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CROP PROTECTION

Toxicity of insecticides used in rice crop on the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) under field conditions

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ABSTRACT: *We evaluated under field conditions the toxicity of insecticides previously identified as harmful in laboratory and semifield bioassays on the parasitoid* Trichogramma pretiosum *(Hymenoptera: Trichogrammatidae). The experiments were conducted during the 2019/20 and 2020/21 harvests in rice fields. Following the recommendations of the International Organization for Biological and Integrated Control (IOBC), four insecticides were applied in 64 m² experimental plots. Subsequently,* T. pretiosum *was released inundatively. To verify parasitism rates, at 1, 2, 4 and 6 days after release (DAR) of the parasitoids, eggs from the host* Ephestia kuehniella *(Lepidoptera: Pyralidae) were offered. After determining the number of parasitized eggs, the data were grouped into a reduction coefficient (Ex) to provide a single result for the effects of the insecticides on parasitoid. For both the 2019/20 and 2020/21 evaluated crops, it was found that at 2 DAR, the highest parasitism rates occurred. In contrast, in 6 DAR, no parasitism rates were observed. Lambda-cyhalothrin, thiamethoxam, and zeta-cypermethrin were classified as moderately harmful; thiamethoxam + lambda-cyhalothrin was classified as harmful. Following IOBC guidelines, the toxicity of these products under field conditions is lower than that obtained in the laboratory or semi-field for the* T. pretiosum. *However, these insecticides should be avoided, or used at times that do not coincide with the release or presence of the parasitoid in the field.*
Key words: *biological control, chemical control, integrated pest management, natural enemies, pesticide selectivity.*

Toxicidade de inseticidas utilizados na cultura do arroz sobre o parasitoide de ovos *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) em condições de campo

RESUMO: *Avaliamos em condições de campo a toxicidade de inseticidas previamente identificados como nocivos em bioensaios de laboratório e semicampo sobre o parasitóide* Trichogramma pretiosum *(Hymenoptera: Trichogrammatidae). Os experimentos foram conduzidos durante as safras 2019/20 e 2020/21 em lavouras de arroz. Seguindo as recomendações da International Organization for Biological and Integrated Control (IOBC), quatro inseticidas foram aplicados em parcelas experimentais de 64 m². Posteriormente, realizou-se uma liberação inundativa de* T. pretiosum. *Para verificar as taxas de parasitismo, aos 1, 2, 4 e 6 dias após a liberação (DAR) dos parasitoides, ovos do hospedeiro* Ephestia kuehniella *(Lepidoptera: Pyralidae) foram ofertados. Após a determinação do número de ovos parasitados, os dados foram agrupados em um coeficiente de redução (Ex) para fornecer um resultado único para os efeitos dos inseticidas sobre o parasitoide. Tanto para as safras 2019/20 quanto 2020/21, verificou-se que em 2 DAR, ocorreram as maiores taxas de parasitismo. Em contraste, aos 6 DAR, não foram observadas taxas de parasitismo. Lambda-cialotrina, tiametoxam e zeta-cipermetrina foram classificados como moderadamente nocivos; tiametoxam + lambda-cialotrina foi classificado como prejudicial. Seguindo as diretrizes da IOBC, a toxicidade desses produtos em condições de campo é inferior à obtida em laboratório ou semi-campo para o* T. pretiosum. *Entretanto, esses inseticidas devem ser evitados, ou utilizados em momentos que não coincidam com a liberação ou presença do parasitoide a campo.*
Palavras-chave: *controle biológico, controle químico, manejo integrado de pragas, inimigos naturais, seletividade de agrotóxicos.*

INTRODUCTION

In Brazil, rice (*Oryza sativa* L.) cultivation is of great economic and social importance, since with a production of over 11 million tons, the country is the largest rice producer outside the Asian continent. Approximately 80% of the national production comes from flood irrigated areas, where the state of Rio Grande do Sul accounts for more than 70% of Brazilian rice production (CONAB, 2021). However, despite high productivity, rice crops are subject to several biotic factors and, among these, arthropod pests stand out because of the significant economic losses they cause.

