Toxicidade de inseticidas a *Plutella xylostella* (Lepidoptera: Plutellidae) parasitadas por *Oomyzus sokolowskii* (Hymenoptera: Eulophidae)

Insecticide toxicity to *Plutella xylostella* (Lepidoptera: Plutellidae) parasitized by *Oomyzus sokolowskii* (Hymenoptera: Eulophidae)

DOI: 10.34188/bjaerv3n4-067

Recebimento dos originais: 20/08/2020
Aceitação para publicação: 20/09/2020

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RESUMO
A traça-das-cruçíferas é um dos principais fatores limitantes para o cultivo de brássicas. Visando reduzir os danos causados ao produto final, é preciso controlar a população deste inseto, o que ocorre principalmente com inseticidas que oferecem riscos aos consumidores e ao ambiente. O uso de parasitoides para o controle populacional deste inseto é uma alternativa viável, e, neste cenário, encontra-se o endoparasitoide, larval-pupal, *Oomyzus sokolowskii* (Kurdjumov, 1912) (Hymenoptera: Eulophidae). Porém, o uso de inseticidas não seletivos pode prejudicar os organismos não alvos. Assim, este trabalho teve por objetivo, investigar os efeitos da exposição a produtos químicos e biológico sobre a fase larval de *O. sokolowskii*. Para tanto, parasitoide e hospedeiro foram criados em laboratório sob condições controladas. Os tratamentos foram compostos por cinco inseticidas químicos (Acefato, Clorantraniliprole, Cyantraniliprole, Imidacloprido e Novaluron), um biológico (*Bacillus thuringiensis*) e um controle (água), através de aplicação via contato e ingestão, em lagartas previamente parasitadas. Cada tratamento foi composto por 10 repetições com cinco insetos cada. Os resultados, indicam que o inseticida de origem biológica promoveu maior mortalidade de lagartas parasitadas, logo, não sendo seletivo para o endoparasitoide. Quando avaliada a emergência de vespas não foi observada diferença significativa, assim como, para a razão sexual. Porém, novos estudos devem ser realizados para se testar a provável resistência que a população estudada possui em relação aos demais inseticidas testados.

Palavras-Chave: Traça das crucíferas, Endoparasitoide, Controle biológico.

ABSTRACT
The diamondback moth is one of the main factors limiting the production of brassica crops. In order to reduce the damage caused to the final product, the population of this insect is controlled mainly with insecticides, posing risks to consumers and the environment. The use of parasitoids for the control of this insect is a viable alternative, such as the larval-pupal endoparasitoid *Oomyzus sokolowskii* (Kurdjumov, 1912) (Hymenoptera: Eulophidae). However, non-selective insecticides can adversely affect non-target organisms. We investigated the effects of exposure to chemical and biological pesticides on the larval phase of *O. sokolowskii*. Parasitoids and hosts were reared under laboratory conditions for bioassays. Treatments consisted of five chemical insecticides (Acephate, Clorantraniliprole, Cyantraniliprole, Imidacloprid, and Novaluron), one biological control agent (*Bacillus thuringiensis*), and a control group (water), with exposure of previously parasitized caterpillars by contact and ingestion. Each treatment consisted of 10 repetitions with five insects per repetition. Our findings revealed a higher mortality of parasitized caterpillars treated with the biological insecticide, thus not selective for *O. sokolowski*. No significant differences in wasp emergence and sex ratio were observed. However, further studies should be carried out to assess insecticide resistance in the study population.

Keywords: Diamondback moth, Endoparasitoid, Biological Control, Selectivity, Insect Pest, Natural Enemy.
1 INTRODUCTION

Current human feeding habits have included the consumption of more fresh vegetables, such as collard greens (*Brassica oleracea* var *acephala*) (Vilela & Henz, 2000; Dantas *et al*., 2005). Brassicas in general are nutritionally important for human consumption because of their high levels of fibers, proteins, iron, calcium, antioxidants, among others (Carvalho *et al*., 2006; Reyes-Munguía *et al*., 2017).

