

Selectivity of organic compounds to the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygastridae)

Seletividade de compostos orgânicos ao parasitoide de ovos *Telenomus remus* Nixon (Hymenoptera: Platygastridae)

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Abstract

The selectivity of insecticides, bio-protective mixtures, and biofertilizers used in organic soybean production was evaluated for adults and pupae of the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygastridae) under laboratory conditions in accordance with protocols proposed by the International Organization for Biological Control (IOBC). The products sprayed (dosage/180L of water) were: 1) Baculovirus anticarsia 140x10⁹ cpi; 2) *Bacillus thuringiensis* 16.8g; 3) Azadirachtin-A, azadirachtin-B, nimbina and salamina 9.6 ppm; 4) Rotenoids 4 liters; 5) Nitrogen 1.3%, phosphorus 3.0% and total organic carbon 8.0% 3 liters; 6) Sodium silicate 2% 4 liters; 7) Copper 7% + calcium 3.3% 1.8 liters; 8) Sulfur 20% + quicklime 10% 1.8 liters; 9) Chlorpyrifos 384g (positive control); 10) Distilled H₂O (negative control). The results of experiments using pupae indicate that the organic compounds were classified as harmless (Class 1), except for the copper 7% + calcium 3.3% and sulfur 20% + quicklime 10%, which were classified as slightly harmful (Class 2). The contact bioassay with adults showed that all products were classified as harmless (Class 1). Only chlorpyrifos (384g) was classified as harmful (Class 4) for both stages of the parasitoid. However, the use of this product (chlorpyrifos) is not permitted in organic farming, and even in conventional farming is recommended, where feasible, replacement of the product with one compatible with the preservation of *T. remus* in nature. Thus, the products tested and used in organic soybean production were considered compatible with the parasitoid eggs of *T. remus*.

Key words: Biological control, IPM, sustainability, toxicity

Resumo

A seletividade de diferentes inseticidas, caldas fitoprotetoras e biofertilizantes utilizados na produção de soja orgânica foi avaliada sobre adultos e pupas do parasitoide de ovos *Telenomus remus* Nixon (Hymenoptera: Platygastridae) em condições de laboratório, de acordo com protocolos propostos pela Organização Internacional para o Controle Biológico (IOBC). Os produtos (dose/180 L de água) foram: 1) Baculovirus anticarsia 140x10⁹ cpi; 2) *Bacillus thuringiensis* 16,8g; 3) Azadirachtin-A, azadirachtin-B, nimbina e salamina 9,6ppm; 4) Rotenoides 4% 4 litros; 5) Nitrogênio 1,3%, fósforo 3,0% e carbono

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orgânico total 8,0% 3 litros; 6) Silicato de Sódio 2% 4 litros; 7) Cobre 7% + Cálcio 3,3% 1,8 litros; 8) Enxofre 20% + cal virgem 10% 1,8 litros; 9) Clorpirifós 384g (controle positivo); 10) Água destilada (controle negativo). O resultado dos experimentos para a fase de pupa indicaram que os compostos orgânicos foram classificados como inócuos (classe 1), exceto para as misturas cobre 7% + cálcio 3,3% e enxofre 20% + cal virgem 10%, que foram classificados como levemente nocivos (classe 2). O teste de contato com os adultos mostrou que todos os produtos foram classificados como inócuos (classe 1). Somente clorpirifós foi classificado como nocivo (classe 4) para ambas as fases de desenvolvimento do parasitoide. Entretanto, a utilização deste produto (clorpirifós) não é permitida em cultivos orgânicos, e mesmo na agricultura convencional recomenda-se, quando viável, a substituição desse produto por outro compatível com a preservação de *T. remus* na natureza. Sendo assim, os produtos testados e utilizados na produção orgânica de soja foram considerados compatíveis com o parasitoide de ovos *T. remus*.