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Therefore, the main form of population suppression of these pests is by the use of chemical control with synthetic insecticides. However, the widespread use of this control strategy, often without considering the foundations of integrated pest management (IPM), can lead to several problems, such as the unbalanced population of beneficial insects, which act as natural control agents (TORRES & BUENO, 2018).

Egg parasitoids of the family Trichogrammatidae are among the main biological control agents of lepidopteran pests on many crops and are within the largest biological control program in the world (PAZINI et al. 2016; PARRA & COELHO, 2019; RAKES et al. 2021a). Among these, *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) is highlighted as, efficient in parasitizing eggs of the leaf caterpillar *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) (SOUSA et al. 2021) and the panicle caterpillar, *Pseudaletia* spp. (Lepidoptera: Noctuidae) (FOERSTER et al. 2015), which are important pest arthropods in the rice production system (RAKES et al. 2021a).

Although, biological control is of great importance because it makes it possible to regulate populations of pest arthropods, chemical control, because of its speed, is often essential. Based on this scenario, to develop a program following the IPM premises and efficiently combining biological and chemical control, the first step to be evaluated is the selectivity of pesticides on the parasitoid (TORRES & BUENO, 2018). For this, the International Organization for Biological and Integrated Control (IOBC) presents a standardized sequential methodology involving laboratory, semifield, and field tests to provide sufficient information to classify the adverse effect of a pesticide on a beneficial organism (HASSAN et al. 2000).

Many factors can interfere with the toxicity of an active ingredient (RAKES et al. 2021b) and the toxicity of the compound to natural enemies along the experimental sequence proposed by the IOBC can be reduced. Thus, we investigated the hypothesis that the toxicity of pesticides to *T. pretiosum* is lower when tested under field conditions. In addition, in the rice production system, few data are available on the subject, since only PAZINI et al. (2016) studied the deleterious effects of 24 pesticides on *T. pretiosum* under laboratory conditions, and showed high toxicity for pyrethroid and neonicotinoid classes. Based on this assumption, this study evaluated the toxicity, under field conditions, of insecticides used in rice crop previously reported as harmful in laboratory and semifield trials on the egg parasitoid *T. pretiosum*.

MATERIALS AND METHODS

The experiments were conducted in two agricultural years of rice cultivation [2019/20 (1) and 2020/21 (2)], in a systematized plot with leveling of the soil surface and total area of approximately 10,000 m^2, located in the municipality of Capão-do-Leão, RS, Brazil (latitude 31°44’13.3” S, longitude 52°28’37.0” W). The area is surrounded by dense vegetation and water supply ditches for irrigation, with no other agricultural crops around (Figure 1A). The soil type is a typical eutrophic haplic planosol, that is common in flood irrigated rice fields in southern Brazil. The region’s climate is of type ‘Cfa’, subtropical, warm temperate, with well-distributed rainfall and well-defined seasons, according to the Köppen-Geiger classification.

The experimental area was sown in dry soil, with approximately 80 kg/ha of rice seeds of the medium cycle cultivar ‘IRGA 424CL’, following the technical recommendations of the crop for southern Brazil (REUNIÃO, 2018). The experiments were conducted in a randomized block design, with five treatments (four insecticides + control) and four replicates (plots). Each plot was an area of 8.0 × 8.0 meters (64 m^2 of usable area). The spacing between plots was 5 m to reduce the probability of dispersal of the parasitoid *T. pretiosum* and avoid contamination between treatments, totaling 4200 m^2 of experimental area (Figure 1A) following the indications proposed by the IOBC. We used four commercial insecticide formulations (treatments) registered for irrigated rice crops, previously reported as harmful (under laboratory conditions) to the egg parasitoid *T. pretiosum* (PAZINI et al. 2016) (Table 1). The control treatment was sprayed with distilled water. The applications of insecticides were made in the period before the beginning of irrigation by flooding, when the plants were in the vegetative development period V2–V3, corresponding to the formation of the collar in the main stem of two to three expanded leaves. A CO_2 pressurized backpack sprayer was used to apply the treatments, with constant pressure maintained at 40 psi. The boom was equipped with four XR 11002 tips, spaced at 0.5 m, calibrated to apply a spray volume of 200 L ha^{-1}.

