REVISTA DELOS DESARROLLO LOCAL SOSTENIBLE

# Seed treatment with essential oils to control *Sclerotium rolfsii* Sacc

# Tratamento de sementes com óleos essenciais para controle de *Sclerotium rolfsii* Sacc

# Tratamiento de semillas con aceites esenciales para el control de *Sclerotium rolfsii* Sacc

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**ABSTRACT**
The sclerotia wilt in cowpea caused by the fungus Sclerotium rolfsii has in chemical control an inefficient option. Essential oils have antifungal properties, constituting an alternative to agrochemicals. Here, we evaluated the efficiency of essential oils in the control of *S. rolfsii*, from seed treatment, as well as their effect on seed germination. Based on the diameter of the colonies of the fungus *S. rolfssi* submitted to a concentration of 8.0 ml/kg of seed$^{-1}$, the oils of *L. sidoides*, *L. origanoides* and *C. zehntneri* reduced mycelial growth by 61.5, 67.2 and 40% respectively. The reduction in the percentage of germination occurred at the highest concentrations. The fungus was sensitive to all evaluated oils, being more sensitive to *Lippia* oils. Seed treatment with essential oils is effective at a concentration of 4.0 ml/kg$^{-1}$.

**Keywords:** alternative control, mycelial growth, plant diseases, natural, pesticides.

**RESUMO**
A murcha de escleródios do feijão-caupi causada pelo fungo *Sclerotium rolfsii* tem no controle químico uma opção ineficiente. Os óleos essenciais possuem propriedades antifúngicas, constituindo uma alternativa aos agroquímicos. Aqui avaliamos a eficiência dos óleos essenciais no controle de *S. rolfsii*, a partir do tratamento de sementes, bem como seu efeito na germinação das sementes. Com base no diâmetro das colônias do fungo *S. rolfssi* submetidas à concentração de 8,0 ml/kg de semente$^{-1}$, os óleos de *L. sidoides, L. origanoides e C. zehntneri* reduziram o crescimento micelial em 61,5, 67,2 e 40 % respectivamente. A redução na porcentagem de germinação ocorreu nas maiores concentrações. O fungo foi sensível a todos os óleos avaliados, sendo mais sensível aos óleos de Lippia. O tratamento de sementes com óleos essenciais é eficaz na concentração de 4,0 ml/kg$^{-1}$.

**Palavras-chave:** controle alternativo, crescimento micelial, doenças de plantas, natural, pesticidas.

**RESUMEN**
La marchitez esclerótica del caupí causada por el hongo Sclerotium rolfsii tiene en el control químico una opción ineficaz. Los aceites esenciales tienen propiedades antifúngicas, constituyendo una alternativa a los agroquímicos. Aquí, evaluamos la eficiencia de los aceites esenciales en el control de S. rolfsii, a partir del tratamiento de semillas, así como su efecto sobre

la germinación de las mismas. En base al diámetro de las colonias del hongo S. rolfssi sometidas a una concentración de 8,0 ml/kg de semilla-1, los aceites de L. sidoides, L. origanoides y C. zehntneri redujeron el crecimiento micelial en un 61,5, 67,2 y 40% respectivamente. La reducción del porcentaje de germinación se produjo en las concentraciones más altas. El hongo fue sensible a todos los aceites evaluados, siendo más sensible a los aceites de Lippia. El tratamiento de las semillas con aceites esenciales es eficaz a una concentración de 4,0 ml/kg-1.

**Palabras clave:** control alternativo, crecimiento micelial, enfermedades de las plantas, natural, plaguicidas.

## 1 INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) deserves to be highlighted among the various legumes consumed in the world, as it is an important source of protein, energy, fiber and minerals in human food, in addition to being used as a raw material for fertilization green (Manaf & Zayed, 2015).

Despite its hardiness, some diseases can affect the cowpea crop, among them, sclerotia wilt, characterized by a tangle of white mycelium and, under this, there is an intense disruption of the parasitized plant tissue (Athayde Sobrinho et al., 2005), leading to to the reduction of the stand and, consequently, in the grain yield.

The scarcity of resistant varieties for the pathosystem has required the search for integrated management measures that promote plant development to the detriment of the pathogen, such as the use of crop rotation, avoidance of high planting densities, biological and chemical treatment (Bedendo, 2018), and the chemical treatment is currently the most adopted in the world. However, its use unfortunately brings several inconveniences, among which the following stand out: the risk of environmental contamination, intoxication of workers and the rural population, in addition to the occurrence of residues in food (Pignati et al., 2017). To minimize these risks, the seed treatment strategy has been used, whose application is quite simple, low cost and highly efficient (Pereira et al., 2016a).

Among the alternatives to the use of synthetic chemicals, the use of essential oils from plants has shown high potential in controlling pests and diseases (Raveau et al., 2020; Chang et al., 2022). This thesis is supported by the fact that plants naturally synthesize several secondary compounds that represent natural means of defense against predators and pathogens, even showing herbicidal action (Alves et al., 2018).

