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ESSENTIAL OIL OF *Thymus vulgaris* ON THE SANITARY AND PHYSIOLOGICAL QUALITY OF SEEDS OF *Caesalpinia ferrea*

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ABSTRACT - As for most plant species occurring in forests, *Caesalpinia ferrea* Mart. Ex Tul. has been presenting some phytosanitary problems, and thus research efforts have been increased considering the use of alternative treatments using as basis essential oils like *Thymus vulgaris* L., which is responsible for antimicrobial activity. The objective of this study was to evaluate the influence of the essential oil from thyme on the sanitary and physiological quality of ironwood seeds. The experiment was carried out in a completely randomized design with four repetitions with 50 seeds. The treatments considered the use of the thyme essential oil at concentrations of 0, 2, 4, 6, 8, and 10%. The ironwood seeds were obtained from matrices taken at random, moved to the laboratory, where sanitary evaluations were carried out using the *Blotter test* method in 25±2°C temperature and in a 12h photoperiod. After 7 days, with the aid of a microscope, fungi were identified. Next, physiological analysis were conducted — germination, first count of germination, and germination speed index. Measurements of the shoot and root parts of the seedlings, shoot dry matter, and root dry matter were taken. According to the obtained results in this work, it was verified that the *T. vulgaris* dry matter in all concentrations presented different results, noting that when the oil concentration increases, the occurrence of pathogens decreases, which proves that the *T. vulgaris* essential oil has specific action on some genera of fungi.
Keywords: Ironwood, thyme, seed pathology.

ÓLEO ESSENCIAL DE Thymus vulgaris NA QUALIDADE SANITÁRIA E FISIOLÓGICA EM SEMENTES DE Caesalpinia ferrea

RESUMO - Como na maioria das espécies florestais, a *Caesalpinia ferrea* Mart. Ex Tul. vem apresentando problemas fitossanitários, por isso tem-se ampliado pesquisas com o uso de tratamentos alternativos a partir de óleos essenciais como o *Thymus vulgaris* L., que é responsável pela atividade antimicrobiana. O objetivo deste estudo foi avaliar a influência do óleo essencial de tomilho sobre a qualidade sanitária e fisiológica em sementes de pau ferro. O experimento foi conduzido em delineamento experimental inteiramente ao acaso com quatro repetições de 50 sementes, os tratamentos foram compostos pela concentração do óleo essencial de tomilho: 0, 2, 4, 6, 8 e 10%. As sementes de pau ferro foram adquiridas de matrizes ao acaso, em seguida, transportadas para o laboratório, onde foram feitas a avaliação sanitária pelo método *Blotter test* em temperatura de 25 2°C e fotoperíodo de 12 h. Após 7 dias, com auxílio de microscópio foi feita a identificação dos fungos. Em seguida, foi realizada as análises fisiológicas: germinação, primeira contagem de germinação e o índice de velocidade de germinação. Foram medidos o comprimento da parte área e das raízes das plântulas, a massa seca da parte aérea e a massa de massa seca de raiz. De acordo com os resultados obtidos no presente trabalho, verificou-se que o extrato de *T. vulgaris* em todas as concentrações, apresentou diferentes resultados, visto que quando aumenta a concentração do óleo, constatou-se a diminuição na incidência de patógenos. Comprovando que o óleo essencial de *T. vulgaris* tem ação específica para determinados gêneros fúngicos.
Palavras-chave: Pau-ferro, tomilho, patologia de sementes.

INTRODUCTION

The *Caesalpinia ferrea* (Benth). Ducke species is a tropical arboreal pea belonging to the Fabaceae family, widely spread with low populational density, highly disseminated throughout the North and Northeast regions in Brazil. In the Center-West region, this crop is being used for reforesting and recovery of areas degraded by the force of nature or by the action of men. The species is also known as Brazilian ironwood (MITSUMORI et al., 2017).

This crop is considered an ideal tree for urban planting and landscaping, in general presenting great decorative characteristics, providing good shadow areas in squares, besides presenting good pharmacological activities, such as the treatment of diabetes, fevers, hepatic affections, among other diseases (CORREA et al., 2018).

According to Cadorin et al. (2021), natural resources from forests have received great pressure over time mainly due to forest clearance for animal husbandry. However, due to the decrease in the availability of these resources, the production of quality seedlings has been

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intensified over time for the recovery of degraded areas and for the restoration of regional landscapes.

Thus, the seedlings quality still depends on how they are produced, which can positively influence the growth and development of the species that is under study in the field after the seedling transplant (HERBELE et al., 2018; CADORIN et al., 2021). To produce quality seedlings, first, good quality seedlings in sanitary and physiological terms must be acquired, presenting good genetic and physical attributes. All these factors directly aid the yielding of quality seedlings, *i.e.*, with high germination percentages, viability, and vigor maintenance (CRUZ et al., 2018).

