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Essential oils of thyme, lemongrass, and rosemary in the control of *Aspergillus flavus* Link inoculated in broccoli seeds

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Abstract: Essential oils of plant origin have been studied as alternatives for managing seed-associated pathogens in organic production systems, although their effects on seed physiological quality remain insufficiently understood. This study evaluated the influence of essential oils on the physiological and sanitary quality of broccoli seeds inoculated with *Aspergillus flavus* Link. Seeds were artificially inoculated and treated with essential oils of thyme (*Thymus vulgaris* L.), lemongrass (*Cymbopogon citratus* (DC.) Stapf), and rosemary (*Rosmarinus officinalis* L.). The experiment followed a 3 × 5 factorial design, consisting of three oils and five concentrations (0; 2.0; 2.5; 3.0; and 3.5 %), totaling 15 treatments. Seeds were immersed in the solutions under agitation for five minutes and dried at 30 °C for one hour. A completely randomized design with four replications was adopted. Thyme and lemongrass essential oils showed high antifungal efficiency against A. flavus from 2.0 %; however, the highest concentration (3.5 %) caused phytotoxic effects and reduced seed physiological quality. Rosemary essential oil maintained seed vigor and seedling development, demonstrating potential as an alternative for integrated seed treatment in organic production systems.

Key words: *Brassica oleracea* L. ssp *oleracea* convar. *botrytis* (L.) Alef var. *italica* Plenk, essential oil, vigor, germination, alternative pathogen control.

Eterična olja timijana, limonske trave in rožmarina pri nadzoru glive *Aspergillus flavus* Link, cepljene v seme brokolija

Izvleček: Eterična olja rastlinskega izvora so preučevali kot alternativo za obvladovanje patogenov, povezanih s semeni, v sistemih ekološke pridelave, čeprav njihovi učinki na fiziološko kakovost semen ostajajo premalo razumljeni. Ta študija je ocenila vpliv eteričnih olj na fiziološko in sanitarno kakovost semen brokolija, cepljenih z *Aspergillus flavus* Link. Semena so umetno cepili in tretirali z eteričnimi olji timijana (*Thymus vulgaris* L.), limonske trave (*Cymbopogon citratus* (DC.) Stapf) in rožmarina (*Rosmarinus officinalis* L.). Poskus je sledil faktorski zasnovi 3 × 5, sestavljeni iz treh olj in petih koncentracij (0; 2,0; 2,5; 3,0; in 3,5 %), skupaj 15 obravnavanj. Semena so bila potopljena v raztopine ob mešanju pet minut in sušena pri 30 °C eno uro. Sprejeta je bila popolnoma naključna zasnova poskusa s štirimi ponovitvami. Eterična olja timijana in limonske trave sta pokazala veliko protiglivično učinkovitost proti glivi *A. flavus* od 2,0 %; največja koncentracija (3,5 %) pa je povzročila fitotoksične učinke in zmanjšano fiziološko kakovost semena. Eterično olje rožmarina je ohranilo moč semen in razvoj sadik, kar je pokazalo potencial kot alternative za integrirano obdelavo semen v sistemih ekološke pridelave.

Ključne besede: *Brassica oleracea* L. ssp. *oleracea* convar. *botrytis* (L.) Alef var. *italica* Plenk, eterično olje, vigor, kalitev, alternativni nadzor patogenov.

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1 INTRODUCTION

The growth of the world's population has intensified the demand for food, stimulating the expansion of agricultural production. In this context, the intensive use of agricultural inputs has caused significant environmental impacts, driving the search for more sustainable production systems, such as organic agriculture (Meena et al., 2017; Hurtado et al., 2019).

This is based on the rational use of natural resources and the adoption of ecologically based practices. In Brazil, this system is regulated by Law No. 10,831, of December 23, 2003, and Decree No. 6,323, of December 27, 2007, in addition to normative instructions that guide the use of permitted inputs, prioritizing products of natural origin and aiming to reduce risks to human health and the environment (Brazil, 2007).

Among the challenges of this system, the management of pathogens associated with seeds stands out, due to the limited availability of alternative products for treatment. In this context, essential oils have been investigated as a promising alternative, as they are secondary metabolites synthesized by plants and have recognized antimicrobial activity (Swamy et al., 2016; Lima et al., 2016; Silva et al., 2024; Gomes et al., 2026).

