JOURNAL OF EDUCATION, SCIENCE AND HEALTH – JESH
Revista de Educação, Ciência e Saúde
DOI: https://www.doi.org/10.52832/jesh.v5i3.660
Home page: https://bio10publicacao.com.br/jesh
e-ISSN: 2763-6119

TREATMENT OF SUNFLOWER SEEDS (*Helianthus annuus*) WITH ESSENTIAL OIL MICROEMULSIONS

TRATAMENTO DE SEMENTES DE GIRASSOL (*Helianthus annuus*) COM MICROEMULSÃO DE ÓLEOS ESSENCIAIS

TRATAMIENTO DE SEMILLAS DE GIRASOL (*Helianthus annuus*) CON MICROEMULSIÓN DE ACEITES ESENCIALES

Arieli Caldeira da Cunha [1]; Sumária Sousa e Silva [2]; Luiz Carlos Pascuali [3]; Alexandre Gonçalves Porto[4*]; José Wilson Pires Carvalho[5*]

Acadêmica do Curso de Engenharia de Produção Agroindustrial, Universidade do Estado de Mato Grosso (UNEMAT), Barra do Bugres, Mato Grosso, Brasil; [2] Doutora Pelo Instituto de Química de São Carlos, Universidade do estado de São Paulo (IQSC/USP). Técnica em Assuntos Educacionais no Instituto Federal de Mato Grosso (IFMT), Alta Floresta, Mato Grosso, Brasil; [3] Doutor pela Universidade Federal de Pelotas, (UFPEL). Docente da Faculdade de Arquitetura e Engenharias, Universidade do estado de Mato Grosso, Barra do Bugres, Brasil; [4] Doutor pela Universidade Federal de Pelotas, (UFPEL). Docente da Faculdade de Arquitetura e Engenharias, Universidade do estado de Mato Grosso, Barra do Bugres, Brasil; [5] Doutor Pelo Instituto de Química de São Carlos, Universidade do estado de São Paulo (IQSC/USP). Docente da Faculdade de Arquitetura e Engenharias, Universidade do estado de Mato Grosso, Barra do Bugres, Brasil.
*Autor correspondente: jwilsonc@unemat.br

Received: 18/02/2026 | **Approved:** 20/05/2026 | **Published:** 25/08/2026

Abstract: Synthetic pesticides have been used to control agricultural pests and diseases. However, these products often harm the natural enemies of pests. In this context, this study aimed to evaluate the effect of essential oil microemulsions of long pepper (*Piper hispidinervum*), lemon balm (*Lippia alba*), lemongrass (*Cymbopogon citratus* Stapf), and citronella (*Cymbopogon nardus* L.), in the combinations lemongrass + citronella + long pepper and lemon balm + citronella + long pepper, on the germination and vigor of *Helianthus annuus* seeds. Sunflower was selected because of its commercial importance in the production of high-quality vegetable oil, animal feed, silage, and crop rotation systems. Microemulsion formulations were prepared with essential oil combinations at concentrations ranging from 1,000 to 7,000 ppm and applied to sunflower seeds. The evaluated parameters were first germination count, germination, soil emergence, emergence speed index (ESI), shoot dry mass, and root dry mass. The control sample received only distilled water. The results were statistically analyzed using Duncan's test at a 5% probability level. The combinations of essential oils did not significantly affect sunflower seed development, as first germination count, germination, emergence speed index (ESI), shoot dry mass, and root dry mass did not differ statistically from the control. Soil emergence decreased at concentrations of 2,000 and 3,000 ppm. Overall, the results indicate that essential oils can be an alternative to synthetic pesticides in seed treatment without significantly affecting seed germination and vigor.

Keywords: Sustainability. Natural product. Organic products. Sustainable agriculture. Seed vigor.

