

## **Toxicity of insecticides for adults of *Diceraeus melacanthus* Dallas, 1851 (Hemiptera: Pentatomidae) in three exposure modes**

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**Abstract.** Phytophagous stink bugs are considered the important pest in second-crop corn cultivation in Brazil, especially when they occur during the early stage of plant development. This study aimed to evaluate the efficiency of insecticides in controlling adults of the *Diceraeus melacanthus* (Dallas, 1851) stink bug when applied separately in three different modes of exposure. The treatments were evaluated in three modes of exposure of insecticides to *D. melacanthus* adults as described below: direct contact (direct application of the insecticide to the insects); tarsal contact (exposure through their walking on the treated surface) and ingestion (contact through their feeding on previously treated fresh bean pods). Mortality was assessed at 1, 5, 24 and 48 hours post-exposure. We observed that the percentage of accumulated mortality of *D. melacanthus* adults was significant through direct contact with chemical treatments. However, if the target insect does not receive direct spraying on its body, indirect contact through its tarsus walking on the treated surface can guarantee a significant final mortality of the stink bugs. In addition, although the mode of exposure through ingestion has shown low mortality, it may also contribute to the final mortality of stink bugs in the field depending on the chemical treatment applied to the crop.

**Key words:** chemical control, green belly stink bug, mortality, neonicotinoids.

### **INTRODUCTION**

Phytophagous stink bugs are the primary entomological issue in second-crop corn cultivation in Brazil from January to July, particularly when they appear during the early stages of plant development (Ávila & Panizzi, 1995). The green-bellied stink bug *Diceraeus melacanthus* Dallas, (Heteroptera: Pentatomidae) is considered a pest in several crops such as soybeans (Panizzi, 1997), corn (Guedes et al., 2017) and wheat (Manfredi-Coimbra et al., 2005). However, in corn, this species inflicts the most damage by inserting its beak into the sheath of the inner leaves of the plants, leading to

deformations, tillering, stunted growth, and even plant death (Bianco, 2005; Roza-Gomes et al., 2011; Duarte; Ávila; Santos, 2015).

The most used and effective way to control *D. melacanthus* in corn is with chemical insecticides applied both in seed treatments and in sprays during the early stages of crop development (Panizzi, 2000; Chiesa et al., 2016). These sprays frequently utilize insecticides from the pyrethroid and neonicotinoid groups, either applied alone or in combination, along with other active ingredients from the organophosphate and carbamate groups (Sósa-Gomez et al., 2001; Sósa-Gómez & Silva, 2010; Furlan & Kreutzweiser, 2015).

In agricultural pest management, insecticides primarily affect pests through three distinct modes of exposure. The most recognized mode is direct contact, where the insecticide upon contact with sprayed plants directly affects insects. Another significant mode is residual contact toxicity, where insects are directly affected by the insecticides upon contact with sprayed plants, such as leaves, branches, and reproductive structures, allowing the insecticide to adhere to their feet. Additionally, ingestion serves as another critical mode of exposure, wherein insects ingest insecticide residues present on vegetative and reproductive plant structures during feeding. This ingestion can affect both sucking and defoliating insects, influencing their population dynamics and control in agricultural settings (Leskey et al., 2012).

Several studies have been conducted involving the direct contact of insecticides with phytophagous stink bugs through topical application (Sosa-Gómes et al., 2009; Sósa-Gómez & Silva, 2010; Somavilla et al., 2020), as well as through tarsal contact in stink bugs (Sosa-Gómez & Corso, 2001; Tillman, 2006), the same occurring involving the mode of exposure through ingestion, which are rarer (Tibola et al., 2021). However, most of these studies involving different modes of exposure of insecticides for stink bugs are aimed at identifying populations of pests or natural enemy's resistant to insecticides, fewer studies have examined exposure through ingestion (Sósa-Gómez & Corso, 2001; Tillman & Mullinix, 2004; Tillman, 2006; Sósa-Gómez & Silva, 2010; Hush & Sósa-Gómez, 2013; Somavilla et al., 2020).

It is crucial that studies on exposure modes carry out when selecting active ingredients for pest control in crops. Understanding the effectiveness of each mode of exposure in controlling specific pests can provide valuable guidance to producers and consultants. This knowledge aids in making informed decisions about the most appropriate products for use across different crops, optimizing pest management strategies.

Therefore, the objective of the present work was to evaluate the efficiency of insecticides in controlling adults of the *D. melacanthus* stink bug when applied separately in three different modes of exposure.

