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Efficiency of insecticides on nymphs and adults of *Dichelops melacanthus* (Dallas, 1851) (Hemiptera: Pentatomidae)

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Abstract

The green-belly stink bug, *Dichelops melacanthus*, stands out as one of the main pests of the corn crop, mainly for its potential damage at the beginning of crop development, due to the sap suction at the base of seedlings, causing tillering and trimming symptoms, decreasing productivity and product quality. Aiming at the control of the stink bug, the objective of the present research was to evaluate the effect of insecticides, as well as the lethal time (LT50) of these products on *D. melacanthus*. To perform the tests, it was used third instar nymphs and adults of *D. melacanthus* aged up to 72 hours, which were individualized in Petri dishes. For the application of the insecticide, corn leaves were collected and placed in plastic trays. With the aid of a pressurized spray, the insecticides Acefathe + Aluminum silicate (5 g/L), Thiamethoxam + Lambda-Cyhalothrin (1 mL/L), Imidacloprid + Bifenthrin (1.75 mL/L), Bifenthrin + Zeta-Cypermethrin (0.75 mL/L) and Azadiractin (10 mL/L) were applied. In the control, only distilled water was applied. Subsequently, the pulverized leaf pieces were individualized in the Petri dishes, where the third instar and adult bedbugs were already allocated. After application, each treatment was evaluated at 15, 30 minutes, 1, 3, 5, 24 and 48 hours, accounting for insect mortality. To correct mortality, the values were transformed using the Scheneider-Orelli formula. The relationship between the periods of action of the insecticides on the mortality rate of insects was verified by nonlinear regression analysis, using the mathematical model of dose-response. All chemical insecticides evaluated were effective in controlling third instar nymphs; however, when applied to adults, Thiamethoxam + Lambda - Cyhalothrin and Acefathe + Aluminum silicate had reductions in bedbug mortality. Azadiractin showed low efficiency in controlling nymphs and adults of *D. melacanthus*. Insecticides showed better LT50 in nymphs when compared to adults. The insecticides Imidacloprid + Bifenthrin, Thiamethoxam + Lambda - Cyhalothrin and Bifenthrin + Zeta-Cypermethrin obtained the best LT50 responses for nymphs and adults of the green-belly stink bug. All chemical insecticides evaluated can be used in the integrated management of *D. melacanthus.*

Keywords: integrated pest management; corn; green-belly stink bug.

Eficiência de inseticidas sobre ninfas e adultos de *Dichelops melacanthus* (Dallas, 1851) (Hemiptera: Pentatomidae)

Resumo

O percevejo barriga-verde, *Dichelops melacanthus*, destaca-se como uma das principais pragas da cultura do milho, principalmente por seu potencial de danos no início do desenvolvimento da cultura, em virtude da sucção da seiva na base de plântulas, causando sintomas de perfilhamento e enfezamento, diminuindo a produtividade e a qualidade do produto. Visando o controle do percevejo, o objetivo da presente pesquisa foi avaliar o efeito de inseticidas, bem como, o tempo letal (TL50) destes produtos sobre *D. melacanthus*. Para a realização dos ensaios, utilizou-se ninfas de terceiro instar e adultos de *D. melacanthus* com idade de até 72 horas, que foram individualizados em placas de Petri. Para a aplicação do inseticida, folhas de milho foram coletadas, cortadas e alocadas em bandejas plásticas. Com auxílio de um borrifador pressurizado, foram realizadas as aplicações dos inseticidas Acefathe + Silicato de alumínio (5 g/L), Tiametoxan + Lambda-Cialotrina (1 mL/L), Imidaclopride + Bifentrina (1,75 mL/L), Bifentrina + Zeta-Cipermetrina (0,75 mL/L) e Azadiractina (10 mL/L). Na testemunha, aplicou-se apenas água destilada. Posteriormente, os pedaços de folhas pulverizados foram individualizados nas placas de Petri, onde já estavam alocados os percevejos de terceiro instar e adultos. Após a aplicação, cada tratamento foi avaliado no tempo de 15, 30 minutos, 1, 3,

5, 24 e 48 horas, contabilizando a mortalidade dos insetos. Para correção da mortalidade os valores foram transformados a partir da fórmula de Scheneider-Orelli. A relação entre o período de tempo de ação do inseticida sobre a taxa de mortalidade dos insetos foi verificada pela análise de regressão não linear, utilizando o modelo matemático de dose-resposta. Todos os inseticidas químicos avaliados foram eficazes no controle de ninfas de terceiro instar, porém, quando aplicados sobre adultos, Tiametoxan + Lambda-Cialotrina e Acephate + Silicato de alumínio tiveram reduções na mortalidade do percevejo. Azadiractina apresentou baixa eficiência no controle de ninfas e adultos de *D. melacanthus*. Os inseticidas apresentaram melhor TL50 em ninfas quando comparado com os adultos. Os inseticidas Imidacloprido + Bifentrina, Tiametoxan + Lambda-Cialotrina e Bifentrina + Zeta-Cipermetrina obtiveram as melhores respostas de TL50 para ninfas e adultos do percevejo barriga-verde. Todos os inseticidas químicos avaliados podem ser utilizados no manejo integrado de *D. melacanthus*.
Palavras-chave: manejo integrado de pragas; milho; percevejo barriga-verde.

