* sp., *Rhizopus* sp. and *Penicillium* sp., were detected and identified. Only the *Fusarium* sp. and *Aspergillus* sp. genera present in the lima bean seeds significantly interacted with the essential oils (Table 1).

A reduction in the percentage of *Aspergillus* sp. was observed when the Raio de Sol Amarela and the Raio de Sol Vermelha varieties were treated with citronella and fennel essential oils (Table 1). The *Fusarium* genus was significantly different between the cultivars and treatments ($p < 0.01$) for the Raio de Sol Amarela seeds, which were treated with citronella and eucalyptus essential oils, resulting in lower incidence values.

Khalid et al. (2015) reported that the basil essential oil more strongly inhibited the fungi present in lima bean seeds, what may be related to major compounds such as estragole, which has a fungitoxic action. In studies with basil essential oil, Özean & Erkmen (2001) reported the inhibition of the *in vitro* growth of *Aspergillus niger*, *A. ochraceus* and *Fusarium culmorum*, with the main major compound being estragole.

Fusarium sp., the causal agent of Fusarium wilt, infects plants at any stage of development

Table 1. Incidence of fungi in seeds of traditional lima bean cultivars treated with 1 % essential oils.

Treatments	CCA	CSA	CSV	CCA	CSA	CSV
	Aspergillus sp. (%)			*Fusarium* sp. (%)		
Control	52.0 aA*	19.0 aB	19.0 abB	2.0 aB	9.0 aA	0.0 aB
Citronella	5.0 cdB	14.0 abA	7.0 deB	0.0 aB	9.0 aA	0.0 aB
Lemongrass	0.0 dB	2.0 dB	8.0 cdeA	0.0 aA	1.0 bcA	0.0 aA
Eucalyptus	9.0 bcB	20.0 aA	11.0 bcdB	0.0 aB	12.0 aA	0.0 aB
Fennel	11.0 bcAB	6.0 cdB	15.0 abcA	3.0 aA	4.0 bA	0.0 aB
Basil	13.0 bB	10.0 bcB	23.0 aA	1.0 aA	2.0 bcA	0.0 aA
Captana	5.0 cdA	9.0 bcdA	4.0 eA	0.0 aA	0.0 cA	0.0 aA
CV (%)		14.41			9.73	

* Means followed by the same lowercase letter in the column and uppercase letter in the row are statistically equal according to the Tukey test ($p \leq 0.05$). CCA: Caramelo; CSA: Raio de Sol Amarela; CSV: Raio de Sol Vermelha. Control: sterilized distilled water; Captana fungicide: 240 g 100 kg^{-1} of seeds. Data previously transformed into $\sqrt{x} + 1$.

(Zhang et al. 2021). Symptoms in seedlings include yellowing, wilting of cotyledons, and subsequent death. In plants at the final development stage, stunting, tissue degradation and plant death occur (Davis et al. 2006).

Fungi of the *Aspergillus* genus are common in seeds, and the most frequent symptoms may be related to deterioration, resulting in the consumption of reserve tissues (Sousa et al. 2021). The results obtained in this study show positive effects, when compared with those of Farias et al. (2020), who, regardless of the concentration of rosemary essential oil (*Rosmarinus officinalis*), reported a reduction in the incidence of *Aspergillus*. Sales et al. (2018) reported that the incidence of *Aspergillus* sp., when the percentage of *Aspergillus* sp. is high, tends to be detrimental to the physiological quality of the seeds, reducing vigor and viability and causing pre and post emergence rot.

Farias et al. (2020) reported the antifungal potential of rosemary essential oil at a concentration of 1 % on broad bean cultivars, with a reduction in the development of *Cladosporium* sp., *Penicillium* sp. and *A. niger*. The substances present in the essential oils in this study, such as thymol (2-isopropyl-5-methylphenol), are monotremes that inhibit the growth of several microorganisms (Marchese et al. 2016). However, it is necessary to study the effects that influence the production of these secondary metabolites in plants and their interactions with biochemical processes (Braga et al. 2020).

In terms of germination percentage, first germination count, germination speed index, percentage of abnormal seedlings, number of infected seedlings and number of dead seeds, no significant differences were detected among the bean cultivars (Table 2).

