

Control of diamondback moth with *Lippia gracilis* essential oil







Abstract – The objective of this work was to evaluate the potential of the essential oils of the LGRA-106 and LGRA-108 *Lippia gracilis* genotypes for the control of diamondback moth (*Plutella xylostella*). The lethal concentrations (LCs) were estimated by two routes of action (residual and spraying), using oil concentrations ranging from 0.5 to 3.0% v v⁻¹, diluted in Tween 80 (1.5%). To determine the effect of the LC to 50% (LC₅₀) on the development of *P. xylostella*, two compounds of the *L. gracilis* genotypes, thymol, and carvacrol, were sprayed on the insects. The repellency of the LC₅₀ was evaluated by residual action, in a free-choice behavioral bioassay. The LGRA-106 genotype showed a greater toxicity via residual action (LC₅₀ = 8.82 mg mL⁻¹), as well as a higher repellency index. LGRA-108 was more toxic via spraying (LC₅₀ = 9.64 mg mL⁻¹). Larval development and viability were reduced in approximately 50% with LGRA-106 or thymol and up to 70% with LGRA-108 or carvacrol, which caused mortality from 1.70 to 1.97 days after spraying. The oils of the LGRA-106 and LGRA-108 genotypes of *L. gracilis* have insecticidal activity in the control of *P. xylostella*.

Index terms: *Plutella xylostella*, alecrim-da-chapada, insecticidal activity.

Controle de traça-das-crucíferas com óleo essencial de *Lippia gracilis*

Resumo – O objetivo deste trabalho foi avaliar o potencial do óleo essencial dos genótipos LGRA-106 e LGRA-108 de *Lippia gracilis* no controle de traça-das-crucíferas (*Plutella xylostella*). As concentrações letais (CLs) foram estimadas por duas vias de ação (residual e pulverização), com uso dos óleos em concentrações de 0,5 a 3,0% v v⁻¹, diluídos em Tween 80 (1,5%). Para determinar o efeito da CL média (CL₅₀) no desenvolvimento de *P. xylostella*, dois compostos dos genótipos de *L. gracilis*, timol e carvacrol, foram pulverizados nos insetos. A repelência da CL₅₀ foi avaliada por ação residual, em bioensaio com chance de escolha. O genótipo LGRA-106 apresentou maior toxicidade via ação residual (CL₅₀ = 8,82 mg mL⁻¹), bem como maior índice de repelência. Já o LGRA-108 foi mais tóxico via pulverização (CL₅₀ = 9,64 mg mL⁻¹). O desenvolvimento e a viabilidade larval foram reduzidos em cerca de 50% com o LGRA-106 ou o timol e, em até 70%, com o LGRA-108 ou o carvacrol, que causaram mortalidade de 1,70 a 1,97 dias após a pulverização. Os óleos dos genótipos LGRA-106 e LGRA-108 de *L. gracilis* possuem atividade inseticida no controle de *P. xylostella*.


Termos para indexação: *Plutella xylostella*, alecrim-da-chapada, atividade inseticida.

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Introduction

Plutella xylostella (Linnaeus, 1758; Lepidoptera: Plutellidae), commonly known as diamondback moth, is the main pest of brassicas and occurs in all producing regions of the world (Trindade et al., 2014). This insect has a high reproductive capacity that facilitates its infestation, with population peaks in hot and dry temperatures (Torres et al., 2006). The foliar injuries caused by *P. xylostella* can substantially reduce plant yield, decrease photosynthetic area, and interfere with plant development (Brandão Filho et al., 2010; Furlong et al., 2013; Filomeno et al., 2017).

To reduce the damage caused by this pest in brassica plantations, it is common to use chemical control with insecticides, generating an annual global expenditure of approximately US\$ 5 billion (Furlong et al., 2013; Silva Filho et al., 2020). However, the inappropriate use of chemical insecticides contributes to the selection of resistant insect populations, as observed for *P. xylostella*, considered a lepidopteran with resistance to a large number of different active components (Sundufu et al., 2006; Chagas Filho et al., 2010).

Therefore, the search for alternative methods to control this and other pests has intensified, particularly focusing on the development of insecticidal products using essential oils due to their effectiveness in controlling various pests and to their multiple mechanisms of action, as well as to the low toxicity of their residues during application (Wang et al., 2019; Chenni et al., 2020) and to their selectivity to natural enemies (Purwatiningsih et al., 2012). *Lippia gracilis* Shauer (Verbenaceae), commonly known as “alecrim-da-chapada”, for example, is rich in essential oils and has a strong potential as a bioprospecting pesticide (Silva et al., 2008; Cruz et al., 2018; Melo et al., 2018). The different genotypes of this species present the monoterpene phenol isomers thymol or carvacrol as major compounds, along with minor quantities of other terpenoids such as limonene, γ -terpinene, *p*-cymene, caryophyllene, and linalool (Cruz et al., 2018).