Introduction

Corn (*Zea mays* L.) is one of the most important cereals in the world, being widely used in human and animal food, biofuel manufacturing and high technology industries, such as production of biodegradable films and packaging (BARROS; CALADO, 2014; GALVÃO *et al*., 2014; MARTINS, 2013; MELO *et al*., 2010; MIRANDA, 2003; NUNES, 2017; SANTOS *et al*., 2014). This sum of factors makes it possible for Brazil to be the third largest world producer of this crop, with an annual production of 91,190.3 thousand tons in the 2018/19 crop year (CONAB, 2019).

Due to this importance, new research in the field of providing an increase in the productivity of corn crops is emerging. As an example, studies related to no-till, hybrids with greater productive potential, increase in cultivated area and studies of integrated management of diseases, weeds and pests (RODRIGUES, 2011). However, some factors related to the inadequate management of the crop and the lack of information and training, triggered the population growth of some species of insect pests, especially the green belly stink bug, *Dichelops melacanthus* (DALLAS, 1851) (Hemiptera: Pentatomidae).

The green-belly stink bug is an important pest that affects the beginning of corn development, causing damage to seedlings, with tillering, stunting and breaking of plants (ÁVILA; PANIZZI, 1995; BIANCO, 2005; ROZA-GOMES *et al*., 2011). During feeding, the stink bug introduces its stylus into young tissues, injecting spittle to facilitate the suction of sap. Depending on the age of the seedling and the intensity of the infestation, stink bug damage can cause anything from mild symptoms to death of the seedling. This begins with withering of the central leaves; symptom of dead heart, and ends with the total dryness of the plant, reducing the crop stand (HORI, 2000; PANIZZI, 2015; RODRIGUES, 2011). The damage caused by the green-belly stink bug in corn is significant. Studies demonstrates that a green-belly stink bug per m^{-2} can cause a reduction of up to 5.98% in grain production/ha^{-1} (DUARTE *et al*., 2015).

Particularly in the southern region of Brazil, where off-season corn is grown after the soybean harvest, *D. melacanthus* finds conditions of climate and food diversity that favors the survival and multiplication of the insect to the point of reaching populations that can cause significant damage in the crop, especially in its early stages of development (CHOCOROSQUI; PANIZZI, 2004; ROZA-GOMES *et al*., 2011).

Due to its high potential for damage, currently the most effective control of green-belly stink bug has been carried out preventively, treating seeds with insecticides (ALBUQUERQUE *et al*., 2006; ÁVILA; DUARTE, 2012; BRUSTOLIN *et al*., 2011; CHOCOROSQUI, 2001; MARTINS *et al*., 2009) or in sprays. In this case, the sprays need to be realized in the initial phase of the crop development (ALBUQUERQUE *et al*., 2006; CHOCOROSQUI, 2001; GRIGOLLI *et al*., 2016, 2017).

Studies indicate that the control of *Dichelops* sp. in wheat and corn, with the use of insecticides, it has become increasingly inefficient, due to the increased resistance of these pests to these chemicals, if there is a need to increase the dosage of the products in relation to the package insert (CHIESA *et al*., 2016; CHOCOROSQUI; PANIZZI, 2004 ; MARTINS *et al* ., 2009).

Due to this context, there is a need for new research, aiming the efficient management and control of this insect pest. Thus, the objective of the present research was to evaluate the effect of insecticides, as well as the lethal time (LT50) of these products on the third instar and adult nymphs of the stink bug *D. melacanthus.*

Material and Methods

COLLECTION OF *D. melacanthus*

In order to obtain the initial population of *D. melacanthus*, collections were carried out in commercial crops with cultivation of soybeans (*Glycine max* L. Merril) and corn (*Z. mays* L.), as well as straw and weeds, such as spiderwort (*Commelina sp.*), sourgrass (*Digitaria insularis* L.), goosegrass (*Eleusine indica* L.) and hairy fleabane (*Conyza sp.*). After collection, the insects were taken to the Entomology laboratory of the Faculdade Educacional de Medianeira - UDC Medianeira. After that, they are placed in cages for mass rearing.

MASS REARING OF *D. melacanthus* IN LABORATORY

After collection in the field, the insects were placed in breeding cages, which consisted of adapting plastic boxes (14 x 11 x 8 cm), with the surface lined with tulle fabric sewn with a zipper.

For mass breeding of *D. melacanthus*, it was made available a diet based on green beans, peanut and okra seeds. In addition, cotton pads soaked with water were distributed in Petri dishes. The boxes were cleaned every two days to remove eggs, dead insects and food exchange (CHOCOROSQUI, 2002; DUARTE *et al*., 2009; MODOLON *et al*., 2016; PANIZZI, 2002; PEREIRA *et al*., 2008; WAGNER, 2017).

The breeding cages were kept in an air-conditioned chamber (BOD) with a temperature of 25 °C ± 1 °C, with relative humidity of 60% ± 10% and a photoperiod of 14 h (COSTA *et al*., 1998; CHOCOROSQUI, 2002; PANIZZI; MOURÃO, 1999).

For oviposition, germitest paper was placed on one side of the breeding cage. The collection of eggs was carried out with the aid of a brush and tweezers, where all the collected eggs were separated in Petri dishes, covered by transparent film paper, and later, placed in the air-conditioned chamber, with a temperature of 25 °C ± 1 °C and a photoperiod of 14 h.