Palavras-chave: Controle biológico, MIP, toxicidade, viabilidade

Introduction

Organic farming has grown rapidly throughout the world during the last decade. According to surveys conducted by the Research Institute of Organic Agriculture (FiBL) and the International Foundation for Organic Agriculture (IFOAM), there are around 37.2 million hectares cultivated with organic crops worldwide (WILLER et al., 2013). The high demand for pesticide-free food and the high prices of organic products have fostered an increase in the production and marketing in this market segment (CAVIGELLI et al., 2013; MCBRIDE; GREENE, 2009). In addition to these economic benefits, organic farming can help mitigate the negative impacts of traditional agriculture, such as pesticide residues in food and water; improve soil quality and organic matter; and increase biodiversity, especially of beneficial insects such as biological control agents (LAIRON, 2010; MCBRIDE; GREENE, 2009).

In order to achieve profitable organic soybean yields, it is important to use pesticides which are not only effective against pests and diseases but also meet the requirements for organic certification (CAVIGELLI et al., 2013; ZEHNDER et al., 2007). Organic pesticides, although formulated for use in organic farming, are not always harmless to natural enemies, and might therefore endanger the success of biological control strategies (SIDI et al., 2012). Less harmful pesticides should always be chosen over the more harmful ones, since they allow the control of the target pest without negatively

affecting beneficial arthropods in the environment (BACCI et al., 2007; BUENO; FREITAS, 2004).

In organic farming, biological control using parasitoids can replace old chemical control strategies that are not permitted in organic production (VAN LENTEREN; BUENO, 2003). Inundative releases of the egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygasteridae) have been used in South and Central American countries, reaching up to 90% parasitism on *Spodoptera* eggs (FERRER, 2001). This is a successful example of parasitoid use and has been identified by different researchers as a promising alternative to chemical control, while also helping to reduce the negative effects of intensive agriculture on the environment and being economically viable for soybean growers (BUENO et al., 2008; VAN LENTEREN; BUENO, 2003).

It is important to note in this context that economically important pests can be controlled by parasitoids such as *T. remus* (BUENO et al., 2008), especially in organic farming, in which chemical control is not allowed. Minerals and other biological insecticides can also be used in organic farming to facilitate pest control (ZEHNDER et al., 2007). However, growers must follow International Foundation for Organic Agriculture (IFOAM) recommendations in order to use such biological insecticides. Possible effects of those biological insecticides to the environment and to human or animal health should be taken into consideration (IFOAM, 2005). Although there are criteria for

using these products of organic and mineral origin, there are few studies on their effects on beneficial arthropods, such as *T. remus* or other egg parasitoids. This knowledge is crucial to integrate these different pest management strategies (ZEHNDER et al., 2007). Therefore, this study evaluated the selectivity to the egg parasitoid *T. remus* of compounds used in organic soybean production.

Materials and Methods

The experiments were conducted in the laboratory under controlled conditions (25 ± 1 °C, $70 \pm 10\%$ RH, and photoperiod of 14/10 h L/D) in a randomized design, with 10 treatments and five repetitions according to standard protocols by the International Organisation for Biological Control (IOBC) as described by Hassan (1992). The selectivity of the products used in organic agriculture to natural enemies was assessed using the maximum recommended dosage for the control of pests and diseases in the production of organic soybean.

Bioassay with pupae. Cardboard squares (approximately 1 cm²) containing *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) laying eggs previously parasitized by *T. remus* were sprayed with various treatments (Table 1) nine days after parasitism using a Potter Tower regulated to a pressure of 1.5 Kg/cm², ensuring a deposit of 1.25 ± 0.25 mg cm⁻² (HASSAN, 1992; MANZONI et al., 2007). The applied volume was controlled using an electronic precision scale before and after spraying treatments (BUENO, 2001). Then, the cards were maintained under environmental conditions for about two hours to eliminate excess moisture. After they were dried, they were placed in contact cages consisting of two glass plates separated by an aluminum frame with ventilation holes (DEGRANDE et al., 2002). Once the parasitoids emerged, cards containing fresh *S. frugiperda* eggs (less than 24 hours old) were placed in the cages for two consecutive days in order to evaluate the parasitism capacity of the surviving parasitoid

females (CARMO et al., 2010). The viability of the sprayed pupae (%), calculated as the number of adults emerging from the sprayed eggs, and the parasitism and parasite viability of the F₁ generation (offspring of the treated pupae) were analyzed.