The toxicity of the oils of *L. gracilis* is dependent on the composition of each genotype, especially of its main compounds (Melo et al., 2018; Santos et al., 2019; Teodoro et al., 2021). Although Silva et al. (2012) have already verified the toxicity of *L. gracilis* oil on *P. xylostella* caterpillars, there is no known information about the assessed genotype or other effects on the insect.

The objective of this work was to evaluate the potential of the essential oils of the LGRA-106 and LGRA-108 genotypes of *L. gracilis* for the control of *P. xylostella*.

Materials and Methods

The bioassays were conducted from September 2018 to August 2019, in the municipality of São Cristóvão, in the state of Sergipe, Brazil, at a Laboratory for Biotechnological Pest Control – Laboratório de Controle Biotecnológico de Pragas (LCBiotec) –, under the following controlled conditions: temperature of 25±2°C, relative humidity of 70±10%, and photophase of 12 hours. *Plutella xylostella* caterpillars were obtained from the breeding stock of LCBiotec. The oils used in the process were extracted from leaves of the LGRA-106 and LGRA-108 *L. gracilis* genotypes, vouchers n° 14733 and 14734, by hydrodistillation in a Clevenger apparatus (Table 1), as described by Cruz et al. (2018). The major compounds of the oils, thymol and carvacrol, were selected according to the chromatographic results obtained by Cruz et al. (2018) and were acquired commercially from Sigma-Aldrich Brasil Ltda. (São Paulo, SP, Brazil) with purities ≥ 98.0%.

The experimental design used to estimate the lethal concentrations (LCs) by spraying and residual action was completely randomized, with eight treatments (seven concentrations and control) and five replicates of 25 caterpillars. Preliminary bioassays were conducted to determine parameters such as surfactant concentration, oil concentration series, application volume, caterpillar age, and exposure time to treatments. For the LC estimation bioassays, *P. xylostella* caterpillars were used 72 hours after hatching.

The LCs of the oils of the LGRA-106 and LGRA-108 *L. gracilis* genotypes were determined via spraying and residual action. In the spraying bioassay, the essential oils were weighed on the BEL analytical balance with a 0.0001 g precision (BEL Engineering, Piracicaba, SP, Brazil) and diluted in Tween 80 (1.5%) solution; the concentrations of milligram of oil per milliliter were obtained at the proportions of 0.5, 0.7, 1.0, 1.3, 1.7, 2.0, and 2.5% v v⁻¹. Distilled water with Tween 80 (1.5 %) was used as the control. The caterpillars were placed in 125×20 mm Petri dishes containing Ø 125 mm cabbage disks and sprayed from

a distance of 10 cm with 1.0 mL of the treatment using an electric microatomizer, at a pressure of 10 pounds. The caterpillars were then transferred to 90×10 mm Petri dishes containing new cabbage disks of Ø 80 mm, used as feeding substrates. All plates were covered with plastic film to avoid caterpillars from escaping and kept under controlled conditions until evaluation. Mortality was recorded after 24 and 48 hours of exposure, when the caterpillars that remained, even after brush simulation, were considered dead.

The bioassay to assess the residual effect of the oil was based on the adapted method of Javier et al. (2019). For this assessment, the proportions of oils used were 0.5, 0.7, 1.0, 1.5, 2.0, 2.5, and 3.0% v v⁻¹. Cabbage discs of Ø 80 mm were immersed in the treatments for approximately 30 s and kept at an ambient temperature of 25±2°C for approximately 1.5 hour for drying. Subsequently, the treated disks were transferred to

90×10 mm Petri dishes with 25 caterpillars. All Petri dishes were covered with plastic film and kept under controlled conditions until evaluation.