After the eggs hatched, the first instar nymphs were transferred in gerbox type boxes with cotton soaked with water. In addition, small corn leaves and pieces of green beans were disposed in the boxes for feeding. The nymphs were kept in the air-conditioned chamber, where food and water were replaced daily, until the emergence of the adults. The adults were put back in the cages for mass rearing.

INSECTICIDES ACTIVITY ON NYMPHS AND ADULTS OF *D. melacanthus*

To verify the efficiency of insecticides in the nymphal and adult stages of *D. melacanthus*, experimental tests were conducted at the Entomology Laboratory of the Faculdade Educacional de Medianeira. The experimental design adopted was a completely randomized design in a 6x6x2 factorial scheme (insecticides x time after application x stage of insect development). Each treatment (insecticides + control) was composed of 12 repetitions.

The treatments included four commercial insecticides recommended to control *D. melacanthus* in corn, a commercial plant extract based on Neem and the control, which are treated with distilled water (Table 1).

To perform the tests, first instar nymphs were individualized in gerbox type boxes, fed with beans and water-soaked pods. The development of insects was monitored daily until they reached the third instar and adult phase, which are phases used to carry out the application of insecticides (PELLIN, 2017, adaptaded).

The identification of the stages of the nymphs was carried out according to the morphological description described by Pereira *et al*. (2007), as well as the monitoring of ecdysis (exuvia). The adult insects were separated at the age of up to 5 days for the experimental tests.

Table 1 Treatments containing the active ingredients, chemical groups, action mode and dosages of insecticides tested.

	ACTIVE INGREDIENT	CHEMICAL GROUP	ACTION MODE	DOSAGE
T1	Acefathe + Aluminum silicate	Organophosphates	Systemic contact and ingestion insecticide	0,8-1 Kg p.c/ha^{-1}
T2	Thiamethoxam + Lambda-Cyhalothrin	Pyrethroid + Neonicotinoid	Systemic contact and ingestion insecticide	200-300 mL p.c/ha^{-1}
T3	Imidacloprid + Bifenthrin	Neonicotinoid + Pyrethroid	Systemic contact and ingestion insecticide	300-400 mL p.c/ha^{-1}
T4	Bifenthrin + Zeta-Cypermethrin	Pyrethroid + Pyrethroid	Contact and ingestion insecticide	100-200 mL p.c/ha^{-1}
T5	Azadiractin	Triterpenoid	Contact and ingestion insecticide	1L p.c/100 L water
T6	Distilled water	———	———	1L of water

When the insects were of adequate age, they were individualized in Petri dishes. For the application of insecticides, corn leaves, cultivar Feroz® (Viptera 3) in stage V2-V4, planted in pots, were collected, cut into small pieces and placed in plastic trays. With the aid of a pressurized spray, in the gas exhaust chamber, insecticides were sprayed on the leaves (SILVA, 2017), according to the dosage indicated on the package insert for each product (Table 1).

The dosage of the insecticides tested was converted to one liter of spray mix, with 5 g, 1 mL, 1.75 mL, 0.75 mL and 10 mL being applied for treatments T1, T2, T3, T4 and T5, respectively. In the control, only distilled water was sprayed. Subsequently, pieces of pulverized leaf were individualized in the Petri dishes, where insects (third-instar nymphs and adults) were also individualized.

Then, the Petri dishes were covered with film paper, perforated with an aeration pin, identified with the numbers of treatments and repetitions, and stored in the air-conditioned chamber.

After the application of the products, each treatment was evaluated in 15 min, 30 min, 1 h, 3 h, 5 h and 24 h, accounting for insect mortality.

To obtain the values of total mortality and lethal time, the observed values of mortality in the respective times were submitted to transformation using the Scheneider-Orelli formula to correct mortality.

Then, the lethal time for mortality of 50% of the population (LT50) was analyzed, as well as the comparison between products, in order to verify its efficiency in total mortality.

To obtain the LT50, a dose-response equation was used, correlating the time period of action of the insecticide on the mortality rate of insects according to equation 1:

In which:

A – Upper limit of the curve;

B - Lower limit of the curve;

LT50$_{\%}$ - time period (decimal time) that corresponds to 50% of the mortality rate reached by the molecule;

C – Slope coefficient of the curve.

To obtain the coefficients of the mathematical model and obtain the statistical parameters, the data were subjected to non-linear logistic regression analysis, with significance by the F test for the model. In this test, the analysis of the regression residues was performed using normality distribution tests estimation errors by the Shapiro-Wilk test and by the homoscedasticity test of the variances of the estimates errors. Spearman's test was also used, at the level of 5% probability of error. The software used for statistical procedures was Sigmaplot version 11.0 (SIGMAPLOT, 2011).

In comparing efficiency between products, the combined proportions comparison test was used. In this test, it was calculated from the mortality percentage values at 48 h of each product, making a comparison where all were combined with all, according to equations 2 and 3:

Equation 1: Equation for obtaining P values between two insecticides.

$$P = \frac{(N1-P1)+(N2-P2)}{(N1+N2)}$$

Equation 2: Z-calculated for analysis of the comparison of proportions between two insecticides.

$$Zcal = \frac{P1-P2}{\sqrt{\frac{P \times (1-P)}{N1} + \frac{P \times (1-P)}{N2}}}$$

In which:

P - Combined proportion;

P1 - Percentage values in decimal form of mortality found for the first product;

P2 – Percentage values in decimal form of mortality found for the second product;

N1 – Decimal values of the desired mortality in product 1;

N2 – Decimal values of the desired mortality in product 2.

