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Article

Mortality of *Rachiplusia nu* (Guenée, 1852) (Lepidoptera: Noctuidae) Caused by *Sophora flavescens* Bioinsecticide and Its Selectivity to *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae)

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Abstract

There is a global demand for reducing the adoption of traditional chemical insecticides in agriculture. Among the most promising alternatives, botanical insecticides have gained increasingly attention due to their efficacy combined with a more environmentally safe impact. Among the different botanical insecticides commercially available, oxymatrine is alkaloid found in the roots of *Sophora flavescens* which exhibits wide insecticide activity. However, their side-effects on non-target organisms have not been extensively evaluated. Therefore, this study aimed to investigate in laboratory conditions the insecticidal potential of a commercial botanical insecticide (Matrine®) based on ethanolic extract of *S. flavescens* roots at 0.2; 0.6; 1.0; 1.4; 1.8; and 2.2 L of commercial product per hectare to control third-instar larvae of *Rachiplusia nu* and its selectivity to the egg parasitoid *Trichogramma pretiosum*. Overall, our results showed that the ethanolic extract of *S. flavescens* is an efficient tool to control *R.nu* from 0.6 to 2.2 L/ha, with similar *R. nu* mortality at 48 and 72 hours after spraying (close 100% mortality) associated with low impact on the egg parasitoid. The botanical insecticide was classified as harmless to the pupae and slightly harmful to the adults of *T. pretiosum* accordingly to the International Organization for Biological Control (IOBC) protocols. Therefore, the slower rates of 0.6 to 1.4 (range also registered and recommended for other caterpillar in soybean – *Anticarsia gemmatalis*) should be tested in field conditions to evaluate possible extension of the botanical insecticide registration and recommendations to be also used to control *R. nu* in the field.

Keywords: botanical insecticides; lepidopteran pest; egg parasitoid; IPM; plant ethanolic extract

1. Introduction

The sunflower looper, *Rachiplusia nu* (Guenée, 1852) (Lepidoptera: Noctuidae), is a polyphagous pest species endemic of Southern of South America [1], reported on 56 different plant species including several important crops as soybean, cotton among others cultivated and non-cultivated plants [2]. Despite being considered a major pest of soybean in Argentine [3] *R. nu* used to be of secondary importance in Brazil, occurring in low levels in soybean fields, restricted to the mid-south

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of the country until the crop season 2019/20 [4]. However, due the abusive adoption of *Bt* soybean (expressing Cry1Ac toxin) in Brazil and, consequently, lower compliance of refuge area (20% of the area cropped with non-*Bt* cultivars), as insect resistance management (IRM), unexpected defoliation caused by *R.nu* in *Bt* soybean (expressing only Cry1Ac) has been recorded from 2021 onwards [5]. Later, it was confirmed as the first case of resistance of a Lepidoptera species to Cry1Ac action [6] bringing back sprays of traditional insecticides to control *R. nu* outbreaks [7].

Insecticide against *R. nu* has been sprayed even before reaching economic thresholds (30% defoliation in the soybean vegetative stage or 15% defoliation in the soybean reproductive stage) [8]. This has endangered the most important benefits from the adoption of soybean-*Bt* technology; the reduction in the use of chemical insecticides [7]. Therefore, the development of eco-friendly pest control strategies is of great theoretical and practical interest that will benefit hundreds of farmers who need to control this pest not only on *Bt* but also on non-*Bt* crops.

Regarded as sustainable pest management strategy, botanical insecticides have been gained increasingly attention [9] due to their overall lower persistence in the environment [10], faster degradation [11] and lower impact on non-target organisms [12] compared to the use of traditional chemical insecticides [13]. Among different botanical insecticides, chemicals from *Sophora flavescens* (Leguminosae, Sophora) include a number of water-soluble alkaloids [14], including oxymatrine ($C_{15}H_{24}N_2O_2$) found in the roots of the plant. Despite its widely recognized insecticide activity [15], the only commercial *Sophora-flavescens*-based insecticide available to be used in soybean Brazil, Matrine®, contains 19.05% of ethanol extract of *S. flavescens* (equivalent to 0.2% of oxymatrine) and 80.95% of other ingredients, and is restricted to control *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae) and *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) at rates from 0.6 to 1.4 liters of commercial product/ha [16], with its non-target effect still poorly understood. Therefore, the present study aimed to expand knowledge about the potential of this commercial bioinsecticide based on *Sophora flavescens* against *R. nu* besides to evaluate its selectivity to the egg parasitoid *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae), the biocontrol agent responsible for more than 90% of natural parasitism of lepidopteran eggs recorded in soybean fields [17].

