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Antifungal effect of essential oils on control of phytopathogens in stored soybean seeds[1]

Efeito antifúngico de óleos essenciais no controle de fitopatógenos em sementes de soja armazenadas

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HIGHLIGHTS:

The treatment of soybean seeds with essential oil of Rosmarinus officinalis *showed efficiency in controlling the storage fungus.*
R. officinalis *essential oil does not alter the germination of stored soybean seeds of the cultivar Brasmax Lance®.*
Soybean seeds treated with Vitavax-Thiram® showed a lower incidence of storage fungi than seeds treated with essential oil.

ABSTRACT: Soybean is subject to occurrences of pathogens transmitted by seeds, requiring phytosanitary treatment, however, it can be toxic to humans and the environment. The objective of this study was to evaluate the effect of essential oils of rosemary (*Rosmarinus officinalis*) and eucalyptus (*Corymbia citriodora*) on the control of phytopathogenic fungi in stored soybean seeds. The experiment was carried out in a completely randomized design, using a 2 × 5 factorial arrangement consisted of seeds with and without fungal inoculation (*Aspergillus flavus* and *Penicillium rubens*) and five seed treatments (three essential oils, positive control, and negative control), with four replicates. Rosemary and eucalyptus essential oils and their combination were applied to soybean seeds at the rate of 4 mL kg^{-1}, at a concentration of 700 μL mL^{-1}. Distilled water with 2% Tween® 80 was used as negative control, and the commercial fungicide Vitavax-Thiram® 200SC was used as positive control, according to the manufacturers' recommendations. The *R. officinalis* essential oil was efficient in the control of *Penicillium* sp. in stored soybean seeds and showed a similar effect to the positive control. The *C. citriodora* essential oil reduced the incidence of *Aspergillus* sp. Therefore, these essential oils have potential to be used as an alternative control of soybean seed pathogens.

Key words: *Glycine max* (L.) Merril, alternative control of pathogens, *Corymbia citriodora*, *Rosmarinus officinalis*, seed treatment

RESUMO: A soja está sujeita à ocorrência de patógenos transmitidos por sementes, necessitando de tratamento fitossanitário, porém, este pode ser tóxico ao homem e ao ambiente. Objetivou-se avaliar o efeito dos óleos essenciais de alecrim (*Rosmarinus officinalis*) e eucalipto (*Corymbia citriodora*) no tratamento contra fungos fitopatogênicos em sementes de soja armazenadas. O experimento foi em delineamento inteiramente casualizado em arranjo fatorial 2 × 5, para sementes com e sem inoculação de fungos (*Aspergillus flavus* e *Penicillium rubens*) e cinco tratamentos de sementes (três óleos essenciais, controle positivo e controle negativo), com quatro repetições. Os óleos essenciais de alecrim, eucalipto e a combinação destes foram aplicados na dose de 4 mL kg^{-1} de sementes de soja, na concentração de 700 μL mL^{-1}. Como controle negativo (testemunha) utilizou-se água destilada com Tween® 80 a 2% e como controle positivo o fungicida comercial Vitavax-Thiram® 200SC, conforme dosagem do fabricante. O óleo essencial de *R. officinalis* foi eficiente no controle de *Penicillium* sp. nas sementes de soja armazenadas e apresentou efeito semelhante ao controle positivo. O óleo essencial de *C. citriodora* reduziu a incidência de *Aspergillus* sp. Portanto, estes óleos essenciais têm potencial para serem utilizados no controle alternativo de patógenos de sementes de soja.

Palavras-chave: *Glycine max* (L.) Merril, controle alternativo de patógenos, *Corymbia citriodora*, *Rosmarinus officinalis*, tratamento de sementes

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Introduction

Soybean (*Glycine max* (L.) Merrill) is one of the most prominent commodities in the Brazilian and international markets. The use of high-quality seeds that include technologies for production of seeds with germination potential and vigor is essential to increase soybean yields (CONAB, 2018). In addition, some treatments are needed to reduce the occurrence of phytopathogens after harvest to improve and maintain the good quality of these seeds (Oliveira et al., 2020; Pereira et al., 2021), especially in the storage period, such as pathogens of the *Aspergillus* and *Penicillium* species (Abd-Allah et al., 2018; Seixas et al., 2020).

Seed treatments with systemic and/or contact fungicides are used to control these diseases (Goulart, 2018; Rodrigues et al., 2019). However, they can cause phytotoxicity, harming the seeds, affecting their physiological quality (Seixas et al., 2020) and contaminating water sources and soil (Souza et al., 2015).

Considering this situation, the production of foods without agrochemical residues and with less environmental impact has been sought (Daronco et al., 2015). In this sense, studies have addressed the management of diseases in soybean crops, such as application of essential oils (Kesho et al., 2020), which have shown promising results and what they are.