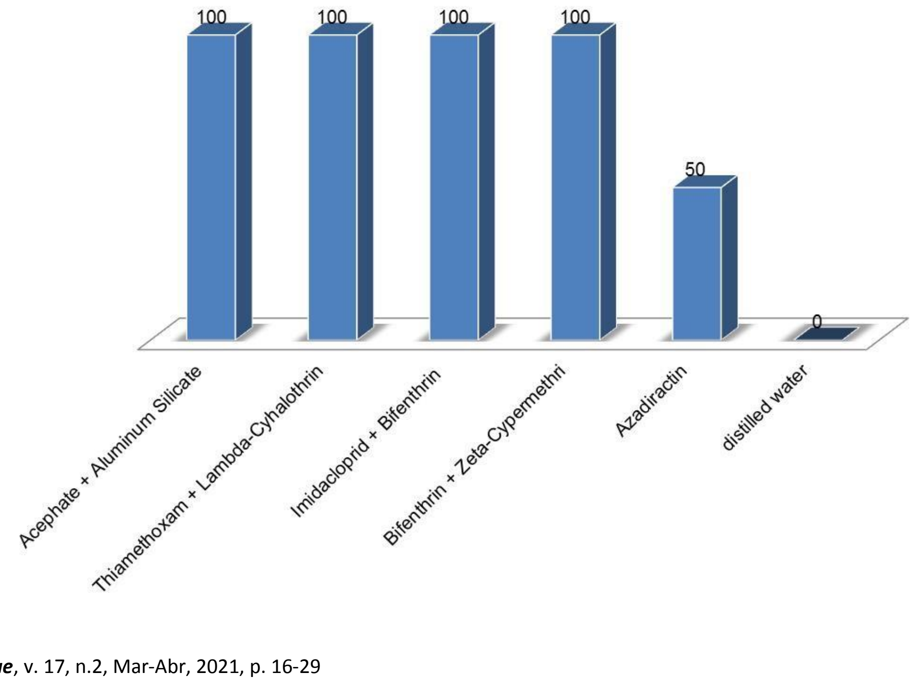
After obtaining the calculated P and Z values (test to compare proportions), they were compared with the tabulated Z values (5% = 1.96) in order to verify statistical differences among the tested products.

Results and Discussion

ACTION OF INSECTICIDES ON NYMPHS AND ADULTS OF *D. melacanthus*

The survival of third instar nymphs and adults of *D. melacanthus* was significantly influenced by the chemical insecticides applied (Figures 1 and 2; Tables 2 and 3). In third-instar nymphs, the insecticides Acephate + Aluminum Silicate, Thiamethoxam + Lambda-Cyhalothrin, Imidacloprid + Bifenthrin and Bifenthrin + Zeta-Cypermethrin caused 100% mortality 48 h after application (Figure 1).

Figure 1. Mortality (%) of nymphs of third instar of *D. melacanthus* after 48 h of the application of insecticides.



Evaluating the insecticides tested, it was found that the treatments Acephate + Aluminum Silicate, Thiamethoxam + Lambda-Cyhalothrin, Imidacloprid + Bifenthrin and Bifenthrin + Zeta-Cypermethrin were statistically equal, and differed only from Azadiractin and the control (Table 2).

The neem-based botanical insecticide (Azadiractin) showed a 50% efficiency in the mortality of third instar nymphs (Figure 1), not differing statistically from the control (Table 2). However, showing a significant difference when compared to the chemical insecticides evaluated (Table 2).

Table 2. Comparison of proportions of Z-calculate and Z-tabeled for mortality values in third instar nymphs of *D. melacnthus* as a function of the active principle.

TREATMENTS	**Value Z calculated**	
T1 vs T2	0	A
T1 vs T3	0	A
T1 vs T4	0	A
T1 vs T5	2.84	B
T1 vs T6	4.14	B
T2 vs T3	0	A
T2 vs T4	0	A
T2 vs T5	2.84	B
T2 vs T6	4.14	B
T3 vs T4	0	A
T3 vs T5	2.84	B
T3 vs T6	4.14	B
T4 vs T5	2.84	B
T4 vs T6	4.14	B
T5 vs T6	1.73	Bc

Letters assigned from Z-tabeled (5%) = 1.96

For adults, the results obtained were similar to those found in immature *D. melacanthus* (Figure 2 and Table 3). Imidacloprid + Bifenthrin and Bifenthrin + Zeta-Cypermethrin reached 100% efficiency in insect mortality (Figure 2). This efficiency decreased to 89.9% in the active ingredients Acephate + Aluminum Silicate and Thiamethoxam + Lambda-Cyhalothrin (Figure 2). However, when analyzing the interaction between the products, these chemical insecticides also did not show statistical difference between them, but they differed significantly from the control and from the neem-based botanical insecticide (Table 3).

Figure 2. Mortality (%) of adults of *D. melacanthus* after 48 h of insecticide application.

