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Vol. 10(40), pp. 3825-3831, 1 October, 2015
DOI: 10.5897/AJAR2014.9083
Article Number: 8ED195C55660
ISSN 1991-637X

http://www.academicjournals.org/AJAR

AfricanJournalof Agricultural Research

Full Length Research Paper

Evaluating the selectivity of registered fungicides for soybean against *Trichogramma pretiosum* Riley, 1879(Hymenoptera: *Trichogrammatidae*)

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Received 19 August, 2014; Accepted 18 September, 2015.

The aim of this study was to evaluate the effects of fungicides registered for soybean on the parasitoid *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: *Trichogrammatidae*). Bioassays wereconducted in laboratory exposing adult insects to dried residues of fungicides, using the methodology proposed by the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC). The experimental design was completely randomized with four replications per treatment. The parameter used for classification of fungicides was based on the reduced parasitism (RP) evidenced by the number of parasitized eggs per female in the control treatment. Based on these results, we found that fungicides are classified in different classes of selectivity to adults of *T. pretiosum* regarding the tested fungicides. The fungicides cyproconazole, epoxyconazole + kresoxim-methyl, metconazole, thiophanatemethyl (CS), pyraclostrobin, difenoconazole, were classified as harmless (Class 1); carbendazim, carbendazim + thiram, tetraconazole, tetraconazole+ azoxystrobin, epoxyconazol + pyraclostrobin, tebuconazole, prothioconazole + trifloxystrobin, tebuconazole + trifloxystrobin, azoxystrobin, azoxystrobin + cyproconazole, and thiophanate methyl (WG) were slightly harmful (Class 2); flutriafol, thiophanate methyl (WP), epoxyconazole + pyraclostrobin, and cyproconazole+ trifloxystrobin were moderately harmful (Class 3); and Kumulus DF was harmful (Class 4) to the adult parasitoids.

Keywords: Chemical control, integrated pest management (IPM), parasitoid eggs, pesticide, International Organization for biological and integrated control of noxious animals and plants (IOBC) methodology, soybean.

INTRODUCTION

Around the world, Brazil is the second largest producer of soybeans, producing around 86 million tonnes of soybean grains, in the past harvest, with expectations to export about 46 million tonnes (CONAB, 2015). As

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soybean is grown commercially for human consumption and animal feed, it is considered a major source of protein and vegetable oil in the world. In addition, soybean is emerging as an alternative for biodiesel production (USDA, 2013).

The occurrence of pest attacks is a biotic factor that presents a greater interference on soybean yield. Among the many diseases that attack soybean, the Asian soybean rust (*Phakopsora pachyrhizi* Syd & P. Syd) identified in Brazil in 2001, stands out as a pathogen capable of causing losses ranging from 10 to 90%, mainly damaging early foliage, which prevents full grain development leading to reduced productivity (Roese, 2011). Brazil has an estimated accumulated loss of 15 million tonnes of soybean grains due to the presence of this pathogen in crops (Goulart, 2010), with chemicals as the main controlling strategy to suppress the pathogen (Sosa-Gómez et al., 2003).

In this context, the use of fungicides for soybean crops has intensified, leading in some cases with up to three applications as needed (Alessio, 2008), due to the spread of Asian soybean rust. According to Carneiro et al. (2012), for the periods between 2002 and 2011, Brazilian soybean crops showed an increase use of 1.6 L ha^{-1} of fungicides.

As of today, there is great difficulty in predicting and monitoring the presence of this pathogen in soybean crops. In an attempt to avoid losses and minimize the spread of this disease, a preventive application of fungicides has become an alternative (Augusti et al., 2014). However, this management, suffering deleterious effects (Sosa-Gómez, 2005), may affect beneficial organisms in the agroecosystem.

Among these beneficial organisms, highlights *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: *Trichogrammatidae*), considered an important egg parasitoid of soybean pests, such as the velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner, 1818), and the soybean looper, *Chrysodeixis includes* (Walker, 1857). Its action is to prevent outbreaks of the host before any damage is done to the plants (Bueno et al., 2011; Bueno et al., 2012).