This crop is susceptible to pest insects, such as the diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) (Talekar & Shelton, 1993). During the larval stage, this microlepidopteran feeds on collard leaves (Medeiros *et al*., 2003), the end-commercial product, consequently hindering plant development and reducing yields (Monnerat *et al*., 2004).

In order to reduce damage, up to three different insecticides can be applied in a given week (Castelo Branco *et al*., 2001). However, indiscriminate applications can have several undesirable effects, such as elimination of natural enemies (Sarfraz *et al*., 2005), selection of resistant individuals (Castelo Branco & Gatehouse, 1997), contamination of food, soil and water bodies, and impact on the local biodiversity and its surroundings (Soares & Porto, 2007).

Among the natural enemies of the diamondback moth, *Oomys sokolowskii* (Kurdjumov, 1912) (Hymenoptera: Eulophidae) is a larval endoparasitoid that attacks second to fourth instar caterpillars (Nakamura & Noda, 2002). In Brazil, it has been reported in Rio Grande do Sul (Ferronato & Becker, 1984), Pernambuco (Loges, 1996), Distrito Federal (Castelo Branco *et al*., 2001), Paraná (Marchioro & Foerster, 2016), and Mato Grosso (Bersani *et al*., 2017). This endoparasitoid has already been used in biological control programs because of high parasitism rates (between 68 and 100%), making it one of the main natural enemies of *P. xylostella* (Cock, 1985).

The insecticides used to control the diamondback moth, however, can be harmful to natural enemy populations, such as *O. sokolowskii*. Laboratory tests indicate that when *P. xylostella* pupae containing developing parasitoids are exposed to the insecticide permethrin, a 37.5% mortality rate of endoparasitoid pupae is observed (Haseeb *et al*., 2005).

Because insecticide applications can reduce or eliminate endoparasitoid populations in the field, information is needed regarding which insecticides are selective to beneficial insects. This study investigated the toxicity of five chemical insecticides and one biological control agent to the immature stages of *O. sokolowskii*. 
2 MATERIALS AND METHODS

2.1 STUDY SITE

The study was carried out at the Laboratory of Entomology of the Center for Research, Studies, and Agro-environmental Development of the Mato Grosso State University, Professor Eugênio Stieler campus -Tangará da Serra.

2.2 INSECT REARING

Caterpillars were collected in a collard crop, taken to the laboratory, monitored for the emergence of moths or parasitoids, and then added to a breeding colony.

Adult moths were kept in acetate cages closed with two lids. An inner lid with a square opening of 4 x 4 cm was fitted with a piece of collard leaf and an outer lid was placed on the first lid and the leaf in between to prevent moths from escaping (Marchioro & Foerster, 2011). The collard leaf between lids was used to stimulate oviposition and was replaced three times a week.

Larvae were kept in 5-L plastic containers with side and upper openings covered with voile fabric. A beaker containing water was placed inside the cage to maintain the turgidity of collard leaves provided as food. Maintenance was carried out three times a week, adding more leaves and removing pupae, which were kept in plastic containers until adult emergence and later transferred to cages.

Collard leaves used to feed caterpillars were grown in a greenhouse, following the recommended practices for the crop, without the use of pesticides. The breeding colony was kept in a room at 25 ± 2 °C, 70 ± 20% air humidity, and 12-h photophase.

The rearing of O. sokolowskii was adapted from Silva-Torres et al. (2009a) and consisted of 4.5-L rectangular plastic containers with openings to allow ventilation, kept in a B.O.D. incubator at 28 ± 1 °C, 70 ± 10% relative humidity, and 12-h photophase. Third instar caterpillars were offered to O. sokolowskii, and after parasitism, placed in cages until the pupal stage, when they were removed and placed in 145 mL plastic containers. On the fifth day of the pupal stage, parasitized pupae were identified by their brown color, and individualized in 5 mL micro tubes. Emergence of parasitoids was monitored and emerged adults were fed a 70% honey solution.