## MATERIALS AND METHODS

The experiment was conducted at Embrapa Agropecuária Oeste, Dourados, Brazil. Seven treatments (six active ingredients + control) were evaluated on adults of *D. melacanthus* stink bug (Table 1). The doses of the insecticides evaluated in the present work correspond exactly to those recommended for the control of

*D. melacanthus* in corn crops (AGROFIT, 2022). The treatments were assessed across three exposure modes of adult insects: direct contact (direct application of the insecticide mixture to the insects); tarsal contact (exposure of stink bugs to insecticides: direct contact (direct application of the insecticide mixture to the insects); tarsal contact (exposure of stink bugs to insecticides through their walking onto treated surface) and ingestion (contact of stink bugs to insecticides through their feeding on previously treated fresh bean pods). The insects were field-collected.

**Table 1.** Treatments used to control adults of the *Diceraeus melacanthus* stink bug in three different modes of exposure (direct contact, tarsal contact and ingestion) under laboratory conditions. Embrapa CPAO

Treatments	g .a. ha <sup>1</sup>
1. Imidacloprid + beta-cyfluthrin	100.0 + 12.5
2. Thiamethoxam + lambda-cyhalothrin	35.2 + 26.5
3. Imidacloprid + bifenthrin	100.0 + 20.0
4. Acetamiprid + bifenthrin	75.0 + 75.0
5. Bifenthrin + carbosulfan	30.0 + 90.0
6. Acephate	750.0
7. Control (water)	-

<sup>1</sup>Doses recorded for the control of *D. melacanthus* in corn crop.

### Direct contact test

In this test, the chemical treatments were sprayed directly on the adult stink bugs using a constant pressure sprayer (CO<sub>2</sub>) equipped with a cone-type nozzle and using a volume of spray equivalent to 150 L ha<sup>-1</sup>. For the control treatment, only water was sprayed onto the insects using the same sprayer and spray volume as in the chemical treatments.

After spraying, the treated insects from each experimental unit (10 stink bug adults) were placed in rectangular trays measuring 0.2 m × 0.4 m, lined at the base with paper towel, thus constituting a repetition of the test. The trays were covered with tulle fabric, secured at the upper edges with elastic bands to contain the insects. Green bean pods were placed inside the trays as food for the stink bugs.

### Tarsal contact assay

For this mode of exposure, trays like those previously described for the direct contact test were lined at their base with clean green soybean leaves. Next, the soybean leaves were sprayed with the same chemical treatments as in the direct contact test, leaving them to settle for 1 hour to dry. After drying the insecticide mixture on the leaves, 10 adult stink bugs were released inside the tray to walk on the treated surface so that the contact of the insects with the products sprayed on the leaves was only through their tarsus. In the control treatment, soybean leaves were sprayed with water. Similar to the direct contact test, the trays were also covered with tulle fixed at the edges with elastic and untreated green bean pods were placed to feed the insects.

### Ingestion assay

In the ingestion test, the same chemical treatments were sprayed on the fresh green pods of common beans, which, after drying from the insecticide, were offered to the stink bugs for food. To do this, 10 adult stink bugs were placed inside a 100 mm diameter PVC cage which was closed at the top with tulle secured by an elastic band. Three green bean pods treated with insecticides were placed on the tulle to feed the stink bugs present in the cage. Untreated bean pods were placed in the control treatment. To avoid the stink

bug's tarsal contact with the treated pods, plastic straws (2.5 mm in diameter) were placed between the pods and the tulle fabric of the cage this ensured that the insect had contact with the bean pods only through their stylet during feeding. In the control treatment, the bean pods were sprayed with just water.

### Experimental conditions

The trays and cages containing the insects from the three tests were kept in laboratory conditions at a temperature of 25 °C and a 14-hour photophase. The three exposure mode trials were conducted in a completely randomized design with seven treatments (active ingredients + control) and four replications (PVC trays or cages with 10 adult insects). Stink bug mortality was evaluated at 1,5, 24 and 48 hours after the insects were first exposed to the insecticides in each mode of exposure.

### Data collection and analysis

The mortality values obtained in each experimental unit were subjected to analysis of variance and, when a significant treatment effect was found, the means were compared using the Scott-Knott test at 5% probability using the Rbio software (Bhering, 2017).

## RESULTS AND DISCUSSION

In the direct contact test, all chemical treatments, except for acephate (750.0) demonstrated significant levels of mortality in *D. melacanthus* adults within the first evaluation period (1 hour). The mortality observed in the acephate treatment did not differ significantly from the control group at this time (Table 2). However, in the subsequent evaluation at 5, 24, and 48 hours, all chemical treatments, including acephate, resulted in significantly higher stink bug mortality compared to the control, without notable differences between the treatments.

