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ESSENTIAL OIL IN THE MANAGEMENT OF THE SEED BANK OF INFESTANT PLANTS

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Abstract

The weed seed bank causes damage to agriculture, due to competition with cultivated plants and raising production costs, *Cenchrus echinatus* has a high seed multiplication and dissemination mechanism and *Conyza bonariensis* glyphosate resistance mechanism. The objective was to evaluate the potential of *Mentha arvensis* essential oil by means of fumigation in the weed seed bank. Soil samples (2 kg per treatment) were collected, homogenized and subjected to fumigation for 24 and 48 hours at concentrations of 2.5% and 5.0% of essential oil. The soil was solarized (control) and dried in the shade for three days as a witness. Then, 500 g of soil were distributed in four trays in a completely randomized design, where they received irrigation, identification and registration of emerging seedlings. Data were checked for normality and homogeneity of variance, transformed when necessary and submitted to analysis of variance at a 5% significance level. The averages were performed by Tukey and Dunnet tests at 5% probability. It was concluded that fumigation reduced grass and horseweed emergence regardless of dose and exposure time, with results similar to solarization.

Keywords: bioherbicide; solarization; *Conyza bonariensis; Mentha arvensis*.

ÓLEO ESSENCIAL NO MANEJO DE BANCO DE SEMENTES DE PLANTAS INFESTANTES

Resumo

O banco de sementes de plantas daninhas ocasiona danos a agricultura, devido a competição com plantas cultivadas e elevando custos de produção, o *Cenchrus echinatus* tem alto mecanismo de multiplicação e disseminação de sementes e a *Conyza bonariensis* mecanismo de resistência ao glifosato. Objetivou-se avaliar o potencial do óleo essencial de *Mentha arvensis* por meio da fumigação sobre o banco de sementes de plantas daninhas. As amostras de solo (2 kg por tratamento) foram coletadas, homogeneizadas e submetidas à fumigação por 24 e 48 horas nas concentrações de 2,5% e 5,0% de óleo essencial, sendo solo solarizado (controle) e solo seco a

sombra por três dias como testemunha. Em seguida, 500 g de solo foi distribuída em quatro bandejas em delineamento inteiramente ao acaso, onde receberam irrigações, identificação e registros das plântulas emergentes. Os dados foram verificados a normalidade e homogeneidade de variância, transformados quando necessário e submetidos à análise de variância ao nível de significância de 5%. As médias foram realizadas pelos testes de Tukey e Dunnet a 5% de probabilidade. Conclui-se que a fumigação reduziu a emergência do capim carrapicho e buva independente da dose e o tempo de exposição, com resultado similar a solarização.

Palavras-chave: bioherbicida; solarização; *Conyza bonariensis; Mentha arvensis*.

Introduction

Weeds are plant species that cause significant reductions in crop areas (COSTA *et al.,* 2019). As a result of the depletion of resources that are essential for the growth and development of cultivated species, causing great losses, increasing production costs and reducing the quality of products (SARDANA *et al.,* 2017).

An important characteristic of these species is the high potential in seed production, with extreme capacity to accumulate propagative materials in the soil. The seed bank results in high regeneration and natural dispersal of plants over the years, ensuring the perpetuation of the species (DINIZ *et al.,* 2017). Information about the dynamics of the seed bank and the identification of the species present in the soil, allow improving management strategies, relating the behavior and propagative potential of the species (OLIVEIRA *et al.,* 2018).

In vegetables, there are few active ingredients registered for the control of invasive species, the control is usually performed by non-chemical methods in order to eliminate competition effects between plants or host species of pests and diseases (PANNACCI *et al.,* 2017), however, when the control is not properly performed the interference of weeds in vegetables cause significant reductions in yields (ADKINS; SHABBIR, 2014). According to results Castro *et al.* (2016), weeds are responsible for large losses in tomato production for both industrial processing and fresh consumption. According to Bachega *et al*. (2013), the okra crop showed losses of more than 95% of productivity due to weed infestation.

The management of weeds in vegetables requires planning and execution, aiming to have an economical and viable activity. One strategy to improve efficiency in crop production is the use of solarization (PANNACCI *et al.,* 2017) and allelopathic extracts (BAJWA *et al.,* 2015).

Soil solarization is a potential method in controlling the weed seed bank for several areas (WILDE *et al.,* 2017; HESS *et al.,* 2018). According to Ghini (2004), one way to perform solarization is by using galvanized steel boxes with tubes of the same material internally and a

cover with transparent glass, which allows the sun's rays to enter and heat the soil to temperatures lethal to microorganisms.

