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Original Article

Physiological quality of Arabica coffee seeds treated with essential oils

Qualidade fisiológica de sementes de café arábica tratadas com óleos essenciais

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Abstract

The preservation of *Coffea arabica* L. seeds is essential to obtain vigorous and healthy seedlings, but there are no registered fungicides for their phytosanitary treatment during the storage phase. Therefore, essential oils appear as promising alternatives due to the presence of bioactive compounds with potential antimicrobial action, in addition to contributing to the sustainability of the production chain. This study aimed to evaluate the physiological quality of Arabica coffee seeds treated with different essential oils during storage. Six essential oils (*Rosmarinus officinalis, Cymbopogon citratus, Cymbopogon winterianus, Syzygium aromaticum, Eucalyptus globulus* and *Melaleuca alternifolia*), a synthetic fungicide (TECTO®) and a control were used, subjected to five storage periods (0, 3, 6, 9 and 12 months). The physiological quality of the seeds was verified by means of standard germination and vigor tests: moisture content, germination, accelerated aging, seedling emergence and emergence speed index. There was a significant interaction between treatments and storage time for all variables analyzed. The essential oils of *Rosmarinus officinalis* and *Cymbopogon winterianus* preserved seed germination and vigor for up to nine months, a performance similar to that of the synthetic fungicide. Thus, these oils stand out as viable alternatives to the conventional fungicide treatment of Arabica coffee seeds.

Keywords: coffee growing, germination, seed production, seed storage, vigor.

Resumo

A conservação das sementes de *Coffea arabica* L. é essencial para a obtenção de mudas vigorosas e sadias, porém não há fungicidas registrados para seu tratamento fitossanitário na fase de armazenamento. Diante disso, os óleos essenciais apresentam-se como alternativas promissoras, devido à presença de compostos bioativos com potencial ação antimicrobiana, além de contribuírem para a sustentabilidade para a cadeia produtiva. Este trabalho objetivou avaliar a qualidade fisiológica de sementes de café arábica tratadas com diferentes óleos essenciais durante o armazenamento. Foram utilizados seis óleos essenciais (*Rosmarinus officinalis, Cymbopogon citratus, Cymbopogon winterianus, Syzygium aromaticum, Eucalyptus globulus* and *Melaleuca alternifolia*), um fungicida sintético (TECTO®) e uma testemunha, submetidos a cinco períodos de armazenamento (0, 3, 6, 9 e 12 meses). A qualidade fisiológica das sementes foi verificada por meio do teste de germinação e vigor: teor de água, germinação, envelhecimento acelerado, emergência de plântulas e índice de velocidade de emergência. Houve interação significativa entre tratamentos e tempo de armazenamento para todas as variáveis analisadas. Os óleos essenciais de *Rosmarinus officinalis* e *Cymbopogon winterianus* preservaram a germinação e o vigor das sementes por até nove meses, desempenho semelhante ao do fungicida sintético. Dessa forma, esses óleos destacam-se como alternativas viáveis ao tratamento fungicida convencional de sementes de café arábica.

Palavras-chaves: cafeicultura, germinação, produção de sementes, armazenamento de sementes, vigor.

1. Introduction

In the Arabica coffee seedling production chain in Minas Gerais, the harvesting of ripe fruits for seed extraction takes place between April and June, while the planting of seedlings, usually aged 6 months or 1 year, takes place from October onwards. Thus, the seeds need to be stored for at least nine months before sowing in the nurseries. During storage, the high-moisture content in the seeds accelerates the loss of embryo viability and the deterioration of the reserve tissues, as it intensifies respiration and favors undesirable fermentations (Afonso Júnior et al., 2006).

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In addition, high humidity creates an environment conducive to the proliferation of microorganisms, insects and mites (Golldfarb and Queiroga, 2013).

Although drying and storage at low temperatures are widely used strategies for seed conservation, Arabica coffee seeds have intermediate tolerance to desiccation, being partially sensitive to both water loss and cold (Dussert et al., 2006). This particularity makes it difficult to simultaneously maintain the sanitary and physiological quality of the seeds throughout storage.

The chemical seed treatment helps preserve seeds during storage and in the initial stages of seedling establishment in nurseries or in the field (Menten & Moraes, 2010). However, to date, there are no records of chemical fungicides in the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) for pre-storage treatment of coffee seeds, via the AGROFIT (2023). Given this lack, some seed producers and nurseries have been using products that are not registered for coffee growing (Penido et al., 2021). Without adequate technical information, there is a risk of phytotoxicity or inefficiency in application, in addition to environmental risks and poisoning of those who handle the treated material.

Some plant species, such as medicinal and aromatic plants, produce substances, or classes of substances, of human interest for multiple uses, which can be directed to agriculture (Silva et al., 2018). Among the alternative phytosanitary treatments, essential oils stand out for their potential in seed preservation, with proven efficacy in controlling pathogens associated with numerous agricultural crops. Furthermore, its use meets the growing market demand for products that are less toxic to humans and the environment (Casali et al., 2015).