However, studies demonstrating the control of soil pathogens using essential oils are scarce. And those that bring information about its use in seed treatment targeting pathogens such as *S. rolfsii* practically do not exist. Assuming that essential oils have proven fungitoxic action, there is strong confidence that essential oils will establish themselves as a viable alternative to the replacement of synthetic chemicals (Dias et al., 2019).

The present work aimed to evaluate the treatment of seeds with essential oils of *Lippia sidoides*, *Lippia origanoides* and *Croton zehntneri* in the control of *S. rolfsii*, the causal agent of sclerotia wilt in cowpea, as well as its effect on seed germination treated.

## 2 MATERIALS AND METHODS

### 2.1 LOCATION

The experiments were carried out in a greenhouse and in the Phytopathology Laboratory of Embrapa Meio-Norte, in Teresina, Piauí, Brazil, with all tests performed in triplicate. *In vitro* and *in vivo* assays were performed sequentially.

### 2.2 ORIGIN OF FUNGAL ISOLATE AND ESSENTIAL OILS

The tests were carried out using the fungus *S. rolfsii* collected in Teresina, PI (-5.036410, -42.797898), isolated from the stem of cowpea plants with symptoms of the disease whose specimen was deposited in the Soil Fungi Collection of Federal Rural University of Pernambuco under number CFS 614. Essential oils extracted from the species *Lippia sidoides* Cham, *Lippia origanoides* Cham and *Croton zehntneri* Pax et Hoffm were used. They are all spontaneously occurring shrubs or subshrubs, none of them are yet cultivated, their propagation being typically vegetative. (Table 1).

Table 1 - Species, origin, registration number and part of the plant used to obtain the tested essential oils.

| Species | Origin | Register | Part of the plant |
|---|---|---|---|
| *Lippia sidoides* | Teresina – PI | CEN 92438 | Leaf |
| *Lippia origanoides* | Jatobá do Piauí - PI | CESJ 70120 | Leaf |
| *Croton zehntneri* | Valença - PI | TEPB 30944 | Leaf |

Source: Elaborated by the authors.

The extraction of essential oils was performed using the hydrodistillation method, in a Clevenger apparatus, coupled to a heating blanket, as a heat source for the system (Gomes et al., 2014). In each extraction, 200g of dehydrated leaves, previously crushed at room temperature, were used.

## 2.3 IN VITRO EFFECT OF SEED TREATMENT WITH ESSENTIAL OILS

This test, considered a pilot and submitted to strict laboratory control, used cowpea seeds, cultivar BRS Tumucumaque, donated by the Improvement Program of Embrapa Meio-Norte. Petri dishes of 90 mm in diameter were used with PDA medium (Potato-Dextrose-Agar) in the center of which discs of *S. rolfsii* mycelium were placed surrounded by four seeds treated with essential oil, according to concentrations 0.5; 1.0; 2.0; 4.0 and; 8.0 ml/kg of seed$^{-1}$ and placed equidistant from the center, 4 mm from the edges of the plates (Figure 1). The control treatment followed the same scheme and, replacing the oils, the seeds received sterile distilled water. The treatments were applied by placing the oils on the seeds in test tubes (15 x 100 mm), agitated for three minutes in a vortex mixer, in order to guarantee the uniform distribution of the oil on the seeds. The tubes with the treated seeds were sealed with plastic film, remaining overnight.

Figure 1 - Detail of the experimental unit showing the radial distribution of the seeds of cowpea BRS Tumucumaque in relation to the inoculum.

Source: Elaborated by the authors.

The test was carried out in a completely randomized design in a 3 x 5 factorial arrangement (oil x concentrations) with three replications. The evaluation of the mycelial growth was carried out five days after the installation of the experiment, when it reached the seeds in at least one of the plates and was obtained by measuring the diameter of the fungus colony in two diametrically opposite directions.

Mycelial growth data were submitted to analysis of variance, with the effect of concentrations evaluated by linear regression analysis, while the effect of oils among themselves was compared by Tukey's test at 5% probability. For that, the statistical program Variance Analysis System for Balanced Data – SISVAR was used, according to Ferreira (2011).

## 2.4 IN VIVO EFFECT OF SEED TREATMENT WITH ESSENTIAL OILS ON SCLEROTIUM ROLFSII

Four seeds were sown per pot, in plastic pots of 1.5 liters, containing vegetable sand and carbonized rice husk (3:1) previously sterilized in an autoclave. Soil infestation was carried out by depositing a colonized autoclaved rice grain with husk in the center of the vase and around which the seeds treated with the essential oils in the respective concentrations were arranged.

The test, similarly to the *in vitro* effect, was organized in a completely randomized design in a 3 x 5 factorial arrangement (oils and concentrations) with five replications. The following concentrations were evaluated: 0.5; 1.0; 2.0; 4.0 and; 8.0 ml/kg of seed$^{-1}$ and a control (without oil). The pots were irrigated daily and the incidence evaluations, carried out daily for 15 days, consisted of counting the plants with symptoms of necrosis, stem constriction and seedling death due to fungus attack (Figure 2).

