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CROP SCIENCE

The action of clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) essential oils in the control of *Acanthhoscelides obtectus* (Coleoptera: Chrysomelidae) in laboratory

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Abstract: The objective of this study was to assess the efficiency of essential oils of cloves (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) on the control of *Acanthoscelides obtectus* in laboratory conditions. The extraction of the oils was executed by the hydro-distillation method in a Clevenger device, for 4 hours and the design used was completely randomized, with five replications, in a 10x8 factorial arrangement (dozes and exposition time) with ten concentrations (20; 10; 5; 2.5; 1.75; 0.75; 0.5; 0.25 and 0.0% and Tween® 5.0%). Each replication was comprised by 10 unsexed insects of *A. obtectus*. The variables evaluated are as follows: control efficiency and CL50 through the Proc Probit analysis. The evaluations were carried out in 1, 2, 3, 12, 24, 48, 72, and 96 hours after the treatment. The results demonstrate that the essential oils of cloves and thyme caused 100% of mortality after 48 and 72 hours, respectively, in the 20% concentration. The Cl50 was estimated at 30.46 µL mL-1 for the oil of cloves and 24.93 µL mL^{-1} for the oil of thyme. The use of essential oils of cloves and thyme represent a viable alternative for use in storehouses for the integrated management of *A. obtectus*.

Key words: Insecticide activity, control efficiency, alternative methods, pest stored grain.

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) represents an important source of proteins, carbohydrates and iron in the human diet, but pest insects cause serious damage during the storage process (Smaniotto et al. 2010). *A. obtectus* is considered the main pest of storage bean. Adults measure 2 to 4 mm in length and are dark brown in color, with reddish spots on the abdomen, legs and antennas; eyes are distinctly emarginated and posterior femurs have a wide ventral thorn (Quintela 2002).

The factor that contributes to the worsening of the problem is the availability of few insecticides registered to control stored grain and seed pests, which in some way limits the alternation of active ingredients, recommended to avoid the selection of resistant insects (Lorini et al. 2015).

For the control of these stored product pests, synthetic chemicals insecticides belonging to different toxicological classes are used, which are not always able to kill the pests or avoid new infestations, which have caused several environmental problems, besides causing damage to the health of the applicator and the consumer (Campos et al. 2014). To solve this problem, it is important using natural chemical defenses of the plants, which can be prepared and applied in the form of dust, extracts and essential oils (Teixeira et al. 2019).

Essential oils are originated from the secondary metabolism of the plants and, basically, they consist of phenylpropanoids and terpenoids (Lovatto 2020). In the control of pest insects, those who have monoterpenes, dieterpenes and sesquiterpenes, which demonstrate ovicidal, larvicidal, repellent, anti-food and toxic effects such as mortality and are safe for their applicators, and contain low added value and become a promising perspective for the development of research and new technologies for the agronomic sector (Dietrich et al. 2011).

Essential oils present properties with insecticide and repellent activity against various species of insects because they are volatile and low-persistence substances and degrade in the environment (Lovatto 2020). The importance of the use of botanical insecticides in the control of stored pests has been reported in a promising, viable and ecologically correct way, and has increasingly gained space and attention from the various segments of science, due to their various effects on insects, In this way new research should be carried out due to different plants presenting potential insecticide (Lovatto 2020).

Although the mentioned species produce essential oils and their composition presents compounds with insecticide properties, little is known about the effectiveness of these oils in the control of insect pests of stored products.

The objective of this study was to evaluate the efficiency of clove (*Syzygium aromaticum* L) and thyme (*Thymus vulgaris*) oils in the laboratory control of *A. obtectus.*

MATERIALS AND METHODS

The experiment was carried out at the Insect Ecology Laboratory of the Universidade Federal de Pelotas (UFPel), Rio Grande Sul, Brazil. The insects of *A. obtectus* used in the experiment were obtained from the creation maintained in this laboratory.

Maintenance of *the breeding of A. obtectus*

A. obtectus was maintained in bean grains (*Phaseolus vulgaris*) in glass pots with a capacity of 1 kg in laboratory, wrapped in the upper part with a vual tissue and fixed with elastic. For the maintenance of insects, 20 non-sexed adult insects were placed in containers with grains, keeping them for 15 days, after this period, they were removed, remaining only eggs for hatching. With this procedure it was possible to obtain insects with similar age for the tests.

Experimental design

The two trials were arranged in a completely randomized accordin Ferreira and Patino (2016), design with five replications, arranged in a 10 X 8 factorial scheme (dosages and exposure time. The experiment was carried out in circular Petri dishes (90 X 15 mm), mixed and shaken manually for two minutes containing essential oils with 20 g of bean grains, at concentrations of 20.0; 10.0; 5.0; 2.5; 1.75, 0.75; 0.5; 0.25 % and control group (Tween and water).

Extraction of essential oils from cloves and thyme

The flower buds of clove and dried leaves of thyme were acquired from the LUAR SUL Food Company located in the municipality of Santa Cruz do Sul Rio Grande do Sul state, Brazil, 2015. The cloves come from cultivation located in the south of Bahia harvested from the crop from September to February 2015, while the dried thyme leaves were imported from Morocco and Egypt in the crop from April to May.

The essential oils of clove and thyme were extracted at the Laboratory of Natural Product Research (LPPN) of the Department of

Organic Chemistry of UFPel. The extraction was performed by distillation with steam carrier by the Clevenger apparatus, coupled to a 2000 mL volumetric flask and, as a heat source, a heating blanket. In the extraction of essential oils, 100 grams of the crushed sample (of the dried floral buds of the clove and dried thyme leaves) were weighed in a knife mill, adding 1500 mL of distilled water for each extraction (Jairoce et al. 2016b).

Then, the temperature of the electrical heating blanket was adjusted to 100 °C. after 4 hours of distillations, the essential oil of each plant sample was collected. The oils were centrifuged for three minutes. Soon, they were dried with anhydrous sodium sulphate. Samples were stored in amber glass under refrigeration to prevent possible losses of volatile constituents (Ascenção & Mouchrek Filho 2013).

Assessment of the chemical composition of essential oils of clove and thyme

The characterization of the major components of the oils was performed by GC/FID using GC-2010 gas chromatography (Shimadzu, Kyoto, Japan) with a column HP1 (30 m x 0.32 mm i.d x 0.25 $^{\mu}$m methylsilanano, Hewlett Packard, USA).