The recommendation for the use of an essential oil in seeds should consider both the efficacy in controlling the pathogen and the assessment of the risks of phytotoxicity. It is known that some constituents of essential oils interfere with germination, either as promoters or inhibitors of seedling growth (Duke et al., 2002). Examples of allelochemicals that can interfere with germination include sesquiterpenes, cyanogenic glycosides, alkaloids, flavonoids, phenolic acids, tannins, and others (King & Ambika, 2002; Oliveira et al., 2011).

Given the lack of information on the effects of essential oils on the physiological quality of Arabica coffee seeds, the objective of this study was to evaluate, during storage, the physiological quality of seeds treated with essential oils extracted from medicinal and aromatic plants popular in Brazil.

2. Material and Methods

This research was conducted between May 2021 and August 2022 at the Seed Research Laboratory of the Federal University of Viçosa, in Viçosa, Minas Gerais State, Brazil. Seeds of *Coffea arabica* L., cultivar Paraíso MG H419-1, 2021 crop, were used. These seeds were purchased from a seed production field registered with MAPA, located in the municipality of Paula Cândido, Minas Gerais (42º54′56.7″W; 20º49′46.3″S).

2.1. Harvest, processing, and seed conditioning

After manual harvesting of the cherries, the processing was carried out using a wet method, with mucilage removal in a natural fermentation tank for 12h00. All operations followed the standard routine of the farm, in compliance with MAPA, Normative Instruction Nº 35, dated November 29, 2012 (Brasil, 2012). In the Drying and Storage of Agricultural Products sector of the Agricultural Engineering Department at the Federal University of Viçosa, drying was performed on mesh screens under direct sunlight, resulting in a reduction of the moisture content from 46% to 25%. The initial moisture content was determined by the oven method at 105 ± 3ºC for 24h00, using four samples of 25 seeds, as described in (Brasil, 2009). The loss of water mass was monitored by weighing on a digital balance every 30 minutes, with manual stirring of the seeds every 15 minutes.

After drying, the seeds were fumigated with aluminum phosphide (Phosphine - Gastoxin®) for 48h00 to control the *Hypothenemus hampei*, a pest detected during the seed turning. Finally, the total mass of the seeds was divided into portions of 300 grams, corresponding to each experimental unit, and stored in polyethylene bags 0.12 mm thick.

2.2. Preparation and application of treatments

The selection of the six essential oil types and the concentration of 1% (v v^{-1}) was based on a literature review, considering their effectiveness in controlling fungi associated with seeds, their effects on physiological quality, and the commercial availability of the products. The essential oils were purchased from the Group LASZLO, with the following species, major chemical composition (>1%), and chromatographic analysis dates: *Rosmarinus officinalis*: α-pinene (4.0%), camphor (28.9%), eucalyptol (37.5%), limonene (20.8%), myrcene (1.1%), p-cymene (1.9%), and β-pinene (1.0%), November 2020. *Cymbopogon citratus*: myrcene (8.9%), 5-hepten-2-one (1.4%), neral (33.9%), geranial (46.1%), and geraniol (2.9%), February 2021. *Cymbopogon winterianus*: limonene (4.6%), linalool (1.3%), citronellal (45.5%), citronellol (1.1%), methyl eugenol (6.2%), geranyl acetate (7.8%), citronellyl formate (6.3%), γ-muurolene (2.1%), α-muurolene (1.4%), elemol (4.2%), and nerolidol (10.4%), March 2021. *Syzygium aromaticum*: eugenol (88.5%), caryophyllene (8.6%), and eugenyl acetate (1.7%), September 2020. *Eucalyptus globulus*: α-pinene (2.94%), p-cymene (5.67%), β-phellandrene (7.42%), and 1,8-cineole (81.53%), December 2020. *Melaleuca alternifolia*: α-pinene (4.4%), β-pinene (1.2%), α-terpinene (5.9%), p-cymene (6.7%), limonene (1.8%), 1,8-cineole (1.9%), γ-terpinene (17.5%), terpinolene (3.4%), terpinen-4-ol (42.4%), and α-terpineol (2.9%), March 2021. For the preparation of the solution, 50 µL of the essential oil were pipetted and diluted in 4.95 mL of distilled water.

Although there is no registered commercial product, the systemic fungicide TECTO® (Tiabendazole, 485 g L^{-1}) has been used by coffee producers and seed nurseries for conservation during storage. The usual dose of 1 mL per kg of seed, diluted in distilled water at 20% concentration, was used for evaluation. The control group received no fungicide, but 5 mL of distilled water were applied to standardize the moisture gain between treatments.