The sanitary quality of seeds is fundamental for crop establishment, and the use of alternative products can contribute to the control of associated microorganisms (Mazzafera, 2003; Brito et al., 2010; Nakada-Freitas et al., 2022). Studies demonstrate that essential oils can act as fungicidal agents, inhibiting mycelial growth and conidia germination, in addition to exhibiting antibacterial and insecticidal activity (Reis et al., 2020; Baiotto et al., 2022; Baiotto et al., 2023; Pereira et al., 2018).

Among the fungi associated with seeds, species of the genus *Aspergillus* stand out, which can compromise germination and vigor by colonizing reserve tissues or the embryo, resulting in abnormal seedlings or reduced growth (Tian et al., 2022). Among these species, *Aspergillus flavus* has a high colonization capacity and potential to affect the physiological and sanitary quality of seeds of all plant species.

Broccoli (*Brassica oleracea* L. ssp. *oleracea* convar. *botrytis* (L.) Alef. var. *italica* Plenck) is a vegetable of high economic and nutritional importance, whose proper establishment depends on the use of high-quality seeds. However, studies evaluating the effects of essential oils in seed treatment and their simultaneous influence on physiological and sanitary quality, especially in the control of *Aspergillus flavus* Link. are still scarce.

Given the above, the objective was to evaluate the physiological and sanitary quality of broccoli seeds inoculated with *Aspergillus flavus* and treated with essential oils of thyme (*Thymus vulgaris* L.), lemongrass (*Cymbopogon citratus* (DC.) Stapf) and rosemary (*Rosmarinus officinalis* L.).

2 MATERIALS AND METHODS

The experiment was conducted at the Seed Analysis Laboratory of the Faculty of Agricultural and Technological Sciences (FCAT), of the São Paulo State University (UNESP), Dracena Campus, SP.

Broccoli seeds (*Brassica oleracea* var. *italica*), cultivar Ninja, untreated with fungicides, were used. The seeds were artificially inoculated with the fungus *Aspergillus flavus*. The pathogen inoculum was identified according to the Embrapa fungi identification manual (Assis, 2015) and multiplied in potato-dextrose-agar (PDA) medium supplemented with mannitol (33 g l^{-1}), used as a water restrictor to obtain a water potential of −0.8 MPa, according to the methodology described by Machado et al. (2001). Subsequently, the seeds were arranged in a single layer on the culture medium and kept in contact for one hour to promote infection (Martins et al., 2016). The experiment was conducted in a 3 × 5 factorial design, consisting of three essential oils: thyme (*Thymus vulgaris*), lemongrass (*Cymbopogon citratus*), and rosemary (*Rosmarinus officinalis*), and five concentrations (0; 2.0; 2.5; 3.0; 3.5%), totaling 15 treatments. The inoculated seeds were immersed in solutions containing the essential oils on an orbital shaker (model MA-376) for five minutes and subsequently dried in a forced-air oven for one hour at 30 °C. The experimental design adopted was completely randomized, with four replications.

The chemical composition of the essential oils was obtained from the supplier's website (Laszlo®, 2019), based on chromatographic analysis. For thyme essential oil, the main components were thymol (46.6 %) and p-cymene (38.9 %). In lemongrass essential oil, geranial (44.4 %) and neral (33.5 %) stood out. For rosemary essential oil (QT cineol GT Morocco), the main constituents were 1, 8-cineole (41.2 %) and α-pinene (14.3 %).

The variables evaluated were:

Germination: conducted according to the Brazilian Rules for Seed Analysis (RAS) (Brazil, 2009a), with the percentage of normal seedlings determined on the fifth day.

First germination count: performed in the same germination test, counting the percentage of normal seedlings on the tenth day (Brazil, 2009a).

Seedling length: at the end of the germination test, ten normal seedlings were selected per replicate, and the lengths of the primary root, the shoot, and the total length of the seedlings were determined.

Seedling dry mass: normal seedlings from the germination test were placed in a forced-air oven at 40 °C until they reached a constant mass, and then weighed on an analytical balance. The results were expressed in milligrams per seedling.

Emergence speed index (ESI): sowing was carried out in polyethylene trays with 162 cells, containing substrate for seedling production. Four replicates of 50 seeds per treatment were used. The trays were kept in a greenhouse and the counts of emerged seedlings were carried out daily from the emergence of the first seedling until stabilization. The index was calculated according to the methodology proposed by Maguire (1962).

Seedling emergence: evaluated in the same experiment used to calculate the emergence speed index, determining the percentage of normal seedlings established in the second count (tenth day after sowing), as recommended by the RAS (Brazil, 2009a).