Resumo: Os defensivos agrícolas sintéticos têm sido utilizados no controle de pragas e doenças da agricultura. Porém, esses produtos sintéticos muitas vezes prejudicam inimigos naturais de pragas. Nesse contexto, o objetivo deste estudo foi avaliar o efeito de microemulsões de óleos essenciais de pimenta-longa (*Piper hispidinervum*), melissa (*Lippia alba*), cidreira (*Cymbopogon citratus* Stapf) e citronela (*Cymbopogon nardus* L.), nas combinações cidreira+citronela+pimenta-longa e melissa+citronela+pimenta-longa, na germinação e vigor de semente de *Helianthus annuus*. O girassol foi utilizado no estudo devido sua importância comercial na produção de óleo vegetal de alta qualidade, ração animal e na rotação de culturas. Foram realizadas formulações de microemulsões com as combinações de óleo essencial com contração entre 1.000 -7.000 ppm. Os parâmetros avaliados foram: primeira contagem de germinação, germinação, emergência em solo, índice de velocidade de emergência (IVE), massa seca aérea e massa radicular. A amostra testemunha recebeu somente água destilada. Os resultados foram analisados estatisticamente com teste de Duncan à 5% de probabilidade. Os resultados mostram que as combinações de óleos essenciais não interferiram de forma significativa no desenvolvimento das sementes de girassol, tendo em vista que primeira contagem de germinação, germinação, índice de velocidade de emergência (IVE), massa seca aérea e massa radicular não diferiram estatisticamente da testemunha. Para a emergência em solo houve uma

diminuição nas concentrações 2.000 e 3.000 ppm. Por fim, esses resultados mostram que os óleos essenciais podem ser uma alternativa aos defensivos sintéticos no tratamento de sementes sem interferir significativamente na germinação e vigor de sementes.

Palavras-chave: Sustentabilidade. Produto natural. Produtos orgânicos. Agricultura sustentável. Vigor de sementes.

Resumen: Los plaguicidas sintéticos se han utilizado para controlar plagas y enfermedades agrícolas. Sin embargo, estos productos sintéticos suelen dañar el medio ambiente y a los enemigos naturales de las plagas. En este contexto, el objetivo de este estudio fue evaluar el efecto de microemulsiones de aceites esenciales de pimienta larga (*Piper hispidinervum*), melisa (*Lippia alba*), hierba limón (*Cymbopogon citratus* Stapf) y citronela (*Cymbopogon nardus* L.), en las combinaciones hierba limón + citronela + pimienta larga y melisa + citronela + pimienta larga, sobre la germinación y el vigor de semillas de Helianthus annuus. El girasol se utilizó en este estudio debido a su importancia comercial y versatilidad en la producción de aceite vegetal de alta calidad, salvado para alimentación animal, ensilaje y rotación de cultivos. Se prepararon formulaciones de microemulsión con combinaciones de aceites esenciales en concentraciones entre 1000 y 7000 ppm y se aplicaron a semillas de girasol. Los parámetros evaluados fueron: recuento de la primera germinación, germinación, emergencia del suelo, índice de velocidad de emergencia (IEE), masa seca del tallo y masa radicular. La muestra de control recibió únicamente agua destilada. Los resultados se analizaron estadísticamente mediante la prueba de Duncan con un nivel de significancia del 5%. Los resultados muestran que las combinaciones de aceites esenciales no interfirieron significativamente en el desarrollo de las semillas de girasol, ya que el recuento de la primera germinación, la germinación, el índice de velocidad de emergencia (IEE), la masa seca del tallo y la masa radicular no difirieron estadísticamente del control. En cuanto a la emergencia del suelo, se observó una disminución en concentraciones de 2000 y 3000 ppm. Finalmente, estos resultados demuestran que los aceites esenciales pueden ser una alternativa a los plaguicidas sintéticos en el tratamiento de semillas sin interferir significativamente en la germinación ni en el vigor de las semillas.

Palabras-clave: Sostenibilidad. Producto natural. Productos orgánicos. Agricultura sostenible. Vigor de las semillas.

1 INTRODUCTION

Helianthus annuus is an annual crop belonging to the Asteraceae family. Plants in this family can reach up to 3 m in height, although some species are approximately 40 cm tall. The leaves are oval, petiolate, and have rough veins, and the flowers are large and showy. The scientific name "Helianthus" means "sunflower," reflecting the plant's appearance (Oliveira et al., 2012; Turchetto et al., 2021).

Sunflower has broad adaptability to different latitude, longitude, and photoperiod conditions. According to the Brazilian Agricultural Research Corporation (EMBRAPA), sunflower originated in North America. However, it is cultivated worldwide and is classified as the fourth most produced oilseed, accounting for approximately 13% of global vegetable oil production (Santos, 2018).