The solutions of each treatment were poured directly onto the seeds inside their packaging, followed by manual agitation until complete spreading. This methodology was chosen due to its practicality, allowing execution in environments with limited infrastructure, such as nurseries, and resembling the application of other seed treatments.

For 2h00 after treatment application, the packages were exposed to natural ventilation in a cool, airy location. They were then sealed and stored for up to 12 months in a refrigerated chamber (13 ± 2ºC, 70 ± 5% RH) provided by Empresa de Pesquisa Agropecuária de Minas Gerais - Sudeste.

2.3. Evaluation of seed moisture and physiological quality

Evaluations of seed physiological quality were carried out at the beginning of storage (month 0) and then every three months, covering the periods of 3, 6, 9, and 12 months of storage. The analyses followed the Seed Analysis Standards (Brasil, 2009), and when applicable, additional referenced and consolidated methodologies were used. Before the tests, the parchment of the seeds was manually removed.

Moisture content: For each plot, 75 seeds were used, distributed into three repetitions of 25 units. The samples were weighed on a digital balance with a precision of 0.001 g, before and after drying in an oven at 105 ± 3 °C for 24h00. The results were expressed as a percentage.

Germination test: For each plot, 200 seeds were used, distributed into four repetitions of 50 units. The seeds were placed on Germitest paper rolls, previously moistened with distilled water in a volume equivalent to 2.5 times their initial mass. The rolls were kept in a germinator at 30 ºC for 30 days, in the absence of light, with weekly re-watering. The results were expressed as the percentage of normal seedlings.

Accelerated aging test: Conducted according to the methodology described by (Carvalho, 2019). For each plot, 200 seeds were used, distributed into four repetitions of 50 units. The seeds were placed uniformly on a stainless-steel screen inside Gerbox containers (11 × 11 × 3.5 cm) containing 40 mL of distilled water and kept in a BOD chamber at 46 ºC for 48h. Afterward, the seeds were subjected to the germination test. The results were expressed as the percentage of normal seedlings.

Sand bed emergence test: The test was conducted following the methodology described by (Brandão Junior et. al, 2002) starting at three months of storage. For each plot, 200 seeds were used, distributed into four repetitions of 50 units. Seeding was performed at 3 cm depth in Styrofoam trays containing sterilized sand as substrate. The trays were kept in a growth chamber at 30 °C in the absence of light, with irrigation applied as needed. The emergence percentage was determined based on the total number of seedlings emerged until the population stabilized, with seedlings considered emerged when the cotyledon was exposed above the substrate in the "matchstick" shape. The results were expressed as the percentage of emerged seedlings.

Emergence Velocity Index: Determined according to the methodology of (Maguire, 1962), together with the sand bed emergence test. For each treatment, the number of emerged seedlings was counted daily, considering those in the "matchstick" shape and excluding those already counted on previous days until the population stabilized. The collected data were used to calculate the index using the following Equation 1.

$$EVI = \frac{N1}{D1} + \frac{N2}{D2} + \ldots + \frac{Nn}{Dn} \tag{1}$$

On, what:
EVI = emergence velocity index;
N = number of seedlings emerged on the counting day;
D = number of days after seeding when the count was performed.

2.4. Experimental statistics

For the tests of moisture content, germination and accelerated aging, a completely randomized experimental design (DIC) was used in an 8 × 5 factorial scheme with three repetitions. The treatments consisted of six essential oils (*R. officinalis, C. citratus, C. winterianus, S. aromaticum, E.s globulus,* and *M. alternifolia*), a synthetic fungicide (TECTO®), and a control group, evaluated at five storage times (0, 3, 6, 9, and 12 months).

For the emergence and emergence velocity index tests in the sand bed, a block experimental design (DBC) was adopted in an 8 × 4 factorial scheme with three repetitions. The treatments were the same as mentioned above, evaluated at four storage times (3, 6, 9, and 12 months). Each of the three shelves used in these tests was considered a block, with average temperature differences of 1.5 °C between them.

The results of the germination and vigor tests were analyzed using analysis of variance with the SAS software (SAS Institute Inc, 2023). When necessary, the effect of the type of product applied was compared using the Dunnett test, and the impact of the storage time factor was evaluated by regression, both at a 5% significance level.

3. Results and Discussion

Tables 1 and 2 present a summary of the analysis of variance of the physiological quality of Arabica coffee seeds treated with essential oils (EO), synthetic fungicide TECTO® and control, during storage times. Table 1 presents the variables analyzed in the DIC, and Table 2 the variables analyzed in the DBC.

Statistical analysis of the data indicated that all variables evaluated were significantly influenced by the type of product applied to the seeds and by the storage time. These two factors significantly impacted the variables both individually and in interaction, which justified the need for additional analysis to better understand the results. It is important to highlight that, as the block effect was not significant, the sand bed emergence data and the emergence speed index were broken down and discussed in the same way as the other variables.