Seed health: determined by the filter paper method (Blotter test), as described in the Manual of Seed Health Analysis (Brazil, 2009b). Eight subsamples of 25 seeds per treatment were evaluated. The plates were incubated for seven days at 20 °C, under a regime of 12 hours of light and 12 hours of darkness. After the incubation period, the fungi were identified using a stereoscopic microscope. The normality of the residuals and the homogeneity of the variances were verified prior to the statistical analysis, and no significant differences were observed in the tests performed ($p > 0.05$).

3 RESULTS AND DISCUSSION

The control treatment showed 100 % contamination of broccoli seeds by *Aspergillus flavus*, demonstrating high pathogen pressure in the absence of treatment with essential oils. The application of the oils promoted a reduction in the incidence of the fungus, with variations associated with the type of essential oil and the concentrations evaluated (Table 1).

The essential oils of thyme and lemongrass showed high efficiency in the sanitary control of the seeds, promoting the absence of *Aspergillus* spp. incidence from a concentration of 2.0 %, an effect maintained up to the highest concentration evaluated (3.5 %). These results show strong antifungal activity and indicate their potential for use in seed treatment in organic production systems.

In contrast, rosemary essential oil showed low efficiency in controlling the pathogen, not promoting a reduction in the incidence of *Aspergillus* spp., since all seeds remained contaminated at all concentrations evaluated. A similar result was observed by Xiang et al. (2020), who reported a relatively weaker inhibitory effect of rosemary essential oil on *Aspergillus* spp., with efficacy only at higher concentrations (50 µl disc^{-1}).

The greater efficiency of thyme and lemongrass essential oils can be attributed to their chemical composition. Thyme oil has thymol as its main constituent, a phenolic monoterpene recognized for its high antifungal activity, capable of destabilizing the fungal cell membrane, increasing plasma membrane permeability and promoting leakage of cellular components (Farsaraei et al., 2017).

Similarly, lemongrass essential oil has geranial and neral (citral) monoterpene aldehydes as its main components, associated with cell membrane disruption and inhibition of metabolic processes essential to fungal growth (Zhang et al., 2022). On the other hand, rosemary essential oil has 1, 8-cineole as its main constituent, a compound that, although possessing antimicrobial activity, generally exhibits less antifungal effect when compared to phenolic compounds, such as thymol (Shahina et al., 2022). This difference in chemical composition may explain the lower efficiency observed in controlling *Aspergillus* spp. under the evaluated conditions.

It has also been observed that thyme essential oil exhibits high antifungal activity, being able to completely inhibit the production of aflatoxins by *Aspergillus flavus* (Kohiyama et al., 2015). Similarly, Nakada-Freitas et al. (2022) verified total control of this fungus in cauliflower seeds treated with thyme essential oil at concentrations below 2 %. Among the 33 substances identified in this oil, thymol, p-cymene, γ-terpyrene, carvacrol, and borneol stand out in greater proportions (Jakiemiu et al., 2010). Thyme is widely recognized as an aromatic and medicinal plant with high antimicrobial activity, with its extracts even being reported to be effective in controlling *Aspergillus* spp. (Yehia & Ali, 2020).

Regarding lemongrass essential oil, Alves et al. (2019) identified neral (34.1 %) and geranial (46.83 %) as the main components, proportions similar to those observed in the present study, confirming its antifungal

Table 1: Seed health in broccoli inoculated with *Aspergillus flavus* following treatment with essential oils of rosemary, lemongrass, and thyme.

Essential oil	*Aspergillus flavus* (%)				
	0,0	2,0	2,5	3,0	3,5
Rosemary	100 a[1]	100 b	100 b	100 b	100 b
Lemongrass	100 a	0 a	0 a	0 a	0 a
Thyme	100 a	0 a	0 a	0 a	0 a

[1]Means followed by the same lowercase letter in the column do not differ from each other using the Tukey test at 5 % probability.

efficacy. Similar results were obtained by El Bayomi et al. (2021), who used this essential oil on beef contaminated with the same fungus as in the study and verified total control at concentrations of 1 and 2 %.

In addition to inhibiting fungal growth, lemongrass essential oil also reduced the production of aflatoxins from *Aspergillus* spp. in corn kernels, being considered a potential natural antifungal for use in the storage of corn and other grains (Xiang et al., 2020).