2.3 BIOASSAYS

Bioassays were carried out using insecticides at the maximum dosages recommended for field application against the P. xylostella (Table 1), totaling five chemical pesticides, one biological insecticide, and a control group with water. For each treatment, 10 repetitions were carried out. Each repetition consisted of a Petri dish with five parasitized third instar caterpillars (totaling 50 caterpillars
per treatment) kept in a B.O.D. incubator at 28 ± 1 ° C, 70 ± 10% relative humidity, and 12-h photophase.

Table 1 - Insecticides used to evaluate toxicity in caterpillars of Plutella xylostella (Lepidoptera: Plutellidae) parasitized by Oomyzus sokolowskii (Hymenoptera: Eulophidae).

| Active ingredient | Chemical group    | Mode of action       | Concentration¹ |
|-------------------|-------------------|----------------------|----------------|
| Acephate          | Organophosphate   | Contact and ingestion| 100 g/100 L water |
| Bacillus thuringiensis | Biologic         | ingestion           | 50 g/100 L water  |
| Chlorantraniliprole | Anthranilamide   | Contact and ingestion| 7.5 mL/100 L water |
| Cyantraniliprole  | Anthranilamide   | Contact and ingestion| 100 mL/800 L water |
| Imidacloprid      | Neonicotinoid     | Systemic            | 200 g/ha         |
| Novaluron         | Benzoilurea       | Physiological       | 50 mL/100 L water |

¹ Maximum recommended dosages.

The methodology used for the bioassays was adapted from De Bortoli et al. (2012). In order to evaluate the toxicity of the treatments with exposure by ingestion, 8.5 cm diameter collard discs were immersed in the treatments for 10 seconds and allowed to air dry at 25 ± 2 ° C. After two hours, disks were placed on Petri dishes containing filter paper, and offered to 5 previously parasitized third instar caterpillars for 24 hours. Parasitized caterpillars were obtained by exposing them to endoparasitoids for 24 hours prior to the assay, following De Bortoli et al. (2012).

In the assay to evaluate contact toxicity, caterpillars were placed on a plastic Petri dish and sprayed with an air compressor (Schulz brand, Jet Fácil model with a maximum operating pressure of 40 lbf/2) coupled to an airbrush gun inside a hood in the laboratory. After spraying, caterpillars were fed untreated collard discs.

Caterpillar mortality was evaluated every 24 hours, as well as maintenance of Petri dishes. When caterpillars reached the pupal stage, P. xylostella pupae were placed in flat-bottomed tubes (Ø = 3 cm, height = 8 cm) sealed with plastic film and monitored for the emergence of O. sokolowskii.

2.4 STATISTICAL ANALYSIS

The results were compared with an analysis of variance (ANOVA) and the means were grouped with the Scott-Knott test at 5% significance level, using the software R Studio (R Core Team, 2018) and the ScottKnott package (Jelihovschi et al., 2014 ). The t-test was also performed to compare modes of application.
3 RESULTS AND DISCUSSION

The exposure by contact revealed that mortality of parasitized caterpillars was higher in the treatment with *B. thuringiensis*, while for ingestion, the highest mortality rates were observed for *B. thuringiensis* and chlorantraniliprole (Table 2).

**Table 2** - Mean (± standard deviation) accumulated mortality of parasitized *Plutella xylostella* caterpillars exposed by contact and ingestion (n = 5 caterpillars).

| Treatments                  | Contact       | Ingestion     | P  |
|-----------------------------|---------------|---------------|----|
| Water                       | 1.2 ± 1.47 b A | 0.6 ± 0.84 b A | 0.27 |
| Acephate                    | 2.0 ± 1.24 b A | 1.2 ± 1.68 b A | 0.24 |
| *Bacillus thuringiensis*    | 3.9 ± 1.28 a A | 3.8 ± 1.13 a A | 0.85 |
| Chlorantraniliprole         | 1.8 ± 1.03 b A | 3.8 ± 1.13 a B | 0.00 |
| Cyantraniliprole            | 1.7 ± 1.33 b A | 0.9 ± 1.10 b A | 0.16 |
| Imidacloprid                | 1.7 ± 1.25 b A | 0.6 ± 0.84 b B | 0.03 |
| Novaluron                   | 2.2 ± 1.03 b A | 0.6 ± 0.84 b B | 0.00 |
| *P*                         | 0.0004        | 6.63⁴²        |