Regarding the interactions observed in Tables 1 and 2, the averages of the treatment factor over time were analyzed. Table 3 presents the averages obtained for the moisture content (MC) variable.

Table 1. Summary of analysis of variance of moisture content (MC), germination (GM), accelerated aging (AA) of treated (T) and stored (M) Arabica coffee seeds.

VF	GL	MEAN SQUARES		
		MC	GM	AA
T	7	2.18	98.79	214.06
M	4	3.14	4,340.94	9,878.95
T x M	28	0.35 *	76.62 **	131.63 **
Error	80	0.20	32.10	51.20
CV	--	1.84%	7.95%	14.46%

VF: source variation; GL: Degrees of freedom; CV: Coefficient of Variation; *($p \leq 0.05$); **($p \leq 0.01$).

Table 2. Summary of analysis of variance of sand bed emergence (SBE) and sand bed emergence speed index (ESI) of treated (T) and stored (M) Arabica coffee seeds.

VF	GL	MEAN SQUARES	
		SBE	ESI
block	2	37.63 ns	0.027 ns
T	7	181.24	0.110
M	3	13,867.28	4.034
T x M	21	105.49 **	0.035 **
Error	62	33.60	0.014
CV	--	8.45%	12.10%

VF: source variation; GL: Degrees of freedom; CV: Coefficient of Variation; **($p \leq 0.01$); ns : not significant by F test at 5% probability.

Table 3. Averages of moisture content tests (%) in Arabica coffee seeds, according to treatments and storage times.

TREATMENT	STORAGE TIME				
	0 month	3 months	6 months	9 months	12 months
R. officinalis	24.67	23.62 ©®	24.41	24.46 ©	24.49
C. citrate	24.22 ©	24.13	23.63	24.47 ©	23.74 ©®
C. Winterianus	25.17	24.74	23.88	25.12	25.01
S. aromático	24.72	24.48	23.95	24.30 ©	24.51
E. globulus	24.23 ©	24.24	23.48	24.02 ©	24.18
M. alternifolia	25.19	24.84	23.65	25.34	25.30
TECTO®	25.17	24.68	24.04	24.40 ©	24.74
Control	25.60	24.65	24.12	25.52 ®	24.91

©Means that differ from the Control, by Dunnett's test, at 5% probability; ®Means that diverge from the TETO®, by Dunnett's test, at 5% probability.

The seed MC is directly related to its storage capacity, influencing physiological quality and microbial activity. Although some significant differences in SM were observed in relation to the control (25.06%), the overall average variation was only 0.46%; a value considered insufficient to compromise the comparison of the physiological potential of the seeds.

These small variations in MC can be attributed to the hygroscopicity of the seeds in equilibrium with the internal air of the packaging, which is classified as impermeable (thickness of 0.12 mm), according to Baudet (2003). Furthermore, the low variation in MC observed is a positive aspect of the methodology used for the application of EO, since both the volume of the solution and the application technique can increase humidity, which is an obstacle to the treatment of seeds in pre-storage.

Before discussing the variables related to germination and vigor, it is important to consider some aspects of the initial quality of the seed lot used. Even though they were recently harvested and subjected to standardized drying processes, the seeds presented relatively low averages. One of the factors that may have negatively influenced these results was the presence of the coffee berry borer (*Hypothenemus hampei*), observed in the samples. In a similar study, Souza (2023) evaluated the initial germination of the cultivar Paraíso MG H 419-1, from the same property in the 2021 harvest, and obtained averages of 79% in seeds with a moisture content of 20%.

Germination power is one of the main indicators of the physiological quality of seeds, and is used as a fundamental criterion for their commercialization. As established in Annex XXII of Normative Instruction N° 35, of November 29, 2012, of the MAPA, Arabica coffee seeds must present at least 70% germination (GM), 98% physical purity and absence of live insects to be commercialized (Brasil, 2012). In this context, it is observed that the seeds used as control maintained satisfactory germination percentages up to nine months of storage (Table 4).

The germination results (Table 4) indicate that the EO did not exert a negative effect on the seeds up to the ninth month of storage, since all treatments presented values statistically similar to those of the control and the fungicide TECTO®. In the last evaluation, only seeds treated with EO of *R. officinalis*, *C. winterianus* and *M. alternifolia* maintained germination percentages comparable to those of the control and TECTO®, standing out from the other oils evaluated.

The EO of *R. officinalis* demonstrated efficacy in controlling fungi without compromising GM in soybean seeds (*Glycine max*) stored in cold storage (Baiotto et al., 2023) and in three broad bean cultivars (*Phaseolus lunatus*) evaluated by Farias et al. (2020). In Arabica coffee seeds, Ribeiro et al. (2016) observed maintenance of germination above 70% for up to 15 months, using dehydrated and ground leaves of the same species. The EO of *C. winterianus* was effective in controlling *Colletotrichum gloeosporioides* in onion seeds, without compromising physiological quality (Lozada et al., 2019), and increased GM in fennel seeds (*Foeniculum vulgare*, according to Araújo Neto et al. (2012).