Therefore, in order to evaluate the selectivity of fungicides on parasitoid eggs Bueno et al., 2008; Carmo et al., 2009; Carvalho et al., 2012); we used the standardized methodologies of International organization for Biological and Integrated control of noxious Animals and Plants (IOBC) on soybean crops. Thus, it is apparent that poor support of IPM in soybean crops, regarding indications of selective products to natural enemies, is impairing the suppression of pests by biological control or its association with chemical control.

In order to assess the effects of fungicides on beneficial insects (non-targets), the IOBC has developed a standard protocol, using egg parasitoid specie of the genus *Trichogramma* based on the cosmopolitan distribution, ease of reproduction, high parasitism capacity, and sensitivity to fungicide exposure (Hassan et al., 2000). Based on the facts presented, the objective of this research was to evaluate the selectivity of registered fungicides for soybean on adult parasitoid *Trichogramma pretiosum.*

MATERIALS AND METHODS

Six bioassays were conducted to evaluate the selectivity in *T. pretiosum* adults, following the methodology adapted to the species (Giolo et al., 2005; Manzoni et al., 2006a), and in accordance with the guidelines proposed by IOBC (Hassan et al., 2000; Hassan and Abdelgader, 2001). The biological material was used in bioassays comprising the egg parasitoid, which were multiplied in the factious host, *Ephestia kuehniella* Zeller,1879 (Lepidotera: *Pieridae*) eggs according to the technique described by Parra (1997). The offspring were maintained in the laboratory, in climate-control chambers of 25±2°C, relative humidity of 70±10%, and photophase of 14 h. Twenty-three fungicides of different chemical groups commonly applied in soybean crops (Table 1) were evaluated. In addition, to these fungicides, a positive control, Lannate BR (Methomyl), which is recognized as a standard toxic pesticide (verify in preliminary tests), is capable of providing 100% of the parasitoid mortality (Magano et al., 2013) and a negative control of distilled water was used.

The parasitoids were then exposed to dry residues of fungicide syrup, which is sprayed on 13×13 cm glass plates, at the maximum recommended rate for use in the field (Agrofit, 2013). Applications were applied using a hand sprayer that provided a syrup deposit of 1.75±0.25 mg cm^{-2} on each glass plate, measured by a precision balance. Toxicity test were conducted in the laboratory, under the same conditions used for the parasitoid offspring.

To start the experiment we first used emergence tubes (transparentglassbulb120mmlongby20mmindiameter at the baseand7mmattheopening),inwhicheach contained a stationary cardboard circle (STC) (1 cm in diameter) with 250±50 eggs of *E. kuehniella*previously parasitized by *T. pretiosum*. Approximately 24 h after emergence, the tubes containing the adults of *T. pretiosum* were in connected cages for 12 h, allowing the entry of insects to be exposed to the fungicides. The feed, which consisted of a honey gelatin mix, of the parasitoid was applied on each STC with eggs viable at 24 (three STCs), 48 (two STCs), and 96 h (one STC). Afterwards, each STC was counted to determine the number of parasitized eggs and the number of females inside the cage, thus, giving the average number of parasitized eggs per female *T. pretiosum* for each treatment.

The reductions, in the average number of parasitized eggs, depending on the tested products were corrected by Equation 1 (Hassan et al., 2000):

$$RP = (1 - Rt / Rc) \times 100 \quad (1)$$

Where: RP corresponds to the % reduction in parasitism; Rt is the average value of parasitism for each product; and Rc is the average parasitism observed for the control treatment (negative). Due to the reduction in parasitism, selectivity classes were defined: Class 1 (RP>30%), Class 2 (30<RP<79%), Class 3 (79<RP<99%), and Class 4 (RP>99%).