Hydrogen was used as carrier gas with a flow of 1 mL min^{-1}. The initial temperature was 40°C, increasing gradually in 10°C until reaching 300°C, maintaining at this temperature for 10 min. The injector and detector temperature were 280°C.

Method of application of essential oils of clove and thyme to *the control of A. obtectus*

Insecticide activity tests were conducted on Petri dishes (90 mm x 15 mm) containing each one 10 non-sexed insects and 20 grams of beans, where they were sprayed with solutions containing Tween® 20 to 0.5% as diluent/emulsifier mixed with essential oils of clove and thyme separately for each experiment in concentrations of: 0.0; 0.25; 0.5; 0.75; 1.25; 2.5; 5.0; 10 and 20% and group control treated with distilled water. The plates containing beans were infested with insects aged between 15 and 17 days. Soon after, they were sealed with Parafilm® and packed in an air-conditioned chamber with a temperature between 25 °C ± 3 °C, RH of 70±10% and photophase of 12 hours.

To verify insecticide activity, the survival of insects was observed in treatments one, two, three, 12 and every 24, 48, 72, 96 hours after the beginning of the experiment.

The genus *Acanthoscelides* presents as characteristic behavior tanatosis – behavior of the insect that pretend to be dead. From this behavior, the insects that did not show movements for two minutes were considered dead according to the methodology described by (Antunes et al. 2013). Control efficiency (EC%) was calculated using the Abbott equation (1925).

Data analysis

The data were submitted to analysis of variance ($p \leq 0.05$). Considering statistical significance, the effects the essential oils were compared by the t test ($p \leq 0.05$); and the dosages by regression analysis, adjusting the data to the linear decay equation ($p \leq 0.05$) and the quadratic decay equation ($p \leq 0.05$) as follows: $Y = y_0 + ax + bx^2$, where: Y = oil content; x = exposure time (hours) and, a, y_0 and b are parameters estimated from the equation, a being the difference between the maximum and minimum points of the variable; y_0 content of the oils corresponding to the minimum point of the curve; and b, the slope of the curve. Model selection was based on: (a) Low residue; (b) Low p-value; and (c) High R^2 and R^2 adj (Jairoce et al. 2016a, b). Control efficiency by Abbott equation (1925). The mean lethal concentration of 50% of the population (CL_{50}) was calculated using PROC PROBIT analysis (Finney 1971).

RESULTS AND DISCUSSION

Evaluation of the chemical composition of *S. aromaticum* essential oil

S. aromaticum has 11 components being six unidentified and five were identified as being methyl salicylic with a concentration of 0.34%, eugenol (83.59%), ß-cariofilene (9.77), α-cariofilene (1.22%) and eugenol acetate (2.99%), Eugenol was identified as a major component at a concentration of 83.59% (peak 8) (Table I).

Eugenol is the major component although the components have not been identified. Eugenol is a volatile phenolic compound being the main oil extracted from the clove using the dry floral buttons, was confirmed by Oliveira et al. (2009) in his research on volatile chemical constituents of spices rich in eugenol, finding 88.38% eugenol, 0.64%, 0.51% and 10.98%, referring to caryophyllene oxide and eugenol acetate, respectivelyb (Figure 1). According to the same author, the composition of the essential clove oil varies according to the part of the plant from which it is extracted, such as fresh leaves, sun-dried leaves and greenhouse dried, dried fruits, peduncles and dried floral buds.

Evaluation of the chemical composition of the essential oil of *T. vulgaris*

T. vulgaris has 23 components, four of which were not identified and 19 were identified, the components that had a concentration above 2% are α-pinene (3.96%), α- terpinetrol (20.98%), ß-linalo (2.43%), borneol (31.34%), canphene (6.9%), carvacrol (9.85%), karyophylenel (4.29%), ortho cimeno (2.22%) and timol (5.84%) (Figure 2). The borneol compound was identified as a major component at a concentration of 31.34% (peak 12) (Table II).

In this gas chromatography of thyme essential oil, dried plants were used (Table II), it showed that borneol is a major compound more sensitive to volatizing (31.34%), followed by α- terpineol (20.98%), carvacrol (9.85%) and thymol (5.84%). Similar borneol values were found by Jakiemiu (2008) when evaluating the thyme plants where values of 32 to 35% of the borneol compound were found.

Table I. Percentages of the chemical components present in the clove oil as a function of the retention time performed by gas chromatography.

Peak	Compound	Retention Time	Area	Height	Concentration (%)
1	Not identified	5.486	428 313	254 129	0.13
2	Not identified	5.923	1670 284	974 641	0.52
3	Not identified	6.341	1 918 509	1 084 778	0.60
4	Not identified	7.476	1 392 526	729 078	0.43
5	Not identified	9.485	702 021	314 076	0.22
6	Not identified	10.909	567 709	263 157	0.18
7	Salicylic methyl	13.888	1 096 382	461 361	0.34
8	Eugenol	18. 571	267 928 994	29 851 479	83.59
9	ß-cariophyllene	20.031	31 300 733	11 473 451	9.77
10	α-cariophyllene	20. 863	3 913 502	1 575 264	1.22
11	Eugenol acetate	22.633	9 597 595	3 854 999	2.99
Total		141, 606	355 716 568	50 836 413	99.99