The absence of phytotoxicity of EO on the germination of Arabica coffee seeds is a crucial factor for their viability as an alternative in the control of storage fungi. This is because, according to Jabran (2017), EO can negatively interfere in the germination process when they come into contact with the seeds, due to the presence of certain chemical constituents capable of affecting the synthesis of phytohormones, the integrity of cell membranes and several essential physiological processes, such as photosynthesis, respiration, enzymatic activity, carbon flow, cell division and biosynthesis.

The accelerated aging (AA) test allows comparing the vigor of seeds that have undergone thermal stress, which can occur during storage or in the field phase (Dutra and Vieira, 2004). Observing the control results (Table 5), it is noted that the seed lot used was not very resilient to the thermal stress imposed by the test, not reaching the minimum germination limit for commercialization in any of the storage times evaluated. The low initial physiological quality of the lot may partly explain these results.

Although the observed averages did not reach the minimum value required for commercialization of coffee seeds, no significant differences were observed between the treatments with EO and the control until month 6 (Table 5). This result reinforces the hypothesis that the applied products did not compromise the physiological quality of the seeds. At nine months, the treatments with *R. officinalis*, *C. winterianus* and *E. globulus* presented higher averages than the control, suggesting a possible protective effect of these EO. At twelve months, the treatments with *C. citratus* and *E. globulus* differed from the control treated with TECTO®, standing out for the absence of normal seedlings.

When studying the influence of plant extracts on the physiological and sanitary quality of bean seeds (*Phaseolus vulgaris*), Silva et al. (2023) found higher percentages of AA in seeds treated with basil extracts (*Ocimum gratissimum*), horsetail (*Equisetum hyemale* L.) and citronella (*Cymbopogon Winterianus*). Regarding the evaluation of the performance of *E. globulus* EO in the AA test, Daronco et al. (2015) did not observe differences between seeds treated with EO (30%) and control.

According to Pertel (2004), both natural and artificial aging promote reduced seed germination and vigor, due to changes in the respiratory rate, soluble sugar and lipid levels. Furthermore, during the evaluations, it was found that tissue damage caused by stress favored the

Table 4. Germination averages (%) in Arabica coffee seeds, according to treatments and storage times.

TREATMENT	STORAGE TIME				
	0 month	**3 months**	**6 months**	**9 months**	**12 months**
R. officinalis	76.67	79.00	83.00	75.33	52.33
C. citrato	75.67	81.33	79.00	68.33	40.67 ©®
C. Winterianus	70.33	78.33	83.33	73.67	55.67
S. aromático	76.00	77.33	85.33	71.33	36.00 ©®
E. globulus	75.00	77.33	84.00	69.33	36.00 ©®
M. alternifolia	74.00	83.33	76.33	78.33	54.33
TECTO®	66.00	83.67	77.67	66.67	54.33
Controle	76.67	81.33	82.67	79.67	56.33

©Means that differ from the Control, by Dunnett's test, at 5% probability; ®Means that diverge from the TETO®, by Dunnett's test, at 5% probability.

proliferation of saprophytic fungi, which may justify the absence of germination in two of the treatments in the last period evaluated. These findings reinforce the importance of adequate control of environmental conditions during storage, since the physiological damage caused is largely irreversible.

Regarding the sand bed emergence (SBE) and emergence speed index (ESI) tests, they aim to simulate the performance of seeds in situations similar to those in the field; the higher the germination percentage, speed and uniformity, the more vigorous the batch (Brasil, 2009). The data from these tests are presented together in Table 6.

Significant differences were observed between treatments with EO, TECTO® and control at month 3, for SBE and EVI, only treatments with EO of *C. Winterianus* maintained SBE and EVI similar to the control and TECTO® at all times evaluated (Table 6). The good performance of the EO of *C. winterianus* in the seedling SBE was also

Table 5. Averages (%) of the accelerated aging test on Arabica coffee seeds, according to treatments and storage times.

TREATMENT	STORAGE TIME				
	0 month	3 months	6 months	9 months	12 months
R. officinalis	51.00	69.00	70.00	52.67 ©	17.00
C. citrate	45.33	69.33	64.00	46.67	00 ©®
C. Winterianus	46.33	68.67	68.67	60.00 ©	26.67
S. aromatic	48.00	68.00	68.00	58.33 ©	17.33
E. globulus	54.00	68.33	61.67	35.67	00©®
M. alternifolia	57.33	64.33	65.67	54.67 ©	21.00
TECTO®	53.33	62.33	62.33	47.00	23.00
Control	51.00	62.33	62.33	33.33	25.33

©Means that differ from the Control, by Dunnett's test, at 5% probability; ®Averages that diverge from the TECTO®, by Dunnett's test, at 5% probability.