2. Materials and Methods

2.1. Insects Rearing

Field-derived colony of *R. nu* were established from larvae collected at the Embrapa Soja field station in Londrina municipality, Paraná state, Brazil (23°11'45.2" S 51°10'54.4" W) from December 2018 to January 2019 on Cry1Ac soybean. Populations were maintained in the laboratory since then with new field insects introduced into the colonies each year to maintain colony quality over time. Larvae were maintained under controlled conditions [25 ± 2ºC, 70 ± 10% RH, and a 14 h light/10 h dark photoperiod] in the Entomology Laboratory and fed an artificial diet [18,19] as methodology previously described in the literature [20]. After hatching, adults were kept inside 32 x 45 x 30 cm transparent acrylic cages (Criartshop, Londrina, Brazil), fed with a 40% brewer's yeast/water solution and covered with sulfite paper (Chamex®, Mogi-Guaçu, São Paulo, Brazil) placed on the inner walls of the cage. Eggs deposited on the sulfite paper were collected daily to start a new cycle of the species. Also, *R. nu* larvae and eggs from the colony were used for experiments and for colony maintenance.

Trichogramma pretiosum rearing and multiplication was performed on eggs of the factitious host, *Ephestia kuehniella* Zeller, 1979 (Lepidoptera: Pyralidae), according to methodology described in literature [21]. Eggs of *E. kuehniella* were glued onto 8.0 x 2.5 cm cards and subsequently exposed to ultraviolet light for 45 minutes for sterilization. Next, the cards were transferred into 8.5 x 2.5 cm glass tubes containing honey droplets, into which parasitoid females were introduced in sequence. The rearing procedure was performed inside climatic chambers set at 25±1ºC, 70±10% RH, and 14/10 hours photophase (L/D). Parasitoids from this colony was then used for the experiments.

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2.2. Mortality of R. nu Caused by Matrine® (Bioassay 1)

The experiment was carried out independently in climate chambers (ELETROLab®, model EL 212, São Paulo, SP, Brazil) at 25°C ± 2°C, 70% ± 10% RH and a photoperiod of 14:10 h (L:D) with seven treatments (**Table 1**) in a completely randomized design with three replicates containing 24 third-instar larvae of *R.nu* per replicate.

Table 1. Description of the treatments evaluated in control bioassays under laboratory conditions (25°C ± 2°C, 70% ± 10% RH, and photoperiod of 14:10 h Light:Dark) with *Rachiplusa nu* (considering a spray volume in the field of 150 liters/hectare).

Commercial Product (cp) (L of cp/ha)	Formulation	Concentration [Grams (g) of Active Ingredient (a.i)/Liter or Kilograms]	(g) a.i./ha	Commercial Product (cp) (L of cp/ha)
Water (control)	-	-	-	-
Matrine® 2.2	Soluble concentrate (SL)	Ethanolic extract of *Sophora flavescens* 190.5	419.1	2.2
Matrine® 1.8	Soluble concentrate (SL)	Ethanolic extract of *Sophora flavescens* 190.5	342.9	1.8
Matrine® 1.4	Soluble concentrate (SL)	Ethanolic extract of *Sophora flavescens* 190.5	266.7	1.4
Matrine® 1.0	Soluble concentrate (SL)	Ethanolic extract of *Sophora flavescens* 190.5	190.5	1.0
Matrine® 0.6	Soluble concentrate (SL)	Ethanolic extract of *Sophora flavescens* 190.5	114.3	0.6
Matrine® 0.2	Soluble concentrate (SL)	Ethanolic extract of *Sophora flavescens* 190.5	38.1	0.2

The studied treatments (**Table 1**) in its respective doses were applied by spraying (volume of 1.25 ± 0.25 mg/cm^{-2}, representing 200 liters/hectare, which is commonly used by soybean farmers) on each replicate (glass plates measuring 13 cm x 13 cm containing 24 third-instar larvae of *R. nu*) using a Potter Spray Tower (Burkard Manufacturing Co Ltd, Hertfordshire County, England) set to a pressure of 1.8 kgf/cm^2. After these sprays, the larvae were left to dry for approximately 10 minutes and subsequently maintained in an ELISA plate, individualized with one caterpillar per cell, containing artificial diet [**19**] and kept in the same climate chambers previously described. Mortality was monitored at 24, 48, and 72 hours post-application. Assessments were performed using a fine brush and larvae that did not respond to mechanical stimulation were considered dead.