Figure 2 - Reference symptom pattern used in the evaluation of the incidence of sclerotia wilt in cowpea.

Source: Elaborated by the authors.

The results were submitted to analysis of variance, with the effect of the concentrations evaluated by linear regression analysis and the means of the data obtained for the oils were compared among themselves using the Tukey test at 5% probability. For that, the statistical program Variance Analysis System for Balanced Data – SISVAR was used, according to Ferreira (2011).

## 2.5 EFFECT OF OIL ON SEED GERMINATION

Once the effective concentration in controlling the pathogen was defined, the seeds were treated and then the germination test was carried out in order to evaluate a possible phytotoxic or allelopathic effect caused by the oils tested at two concentrations (4 and 8 mL/Kg of seed$^{-1}$) and a control (without oil), using the methodology as previously described.

To evaluate the influence of oil on the seed, the standard Germination Test (Brasil, 2014) was used, with the effect of treatments being compared by analysis of variance and the means of germination percentages by Tukey's test at 5% probability, in a completely randomized design, with four treatments (oil from *L.cidoides*, *L. origanoides* and *C. zehntneri* and the control without oil) and eight replications. In all evaluations, the statistical program Analysis of Variance for Balanced Data – SISVAR was used, according to Ferreira (2011).

## 3 RESULTS AND DISCUSSION

Sclerotia wilt is a disease that causes significant damage to cowpea crops. Its severity is such that, in addition to killing the affected plants, it produces resistance structures, the sclerotia (Figure 3), which can remain viable in the soil for several months (Marcuzzo, 2014), decreasing the plant population and reducing production of grains, with the consequent economic loss to the rural producer (Antwi-Boasiako et al., 2022). This results in a great effort to identify a natural and efficient way to control it.

Figure 3 - Aspects of sclerotia wilt in cowpea.

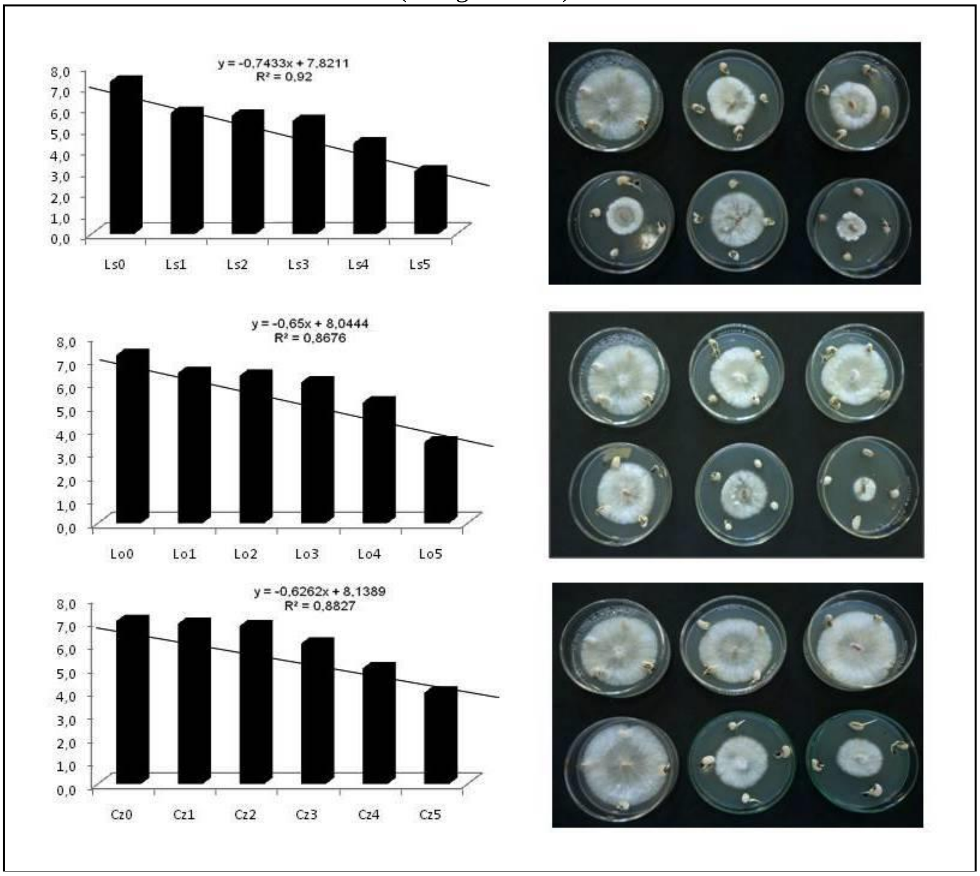
Source: Elaborated by the authors.