In Brazil, the Central-West region stands out in sunflower production, with the crop used not only for oil but also for human and animal feed. In the state of Mato Grosso, sunflower has been a good option for crop rotation, and the planted area has varied across cropping seasons. In addition, this crop has several uses, including vegetable oil production, bran for animal feed, and silage, because almost the entire plant can be used. For this reason, it has gained national and international prominence (Turchetto et al., 2021).

Sunflower oil stands out among vegetable oils because of its beneficial physicochemical and nutritional characteristics. Its composition includes fatty acids of high nutritional quality, with approximately 65.3% polyunsaturated fatty acids, including 65% linoleic acid, a compound beneficial to the human body (Castro, Castiglioni & Balla, 1996).

However, sunflower crops are attacked by more than 35 species of phytopathogenic organisms, most of which are fungi. It is estimated that approximately 12% of annual global losses in sunflower production are caused

by diseases. This is the main limiting problem faced by the crop in most sunflower-producing regions (Gomes et al., 2008; Gomes et al., 2024).

Thus, the search for alternative products with formulations based on natural substances and lower environmental impact, as replacements for synthetic pesticides, has been increasing. However, studies on the application of essential oils are still limited, and most of the available studies have been conducted in vitro (Silva et al., 2019; Monteiro et al., 2020; Queiroz et al., 2020; Pinheiro, Rezende & Krinski, 2022; Oliveira et al., 2024).

Accordingly, this study aimed to evaluate the application of microemulsions containing essential oils of long pepper (*Piper hispidinervum*), lemon balm (*Lippia alba*), lemongrass (*Cymbopogon citratus* Stapf), and citronella (*Cymbopogon nardus* L.), in the combinations lemongrass + citronella + long pepper and lemon balm + citronella + long pepper, on the germination and vigor of *Helianthus annuus* seeds.

2 MATERIALS AND METHODS

This research was conducted at the State University of Mato Grosso (UNEMAT), Deputado Estadual Renê Barbour campus, in the municipality of Barra do Bugres, Mato Grosso (MT), Brazil. The laboratory experiments were carried out at the Laboratory of Agroindustrial Engineering and Processing (LEPA). The field experiments were conducted in the facilities of Unit II of UNEMAT Barra do Bugres.

2.1 Extraction of essential oils and acquisition of sunflower seeds

Essential oils were extracted from long pepper (*Piper hispidinervum*), lemon balm (*Lippia alba*), lemongrass (*Cymbopogon citratus* Stapf), and citronella grass (*Cymbopogon nardus* L.) plants grown in the experimental area of UNEMAT Unit II, Barra do Bugres.

The plant material used for essential oil extraction was harvested in the morning and kept in the shade until extraction. The essential oils were extracted by steam distillation using an extractor with a capacity of 60 kg of plant material (Linax). The essential oil obtained was stored in amber glass bottles and kept in a temperature-controlled environment at 25 °C until the studies were conducted.

Sunflower seeds (*Helianthus annuus*) were harvested from a plantation located at UNEMAT Unit II. After harvest, the seeds were manually selected, stored in PET (polyethylene terephthalate) containers, and kept in a BOD (Biological Development of Microorganisms) incubator at 10 °C until the studies were conducted.

2.2 Studies with essential oils

For seed testing and preparation, the procedures described in the Rules for Seed Analysis (Brasil, 2009) were followed. Eight samples containing 100 seeds each were manually counted, weighed on a semi-analytical balance, and used to calculate the thousand-seed weight.

Seed treatment was performed with microemulsions of essential oils of long pepper (*Piper hispidinervum*), lemon balm (*Lippia alba*), lemongrass (*Cymbopogon citratus* Stapf), and citronella grass (*Cymbopogon nardus* L.), in ternary combinations of lemongrass + citronella + long pepper and lemon balm + citronella + long pepper, at equal volume proportions. The microemulsions were prepared in the concentration range of 1,000 to 7,000 ppm

(parts per million) of essential oils, using Tween 80 at 500 ppm as the emulsifying agent. The microemulsion was applied under agitation, with circular movements, until the seed surface was completely covered, at a volume of 1.5 mL per 15 g of sunflower seeds (Pascuali et al., 2018; Silva et al., 2019; Queiroz et al., 2020; Oliveira et al., 2024).

The soil emergence test was conducted with four replicates of 50 seeds each, sown in rows at a depth of 4 cm. One day before sowing, the seedbeds were prepared and irrigated to maintain adequate moisture. After sowing, the seedbeds were watered once a day. Counts were performed from the beginning of emergence, from the 4th to the 15th day after sowing. The results were expressed as the percentage of emerged seedlings (Brasil, 2009).