However, few studies have focused on the application of essential oils, including those of rosemary and eucalyptus, for antifungal treatment of soybean seeds, which is a differential of this study. Therefore, the objective was to evaluate the effect of essential oils of rosemary (*Rosmarinus officinalis*) and eucalyptus (*Corymbia citriodora*) on the control of phytopathogenic fungi in stored soybean seeds.

Material and Methods

The experiment was conducted at the Seed Analysis Laboratory of the Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ), in Ijuí, RS, Brazil, from May to October 2021, using soybean seeds of the cultivar Brasmax Lance®. A completely randomized design was used, in a 2 × 5 factorial arrangement (seeds with and without fungal inoculation × three essential oils + positive control + negative control), with four replicates.

The evaluation of seed infection was carried out before the beginning of the experiment, through a sanity test in a batch of seeds from the field, which showed that the natural level of infection by the storage fungi *Aspergillus* sp. and *Penicillium* sp. was 18 and 2%, respectively; thus, it was proceeded with the artificial fungal inoculation of the seeds (Brasil, 2009b). Moreover, this initial evaluation showed that less than 10% of seeds were contaminated with *Fusarium*, *Rhizopus*, or *Cladosporium* fungi.

A sterile saline solution (0.85%) from young colonies of filamentous fungi grown on potato-dextrose agar medium for 7 to 12 days was prepared for the inoculation with phytopathogenic storage fungi (*Aspergillus flavus* and *Penicillium rubens*) in half of the soybean seed samples. This fungal suspension was adjusted for a turbidity of 0.5 McFarland, corresponding to $1 \times 10^6 – 5 \times 10^6$ CFU mL^{-1}, which was calibrated with the aid of spectrophotometer readings. This suspension was diluted in sterile distilled water until obtaining 5×10^6 CFU L^{-1}; the soybean seeds were inoculated with 1 mL of each fungal strain and shade-dried in an aseptic container for four hours in the laboratory.

The different treatments used in the experiment are shown in Table 1. The method used to apply the seed treatments consisted of manually mixing them with 500 g of soybean seeds of the cultivar Brasmax Lance® in transparent plastic bags and shaking them for 5 min for a better covering and homogenization of the seeds with the treatments. The treatments were applied individually with the addition of the essential oil solutions at the rate of 4 mL kg^{-1} of seed, with the essential oils (*Rosmarinus officinalis* and *Corymbia citriodora*) at concentrations of 700 µL mL^{-1} (concentration of essential oil for each mL of water), and a proportional fraction of both for their combination. The negative control consisted of sterile distilled water at the same rate used for the essential oils, combined with the emulsifying agent Tween® 80 (Polysorbate-80), and the positive control consisted of the synthetic fungicide Vitavax-Thiram® 200SC (a. i. Carboxin 200 g L^{-1} + Tiram 200 g L^{-1}) at the rate of 4 mL kg^{-1} of seed, according to the manufacturer's instructions.

After applying the treatments, the soybean seeds were shade-dried at room temperature in an open container in an aseptic place for four hours and, then, stored in polypropylene bags, identified by treatment, and taken to a room where they were stored for four months under controlled temperature (18 ± 2 °C) and relative air humidity below 70%.

Table 1. Description of the treatments used in the experiment to evaluate fungal incidence in soybean seeds of the Brasmax Lance® cultivar inoculated or not with the fungi *Aspergillus flavus* and *Penicillium rubens*

Treatments	Description	Concentration /Rate	Application volume per kg of soybean seed (mL kg^{-1})
T1	Seeds without inoculation + sterile distilled water with Tween® 80	-	4
T2	Seeds without inoculation + *Rosmarinus officinalis* EO	700 µL mL^{-1}	4
T3	Seeds without inoculation + *Corymbia citriodora* EO	700 µL mL^{-1}	4
T4	Seeds without inoculation + *Rosmarinus officinalis* EO (50%) + *Corymbia citriodora* EO (50%)	700 µL mL^{-1}	4
T5	Seeds without inoculation + Vitavax-Thiram® 200SC + sterile distilled water	2 g mL^{-1}	4
T6	Seeds inoculated with fungi (*Aspergillus flavus* and *Penicillium rubens*)	5 x 10^6 CFU L^{-1}	-
T7	Seeds inoculated with fungi + *Rosmarinus officinalis* EO	700 µL mL^{-1}	4
T8	Seeds inoculated with fungi + *Corymbia citriodora* EO	700 µL mL^{-1}	4
T9	Seeds inoculated with fungi + *Rosmarinus officinalis* EO (50%) + *Corymbia citriodora* EO (50%)	700 µL mL^{-1}	4
T10	Seeds inoculated with fungi + Vitavax-Thiram® 200SC+ sterile distilled water	2 g mL^{-1}	4

EO - Essential oil; CFU - Colony forming units

Analyses of seed germination and moisture content were carried out using samples of soybean seeds from the field, before the inoculation with the fungi and application of the treatments, which were taken to the Laboratory of Seed Analysis of the Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUÍ) to monitor their conditions at the end of the storage period. The seed moisture was measured on the first and last day of storage, using a Gehaka portable moisture meter, for the physical characterization of the seeds.