### 3.1 IN VITRO EFFECT OF SEED TREATMENT WITH ESSENTIAL OILS ON S. ROLFSII

Seed treatment with the three oils studied proved to be highly effective in controlling the fungus *S. rolfsii* by demonstrating significant inhibition ($P < 0.05$) of mycelial growth. Treatment response followed a decreasing linear model, as shown in Figure 4. This bioassay model is a pioneer, since no work was found in the literature that used the same model, whose structure has been used to carry out screening of other seed treatment products, in a controlled environment and under low risk of environmental exposure.

The results obtained are indicative of the real chance that the oils will become viable as natural agents for controlling the fungus. In principle, these results confirm the observations of some authors when they highlight that the incidence of diseases tends to decrease in the presence of essential oils (Dias et al., 2018; Peixinho et al., 2019). On the other hand, there are works that prove the reduction of the incidence of different pathogens in seeds (Baiotto et al.; 2023; Lozada et al.; 2019), however, there are still few that present the seed as an efficient vehicle for the application of natural "protectors" similar to essential oils and C. *zehntneri*. This, in turn, was significantly lower (P<0.05) than the oil of *L.cidoides*

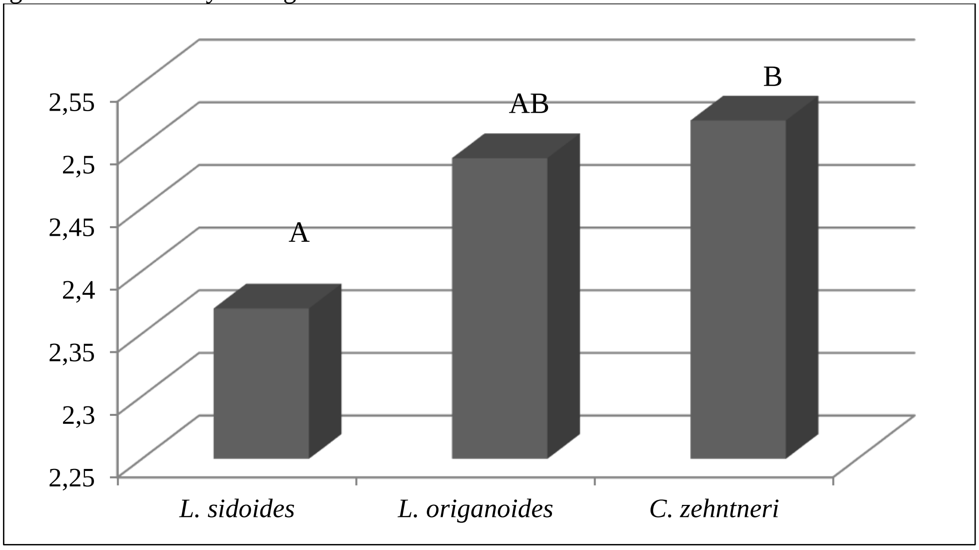
Figure 4 - In vitro effect on mycelial growth of Sclerotium rolfsii in PDA medium containing seeds treated with essential oil of Lippia sidoides (Ls), Lippia origanoides (Lo), and Croton zehntneri (Cz) in five concentrations (ml/kg of seeds).




* On the right, Petri dishes, showing the behavior of the fungus when subjected to essential oils at different concentrations. Mycelial growth in cm (y-axis), concentration (x-axis). Source: Elaborated by the authors.

The result of the qualitative effect demonstrated by the three evaluated oils is shown in Figure 5. Based on the measurement of the diameter of the colonies of the fungus *S. rolfssi* submitted to a concentration of 8.0 ml/kg of seed$^{-1}$, the oils of *L. sidoides*, *L. origanoides* and *C. zehntneri* reduced mycelial growth by 61.5, 67.2 and 40% respectively. When compared to each other, it was possible to verify that the oil of *L. origanoides* was similar to the oils of *L. sidoides* and *C. zehntneri*. This, in turn, was significantly lower (P<0.05) than the oil of *L.cidoides*.

Figure 5 - *In vitro* mycelial growth of Sclerotium rolfsii after seed treatment with essential oil.