The LC to 50% (LC₅₀) values estimated in the spray bioassay were used to verify the effect of the oils of the LGRA-106 and LGRA-108 *L. gracilis* genotypes on the development of *P. xylostella*. Carvacrol and thymol, the major compounds of those genotypes, were also used as treatments at concentrations corresponding to the proportion of the estimated LC₅₀ for their respective genotypes. The two essential oils and carvacrol were diluted in Tween 80 (1.5%) solution, and thymol was diluted in DMSO (1.0%) with Tween 80 (1.0%). The experimental design used to determine the effect of *L. gracilis* on the development of *P. xylostella* was completely randomized and composed of five treatments (LGRA-106, LGRA-108, carvacrol, thymol, and control) and five replicates. Distilled water with Tween 80 (1.5%) was used as the control. The experiment was conducted according to the bioassay method for estimating the LCs by spraying. Insect development was monitored daily, and, when the caterpillars reached the pupal stage, they were transferred to glass tubes and evaluated daily until their emergence as adults. Assessed parameters included the viability and duration of the larval and pupal stages.

To verify the possible existence of the repellency action of the oils and feeding deterrence by *P. xylostella*, a free-choice behavioral bioassay was performed (Javier et al., 2019), using a completely randomized design with two treatments (treated and untreated cabbage disks) and 40 replicates. The concentration used in this evaluation corresponded to the LC₅₀ of the LGRA-106 and LGRA-108 genotypes estimated by residual action. The oils were diluted in a solution of Tween 80 (1.5%), and the control was composed only of a solution of Tween 80 (1.5%). Two cabbage disks of Ø 35 mm were positioned equidistantly inside a 90×10 mm Petri dish, one of which was previously treated with the oil to determine the residual LCs. One caterpillar, 96 hours after hatching and deprived of food for 7 hours, was then placed in the center of the plate. The assessment was performed by observing the caterpillar's food preference every 20 min in the first hour and at 12, 24, and 48 hours after the start of the bioassay.

Table 1. Chemical composition of essential oils of two genotypes of *Lippia gracilis*.

Constituent	LGRA-106	LGRA-108
α-thujene	0.44	0.56
α-pinene	0.21	0.00
Myrcene	2.18	1.61
α terpinene	0.94	1.33
p-cymene	6.31	14.44
Limonene	0.50	0.58
1.8 cineol	3.80	3.21
γ-terpinene	4.37	6.68
Linalol	0.44	0.22
Terpinen-4-ol	0.66	0.75
Methyl thymol	9.81	7.43
Thymol	60.47	3.09
Carvacrol	0.38	46.10
β-cariofilene	6.55	2.93
α-humulene	0.34	0.71
Viridiflorene	0.32	0.51
Bicyclogermacrene	0.39	1.63
Spathulenol	0.00	1.85
Caryophyllene oxide	1.15	1.03
Monoterpenes (%)	90.51	86.00
Sesquiterpenes (%)	8.75	8.66
Total (%)	99.26	94.66
Essential oil content (%)	1.42	2.17

The mortality results of the bioassays used to obtain the LCs were subjected to Probit analysis, in order to determine the LCs of the genotypes, using the PoloPlus, version 1.0, software (LeOra Software LLC, Parma, MO, USA), at 5% probability.

The results of the parameters evaluated in the developmental bioassay (larval and pupal viability) were subjected to the analysis of variance using the ANOVA procedure of the SAS software, versions 8.0, 8.1, and 8.2 (SAS Institute Inc., Cary, NC, USA), and means were compared by Duncan's test ($p < 0.05$). Using caterpillar mortality data, average survival was determined. To determine survival curves and lethal times, the obtained data were subjected to the log-rank test using Kaplan-Meier estimators by pairs of isolates with the Proc Lifetest of the same versions of SAS (SAS Institute Inc., Cary, NC, USA).

For the analyses in the free-choice test, means were compared using the null hypothesis of choice of the PROC FREQ of SAS, versions 8.0, 8.1, and 8.2 (SAS Institute Inc., Cary, NC, USA), interpreted by the chi-square test, at 5% probability.

Results and Discussion

In the spraying bioassay, the LCs of the essential oils of the LGRA-108 and LGRA-106 genotypes of *L. gracilis* were 9.64 and 13.79 mg mL⁻¹, respectively (Table 2). The susceptibility of the caterpillars varied according to the tested genotype. LGRA-108 was more toxic and showed less variation in the confidence interval, besides presenting a greater slope of the line (4.65±0.35), which is indicative that smaller variations in oil concentrations result in larger variations in *P. xylostella* mortality. This homogeneous response of *P. xylostella* to LGRA-108 indicates a lower probability of there being resistant individuals to the tested substance (Hoskins & Gordon, 1956), which

shows the potential of the essential oil of *L. gracilis* to securely control the high tolerance of this pest.