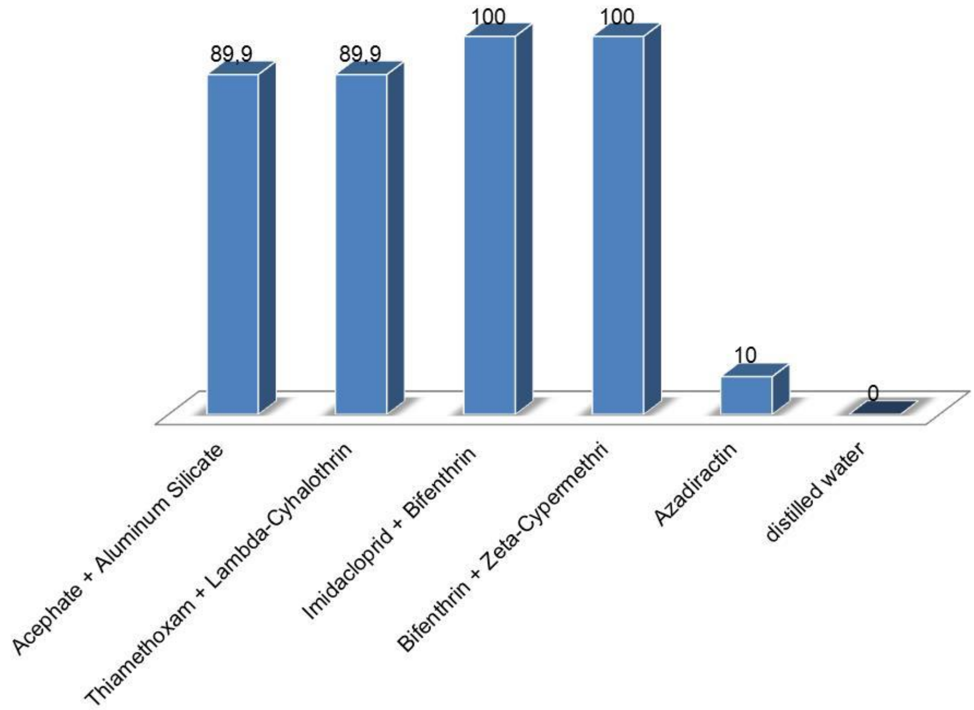


Table 3. Comparison of proportions of Z-calculated and Z-tabled for the mortality values for adults of *D. melacanthus* as a function of the active principle.

TREATMENTS	**Values Z-calculated**	
T1 vs T2	0	A
T1 vs T3	1.13	A
T1 vs T4	1.13	A
T1 vs T5	3.91	B
T1 vs T6	4.42	B
T2 vs T3	1.13	A
T2 vs T4	1.13	A
T2 vs T5	3.91	B
T2 vs T6	4.42	B
T3 vs T4	0	A
T3 vs T5	4.43	B
T3 vs T6	4.90	B
T4 vs T5	4.43	B
T4 vs T6	4.90	B
T5 vs T6	1.13	Bc

Letters assigned from Z-tabled (5%) = 1.96

With the exception of Acephate + Aluminum Silicate and Thiamethoxam + Lambda-Cyhalothrin for adults, the other chemical insecticides, at the recommended dosages, showed excellent levels of mortality in nymphs and adults of the green-belly stink bug (Figures 1 and 2; Tables 2 and 3).

Similar results were verified in the adult mortality of the brown stink bug - *Euschistos heros*, with the application of the insecticides Thiamethoxam + Lambda-Cyhalothrin (76.47%), Imidacloprid + Bifenthrin (78.99%) and Acephate (RIBEIRO *et al*., 2016).

In addition, for *E. heros*, in soybean, Thiamethoxam + Lambda-Cyhalothrin had a lower mortality percentage in adults (84.56%) compared to nymphs (94.67%) of this species (ENGEL *et al*., 2018). The same result was found for Acephate (83.68% in nymphs and 75.31% in adults) and Imidacloprid + Bifenthrin (94.91% in nymphs and 84.49% in adults) (ENGEL *et al*., 2018). Thus, it appears that in adults the efficiency in the control of stink bugs using such active ingredients was reduced, according to the results found in the present study, using Acephate + Aluminum Silicate and Thiamethoxam + Lambda-Cyhalothrin in *D. melacanthus* (Figure 2).

Tests with applications of different doses of Thiamethoxam + Lambda-Cyhalothrin (37.05 g i.a/ha^{-1} and 61.75 g i.a/ha^{-1}) at eight days after corn emergence (DAE), showed low efficiency (34 and 49%, respectively, according to dosages) in the control of *D. melacanthus* adults (ALBUQUERQUE *et al*., 2006), diverging from the results of this study. However, when allied seed treatment was applied after the crop emerged, the control was satisfactory, causing mortality of 84% (61.75 g i.a/ha^{-1}) of stink adults (ALBUQUERQUE *et al*., 2006).

In a study using the active ingredient Thiamethoxam + Lambda-Cyhalothrin, mortality values of 58.3% to 87.5% were found at three days after application on *D. melacanthus* in the dosages 150 mL/ha^{-1} and 300 mL/ha^{-1}, respectively. Again, when combining leaf spray together with seed treatment with Thiamethoxam, the mortality values increased to 95.8% and 100%, according to the respective dosages (ÁVILA; DUARTE, 2012).

Working with the control of the green-belly stink bug, it was found that the mixture of Thiamethoxam + Lambda-Cyhalothrin (450 mL p.c./100 mL water) was more effective in controlling adults of *D. melacanthus* (56.57%) in comparison with the active ingredient Imidacloprid + Beta-cyfluthrin (450 mL p.c/100 mL water) (45%) 24 hours after application (WAGNER, 2017). It was also found that, in longer evaluations (48 hours after application), the active ingredient Thiamethoxam + Lambda-Cyhalothrin, had higher mortality results as a function of time, reaching 86.57% efficiency in pest control (WAGNER, 2017).