Bioassay with adults. Treatments were applied directly onto glass plates (13 cm × 13 cm) using a Potter Tower, following the same methodology described for the bioassay with pupae (BUENO, 2001). When dry, the plates were set in aluminum frames, using the cage arrangement described by Degrande et al. (2002). Vials containing the parasitoids were then connected to the cages, exposing the parasitoids to the glass plates (CARMO et al., 2010). Twenty-four hours after parasitoid release into the cages, numbered cards containing *S. frugiperda* egg masses (400 eggs) and honey droplets were inserted. A second card was placed inside each cage 24 h after the placement of the first. Since viable eggs were used in order to avoid any change in *T. remus* behavior, the trials needed to be stopped on the third day to prevent *S. frugiperda* from hatching.

Evaluations and analyses. Parasitism (%) and parasitoid emergence (%) were evaluated. The data were tested for normality (SHAPIRO; WILK, 1965) and homogeneity of variance (BURR; FOSTER, 1972) of treatments, and if necessary were transformed into $\arcsin \sqrt{x/100}$ to allow for an ANOVA. The means were compared using Tukey's test (5% probability). The reduction in parasitism relative to the control treatment (distilled water) was computed using the equation: $E (\%) = (1 - V_t/V_c) \times 100$, in which E (%) is the percent reduction in parasitism; V_t is the mean parasitism for the treatment tested; and V_c is the mean parasitism observed for the control treatment. The products were classified according to the IOBC standards, as follows: Class 1, harmless ($E < 30\%$); Class 2, slightly harmful ($30\% \leq E \leq 79\%$); Class 3, moderately harmful ($80\% \leq E \leq 99\%$); and Class 4, harmful ($E > 99\%$) (CARMO et al., 2010; HASSAN, 1992).

Table 1. Treatments tested against pupae and adults of the egg parasitoid *Telenomus remus* (Hymenoptera: Platygasteridae).

Commercial Product	Formulation	Provider/manufacturer	Dosage
			180 L H ₂ O
Baculovirus AEE®	Baculovirus anticarsia 7 x 10 ⁹ cpi.g ⁻¹	Embrapa Employees Association AEE/CNPSoja	140 x 10 ⁹ cpi
Dipel®	<i>Bacillus thuringiensis</i> 33.6 g.l ⁻¹	Sumitomo Chemical of Brazil Representatives Ltd	0.5 L
Neemseto®	Neem oil (Azadirachtin-A, Azadirachtin-B, Nimbina and Salamina 2.389 ppm.l ⁻¹)	Cruangi Neem of Brazil Ltd	4.0 L
Arrast®	Deguelia utilis extract (Rotenoids 4%)	Humanita Chemical and Pharmaceutical S.A.	4.0 L
Fish-Fertil Indure®	Derivatives from crustaceans shells (nitrogen 1.3%, phosphorus 3.0% and total organic carbon 8.0%)	Fish Industries and Commerce of Fertilizers Ltd	3.0 L
Na Silicate	Sodium silicate 2% dissolved in H ₂ O	*	4.0 L
Borda-Ferti pH7®	Copper 7% + Calcium 3.3%	Planalquímica of Brazil Chemical Industry Ltd	1.8 L
Lime-sulfur mixture	Sulfur 20% + quicklime 10% (POLITO, 2000)	*	1.8 L
Lorsban 480 CE®	chlorpyrifos 480g.l ⁻¹	Dow AgroSciences Industrial Ltd	0.8 L
Control	Distilled H ₂ O	-	-

*Mixture prepared at EMBRAPA Soybean to be used in the experiment.