In the bioassay using residual action, there was an inversion in the toxicity of the genotypes: the concentration found for LGRA-106 was of 8.82 mg mL⁻¹, lower than that of 14.77 mg mL⁻¹ for LGRA-108 (Tables 2 and 3). However, once again, the LGRA-108 genotype showed a greater slope of the line and a smaller variation in the confidence interval.

The greater toxicity of the LGRA-108 genotype via spray application can be related to the ability of its constituents to penetrate the insect cuticle. Tak & Isman (2017) found that *p*-cymene increased the penetration of thymol through the integument, resulting in a synergistic effect when applied topically. The chemical characterization of the *L. gracilis* essential oils used in the present study showed the presence of *p*-cymene in both genotypes, but LGRA-108 had a greater amount of this compound (Table 1), which may have facilitated the penetration of carvacrol through the integument.

Silva et al. (2012) assessed the insecticidal effect of an essential oil from the genus *Lippia* on *P. xylostella* through topical application, finding a LD₅₀ of 11.67 mg g⁻¹ insect. Niculau et al. (2013) obtained LD₅₀ values between 1.20 and 1.56 µg mg⁻¹ insect when studying the effect of different genotypes of *Lippia alba* (Mill.) N.E.Br. ex. Britton & P. Wilson (Verbenaceae) on *Spodoptera frugiperda* (J.E. Smith, 1797; Lepidoptera: Noctuidae) caterpillars.

In a study with *Aceria guerreronis* (Keifer, 1965) Acari: Eriophyidae, a greater toxicity of LGRA-106, evaluated using the residual effect, was also observed (Santos et al., 2019; Teodoro et al., 2021). According to these authors, the LC₅₀ of carvacrol (6.84 mg mL⁻¹) was slightly higher than that of thymol (5.34 mg mL⁻¹), and the LC₅₀ of genotype LGRA-109 (carvacrol chemotype) was approximately 6-fold higher than that of LGRA-106.

Table 2. Lethal concentration (LC) to *Plutella xylostella* caterpillars, by spraying, estimated for the essential oils of two genotypes of *Lippia gracilis*⁽¹⁾.

Treatment (genotype)	n	DF	LC ₅₀ (95% CI) mg mL ⁻¹	Inclination (β±SE)	χ ²	H
LGRA-106	845	4	13.79 (10.80–16.16)	2.84±0.31	4.92	1.23
LGRA-108	961	4	9.64 (7.86–10.38)	4.65±0.35	8.60	2.15

⁽¹⁾n, total number of caterpillars; DF, degree of freedom for the chi-square test; LC₅₀ (95% CI), lethal concentration to 50% at a 95% confidence interval; β±SE, slope coefficient ± standard error; χ², chi-square test; and H, heterogeneity.

In the bioassay to assess the residual effect of the oils, two modes of action were tested: contact and ingestion. In a previous work, Nitbani et al. (2012) had already recorded the toxicity of the essential oil of *Acorus calamus* L. (Acoraceae) on *P. xylostella* by both methods. This type of procedure is adopted since the insecticidal action of essential oils can occur either through the interaction with the integument and interference with the neurotransmitter system or through associations with digestive enzymes (Isman et al., 2006). For example, when larvae of *Xanthogaleruca luteola* (Müller, 1766) (Coleoptera: Chrysomelidae) fed on leaves treated with essential oils of thyme (*Thymus vulgaris* L.) and lavender (*Lavandula angustifolia* Mill.) plants, the activity level of digestive enzymes decreased in their midguts (Khosravi & Sendi, 2013). Therefore, given the premise that, in this form of application, the action of the oil can occur through ingestion and thymol can reduce the activity of the proteins responsible for the detoxification system (Waliwitiya et al., 2012), it is possible that the action of the LGRA-106 genotype is potentiated by ingestion.

The oils of the two genotypes of *L. gracilis* and their major compounds reduced the larval viability of *P. xylostella*, but had no significant effect on the other biological parameters (Table 4). Viability was reduced by LGRA-106 and thymol in approximately 50% and by LGRA-108 and carvacrol by up to 70%.

The *L. gracilis* genotypes showed their insecticidal potential by causing caterpillar mortality and, consequently, by leading to a reduction in larval viability; the insecticidal action of these genotypes was correlated to their major compounds. A higher level of toxicity has also been observed for the major compounds of chemotypes other than *L. gracilis*, as well as for thymol and carvacrol, when applied to *Diaphania hyalinata* (L., 1958) (Lepidoptera: Crambidae) (Melo et al., 2018).