The sanitary quality of the seeds is another important factor for the production of seedlings, since the seeds used for propagation need to be healthy and pathogen-free. Seeds infected by diseases may not present viability or may have low vigor. The seed is a pathogen dissemination vector, which can, sometimes, cause disease surges in plants, since low quantities of inoculum in the seed may have great epidemical significance (PESKE et al., 2016).

The damage a pathogen could cause, considering each of the plants, from their seeds can be enumerated — pre-emergence death; root rot; damping-off; necrotic spots in leaves, stems, fruit, and vascular system; malformations (hypertrophy and subdevelopment); bleaching (deviations from the regular colors); latent infections, *etc.* However, it is recommended that the sanity tests and the physiological quality of seeds should be integrated using specific and efficient methods. For organic agriculture of seeds and seedlings of forest species, alternative methods have been implemented with the usage of essential oils that have an important function in the seed-pathogen interactions that might harm pre-emergence, among others (STANGARLIN et al., 2011).

Among the alternative methods that can be used in organic agriculture to reduce pathogens in forest seeds, the essential oil from thyme (*Thymus vulgaris* L.), a plant that belongs to the Lamiaceae family, aromatic native from the Mediterranean countries, presents high concentrations of chemical constituents such as thymol and carvacrol, substances that are responsible for antimicrobial activity against a wide microorganism spectrum, having carvacrol been studied for its bactericide effects (MERLIN; CRUZ, 2021).

According to Pereira et al. (2015), the effect of the thyme essential oil on *Oidio psistaurica* on the bell pepper crop reduces the severity of the fungus in 60.78%.

Tagami et al. (2009) say that the essential oil presented fungitoxic action on the micellar growth of the *Alternaria alternata* (Fr.) Kiessler, *Colletotrichum graminicola* (Ces.) Wils., *Rhizoctonia solani* Kuhn, *Sclerotium rolfsii* Sacc, and *Fusarium moniliforme* Sheldon fungi. Thus, the objective of this work was verifying the influence of the thyme essential oil on the sanitary and physiological quality of ironwood seeds.

MATERIAL AND METHODS

The experiment was carried out in a laboratory environment at the Mato Grosso State University (*Universidade do Estado do Mato Grosso* — UNEMAT), at the Cáceres *Campus*. The thyme essential oil was obtained from a live drugstore (concentrated), diluted for the preparation of the treatments. After the oil has been prepared, the seeds were dipped into the oil for 15 minutes. For each of the concentrations, the samples for each treatment were evaluated for their sanitary quality.

The ironwood seeds were acquired from matrix plants in the neighboring cities in the state of Mato Grosso and sent to the laboratory for manual enhancement. The drying of the seeds was carried out in ambient temperature (28-30°C) and average air relative humidity of 45-55%, packed into plastic bags (1 kg), and stored in a fridge at 4°C for 7 days.

After storage, the seeds were distributed in a double-layer filter paper, moistened with deionized distilled water, and incubated in Petri dishes under the ambient temperature of 25±2°C and 12 h photoperiod during 7 days (BRASIL, 2009). On the seventh day after the deployment of the test, with aid from an electronic microscope, fungi were identified via the observation of the structures, with further aid from specialized literature (BARNETT; HUNTER, 1972). The evaluation of the sanitary quality was carried out via the Blotter test (ISTA, 2017). After this period, the pigmentation of the structures was made with methylene blue and analysis of the texture and consistency of the verse and reverse of the developed colonies. The microstructures were put in microscope slides for observation using an electronic microscope (100x).

The characterization of fungi genera was carried out according to morphological criteria described in specialized literature (NITHIYAEATE et al., 2012; HAFIZI et al., 2013; EHGARTNER et al., 2017; NAYYAR et al., 2017; SANTOS et al., 2018). In order to verify the effects of the concentrations on the infested seeds percentage, the results were calculated according to Sangoi et al. (2000) (Equation 1) and expressed in percentages (%).

$$\%\text{ of infested seeds} = \frac{100 \times \#\text{ of infested seeds}}{\text{total }\#\text{ of seeds}} \quad \text{(Equation 1)}$$

Germination was carried out with four repetitions with 50 seeds for each treatment, planted on paper towel, moistened with water equivalent to three times the seed dry weight, and kept in Biochemical Oxygen Demand (BOD) at a constant temperature of 18°C with 8h of light per day. The count of normal seedlings was carried out on the seventh day after the deployment of the test (BRASIL, 2009). The first germination count and the germination speed index (GSI) were determined along with the germination test, being the count done on the fourth day after sowing, with results expressed as percentages (%) (BRASIL, 2009). For the GSI determination, the number of normal seedlings that emerged daily was counted, until complete stability was reached (MAGUIRE, 1962).