Nascimento et al. (2020), when evaluating the effects of candeia (*Eremanthus erythropappus*) and palmarosa (*Cymbopogon martini*) essential oils on common bean (*Phaseolus vulgaris*) and corn (*Zea mays* L.) seeds, reported that there was no change in the germination potential of the seeds, similarly to the Caramelo variety, for which the same results were obtained. The effects of essential oils on germination may be related to their chemical composition, as noted by Anjos et al. (2022). Gomes et al. (2016) reported that copaiba and basil essential oils did not compromise the quality of lima bean and reduced the incidence of fungi.

For the Raio de Sol Vermelha variety, a higher percentage of germination speed index (18.43 %) was

Table 2. Average values for germination (G), first germination count (FGC), germination speed index (GSI) and dead seeds (DS) of traditional lima bean cultivars treated with 1 % essential oils.

Treatments	CCA	CSA	CSV	Mean	CCA	CSA	CSV	Mean
	G (%)				FGC (%)			
Control	100 aA*	100 aA	100 aA	100	100 aA	100 aA	100 aA	100
Citronella	100 aA	99 aA	96 bB	98.33	100 aA	99 aA	96 bB	98.33
Lemongrass	100 aA	100 aA	98 abB	99.33	100 aA	100 aA	98 abB	99.33
Eucalyptus	100 aA	100 aA	100 aA	100	100 aA	100 aA	100 aA	100
Fennel	100 aA	100 aA	100 aA	100	100 aA	100 aA	100 aA	100
Basil	100 aA	100 aA	100 aA	100	100 aA	100 aA	100 aA	100
Captana	100 aA	99 aA	100 aA	99.67	100 aA	99 aA	100 aA	99.67
Mean	100	99.71	99.14		100	99.71	99.14	
CV (%)		1.07				1.07		
Treatments		GSI		Mean		DS (%)		Mean
Control	14.62 bB	14.25 aB	19.75 aA	16.21	0.0 aA	0.0 aA	0.0 bA	0.0
Citronella	13.13 bB	14.23 aAB	16.87 abA	14.74	0.0 aB	1.0 aB	4.0 aA	1.67
Lemongrass	15.71 abA	15.46 aA	14.35 bA	15.17	0.0 aB	0.0 aB	2.0 abA	0.67
Eucalyptus	14.21 bB	14.54 aB	19.33 aA	16.03	0.0 aA	0.0 aA	0.0 bA	0.0
Fennel	13.67 bB	14.52 aB	19.42 aA	15.87	0.0 aA	0.0 aA	0.0 bA	0.0
Basil	18.25 aA	14.86 aB	19.93 aA	17.68	0.0 aA	0.0 aA	0.0 bA	0.0
Captana	12.54 bB	14.81 aB	19.37 aA	15.58	0.0 aA	1.0 aA	0.0 bA	0.33
Mean	14.59	14.67	18.43		0.0	0.29	0.86	
CV (%)		10.46				27.46		

* Averages followed by the same lowercase letter in the same column and uppercase letter in the same row are statistically significant according to the Tukey test ($p \leq 0.05$). CCA: Caramelo; CSA: Raio de Sol Amarela; CSV: Raio de Sol Vermelha. Control: sterilized distilled water; Captana fungicide: 240 g 100 kg^{-1} of seeds.

observed, with the citronella essential oil promoting a lower germination speed index than the other oils for this cultivar. The Caramelo and Raio de Sol Amarela varieties did not differ among the treatments.

With respect to the root length of the seedlings originating from treated seeds, there was a significant interaction between treatments and cultivars, where the Raio de Sol Amarela cultivar presented superior results for both root and shoot length, with averages between 20.39 % (shoot length) and 14.09 % (root length) (Table 3).

When comparing the cultivar varieties, it was observed that the germination traits significantly differed among the individual factors. Raio de Sol Amarela presented the highest averages, with 20.39 cm for root length and 14.09 cm for shoot length. For root dry matter, Raio de Sol Vermelha presented a greater value (2.26 g) than the other varieties, whereas Raio de Sol Amarela showed 2.57 g for shoot dry matter (Table 3).

Seed germination is considered a vital process for the maintenance of species (Mohamed et al. 2022), and, in semiarid regions, under high water stress conditions, to achieve a successful production, it is necessary to use healthy seeds that present high vigor in order to ensure a good germination. The use of essential oils for the treatment of lima bean seeds provided similar results for the Raio de Sol Vermelha and Raio de Sol Amarela cultivars. The shoot length was greater in seedlings from seeds treated with lemongrass essential oil, with an average of 17.33 % for the Raio de Sol Amarela cultivar (Table 3).