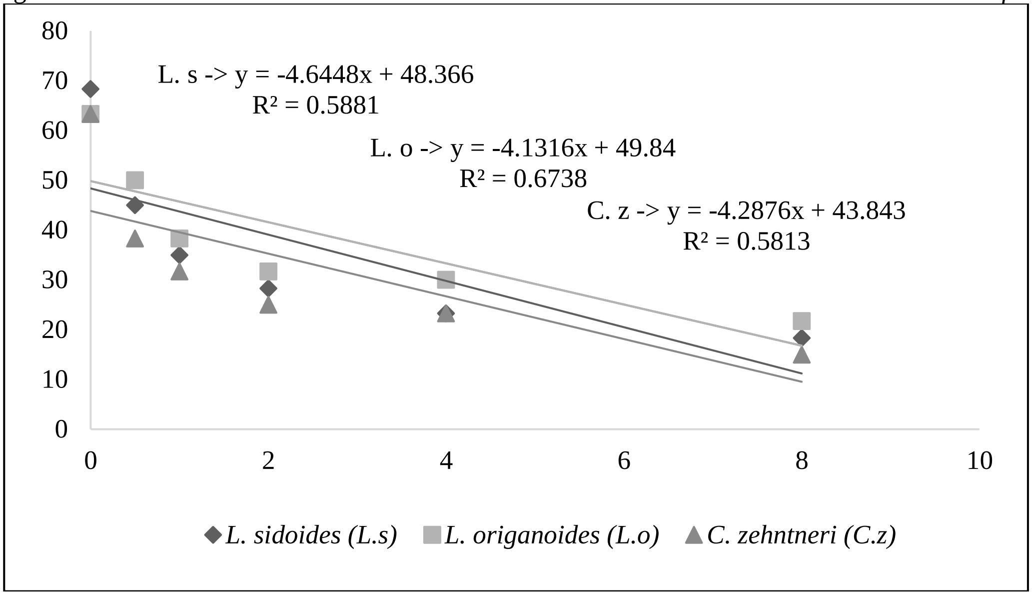



* Mycelial growth in cm (y-axis), evaluated oil (x-axis).
Source: Elaborated by the authors.

## 3.2 IN VIVO EFFECT OF SEED TREATMENT WITH ESSENTIAL OILS ON SCLEROTIUM ROLFSII

The results of the in vivo assays indicate that all the oils had a significant effect ($P < 0.05$) on the control of the fungus with a significant difference for doses, whose behavior obeys a decreasing linear model as the concentration of the oils studied increases (Figure 5). On the other hand, no differences were observed regarding the effect of the oil variable, nor for the oil x concentration interaction. The comparative analysis of the general behavior of the results, generated in triplicate in the two studied systems, *in vitro* and *in vivo*, reveals the tendency of close similarity between them, which consolidates the coherence of the obtained data. Despite this observation, there was a slight difference between the systems, as, in the *in vitro* assays, the essential oil of *L. sidoides* was superior to that of *C. zehntneri* (Figure 5), which was not observed in the *in vivo* treatment (Figure 6). Probably this subtle difference is explained because in the *in vivo* condition, with the work being conducted in the soil, where there are interacting multifactors, it may have inhibited the differentiation of the effects between the oils.

Figure 6 - *In vivo* effect of seed treatment with essential oils on the incidence of *Sclerotium rolfsii*.




* Incidence (y axis), concentration (x axis).
Source: Elaborated by the authors.

As mentioned, studies that relate seed treatment with essential oils, as a strategy for managing soil fungi, are scarce, which reinforces the innovative character of this research. The works that deal with this subject are restricted to the treatment of seeds, targeting pathogens that are associated with the seeds themselves, without, however, intending to have them as a vehicle for the transport of natural substances to control preexisting pathogens in the soil, regardless of whether they are present in the soil. or not infected.

In this perspective, several works are found that, although not directly targeting targets external to seeds, validate, in a broad sense, the strategy of using essential oils in the management of plant diseases (Lozada et al.; 2019; Moumni et al., 2021; Grzanka et al., 2021) and support the present study.

From a practical point of view, the use of essential oils from *L. sidoides*, *L. origanoides* and *C. zehntneri* in seed treatment becomes perfectly viable. The information from Santos et al. (2020) related to oil yield rates of these species, 1.38; 0.4 and 1.2%, respectively, partially enable the technology. The authors also point out that none of them is cultivated rationally. Another favorable aspect is that these species, being native, occur spontaneously in fragile ecosystems (caatinga and cerrado). Thus, the possibility of its use as a raw material for “natural fungicides” constitutes a strong ecological appeal, in the sense of preserving these species and their biomes, insofar as it can inhibit the pressure of deforestation in these environments.

There are few works that associate the use of essential oils in the treatment of cowpea seeds. Farias et al. (2016a) evaluated the effect of andiroba (*Carapa guianensis* Aubl.) and copaiba (*Copaifera langsdorffi* Desf) essential oils on the health quality of macassar bean cultivars and found a reduction in the occurrence of *Aspergillus flavus* and *Fusarium* sp. in the BRS Gurgueia cultivar and in *Cladosporium* sp. and *Fusarium* sp. in Marataoã. On the other hand, in the consulted literature, no works were found related to the action of essential oils, applied in seed treatment, in the control of soil pathogens, notably on the fungus *S. rolfsii*. This is, therefore, the first report in this sense, and, as discussed, it revealed very promising results.