The physical quality characterization of soybean seeds of the cultivar Brasmax Lance® showed that the seeds presented, immediately, before their use in the tests, 12% moisture content and 90% germination. After the four-month period, the moisture content of the samples decreased to 10%, an average decrease of 0.5 percentage points per month of storage.

After the storage period (120 days), the samples of soybean seeds were subjected to germination, vigor, and pathological analyses in the laboratory. The germination test was carried out using 200 soybean seeds from the field for each treatment, consisting of four replicates of 50 seeds. The germination test was carried out according to the Rules for Seed Analysis (RSA) (Brasil, 2009a): the seeds were incubated in a Germitest® paper roll, moistened with distilled water, using a volume of 3-fold the weight of the dry paper, and incubated in a germination room under temperature of 25 ± 2 °C and photoperiod of eight hours. The germination was evaluated on the seventh day of incubation and consisted of counting normal germinated seedlings (those that presented perfect vegetative structures), abnormal germinated seedlings (those with imperfect vegetative structures), and dead seeds (those that did not germinate).

The seed vigor test, or traditional accelerated aging, was carried out using treated seed samples placed on a screen tray attached to acrylic germination boxes (Gerbox®), containing 40 mL of distilled water at the bottom. The boxes were placed in a BOD chamber for 24 hours at 41 °C for accelerated aging (Ferreira & Borghetti, 2004). Four replicates of 200 seeds were used for each treatment. After this period, the seeds were subjected to the germination test, according to the Rules for Seed Analysis (RSA) (Brasil, 2009a).

The pathology test was carried out using 100 seeds per treatment, consisting of four replicates of 25 seeds. The seeds were equidistantly distributed in Gerbox® boxes (11 × 11 × 3.5 cm) on three sheets of filter paper previously moistened with 15 mL of distilled water. The boxes with seeds were incubated in the chamber at a temperature of 25 ± 2 °C) with a photoperiod of 12 hours (white light with a photon flux of 50-100 W m^{-2}) for seven days. After this period, each seed was individually evaluated with the aid of stereoscopic and optical microscopes, identifying the fungi through morphological characteristics of their structures (Goulart, 2018), according to the Manual for Seed Analysis (Brasil, 2009b). Results were expressed as percentage of contaminated seeds for each fungus.

The data obtained with these analyses were entered into a database and subjected to analysis of variance; the analyses of means of qualitative variables were carried out using plus or minus one standard deviation and coefficient of variation, and compared by the Scott-Knott test at $p \leq 0.05$, using the free software GENES (Quantitative Genetics and Experimental Statistics - version 1990.2019.91). In the absence of normality, the data were transformed into $\sqrt{x + 0.5}$. Therefore, the data referring to the abnormal and dead seeds were not submitted to the test of means and to the analysis of variance because they did not present normal distribution.

Results and Discussion

The result of the analysis of variance (Table 2) indicated no interaction between the sources of variation Inoculation (inoculated and uninoculated seeds) and treatments (I × T) nor for the Inoculation, when evaluating the results of seed germination and vigor. Only the treatments showed significance for the variables at $p \leq 0.05$ by the F test. Therefore, the comparison of means of the different treatments applied to soybean seeds for germination and vigor was carried out (Table 3).

The germination percentages of stored seeds in all treatments were satisfactory (Table 3), and remained above the minimum established by the legislation, being suitable for cultivation. The percentage values of the inoculate seeds were similar to those of the positive control, with low variation between treatments, and statistically lower than those of the negative control.

Table 3 shows the comparisons of germination and vigor percentages of normal soybean seeds subjected to the different treatments. The results showed that the three treatments with essential oils and the positive control (synthetic fungicide) did not differ, statistically, from each other, indicating no phytotoxic effect on germination. However, they presented lower germination percentages than the control (untreated seeds), which may indicate a failure in the coating of the seeds

Table 2. Analysis of variance of results of germination and vigor of soybean seeds with and without inoculation with the fungi *Aspergillus flavus* and *Penicillium rubens* and subjected to different treatments

Source of variation	Degrees of freedom	Mean square	
		Germination	Vigor
Inoculation (I)	1	78.4^{ns}	48.4^{ns}
Treatments (T)	4	119.0*	260.3*
I × T	4	91.4^{ns}	71.1^{ns}
Error	30	43.2	12.02
Overall mean	-	79.0	77.8
Coefficient of variation (%)	-	8.3	8.6

*, ^{ns} - Significant and not significant at $p \leq 0.05$ by the F test, respectively

Table 3. Comparison of means of percentages of normal seedlings, in germination and vigor tests of soybean seeds of the cultivar Brasmax Lance® subjected to different treatments with essential oils and controls

Treatments	Germination	Vigor
	(%)	
Negative control	85.50 a	81.50 a
Positive control*	79.50 b	85.50 a
Rosemary	77.00 b	76.70 b
Eucalyptus	77.00 b	71.50 b
Rosemary + Eucalyptus	76.00 b	73.70 b

Means followed by the same lowercase letter in the columns do not differ by the Scott & Knott test at $p \leq 0.05$; * Positive control with Vitavax-Thiram® 200 SC fungicide by the treatments applied, facilitating the enzymatic activity and fungal incidence, and decreasing the germination of the seeds during the storage.