Results

A statistical difference ($p < 0.0001$) was observed in the evaluated parameters both in the pupal and adult trials (Tables 1 and 2), indicating the influence of the tested products on the emergence of pupae, parasitism capacity, and viability of *T. remus* on *S. frugiperda* eggs. *T. remus* pupae in the Baculovirus anticarsia treatment group had the highest viability, which remained above 90%, similar to that of the negative control (H₂O). The other treatments, *Bacillus thuringiensis*, neem oil, derivatives from crustaceans, Na silicate, and sulfur 20% + quicklime 10%, although having a slight reduction in parasitism, were considered harmless (Class 1) according to the IOBC classification, the same as for the Baculovirus anticarsia (Table 2). The lowest

parasitoid emergence index (Table 2), excluding chlorpyrifos (384g), was observed in the treatment with *Deguelia utilis* extract (58.98%), which was classified as slightly harmful to parasitoids (Class 2).

T. remus parasitism in *S. frugiperda* eggs offered to females one day after emergence (DAE) remained above 99% for most of the treatments (Table 2) and did not differ from the negative control treatment (H₂O), except for Na silicate and the sulfur 20% + quicklime 10%, which were classified as harmless even though they reduced the parasitism of *T. remus* compared to water treatment. The viability of parasitism for most treatments did not differ from that of chlorpyrifos (384g); only the sulfur 20% + quicklime 10% reduced the viability of parasitoids

(Table 2) and was rated as slightly harmful (Class 2). The other treatments were classified as harmless to *T. remus* (Class 1).

Eggs of *S. frugiperda* offered two DAE to *T. remus* pupae had lower levels of parasitism than the negative control (H₂O) in the treatments derived from crustaceans and the sulfur 20% + quicklime 10% (Table 2). The viability of parasitism was higher in eggs offered to adults from the Baculovirus anticarsia treatment (82%); other products caused a reduction in viability when compared to the negative control (H₂O) (Table 2). Despite the viability reductions, all treatments were classified as harmless to *T. remus* (Class 1), except for chlorpyrifos (384g), which was classified as harmful (Class 4).

In bioassays with *T. remus* adults, *S. frugiperda* eggs inserted one day after application (DAA) of the showed reduced parasitism by *T. remus* in the treatments with *Deguelia utilis* extract and derivatives of crustaceans, while others (*B. anticarsia*, azadirachtin, *B. thuringiensis*, copper 7% + calcium 3.3% and the sulfur 20% + quicklime 10%) were similar to the negative control (H₂O) (Table 3). The treatments with Baculovirus anticarsia, *B. thuringiensis*, neem oil, *Deguelia*

utilis extract and derivatives of crustaceans had the highest viability rates (Table 3). Treatment with Na silicate and sulfur 20% + quicklime 10% reduced the viability of parasites by approximately 29% and 15%, respectively, compared to the control (H₂O). However, even with the reduction in viability, all treatments were classified as harmless (Class 1), except for chlorpyrifos (384g), which was slightly harmful (Class 2).

Spodoptera frugiperda eggs offered to *T. remus* adults two days after the applications were parasitized to a lesser extent only in the treatments with neem oil, *Deguelia utilis* extract, and derivatives of crustaceans (Table 3). The remaining products did not influence the parasitism, similar to the negative control. The viability of eggs parasitized by adults who were exposed to the products was similar to the negative control in the treatments with Baculovirus anticarsia, derivatives of crustaceans, *Deguelia utilis* extract, and the sulfur 20% + quicklime 10%, remaining above 79%. The other products, *B. thuringiensis*, neem oil, Na silicate, and the copper 7% + calcium 3.3%, reduced the viability of the parasitoid. Unlike chlorpyrifos (384g) (Class 4), all of these products were harmless (Class 1) to *T. remus*.

Table 2. Side effects of organic compounds above *Telenomus remus* (Hymenoptera: Platygasteridae) pupae and parasitism and viability of F₁ generation.