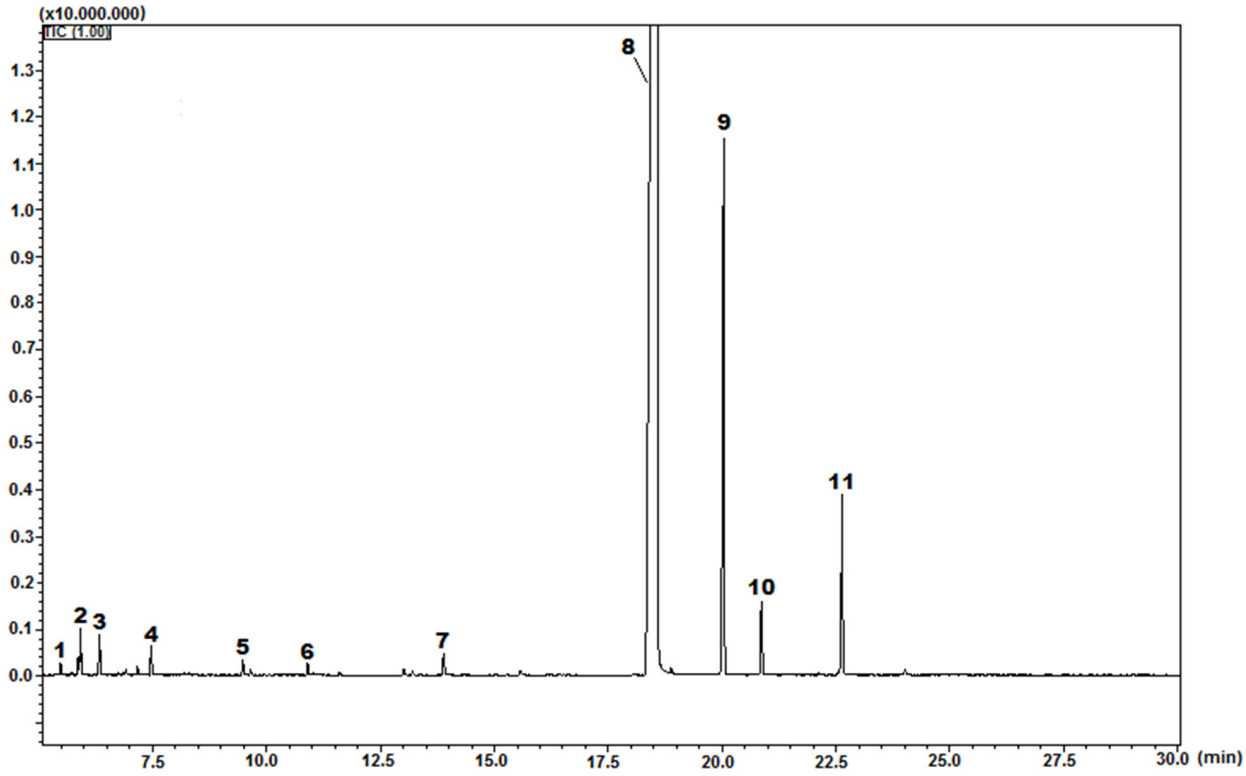

Figure 1. Characterization of eugenol and other essential oil components of clove (*Syzygium aromaticum*) (dried floral buttons) obtained in the trade in the municipality of Pelotas/RS by GC/FID using GC-2010 gas chromatography.

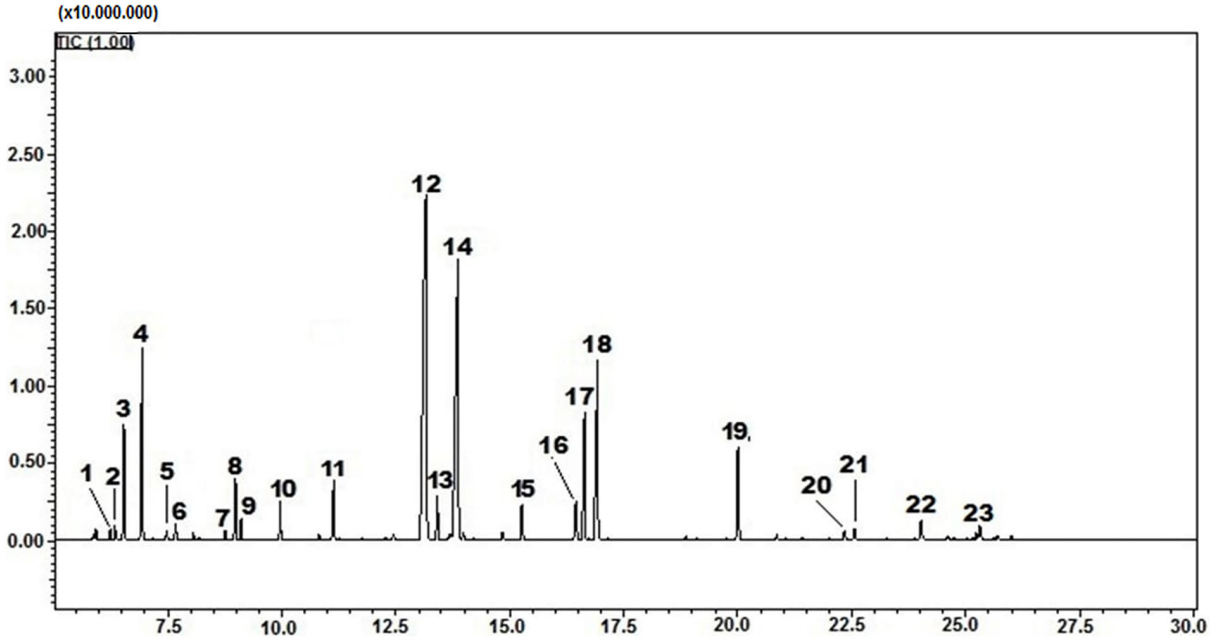

Figure 2. Characterization of thyme essential oil components (*Thymus vulgaris*) (dried floral buds) obtained in the trade in Pelotas/RS by GC/FID using GC-2010 gas chromatography.

Table II. Percentages of the chemical components present in thyme oil according to the retention time performed by chromatography.

Peak	Compound	Retention time	Area	Height	(%)
1	Not identified	6.239	1153688	652045	0.32
2	Not identified	6.343	2214315	969531	0.62
3	Alpha pinene	6.545	14130091	7419861	3.96
4	Camphene	6.932	24628193	12450619	6.90
5	Not Identified	7.477	1183340	600902	0.33
6	β-pinene	7.673	2064230	1053921	0.58
7	Alpha terpineno	8.761	1199142	612994	0.34
8	Orto cimeno	8.984	7911573	4067790	2.22
9	Limonene	9.100	2843043	1376390	0.80
10	γ-terrpinene	9.965	5243361	2550909	1.47
11	β-linalol	11.138	8687131	3912705	2.43
12	Borneol	13.163	111813906	22643858	31.34
13	4- terpineol	13.407	6529027	2874568	1.83
14	α- terpineol	13.855	74853905	18107033	20.98
15	Thymol methyl ester	15.265	5306390	2298798	1.49
16	Borneol acetate	16.443	6757087	2438115	1.89
17	Thymol	16.633	20842183	8264544	5.84
18	Carvacrol	16.914	35160977	11785531	9.85
19	Karyophyllene	20.006	15307253	6007001	4.29
20	γ-cadinene	22.337	1434155	561097	0.40
21	Δ- Cadinene	22.553	1782665	685254	0.50
22	Not Identified	24.016	3250341	1216850	0.91
23	γ-mauroleno	25.308	2537429	889282	0.71

Insecticide activity of the essential oil of cloves and thyme in the control of *A. obtectus* in beans

The effects of the factors studied in the present study on the control efficiency for bean weevil, *A. obtectus*, according to the analysis of variance, indicated that the dosages and exposure time of the clove and thyme oils were highly significant from 12 hours of exposure. The interaction of oils and dosages was significant from 12 hours of exposure, indicating both the oil dose and the exposure time influenced *the control efficiency of A. obtectus* (Table III).