The emergence speed index (ESI) was evaluated together with the soil emergence test. The emerged seedlings were counted daily, always at the same time, to minimize variation in the data. Counts were performed from the fourth day after sowing until the fifteenth day. The emergence speed index was calculated according to Maguire (1962), using the formula: ESI = N1/D1 + N2/D2 + ... + Nn/Dn, where N is the number of seedlings recorded on each counting day and D is the number of days after sowing on which the count was performed.

On the eleventh day, normal seedlings from the germination test were carefully removed to avoid loss of the root system. They were washed, separated into shoot and root portions, placed in Kraft paper bags, and dried in a forced-air oven at 70 °C for 48 h. After drying, they were weighed on a semi-analytical balance, and the results were expressed in milligrams per seedling.

Finally, moisture content was determined according to the Rules for Seed Analysis (Brasil, 2009), using 5 g of seeds. The samples were weighed on a semi-analytical balance and kept in a forced-air oven at 105 °C for 24 h. The results were expressed as moisture percentage on a wet basis.

2.3 Statistical analysis

The experimental design was completely randomized, with four replicates per treatment. The data obtained were subjected to analysis of variance (ANOVA), and means were compared using Duncan's test at a 5% probability level with the WinStat statistical software (Machado & Conceição, 2005).

3 RESULTS AND DISCUSSION

Sunflower seed water content was determined before seed treatment with the microemulsions, and the moisture percentage was 4.6 ± 0.1%. José et al. (2010), using sunflower seeds supplied by a seed production company located in Uberlândia, Minas Gerais, found a moisture content of 4.7%, which is similar to the value found in the present study. In addition, Alves et al. (2012), using seeds of the Hélio 360 sunflower hybrid obtained from plots sown with different proportions of high- and low-vigor seeds and cultivated on an experimental farm in Uberlândia, Minas Gerais, found moisture contents between 6.44% and 6.63%. Rodrigues et al. (2018), using sunflower grains acquired from the local market in Campina Grande, Paraíba, found a moisture content of 9.07 ± 0.01%. Therefore, the moisture content found in the sunflower seeds used in the present study is close to values reported in the literature (José et al., 2010; Alves et al., 2012; Rodrigues et al., 2018). It is worth noting that seed

moisture content can directly affect the interaction between the essential oil and the seed surface, consequently affecting seed treatment and viability (José et al., 2010; Alves et al., 2012; Rodrigues et al., 2018).

3.1 First germination count and germination

Table 1 presents the percentages of first germination count and germination of sunflower seeds treated with essential oil microemulsions. For the microemulsion containing lemongrass + citronella + long pepper, no significant difference was observed in the essential oil concentration range evaluated compared with the control, which received only water (53–54%). The same was observed for the microemulsion containing lemon balm + citronella + long pepper (44–53%), with coefficients of variation of 10.2% for the first count and 9.4% for germination (Table 1). The same trend was observed for germination, with no statistical difference between the two microemulsion formulations in relation to the control or to the essential oil concentration. These results suggest that the different essential oils did not significantly influence sunflower seed germination within the concentration range of 1,000 to 7,000 ppm (Table 1).

Table 1. First germination count and germination of sunflower seedlings treated with essential oil microemulsions.

Concentration (ppm)	Lemongrass + Citronella + Long pepper		Lemon balm + Citronella + Long pepper	
	First count	Germination	First count	Germination
0	53[a]	84[a]	53[a]	84[a]
1,000	52[a]	76[a]	53[a]	84[a]
2,000	47[a]	84[a]	53[a]	77[a]
3,000	50[a]	84[a]	50[a]	79[a]
4,500	54[a]	84[a]	46[a]	81[a]
7,000	54[a]	87[a]	44[a]	73[a]
Coefficient of variation	10.2	9.4	10.2	9.4

Note: Means followed by the same letters do not differ from one another according to Duncan's test at a 5% probability level.
Source: Own authors.

Dias et al. (2021) reported that citronella essential oil at 1% (10,000 ppm), using Tween 80 as an emulsifier, reduced the first germination count and germination of corn seeds (*Zea mays* L.).