**Table 2.** Average number of dead *Diceraeus melacanthus* adults accumulated at 1, 5, 24 and 48 hours after spraying with different insecticides in the direct contact test under laboratory conditions. Embrapa CPAO

Treatments	1 hour	5 hours	24 hours	48 hours
Imidacloprid + beta-cyfluthrin	4.8 ± 1.25 a	6.3 + 0.40 a	9.8 + 0.30 a	10.0 + 0.00 a
Thiamethoxam + lambda-cyhalothrin	5.8 ± 1.63 a	8.7 + 0.40 a	10.0 + 0.0 a	10.0 + 0.00 a
Imidacloprid + bifenthrin	3.3 ± 2.38 a	5.3 + 1.70 a	9.5 + 0.40 a	10.0 + 0.00 a
Acephate	0.0 ± 0.00 b	6.0 + 1.3 a	9.8 + 0.30 a	10.0 + 0.00 a
Acetamiprid + bifenthrin	7.0 ± 1.00 a	7.3 + 0.80 a	10.0 + 0.00 a	10.0 + 0.00 a
Bifenthrin + carbosulfan	5.5 + 0,60 a	6.,0 + 0.00 a	10.0 + 0.00 a	10.0 + 0.00 a
Control	0.0 ± 0.00 b	0.0 + 0.00 b	1.0 + 0.50 b	1.5 + 1.20 b

<sup>1</sup>Recommended doses in corn crop to control *Diceraeus melacanthus*. Means followed by the same letter in the column do not differ statistically from each other using the *Scott-Knott test* at 5% probability.

Hush & Sosa-Gómez (2013) also found a slower action of the acephate insecticide to control the *Euschistus heros* stink bug in soybean crops compared to the other active ingredients tested, supporting the findings of this study in the direct contact exposure mode. Sosa-Gómez & Silva (2010), studying the resistance of *E. heros* adults to the methamidophos insecticide, found that direct contact (topical application) was effective in identifying resistant populations of this bug. Furthermore, Sosa-Gómez et al. (2009) also

found that direct contact through topical applications on *E. heros* adults was effective in identifying variations in the susceptibility of populations of this bug to the methamidophos and endosulfan insecticides. In another study, Lardeux et al. (2010), evaluating different insecticides for the control of the *Triatoma infestans* stink bug through direct contact exposure mode (topical application), successfully identified effective active ingredients for the control of this insect vector of Chagas disease in Bolivia.

In the tarsal contact exposure mode, only the acetamiprid + bifenthrin (75.0 + 75.0) and bifenthrin + carbosulfan (30.0+90.0) treatments resulted in significant mortality of *D. melacanthus* adults during the first hour of exposure (Table 3). For the other treatments, no significant mortality was observed compared to the control. In the 5, 24 and 48 h evaluations, only the mixtures containing the bifenthrin insecticide and the acephate insecticide (750.0) showed significant mortality of *D. melacanthus* adults (Table 3), when compared to the control, although the mixture with the lowest dose of bifenthrin had lower mortality in these last two evaluations (Table 3).

**Table 3.** Average number of dead *Diceraeus melacanthus* adults accumulated at 1, 5, 24 and 48 hours after spraying soybean leaves with different insecticides in the tarsal contact test by walking on the treated surface under laboratory conditions. Embrapa CPAO

Treatments	1 hour	5 hours	24 hours	48 hours
Imidacloprid + beta-cyfluthrin	0.0 + 0.00 b	0.3 + 0.35 b	0.8 + 0.67 c	2.8 + 0.67 c
Thiamethoxam + lambda-cyhalothrin	0.0 + 0.00 b	0.0 + 0.00 b	0.5 + 0.40 c	3.5 + 0.90 c
Imidacloprid + bifenthrin	0.0 + 0.00 b	2.8 + 1.93 a	3.0 + 2.21 b	6.3 + 1.84 b
Acephate	0.8 + 1.05 b	4.5 + 1.76 a	5.3 + 1.75 a	8.3 + 1.20 a
Acetamiprid + bifenthrin	1.5 + 0.70 a	2.8 + 0.67 a	6.3 + 1.75 a	10.0 + 0.00 a
Bifenthrin + carbosulfan	1.8 + 0.35 a	3.8 + 0.88 a	6.5 + 0.90 a	9.3 + 0.35 a
Control	0.0 + 0.00 b	0.0 + 0.00 b	0.0 + 0.00 c	0.8 + 0.35 d

<sup>1</sup>Recommended doses in corn crop to control *Diceraeus melacanthus*. Means followed by the same letter in the column do not differ statistically from each other using the *Scott-Knott test* at 5% probability.