Hlebová et al. (2021), when evaluating different fungal strains treated with combinations of essential oils, observed that *Aspergillus* spp. was one of the most sensitive species to the treatments. The authors also reported that the combination of thyme and lemongrass oils showed a strong synergistic antifungal effect, suggesting that the association of essential oils can be used to control fungal growth or enhance the action of synthetic antifungals, allowing for a reduction in the doses applied.

Although rosemary essential oil did not show efficiency in controlling the fungus, better performance was observed in the physiological quality of the seeds compared to the other oils evaluated, at all concentrations tested (Table 2). Similar behavior was observed for vigor, assessed by the first germination count, including at the highest concentration. On the other hand, lemongrass essential oil showed the lowest values in the germination and first germination count tests. However, this trend was not observed in the emergence test, in which there were no significant differences between the oils at the respective concentrations, observed only at the 3.5 % concentration, with higher values for lemongrass and thyme standing out. This result may be related to the conditions of the emergence test, in which the substrate was irrigated, allowing for the dilution or removal of possible phytotoxic compounds. In contrast, in the germination test there is no water renewal, which may intensify the effect of these compounds on seedling development.

Considering the efficient control of *Aspergillus* spp. at the lowest concentration evaluated (2.0 %) with the use of lemongrass essential oil (Table 1), even with a reduction in seedling performance in a controlled environment, its potential for use in the seedling production process stands out (Table 2). For thyme essential oil, no differences were observed in relation to rosemary oil at concentrations of 2.0 and 3.0 % for both the germination test and the first germination count.

Figure 1 shows the results of germination, first germination count, and emergence of broccoli seeds inoculated with *Aspergillus flavus* and treated with rosemary essential oil. It can be observed that the treatment maintained high physiological performance of the seeds, regardless of the concentration applied, with high germination values and first germination count. These results indicate the absence of significant phytotoxic effects on embryonic tissues, preserving the metabolic processes associated with imbibition and initial growth of the embryonic axis.

Similar results were reported by Baiotto et al.

Table 2: Germination, first germination count and emergence of broccoli seeds treated with essential oils at concentrations (%) of rosemary, lemongrass and thyme.

			Germination (%)	
Essential oil	2,0	2,5	3,0	3,5
Rosemary	64 a[1]	73 a	74 a	81 a
Lemongrass	0 b	35 b	12 b	6 c
Thyme	50 a	39 b	66 a	38 b
			First germination count (%)	
Essential oil	2,0	2,5	3,0	3,5
Rosemary	27 a[1]	68 a	52 a	63 a
Lemongrass	0 b	13 b	0 c	0 b
Thyme	29 a	23 b	20 b	15 b
			Emergence (%)	
Essential oil	2,0	2,5	3,0	3,5
Rosemary	56 a	49 a	54 a	38 b
Lemongrass	54 a	42 a	44 a	58 a
Thyme	60 a	49 a	54 a	55 a

Means followed by the same lowercase letter in the column do not differ from each other using the Tukey test at 5 % probability.

(2023), who observed, in soybean seeds, maintenance of germination and vigor in treatments with essential oils of thyme, lemongrass, and rosemary, with performance comparable to the positive control with the fungicide Vitavax-Thiram®. Similarly, Nakada-Freitas et al. (2022) found that rosemary essential oil did not compromise germination and vigor, such as first germination count and emergence speed index, even with increasing concentrations evaluated (0.4; 0.8; 1.6 and 2.0 %).

Similarly, Farias et al. (2020), when evaluating *Phaseolus lunatus* L. seeds, observed that rosemary essential oil did not affect germination, first count, germination speed index, or dry mass accumulation of the aerial part and root of the cultivars 'Raio de Sol', 'Rosinha' and 'Roxinha', even under increasing concentrations of the oil (0.25; 0.5; 0.75 and 1.0 ml l^{-1}).

Despite maintaining high germination and first germination count values, a reduction in emergence values is observed in Figure 1. This difference may be attributed to the evaluation conditions. While germination tests are conducted under controlled laboratory conditions, favoring the expression of the physiological potential of the seeds, emergence tests occur under less controlled conditions, in which factors such as substrate, temperature, and irrigation can influence seedling establishment.

Rosemary essential oil showed the highest values for seedling dry mass (2.6 mg), primary root length (3.4 cm), shoot length (5.5 cm), and total seedling length (8.9 cm), indicating that the treatment did not compromise the physiological vigor of the seeds and favored the initial development of the seedlings compared to lemongrass and thyme oils (Table 3).