Aiming only at seed treatment, Imidacloprid + Thiodicarb (97.5%) showed better results in the control of *D. melacanthus* when compared to Thiamethoxam (82.5%) (FERNANDES, 2017). In addition, in wheat, for the same species, it was also found that Imidacloprid used in seed treatment had higher production rates (237.9 g/m^{-2}) when compared to Thiamethoxam (240.3 g/m^{-2}) (CHOCOROSQUI; PANIZZI, 2004).

The results found in the present research show that mixtures of active ingredients are highly effective in controlling *D. melacanthus* (Figures 1 and 2). The fact that the treatments Thiamethoxam + Lambda-Cyhalothrin and Acephate + Aluminum Silicate in adults cause a small reduction in its effectiveness, can be corrected with the use of seed treatment, as already verified in other researches with the respective pest (ALBUQUERQUE *et al*., 2006; ÁVILA; DUARTE, 2012; BRUSTOLIN *et al*., 2011; CHIESA *et al*., 2016; CHOCOROSQUI; PANIZZI, 2004; ENGEL *et al*., 2018; FERNANDES, 2017; GRIGOLLI *et al*., 2016; 2017).

In the evaluation of the botanical insecticide, the mortality of nymphs (50%) was higher when compared to adults (10%) (Figures 1 and 2). However, both treatments resulted in a significant difference compared to chemical insecticides (Tables 2 and 3).

Similar results to that found in this research, using neem insecticide-based products, it was reported for the bug *E. heros*, however, with longer evaluation times (7 days), which resulted in higher mortality results (24.76%) of adults of this species (SILVA *et al*., 2013).

Studies prove the efficiency of neem insecticides directly on pests when used in higher concentrations. In this context, it was found that Azadiractin plant extract at a concentration of 32 mL p.c/L resulted in better efficiency in the control (84.78%) of whitefly nymphs (*Bemisia argentifolii* Bellows & Perring) when compared to the concentration of 4 mL p.c/L (25.25%) (SILVA *et al*., 2003).

It is noteworthy that in the present study, the low mortality caused by Azadiractin in nymphs and adults (Figures 1 and 2) may have been a reflection of the dosage used for worked concentration (1%). In addition, another important factor to be mentioned is that in several studies the evaluations take place over a longer period of time (SILVA *et al*., 2003; SILVA *et al*., 2013), and in this study, the observations occurred only until 48h after application of the products.

EVALUATION OF LETHAL TIME (LT50) OF INSECTICIDES ON NYMPHS AND ADSULTS OF *D. melacanthus*

After checking the total accumulated mortality of the green-belly stink bug, the efficiency of insecticides was analyzed through the time necessary to occur at the lethal dose of

50% of the population (LT50) of third instar nymphs and adults of *D. melacanthus*.

The treatment Imidacloprid + Bifenthrin showed the LT50 most effective (0.23 h) in the control of third instar nymphs, followed by Thiamethoxam + Lambda-Cyhalothrin (0.24 h) and Bifenthrin + Zeta-Cypermethri (0.31 h) (Figure 3). The treatments Azadiractin and Acephate + Aluminum Silicate showed a higher LT50 when compared to the other insecticides tested, with 3.05 and 6.54 h, respectively (Figure 3).

For adult bedbugs, the LT50 increased significantly when compared to immature ones (Figures 3 and 4). The active ingredients that had the best responses in relation to time were Imidacloprid + Bifenthrin (1.74 h), Bifenthrin + Zeta-Cypermethri (2 h) and Thiamethoxam + Lambda-Cyhalothrin (2.82 h). For Azadiractin and Acephate + Aluminum Silicate, the LT50 was 7.51 and 8.30 h, respectively (Figure 4).

Figure 3. Lethal time (LT50) in *D. melacanthus* third instar nymphs for the active ingredients Acephate + Aluminum Silicate; Thiamethoxam + Lambda-Cyhalothrin; Imidacloprid + Bifenthrin; Bifenthrin + Zeta-Cypermethrin; Azadiractin.