2.3. Impact of Matrine® over the Pupae of Trichogramma pretiosum (Bioassay 2)

The selectivity of Matrine® (**Table 1**) to *T. pretiosum* pupae was tested accordingly to the standard protocols established by "International Organization for Biological Control" - IOBC [22–24]. Cards measuring 3 cm^2 (1 card per replicate) containing approximately 100 24-h-old *R. nu* eggs were exposed to newly emerged parasitoid females (≤ 24 h). Parasitism was allowed for 24 h. Subsequently, the cards were transferred to plastic cages (8.5 cm in height and 7 cm in diameter) (Plasvale Ltda., Gaspar, SC, Brazil) until pupation (168 to 192 h after parasitism) [25]. Then, the parasitoid pupae were sprayed with the treatments (**Table 1**) with the aid of a Potter Tower as already explained in the previous experiment (bioassay 1) and were left to dry for approximately 2 h. Then, each card contained the sprayed parasitoid pupae were placed in cages [22] until adult emergence, which were fed with honey during the experiment.

After adult emergence from sprayed pupae, new cards containing approximately 100 eggs of *R. nu* (≤ 24 h), on the first day (24 hours) and second day (48 hours), and a card containing approximately 50 eggs on the third day (72 hours) after parasitoid emergence were introduced into the cages. Honey droplets were provided daily as food source for the adults of the parasitoid. The cards remained in the cages until the fourth day after parasitoid emergence, when they were removed and stored in

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cylindrical tubes inside a climate chamber (ELETROLab®, model EL 212, São Paulo, SP, Brazil) at 25°C ± 2°C, 70% ± 10% RH and a photoperiod of 14:10 h (L:D) until adult emergence of the second generation (F2). The evaluated parameters were parasitoid emergence from sprayed pupae (F1) and parasitism (%) and emergence (%) of the second generation (F2) with the aid of a stereoscopic microscope (Leica-Wild M10, Wetzlar, Germany). The emergence of sprayed pupae (F1) was calculated using the numbers of parasitized eggs of *R. nu* of each replicate that had adult parasitoid emerged divided by the total number of parasitized eggs of each replicate multiplied by 100. Parasitism (%) of F2 was the number of parasitized eggs divided by the total number of eggs offered to the parasitoid, multiplied by 100 and emergence (%) calculated as the number of parasitized eggs that adults had emerged from (identified by the emergence hole), divided by the number of parasitized eggs, multiplied by 100 [24].

2.4. Impact of Dry Residue of Matrine™ to Adults of Trichogramma pretiosum (Bioassay 3)

Approximately 100 *R. nu* eggs were glued onto cards. These cards were then offered to newly emerged *T. pretiosum* (≤ 24 h) for oviposition for 24 h. After that, the parasitized *T. pretiosum* eggs were placed into Duran® tubes (emergence vials, 0.6 cm in diameter × 6 cm in height) containing a drop of honey. The Duran® tubes were then sealed with plastic film and stored in a climate chamber (ELETROLab®, model EL 212, São Paulo, SP, Brazil) at 25 °C ± 2 °C, 70% ± 10% RH, and a 14:10 h (L:D) photoperiod until parasitoid emergence. Glass plates (13 x 13 cm) received the treatments by spraying the products (**Table 1**), accordingly to methodology proposed by IOBC previously described [22–24].