Similar results were observed by Nakada-Freitas et al. (2022), who reported that treatments with essential oils did not affect the dry mass or initial growth of the seedlings. The authors also found better performance of rosemary and thyme oils compared to lemongrass in the first germination count, while thyme showed a higher emergence speed index, also highlighting that rosemary essential oil had a low impact on seed vigor, following the same results as the present study.

Similarly, Farias et al. (2020) observed that rosemary essential oil did not compromise seed vigor and even promoted an increase in shoot length (SLL) and root length (RL) in different *Phaseolus lunatus* cultivars. For the 'Rosinha' cultivar, from a concentration of 0.5 ml l^{-1}, the shoot and primary root length values were higher than those observed in the fungicide treatment and in the control.

On the other hand, Alves et al. (2004) demonstrated that compounds present in essential oils may not prevent germination at lower concentrations, but negatively interfere with the initial growth of seedlings, evidencing potential allelopathic effects.

Although lemongrass essential oil did not significantly reduce seedling emergence (Table 2), the values for dry mass (0.6 mg), root length (0.8 cm), shoot length (1.3 cm), and total seedling length (2.0 cm) were significantly lower than those obtained with rosemary and thyme (Table 3). This behavior may be related to the bioactive compounds present in this oil, which may exert allelopathic effects or mild phytotoxicity at certain concentrations.

However, Nakada-Freitas et al. (2022) observed that lower concentrations of lemongrass essential oil (1.6 %) did not affect the physiological quality of the seeds and that the concentration of 1.9 % showed a fungistatic effect against *Aspergillus flavus*, indicating potential for use in seed treatment in organic production systems.

Evaluating the effect of concentrations on seedling growth as a function of different essential oils allows us to understand the physiological response of broccoli seeds in each treatment (Table 4). For rosemary essential oil, superior performance was observed in all variables analyzed, with seedling dry mass values ranging from 2.1 to 2.9 mg, primary root length between 3.1 and 3.6 cm, shoot length between 4.7 and 6.1 cm, and total seedling length between 7.8 and 9.5 cm. These results show physi-

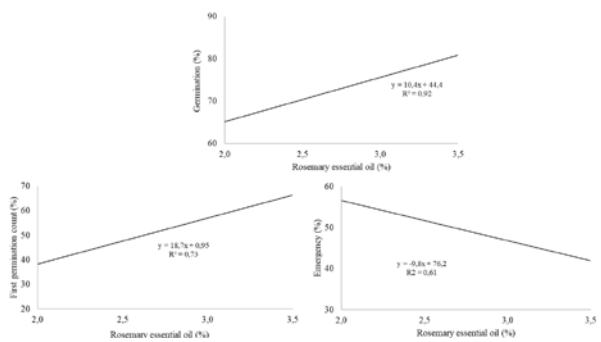


Figure 1: Germination, first germination count, and emergence of broccoli seeds inoculated with Aspergillus flavus and treated with rosemary essential oil.

Table 3: Seedling dry mass (SDM), root length (RL), shoot length (SLL) and seedling length (SPL) of broccoli seeds treated with essential oils of rosemary, lemongrass and thyme.

Essential oil	SDM	RL	SLL	SPL
	(mg)	(cm)	(cm)	(cm)
Rosemary	2.6 a[1]	3.4 a	5.5 a	8.9 a
Lemongrass	0.6 b	0.8 c	1.3 c	2.0 c
Thyme	2.2 a	1.9 b	3.1 b	5.0 b

Means followed by the same lowercase letter in the column do not differ from each other using the Tukey test at 5 % probability.

ological stability at the concentrations evaluated (2.0, 2.5, 3.0, and 3.5 %), indicating the absence of significant phytotoxic effects even with increasing doses. The concentrations of 2.5 and 3.0 % stood out for presenting a better balance between root growth and dry mass accumulation, suggesting greater physiological efficiency under these conditions.

Unlike this result, lemongrass essential oil showed inferior performance, characterized by a marked reduction in root growth (0.0–0.6 cm), especially at higher doses, demonstrating high sensitivity of the root system to the compounds present in the oil. The low values of seedling dry mass (0.0–0.2 mg), shoot length (0.0–0.83 cm), and total seedling length (0.0–1.4 cm) indicate that increasing the concentration intensified the phytotoxic effects. This behavior may be associated with the presence of monoterpenic aldehyde compounds, such as geranial and neral (citral), which have high biological activity and can interfere with physiological processes related to cell division and elongation, directly affecting the initial growth of seedlings.