Solarization in turn has been employed by researchers such as Cohen *et al.* (2019), who affirm the effectiveness of solarization in reducing the seed bank of various invasive trees, including *Pereskia aculeata* and *Leucaena leucocephala*. According to Fragoso *et al.* (2016), it was observed in the field that soil solarization promoted greater control over (*Megathyrsus maximus*) colonião grass compared to (*Cyperus rotundus*) tiririca, however being efficient on the species studied.

Another potential method in the control of weeds present in the seed bank is the use of allelopathic extracts, because they can inhibit or delay the germination of invasive species. Essential oils emerge as an alternative, as they are aromatic compounds extracted from secondary metabolism of plants (SARTO; ZANUSSO-JUNIOR, 2014), through steam-drag distillation using solvents (TRANCOSO, 2013). The essential oils of melaleuca (*Melaleuca alternifolia* Cheel), carqueja (*Baccharis trimera* Less.) and laurel (*Laurus nobilis* L.) were shown to be efficient on the germination of the invasive species annoni grass (*Eragrostis plana* Nees) Maldaner *et al.* (2018).

Mentha arvensis belongs to the family Lamiaceae, the essential oil has menthol (over 60%) and mentone (4-18%) as main constituents (VIVEK *et al.,* 2009). The oil holds antibacterial and antifungal activities in controlling microorganisms conferring protection to agriculture according to Kalemba and Synowiec (2020).

To understand the toxic potential of essential oils for the control of weed seeds along with information about the use of *M. arvensis* essential oil and solarization in the management of weed seed bank in soils cultivated with vegetables. The objective of the experiment was to evaluate the application of allelopathic extract in the form of essential oil of *M. arvensis* on the weed seed bank in soil from vegetable production area.

Material and Methods

The trial was conducted from soil samples in a fallow state of a vegetable production area of the Federal Institute of Mato Grosso do Sul, Nova Andradina Campus, located at 22°04'58.3" S and 53°28'09.4" W, at approximately 346.5 m of altitude, with LVd26 classification- Dystrophic Red Latosols + Dystrophic Red Argissolos + Dystrophic Red-Yellow Argissolos (SANTOS, 2018). The climate of the region, according to Köppen, is classified as Aw (tropical climate with winter dry season) and the soil with chemical characteristics as described in Table 1.

Table 1. Physical-chemical characterization of the soil of agricultural area, Nova Andradina - MS, 2017.

Samples	P (Melich1)	pH	K	Ca	Mg	H+Al	CT_e	V	Clay
	mg dm^{-3}	H_20	.....cmolc dm^{-3}.....					.....%.....	
0-20cm	45,89	5,41	0,26	5,68	2,06	1,75	8,02	82	19

Previously, the soil was identified with the presence of seed bank weeds by Araújo *et al.* (2019) by collecting samples at a depth of five cm using a hoe. The population of weeds from the seed bank is contained in Table 2.

Table 2. Identification of initial weed population (density and relative density) from seed bank in vegetable growing area, Nova Andradina-MS.

Scientific Name	Family	Common name	Density (ind. emersed per kg soil)	relative density (%)
Conyza canadenses	Asteraceae	Buva avoadinha	365	42,2
Cenchrus echinatus	Gramineae	Capim-carrapicho	181,5	21,1
Conyza bonariensis	Asteraceae	Buva arranha-gato	172	20,0
Chamaesyce hyssopifolia	Euphorbiaceae	Burra leiteira	3,5	0,4
Espécie não identificada	Unidentified Species	Unidentified Species	1	0,1
Aeschynomene denticulata	Fabaceae	Angiquinho	97,5	11,3
Alternanthera tenella	Amaranthaceae	Apaga-fogo	0,5	0,1
Emilia fosbergii	Asteraceae	Falsa serralha	12	1,4
Digitaria horizontalis	Poaceae	Capim-colchão	9,5	1,1
Tagetes minuta	Asteraceae	Cravo-de-defunto	1,5	0,2
Artemisia verlotorum	Asteraceae	Losna-brava	18	2,1
	Totals		862	100

For soil sampling, 24 single samples from the previously identified area were collected using a hoe (L=210.0 mm x H=225.0 mm) at a depth of five centimeters. Afterwards, the samples were homogenized in a bucket, forming a composite sample, divided into equivalent portions, dried in the shade and stored in a paper bag in a protected place until they received the treatments.

The trial was set up in an entirely randomized design with four treatments combined in a 22 factorial scheme (exposure times of 24 and 48 hours x essential oil concentrations of 2.5% and 5.0%), plus two controls (soil without weed control and solarized soil), with four repetitions each.