After spraying, the plates were kept at room conditions for 2 h to dry, after which they were fixed to aluminum frames to form the exposure cage, where a circulating air flow allowed the elimination of possible toxic gases [22–24]. Then, the tubes containing adult parasitoids were covered with aluminum foil and connected to holes in the cages to introduce the insects, according to methodology described in literature [26]. One (24 hours), two (48 hours), and three days (72 hours) after exposing the parasitoids to the dry residues of the products on the glass plates, cards (1 x 2 cm) containing approximately 200 eggs of R. nu (≤ 24 h), on the first (24 hours) and second day (48 hours)), and cards containing approximately 50 eggs on the third day (72 hourns), and honey droplets were introduced on a daily basis into the cages. The cards containing eggs of the parasitized host were removed on the fourth day of exposure, placed in Duran tubes and stored in a climate chamber at 25°C ± 2°C, 70% ± 10% RH and a photoperiod of 14:10 h (L:D). The number of parasitized eggs and the number of insects that emerged in each treatment were evaluated using a stereoscopic microscope (Leica-Wild M10, Wetzlar, Germany).

2.5. Statistical Analysis

The effects of botanical insecticide on the survival of *R. nu* in each time interval (24, 48 and 72h) were analyzed with the Tukey test at 5% probability. To analyze the effects of Matrine® on *T. pretiosum* during each time interval (24, 48 and 72h) either in the experiment involving exposition of pupae or adults, we used two statistical procedures. If data assumed normal distribution of residues and homoscedasticity, we used 1) two-way variance analysis (ANOVA) followed by Tukey post-hoc analysis, with a Boneferroni correction, to pairwise comparisons when $p < 0.05$; otherwise, 2) non-parametric Kruskal-Wallis analysis were performed and when $p<0.05$ Dunn tests to generate pairwise comparisons were carried out. Normal distribution was checked with Shapiro-Wilk tests and homoscedasticity with Levene tests from 'car' package. Statistical analysis was performed using R and Agro R fisher 4.0.0 software (R Project for Statistical Computing. https://fisher.uel.br/AgroR_shiny.pt/).

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3. Results

3.1. Mortality of R. nu Caused by Matrine™ (Bioassay 1)

The number of dead *R. nu* larvae was higher than control (water) at all treatments and evaluation timing (24, 48, and 72 hours after spraying) except at the lower treatment of Matrine™ (0.2 L of cp/150 L of H_2O) at the first evaluation (24 hours after spraying) which did not differ from control. Overall, the botanical insecticide had high lethal effect against *R. nu*, being a promising control tool against *R. nu* at studied rates from 0.6 L to 2.2 L of cp/150 L of H_2O, with good knockdown effects (control 48 hours after treatment). At 48 h after spraying, Matrine™ at 0.6, 1.0, 1.4, 1.8, and 2.2 L of cp/150 L of H_2O triggered mortality of *R. nu* higher than 88%, which increased to higher than 98% at 72 hours after spraying. Only the lower Matrine™ rate of 0.2 L of cp/150 L of H_2O presented low initial mortality, being inferior than 36% and 64% at 24 and 48 hours after treatment, respectively. Nevertheless, even Matrine™ 0.2 L of cp/150 L of H_2O triggered 88.8% mortality at 72 hours after spraying, however, statistically inferior than the other studied botanical insecticide treatments (**Table 2**).

Table 2. Number of dead *Rachiplusia nu* larvae (N=24) (mortality%) at different periods after topical application of the studied treatments (bioassay 1).

Treatment (L of cp/150 L H_2O)		Number of de *R. nu* Larvae (Mortality%)		
		24 Hours	48 Hours	72 Hours
Water (control)		0.7 ± 0.8 c (2.9%)	2.3 ± 0.8 c (9.6%)	2.3 ± 0 .0 c (9.6%)
Matrine® 2.2		23.0 ± 4.2 a (95.8 %)	23.3 ± 1.6 a (97.1%)	24.0 ± 0.0 a (100%)
Matrine® 1.8		24.0 ± 0.0 a (100%)	24.0 ± 0.0 a (100%)	24.0 ± 0.0 a (100%)
Matrine® 1.4		15.6 ± 2.1 b (65.0%)	21.3 ± 0.8 a (88.8%)	24.0 ± 0.0 a (100%)
Matrine® 1.0		22.3 ± 2.1 a (92.9%)	22.6 ± 1.6 a (92.9%)	23.6 ± 0.8 a (98.3%)
Matrine® 0.6		19.3 ± 3.4 ab (84.4%)	22.0 ± 2.4 a (91.7%)	23.6 ± 0.8 a (98.3%)
Matrine® 0.2		8.6 ± 5.7 c (35.8%)	15.3 ± 2.1 b (63.8)	21.3 ± 2.8 b (88.8%)
Statistics	F	58.43	153.01	256.28
	P	0	0	0

Means ± Standard Error (SE) in each column followed by the same letter did not differ from each other according to the Tukey test (5% probability).