Olinto et al. (2023) analyzed the effect of citronella essential oil at 1,000 ppm, using Tween 80 as an emulsifier, on the physiological quality of seeds of native species such as craibeira (*Tabebuia aurea* (Manso) Benth. & Hook. f. ex S. Moore), pink ipê (*Handroanthus heptaphyllus*), and jurema-branca (*Piptadenia stipulacea* (Benth.) Ducke). They observed that the essential oil did not significantly affect seed germination.

The results obtained for sunflower seeds (Table 1) indicate that the microemulsions did not significantly affect seed physiological quality, vigor, or viability. Moreover, according to the literature, the effect of essential oils on physiological quality, vigor, and viability may vary depending on the seed type or species. In the present study, no negative interference of essential oils was observed in sunflower seeds (Silva et al., 2019; Dias et al., 2021; Olinto et al., 2023).

Table 2 presents the emergence speed index and soil emergence results for sunflower seeds treated with the microemulsion formulations lemongrass + citronella + long pepper and lemon balm + citronella + long

pepper at concentrations from 1,000 to 7,000 ppm.

The coefficients of variation were 17.5% for emergence speed index and 11.9% for soil emergence. The emergence speed index did not differ significantly for sunflower seeds treated with the two formulations compared with the control, which received only water (Table 2). However, soil emergence differed significantly for seeds treated with 3,000 ppm of the lemongrass + citronella + long pepper formulation and with 2,000 ppm of the lemon balm + citronella + long pepper formulation, while the other concentrations did not differ significantly from the control, which received only distilled water (Table 2).

Table 2. Emergence speed index and soil emergence of sunflower seedlings treated with essential oil microemulsions.

Concentration (ppm)	Lemongrass + Citronella + Long pepper		Lemon balm + Citronella + Long pepper	
	Emergence speed index (%)	Soil emergence (%)	Emergence speed index (%)	Soil emergence (%)
0	5.7[a]	61.5[ab]	5.7[a]	61.5[ab]
1,000	5.4[a]	58.8[b]	5.8[a]	63[ab]
2,000	6.6[a]	70.8[ab]	5.3[a]	60.1[b]
3,000	5.5[a]	60.1[b]	5.6[a]	62.1[ab]
4,500	6.0[a]	65.3[ab]	6.5[a]	69.9[ab]
7,000	6.0[a]	65.6[ab]	6.6[a]	73.9[a]
Coefficient of variation	17.5	11.9	17.5	11.9

Note: Means followed by the same letters do not differ from one another according to Duncan's test at a 5% probability level.

Source: Own authors.

Duarte (2018), using citronella essential oil at 500 µL/100 mL of sterile distilled water and three drops of concentrated Tween (approximately 5,000 ppm), observed a significant reduction in the germination speed index for the sunflower cultivars BRS 324, Aguará 4, and CF 11, from 24.76% to 8.54%, from 24.66% to 0.89%, and from 25.00% to 5.26%, respectively.

Silva et al. (2019) evaluated the vigor of Caiapó rice seeds (*Oryza sativa* L.) treated with citronella essential oil, lemon balm essential oil, and the citronella–lemon balm combination at 5,000 ppm, using 1,000 ppm of Tween 80 as an emulsifier. The results showed that citronella and lemon balm essential oils, as well as the citronella–lemon balm combination, completely inhibited Caiapó rice seed development.

The results obtained with the essential oil combinations are relevant because the emergence speed index (ESI) and soil emergence indicate the vigor of treated seeds and the crop establishment potential, which can directly influence plant productivity. In this study, decreases in these parameters were observed only at concentrations of 2,000 and 3,000 ppm (Table 2). Moreover, each crop has specific characteristics and sensitivity to essential oils, as demonstrated by different studies (Duarte, 2018; Silva et al., 2019; Dias et al., 2021; Olinto et al., 2023; Oliveira et al., 2024).

Table 3 presents the shoot and root dry mass results obtained with sunflower seeds. Shoot dry mass ranged from 25.8 to 29.0 mg/plant, and root dry mass ranged from 11.5 to 14.2 mg/plant, with coefficients of variation of 9.6% and 13.4%, respectively, within the essential oil concentration range of 1,000 to 7,000 ppm. The shoot

and root dry mass data show no statistically significant difference between treatments with the two essential oil formulations or in relation to the control, which received only water. Thus, the results (Table 3) indicate that the essential oil formulations at concentrations of 1,000 to 7,000 ppm did not significantly interfere with sunflower seedling growth, as there was no significant difference compared with the control, which received only distilled water during the study.