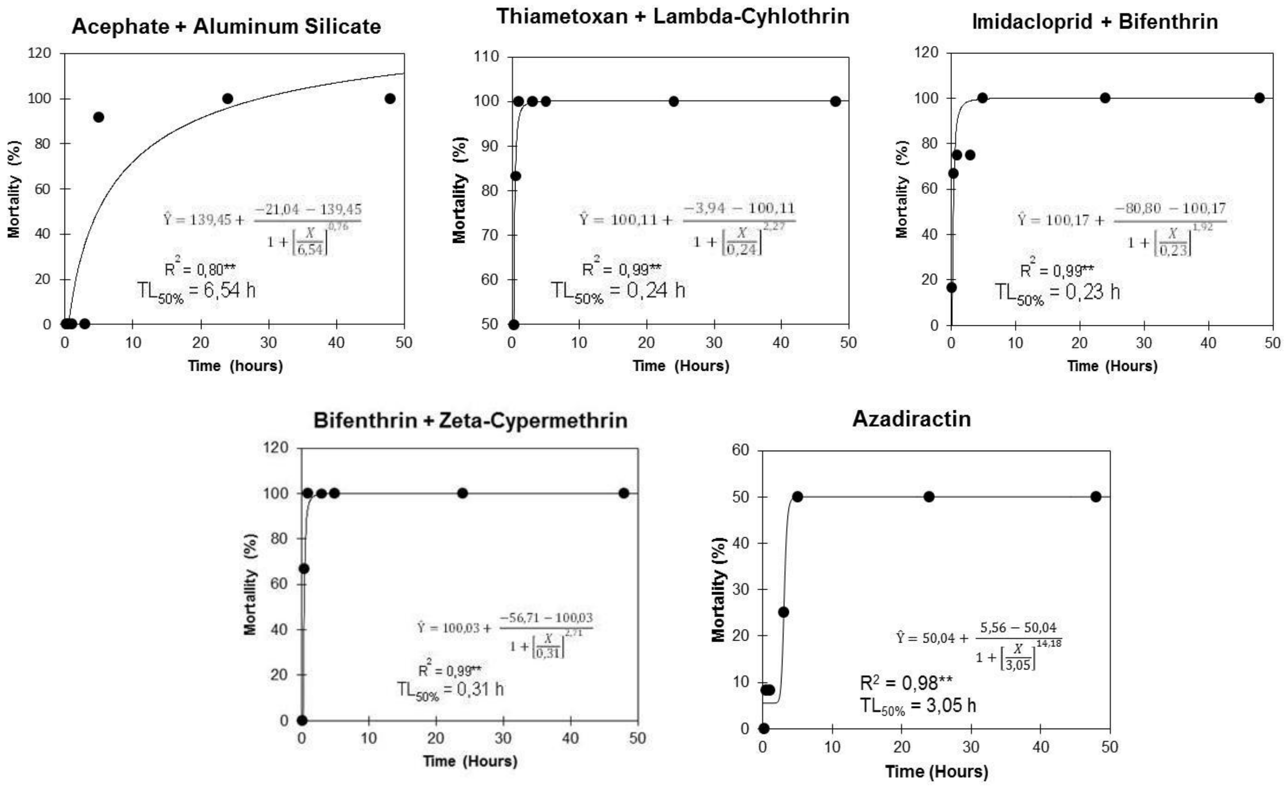
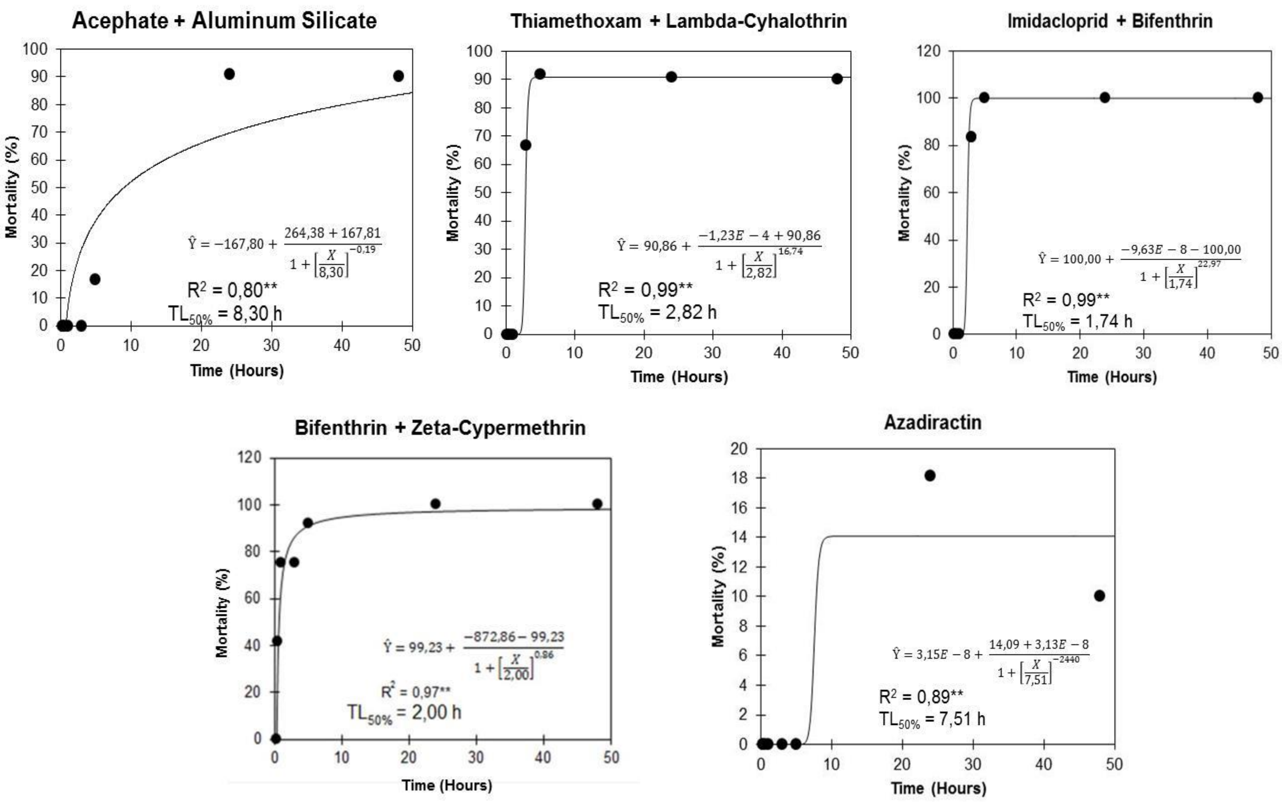


Figure 4. Lethal time (LT50) in *D. melacanthus* adults for the active ingredients Acephate + Aluminum Silicate; Thiamethoxam + Lambda-Cyhalothrin; Imidacloprid + Bifenthrin; Bifenthrin + Zeta-Cypermethrin; Azadiractin.