For the survival and efficiency variables evaluated at 1, 2 and 3 hours after treatment application, only significance was observed at $p \leq 0.05$ of the dose treatment factor (Figure 3). The control efficiency data of *A. obtectus* at one hour were adequately adjusted to the quadratic polynomial regression model ($F = 279.9503$; $p < 0.0001$). At this time, only the 20% dose presented efficiency, with an increase of more than 100% when compared to the 10% dose (Figure 3d).

In survival at 48 hours after treatment application, there was also a significant difference between cloves and thyme at a dose of 20.0%. When the comparison was made with the control, both in the clove and in the thyme, the differences were observed from the 0.75% dose. For the doses, the data adjusted

Table III. Mean values of living adult insects of Acanthoscelides obtectus according to the time of exposure (12, 24, 48, 72 and 96 hours after treatment), of the dosages of the clove and thyme oils (0,0, 0,25, 2,50, 5,00, 10,00, and 20% and control).

Doses (%)	Hours after application									
	12h		24h		48h		72h		96h	
	Clove	Thyme	Clove	Thyme	Clove	Thyme	Clove	Thyme	Clove	Thyme
Group Control	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
0.00	10.0a^{ns}	10.0a	10.0a^{ns}	10.0a^{ns}	10.0a^{ns}	10.0a^{ns}	10.0a^{ns}	10.0a^{ns}	10.0a^{ns}	10.0a^{ns}
0.25	10.0a^{ns}	10.0a	10.0a^{ns}	10.0a^{ns}	9.2a^{ns}	9.6a^{ns}	8.8a*	9.4a^{ns}	8.6a*	9.2a^{ns}
0.50	10.0a^{ns}	10.0a	9.6a^{ns}	9.4a^{ns}	8.6a^{ns}	9.0a^{ns}	8.2a*	8.8a^{ns}	7.8a*	8.2a*
0.75	9.8a^{ns}	9.8a	9,4a^{ns}	9.4a^{ns}	8.4a*	8.4a*	7.8a*	7.8a*	7,6a*	7.6a*
1.25	9.4a^{ns}	9.4a	9.4a*	8.6a^{ns}	8.2a*	7.6a*	7.2a*	7.4a*	6.4a*	6.8a*
2.50	9,0a^{ns}	9.0a	7.6b*	9.0a^{ns}	6.2a*	6.0a*	5.6a*	4.2b*	4.2a*	2.6b*
5.00	8.0a*	8.0a$^{\alpha}$	6.2b*	8.0a*	4.2a*	4.0a*	3.4a*	1.0b*	1.4a*	0.4a*
10.00	6.0a*	5.2a$^{\alpha}$	3.8a*	2.8a*	2.0a*	2.0a*	1.2a*	1.0a*	0.6a*	0.4a*
20.00	2.0b*	4.2a$^{\alpha}$	1.6b*	3.6a*	0.0b*	1.8a*	0.0a*	0.0a*	0.0a*	0.0a*
CV (%)	**7.16**		**7.69**		**11.35**		**10.87**		**11.19**	

CV – coefficient of variation. Means followed by the same lower-case letter in the line do not differ statistically from each other by the t test (p≤0.05) comparing the oils over the time of exposure, comparing the oils *, significant and non-significant ns, respectively, in relation to the control by Dunnett test (p≤0.05).

adequately to the quadratic regression model in the clove (F = 421.0544; p < 0.0001) as for the thyme (F = 296.7694; p < 0.0001). In the clove, when comparing the dose of 2.5 with 5, 10 and 20%, there were decreases in survival percentage of 33.83 and 115%, respectively.

For thyme, these percentages were lower, from 35, 84 and 90%, for the same comparisons (Table III and Figure 4c).

In survival at 72 hours after treatment application, there was a significant difference between cloves and thyme at 2.5 and 5.0% doses. When the comparison was made with the control, for clove differences were observed from the 0.25% dose and for the thyme from the 0.75% dose. For the doses, the data adjusted adequately to the quadratic regression model in the clove (F = 445.7281; p < 0.0001) as for the thyme (F = 192.0526; p < 0.0001). In the clove, when comparing the dose of 2.5 with 5. 10 and 20%, there were decreases in survival percentage of 38.91 and 97%, respectively.

For thyme, these percentages were lower, 50.11 and 68.0%, for the same comparisons (Table III and Figure 4d).

For the variable control efficiency evaluated at 12, 24, 48, 72 and 96 hours after treatment application, there was significance for the interaction between treatment factors (Figures 4 and 5). In the efficiency at 12 hours after the treatment application, only a significant difference between cloves and thyme was observed at a 20% dose. For the doses, the data were adjusted adequately to the linear regression model in the cloves (F = 867.4916; p < 0.0001) as for the thyme (F = 295.3199; p < 0.0001). In the clove, when comparing the dose of 2.5 with 5.10 and 20%, there were decreases in survival percentage of 105.315 and 736%, respectively. For thyme, these percentages were lower, from 62.247 and 576%, for the same comparisons (Table IV and Figure 5a).

In the efficiency at 48 hours after the treatments, there was a significant difference between cloves and thyme at a dose of 20%. For the doses, the data adjusted adequately to the quadratic regression model in the clove (F = 421.0544; p < 0.0001) as for the Thyme (F = 296.7694; p < 0.0001). In the carnation, when comparing the dose of 2.5 with 5, 10 and 20%,