Thyme essential oil showed intermediate behavior, with seedling dry mass values between 1.9 and 2.5 mg, lower than those observed for rosemary, but higher than those obtained with lemongrass. Root growth varied between 1.8 and 2.0 cm, indicating moderate limitation, but without worsening with increasing concentration. Among the doses evaluated, the 3.0 % concentration showed the best balance between root growth and shoot development. This behavior may be related to the presence of phenolic compounds, such as thymol and carvacrol, known for their high antifungal activity (Farsaraei et al., 2017), but which at certain concentrations can also exert phytotoxic effects on plant tissues (Pinheiro et al., 2015; Zhou et al., 2021).

In general, the response of broccoli seeds varied according to both the essential oil type and concentration, demonstrating that seedling growth is more sensitive to phytotoxic effects than the germination process itself. It has important practical implications for the development of seed treatment protocols in organic production systems, where the use of synthetic fungicides is restricted and the balance between pathogen control and seed physiological quality becomes essential.

Although thyme and lemongrass essential oils completely controlled *Aspergillus flavus* at concentrations

Table 4: Dry mass, primary root length, aerial part length , and total seedling length of broccoli seeds inoculated with *Aspergillus flavus* as a function of rosemary, lemongrass, and thyme essential oils at each concentration.

	Seedling dry mass (mg)			
Essential oil	2.0	2.5	3.0	3.5
Rosemary	2.1 a[1]	2.6 a	2.7 a	2.9 a
Lemongrass	0.0 b	1.5 a	0.7 b	0.2 b
Thyme	2.5 a	2.1 a	1.9 ab	2.4 a
Essential oil	Primary root length (cm)			
	2.0	2.5	3.0	3.5
Rosemary	3.4 a	3.4 a	3.6 a	3.1 a
Lemongrass	0.0 c	1.7 b	0.7 b	0.6 b
Thyme	1.8 b	1.8 a	1.8 b	2.0 a
Essential oil	Aerial part length (cm)			
	2.0	2.5	3.0	3.5
Rosemary	5.7 a	6.1 a	5.4 a	4.7 a
Lemongrass	0.0 c	2.9 b	1.2 b	0.83 b
Thyme	3.0 b	2.6 b	3.5 a	3.0 ab
Essential oil	Seedling length (cm)			
	2.0	2.5	3.0	3.5
Rosemary	9.1 a	9.5 a	9.1 a	7.8 a
Lemongrass	0.0 c	4.7 b	1.9 c	1.4 b
Thyme	4.8 b	4.5 b	5.4 b	5.0 a

Means followed by the same lowercase letter in the column do not differ from each other using the Tukey test at 5 % probability.

from 2.0 %, their phytotoxic effects indicate that sanitary efficiency alone is insufficient to define their practical applicability. In commercial seed treatment programs, excessive reductions in seedling growth may compromise field establishment and stand uniformity, especially under adverse environmental conditions. Therefore, the use of these oils should prioritize concentrations capable of maintaining antifungal activity while minimizing physiological damage.

In contrast, rosemary essential oil preserved seed vigor and seedling development, although without effective fungal control. This result suggests that rosemary oil may be more suitable as a complementary component in integrated seed treatment strategies rather than as an isolated antifungal treatment. In this context, future studies should investigate combinations of rosemary with more active oils, such as thyme or lemongrass, aiming to reduce phytotoxicity while maintaining satisfactory pathogen suppression.

Furthermore, the results highlight the need for additional studies under nursery and field conditions, since responses observed under laboratory conditions may differ during seedling establishment in commercial organic production systems. Evaluations involving storage stability, compatibility with biological treatments, and effects on field emergence are also necessary to support the practical adoption of essential oils in sustainable seed technology.

4 CONCLUSIONS

Rosemary essential oil stood out for preserving seed vigor and initial seedling growth, although it did not show effective antifungal activity against *Aspergillus flavus*.

Thyme and lemongrass essential oils showed high antifungal efficiency against *Aspergillus* spp., promoting complete pathogen control from the concentration of 2.0 %. However, higher concentrations, especially 3.5 %, caused phytotoxic effects and negatively affected seed physiological quality.

Data availability statement:

The data supporting the findings of this study are openly available in Harvard Dataverse (NAKADA-FREITAS, 2026) at https://doi.org/10.7910/DVN/JB8MUZ

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