According to Dorneles et al. (2019), seed treatments should form a film on the seeds, which causes a temporary physical barrier that makes gas exchange and imbibition difficult. In this sense, Nerilo et al. (2020) reported that essential oils remain bioactive as vapors after seed treatment, also enabling protection during storage.

Regarding the physiological quality of stored seed, Santos et al. (2020) reported that germination is a phenomenon affected by environmental conditions, mainly availability of water, and decreases in germination rates are directly related to presence of microorganisms in the seeds; according to the data presented, the resistance of several fungi was adjusted due to these factors.

Costa et al. (2020) found that the higher the incidence of *Aspergillus* sp. in seeds, the lower the germination percentage, which was similar to that found in the present study for seeds treated with the combination of essential oils (rosemary + eucalyptus). Other studies also show that this fungus is necrotrophic and, thus, needs to kill the host cell before infecting it, which leads to a lower seedling development (Shao et al., 2021).

Similar results were found by Daronco et al. (2015) for soybean seeds treated with essential oils of *Baccharis trimera* at 20% and *Eucalyptus globulus* at 30%, which presented lower germination (63 and 68%, respectively) than those treated with synthetic fungicide (Vitavax-Thiram) and those in the negative control, as they presented higher incidences of *Aspergillus* sp. and *Penicillium* sp. Therefore, the effect of an essential oil depends not only on individual effects of its few key constituents, but also on the interaction between their compounds.

Hillen et al. (2012) evaluated soybean seeds treated with fumigation of pure rosemary essential oil for 10 min and found 57% germination, indicating a phytotoxic effect. A higher concentration of the essential oil and another treatment method was used in the present study, which explains the better physiological conditions of the seeds.

Domene et al. (2016) found a decrease in germination of corn seeds treated with *Corymbia citriodora* essential oil. In the present study, the *C. citriodora* essential oil showed similar results to the positive control (synthetic fungicide), but the amount of oil used in the treatment was lower due to the different methodology used. However, seed germination was not directly affected by the essential oils, but by the fungal incidence and long storage period in the present experiment, validating the methodology as adequate for the evaluation of the essential oil, and therefore, this explains the higher percentage of germination in the negative control than in the other treatments.

In addition to fumigation and immersion methods, seeds can also be treated by direct contact with the film-forming active agent. According to some studies, excessive imbibition of seeds in the immersion method can compromise germination by damaging the seed coat. However, a short period of exposure to essential oils can promote the control of phytopathogens without causing significant damages to the seeds (Nascimento et al., 2021). In the present study, seed coating was used through the dispersion of treatments, using smaller amounts of essential oil in contact with them, and, according to the data presented (Table 3), the essential oils cause no damage or phytotoxicity; however, this manual treatment method may result in failures in the full cover of the surface of some seeds, which consequently contributes to incidence of pathogens.

According to Parisi et al. (2019), pathogenic microorganisms can cause the death of seeds before the beginning of germination, during the germination process, or after the establishment of the seedlings through the action of enzymes and toxins produced by these pathogens. This also explains the high rate of abnormal and dead seeds found in the treatments and in the negative control, according to the germination and vigor tests (Table 4).

The same result was found for vigor of normal seeds (Table 3), for which the positive control (Vitavax-Thiram®) showed better results than the essential oils, however it did not differ from the negative control, and it did not affect the seed physiology. Therefore, the harmful effect on the seeds was probably caused by the fungi and long storage period. It corroborates the findings of Dan et al. (2010), who explains that decreases in physiological quality of seeds caused by chemical treatments intensifies with the prolongation of the storage period of the treated seeds. It explains the decrease in seed germination found in the present experiment at the end of the storage period in relation to the percentage at the initial analysis, and indicates the need for further research to verify and adjust the time period for optimal storage of soybean seeds.

According to Santos et al. (2020), fungi of the genera *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp. can damage stored seeds, reducing their quality, as these fungi develop in tissues of embryos, causing discoloration and seed rot, thus reducing the seed germination rate and vigor. In the present study, a lower incidence of these fungi was found in the previous analysis carried out with seeds from the field. In addition, according to the bibliographic research, so far, there is no maximum level of infection/incidence established by the Brazilian legislation for fungi detected in soybean seed lots, despite their potential to decrease agricultural yields.