Insecticidal action can occur, among other ways, through an interference in the neurotransmitter system. For example, carvacrol is up to ten times more efficient than the thymol isomer, present in LGRA-106, in inhibiting the acetylcholinesterase (AChE) enzyme in vitro (Jukic et al., 2007). This

Table 3. Lethal concentration (LC) to *Plutella xylostella*, by residual action, estimated for the essential oils of two genotypes of *Lippia gracilis*⁽¹⁾.

Treatment (genotype)	n	DF	LC ₅₀ (95% CI) mg mL ⁻¹	Inclination (β±SE)	χ ²	H
LGRA-106	902	4	8.82 (7.61–10.06)	5.40±0.33	8.37	2.09
LGRA-108	995	4	14.77 (13.87–15.51)	15.65±1.47	6.18	1.54

⁽¹⁾n, total number of caterpillars; DF, degree of freedom for the chi-square test; LC₅₀ (95% CI), lethal concentration to 50% at a 95% confidence interval; β±SE, slope coefficient ± standard error; χ², chi-square test; and H, heterogeneity.

Table 4. Averages (± standard error) for the viability and duration of the larval and pupal stages of *Plutella xylostella* treated with the oils of two genotypes of *Lippia gracilis* (LGRA-106 and LGRA-108) and their major compounds, using the values of the lethal concentration to 50% (LC₅₀) estimated by spraying⁽¹⁾.

Biological parameter	Larval viability (%)	Duration of larval stage (days)	Pupal viability (%)	Duration of larval stage (days)
Control	78.20±2.21a	5.42±0.14a	51.06±3.96a	5.62±0.11ab
LGRA-106	46.73±4.02b	5.48±0.09a	60.51±5.35a	4.87±0.32b
Thymol	44.77±3.73b	5.63±0.12a	64.70±5.11a	5.80±0.06a
LGRA-108	29.14±5.21c	5.43±0.18a	58.16±5.78a	4.88±0.33b
Carvacrol	32.22±2.98c	5.80±0.11a	60.88±7.91a	6.13±0.19a
P-value	<0.0001	0.5745	0.5238	0.0048
F-value	25.51	0.74	0.82	4.83
CV (%)	20.55	8.01	23.76	11.70

⁽¹⁾Values followed by equal letters, in the columns, do not differ by Duncan's test, at 5% probability. CV, coefficient of variation.

activity of carvacrol on AChE has been reported for houseflies, ticks, and cockroaches (Anderson & Coats, 2012). Another action of the insecticidal activity of the thymol and carvacrol compounds is related to the interaction with γ -aminobutyric acid (GABA), and the efficiency of the binding of these monoterpenoids with GABA receptors has been verified in fruit flies and house flies (Priestley et al., 2003; Tong & Coats, 2010). However, with this information alone, it was not possible to determine whether the GABA system was affected positively or negatively; therefore, it was necessary to evaluate the GABA-induced uptake of Cl^- , which, in this case, had an increased efficiency with carvacrol compared with thymol (Tong & Coats, 2010). However, thymol can reduce the action of cytochrome P450 and glutathione S-transferase, which are proteins responsible for the detoxification system and the metabolism of endogenous and exogenous compounds (Waliwitiya et al., 2012).

The effect of essential oils on insects can also be assessed through developmental studies. Essential oils of thyme, composed of 41.2% thymol, caused the death of larvae and pupae and decreased the weight of pupae of *Anticarsia gemmatalis* (Hübner, 1818) Lepidoptera: Erebididae, whereas oregano (*Origanum vulgare* L.) oil, composed of 74.9% carvacrol, reduced the number and viability of eggs/females (Marinho-Prado et al., 2019). However, in the present study, there was no significant change in the pupal and larval development of *P. xylostella* caused by *L. gracilis* oils.

Through the evaluation of daily larval mortality, it was possible to draw a survival curve of the larval stage of *P. xylostella* as a function of time, showing the intensity of the toxicity of the treatments on the insects (Figure 1). Although the genotypes and their major compounds caused different larval mortality levels, there was no difference regarding the survival time of the caterpillars after treatment, which varied from 1.70 to 1.97 days, contrasting from the 4.37 days for the control. The difference in larval survival between the control and treatments reinforces the insecticidal activity of the tested *Lippia* oils and their compounds on *P. xylostella* caterpillars.

When using insecticidal plants in pest control, secondary effects to mortality, such as oviposition and/or feeding reduction, can be observed with repellency tests (Brito et al., 2015).