The results of the emergence test revealed that the Raio de Sol Vermelha cultivar presented the greatest degree of seedling emergence, at approximately 99.14 % (Table 4). When the first emergence count was evaluated, average values ranging from 77 to 90 % were obtained for cultivars treated with lemongrass or basil oil. For the emergence speed index and shoot length, no significant differences were observed.

The results obtained for seedlings, shoot and root length were significantly different among the treatments with citronella, eucalyptus, fennel and basil essential oils for the studied varieties. The Raio de Sol Amarela and Raio de Sol Vermelha varieties presented average values of 12.30 and 18.41 %, respectively (Table 5).

When the root dry mass content was evaluated, the average weights were 0.827 g for the Caramelo variety and 0.053 g for the Raio de Sol Vermelha variety, which were significantly different (Table 5).

Table 3. Average values for root (RL) and shoot (SL) length, root (RDM) and shoot dry matter (SDM) in the germination test of traditional lima bean cultivars originating from seeds treated with 1 % essential oils.

Treatments	CCA	CSA	CSV	Mean	CCA	CSA	CSV	Mean
		RL (cm)				SL (cm)		
Control	13.96	21.55	12.57	16.03 a*	6.33	14.97	15.00	12.10 a
Citronella	13.62	19.26	12.80	15.23 a	5.76	13.15	14.95	11.28 ab
Lemongrass	15.39	19.68	10.28	15.12 a	5.97	17.33	12.87	12.05 a
Eucalyptus	14.24	21.40	11.39	15.68 a	5.62	12.79	11.13	9.85 ab
Fennel	14.85	22.60	10.88	16.11 a	5.61	12.24	10.78	9.54 b
Basil	14.08	19.16	11.20	14.81 a	3.62	14.25	12.11	9.99 ab
Captana	14.29	19.06	11.94	15.10 a	6.09	13.88	14.56	11.51 ab
Mean	14.35 B	20.39 A	11.58 C		5.57 B	14.09 A	13.06 A	
CV (%)		13.37				17.83		
Treatments		RDM (g)		Mean		SDM (g)		Mean
Control	0.824	0.783	2.342	1.317 ab	2.385	2.518	1.146	2.016 a
Citronella	0.838	0.685	2.689	1.404 a	1.578	2.578	2.108	2.088 a
Lemongrass	0.855	0.405	1.516	0.925 b	2.131	2.592	1.172	1.965 a
Eucalyptus	0.827	0.831	2.650	1.436 a	2.157	2.706	1.324	2.062 a
Fennel	0.842	0.610	1.986	1.146 ab	2.158	2.741	0.882	1.927 a
Basil	0.713	0.632	2.548	1.298 ab	2.219	2.599	1.513	2.110 a
Captana	0.825	0.787	2.129	1.247 ab	2.256	2.315	1.043	1.871 a
Mean	0.818 B	0.676 B	2.266 A		2.126 B	2.579 A	1.313 C	
CV (%)		27.87				25.09		

* Averages followed by the same lowercase letter in the column and uppercase letter in the row are statistically equal according to the Tukey test ($p \leq 0.05$). CCA: Caramelo; CSA: Raio de Sol Amarela; CSV: Raio de Sol Vermelha. Control: sterilized distilled water; Captana fungicide: 240 g 100 kg^{-1} of seeds.

Table 4. Average values for emergence (EM), first emergence count (FEC), emergence speed index (ESI) and root length (RL) of traditional lima bean cultivar seedlings originating from seeds treated with 1 % essential oils.