The analysis of variance of fungal incidence (Table 5) showed significant effect of the inoculation (I) (seeds with and

Table 4. Mean percentages of abnormal and dead seedlings in germination and vigor tests of soybean seeds subjected to different treatments with essential oils and synthetic fungicide

Treatments	Germination (%)		Vigor (%)	
	Abnormal seeds	Dead seeds	Abnormal seeds	Dead seeds
Negative control	10.0 I	4.5 I	11.5	7.0
Positive control**	13.8 S	6.7	10.8 I	3.7 I
Rosemary	13.3	9.7	13.8	9.5
Eucalyptus	11.8	11.2	13.3	15.2 S
Rosemary + Eucalyptus	12.2	11.8	15.8 S	10.5
Overall mean	12.2	8.8	13.0	9.2
Coefficient of variation (%)	2.2	9.6	3.9	18.2
Standard deviation (SD)	1.5	3.1	2.0	4.3

S - Greater than the mean plus standard deviation (mean + 1SD); I - Lower than the mean minus standard deviation (mean – 1SD). ** Positive control with Vitavax-Thiram® 200 SC fungicide

Table 5. Analysis of variance of fungal incidence by the sources of variation: inoculation (with and without inoculation) and different treatments of soybean seeds of the cultivar Brasmax Lance®

Source of variation	Degree of freedom	Mean square			
		Aspergillus sp.	*Penicillium* sp.	*Fusarium* sp.	*Rhizopus* sp.
Inoculation (I)	1	3385.6*	3.6*	1177.2*	1345.6*
Treatments (T)	4	2990.6*	6.4*	1164.5*	776.6*
I × T	4	162.6*	1.6*	615.5*	668.6*
Error	30	51.0	0.46	116.5	105.5
Overall mean	-	75.8	1.1	62.6	84.2
Coefficient of variation (%)	-	9.4	62.1	17.2	12.2

* - Significant at $p \leq 0.05$ by the F test

without inoculation), treatments (T), and the I × T interaction - for all fungi, at $p \leq 0.05$ by the F test.

According to the results presented in Tables 6 and 7, the fungi found in the samples after the seed storage period were *Aspergillus* sp., *Fusarium* sp., *Rhizopus* sp., and *Penicillium* sp. The experiment did not include evaluation of fungal incidence at different seed storage times, thus, it was not possible to verify whether the effect of essential oils and fungicide on the control of the pathogens was greater at initial storage months, which can be assessed in further studies. However, after four months of storage of the treated seeds, the results of the sanity test (Table 6) show that the positive control (fungicide Vitavax-Thiram®) was the best treatment to control the pathogen *Penicillium* in soybean seeds stored with and without inoculation, and also the fungus *Aspergillus* sp. in uninoculated seeds. However, among the treatments with essential oil, only the application of *R. officinalis* in seeds inoculated with *Penicillium* sp. obtained the same result as Vitavax-Thiram® controlling the fungus. In this same condition, the essential oils *C. citriodora* and combined oil (*R. officinalis* + *C. citriodora*) showed a reduction in infestation by *Penicillium* sp. when compared to the negative control. And for the seeds without inoculation of this fungus, it was observed that there was no satisfactory control by the essential oils.

According to data in Table 6, the treatments did not obtain good results in the control of *Aspergillus* sp. in seeds inoculated with this fungus, however, in uninoculated seeds the incidence of the pathogen was lower in the treatment with Vitavax-Thiram (30%), and in the sequence, the combined essential oil (*R. officinalis* + *C. citriodora*) and *C. citriodora* showed a fungal incidence of 69 and 73%, respectively, which demonstrates a reduction in the pathogen infestation in seeds treated with these oils.

However, it should be noted that the treatments presented different results for each fungus and these results are independent of the condition of the seeds (with or without inoculation), because in the infestation of *Aspergillus* sp., it can be seen that there was a reduction of the pathogen in non-inoculated seeds, while the opposite occurred for *Penicillium* sp.

Table 7. Incidence of phytopathogens in soybean seeds stored for four months as a function of the application of different treatments

Treatments	Fungi (%)	
	Fusarium sp.	*Rhizopus* sp.
Control	51 a	79 a
Vitavax-Thiram	64 b	71 a
Rosemary	68 b	88 b
Eucalyptus	51 a	87 b
Rosemary + Eucalyptus	79 c	97 b

Means followed by the same lowercase letter in the columns do not differ by the Scott & Knott test at $p \leq 0.05$; Rosemary - *Rosmarinus officinalis*; Eucalyptus - *Corymbia citriodora*

In the control of the *Fusarium* sp. fungus, no treatment proved to be effective (Table 7). According to Nascimento et al. (2021), essential oils and their compounds are considered efficient in treatment of seeds for controlling phytopathogens when they efficiently control the target pathogen with no adverse effects on seed germination and seedling development. Therefore, the present study showed satisfactory results in reducing the infestation of storage pathogens.

However, the use of the fungicide Vitavax-Thiram resulted in lower incidence of the pathogen *Rhizopus* sp. in the seeds when compared to the treatments with essential oils (Table 7), which are not adequate for the control of this pathogen, but with good inhibition of the fungus *Fusarium* sp. According to Nascimento et al. (2021), the mechanism of action of essential oils on phytopathogens is not exactly known, although several studies focus on elucidate this question. Considering the chemical constituents of these oils and the physiological characteristics of the fungi, it was found that the same essential oil can cause irreversible damage to a pathogen, but does not affect others.