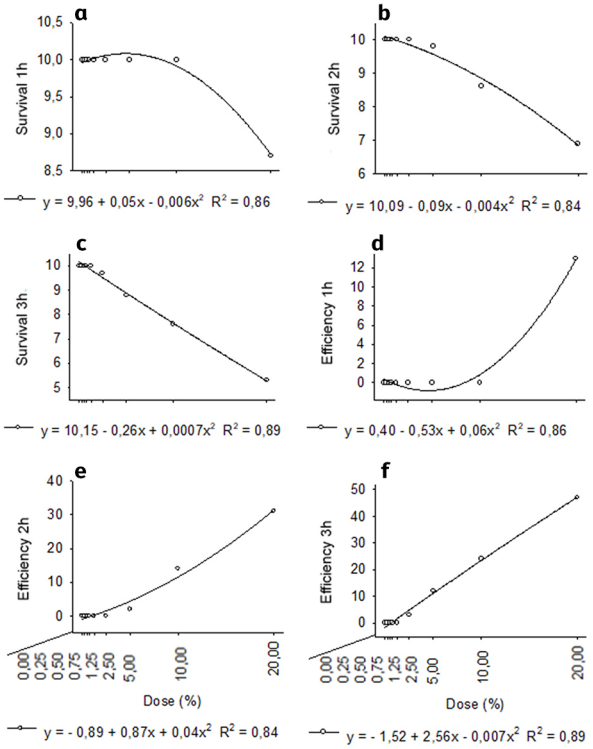

Figure 3. Survival at 1 (a), 2 (b) and 3 (c) hours and control efficiency (%) of *Acanthoscelides obtectus* at 1 (d), 2 (e) and 3 (f) hours after application of different doses of essential cloves and thyme oils on bean grains.

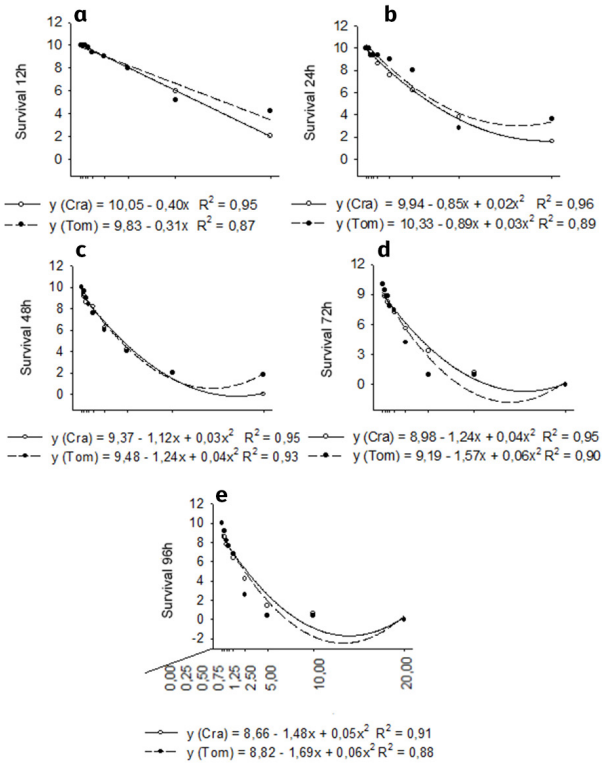

Figure 4. Survival of *Acanthoscelides obtectus* at 12 (a), 24 (b), 48 (c), 72 (d) and 96 (e) hours after application of different doses of essential oils of clove and thyme on bean grains.

there were decreases in survival percentage of 84, 211 and 304%, respectively. For thyme, these percentages were 99, 239 and 286% for the same comparisons (Table III and Figure 4c).

In survival at 72 hours after treatment application, there was a significant difference between cloves and thyme at 2.5 and 5.0% doses. For the doses, the data adjusted adequately to the quadratic regression model in the clove ($F = 445.7281$; $p < 0.0001$) as for the thyme ($F = 192.0526$; $p < 0.0001$). In the clove, when comparing the dose of 2.5 with 5, 10 and 20%, there were decreases in survival percentage of 61, 144 and 154%, respectively.

For thyme, these percentages were 66, 149 and 124% for the same comparisons (Table III and Figure 4d).

Control efficiency of 100% was achieved at a dose of 20% after 48 hours and 72 hours of exposure for clove and thyme oils, respectively (Table IV).

The results of (Table III and Fig. 4; Table IV and Fig. 5), show that the insect mortality increases according to the period by which the insect is exposed to the extract, data similar to a research carried out by Carvalho et al. (2014) that by using essential oil from *Thuja occidentalis* on the mortality of adult insects of *Callosbruchus macula* (Coleoptera: Bruchidae), found that the efficiency was when the insect exposure was 9 and 12 hours at concentrations of 20 and 25 µl.

The insecticide activity of the essential oils of clove and thyme for *the control of A. obtectus* began to be observed in the first hours after application in the concentration, and the control

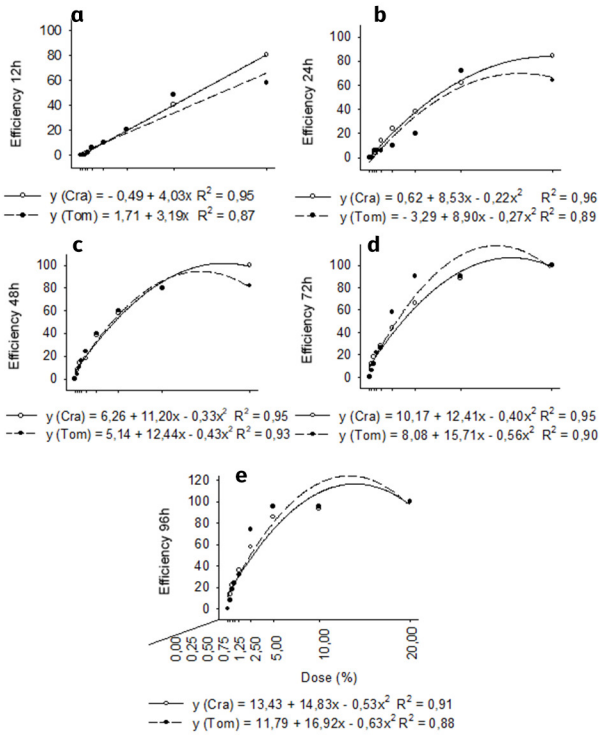

Figure 5. Control efficiency (%) of *Acanthoscelides obtectus* at 12 (a), 24 (b), 48 (c), 72 (d) and 96 (e) hours after application of different doses of essential cloves and thyme oils on bean grains.

efficiency above 50% was observed at 12 hours at a dose of 20% for both oils (Table IV).

The death of a given without respiratory failure results from the fumigant effect which is the most common mode of action among essential oils, due to the presence of compounds such as terpenoids, they manifest in different forms through interaction with the insect's coat and/or digestive enzymes (Isman 2006).