The average environmental conditions during spraying in the first harvest were: temperature 29 °C; relative humidity of the air 54%, and wind speed 2.7 km h^{-1}; the conditions during the second crop were: temperature 29.8 °C, relative humidity of the air 46%, and wind speed 3.1 km h^{-1}. Six hours after the application of the treatments, *T. pretiosum* was flood-released. The insects came from the company Promip – Manejo Integrado de Pragas (Engenheiro Coelho, São Paulo, Brazil), registered as commercial product Trichomip-P. For this, cards containing eggs parasitized by *T. pretiosum* were distributed uniformly within the experimental area, following the recommendations of the manufacturer's package insert (Figure 1B). The equivalent of 100,000 parasitoids/ha was released, following the registration recommendations for control of the larvae (*S. frugiperda*).

Eggs of the alternative host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), reared in laboratory, were offered to evaluate the possible effects of the insecticides on the parasitism capacity of *T. pretiosum*. For this purpose, cardboard cards (5.0 × 2.0 cm) were made with three circles (1 cm in diameter) containing 250 ±50 fresh (~12 hours) and unviable eggs of *E. kuehniella*. Subsequently, the cardboard paper was glued onto bamboo sticks (30 cm long), which were fixed to the ground at a depth of approximately 5 cm (Figure 1C). Eight cards containing the host eggs were placed in each experimental plot using an X-walk within the plot (Figure 1D). The offering of the cards containing host eggs occurred at 1, 2, 4, and 6 days after release (DAR) of the parasitoids, with each offering remaining in the field for a period of 24 h. After 24 h, the cards were removed from the field, identified and stored in Petri dishes (9.0 × 1.5 cm) and taken to the laboratory under controlled conditions of temperature 25 ±1 °C, RH 70±10%, and photophase 14 h to check the number of parasitized eggs. The weather conditions (average, maximum and minimum temperature, rainfall, and relative humidity) during the field bioassays were monitored, and they are detailed in figure 2. It is noteworthy that before the beginning of the bioassays, *E. kuehniella* eggs were offered in the total area, following the same methodology described above, to assess the presence of naturally occurring parasitoids in the area. However, no parasitism rates were observed in either of the two crops, indicating the absence of natural biological control.

The data were analyzed with a generalized linear model with a quasi-Poisson distribution. The model goodness of fit was checked using a half-normal plot with the package *hnp* (MORAL et al. 2017) of R Development Core Team (2020). The means were contrasted using the 'glht' function (HOTHORN et al. 2008) of the R Core Team (2020) program. The reduction in parasitism compared to the control was calculated by the equation PR (%)

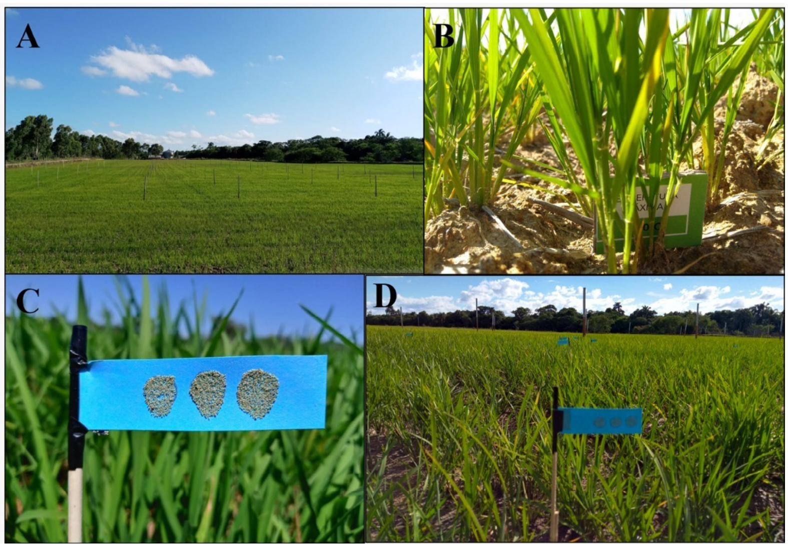

Figure 1 - Details of the bioassay under field conditions with the egg parasitoid *Trichogramma pretiosum*. (A) Experimental area implemented with the irrigated rice culture and details of the demarcation of the plots; (B) cards containing the parasitoids used in the flooding release inside each plot; (C) cards containing eggs from the alternative host *Ephestia kuehniella* inside the parcel; (D) arrangement of the cards in the experimental portion in the experimental portion.