Table 6. Comparison of means of the effect of different treatments on control of two phytopathogens, with and without inoculation, in soybean seeds of the cultivar Brasmax Lance® stored for four months

Condition	Treatments/fungal incidence (%)				
	Negative control	Rosemary	Eucalyptus	Rosemary + Eucalyptus	Vitavax-Thiram® (Positive control)
			Aspergillus sp.		
Without inoculation	82 aC	79 aC	73 bB	69 aB	30 aA
With inoculation	94 bC	95 bC	82 bB	100 bC	54 bA
			Penicillium sp.		
Without inoculation	2 aC	1 bB	3 bD	1 aB	0 aA
With inoculation	2 aC	0 aA	1 aB	1 aB	0 aA

Means followed by the same lowercase letter in the columns, or uppercase in the rows, do not differ by the Scott & Knott test at $p \leq 0.05$; Rosemary - *Rosmarinus officinalis*; Eucalyptus - *Corymbia citriodora*

In this sense, the characteristics of essential oils, such as high volatility, instability, rapid degradation, and low miscibility in aqueous solutions, have been an obstacle to their use, especially in seed treatment formulations (Cadena et al., 2018; Nascimento et al., 2021). Therefore, new formulations are needed to improve stability and preserve the compounds of essential oils, such as nanoencapsulation (Worrall et al., 2018). According to Nerilo et al. (2020), this method maintains the oils bioactive, thus enabling the protection of stored grains.

Silva et al. (2019) developed and characterized nanoemulsions with neem oil (*Azadirachta indica*) at concentrations of 0.5, 1, 2, and 3% (w v^{-1}), which were applied to the fungi *Aspergillus flavus* and *Penicillium citrinum* in soybean seeds. They found an inhibitory effect on the growth of fungal isolates, with the best results found for the concentration of 3%. In addition to being efficient, the neem oil nanoemulsions did not present phytotoxic effects to the seeds. These findings indicate that the use of this product in agriculture is economically viable, as it is easily accessible, and less toxic than common agrochemicals. According to Kumar et al. (2022), neem oil nanoemulsions brought new perspectives to phytochemical applications, improving their bioavailability and specific targeting, reducing undesirable side effects, and minimizing non-specific absorption. Therefore, nanoemulsions could be used in new experiments with the essential oils tested in the present study, as they potentiate and improve the essential oil action.

Based on the results found, the use of rosemary essential oil (*R. officinalis*) at a dose of 700 μL mL^{-1} (concentration of essential oil for each mL of water) was efficient in the control of *Penicillium* sp., while eucalyptus oil (*C. citrodora*) and the combination of essential oils (rosemary + eucalyptus), at the same dose, showed only a reduction in seed infestation in the control of storage fungi for soybean seeds. Therefore, this result is unprecedented, since no other studies were found in the scientific literature with the use of these essential oils in the treatment of stored soybean seeds, indicating the possibility of further studies with the use of rosemary and eucalyptus essential oils in order to consolidate them in the future as a product in the reduction or control of storage fungi applicable in agriculture.

The interest in sustainability and organic production for a better-quality food is increasing, and the use of essential oils is an option for integration with other disease management techniques, which can reduce environmental impacts generated by the exclusive use of agrochemicals, as the bioactive substances present in essential oils degrade rapidly in the environment and are generally considered harmless (Raveau et al., 2020; Dawood et al., 2021). However, despite essential oils have shown antimicrobial activity against a wide range of phytopathogens, studies on their use in seed treatments are still scarce (Nascimento et al., 2021), denoting the differential of the present study.

In view of the above, the importance of this study is noticeable by contributing with new results about the essential oils tested, with a view to the development and application of more stable formulations for long periods, thus reducing the amount of essential oil applied. Therefore, the present study sought to improve their antifungal and allelopathic potential as an alternative for a sustainable agriculture with less environmental impact.

Therefore, the association of biological assays with the phytochemical characterizations of these essential oils, as in the presented study, can be useful for industries to development biocompatible products, either as in natura compounds or models for chemical synthesis or semi-synthesis of products with the necessary characteristics, i.e., low cost, ease of application, small spectrum of action, low residual persistence, and little or no toxicity to humans, animals, and the environment (Alonso-Gato et al., 2021).

Conclusions

1. Soybean seed treatment with Vitavax-Thiram fungicide proved to be efficient in the control of *Penicillium* sp., both in inoculated and non-inoculated seeds with this fungus; and a similar result was found for the essential oil of *Rosmarinus officinalis*, with a significant reduction in seed infestation by the same fungus.