The best lethal times (LT50) in nymphs and adults of *D. melacanthus* occurred with the active ingredients Imidacloprid + Bifenthrin, Thiamethoxam + Lambda-Cyhalothrin and Bifenthrin + Zeta-Cypermethrin (Figures 3 and 4). This fact is possibly justified by its chemical groups (neonicotinoids and pyrethroids), as well as mechanisms of action of these insecticides.

The mechanism of action of the pyrethroids (Bifenthrin, Zeta-Cypermethrin Bifenthrin and Lambda-Cyhalothrin) occurs by binding to one of the subunits of sodium channels present in the nerve cells of the central nervous system of insects, keeping them open for a longer period. It causes the increase in sodium flow into the nerve cell membrane, prolonging the depolarization phase. Therefore, a rapid hyperexcitation of the nervous system occurs, causing loss of muscle coordination, seizures and finally to rapid death (GULLAN; CRANSTON, 2017; SANT'ANNA, 2009).

Neonicotinoids (Imidacloprid and Thiamethoxam), on the other hand, are derived from the nicotine molecule and act as mimicking of the neurotransmitter acetylcholine (ACh), competing with it for its receptors. Unlike ACh, neonicotinoid compounds are susceptible to enzymatic hydrolysis by the enzyme acetylcholinesterase and therefore, the continuous interaction with receptors also leads to hyper excitation of the nervous system, causing the same symptoms as pyrethroids (GULLAN; CRANSTON, 2017; SANT'ANNA, 2009).

Thus, insecticides composed of pyrethroids and neonicotinoids (Imidacloprid + Bifenthrin and Thiamethoxam + Lambda-Cyhalothrin) are tested in this research, at high control rates, since those using the latest tests of active use and with high shock action in the insects. In addition, they are advantageous, as they allow the potentiation of their action through the addition of a synergistic ingredient, further reducing the reduction and decreasing risk of insect resistance (FARIA, 2009; GULLAN; CRANSTON, 2017; MOREIRA *et al*., 2012).

The active ingredient Acephate + Aluminum Silicate, belonging to the chemical group of organophosphates, is classified as AChE inhibitors, since they bind to the enzyme acetylcholinesterase, inhibiting their action and providing accumulation of acetylcholine in the synapse. Due to the continuous and uncontrolled transmission of nerve impulses, it causes hyperexcitability, such as tremors, convulsions, collapse of the central nervous system and death. Thus, there is a paralysis of muscles, especially intercostal, which prevents breathing, leading to the death of the insect due to the lack of oxygen in the brain, being a slower process (GULLAN; CRANSTON, 2017; IRAC, 2016). This factor justifies the higher LT50 in nymphs and adults of *D. melacanthus* for this research (Figures 3 and 4).

Azadiractin has different mechanisms of action than the chemical insecticides evaluated, which possibly led this product to have presented inefficient control in conjunction with high LT50 in nymphs and adults of *D. melacanthus* (Tables 2 and 3; Figures 1 and 2). Its mechanism of action is related to the anti-food effect, growth regulator, effects on reproduction, dermal and repellent action (MARAGONI *et al*., 2012; MOSSINI; KEMMELMEIER, 2005; SCHMUTTERER, 1990), characteristics these not observed in this study.

It was also observed that, in general, the insecticides used in the present study had better responses when applied to third instar nymphs compared to adults (Figures 3 and 4). This result is possibly related to the greater susceptibility of the insect in the immature stages, since the integument is not fully developed, making it more fragile. According to the process called ecdysis, the cuticle creates thicker and more rigid layers, making the insect more resistant to adversity. This factor may have interfered with a higher LT50 in adult insects, since in most cases, the chemical groups used penetrate the insect through its exoskeleton (FARIA, 2009; GULLAN; CRANSTON, 2017).

The characterization of the lethal time of insecticides on nymphs and adults of *D. melacanthus* provided the verification of the performance of the active principles in the mortality of the bedbug according to their respective mechanisms of action. In addition, these results help in the context of resistance to insecticides according to the performance of each chemical researched.

Conclusions

In third instar nymphs, the insecticides Acephate + Aluminum Silicate, Thiamethoxam + Lambda-Cyhalothrin, Imidacloprid + Bifenthrin and Bifenthrin + Zeta-Cypermethrin caused 100% mortality for this pest.

For adults, Imidacloprid + Bifenthrin and Bifenthrin + Zeta-Cypermethrin reached 100% efficiency in insect mortality, while active ingredients Acephate + Aluminum Silicate and Thiamethoxam + Lambda-Cyhalothrin decreased the control efficiency to 89.9%. The botanical insecticide Azadiractin showed low efficiency in controlling nymphs and adults of *D. melacanthus*.

The active ingredients Imidacloprid + Bifenthrin, Thiamethoxam + Lambda-Cyhalothrin and Bifenthrin + Zeta-Cypermethrin presented the best LT50 in nymphs and adults of *D. melacanthus*, respectively. The insecticides evaluated had better lethal times when applied to third instar nymphs compared to adults of the green-belly stink bug.

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