Treatment (dosage/180 L-H ₂ O)	1 day after adult emergence (F ₁)					2 days after adult emergence (F ₁)					
	Viability of pupae (%)	4E%	Class	Parasitismo (%)	Viability (%)	E%	Class	Parasitismo (%)	Viability (%)	E%	Class
Baculovirus anticarsia (140x10 ⁹ cpi)	90.14 ± 1.81 A ^{1,2}	3.65	1 ³	99.22 ± 0.58 A	80.72 ± 6.33 AB	0.78	1	79.28 ± 1.02 AB	82.00 ± 4.08 AB	6.83	1
<i>Bacillus thuringiensis</i> (16.8g)	70.28 ± 6.95 AB	19.2	1	99.41 ± 0.59 A	56.91 ± 8.54 BC	0.59	1	77.95 ± 2.89 AB	70.27 ± 3.89 B	8.39	1
Neem oil (9.6 ppm)	65.63 ± 6.81 AB	24.5	1	99.12 ± 0.78 A	58.98 ± 4.69 ABC	0.88	1	70.92 ± 5.54 AB	68.68 ± 7.54 B	16.65	1
<i>Degevelia utilis</i> extract (4 L)	73.62 ± 3.25 AB	15.4	1	99.71 ± 0.29 A	54.26 ± 6.41 BC	0.28	1	72.62 ± 4.63 AB	72.18 ± 6.25 B	14.65	1
Chlorpyrifos (384g)	31.41 ± 7.59 C	63.9	2	1.69 ± 0.99 D	31.25 ± 6.25 C	98.3	3	0.00 ± 0.00 C	0.00 ± 0.00 B	100	4
Control (H ₂ O)	86.96 ± 2.25 A	-	1	100 ± 0.00 A	89.73 ± 1.67 A	-	1	85.09 ± 3.03 A	100 ± 0.00 A	-	1
Derivatives from crustaceans (3L)	73.12 ± 6.73 AB	15.9	1	100 ± 0.00 A	67.23 ± 6.08 AB	0	1	65.66 ± 2.65 B	72.78 ± 5.10 B	22.83	1
Na silicate 2% (4L)	84.25 ± 3.69 AB	3.12	1	91.59 ± 3.03 B	54.75 ± 8.48 BC	8.41	1	68.55 ± 6.05 AB	73.79 ± 4.29 B	19.44	1
Copper 7% + calcium 3.3% (1.8L)	58.98 ± 7.08 B	32.2	2	100 ± 0.00 A	71.83 ± 3.63 AB	0	1	69.33 ± 4.16 AB	63.80 ± 3.46 B	18.52	1
Sulfur 20%+quicklime 10% (1.8L)	76.55 ± 7.37 AB	12.0	1	63.39 ± 2.65 C	29.99 ± 5.68 C	36.6	2	64.13 ± 4.22 B	59.74 ± 6.71 B	24.64	1
P	< 0.0001			< 0.0001	< 0.0001			< 0.0001	< 0.0002		
df model	9			9	9			9	8		
df residue	40			39	37			40	36		
F	8.56			191.88	8.5			38.11	5.36		
CV	18.19			5.23	22			13.22	15.31		

¹Means±SE followed by the same letter in each assay column are not statistically different by Tukey test (P>0.05); ²Original data followed by statistics performed on data transformed into arcsin $\sqrt{X/100}$ to allow for ANOVA according to BURR & FOSTER (1972); ³E(%) = (1-Vi/Vc)×100, in which E(%) is the percent reduction in parasitism or parasitoid emergence; Vi is the median parasitism for the treatment tested; and Vc is the average parasitism observed for the control treatment (HASSAN, 1992). ³Classes: 1 = harmless (E<30%), 2 = slightly harmful (30%E<79%), 3 = moderately harmful (80%E<99%), 4 = harmful (E>99%).

Table 3. Side effects of organic compounds above *Telenomus remus* (Hymenoptera: Platygastridae) adults.