Table 1 - Insecticides registered for rice culture, tested for their effects on *Trichogramma pretiosum* parasitism under field conditions.

Active ingredient	Trade name	D.C.[1]	Chemical group (IRAC MoA)
zeta-cypermethrin	Mustang 350 EC	40	Pyrethroid (3A)
thiamethoxam+lambda-cyhalothrin	EngeoPleno	250	Neonicotinoid (4A) + Pyrethroid (3A)
thiamethoxam	Actara	150	Neonicotinoid (4A)
lambda-cyhalothrin	Karate Zeon 50 CS	150	Pyrethroid (3A)

[1]D.C. = Dosage of commercial formulation (g or mL. ha^{-1}).

= [(1 – Vt/Vc) × 100], where PR is the percentage reduction in parasitism, Vt is the average parasitism for the treatment, and Vc is the average parasitism of the control. In addition, to provide a single result for the effects of insecticides on *T. pretiosum* under field conditions, encompassing the reduction in parasitism caused during the evaluation dates in both seasons, a reduction coefficient (*Ex*) was calculated. The *Ex* was estimated based on the average parasitism values to each product and evaluation date. Based on the *Ex* values, the insecticides were classified according to the IOBC standards for field conditions: class 1 - inoffensive ($E_x < 25\%$), class 2 - slightly toxic ($25\% \leq E_x \leq 50\%$), class 3 - moderately toxic ($51\% \leq E_x \leq 75\%$), and class 4 - toxic ($E_x > 75\%$).

RESULTS

During the first harvest (2019/20), there was a significant difference between the treatments studied (F=5.3582, df=4, 55, P < 0.0001) (Table 2). At 1 DAR, only the insecticide thiamethoxam (34 eggs parasitized) did not differ significantly from the control (56.25 eggs parasitized) (Table 2). The insecticides zeta-cypermethrin (4.75 eggs parasitized), thiamethoxam + lambda-cyhalothrin (8.75 eggs parasitized) and lambda-cyhalothrin (15.50 eggs parasitized) showed the lowest parasitism values, differing statistically from the control (Table 2). At 2 and 4 DAR, the plots treated with zeta-cypermethrin had the highest parasitism rates among the evaluated insecticides, with 92.00 and 9.00 parasitized eggs, respectively, which did not differ from the control treatments (Table 2). In contrast, on these same evaluation dates, the plots where the mixture of thiamethoxam + lambda-cyhalothrin was applied had parasitism rates (45.75 and 5.50 parasitized eggs, respectively) significantly lower than the control (Table 2). At 6 DAR, parasitism was not observed in any of the treatments (Table 2).

Similar results were observed during the 2020/21 crop, where significant differences were found among the treatments studied (F = 23.442, df = 4, 35, P < 0.0001) (Table 3). At 1 DAR, all insecticides