¹Values followed by different lowercase letters in columns and uppercase letters in lines indicate a significant difference between treatments at 5% significance level.

Because the mode of action of the biological insecticide requires ingestion, dead caterpillars exposed by contact may have ingested the product during spraying, given the mortality rate observed in this treatment, whereas the mode of action of chlorantraniliprole occurs by contact and ingestion.

When assessing the number of parasitoids emerged from surviving caterpillars, no differences were observed among treatments regardless of exposure by ingestion or contact, or when comparing modes of application (Table 3).

Regarding the sex ratio of emerged wasps, differences were observed in the treatment with chlorantraniliprole, but when modes of application were compared, no significant differences were found (Table 4).

**Table 3** - Mean ± standard deviation of wasps emerged from *Plutella xylostella* caterpillars exposed by ingestion and contact.

| Treatments                  | N   | Ingestion NS | N   | Contact NS | P    |
|-----------------------------|-----|--------------|-----|------------|------|
| Water                       | 19  | 14.38 ± 7.97 | 30  | 15.14 ± 2.66 | 0.58 |
| Acephate                    | 32  | 11.09 ± 5.07 | 25  | 13.38 ± 2.93 | 0.39 |
| *Bacillus thuringiensis*    | 5   | 11.33 ± 6.11 | 6   | 10.20 ± 3.96 | 0.92 |
| Chlorantraniliprole         | 4   | 16.33 ± 8.50 | 20  | 11.42 ± 2.73 | 0.45 |
| Cyantraniliprole            | 29  | 14.03 ± 4.41 | 9   | 11.85 ± 4.25 | 0.09 |
| Imidacloprid                | 21  | 8.50 ± 2.65  | 22  | 12.41 ± 6.88 | 0.13 |
| Novaluron                   | 23  | 12.67 ± 2.91 | 17  | 10.72 ± 3.49 | 0.34 |
| *P*                         | 0.21 |               | 0.24 |            |      |

NS: not significant according to the t-test at 5% significance level and analysis of variance. N: number of caterpillars from which wasps emerged after the treatment.
Table 4 - Mean ± standard deviation of the sex ratio of wasps emerged from *Plutella xylostella* caterpillars exposed by ingestion and contact.

| Treatments              | N | Ingestion         | N  | Contact         | P   |
|-------------------------|---|-------------------|----|----------------|-----|
| Water                   | 19| 0.74 ± 0.16 aA²   | 30 | 0.64 ± 0.11 aA | 0.06|
| Acephate                | 32| 0.80 ± 0.12 aA    | 25 | 0.71 ± 0.11 aA | 0.09|
| *Bacillus thuringiensis*| 5 | 0.77 ± 0.11 aA    | 6  | 0.65 ± 0.33 aA | 0.82|
| Chlorantraniliprole     | 4 | 0.64 ± 0.15 bA    | 20 | 0.80 ± 0.07 aA | 0.12|
| Cyantraniliprole        | 29| 0.77 ± 0.11 aA    | 9  | 0.76 ± 0.12 aA | 0.58|
| Imidacloprid            | 21| 0.81 ± 0.06 aA    | 22 | 0.78 ± 0.10 aA | 0.41|
| Novaluron               | 23| 0.83 ± 0.06 aA    | 17 | 0.75 ± 0.12 aA | 0.21|

1Means followed by the same lower case letters in the columns did not differ statistically according to the analysis of variance; ² Means followed by the same upper case letters on the line were not significantly different according to the t-test at 5% significance level. N: number of parasitized caterpillars from which wasps emerged.