Treatments	CCA	CSA	CSV	Mean	CCA	CSA	CSV	Mean
		EM (%)				FEC (%)		
Control	65	90	100	85.00 ab*	64	95	100	86.33 a
Citronella	64	100	96	86.67 ab	60	83	96	79.67 a
Lemongrass	53	92	98	81.00 b	47	86	98	77.00 a
Eucalyptus	72	98	100	90.00 ab	59	91	100	83.33 a
Fennel	86	100	100	95.33 a	76	89	100	88.33 a
Basil	59	100	100	86.33 ab	79	91	100	90.00 a
Captana	54	91	100	81.67 b	60	92	100	84.00 a
Mean	64.71 B	95.86 A	99.14 A		63.57 C	89.57 B	99.14 A	
CV (%)		12.43				14.03		
Treatments		ESI		Mean		RL (cm)		Mean
Control	9.91	12.13	19.75	13.93 ab	14.22	21.55	12.57	16.11 a
Citronella	8.20	11.62	16.87	12.23 bc	15.15	19.26	12.80	15.74 a
Lemongrass	7.01	10.76	14.35	10.71 c	20.68	19.68	10.28	16.88 a
Eucalyptus	10.24	11.91	19.33	13.83 ab	14.66	21.35	11.40	15.80 a
Fennel	13.00	12.68	19.42	15.03 a	13.01	22.60	10.88	15.50 a
Basil	9.98	12.97	19.79	14.25 ab	13.61	19.16	11.20	14.65 a
Captana	8.45	14.06	19.37	13.96 ab	11.85	19.06	11.94	14.28 a
Mean	9.54 C	12.30 B	18.41 A		14.74 B	20.38 A	11.58 C	
CV (%)		16.04				21.92		

* Averages followed by the same lowercase letter in the column and uppercase letter in the row are statistically equal according to the Tukey test ($p \leq 0.05$). CCA: Caramelo; CSA: Raio de Sol Amarela; CSV: Raio de Sol Vermelha. Control: sterilized distilled water; Captana fungicide: 240 g 100 kg^{-1} of seeds.

Table 5. Average values for shoot length (SL), root dry matter (RDM) and shoot dry matter (SDM) in the seedling emergence test of traditional lima bean cultivars originating from seeds treated with 1% essential oils.

Treatments	CCA	CSA	CSV	Mean	CCA	CSA	CSV	Mean	CCA	CSA	CSV	Mean
		SL (cm)				RDM (g)				SDM (g)		
Control	8.46 aB*	14.94 abA	15.00 aA	12.80	0.891	0.783	0.030	0.568 a	2.118	2.518	0.343	1.660 a
Citronella	12.61 aA	13.15 abA	14.95 aA	13.57	0.822	0.685	0.050	0.519 a	2.044	2.578	0.416	1.679 a
Lemongrass	11.56 aB	17.33 aA	12.87 aB	13.92	0.910	0.405	0.097	0.471 a	2.555	2.592	0.503	1.883 a
Eucalyptus	10.48 aA	12.79 abA	11.13 aA	11.47	0.786	0.831	0.036	0.551 a	1.756	2.706	0.317	1.593 a
Fennel	11.43 aA	12.24 bA	10.78 aA	11.48	0.967	0.610	0.041	0.539 a	1.955	2.741	0.232	1.643 a
Basil	11.75 aA	14.25 abA	12.11 aA	12.70	0.713	0.632	0.067	0.471 a	2.219	2.599	0.212	1.677 a
Captana	10.69 aA	13.88 abA	14.56 aA	13.04	0.699	0.787	0.047	0.511 a	2.069	2.315	0.252	1.545 a
Mean	10.99	14.08	13.06		0.827 A	0.676 A	0.053 B		2.102 B	2.579 A	0.325 C	
CV (%)		18.30				54.15				21.44		

* Averages followed by the same lowercase letter in the column and uppercase letter in the row are statistically equal according to the Tukey test ($p \leq 0.05$). CCA: Caramelo; CSA: Raio de Sol Amarela; CSV: Raio de Sol Vermelha. Control: sterilized distilled water; Captana fungicide: 240 g 100 kg^{-1} of seeds.

For shoot dry mass, differences were observed among the cultivars, with Raio de Sol Amarela showing a greater weight (2.579 g), and a lower one for the Raio de Sol Vermelha cultivar (0.325 g). Seed quality is correlated with factors such as physical, genetic, physiological and sanitary factors, which influence the quality of the batch, ensuring uniformity in the field, with vigorous plants being free from diseases (Oliveira et al. 2017).

The identification of the major components of the citronella essential oil revealed that it is composed of monoterpenes (92.86 %), dodecanes (4.22 %) and sesquiterpenes (2.93 %), corroborating the findings of previous studies (Flamini et al. 2007); however, the 2.6-octadien-1-ol-3.7-dimethyl component was responsible for 35.73 % of the composition, according to the comparison of the retention indices/mass spectra (Table 6).