As there is no parallel in the literature that allows comparisons, the effect of oils on this pathosystem can probably be explained by the protective capacity that essential oils lent to seeds, conditioning, in their surroundings, an unfavorable environment for the establishment of pathogen-host relationships, by interposition of an effective barrier between the host tissues and the inoculum of the pathogen present in the cultivation area (Machado, 2000). Similar to what happens with conventional treatment based on synthetic fungicides, seed treatment is the most used and efficient technology, which makes it a strategic tool within the context of integrated disease management (Mesquita et al., 2017), also ensuring, according to Oliveira et al. (2019), adequate plant populations, when soil and climate conditions, during sowing, are unfavorable to germination and rapid emergence.

Studies of the centesimal composition of the studied essential oils were carried out by Santos et al. (2020) and revealed the presence of the major compounds thymol (33.2%) and p-cymene (13.1%) in *L. sidoides*, carvacrol and p-cymene in L. origanoides, representing 67.5% of the constituents and, trazol in *C. zehntneri*, representing 90.1% of the identified compounds. Similar studies related to essential oils of the genus *Lippia* have been extensively carried out. Dias et al. (2019) analyzing essential oil of *Lippia sidoides* found thymol and p-cymene, corroborating the results found in this work.

## 3.3 EFFECT OF OIL ON SEED GERMINATION

The fundamental requirement for a substance to be suitable for use as a fungicide in seed treatment is that it does not have phytotoxic effects (Machado, 2000). The same should occur in relation to essential oils. Therefore, after demonstrating promising data of the oils controlling the target fungus *S. rolfsii*, we sought to evaluate the possible interference in the germination and vigor of the seeds/seedlings.

As shown in Table 2, it is possible to verify a significant phytotoxic action ($P < 0.01$) of the three essential oils tested on seed germination. In general, the reduction in the percentage of germination occurred at the highest concentrations, with the lowest values being verified at the concentration of 8 mL/kg[1] for the oils of *L. origanoides* and *L.cidoides*, respectively.

These results are in agreement with those of Nagao et al. (2002) who observed the inhibition of the germination process of lettuce seeds, proportionally to the increase in the concentrations of the essential oil of *L.cidoides*. This effect may be related to the high concentration of the oil, or to some compound or substance present in it capable of preventing the germination process, since among the constituents of the essential oils studied, it is possible to observe the presence of proven allelopathic compounds, such as terpenoids geraniol, limonene, β-ocimene, linalool, geraniol acetate and β-caryophyllene (Marco et al., 2015).

Table 2 - Percentagem de germinação de sementes de feijão-caupi cv. BRS Tumucumaque tratadas com óleo essencial de *Lippia sidoides, Lippia origanoides* e *Croton zehntneri* em diferentes concentrações em rolo de papel.

| **Treatment** | **% of germination*** |
|---|---|
| Witness (sem óleo) | 90.50 a |
| *Croton zehntneri* (4 mL $Kg^{-1}$) | 91.00 a |
| *Lippia sidoides* (4 mL $Kg^{-1}$) | 87.5 a |
| *Lippia origanoides* (4 mL $Kg^{-1}$) | 77.00 ab |
| *Croton zehntneri* (8 mL $Kg^{-1}$) | 57.00 b |
| *Lippia origanoides* (8 mL $Kg^{-1}$) | 35.00 c |
| *Lippia sidoides* (8 mL $Kg^{-1}$) | 23.00 c |
| | **CV % = 11.30** |

***** The media followed by the same letter does not differ statistically from each other by the Tukey test to 1% probability.
Source: Elaborated by the authors.

The observation made by Hillen et al. (2012), about the probable toxic action of essential oils applied directly to seeds, provoked the need to study this phenomenon in the present work. This is because for a substance to be used in seed treatment, it is not enough that it has effective action against biological targets, but also that it does not exert any deleterious action on plant development (Almeida et al., 2019).

In this regard, there are studies aimed at using essential oil to control phytopathogens existing in the seed itself, even verifying its possible phytotoxic effect (Freddo et al., 2016; Farias et al., 2020b). Aiming to evaluate the influence of treating cowpea seeds with citronella essential oil (Cymbopogon winterianus), Xavier et al. (2012) observed that the seeds treated with the essential oil had a lower percentage of germination, therefore being contraindicated for seed treatment.

Research with essential oils aims, among other things, to develop products that can be effective and safe for treating seeds and that can reduce the environmental impact resulting from the use of fungicides, alone or in combination with other methods (Machado, 2000). However, as studies related to the treatment of seeds with essential oils are relatively recent and not much information is available on the effect caused by essential oils, works like this bring a new perspective, and should stimulate further studies in this area. With this, it is hoped that in the near future, all doubts regarding the feasibility of practical application of essential oils in different agricultural systems will be resolved.

## 4 CONCLUSION

To the best of our knowledge, no study has been carried out so far showing the control of the fungus S. rolfsii by using essential oils from L. sidoides, L. origanoides and C. zehntneri through seed treatment.

The best control is obtained with essential oils of the genus Lippia at a concentration of 4.0 ml kg-1. Although higher doses provide better rates of pathogen control, they cause an adverse effect on the seeds, leading them to present unfavorable rates of germination and vigor.