3.2. Impact of Matrine® over the Pupae of Trichogramma pretiosum (Bioassay 2)

No negative side effects on the emergence of adults of *Trichogramma pretiosum* from treated pupae (F1), or on the parasitism capacity of emerged adults, progeny (F2), were recorded at any of tested rates of Matrine™ (0.2, 0.6, 1.0, 1.4, 1.8, and 2.2 L of cp/150 L of H_2O), never differing from the treatment control (water) (**Tables 3 and 4**). Emergence from treated pupae with Matrine™ was higher than 72%. Parasitism capacity (%) and emergence (viability %) of the progeny at 24 and 48 hours were always higher than 61% and 78%. Therefore, Matrine™ at all studied rates were classified as harmless (class 1) to pupae of *T. pretiosum* at 24 hours and 48 hours after treatments (**Table 3**). Only 72 hours after treatment, which presented an overall lower parasitism, Matrine™ treatment presented a lower numerical parasitism (**Table 4**) which lead to the classification of the bioinsecticide as slightly harmful (class 2), especially at the higher studied rates of 1.8 and 2 L of cp/150 L of H_2O (**Table 3**).

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Table 3. Classification of the selectivity of insecticides to *Trichogramma pretiosum* according to the "International Organization for Biological Control" (IOBC) for pupae and adults, at different periods after spraying of the studied treatments.

Treatment (L of cp/150 L H_2O)	Bioassays with Pupae (Bioassay 2)								Bioassays with Adults (Bioassay 3)					
	Sprayed Pupae		24 Hours		48 Hours		72 Hours		24 Hours		48 Hours		72 Hours	
	EP[a]	C[b]	E[b]	C[c]	E[b]	C[c]	E[b]	C[c]	E[b]	C[c]	E[b]	C[c]	E[b]	C[c]
Matrine® 2.2	0.36	1	8.7	1	22.1	1	55.2	2	27.3	1	32.3	2	100.0	4
Matrine® 1.8	2.45	1	2.5	1	11.0	1	63.5	2	70.6	2	92.3	3	97.0	3
Matrine® 1.4	0	1	0	1	8.3	1	0	1	30.1	2	48.3	2	93.4	3
Matrine® 1.0	9.83	1	0	1	3.7	1	0	1	26.2	1	41.4	2	73.3	2
Matrine® 0.6	0.84	1	0	1	11.9	1	36.8	2	31.5	2	31.3	2	86.5	3
Matrine® 0.2	4.23	1	0	1	0	1	0	1	17.4	1	27.6	1	91.2	3

[a]EP (Effects on pupae %) = (1– adult emergence observed for the tested treatment/ adult emergence observed for the control treatment)×100; [b]Classes: 1 = harmless (EP or E < 30%), 2 = slightly harmful (30 ≤ EP or E ≤ 79%), 3 = moderately harmful (80 ≤ EP or E ≤ 99%), 4 = harmful (EP or E > 99%); [c]E(Effects on adults %) = (1–parasitism observed for the tested treatment/parasitism observed for the control treatment)×100.

Table 4. Effects of exposing parasitized host eggs to Matrine during the pupal stage of *Trichogramma pretiosum* on adult emergence rate (%) of sprayed pupae (F1), parasitism rate, and progeny survival of the second generation (F2) at different periods after spraying of the studied treatments (bioassay 2).