The length of the shoot and of the root parts of the ironwood seedlings were evaluated by measuring the shoot part up to the apex of the seedlings and by measuring the roots below the root node with aid of a millimeter ruler, calculating the mean results on each repetition, with results in mm plant^{-1}. The shoot part of the seedlings and the root system were placed in paper bags and taken to a greenhouse for forced convection at 65°C for 72 h until constant biomass has been reached for the calculation of the dry matter, and the results were expressed in mg plant^{-1}.

The completely randomized experimental design was used with five different concentrations of essential oil (0, 2, 4, 6, 8, and 10%) with four repetitions with 50 seeds. The obtained data were tabulated and submitted to the normality and homogeneity test via the Shapiro-Wilk and Bartlett's tests, respectively. The averages of the treatments were compared via the Scott-Knott test at 5% error probability via the R CORE TEAM statistical software (2017).

RESULTS AND DISCUSSION

According with the variance analysis, statistical differences were present when the ironwood seeds were immersed in essential oil from *Thymus vulgaris*, for which the concentrations of 2 and 4% presented higher occurrence of *Aspergillus* sp., A. niger, *Penicillium* sp., *Alternaria* sp., and *Fusarium* sp. At 6% concentration, there was an intermediate occurrence of the *Aspergillus* sp., *A. niger*, *Penicillium* sp., and *Alternaria* sp. fungi, with *Fusarium* sp. presenting a low incidence. Seeds immersed in thyme essential oil at concentrations of 8 and 10% presented lower occurrence of *Aspergillus* sp., *A. niger*, *Penicillium* sp., *Alternaria* sp., and *Fusarium* sp., however at the 10% concentration, there was a higher occurrence of *Fusarium* sp. when compared to the witness (0%) (Table 1).

TABLE 1 - Sanitary evaluation of ironwood seeds treated with different concentrations of *Thymus vulgaris* essential oil.

Treatments	*Aspergillus* sp.	*Aspergillus niger*	*Penicillium* sp.	*Alternaria* sp.	*Fusarium* sp.
Witness 0%	26.0 a*	20.0 a	24.0 a	10.0 a	10.0 a
T. vulgaris 2%	30.0 a	10.0 b	20.0 a	10.0 a	8.0 a
T. vulgaris 4%	8.0 b	10.0 b	18.0 a	8.0 a	12.0 a
T. vulgaris 6%	6.0 c	10.0 b	2.0 b	2.0 b	1.0 c
T. vulgaris 8%	0.0 d	0.0 c	0.0 c	1.0 c	0.0 c
T. vulgaris 10%	0.0 d	0.0 c	0.0 c	1.0 c	2.0 b
CV(%)	2.0	1.0	2.0	3.0	1.7

*Averages followed by the same letter within a column have no statistical difference, by the Scott-Knott test at 5% error probability.

Results similar to the ones presented in this work were obtained by Oliveira et al. (2008) by studying the effect of the *Lipia gracilis* (Verbanaceae) essential oil on ironwood seeds. The authors validated the control of fungi of the *Apergillus* sp. and *Penicillium* sp. genera. It is worth noting that, even though the *L. gracilis* oil is not of the same species or family of *Thymus vulgaris*, it presents in its constitution significant thyme (10%) and carvacrol (41.7%) contents, which are responsible for the antimicrobial activity (RASSOLI; MIRMOSTAFA, 2013). The essential oil of *T. vulgaris*, in the concentrations of 8 to 10%, was used in the control of *Colletotrichum gloeosporioides* in onion (*Allium cepa* L.) seeds, presenting some reduction in the occurrence of this fungus (LOZADA et al., 2019).

According to the results obtained in this work, it has been verified that the extract of *T. vulgaris* in all evaluated concentrations presented different results, highlighting that when the concentration of the essential oil is increased, there is a decrease in the occurrence of pathogens, proving that this species has specific action for some specific genera of fungus. Such fact can be explained by the antifungal activity of the thyme essential oil that results from the presence of thyme and carvacrol, which have antimicrobial activity against a wide spectrum of microorganisms. These terpenes bond with the amines and hydroxylamenes of proteins, present in fungi membranes, explained by the degeneration of hyphae that control the release of the cell content (DEMARTELAERE et al., 2021).

Hence, it is verified that the thyme essential oil can be used for the alternative control of fungi-based diseases that attack seeds, since the oil presents low toxicity to the environment and to men, and has high antifungal activity (MERLIN; CRUZ, 2021).