Mortality rates *A. obtectus* from 95 to 100% were verified by Santos et al. (2007) 48 h after the application of the essential oil of *Schinus terebinthifolius* Radi on *A. obtectus* and *Z. subfasciatus* observed 100% mortality efficiency at higher doses and by Savaris et al. (2012), after 24 h of *Cunila angustifolia* essential oil application with doses greater than 0.001 ml. Scariot et al. (2016) observed, after 6 h of insect infestation, a mortality rate higher than 95% when they applied *Salvia officinalis* essential oil to control bean weevil at doses higher than 0.5 L t^{-1}

Smaniotto et al. (2010), present similar results to this study when crude extract, hexanic fraction, chloroform, ethyl acetate and essential oil of C. *canjerana leaves* at 10%, 5% and 1% concentration to control *A. obtectus* were used, with an efficiency of 100% for crude extract.

The fumigant and contact effect have as its main property the diffusion capacity in the grain mass since the diffusion of a gas depends mainly on its molecular weight and its boiling point. Thus, the lower these values, the higher the diffusion velocity makes the control more efficient.

A few minutes after the product was applied to control *A. obtectus* in petri dishes, there was an unordered and intense agitation inside the container where they moved toward the plate surface. After 3 hours, almost all insects were apparently dead and at 48 hours after the insect was exposed to the compound, mortality of 100% was observed at the highest concentration (20%) (Table IV). This behavior was probably caused by the fumigating and contact effect of the essential oil of clove. Carvalho et al. (2014), in a study on the mortality and behavior of *the development of Z. sufasciatus* induced by the extract of water sagra (*Croton urucurana*), they found that the mortality of this insect is directly related to the increase in the period of exposure of the oil.

Campos et al. (2014) when testing the activity of sweet carqueja essential oil on *A. obtectus*, verified that with the 32-hour exposure period the morality achieved was 90%, a fact that is in agreement with the results presented in the present study, however, Karabörklü et al. (2010) when studying the bioactivity of the essential oil of 10 plants on *A. obtectus*, verified that there was a relationship between the applied dose

Table IV. Control efficiency of *Acanthhospelides obtectus* as a function of the essential oils of *Syzgium aromaticum* and *Thymus vulgaris* applied to bean grains during (12, 24, 48, 72 and 96 hours) after treatment.

***A. obtectus* control efficiency (%)**										
Hours after application										
Doses (%)	12h		24h		48h		72h		96h	
	C	T	C	T	C	T	C	T	C	T
0.00	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
0.25	0.0a	0.0a	0.0a	0.0a	8.0a	4.0a	12.0a	6.0a	14.0a	8.0a
0.50	0.0a	0.0a	4.0a	6.0a	14.0a	10.0a	18.0a	12.0a	22.0a	18.0a
0.75	2.0a	2.0a	6.0	6.0a	16.0a	16.0a	22.0	22.0a	24.0a	24.0a
1.25	6.0a	6.0a	14.0a	6.0a	18.0a	24.0a	28.0a	26.0a	36.0a	32.0
2.50	10.0a	10.0a	24.0a	10.0b	38.0a	40.0a	44.0a	28.0a	58.0a	74,0b
5.00	20.0a	20.0a	38.0a	20.0b	58.0a	60.0a	66.0a	90.0a	86.0a	96.0a
10.00	40.0a	48.0a	62.0a	64.0a	80.0a	80.0a	88.0a	90,0b	94.0a	96.0a
20.00	80.0a	58.0a	84.0a	72.0a	100a	82.0b	100a	100.0a	100a	100.0a
CV (%)	**35.54**		**25.60**		**20.18**		**14.14**		**12.45**	

CV – coefficient of variation. Means followed by the same lower case letter in the line do not differ statistically from each other by the t test (p≤0.05), comparing the oils over the time of exposure, comparing the oils. C = Clove. T = thyme.

and longevity. When the grains were treated with essential oils of *Myrtus communis*, *Laurus nobilis* and *Tanacetum armenum* the mortality of this insect was 100% in all concentrations, except for *M. communis* that caused 100% mortality with doses from 100 µl.

The insecticide activity of thyme oil was also confirmed by Castro et al. (2006) in their research on the non-preference of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) for essential oils of *Achillea millefolium* L. and *Thymus vulgaris*, having attributed to this biological activity to the thymol compound.

When evaluating the insecticide activity of the essential oil of *Tagetes patula* L. on *S. zeamais*, Restello et al. (2009) observed that the concentration of 0.12% oil had an insecticide effect on the specimens. These results are similar to those obtained in the present study in which interference was observed in the survival of insects at the concentration of 0.25% *of S. aromaticum* and *T. vulgaris oils* after 48 hours of exposure.

The highest mortality accumulated in the present study was obtained after 72 hours in concentrations of 20% and 10%, with 100 and 90% mortality, respectively (Table IV). According to Santos et al. (2007), the cause of the mortality of these insects in exposure to essential oil on contact surface is due to tracheal breathing of the insect-pest that is located laterally through small orifices, allowing the absorption of the oil resulting in death of the insect by asphyxiation.

Control efficiency at the lowest doses (1.25; 0.75; 0.50; and 0.25%) was not significant 3 hours after treatment (Figure 3c and f). In this context, In the present study, it was possible to verify that the contact of insects with the concentrations of essential oil of *T. vulgaris* promoted faster mortality efficiency of insects (Table III and IV). Isman (2006) cites that essential oils and their major components through contact, may interact with the insect coat, in addition to acting on digestive enzymes.

Ahmed & El-Salam (2010) in order to evaluate the toxicity of essential oils of seven plants, applied by fumigation to control *Callosobruchus*

maculatus, they found *that Cinnamomum zelynicum*, *Melaleuca alternifolia* and *T. vulgaris* caused 100% mortality at concentrations of 8.0, 16.0 and 16.9 µL/50 mL. Monoterpenes are found in a higher number of essential oils and are responsible for the behavioral and physiological disorders of insects, which explains in a certain way the choice of insect in beans not treated with essential oil, as well as its preference for oviposition and feeding (Coitinho et al. 2010).

According to Baldin & Pereira (2010), the biological activity of essential oils in the mass of the grains in storage may be linked to the potential of the vapors that enter the grains and cause a Toxic effect on the larvae of *Z. subfasciatus* stage that the insect feeds on the cotyledons.

Determination of mean lethal concentration (CL50) of clove oil for *control of A. obtectus*

The determination of the CL_{50} was carried out through THE PROBIT analysis (Finney 1971). The procedure was PROC PROBIT, program R- 3.1.1, 2014.