Table 3. Mean results of shoot and root dry mass tests of sunflower seedlings treated with essential oil microemulsions.

Concentration (ppm)	Lemongrass + Citronella + Long pepper		Lemon balm + Citronella + Long pepper	
	Shoot dry mass (mg/plant)	Root dry mass (mg/plant)	Shoot dry mass (mg/plant)	Root dry mass (mg/plant)
0	26.3$_a$	11.6^a	26.3^a	11.6^a
1,000	26.9$_a$	13.2^a	28.8^a	13.5^a
2,000	25.8$_a$	13.7^a	29.0^a	14.2^a
3,000	28.0$_a$	11.5^a	27.5^a	13.3^a
4,500	28.2$_a$	13.0^a	27.0^a	12.4^a
7,000	26.6$_a$	11.6^a	27.0^a	12.9^a
Coefficient of variation	9.6	13.4	9.6	13.4

Note: Means followed by the same letters do not differ from one another according to Duncan's test at a 5% probability level.

Source: Own authors.

Silva et al. (2019) evaluated the vigor of Caiapó rice seeds (*Oryza sativa* L.) treated with citronella essential oil, lemon balm essential oil, and the citronella–lemon balm combination at 5,000 ppm, with 1,000 ppm Tween 80 as an emulsifier. The results showed a significant reduction in shoot and root length in the presence of citronella and lemon balm essential oils, as well as complete inhibition of shoot development in the presence of citronella essential oil. In the treatment using the citronella–lemon balm combination, the results were better, as both shoot and root portions of Caiapó rice seedlings developed without differing significantly from the control.

It is worth highlighting that the results obtained with the combinations lemongrass + citronella + long pepper and lemon balm + citronella + long pepper represent a significant step toward understanding their effects on seeds of commercial interest, such as sunflower seeds. Essential oils are important natural substances for seed treatment, especially in organic and sustainable crops, because they are natural phytosanitary agents with reported activity against fungal pathogens such as *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* and bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*; therefore, they may replace synthetic chemical products (Andrade et al., 2012; Queiroz et al., 2020; Chen & Viljoen, 2022). Furthermore, essential oils are considered a promising alternative because they show high capacity to control pathogens, low toxicity to the environment and humans, and a low probability of inducing pathogen resistance (Martins, 2005).

The antipathogenic activity of essential oils is associated with the presence of bioactive molecules in these

oils. Citronella contains beta-citronellal, geraniol, and beta-citronellol (Scherer et al., 2009); long pepper contains safrole and the monoterpenes α-pinene, α-3-carene, and α-terpinolene (Nascimento et al., 2008); lemongrass contains citral, linalool, nerol, geraniol, and acetate (Silva et al., 2006); and lemon balm contains geraniol, nerol, and neryl acetate (Reis et al., 2009). These compounds are recognized as effective in controlling different pathogens and are responsible for the efficacy of essential oils (Silva et al., 2006; Nascimento et al., 2008; Scherer et al., 2009; Reis et al., 2009). These bioactive molecules act against pathogens such as fungi and bacteria by damaging cell membrane integrity, altering fungal morphology, and disrupting the functional structure of bacterial cells (Andrade et al., 2012; Chen & Viljoen, 2022).

4 CONCLUSION

The application of microemulsions containing essential oils of long pepper (*Piper hispidinervum*), lemon balm (*Lippia alba*), lemongrass (*Cymbopogon citratus* Stapf), and citronella (*Cymbopogon nardus* L.) to sunflower seeds (*Helianthus annuus*), in two combinations—long pepper + lemon balm + citronella and long pepper + lemongrass + citronella—at concentrations from 1,000 to 7,000 ppm, showed promising results, as the treatments did not significantly affect seed germination or vigor. Overall, the results represent an important advance in the use of essential oils for seed treatment and indicate that these natural substances may be an alternative product for seed treatment, since they did not significantly affect sunflower seed development.

Acknowledgments

The authors thank the State University of Mato Grosso (UNEMAT), the Technological Center of Mato Grosso (TCMAT), and the Foundation for Research Support of the State of Mato Grosso (FAPEMAT) for their structural and financial support for this research.

Conflicts of interest

The authors declare no conflicts of interest. All authors are aware of the submission.

Authors' Contributions

All authors contributed to all stages of the study.

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