Table 6. Averages of emergence tests in sand bed (%) and emergence speed index in Arabica coffee seeds, according to treatments and storage times.

TREATMENT	STORAGE TIME				
	0 month	3 months	6 months	9 months	12 months
	SANDBED EMERGENCY (%)				
R. officinalis	--	79.33 ©®	88.00	63.33	36.67
C. citrate	--	91.33	82.00	59.33	37.00
C. Winterianus	--	92.00	92.00	70.00	32.00
S. aromatic.	--	89.33	76.67	76.00	36.00
E. globulus	--	79.00 ©®	77.33	52.67	33.00
M. alternifolia	--	91.67	77.33	75.67	30.67
TECTO®	--	93.33	94.33	64.00	35.67
Control	--	92.67	82.33	73.00	42.33
	EMERGENCY SPEED INDEX				
R. officinalis	--	1.25	1.21®	0.73	0.56
C. citrate	--	1.37	1.23®	0.78	0.25 ©
C. Winterianus	--	1.42	1.42	0.99	0.41
S. aromatic.	--	1.37	1.09 ®	1.01	0.44
E. globulus	--	1.13 ©®	1.03 ®	0.64 ©	0.32 ©
M. alternifolia	--	1.40	1.09 ®	1.01	0.44
TETO®	--	1.45	1.50	0.82	0.49
Control	--	1.44	1.22 ®	0.98	0.58

© Means that differ from the Control, by Dunnett's test, at 5% probability; ®Means that diverge from the TECTO®, by Dunnett's test, at 5% probability.

reported by Nascimento (2017). When evaluating the application of this EO, at a concentration of 0.25%, on pepper seeds (*Capsicum annuum*), the author observed ESI, final emergence and dry mass indexes of seedlings similar to those of the control treatment, indicating the absence of phytotoxic effects. On the other hand, Xavier et al. (2012), when testing different dosages of this same EO (20 µL, 15 µL, 10 µL and 5 µL) in cowpea seeds (*Vigna unguiculata*), using the same application technique adopted in the present research, observed negative allelopathic effects . The treatments resulted in reductions in the emergence speed indexes, germination speed and seedling emergence speed coefficient.

Pierre (2009), when treating freshly harvested Arabica coffee seeds with OE and extract of *S. aromaticum*, also observed a positive effect on physiological quality. In the ESI test, conducted with 200 seeds, the highest values were recorded with EO concentrations of 0.25% (0.712), 0.5% (0.849) and 1.0% (0.770), in addition to the extract concentration at 20% (0.747). Although the EI was not evaluated at time 0 in this study, the values obtained at 3 and 9 months (Table 6) for the EO treatments were higher than those reported by Pierre (2009), reinforcing the potential of some EO to maintain or even improve the physiological performance of seeds during storage.

E. globulus EO was the treatment that differed most from the SBE and ESI of the control and TECTO®, presenting lower averages than them in almost all evaluated times (Table 6). According to Miranda et al. (2015), the variation in the results of the influence of EO on seed quality depends on many factors, including dosage. Retention of EO in the substrate can increase the contact time with the seedling, allowing the promotion of beneficial effects, such as seedling growth, or deleterious effects, such as membrane disorganization and dysregulation of respiration.

The breakdown of the effect of time within each treatment, obtained by regression analysis, is presented in Table 7. It is worth noting that three adjustment models were tested for the regression equation: linear, polynomial of degree 2 and degree 3. To validate the model, a coefficient of determination ≥ 60% was considered, in addition to the significance of all characteristics. Due to the proximity of the models, it was decided to present them as equations and not as graphs.

Regarding the behavior of the treatments during storage, the germination and vigor variables, except SBE and ESI, were adapted to the second-degree polynomial models (Table 7). Although the quadratic model is not ideal for biological behavior, the lower averages in month 0, in relation to months 3 and 6, may justify this mathematical trend. The fact that SBE and ESI were not evaluated in month 0, and presented a decreasing linear model, reinforces that the expected behavior for the physiological quality characteristics is a progressive reduction throughout storage.

The models tested did not adjust the behavior of the control, presenting an acceptable coefficient of determination for MC. However, as in the other treatments, no variations in moisture content were observed that were sufficient to interfere with the physiological quality. As an example, we have the seeds treated with the EO of *R. officin*alis, which reached the maximum MC (23.92%) at 5.2 months, a variation of only 0.7% in relation to the initial value (24.91%). This low variation in MC during storage can be justified by the high impermeability of the packaging used to store the samples. Although 0.12 mm polyethylene plastic packaging is considered semipermeable, this thickness is very close to the minimum limit to be classified as impermeable (≥0.125 mm), as proposed by Baudet (2003).