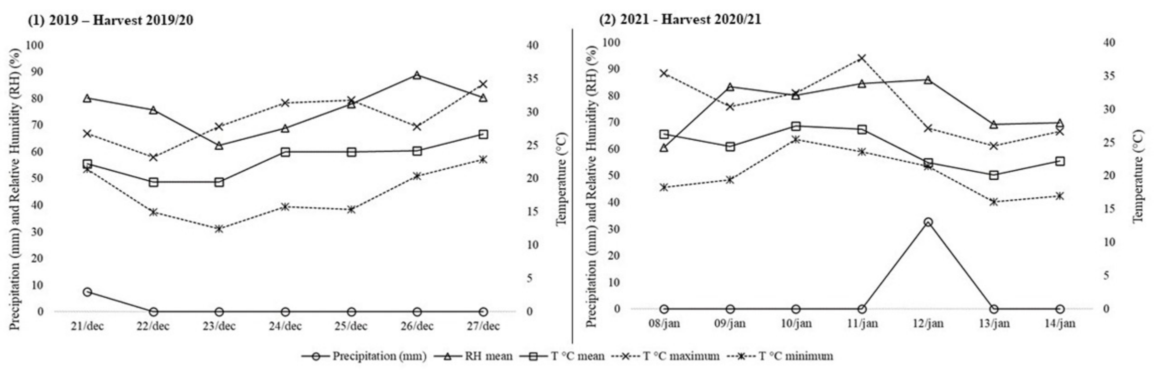

Figure 2 - Meteorological conditions of the 2019/20 and 2020/21 crops during the insecticide toxicity bioassays on the egg parasitoid *Trichogramma pretiosum* under field conditions.

Table 2 - Parasitism (number of parasitized eggs) of *Trichogramma pretiosum* when adult insects were released in plots treated with insecticides registered for irrigated rice culture under field conditions during the 2019/20 harvest.

Treatment	---------------------------Days After Release (DAR)--------------------			
	1	2	4	6
	--------Parasitism ($\bar{x}$ ± SE)--------			
zeta-cypermethrin	4.75±2.92c	92.00±11.65ab	9.00±3.51ab	0.00±0.00
thiamethoxam+lambda-cyhalothrin	8.75±2.05c	45.75±5.72c	5.50±3.88b	0.00±0.00
thiamethoxam	34.00±11.03ab	63.00±12.36bc	4.00±2.61b	0.00±0.00
lambda-cyhalothrin	15.50±8.39bc	69.50±11.67bc	4.25±3.32b	0.00±0.00
Control	56.25±10.29a	107.75±22.19a	40.75±10.92a	0.00±0.00

*Averages followed by the same letter do not differ statistically by contrast from the glm model (P < 0.01).

tested showed significantly lower numbers of parasitized eggs than the control (Table 3). However, even though it differed significantly from the control treatment (88 eggs parasitized), the insecticide thiamethoxam (51.25 eggs parasitized) had the highest parasitism rate on this date (Table 3). In contrast, plots treated with lambda-cyhalothrin caused the lowest parasitism rates (2.75 parasitized eggs), differing significantly from the other treatments (Table 3). As in crop 1, at 2 DAR, zeta-cypermethrin (109.50 eggs parasitized) did not differ significantly from the control treatment (118.25 eggs parasitized) (Table 3). However, at this date, the insecticides thiamethoxam + lambda-cyhalothrin and thiamethoxam caused the lowest numbers of parasitized eggs, with 48.50 and 57.50, respectively (Table 3). In addition, at 4 and 6 DAR, no parasitism was observed (Table 3). It is worth noting that in both years of the experiment, the supply at 2 DAR was the one that obtained the highest parasitism rates of *T. pretiosum*. Parasitism decreased at 4 DAR, in 2019/20 it presented reduced parasitism values (Table 2), and was reduced to zero in 2020/2021 (Table 3).

The reduction coefficients (Ex) for thiamethoxam, zeta-cypermethrin, and lambda-cyhalothrin showed values of 52.87, 55.5, and 68.59%, respectively, so they were classified according to IOBC as moderately harmful (Class 3) (Figure 3). The mixture of thiamethoxam + lambda-cyhalothrin was classified as harmful (Class 4), with Ex > 75% (Figure 3). The parasitism reduction values for each insecticide at the different evaluation dates are shown in table 4.

DISCUSSION

Insecticide applications after rice emergence and before the flood period are considered frequent practices in irrigated rice fields in southern Brazil to manage *S. frugiperda*, which is exactly the period when the pest insect is most damaging to the crop (RAKES et al. 2021b). During these periods, inundative releases and/or increases in the natural population density of the parasitoid in production areas should occur. With the entry of irrigation water

Table 3 - Parasitism (number of parasitized eggs) of *Trichogramma pretiosum* when adult insects were released in plots treated with insecticides registered for irrigated rice culture under field conditions during the 2020/21 harvest.