The bioassay model used in studies conducted *in vitro* proved to be effective and has been used in preliminary evaluations for screening new alternative products with potential indication for use in seed treatment.

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## REFERENCES

Almeida L, Teixeira MC, Lemos JR, Lacerda MN, Silva TC. 2019. Bioatividade de óleos essenciais na germinação e no vigor em sementes de tomate. **Biotemas** 32:13-21. https://doi.org/10.5007/2175-7925.2019v32n2p13

Alves TA, Pinheiro PF, Praça-Fontes MM, Andrade-Vieira LF, Corrêa KB, Alves TA, Cruz FA, Lacerda Júnior V, Ferreira A, Soares TCB. 2018. Toxicity of thymol, carvacrol and their respective phenoxyacetic acids in *Lactuca sativa* and *Sorghum bicolor*. **Ind Crop Prod** 114:59-67. https://doi.org/10.1016/j.indcrop.2018.01.071

Antwi-Boasiako A, Wang Y, Dapaah HK. Zhao T. 2022. Mitigação contra doenças de esclerotinia em culturas de leguminosas: uma revisão abrangente. **Agronomy** 12:3140. https://doi.org/10.3390/agronomy12123140

Athayde Sobrinho C, Viana FMP, Santos AA. 2005. Doenças Fúngicas e Bacterianas, p. 461-484. In: Freire Filho FR, Lima JAA, Ribeiro VQ: **Feijão-caupi: avanços tecnológicos**. 1 ed. Brasília: Embrapa Meio-Norte, Teresina.

Baiotto CS, Baiotto LMC, Beber SC, Kleibert RU, Fell PW, Babeski CM, Bandeira WJA, Basso NCF, Silva JAG, Colet CF. 2023. Antifungal effect of essential oils on control of phytopathogens in stored soybean seeds. **Revista Brasileira de Engenharia Agrícola e Ambiental** 27(4):272-278. https://doi.org/10.1590/1807-1929/agriambi.v27n4p272-278

Bedendo I.P. 2018. Podridões de raiz e colo, p. 329-332. In: Amorim L, Rezende JAM, Bergamim Filho A.: **Manual de fitopatologia**. Piracicaba: Agronômica Ceres. São Paulo.

BRASIL. 2009. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. Secretaria de Defesa Agropecuária. Brasília, DF: MAPA/ACS, p. 395 p.

Chang Y, Harmon PF, Treadwell DD, Carrillo D, Sarkhosh A, Brecht JK. 2022. Biocontrol Potential of Essential Oils in Organic Horticulture Systems: From Farm to Fork. **Front Nutr** 13(8):805138. https://doi.org/10.3389/fnut.2021.805138

Dias L, Santos A, Ribeiro-Paz-Filho E, Soares-da-Silva P, Athayde-Sobrinho C. 2018. Óleo essencial de Cham (alecrim-pimenta) no controle de *Macrophomina phaseolina* em feijão-caupi. **Revista Cubana de Plantas Medicinales** 24(1):1-17.

Farias OT, Nascimento LC, Oliveira FS, Santos MDR, Bruno RLA. 2016a. Óleo essencial de andiroba (*Carapa guianensis* Aubl.) e copaíba (*Copaifera langsdorffi* Desf) sobre a sanidade e fisiologia de sementes de feijão macassar (*Vigna unguiculata* L. Walp). **Rev. Bras. Pl. Med** 18(3):629-635. https://doi.org/10.1590/1983-084X/0112

Farias OR, Cruz JMFL, Gomes RSS, Silva HAO, Nascimento LC. 2020b. Atividade antifúngica do óleo de alecrim sobre sementes de *Phaseolus lunatus*. **Rev. Cienc. Agrar** 43:23-30. https://doi.org/10.19084/rca.18742

Ferreira DF. 2011. Sisvar: a computer statistical analysis system. **Ciênc. agrotec** 35:1039-1042.
Freddo AR, Mazaro SM, Borin MSR, Busso C, Possenti JC, Cechin FE, Zorzzi IC, Dalacosta NL. 2016. Redução no tombamento de *Fusarium* sp. em plântulas de beterraba, pelo tratamento das sementes com óleo essencial de *Aloysia citriodora* palau. **Sci. Agrar. Parana** 15:453-459. https://doi.org/10.18188/sap.v15i4.13451

Gomes MS, Cardoso MG, Soares MJ, Batista LR, Machado SMF, Andrade MA, Azeredo CMO, Resende JMV, Rodrigues LMA.2014. Use of essential oils of the genus Citrus as biocidal agents. **Am J Plant Sci** 5:299-305. https://doi.org/10.4236/ajps.2014.53041

Grzanka, M, Sobiech Ł, Danielewicz J, Horoszkiewicz-Janka J, Skrzypczak G, Sawinska Z, Radzikowska D, Świtek S. 2021. Impact of essential oils on the development of pathogens of the *Fusarium* genus and germination parameters of selected crops. **Open Chemistry** 19(1):884-893. https://doi.org/10.1515/chem-2021-0079