Regarding the other physiological quality characteristics of the control, the maximum values were observed in the first 6 months of storage, with GM (84.99%) at 4.5 months and AA around 3.4 months. SBE and ESI fell 5.33% and 0.09 per month (Table 7). Ribeiro et al. (2016) found different values for the cultivar Catuaí Vermelho IAC 44, with 42% moisture content, satisfactorily preserving the seeds in a cold chamber for up to 15 months, and in ambient conditions for 6 months. However, these differences may be associated with the different initial AT, type of cultivar, initial physiological quality, among other factors that influence seed storability (Marcos Filho, 2015).

R. officinalis EO, the maximum GM values (83.71%) were adjusted at times close to the control, around 4 months of storage. AA presented the highest $\hat{Y}$ value (71.87%), at 4.5 months (Table 7). Some chemical constituents of EO are capable of altering some metabolic processes of germination, such as citral and its derivatives, which interfere in the production of auxin, a hormone involved in cell division and elongation (Jabran, 2017; Graña et al., 2013). Analyzing the angular coefficient of the model adjusted for SBE and ESI, it is clear that this treatment had the smallest monthly drop, reducing 5.08% and 0.085, respectively.

Seeds treated with *C. citratus EO* presented better physiological performance before completing 5 months of storage. Germination was the variable that presented the maximum value with the shortest storage time, with 3.3 months (Table 7). According to the model adjusted for AA, at 12 months the estimated value was only 0.49%, the lowest among the treatments evaluated (Table 7). However, the natural loss of viability of coffee seeds during storage, which occurs mainly due to the reduction in total antioxidant capacity, associated with the thermal stress imposed during this test, may have contributed to this low value (Ferreira et al., 2020). The reduction in physiological quality in this treatment was also observed in ESI, which presented the sharpest drop (0.127%).

Although the effect of *C. citratus* EO did not surpass that of the other EOs during storage, the performance did not differ from the control (Table 7). According to Nascimento et al. (2021), the effectiveness of an EO in seed treatment depends on the chemical composition, exposure time, application technique, among others. Gomes et al. (2019) highlight that the variations in the effects of the use of EO on seeds, whether positive or not, depend mainly on the combination and proportion of the chemical constituents of the EO and the interaction between the species studied. In addition, the potential use of the EO of *C. citratus* for use in alternative seed treatments must be evaluated in conjunction with the results of sanitary control.

Table 7. Adjusted regression equations of germination and vigor variables in stored Arabica coffee seeds, with respective coefficients of determination (R^2/r^2) and maximum adjusted values ($\hat{Y}$) for storage time (M^o).

TREATMENT	EQUATION	R^2/r^2	$\hat{Y}$	M^o
		MOISTURE CONTENT (%)		
R. officinalis	$\hat{Y}= 24.691 - 0.2953^{*} x + 0.0284^{**} x^2$	0.920	23.92	5.20
S. S. aromatic.	$\hat{Y}= 24.769 - 0.19160^{*} x + 0.0143^{*} x^2$	0.792	24.13	6.70
		GERMINATION (%)		
R. officinalis	$\hat{Y}= 74.829 + 4.1921^{**} x - 0.4947^{**} x^2$	0.948	83.71	4.24
C. citrato	$\hat{Y}= 74.886 + 4.3762^{**} x - 0.5952^{**} x^2$	0.992	82.93	3.68
C. Winterianus	$\hat{Y}= 69.543 + 5.2159^{**} x - 0.5291^{**} x^2$	0.983	82.40	4.93
S. aromático	$\hat{Y}= 72.781 + 6.2127^{**} x - 0.7566^{**} x^2$	0.939	85.53	4.11
E. globulus	$\hat{Y}= 72.295 + 5.9587^{**} x - 0.7354^{**} x^2$	0.955	84.37	4.05
M. alternifolia	$\hat{Y}= 73.895 + 4.0142^{**} x - 0.4577^{**} x^2$	0.876	82.70	4.39
TECTO®	$\hat{Y}= 68.448 + 4.8460^{**} x - 0.5159^{**} x^2$	0.897	79.83	4.70
Control	$\hat{Y}= 75.181 + 4.3349^{**} x - 0.4788^{**} x^2$	0.932	84.99	4.53
		ACCELERATED AGING (%)		
R. officinalis	$\hat{Y}= 50.848 + 9.1571^{**} x - 0.9973^{**} x^2$	0.999	71.87	4.59
C. citrate	$\hat{Y}= 45.826 + 10.826^{**} x - 1.2170^{**} x^2$	0.994	69.90	4.45
C. Winterianus	$\hat{Y}= 46.523 + 9.8286^{**} x - 0.9524^{**} x^2$	0.990	71.88	5.16
S. aromatic.	$\hat{Y}= 47.324 + 10.173^{**} x - 1.0450^{**} x^2$	0.985	72.08	4.87
E. globulus	$\hat{Y}= 55.018 + 6.6758^{**} x - 0.9470^{**} x^2$	0.995	66.78	3.52
M. alternifolia	$\hat{Y}= 55.685 + 6.1759^{**} x - 0.7433^{**} x^2$	0.978	68.51	4.15
TECTO®	$\hat{Y}= 53.178 + 5.2135^{**} x - 0.6455^{**} x^2$	0.998	63.70	4.04
Control	$\hat{Y}= 53.266 + 3.7658^{*} x - 0.5370^{**} x^2$	0.851	59.87	3.51
		SANDBED EMERGENCY (%)		
R. officinalis	$\hat{Y}= 105.000 - 5.0889^{**} x$	0.763	--	--
C. citrate	$\hat{Y}= 113.833 - 6.1889^{**} x$	0.971	--	--
C. Winterianus	$\hat{Y}= 122.000 - 6.7333^{**} x$	0.849	--	--
S. aromatic.	$\hat{Y}= 109.667 - 5.3556^{**} x$	0.802	--	--
E. globulus	$\hat{Y}= 101.167 - 5.4222^{**} x$	0.917	--	--
M. alternifolia	$\hat{Y}= 115.000 - 6.1556^{**} x$	0.813	--	--
TECTO®	$\hat{Y}= 122.667 - 6.7778^{**} x$	0.884	--	--
Control	$\hat{Y}= 112.667 - 5.3444^{**} x$	0.909	--	--
		EMERGENCY SPEED INDEX		
R. officinalis	$\hat{Y}= 1.575 - 0.0851^{**} x$	0.906	--	--
C. citrate	$\hat{Y}= 1.860 - 0.1269^{**} x$	0.947	--	--
C. Winterianus	$\hat{Y}= 1.918 - 0.1144^{**} x$	0.871	--	--
S. aromatic.	$\hat{Y}= 1.697 - 0.0960^{**} x$	0.901	--	--
E. globulus	$\hat{Y}= 1.482 - 0.0933^{**} x$	0.953	--	--
M. alternifolia	$\hat{Y}= 1.727 - 0.0987^{**} x$	0.913	--	--
TECTO®	$\hat{Y}= 1.953 - 0.1186^{**} x$	0.866	--	--
Control	$\hat{Y}= 1.753 - 0.0932^{**} x$	0.978	--	--