2. The treatments with *Corymbia citriodora* and the mixture of essential oils (*C. citriodora* + *R. officinalis*) did not present fungal control for *Aspergillus* sp. and *Penicillium* sp. in soybean seeds when compared to the fungicide Vitavax-Thiram; however, there was a reduction in the infestation of seeds with and without inoculation with *Aspergillus* sp. by the essential oil of *C. citrioroda* and the infestation of *Penicillium* sp. with the application of *R. officinalis*.

3. *Corymbia citriodora* and *Rosmarinus officinalis* essential oils and their combination can be used to reduce seed storage fungus infestation. However, further research must be carried out for the development and application of more stable formulations based on essential oils to improve the antifungal potential.

Literature Cited

Abd-Allah, H. H.; Mahdy, A. M. M.; Gad, M. F.; Hassan, E. O.; Embaby, E. M.; Yaseen, A. A. Legume seed deterioration caused by some mould fungi affecting seed quality. Middle East Journal of Applied Sciences, v.8, p.325-336, 2018.

Alonso-Gato, M.; Astray, G.; Mejuto, J. C.; Simal-Gandara, J. Essential oils as antimicrobials in crop protection. Antibiotics, v.10, p.34, 2021. https://doi.org/10.3390/antibiotics10010034

Brasil - Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Brasília, 2009a. 399p.

Brasil - Ministério da Agricultura, Pecuária e Abastecimento. Manual de análise sanitária de sementes. Brasília, 2009b. 200p.

Cadena, M. B.; Preston, G. M.; Van Der Hoorn, R. A. L.; Flanagan, N. A.; Townley, H. E.; Thompson, I. P. Enhancing cinnamon essential oil activity by nanoparticle encapsulation to control seed pathogens. Industrial Crops and Products, v.124, p.755-764, 2018. https://doi.org/10.1016/j.indcrop.2018.08.043

CONAB - Companhia Nacional de Abastecimento. Acompanhamento da safra brasileira de grãos: Safra 2017/2018. Available on: <https://www.conab.gov.br/info-agro/safras/graos/boletim-da-safra-de-graos?start=50>. Access on: Abr. 2022.

Costa, M. L. N.; Goncalves, D. da S. F.; Machado, J. da C. Controle de *Fusarium verticillioides* em sementes de milho com o óleo essencial de gengibre. Summa phytopathol, v.46, p.250-254, 2020. https://doi.org/10.1590/0100-5405/233888

Dan, L. G. de M.; Dan, H. de A.; Barroso, A. L. de L.; Braccini, A. de L. Qualidade fisiológica de sementes de soja tratadas com inseticidas sob efeito do armazenamento. Revista Brasileira de Sementes, v.32, p.131-139, 2010. https://doi.org/10.1590/S0101-31222010000200016

Daronco, M. V.; Schneider, A.; Viau, L. V. M.; Colet, C. de F. Avaliação da eficácia de óleos essenciais no tratamento de sementes de soja. Ciência Agrícola, v.13, p.49-58, 2015. https://doi.org/10.28998/rca.v13i1.1870

Dawood, M. A. O.; Basuini, M. F. E.; Zaineldin, A. I.; Yilmaz, S.; Hasan, M. T.; Ahmadifar, E.; Asely, A. M. E.; Abdel-Latif, H. M. R.; Alagawany, M.; Abu-Elala, N. M.; Doan, H. V.; Sewilam, H. Antiparasitic and antibacterial functionality of essential oils: an alternative approach for sustainable aquaculture. Pathogens, v.10, p.1-38, 2021. https://doi.org/10.3390/pathogens10020185

Domene, M. P.; Glória, E. M. da; Biagi, J. D.; Benedetti, B. C; Martins, L. Efeito do tratamento com óleos essenciais sobre a qualidade fisiológica e sanitária das sementes de milho (*Zea mays*). Arquivos do Instituto Biológico, v. 83, p.1-6, 2016. https://doi.org/10.1590/1808-1657000072014

Dorneles, G. de O.; Silveira, R. G.; Guesser, V. P.; Radmann, E. B.; Missio, E. Desempenho de sementes de soja submetidas a tratamento com fungicida/inseticida e períodos de armazenamento. Brazilian Journal of Development, v.5, p.2303-2310, 2019.

Ferreira, A. G.; Borghetti, F. Germinação: Do básico ao aplicado. Porto Alegre: Artmed, 2004. 324p.

Goulart, A. C. P. Fungos em sementes de soja: Detecção, importância e controle. 2.ed. Brasília: Embrapa, 2018. 71p.

Hillen, T.; Schwan-Estrada, K. R. F.; Mesquini, R. M.; Cruz, M. E. S.; Stangarlin, J. R.; Nozaki, M. Atividade antimicrobiana de óleos essenciais no controle de alguns fitopatógenos fúngicos in vitro e no tratamento de sementes. Revista Brasileira de Plantas Medicinais, v.14, p.439-445, 2012. https://doi.org/10.1590/S1516-05722012000300003

Kesho, A.; Chala, A.; Gebremariam, E. S. Efficacy of essential oils as management option against storage fungi of wheat. International Journal of Multidisciplinary Sciences and Advanced Technology, v.1, p.76-84, 2020.