The CL_{50} of clove oil was estimated at 30.46 (24.93 - 36.0) ppm (Table V).

Several studies have demonstrated the determination of the acute lethal concentration of essential oils that cause mortality of 50% of the insect population, such as the study carried out by Carvalho et al. (2014) where they found that *Croton urucurana* Bail oils are also toxic to adults of *Zabrotes subfasciatus* Boh. Having a CL_{50} of 2560 ppm (2219 ± 3009).

In evaluating the insecticide activity of *the essential oil of Ostericum sieboldii* on *S. zeamais*, they verified a strong activity, in fact, contact-toxic and fumigation with CL_{50} of 13.82 µg. $adult^{-1}$ and a CL_{50} of 27;39 mg. L^{-1}, respectively (Liu et al. 2011).

Determination of mean lethal concentration (CL_{50}) of thyme oil for control of *A. obtectus*

The determination of the CL_{50} was carried out through THE PROBIT analysis (Finney 1971).

Table V. Lethal concentration of essential clove oil required to kill 50% *of Acanthhoscelides obtectus adults* applied in beans.

Dosages (%)	Efficiency of *A. obtectus* control (%) at 96 hours	Lethal concentration (CL_{50}) (95%IC)	GL	X^2	P
20.0	100±0.0a				
10.0	94.0±2.4a				
5.0	86.0±4.0a				
2.5	58.0±3.7b				
1.25	36.0±2.4c	30.46 (24.93 – 36.0) ppm	9	53.24	1.70
0.75	24,0±2.4cd				
0.5	22.0±3.7cd				
0.25	14.0±5.1de				
0.0	0.0±0.0e				
Group Control	0.0±0.0				

GL – degrees of freedom; IC – confidence interval; X^2 – Chi square; P – probability. Means followed by the same lower-case letter in the line do not differ statistically from each other by the t test (p≤0.05), comparing the oils over the time of exposure, comparing the oil.

CL_{50} of thyme oil after 96 hours was estimated at 24.93 (20.75 - 29.10) ppm (Table VI).

Li et al. (2010) when testing the chemical composition of the *essential* oil of the exotic *Murraya* in the *control of S. zeamais*, they found CL_{50} of 8.29 $mg.L^{-1}$ and 11.41 $\mu g.adult^{-1}$, by fumigation and contact, respectively.

In the present study, the concentrations of 5.0 %, 10.0 % and 20 % of essential oils of cloves and thyme did not differ significantly in relation to the mortality efficiency of *A. obtectus* populations after 96 hours after treatment, with the concentration below 5 and 10% being considered the best for *A. obtectus*.

Thus, some theories demonstrate that increased dosage applied is one of the factors that promotes the evolution of the resistance of stored grain insects to insecticides. Studies on the repellent and insecticide activity of the sweet gorse essential oil on the bean weevil have shown that the mortality rate of the bean weevil is directly influenced by the increase in dosage and by the increase in exposure time (Campos et al. 2014).

Some studies have been carried out with essential extracts and oils on the toxic activity for stored pests, verifying that further information is needed in the process of extracting oils from different plants for conducting fumigation and contact tests (Neves & Camara 2011).

In this way, it is possible to state that the essential oils of *Syzygium aromaticum* and *Thymus vulgaris* were efficient in relation to the insecticide effect, but at different exposure times, being 48 and 72 hours, respectively, for the control of bean weevil (*A. obtectus*) in bean grains, however, further research is needed in view of the fact that such research is still scarce, aiming at the phytochemical investigation of plant extracts, deficiency, residue, costs, organoleptic alterations so that the producer can safely use these oils as an alternative in the control of pests of stored products.

Table VI. Lethal concentration of thyme essential oil required to kill 50% of *Acanthoscelides obtectus adults* applied in beans.

Dosages (%)	Efficiency of *A. obtectus* control (%) at 96 hours	Lethal concentration (CL_{50}) (95%IC)	GL	X^2	P
20.0	100±0.0a				
10.0	94.0±2.4a				
5.0	86.0±4.0a				
2.5	58.0±3.7b				
1.25	36.0±2.4c	24.93 (20.75 – 29.1) ppm	9	66.41	2.50
0.75	24.0±2.4cd				
0.5	22.0±3,7cd				
0.25	14.0±5.1de				
0.0	0.0±0.0e				
Group Control	0.0±0.0				

GL – degrees of freedom; IC – confidence interval; X^2 – Chi square P – probability. Means followed by the same lower-case letter in the line do not differ statistically from each other by the t test (p≤0.05), comparing the oils over the time of exposure, comparing the oil.

CONCLUSIONS

According to the results obtained in this work, it can be concluded that:

Eugenol and borneol were the compounds responsible for the insecticide activity of thyme essential oil, applied in bean grains to control *A. obtectus*.

The highest mortality rates of bean weevil were obtained in the period 48 and 72 hours for clove and thyme oils, respectively.

After 96 hours of exposure, the 5% concentration had control efficiency above 80% for both oils.

The mean lethal concentration (CL_{50}) of clove oil is 30,46 ppm and that of thyme oil is 24,93 ppm.

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REFERENCES

ABBOTT WS. 1925. A method of computing the effectiveness of an insecticide. J Econ Entomol 18: 265-267.

AHMED ME & EL-SALAM ABD. 2010. Fumigant toxicity of seven essential oils against the cowpea weevil, *Callosbruchus maculates* (F.) and the rice weevil, *Sitophilus oryzae* (L.). Egypt Acad J Biol Sci 2: 1-6.

ANTUNES LEG, FERRARI FILHO EF, GOTTARDI R, SANT ANA J & DIONELLO RG. 2013. Efeito da dose e exposição à terra de diatomácea de diferentes insetos em milho armazenado. Arq Inst Biol 80: 169-176.

ASCENÇÃO VL & MOUCHREK FILHO VE. 2013. Extração, caracterização química e atividade antifúngica de óleo essencial *Syzygium aromaticum* (cravo-da- Índia). Cad Pesqui 20: 137-144.

BALDIN ELL & PEREIRA JM. 2010. Resistência de genótipos de feijoeiro a *Zabrotes subfasciatus* (Bohemann, 1833) (Coleoptera: Bruchidae). Ciênc Agrotec 34: 1507-1513.

CAMPOS ACT, RADUNZ LL, RADÜNZ AL, MOSSI AJ, DIONELLO RG & ECKER SL. 2014. Insecticide and repellent activity of the essential oil from carqueja doce on bean weevil. Rev Bras de Eng Agrícola e Ambient 18: 861-865.