Our findings revealed a higher mortality of parasitized caterpillars in the group treated with *B. thuringiensis*. Hill and Foster (2000) observed that after 72 hours of exposure to *B. thuringiensis* in laboratory bioassays, approximately 90% of caterpillars were dead. Monnerat *et al.* (2000) found that plots in a field experiment treated with *B. thuringiensis* var. *aizawai* yielded 100% marketable cabbage heads. Similarly, Dias *et al.* (2004), reported that 76.5% of cauliflower heads were marketable with applications of this biological insecticide, while Castelo Branco *et al.* (2001, 2003) obtained mortality rates between 75 and 100% of moths when treated with *B. thuringiensis*.

De Bortoli *et al.* (2012) evaluated the biology of diamondback moth treated with *B. thuringiensis* and observed changes in viability rates of larval and pupal stages, indicating that caterpillars stop feeding after ingesting bacteria. In a greenhouse study carried out by Moraes and Foerster (2012), a similar result was observed, with high mortality rates of first and third instar caterpillars when treated with *B. thuringiensis* var. *aizawai*. Ayalew (2011) observed that the application of this biological insecticide in field tests was able to control the diamondback moth, increasing cabbage yields.

Biological products containing *B. thuringiensis* act after ingestion of bacteria, when the toxins produced rupture the peritrophic membrane that lines the mesenteric region, where nutrient absorption occurs (Aronson *et al.*, 1986). Shortly after ingestion, insects stop feeding, resulting in death from starvation. These toxins are selective and therefore not harmful to beneficial insects (Herrero *et al.*, 2001).

Because the mode of action of the biological insecticide occurs by ingestion, caterpillars exposed by contact may have died as their mouthparts (mandibles) entered in contact with the insecticide and thus, probably ingesting the product, as reported by Hill and Foster (2000).
In this study, the systemic insecticide imidacloprid did not differ significantly from the control treatment. Similar results were observed by Hill and Foster (2000) that reported larval mortality rates below 25% after ingesting food treated with this insecticide. Djomaha et al. (2016) did not observe significant mortality when testing an imidacloprid-based insecticide in the field, in agreement with the reported by Dotasara et al. (2017) in field tests in cauliflower. *P. xylostella* mortality was not significant for novaluron, similar to the reported by Nikam et al. (2015) in third and fourth instar larvae. On the other hand, Ayalew (2011) observed that novaluron was efficient for the control of this pest in cabbage in the field in Ethiopia and did not affect the parasitoid *Diadegma* sp. (Hymenoptera: Ichneumonidae).

Chlorantraniliprole was effective in the bioassay by ingestion. In India, Nikam et al. (2015) observed that this insecticide was toxic for third and fourth instar *P. xylostella*, as also reported by Dotasara et al. (2017). These authors obtained a high mortality rate of caterpillars treated with chlorantraniliprole in field tests. However, the insecticide cyantraniliprole, belonging to the same chemical group as chlorantraniliprole, was not effective to control the diamondback moth. This diamide was released in Brazil for the control of different insect pests; however, Ribeiro (2014) observed that populations of *P. xylostella* in laboratory experiments were cross-resistant to chlorantraniliprole and cyantraniliprole, making this molecule ineffective in the control of this pest. Similarly, the low mortality observed in caterpillars treated with this compound in the present study suggests that this population is resistant.

The organophosphate acephate is extremely toxic to many species; however, this was not observed to the diamondback moth, as the results were similar to those of the control treatment. Castelo Branco et al. (2003) assessed the susceptibility of two populations of *P. xylostella* to acephate in Federal District and did not observe significant mortality. On the other hand, Castelo Branco et al. (2001) reported a mortality rate of 79% in a *P. xylostella* population from Rural Nucleus of Taquara for this insecticide in laboratory tests.