Treatment (dose/180 L – H ₂ O)	1 day after sprayed				2 days after sprayed			
	Parasitism	Viability	E%	Class	Parasitism	Viability	E%	Class
Baculovirus antioctaria (140x10 ⁹ cpi)	77.80 ± 6.52 AB ^{1,2}	83.24 ± 1.56 AB	14.18	1 ³	88.98 ± 1.22 A	83.53 ± 2.48 A	5.29	1
<i>Bacillus thuringiensis</i> (16.8g)	86.75 ± 2.79 A	59.48 ± 2.04 C	4.32	1	63.32 ± 4.52 C	61.15 ± 1.61 CD	25.07	1
Neem oil (9.6 ppm)	79.16 ± 3.59 AB	62.45 ± 2.39 BC	12.69	1	59.80 ± 3.70 C	63.66 ± 3.56 BCD	29.24	1
<i>Deguelia utilis</i> extract (4 L)	64.23 ± 5.05 BC	61.52 ± 6.29 C	29.15	1	78.44 ± 4.42 AB	79.44 ± 5.31 AB	7.19	1
Chlorpyrifos (384g)	47.05 ± 4.99 C	49.26 ± 8.39 C	48.11	2	0.00 ± 0.00 D	0.00 ± 0.00 E	100.00	4
Control (H ₂ O)	90.67 ± 1.73 A	93.75 ± 0.75 A	-	1	84.51 ± 2.69 AB	81.18 ± 4.08 A	-	1
Derivatives from crustaceans (3L)	64.42 ± 5.85 BC	55.41 ± 9.27 C	28.95	1	71.84 ± 3.24 BC	81.92 ± 1.54 A	14.99	1
Na silicate 2% (4L)	78.91 ± 2.36 AB	63.43 ± 3.16 BC	12.97	1	80.59 ± 1.36 AB	73.40 ± 2.92 ABC	4.64	1
Copper 7% + calcium 3.3% (1.8L)	71.77 ± 5.01 AB	52.69 ± 3.82 C	20.84	1	83.95 ± 1.97 AB	56.07 ± 4.35 D	0.66	1
Sulfur 20% + quicklime 10% (1.8L)	76.76 ± 2.52 AB	66.33 ± 3.29 BC	15.34	1	77.92 ± 2.75 AB	79.61 ± 3.51 AB	7.79	1
P	< 0.0001	< 0.0001			< 0.0001	< 0.0001		
df model	9	9			9	8		
df residue	40	39			39	37		
F	8.45	10.7			76.38	8.87		
CV%	13.15	11.67			9.63	10.59		

¹Means±SE followed by the same letter in each assay column are not statistically different by Tukey test ($P>0.05$); ²Original data followed by statistics performed on data transformed into arcsin $\sqrt{X/100}$ to allow for ANOVA according to BURR & FOSTER (1972); ³E(%) = $(1-Vt/Vc) \times 100$, in which E(%) is the percent reduction in parasitism or parasitoid emergence; Vt is the median parasitism for the treatment tested, and Vc is the average parasitism observed for the control treatment (HASSAN, 1992); ⁴Classes: 1 = harmless ($E<30\%$), 2 = slightly harmful (30EE79%), 3 = moderately harmful (80EE99%), 4 = harmful ($E>99\%$).

Discussion

In this study, only chlorpyrifos (384g) was able to penetrate the pupae and affect the development of *T. remus*. It is possibly because pupae is the most resistant stage to pesticides due to the sheltering offered by its cocoon (BUENO et al., 2008; CARMO et al., 2010; SANTOS et al., 2006). Chlorpyrifos works via the phosphorylation of the enzyme acetylcholinesterase (AChE) in the nerve endings, which reduces available AChE and increases activity in the organs due to excess acetylcholine, which similarly affects both beneficial insects and pests. Several surveys have reported low selectivity of organophosphates in Integrated Pest Management (IPM) (BUENO et al., 2008; TOSCANO et al., 2012.).

In the bioassays with pupae and adults of *T. remus*, parasitism and its viability were not affected by Baculovirus anticarsia, which is used to combat the velvetbean caterpillar, *Anticarsia gemmatilis*. The same selectivity was reported for *B. thuringiensis*, also used to control lepidopterans, but with faster mortality of its target. The harmless effect of these compounds is probably because these entomopathogens (viruses and bacteria) require that the pH of the midgut of insects is appropriate for the activation of their toxins, which does not occur in parasitoids (KNUTSON, 1998; TAKADA et al., 2001; VALICENTE; TUELHER, 2009).