According to Table 2, the treatments presented significant difference at 5% error probability, since, when the thyme essential oil concentrations were used, the evaluations of the physiologic quality presented standard behavior for the germination percentage (G), first germination count (FGC), germination speed index (GSI), shoot part length (SPL), root part length (RPL), shoot part dry matter (SPDM), and root part dry matter (RPDM), for ironwood seeds and seedlings (Table 2).

Behavior similar to the presented in this work was verified by Demartelaere et al. (2021) in a study with *Caesalpinia ferrea* seeds treated with *Thymus vulgaris* essential oil, which presented an increase on the germination when compared to untreated seeds. Pereira et al. (2015), when evaluating the effect of thyme essential oil on the physiologic quality of ironwood seeds, observed that concentrations above 7% yielded longer shoot and root parts, shoot and root dry matter of *ferrea* seedlings, highlighting a positive effect of this oil on the development of seedlings. The high physiologic quality obtained in this research shows that the thyme essential oil presents substances that are volatile, lipophilic, with low molecular weight, generally presenting smell and in liquid form, comprised by terpenoid molecules, complex from several classes of substances, among them the phenylpropanoids, mono and sesquiterpenes, belonging to the secondary metabolism of plants (MORAIS, 2019).

TABLE 2 - Physiological quality of ironwood seeds immersed in different concentrations of *Thymus vulgaris* (*T. vulgaris*) essential oil.

Treatments	G	FGC	GSI	SPL	RPL	SPDM	RPDM
	---------------%-------------			mm seedling^{-1}		mg seedling^{-1}	
Witness 0%	30.0 b*	20.0 c	20.0 b	14 b	1.8 b	30.0 b	15.0 b
T. vulgaris 2%	33.0 b	32.0 b	14.0 c	14 b	2.0 b	30.0 b	13.0 b
T. vulgaris 4%	35.0 b	35.0 b	6.0 d	16 b	1.7 b	26.0 b	14.0 b
T. vulgaris 6%	35.0 b	35.0 b	7.0 d	15 b	1.8 b	20.0 b	12.0 b
T. vulgaris 8%	75.0 a	72.0 a	50.0 a	25 a	9.0 a	50.0 a	23.0 a
T. vulgaris 10%	13.0 c	13.0 d	12.0 c	8.0 c	3.0 c	15.0 c	7.0 c
CV(%)	5.0	2.0	2.8	2.0	3.0	2.0	1.0

*Averages followed by the same letter within a column have no statistical difference by the Scott-Knott test at 5% error probability.

The germination percentage obtained for the ironwood seeds varied from 13 to 75% (Table 2). It is important to emphasize that when the seeds were immersed into thyme essential oil at 8% concentration, they obtained the highest averages in all physiological evaluations, while with no oil or at concentrations of 2, 4 or 6%, they presented intermediate results on the same evaluations. At the concentration of 10% of essential oil, the physiological evaluations on ironwood seeds and seedlings presented the lowest averages. These results agree with the ones obtained by Lorenzi; Matos (2012), which verified that the germination of ironwood seeds was influenced by the concentrations of the considered chemical compounds, such as thyme and carvacrol, which can interfere directly on the germination potential.

Thyme and carvacrol, depending on their concentrations, can drastically inhibit the germination and the development of ironwood, since the seedlings did not present enough growth after emerged for the majority of the concentrations considered in this research. According to Oavyum et al. (2018), the allelochemical effects on different physiological processes of a plant depend on the applied concentrations. This sentence proves the results obtained in this work, since ironwood is a forest species susceptible to allelochemical compounds, also used as a reference in similar studies (FERREIRA; AQUILA, 2010).

It is worth noting another important aspect for the bad physiological performance of the treatments in the present study - the plants exposed to allelochemicals might present a decrease in their photosynthetical processes, however, the mechanisms affected by the action of these secondary compounds are still unknown, which might be associated to the chlorophyl metabolism, compromising photosynthesis (COUTINHO et al., 2020; ROCHA et al., 2019).

Modern agriculture has been searching for ecological alternatives, such as the utilization of essential oils on forest species for the control of pathogens, since, besides reducing the use of chemicals, it also reduces the risk of contamination of seeds, also protecting the environment. Moreover, all physiological and sanitary aspects of seeds are preserved, which favors uniformity and production growth, raising the value and quality of the byproducts, also aiding on the biome diversity and on the protection of the ecosystem (BARROCAS; MACHADO, 2010).

Studies concerning the *Thymus vulgaris* essential oil should continue - it should be tested on other species at other concentrations because the oil should have different action on the treatment of other forest species.

CONCLUSION

The concentration of 8% of the *Thymus vulgaris* essential oil presented the lowest percentages of *Aspergillus* sp., *Aspergillus niger*, *Penicillium* sp., *Alternaria* sp., and *Fusarium* sp. fungi and helped *Caesalpinia ferrea* seeds and seedlings reach higher efficiency on their physiological potential.

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