The low mortality rates of *P. xylostella* observed for treatments with acephate, imidacloprid, cyantraniliprole, and novaluron indicate that the study population of the diamondback moth is resistant to these chemical insecticides, as also reported in the literature for different types of insecticides (Castelo Branco et al., 2003; Liu et al., 2015; Wang et al., 2012).

Regarding the effect of insecticides on wasps, no significant differences were observed in number of wasps emerging from bioassays by ingestion and contact. However, a significant difference in sex ratio was found for the bioassay by ingestion with the insecticide chlorantraniliprole, although this was similar to that found for other populations of *O. sokolowskii* (Ferreira et al., 2003; Silva-Torres et al., 2009b).
Shi et al. (2004) evaluating the effect of fenvalerate, avermectin, and chlorfluazuron on P. xylostella pupae with O. sokolowskii pupae, did not observe a reduction in the number of parasitoids and their emergence. However, wasp mortality after 24 hours of emergence was 42.1 and 33.4% for the insecticides fenvalerate and avermectin, respectively. For permethrin, mortality of P. xylostella pupae was 37.5% (Haseeb et al. 2005), which was classified as slightly harmful to O. sokolowskii according to IOBC (Hassan, 1994).

Haseeb et al. (2004), evaluating the effect of insecticides on the pupal stage of Cotesia plutellae (Kurdjumov, 1912) (Hymenoptera: Braconidae), reported low toxicity of the insecticides tested to this endoparasitoid, with no significant differences in emergency rates, when compared with the control group.

Luana-Cruz et al. (2011), evaluating the effect of the insecticides azadiractin, spinosad, imidacloprid, and abamectin to pupae of the parasitoid Tamarixia triozae (Burks, 1943) (Hymenoptera: Eulophidae), observed that imidacloprid prevented the emergence of 100% of the parasitoids. In the treatments with abamectin and spinosad, emergence rates were 74 and 58% respectively, while no significant differences were observed for azadiractin compared to the control group. Beloti et al. (2015) tested 14 insecticides sprayed on pupae of Tamarixia radiata (Waterston, 1922) (Hymenoptera: Eulophidae) and observed significant differences in emergence rate for dimethoate and chlorpyrifos and duration of egg-adult cycle for spinosad. However, sex ratio and longevity were not affected.

The results obtained regarding the effect on wasps indicate that the products tested do not interfere in the development of the endoparasitoid O. sokolowskii. Insecticides containing B. thuringiensis and chlorantraniliprole, on the other hand, should not be recommended for use in the field, given the high mortality of wasps observed.

Contrary to the assumption that parasitized caterpillars would be more susceptible to the tested pesticides, mortality rates of parasitized caterpillars were lower than those of non-parasitized ones. However, our findings indicate that the study population acquired resistance to the chemical insecticides used and further studies are needed.

Bacillus thuringiensis and chlorantraniliprole caused higher mortality of parasitized caterpillars among treatments. Therefore, these products are not selective for endoparasitoids, as they did not emerge from dead caterpillars. In areas where O. sokolowskii has been released or is being preserved, the use of Bacillus thuringiensis and chlorantraniliprole should not be recommended.
4 CONCLUSION

No significant effects on endoparasitoid adults were found. Of the caterpillars that survived the treatments, emergence of wasps was recorded, but of the caterpillars that died, no emergence of parasitoids was observed, demonstrating that *Bacillus thuringiensis* and chlorantraniliprole are not selective to *O. sokolowskii* during embryonic development.

Thus, *B. thuringiensis* and chlorantraniliprole were effective insecticides in the control of *P. xylostella*. However, the parasitoid *O. sokolowskii* did not complete its development in insecticide-treated caterpillars that died.

ACKNOWLEDGMENTS

The authors thank the Coordination for the Improvement of Higher. Education Personnel (CAPES) for the graduate fellowship, the team of the Laboratory of Entomology of the Mato Grosso State University, Professor Eugênio Carlos Stieler campus in Tangará da Serra, and to the landowners in Tangará da Serra – MT where the study where carried out.

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