Treatment (L of cp/150 L H_2O)	Sprayed Pupae Adult Emergence (%)	24 Hours		48 Hours		72 Hours	
		Parasitism (%)	Progery Viability (%)	Parasitism (%)	Progery Viability (%)	Parasitism (%)	Progery Viability (%)
Water (control)	89.2 ± 1.5 a	79.3 ± 5.6 a	70.2 ± 8.3 a	78.7 ± 2.9 a	75.3 ± 5.6 a	59.6 ± 12.3 a	98.0 ± 0.9 a
Matrine® 2.2	90.7 ± 2.0 a	72.4 ± 4.9 a	94.5 ± 0.6 a	61.3 ± 6.6 a	78.6 ± 6.3 a	25.7 ± 13.1 a	87.2 ± 1.8 a
Matrine® 1.8	87.0 ± 5.8 a	77.3 ± 4.0 a	92.0 ± 0.9 a	70.0 ± 11.9 a	91.6 ± 1.1 a	21.7 ± 4.0 a	85.0 ± 6.7 a
Matrine® 1.4	72.4 ± 18.3 a	82.6 ± 5.9 a	87.2 ± 1.4 a	72.2 ± 5.9 a	91.7 ± 1.6 a	69.1 ± 12.0 a	88.3 ± 2.2 a
Matrine® 1.0	80.5 ± 3.0 a	85.9 ± 4.3 a	89.1 ±1.7 a	75.8 ± 3.2 a	92.3 ± 1.6 a	58.9 ± 11.4 a	90.1 ± 2.5 a
Matrine® 0.6	88.5 ± 2.1 a	82.4 ± 5.2 a	88.9 ± 0.7 a	69.3 ± 11.1 a	89.3 ± 1.2 a	37.6 ± 17.4 a	91.0 ± 2.1 a
Matrine® 0.2	85.5 ± 3.3 a	85.9 ± 6.2 a	86.5 ± 1.6 a	83.4 ± 5.0 a	83.8 ± 1.6 a	67.1 ± 12.2 a	81.3 ± 3.4 a
Statistics F	-	0.89		2.03		2.34	
Statistics *P*	-	0.51		0.08		0.05	
Statistics X^2	6.67	-	16.29	-	6.74	-	5.77
Statistics *P*	0.46	-	0.0001	-	0.4	-	0.56

Means (± SE) followed by different letters indicate significant differences according to Tukey's test after ANOVA (F) or Dunn's test following Kruskal–Wallis analysis (χ^2), both at $p < 0.05$.

3.3. Impact of Dry Residue of Matrine™ to Adults of Trichogramma pretiosum (Bioassay 3)

When *T. pretiosum* adults were exposed to the tested treatments, it was recorded that only water (control) and Matrine™ 0.2 L of cp/150 L of H_2O obtained the highest parasitism rate after 24 hours after treatment. Higher tested rates of Matrine™ caused lower parasitism rates (**Table 5**), however, the botanical insecticide impact on the parasitoid adult stage was still classified as harmless (class 1) or only slightly harmful (class 2) at 24 hours after treatment (**Table 3**). At 48 hours and 72 hours after treatment, the parasitism recorded at the different botanical insecticide treatments were even lower than parasitism recorded at 24 hours after treatment (**Table 5**), being, then, classified as slightly harmful (class 2) or moderately harmful (class 3) at rates of Matrine™ 0.2, 0.6, 1.0, 1.4, 1.8, and 2.2 L

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of cp/150 L of H_2O and even as harmful (class 4) for Matrine™ 2.2 L of cp/150 L of H_2O 72 hours after treatment (**Table 3**).

Table 5. Effects of Matrine adults of *Trichogramma pretiosum* on adult parasitism and progeny survival of the at different periods after spraying of the studied treatments (bioassay 3).

Treatment (L of cp/150 L H_20)		24 Hours		48 Hours		72 Hours	
		Parasitism (%)	Progeny Viability (%)	Parasitism (%)	Progeny Viability (%)	Parasitism (%)	Progeny Viability (%)
Water (control)		74.8 ± 2.5 a	93.7 ± 0.9 a	66.1 ± 1.2 a	87.4 ± 2.1 a	26.4 ± 11.0 a	75.2± 6.12 a
Matrine® 2.2		54.3 ± 0.8 b	83.0 ± 2.3 a	44.7 ± 1.7 b	79.09 ± 1.2 a	0.0 ± 0.0 c	No existent
Matrine® 1.8		21.9 ± 14.8 b	50.4 ± 9.6 b	5.0 ± 3.1 d	32.5 ± 9.3 b	0.7 ± 0.7 b	20.0 ± 8.9 a
Matrine® 1.4		52.2 ± 5.5 b	63.4 ± 3.0 b	34.1 ± 8.9 c	56.2 ± 8.3 a	1.7 ± 1.7 b	56.0 ± 10.4 a
Matrine® 1.0		55.1 ± 4.6 b	84.96 ± 1.5 a	35.1 ± 9.4 c	56.06 ± 10.4 a	7.0 ± 7.0 b	10.0 ± 4.5 b
Matrine® 0.6		51.1 ± 5.1 b	87.4 ± 0.7 a	45.4 ± 8.1 b	28.57 ± 8.8 b	3.5 ± 2.8 b	28.6 ± 8.8 a
Matrine® 0.2		61.7 ± 2.1 a	83.8 ± 1.3 a	47.8 ± 5.1 b	20 ± 8.9 b	2.3 ± 1.7 b	40.0 ± 11.0 a
Statistics	F	-	-	-	3.02	-	-
	P	-	-	-	0.04	-	-
	X^2	19.56	18.26	24.9	-	16.5	29.7
	P	0.006	0.01	0.0007	-	0.02	0.002