CARVALHO GS, SILVA LS, SILVA LB, ALMEIDA MLS, PAVAM BE & PERES MTLP. 2014. Mortalidade e comprometimento do desenvolvimento de *Zabrotes subfasciatus* Boh. (Coleoptera: Chrysomelidae), induzido pelo extrato de sagra d'água *Croton urucurana* Baill (Euphorbiaceae). Comun Sci 5: 331-338.

CASTRO DP, CARDOSO MG, MORAES JC, SANTOS NM & BALIZA DP. 2006. Não-preferência de *Spodoptera frugiperda* (Lepidoptera:Noctuidae) por óleos essenciais de *Achillea millefolium* L. e *Thymus vulgaris* L. Rev Bras Pl Med 8: 27-32.

COITINHO RLBC, OLIVEIRA JV, GONDIM JÚNIOR MGC & CÂMARA CAG. 2010. Persistência de óleos essenciais em milho armazenado, submetido à infestação de gorgulho do milho. Ciênc Rural 40: 1492-1496.

DIETRICH F, STROHSOEN AAG, SCHULTZ G, SEBBEN AD & REMPEL C. 2011. Utilização de inseticidas botânicos na agricultura orgânica de arroio do meio/RS. Rev Bras de Agrociênc 17: 251-255.

FERREIRA JCD & PATINO C. 2016. Randomização: mais do que o lançamento de uma moeda. J Bras Pneumol 42: 310-310.

FINNEY DJ. 1971. Probit analysis. 3.ed. London: Cambridge Press, 338 p.

ISMAN MB. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and increasingly regulated world. Annu Rev Entomol 51: 45-66.

JAIROCE CF, TEXEIRA CM, NUNES AM, HOLDEFER DR, KRÜGER AP & GARCIA FRM. 2016a. Efficiency of inert mineral dusts in the control of corn weevil. Rev Bras de Eng Agricola e Ambient 20: 158-162.

JAIROCE CF, TEXEIRA CM, NUNES CFP, NUNES AM, PEREIRA CMP & GARCIA FRM. 2016b. Insecticide activity of clove essential oil on bean weevil and maize weevil. Rev Bras de Eng Agricola e Ambient 20: 72-77.

JAKIEMIU EAR. 2008. Uma contribuição ao estudo do óleo essencial e do extrato de tomilho (*Thymus vulgaris*). Thesis (Master's degree) - Federal University of Paraná, Graduate Program in Food Technology, 89 p. (Unpublished).

KARABÖRKLÜ S, AYVAZ A & YILMAZ S. 2010. Bioactivities of different essential oils against the adults of two stored product insects. Pak J Zool 42: 679-686.

LI WQ, JIANG CH, CHU SS, ZUO MX & LIU ZL. 2010. Chemical composition and toxicity against *Sitophilus zeamais* and *Tribolium castaneum* of the essential oil of murraya exotica aerial parts. Molecules 15: 5831-5839.

LIU ZL, CHU SS & JIANG GH. 2011. Insecticidal Activity and Composition of Essential Oil of *Ostericum sieboldii* (Apiaceae) Against *Sitophilus zeamais* and Tribolium castaneum. Rec Nat Prod 5: 74-81.

LORINI I, KRZYZANOWSKI FC, FRANÇA-NETO JB, HENNING AA & HENNING FA. 2015. Manejo integrado de pragas de grãos e sementes armazenadas. 1ª ed. Embrapa Brasília, Distrito Federal, 81 p.

LOVATTO PB. 2020. Fitoprotetores botânicos: União de saberes e tecnologias para transição agroecológica, Curitiba: Editora Appris, 283 p,

NEVES IA & CAMARA CAG. 2011. Volatile Constituents of Two Croton Species from Caatinga Biomeof Pernambuco-Brasil. Rec Nat Prod 6: 161-165.

OLIVEIRA RA, REIS TV, DO SACRAMENTO CK, DUARTE LP & OLIVEIRA FF. 2009. Constituintes químicos voláteis de especiarias ricas em eugenol. Braz J Pharmacogn 19: 771-775.

QUINTELA ED. 2002. Manual de identificação dos insetos e outras invertebradas pragas do feijoeiro. Documentos 142. Santo António de Goiás: Embrapa Arroz e Feijão, 51 p.

RESTELLO RM, MENEGATT C & MOSSI AJ. 2009. Efeito do óleo essencial de *Tagetes patula* L. (Asteraceae) sobre *Sitophilus zeamais* Motschulsky (Coleoptera, Curculionidae). Rev Bras Entomol 53: 304-307.

SANTOS MRA, LIMA RA, SILVA AG, FERNANDES CF, LIMA DKS, SALLET LAP, TEIXEIRA CAD & FACUNDO VA. 2007. Atividade inseticida do óleo essencial de Schinus terebinthifolius Raddi sobre *Acanthoscelides obtectus* Say e *Zabrotes subfasciatus* Boheman. Boletim de pesquisa e desenvolvimento, 13 p.

SAVARIS M, LAMPERT S, GARCÍA FRM, SABEDOT-BORDIN SM & MOURA NF. 2012. Atividade inseticida de *Cunila angustifolia* sobre adultos de *Acanthoscelides obtectus* em laboratório. Rev Cienc Tecnol 5: 1-5.

SCARIOT MA, FRANSCISCO JÚNIOR WR, RADÜNZ LL, BARRO JP & MOSSI AJ. 2016. *Salvia officinalis* essential oil in bean weevil control. Pesq Agropec Trop 46: 177-182.

SMANIOTTO L, MOURA NF & DENARDIN RBN & GARCIA FRM. 2010. Bioatividade da *Cabralea canjerana* (Vell.) Mart. (Meliaceae) no controle de adultos de *Acanthoscelides obtectus* (Coleoptera, Bruchidae) em laboratório. Biotemas 23: 31-35.

TEIXEIRA CM & SCAPINELLO J, MAGRO JD & GARCIA FRM. 2019. Efeitos do extrato supercrítico de *Melia azedarach* (Meliaceae) no controle de *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera, Curculionidae). Rev Ciênc Agrovet 18: 274-280.

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AZM, CMT, CMP and FRMG0 conceived and planned the experiments; carried out the experiments; contributed to the interpretation of the results; took the lead in writing the manuscript; provided critical feedback and helped shape the research, analysis and manuscript.