Treatment	-----------------------Days After Release (DAR)--------------------			
	1	2	4	6
	--------Parasitism ($\bar{x}$ ± SE)--------			
zeta-cypermethrin	12.25±3.42c	109.50±10.53a	0.00±0.00	0.00±0.00
thiamethoxam+lambda-cyhalothrin	17.25±3.83c	48.50±11.37c	0.00±0.00	0.00±0.00
thiamethoxam	51.25±4.87b	57.50±6.99bc	0.00±0.00	0.00±0.00
lambda-cyhalothrin	2.75±2.13d	60.75±9.88b	0.00±0.00	0.00±0.00
Control	88.00±10.55a	118.25±14.41a	0.00±0.00	0.00±0.00

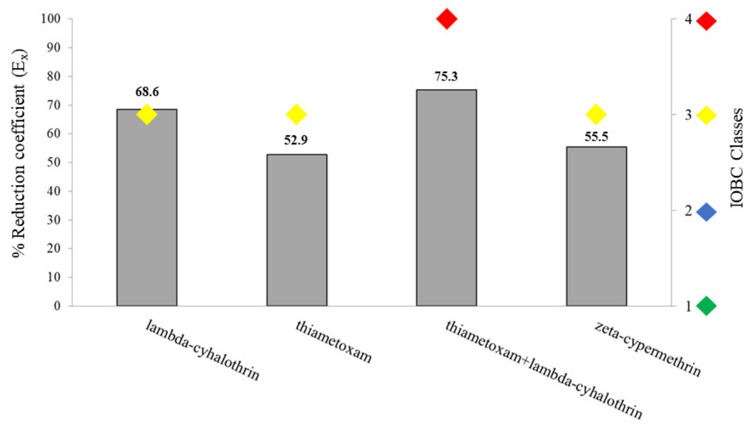

Figure 3 - Reduction coefficient (E_x) caused by the contamination of adults of *Trichogramma pretiosum* by insecticides registered in the irrigated rice culture. *IOBC classes: class 1: harmless (E_x<25%); class 2: little toxic (25%≤ E_x≤50%); class 3: moderately toxic (51%≤ E_x≤75%); class 4: toxic (E_x >75%).

in crops and after the vegetative stage of rice, the attack of the panicle caterpillar (*Pseudaletia* spp.) begins in borders and the driest parts of the crop, leading the parasitoid to be efficient in its regulation.

Thus, for an efficient integration of chemical and biological control within an IPM program, it is necessary to study the compatibility of pesticides with beneficial entomofauna. To date, for rice crops, only experiments under laboratory and semifield conditions have been conducted to evaluate the effects of insecticides on beneficial insects. Faced with the need to obtain information on the classification of the adverse effect of pesticides on natural enemies under field conditions, we developed a methodology and proved the effects on *T. pretiosum* parasitism after the application of insecticides used in irrigated rice.

According to the results reported, the insecticides thiamethoxam, lambda-cyhalothrin,

Table 4 - Reductions in parasitism (%) caused by contamination of *Trichogramma pretiosum* by insecticides recorded in the irrigated rice culture during experiments carried out in the 2019/20 and 2020/21 harvests.

Treatment	-----------------------Days After Release (DAR)--------------------			
	1	2	4	6
-----Reduction of Parasitism (%)-----				
-----Harvest 2019/20-----				
zeta-cypermethrin	91.55	14.61	77.91	-
thiamethoxam+lambda-cyhalothrin	84.44	57.54	86.50	-
thiamethoxam	39.55	41.53	90.18	-
lambda-cyhalothrin	72.44	35.49	89.57	-
Control	-	-	-	-
-----Harvest 2020/21-----				
zeta-cypermethrin	86.07	7.39	-	-
thiamethoxam+lambda-cyhalothrin	80.39	67.44	-	-
thiamethoxam	41.76	51.37	-	-
lambda-cyhalothrin	96.87	48.62	-	-
Control	-	-	-	-