Kumar, S.; Singh, N.; Devi, S.; Kumar, S.; Kamle, M.; Kumar, P.; Mukherjee, A. Neem oil and its nanoemulsion in sustainable food preservation and packaging: Current status and future prospects. Journal of Agriculture and Food Research, v.7, e100254, 2022. https://doi.org/10.1016/j.jafr.2021.100254

Nascimento, D. M. do; Ribeiro Junior, M. R.; Santos, P. L. de; Pereira, A. E.; Kronka, A. Z. Óleos essenciais no tratamento de sementes. Revisão Anual de Patologia de Plantas, v.27, p.77-90, 2021. https://doi.org/10.31976/0104-038321v270004

Nerilo, S. B.; Romoli, J. C. Z.; Nakasugi, L. P.; Zampieri, N. S.; Mossini, S. A. G.; Rocha, G. H. O.; Gloria, E. M. da; Abreu Filho, B. A. de; Machinski Junior, M. Antifungal activity and inhibition of aflatoxins production by *Zingiber officinale* Roscoe essential oil against *Aspergillus flavus* in stored maize grains. Ciência Rural, v.50, p.1-10, 2020. https://doi.org/10.1590/0103-8478cr20190779

Oliveira, A. M. de; Santos, A. M. G.; Silva, L. B.; Teixeira, H. R. S.; Prochnow, J. T.; Almeida, F. A. de; Morais, E. M.; Costa, M. R. da. Soybean seeds treated with vinasse and cassava wastewater. Archives of Agronomy and Soil Science, v.67, p.1-27, 2020. https://doi.org/10.1080/03650340.2020.1799982

Parisi, J. J. D.; Santos, A. F. dos; Barbedo, C. J.; Medina, P. F. Patologia de sementes florestais: danos, detecção e controle, uma revisão. Summa Phytopathol, v.45, p.129-133, 2019. https://doi.org/10.1590/0100-5405/188545

Pereira, R. C.; Pereira, L. C.; Braccini, A. L.; Correia, L. V.; Pelloso, M. F.; Matera, T. C.; Silva, B. G. da; Borges, Y. M.; Coppo, C.; Santos, R. F. dos. Physiological quality of soybean seeds stored after industrial treatments with different chemicals. Research, Society and Development, v.10, p.1-7, 2021. https://doi.org/10.33448/rsd-v10i2.12279

Raveau, R.; Fontaine, J.; Sahraoui, A. L. H. Essential oils as potential alternative biocontrol products against plant pathogens and weeds: A Review. Foods, v.9, p.1-31, 2020. https://doi.org/10.3390/foods9030365

Rodrigues, V. O.; Penido, A. C.; Pereira, D. de S.; Oliveira, A. M. S.; Mendes, A. E. S.; Oliveira, J. A. Sanitary and physiological quality of soybean seeds treated with Ozone. Journal of Agricultural Science, v.11, p.183-196, 2019.

Santos, T. M.; Albuquerque, Á. R.; Raimam, M. P. Qualidade fisiológica e sanitária de sementes de *Cenostigma tocantinum* Ducke (Fabaceae). Scientia Plena, v.16, p.1-8, 2020. https://doi.org/10.14808/sci.plena.2020.120202

Seixas, C. D. S.; Neumaier, N.; Balbinot Junior, A. A.; Krzyzanowski, F. C.; Leite, R. M. V. B. de C. Tecnologias de produção de soja. Londrina: Embrapa Soja, 2020. 347p.

Shao, D.; Smith, D. L.; Kabbage, M.; Roth, M. G. Effectors of plant necrotrophic fungi. Frontiers in Plant Science, v.12, p.1-14, 2021. https://doi.org/10.3389/fpls.2021.687713

Silva, P. de C.; Pereira, L. A. S.; Rezende, É. M. de; Reis, M. V. dos; Lago, A. M. T.; Carvalho, G. R.; Paiva, R.; Oliveira, J. E.; Marconcini, J. M. Production and efficacy of neem nanoemulsion in the control of *Aspergillus flavus* and *Penicillium citrinum* in soybean seeds. European Journal of Plant Pathology, v.155, p.1105-1116, 2019. https://doi.org/10.1007/s10658-019-01838-4

Souza, Â. R. L. de; Machado, J. A. D.; Dalcin, D. Análise de estudos internacionais sobre os fatores que influenciam a decisão dos agricultores pela produção orgânica. Revista em Agronegócio e Meio Ambiente, v.8, p.563-583, 2015. http://dx.doi.org/10.17765/2176-9168.2015v8n3p563-583

Worrall, E. A.; Hamid, A.; Mody, K. T.; Mitter, N.; Pappu, H. R. Nanotechnology for plant disease management. Agronomy, v.8, e285, 2018. https://doi.org/10.3390/agronomy8120285