Soil solarization consisted in the allocation of a sample, moistened to field capacity, inside the prototype solarizer with dimensions 60x40x10 cm built in galvanized steel cylinders painted black inside a box of the same material with a glass cover for temperature elevation. The equipment was positioned according to Ghini's (2004) recommendation, with exposure on the north face and an inclination angle plus 10° to the latitude of Nova Andradina, MS.

Soil fumigation occurred by allocating the samples inside plastic containers (polyethylene terephthalate) connected by plastic tubes (polyvinyl chloride) to an ultrasonic nebulizer equipment (2.4 L capacity) containing two concentrations of M. arvensis essential oil (2.5% and 5.0%), for 24 and 48 hours + Tween 20 (0.5%).

The *Mentha arvensis* essential oil was purchased from the company FERQUIMA, the oil was obtained from steam distillation of the leaves, the main components are menthol (41%), mentone (23%), isomentone (10%), neomenthol (7%), menthol acetate (4%).

After the application of treatments, four repetitions of 500g of soil were distributed in aluminum trays of dimensions 15.2 x 20 x 5.6 cm containing 10 holes of 7.3 mm in the bottom. The samples were kept in a protected site from June to July under irrigation with a 12 hours watering shift and, daily, subjected to counting and recording of emerging weeds for 50 days. The quantification and morphological identification of the seedlings occurred according to Lorenzi (2014), by comparative method and, in case of non-recognition in the seedling stage, the species were transplanted in a pot until it had other morphological characteristics that allowed the identification. The results were expressed in number of individuals per kilogram of soil and by the germination speed index, as proposed by Maguire (1962).

The data obtained were tabulated in Microsoft Excel spreadsheets and subjected to verification of the mathematical model assumptions for normality and homogeneity of variance. When the assumptions were not met, the data were transformed into $\sqrt{x+1}$ and submitted to analysis of variance at a 5% significance level. The means of the parameters analyzed in a factorial scheme and comparisons between the controls (no control and solarization) were performed using Tukey's test for comparison of means and the controls with the common treatments using Dunnet's test, both at 5% significance level.

Results and Discussion

After the 50-day period, only two weed species were found to exist: *Cenchrus echinatus* and *Conyza bonariensis* (Table 3), unlike what was observed previously by Araújo *et al*. (2019), as it is believed that the seeds of the weeds may have been unviable due to the soil storage time until the start of the trial.

Table 3. Summary of the analysis of variance of treatments (G.L. - degrees of freedom; QM - root mean square) of a trial conducted in a 22 factorial scheme with two additional controls.

Variation Factor	G.L	.....C. Carrapicho.....		.....Buva.....	
		QM dens	QM ivg	QM dens	QM ivg
(Trat.)	5	35,3369	1,5782	6,602	1,32
Test.	1	112,138*	4,7944*	19,805*	19,8047*
Test vs. Common	1	64,0902*	3,0955*	13,203*	13,2031*
(Factorial)	3	0,15194	0,0003	0,000	0,000
Time (T)	1	0,28790$^{n.s.}$	0,00072$^{n.s.}$	0,000 $^{n.s.}$	0,000 $^{n.s.}$
Concentration (C)	1	0,05937$^{n.s.}$	2,7E-05$^{n.s.}$	0,000 $^{n.s.}$	0,000 $^{n.s.}$
T x C	1	0,10854$^{n.s.}$	0,00029$^{n.s.}$	0,000 $^{n.s.}$	0,000 $^{n.s.}$
Erro	14	0,23248	0,019136	0,087	0,01

* significant at 5%. $^{n.s.}$ not significant

In Table 3 it was verified that there were statistical differences between the controls and the treatments with essential oils. The treatments (control and comparison of control and common treatments) showed high mean square values revealing the significance of the data, on the other hand, the proposed factorial (time, concentration and interaction) showed low mean square values not significant.

According to the results obtained for weed control (Table 4), it was observed that the seed bank of tick weed in the vegetable production area showed a density of 71.5 immersed individuals per kg of soil in the control trial without control and that with the solarization procedure the value was reduced to zero individual, indicating that solarization is a highly efficient control for tick weed seeds. In the case of the weed, a density of 16.5 immersed individuals per kg of soil was observed in the control soil without control, however, with the use of the solarization method there was a reduction of the value of immersed weed seedlings to zero, reinforcing the efficiency of the method.