Analyzing the mortality results from tarsal contact, it appears that acephate exhibited a slight delay in its toxic action against the stink bug, as it showed a significant mortality effect only after the 5-hour evaluation (Table 2). However, López et al. (2012), evaluating the mode of tarsal contact exposure on surfaces treated with acephate, found high toxicity of this insecticide for the *Euschistus servus* stink bug, similar to what was found in the present work, although the target species were different.

The effects of the tarsal contact exposure mode have also been applied in studies monitoring insecticide resistance to stink bugs (Sosa-Gómez & Corso, 2001) or even natural enemies (Tillman & Mullinix 2004; Tillman 2006). Studies with tarsal contact exposure can also be used to select effective insecticides for field use. Gradish et al. (2019) evaluated the effectiveness of twelve insecticides in controlling the Asian stink bug, *Halyomorpha halis*, through tarsal contact, verifying that the majority of the products tested were effective in controlling nymphs and adults of this pest. In a similar study, Leskey et al. (2012) evaluated the effectiveness of 37 insecticides aimed at controlling the *H. halis* using tarsal contact, and found that this mode of exposure allowed the selection of 14 effective active ingredients for pest control.

Regarding the toxicity of the insecticides tested by the ingestion mode of exposure, low levels of mortality of *D. melacanthus* adults were generally found, especially in the first three evaluations of the trial when none of the chemical treatments tested showed significant mortality of this pest (Table 4). A significant effect in this mode of exposure was only verified 48 hours after insect exposure when the imidacloprid + bifenthrin (100.0 + 20.0) and acephate (750.0) insecticide showed significant levels of stink bug mortality when compared to the mortality observed in the control (Table 4).

**Table 4.** Average number of dead *Diceraeus melacanthus* adults accumulated at 1, 5, 24 and 48 hours after spraying bean pods with different insecticides in the ingestion contact test under laboratory conditions. Embrapa CPAO

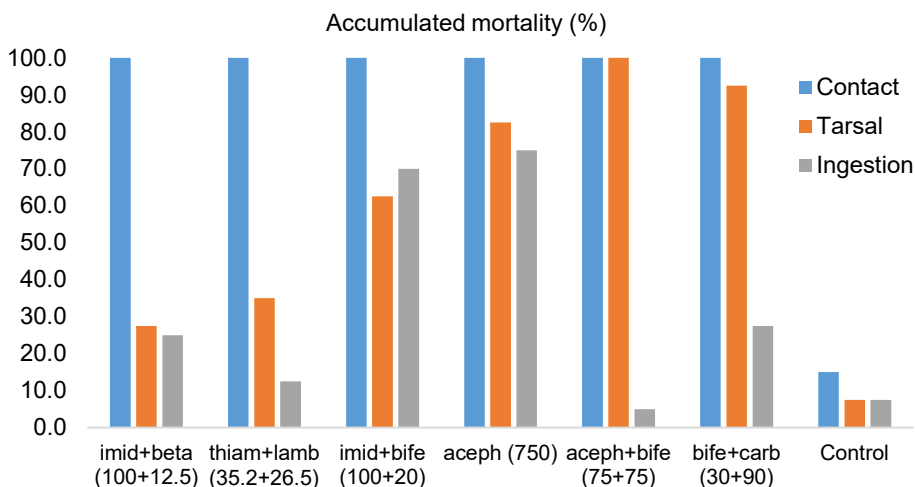
Treatments	1 hour	5 hours	24 hours	48 hours
Imidacloprid + beta-cyfluthrin	0.0 + 0.00 ns <sup>2</sup>	0.5 + 0.70 ns	1.0 + 0.99 ns	2.5 + 0.70 b
Thiamethoxam + lambda-cyhalothrin	0.0 + 0.00	0.3 + 0.35	0.3 + 0.35	1.3 + 1.33 b
Imidacloprid + bifenthrin	0.0 + 0.00	1.0 + 0.81	3.8 + 1.75	7.0 + 1.71 a
Acephate	0.0 + 0.00	0.3 + 0,35	2.8 + 1.20	7.5 + 1.21 a
Acetamiprid + bifenthrin	0.0 + 0.00	0.0 + 0.00	0.5 + 0.40	0.5 + 0.40 b
Bifenthrin + carbosulfan	0.0 + 0.00	0.0 + 0.00	0.8 + 0.67	2.8 + 1.44 b
Control	0.0 + 0.00	0.1 + 0.21	0.8 + 0.35	0.8 + 0.35 b

<sup>1</sup>Recommended doses in corn crop to control *Diceraeus melacanthus*. Means followed by the same letter in the column do not differ statistically from each other using the *Scott-Knott test* at 5% probability.