Means (±SE) followed by different showed significant differences according to Tukey test after ANOVA (F) or Dunn test after Kruskall-Wallis analysis (X).

4. Discussion

The tested bioinsecticide (Matrine™), based on the ethanolic extract of *Sophora flavescens*, was effective in controlling *Rachiplusia nu* at rates of 0.6, 1.0, 1.4, 1.8, and 2.2 L of p.c./ 150 L of H_2O in laboratory conditions with remarkable knockdown effect, achieving more than 84% and 91% control of *R. nu* just 24 hours and 48 hours after treatment, respectively. This result can be attributed to the composition of the commercial product used in this study (Matrine®), which contains the equivalent of 2 g of oxymatrine per liter of insecticide (0.2%) [16]. Oxymatrine acts on the nervous system of insects, interfering with acetylcholine receptors and interrupting the transmission of nerve impulses, which leads to fast paralysis and, subsequently, death [27,28].

Not only effectiveness of insecticides in controlling the target pest but also the selectivity of them on non-target organisms should be taken into consideration when choosing an insecticide to be adopted in Integrated Pest Management (IPM), which brings remarkable benefits to the pest management success [29]. In general, botanical insecticides have less impact on beneficial organisms [30,31], reinforcing their potential as a sustainable tool in IPM. However, the action of a given insecticides may vary between different species of biocontrol agents [32]. In addition, as far as we know, this is the first report of the selectivity of the ethanolic extract of *S. flavescens* to *T. pretiosum*, one of the most important natural biocontrol agents of Lepidoptera in soybean fields in the Neotropics [17].

Overall, the ethanolic extract of *S. flavescens* (Matrine™) was selectivity to *T. pretiosum*, especially to pupae of the parasitoid. The higher tolerance of pupae of *T. pretiosum* to the ethanolic extract of *S. flavescens* in comparison with adults might be linked to the location of the parasitoid inside the host egg, which is protected against botanical insecticide contact by the chorion of the eggs [**33**]. The ability of a product to penetrate the chorion of an insect egg can depend on their physicochemical properties and vary from insecticide to insecticide as well as species to species [**32**] illustrating the importance of the findings herein reported to the management of *R. nu*.

Taking into consideration the negative side effects recorded for adult parasitoids at the higher rates of 1.8 and 2.2 L of Matrine™/ 150 L of H_2O, the most promising results should be between Matrine™ 0.6, 1.0, or 1.4 L of p.c./ 150 L of H_2O. These findings are important, especially considering

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that *R. nu* has stood out as a key pest in soybean crops, severely impacting yield when not properly managed [34], consequently, bringing back the overspray of traditional chemical insecticides to control Lepidoptera in *Bt* soybean cultivars due to its outbreaks [7].

Thus, in conclusion the use of the ethanolic extract of *S. flavescens* emerges as a relevant alternative to reduce traditional chemical insecticides to control *R. nu*, contributing to the reduction of the negative impacts that these synthetic products can cause on biocontrol agents [35] besides other negative effects [36]. Botanical insecticides, in general, present greater environmental compatibility [37,38] and lower persistence in the environment [39], reducing risks such as food contamination, secondary outbreaks and the selection of resistant populations [40,41]. Nevertheless, it is important to emphasize that these experiments were carried out under laboratory controlled environmental conditions, where parasitoids were subjected to the highest possible pressure from the tested botanical insecticide. Under field conditions, however, the negative impact recorded in laboratory may be reduced because *T. pretiosum* can benefit from refuge areas or may avoid treated areas [23,42].

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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