In the soil fumigation (Table 4), the time and concentration of exposure of the seed bank to the essential oil was evaluated. For the species carrapicho grass in the period of 24 h and 48 h showed density of individuals of 1.25 and 1.12 respectively, for the concentrations of 2.5% and 5.0% of the essential oil showed density of individuals 0.62 and 0.75 respectively, action of mint essential oil that reduced the emergence of weeds and the germination speed index. On the other

hand, for the species of cattail, in the period of 24 and 48 hours of exposure to the essential oil in concentrations of 2.5% and 5.0% showed density of zero individuals, indicating that this is a highly efficient method for the control of the cattail seed bank.

Table 4. Density of individuals and germination velocity index (GVI) of carrapicho and buva weeds present in vegetable-growing soil, Nova Andradina-MS.

Treatments		.....Carrapicho.....		.....Buva.....	
		Dens. (ind. per kg)	IVE	Dens. (ind. per kg)	IVE
Witnesses					
No control		71,5 A	5,56 A	16,5 A	1,46 A
Solarized		0,0 B	0,0 B	0,0 B	0,0 B
***Mentha arvensis* essential oil**					
Time	**24 hours**	1.25* a	0.040* a	0,0* a	0,0* a
	48 hours	1.12* a	0.035* a	0,0* a	0,0* a
	-	-	-	-	-
Concentration	**2,5%**	0.62* a	0.020* a	0,0* a	0,0* a
	5,0%	0.75* a	0.020* a	0,0* a	0,0* a
C.V.$_1$ (%)		49.39	72.36	91.68	120.78
C.V.$_2$ (%)		19.82	10.92	19.33	8.54

Averages followed by the same capital or lowercase letters do not differ by the Tukey test at 5%; Averages followed by * differ from the control treatments without control, by the Dunnet test at 5% significance level. CV1 and CV2: coefficients of variation of data without and with transformation, respectively.

The carrapicho grass found in this trial is a weed species that has high seed production (GOMES; KARAM, 2018) and due to its dormancy, it has high species perpetuation efficiency along with competition ability, consequently causing increasing damage (MACHADO, 2013).

The boxgrass found in this trial, the annual weed, has become a difficult plant to control due to its propensity to resist herbicides (GALON *et al.,* 2013). Managing glyphosate-resistant boxgrass demands the use of strategies aimed at avoiding the production of new seeds (KASPARY *et al.,* 2021).

According to Birthisel *et al.* (2019), solarization inhibits weed emergence, the same found by Maia-Júnior *et al.* (2019), where they found that solarization for 30 days reduced weed incidence and favored cocoa bean development. According to Pereira *et al*. (2019) the delay in germination

rate is an indicator of the allelopathic effect on cell elongation and division. The lower the germination rate, the greater the damage to the seeds and time required to germinate.

In accordance with Moura *et al.* (2013), demonstrated allelopathic effect of *Crotalaria juncea* on the germination of *Ipomea grandifolia*, with the extract of *Brachiaria plantaginea* inhibited the germination and caused the reduction of the root system of *Commelina bengalensis* under laboratory conditions.

According to Araújo *et al.* (2021) the mixture of the essential oil of *Cymbopogon nardus* with pyrolenoic acid showed bioherbicidal characteristics on the species crabgrass (*Cenchrus echinatus*).

According to Elshamy *et al.* (2019), the essential oil of *Euphorbia heterophylla* reduced the germination of *C. echinatus* by about 93.95%, according to Ootani *et al.* (2017), the essential oils of *E. citriodora* and *C. nardus* and citronellal compound, reduced chlorophyll and protein content of weeds by 80% and 90% respectively, being potent bioherbicides.

The alternative methods tested showed control in the seed bank, and solarization can be used for small farmers, while for large farmers it is recommended to adapt the method with tarpaulins to cover the soil and consequently perform solarization. The method of soil fumigation performed shows to be viable for small farmers, while for large farmers it would be recommended to synthesize these molecules to later produce a new synthetic molecule of herbicide, being feasible to apply in large areas.

Alternative methods of weed seed bank control are important in decision making, such as rotation of active ingredients (herbicides) to avoid resistant populations. To date, there are few studies that prove the efficiency of the methods, being alternatives that can be further studied, aiming to improve the control of the seed bank.

Conclusion

The application of essential oil by fumigation of *M. arvensis* reduces the emergence of carrapicho and buva weeds regardless of the dose and exposure time, even with results similar to solarization.

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Authors contribution

All authors contributed from the conception of the study to the writing of the manuscript, participating directly in the experimental planning, setting up and conducting the experiment, data analysis, writing and correction of the manuscript.

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