<sup>2</sup>Not significant by the *F test*.

Considering the three modes of exposure together, it appears that the imidacloprid + bifenthrin (100.0 + 20.0) mixture and the acephate (750.0) insecticide were the only chemical treatments that presented high or moderate mortality levels of the stink bugs in the three exposure modes (Fig. 1). These results enable these three treatments to present high mortality of this pest when sprayed in the field. Paz et al. (2021) applied the imidacloprid + bifenthrin mixture in spray form after sowing corn using the same dose as in the present work and found that this mixture was efficient in controlling *D. melacanthus* nymphs and adults, corroborating the results obtained in the present research with this chemical treatment.

On the other hand, the effects of direct contact and tarsal exposure modes indicate that the mixtures of acetamiprid + bifenthrin (75.0 + 75.0) and bifenthrin + carbosulfan (30.0 + 90.0) have a comparative advantage for controlling stink bugs compared to the other products tested, as both treatments demonstrated high levels of pest control in these two exposure modes (Fig. 1). These two mixtures were also the only treatments that showed a significant knockdown effect through tarsal contact to control stink bugs after 1 hour of exposure (Table 3). Guerreiro et al. (2017), evaluating the efficiency of different insecticides in controlling *D. melacanthus* in corn, concluded that the bifenthrin + carbosulfan mixture also showed a greater population reduction of this pest. These results reinforce our hypothesis of the good performance of bifenthrin in controlling *D. melacanthus* adults (Fig. 1). In another study, Martins et. al. (2009) evaluated the thiamethoxan + cypermethrin mixture at the recommended dose for controlling *D. melacanthus* in corn and found that this mixture did not provide good control of this pest. These results show that the variability in mortality levels of insecticide mixtures for the control of *D. melacanthus* is probably associated with the type of pyrethroid insecticide present in its formulation.



**Figure 1.** Accumulated mortality of *Diceræus melacanthus* up to 48 hours after contact of insects with different insecticides in the direct contact exposure modes in the insecticide spray, tarsal contact by walking on the treated surface and indirectly by ingestion through feeding on bean pods previously treated with the different chemical treatments. Embrapa CPAO.

The low efficiency observed with the ingestion exposure method may be due to the behaviour of the active ingredients in the bean pod where the insecticide syrup was previously sprayed. It may be that the imidacloprid + bifenthrin (100.0 + 20.0) mixture and the acephate (750.0) insecticide were more available for the stink bugs' stylet access during their feeding and thus provided the highest levels of control of this pest for 48h of exposure. However, for an insecticide sprayed on crops to be translocated to other plant tissues, it must be systemic (Bassso & Panizzi, 2016). Studying the monitoring of resistance of the *E. heros* stink bug to insecticides, Tibola et al. (2021) evaluated an artificial ingestion diet comparing it with topical applications of thiamethoxam and imidacloprid and lambda-cyhalothrin. The authors concluded that the most effective method for detecting resistance to neonicotinoid insecticides was through ingestion, whereas for pyrethroids, the best method was topical application. Pereira et al. (2005) studied the toxicity of the gamma-cyhalothrin insecticide to the *Podisus nigrispinus* predator through topical application and ingestion, concluding that the topical application exposure mode was less toxic to the predator than the ingestion exposure mode.

## CONCLUSIONS

The percentage of accumulated mortality of *D. melacanthus* adults up to 48 hours after exposure of the insects in the three modes of exposure, it appears that the most significant effect observed was through direct contact with chemical treatments when compared to the tarsal and ingestion. It is important to consider that when spraying is carried out in the field to control stink bugs, direct contact usually occurs first and then tarsal contact. However, if the insect pest is not exposed at the time of spraying, tarsal contact could occur first, while in ingestion mode, it would occur last. With this, we can also infer that: if the target insect does not receive direct spraying on its body, indirect

contact through its tarsi walking on the treated surface can guarantee a significant final mortality of the stink bugs. In addition, although the mode of exposure through ingestion has shown low mortality, it may also contribute to the final mortality of stink bugs in the field depending on the chemical treatment applied to the crop.

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