Different plant extract-based products are successfully used as insecticides (ISMAN, 2006; VENZON et al., 2008). However, some studies show that plants such as neem oil, *Azadirachta indica*, adversely affect the oviposition, fecundity, feeding, and growth of various beneficial insects (GONCALVES-GERVASIO; VENDRAMIM, 2004; MORDUE; NISBET, 2000; SCHMUTTERER, 1990). Differently, one of the first studies on the selectivity of natural products was carried out in India by Joshi et al. (1982), who sprayed aqueous solutions of 2% neem seed on *Spodoptera litura* Fabricius (Lepidoptera:

Noctuidae) eggs, which did not negatively impact *T. remus* parasitism, but did change the longevity and fecundity of adult parasitoids. The reduction in the viability of *T. remus* parasitism during the pupal stage reported in our study may be because the concentration of azadirachtin was twice that used by Joshi et al. (1982). Commercial formulations of neem lack standardization in the amount of active ingredient (azadirachtin), which can cause differences in selectivity and product performance.

Deguelia utilis extract, used for formulation of Arrast®, reduced the viability of parasitism when sprayed on pupae and adults. This reduction can be attributed to the mechanism of action of rotenone, which is the active ingredient of this plant (MOREIRA et al., 2005). Rotenone is a very toxic compound and acts against a wide range of insects by blocking the electron transport chain and preventing energy production in the mitochondria (HOLLINGWORTH et al., 1994). The use of rotenone as an insecticide is limited in the production of organic foods due to its side effects in humans (ISMAN, 2006). However, studies on the side effects of these extracts on non-target organisms are scarce.

Bio-protective mixtures such as the copper 7% + calcium 3.3% and the sulfur 20% + quicklime 10% are being increasingly used by small farmers of organic crops (GONÇALVES et al., 2004; VENZON et al., 2005) as a source of nutrients for plants and for fighting diseases (POLITO, 2001; SILVA; CARVALHO, 2000). There are reports that some bio-protective mixtures can also act as insecticides; however, the effects of these products on natural enemies must still be determined by larger studies (EFROM et al., 2011; GONÇALVES et al., 2004). The sulfur 20% + quicklime 10% and copper 7% + calcium 3.3% had negative effects on *T. remus*, reducing its parasitism, however, the mechanisms of action of these bio-protective mixtures are not well known. Silva (2005) and Dal Soglio et al. (2007) showed that the lime-sulfur mixture also had a negative influence on *Apis mellifera* (Hymenoptera:

Apidae), a pollinator insect in citrus orchards of the same order as *T. remus*. The authors attributed this impact to the number of spray applications made during the study period. According to Polito (2000), the toxic effect of the lime-sulfur mixture is due to the reaction between its components, water and carbon dioxide, to form hydrogen sulfide, which makes the spray insecticidal.

Other products used in organic agriculture are biofertilizers, such as derivatives of crustaceans and Na silicate. Derivatives from crustaceans, which is applied to leaves, comes from the natural enzymatic fermentation of marine fish, enriched with chitosan from the shells of crustaceans. This product acts as a regulator of the nutritional balance of the plants, improving photosynthetic rate (DI PIERO; GARDA, 2008). Another benefit reported by the manufacturers of this biofertilizer is that chitosan induces genes involved in plant resistance to disease and pests (DI PIERO; GARDA, 2008). In this study, derivatives from crustaceans reduced the parasitism and viability of *T. remus* in both pupa and adults, as did Na silicate (2%). Studies show that silicate in plants increases cell wall strength and the number of plant trichomes, which protect the plant against attack by pathogens such as fungi and insects (GOUSSAIN et al., 2002; MORAES et al., 2004). Therefore, due to their negative effects on *T. remus*, caution is required regarding the dosage and concentration of these products when using them as biofertilizers.

Assays for the pupal stage were performed under extreme spray exposure to the recommended doses for soybean, and the products that were considered selective and harmless to the parasitoids will therefore be so in any field conditions to which they are exposed. According to Hassan (1992), products that have been classified as slightly harmful, moderately harmful, or harmful should be re-tested to verify the effects on parasitoids in field conditions. Adult parasitoids, despite having higher mobility, may be more exposed to the negative effects of

spray products used in pest control (BUENO et al., 2008), and it is therefore important to study these products to inform decisions on their use in the IPM.

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