$^{*/**}$ Significant for $p \leq 0.05$ and $p \leq 0.01$, respectively.

S. Aromatum EO, the maximum adjusted values for the germination and vigor variables occurred between the fourth and sixth months of storage, with GM (85.53%) and AA (72.08%) being higher than the other treatments. The angular coefficients of the SBE indicate that the monthly decrease of this treatment was similar to that of *E. globulus* and the control, with an average reduction of 5.35% (Table 7). Regarding ESI, the reduction was similar to *E. globulus, M. alternifolia* and control, with an average monthly drop of 0.096. Silva et al. (2013) evaluated the viability and degree of infestation of cowpea seeds (*Vigna unguiculata* L. Walp), treated with different concentrations of *S. Aromatium* EO and stored in different types of environments; and also obtained better results in physiological quality up to 6 months, with the exception of the highest concentration (8%) which promoted phytotoxic effects.

For seeds treated with *E. globulus* EO, the maximum estimated GM (84.36%) was at 4 months (Table 7). As occurred with the other EOs and the control, the best physiological quality values were between the third and fifth month of storage. Similar observations occurred in the treatment with *M. alternifolia* EO, in which most variables presented maximum values between 3 and 5 months.

Seeds treated with TECTO® maintained physiological quality similar to the control throughout storage. It should be noted that, although this treatment preserved the physiological quality of the coffee seeds, the risks of using a phytosanitary product not recommended for the crop, a practice used by nurseries and coffee seed producers, include possible phytotoxicity, inefficiency of application, in addition to environmental damage and poisoning to those who handle the treated material (Penido et al., 2021).

Although no toxic effects of this fungicide dose were observed in Arabica coffee seeds, given the promising results found in EO treatments and the environmental advantages that this natural product offers, it is important to encourage the use of these more natural products in coffee agriculture. Another relevant factor that encourages the use of these natural products instead of synthetic ones is that they can be extracted from medicinal and aromatic plants of the Brazilian flora, facilitating access for rural producers, who can even grow them on their properties (Cunico et al., 2006).

4. Conclusions

The essential oils of *Rosmarinus officinalis* and *Cymbopogon winterianus* preserved the germination and vigor of Arabica coffee seeds during nine months of storage.

The essential oils of *Rosmarinus officinalis*, *Cymbopogon citratus*, *Cymbopogon winterianus*, *Syzygium aromaticum*, *Eucalyptus globulus*, and *Melaleuca alternifolia* did not negatively affect the germination behavior or seed vigor of Arabica coffee over a twelve-month storage period.

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Data Availability Statements

The entire data set that supports the results of this study was published in the article itself.

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