The effect of the repellency of the LGRA-106 oil on *P. xylostella* caterpillars was stronger after 1 hour of exposure, with 100% repellency of the caterpillars after 12 hours (Figures 2 and 3). For LGRA-108, repellency was 94% in the first hour of exposure and 83.3% at 48 hours after the sample was released (Figure 3).

In the free-choice test, where the presence of the caterpillar was observed either on or under the cabbage disk, feeding activity was evident by the presence

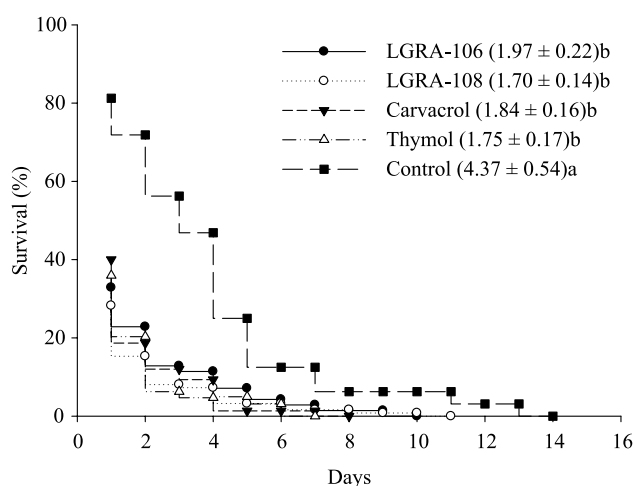


Figure 1. Daily (%) and average (\pm standard error) survival curve of *Plutella xylostella* caterpillars exposed to oils from the LGRA-106 and LGRA-108 *Lippia gracilis* genotypes and to their major compounds (long-rank: $\chi^2 = 33.32$; $p < 0.0001$).

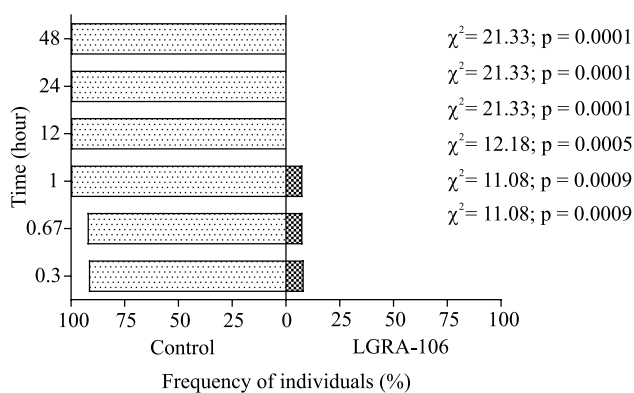


Figure 2. Frequency of choice of *Plutella xylostella* caterpillars treated with the essential oil of the LGRA 106 *Lippia gracilis* genotype.

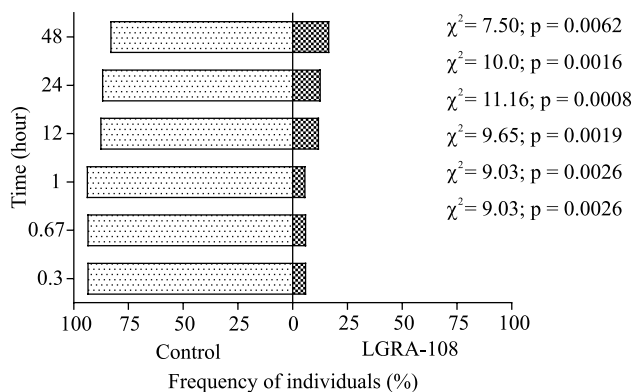


Figure 3. Frequency of choice of *Plutella xylostella* caterpillars treated with the essential oil of the LGRA 108 *Lippia gracilis* genotype.

of perforations and excrements in and on the disk, respectively.

Conclusions

1. The oils of the LGRA-106 and LGRA-108 *Lippia gracilis* genotypes have insecticidal activity in the control of *Plutella xylostella*, the diamondback moth.
2. Genotype LGRA-108 shows a greater insecticidal activity by spraying and LGRA-106 through residual action.
3. In the laboratory, the lethal concentration to 50% (LC₅₀) of the essential oils of *L. gracilis* and their major compounds reduces the larval viability of *P. xylostella*.
4. The LC₅₀ of the LGRA-106 genotype is 100% repellent after 12 hours of exposure of the caterpillars to the treatment.

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