zeta-cypermethrin and the mixture thiamethoxam + lambda-cyhalothrin promoted significant reductions in the parasitism rate of *T. pretiosum* adults when flood-released in the field. These insecticides have been reported to have high toxicity on *T. pretiosum*, causing a 100% reduction in parasitism when compared to the control treatment under laboratory conditions (PAZINI et al. 2016). Furthermore, STEFANELLO JÚNIOR et al. (2012) demonstrated that lambda-cyhalothrin and the mixture of thiamethoxam + lambda-cyhalothrin showed biological persistence on *T. pretiosum* adults, causing significant effects for a period exceeding 30 days when evaluated on corn leaves under semifield conditions. These compounds have neurotoxic action on insects and, through nerve impulses, act on synaptic transmission mechanisms and axonal neurotransmission by modulating sodium channels (PAIVA et al. 2018). Since the mechanism of impulses in the nervous system is similar in different insect orders, neurotoxic insecticides are generally classified as less selective compounds to natural enemies. In the field, even if classified as moderately harmful (lamba-cyhalothrin, thiamethoxam and zeta-cypermethrin) and harmful (thiamethoxam + lambda-cyhalothrin), the toxicity indices reported were lower than those described in the laboratory. The lower toxicity in the field situation may be associated with a greater degradation of the compounds by the action of climatic conditions (e.g., temperature and solar radiation), as well as related to product application, dosage, and plant metabolism (RAKES et al. 2021b).

In addition, insects parasitoids may reduce oviposition when in contact with plants contaminated with agrochemicals (RAKES et al. 2021b) since insecticides can cause repellent effects through “irritations” by acting on the central or peripheral nervous system, making individuals avoid these areas because of the chance of choice (PAIVA et al. 2018). This is not observed in laboratory selectivity bioassays, where natural enemies are submitted to extreme conditions, i.e., with maximum contact with pesticides and no chance to choose and/or escape from contamination areas (HASSAN et al. 2000).

When verifying a possible repellent effect, PAIVA et al. (2018) concluded that insecticides based on lambda-cyhalothrin and thiamethoxam, as well as the formulated mixture (thiamethoxam + lambda-cyhalothrin) significantly reduced the number of eggs parasitized by *T. pretiosum* in the laboratory. Furthermore, these authors concluded that parasitism was lower for lambda-cyhalothrin than for the formulated mixture, indicating that the compound belonging to the pyrethroid class is the main compound responsible for the repellent effect. This fact may be related to effects caused by olfactory orientation, resulting in lower ability to find their hosts. Based on our results, the deleterious effect of the ready-to-use mixture of thiamethoxam + lambda-cyhalothrin was greater, so under our conditions there is the possibility of synergism occurring.

It is also worth noting that in flood releases following the manufacturer’s label recommendations, *T. pretiosum* is placed in the field in its pupal stage, just before the onset of emergence. Therefore, after emergence (approximately up to 24 h of age) there is a need for copulation, explaining the higher parasitism values found at 2 DAR and, in general, in the second crop, in which the insects were released at a more advanced stage of development. In addition, adult parasitoids can migrate and even increase in number in refuge areas, where insecticides are not sprayed (PARRA & COELHO, 2019). This fact may be related to the non-occurrence of parasitism after 4 DAR in this study, since at the vegetative stage of rice, where the parasitoids were released, there were no flowers to feed on, corroborating with the recommendations of the manufacturer of the biological product of the need for a second release three days after the first. For use in an IPM program, the information obtained in this research presents itself as an extremely important and pioneering step in identifying the compatibility of pesticides with natural enemies under field conditions.

CONCLUSION

The insecticides lambda-cyhalothrin, thiamethoxam and zeta-cypermethrin are moderately harmful (class 3); thiamethoxam + lambda-cyhalothrin is harmful (class 4) to the parasitoid egg. Following IOBC guidelines, pesticides classified as inoffensive (class 1) or slightly toxic (class 2) should have their use preferred in Integrated Pest Management (IPM) programs. Thus, despite the toxicity of the pesticides evaluated under field conditions being lower than the results obtained in the laboratory and semi-field to the parasitoid, the use of these active ingredients should be avoided, or used with extreme caution during the release periods and/or presence of *T. pretiosum* to control *S. frugiperda* in irrigated rice areas.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. Funding sponsors had no role in the study design, collection, analysis, and data interpretation; during the writing of this manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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