Table 6. Gas chromatography analysis of the essential oils from the plant species.

Essential oils	Retention index compounds	Contribution to the composition			
		Calculated	Literature	± Standard deviation	Contribution to composition as % of oil
Citronella (*Cymbopogon winterianus*)	Limonene	10.48	10.38	2.14	10.2
	Linalool	11.42	11.12	1.10	29.6
	Citronella	10.37	11.52	32.21	114.8
	Citronellol	12.34	12.36	9.59	2.1
	Citronellyl acetate	15.38	15.22	1.11	15.7
	Geranyl acetate	1,444	1,438	1.82	5.6
	Caryophyllene	1,538	1,522	1.11	15.7
Lemongrass (*Cymbopogon citratus*)	2,6-Octadienal	12.52	12.43	35.82	8.6
	Trans-Geraniol	12.63	12.68	0.96	5.0
	Citral	12.81	-	56.89	-
	Geranyl acetate	13.88	13.83	2.64	4.7
	Naphthalene	1,538	1,524	1.04	13.9
	Caryophyllene oxide	16.14	16.17	1.62	3.3
	1-Naphthalenol	16.49	16.88	1.03	39.1
Fennel (*Pimpinella anisum* L.)	α-pinene	9.42	9.41	5.41	0.7
	β-pinene	9.90	98.64	0.56	3.4
	Benzene	10.42	1,033	0.46	9.4
	Limonene	10.48	10.39	4.10	9.2
	Eucalyptol	10.54	10.45	0.49	8.6
	Fechone	11.34	10.96	14.16	37.3
	Estragole	12.09	12.01	1.56	7.5
	Benzene; 1-methoxy-4-(1-propenyl)	13.01	12.67	73.25	33.6
Eucalyptus (*Eucalypts* sp.)	α-pinene	9.42	9.41	2.13	1.3
	β-pinene	9.90	9.86	0.43	3.8
	β-Myrcene	10.29	9.99	0.40	29.8
	Benzene	10.43	10.33	5.49	9.8
	Limonene	10.49	1,039	6.67	10.0
	Eucalyptol	10.54	10.45	84.89	9.0
Basil (*Ocimum basilicum* L.)	Linalool	11.41	11.12	19.72	29.2
	Estragole	12.09	12.03	77.34	5.9
	Caryophylene	14.44	14.42	0.46	2.0
	Trans-α-Bergamotene	14.52	14.41	0.87	10.5

The identification of the components of the lemongrass essential oil (Table 6) revealed that it is composed of monoterpenes (96.31 %) and sesquiterpenes (Zhao et al. 2006, Flamini et al. 2007). However, one component was responsible for 56.89 % of the composition, and it was not possible to identify it. Thus, the compounds are identified through their mass spectra, which can be confronted with a spectral library, where the fragments are compared to already cataloged spectra. Failure to identify them may result in nonrecognition of the molecule and its functionality within a given compound (Adams 2007). As an inhibitor of the growth of bacteria and fungi, the lemongrass essential oil has high contents of geraniol and citronella (Braga et al. 2020), with classes of terpenes, monoterpenes and sesquiterpenes predominating (Ribeiro et al. 2018).

The fennel essential oil is completely composed of monoterpenes (100 %) (Choi 2003, Zhao et al. 2006, Zeng et al. 2007, Mallard et al. 2014). However, a component that accounted for 73.25 % of the composition could not be identified by chromatography. Eucalyptus essential oils were reported to contain monoterpenes (100 %) (Choi et al. 2003, Zhao et al. 2006, Flamini et al. 2007, Zeng et al. 2007). The identification of the components of the basil essential oil corresponded to monoterpenes (98.67 %) and sesquiterpenes (1.79 %) (Zhao et al. 2006).

CONCLUSIONS

1. Citronella, eucalyptus, fennel and basil essential oils effectively reduce the incidence of *Aspergillus* sp. and *Fusarium* sp. fungi in lima bean seeds of the Caramelo, Raio de Sol Amarela and Raio de Sol Vermelha cultivars;
2. In addition to their antifungal effects, these essential oils also enhanced the growth of roots and shoots of the studied varieties, indicating that these compounds not only act to protect the seeds against fungi, but also stimulate the initial development of the plants. This dual effect is promising for increasing crop yield.

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