Hillen T, Schwan-Estrada KRF, Mesquini RM, Cruz MES, Stangarlin JR, Nozaki M. 2012. Atividade antimicrobiana de óleos essenciais no controle de alguns fitopatógenos fúngicos in vitro e no tratamento de sementes. **Rev. Bras. Plantas Med** 14:439-445. https://doi.org/10.1590/S1516-05722012000300003

Lozada MS, Patrícia PP, Warley RN. 2019. Essential oils in the control of *Colletotrichum gloeosporioides f. sp. cepae* in onion seeds. **Revista ciência agronômica** 50(3):510-518 https://doi.org/10.5935/1806-6690.20190060

Machado JC. 2000.Tratamento de sementes no controle de doenças. Lavras: LAPS/FAEPE, p. 138.

Manaf HH, Zayed MS. 2015. Productivity of cowpea as affected by salt stress in presence of endomycorrhizae and *Pseudomonas fluorescens*. **Ann of Agricul Sci** 60:219-226. https://doi.org/10.1016/j.aoas.2015.10.013

Marco CA, Santos HR, Feitosa AGS, Feitosa JV, Costa JGM. 2015. Teor, rendimento e qualidade do óleo essencial de *Vanillosmopsis arbórea* (Gardner) Baker (candeeiro) e sua ação alelopática. **Rev. Cub. Pl. Medic** 20(1):131-141.

Marcuzzo LL, Schuller A. 2014. Survival and viability of sclerotia from *Sclerotium rolfsii* on the soil. **Summa Phytopathol** 40:281- 283. https://doi.org/10.1590/0100-5405/1951

Mesquita FS, Coiado LR, Freitas AS, Reis CR, Alcântara E, Resende RM. 2017. Tratamento de sementes de feijoeiro-comum com fungicida, inseticida e promotores de crescimento. **Rev. Univ. Vale Rio Verde** 15:769-776. http://dx.doi.org/10.5892/ruvrd.v15i2.3139

Moumni M, Allagui MB, Mezrioui K, Ben Amara H, Romanazzi G. 2021. Evaluation of Seven Essential Oils as Seed Treatments against Seedborne Fungal Pathogens of Cucurbita maxima. Molecules 26(8):2354. http://dx.doi.org/10.3390/molecules26082354

Nagao EO, Innecco R, Medeiros Filho S, Mattos SH. 2002. Efeito do óleo essencial de Alecrim pimenta (*Lippia sidoides* Cham) na germinação de alface. **Horticultura Brasileira** 20(2):1-4.

Oliveira FS, Dias MFS, Pereira RC, Andrade CAB.2019. Produção de sementes de feijão (*Phaseolus vulgaris* L.) **Rev. Terra & Cult** 35:99-116.

Peixinho GS, Ribeiro VG, Amorim EPR, Morais ACM. 2019. Ação do óleo essencial de Citronela (*Cymbopogon nardus* L) sobre o patógeno *Lasiodiplodia theobromae* em cachos de videira cv. Itália. **Summa Phytopathol** 45:428-431. https://doi.org/10.1590/0100-5405/206511

Pereira CL, Garcia MM, Braccini AL, Piana SC, Ferri GC, Matera TC, Felber PH, Marteli DCV. 2016. Efeito da adição de biorregulador ao tratamento industrial sobre a qualidade de sementes de soja (*Glycine max* (L.) Merr.) aos sessenta dias de armazenamento convencional. **Rev. Colomb. Investig**. ***Agroindustriales*** 3:15-22. http://dx.doi.org/10.23850/24220582.347

Pignati WA, Lima FANSL, Lara SS, Correa MLM, Barbosa JR, Leao LHC, Pignatti MG. 2017. Spatial Distribution of Pesticide Use in Brazil: A Strategy for Health Surveillance. **Ciência Saúde** 22:3281-3293. https://doi.org/10.1590/1413-812320172210.17742017

Raveau R, Fontaine J, Lounès-Hadj SA. 2020. Essential Oils as Potential Alternative Biocontrol Products against Plant Pathogens and Weeds. **A Review. Foods** 9(3):365. http://10.3390/foods9030365

Santos ARB, Laranjeira D, Dias LRC, Sousa ES, Melo CDS, Lima SG, Silva PHS, Athayde Sobrinho C. 2020. Chemical composition and control of *Sclerotium rolfsii* Sacc by essential oils. **Afr. J. Microbiol. Res** 14: 119-128. http://10.5897/AJMR2020.9286

Xavier MVA, Oliveira CRF, Brito SSS, Matos CHC, Pinto MADSC.2012. Viabilidade de sementes de feijão caupi após o tratamento com óleo essencial de citronela (*Cymbopogon winterianus* Jowitt). **Rev. Bras. Plantas Med** 14: 250-254. https://doi.org/10.1590/S1516-05722012000500021