For data analysis, the completely randomized design was used with four replications; each cage exposed was considered an experimental unit. The data obtained were tested for normality, after subjected to variance analysis, and average significant were compared by the Tukey test ($p<0.05$) with the software SASM agri (Canteri et al., 2001).

Table 1. Fungicides evaluated in tests of selectivity for adults of *Trichogramma pretiosum* using maximum dosage of the commercial product registered for soybean.

Commercial product	Active Ingredient	Chemical group	DC	Cia[2]
Alto 100	Cyproconazole	Triazol	0.300	0.0150
Brio	Epoxyconazole + kresoxim-methyl	Triazol+ strobilurin	0.600	0.0375+0.0375
Caramba 90	Metconazole	Triazol	0.600	0.0270
Cercobin 500 SC	Thiophanate methyl	Benzimidazole	0.800	0.2000
Comet	Pyraclostrobin	Strobilurin	0.300	0.0370
Derosal 500 SC	Carbendazim	Benzimidazole	0.500	0.1250
Derosal Plus	Carbendazim + thiram	Benzimidazole + Triazol	0.200	0.0150 +0.0350
Domark 100 EC	Tetraconazole	Triazol	0.500	0.0250
Domark XL	Tetraconazole+ azoxystrobin	Triazol+ strobilurin	0.600	0.0240 +0.0300
Envoy	Epoxyconazol + pyraclostrobin	Triazol+strobilurin	0.700	0.0218+0.0297
Folicur 200 EC	Tebuconazole	Triazol	0.750	0.0750
Fox	Prothioconazole +trifloxystrobin	Triazol+strobilurin	0.400	0.0350+0.0300
Impact125 SC	Flutriafol	Triazol	1.000	0.0625
Kumulus DF	Sulfur	Inorgânic	2.500	1.0000
Metiltiofan	Thiophanate methyl	Benzimidazole	0.600	0.2100
Nativo	Tebuconazole + trifloxystrobin	Triazol+ strobilurin	0.600	0.0600+0.0300
Opera	Epoxyconazole + pyraclostrobin	Triazole+ strobilurin	0.600	0.0150+0.0399
Priori	Azoxystrobin	strobilurin	0.200	0.0250
Priori Xtra	Azoxystrobin +cyproconazole	strobilurin +Triazole	0.300	0.0300+0.0120
Score	Difenoconazole	Triazole	0.300	0.0375
Sphere Max	Cyproconazole+trifloxystrobin	Triazole+ strobilurin	0.200	0.0160+0.0375
Support	Thiophanate methyl	Benzimidazole	0.900	0.2250
Support WG	Thiophanate methyl	Benzimidazole	0,700	0,2975

[1]Dosage field (L ou Kg.ha^{-1} of commercial product) considering a spray volume of 200l.ha^{-1} [2]Concentracion (%) active ingredient present in the syrup used in bioassays.

RESULTS AND DISCUSSION

The test of toxicity in *T. pretiosum* adults observed in fungicides Alto 100 (cyproconazole), Brio (epoxyconazole + kresoxim-methyl), Caramba 90 (metconazole), Cercobin 500 SC (thiophanate methyl), Comet (pyraclostrobin), Score (difenoconazole), and Support (thiophanate methyl- SC) were classified as harmless (Class 1), totaling 30.43% of the fungicides tested (Figure 1). Thus, the safety of *T. pretiosum* during adulthood, the most sensitive stage of the parasitoid, was harmless by the fungicides (Hassan, 1998).

For cyproconazole (Table 2, bioassay III), classified as harmless (Class 1), it showed a reduction of 15.28% in parasitism, even with the studies by Hassan (1998) and Sterk et al. (1999) that utilized fungicide Alto 100 in concentrations of 0.08 and 0.0025%, respectively. The acting mechanism on fungi is due to the demethylation of lanosterol at position 14 or at position 24 of dihidrosterol methylene, which are sterol precursors, one of the basic components in the formation of plasma membrane of organism (Reis et al., 2007).

The commercial product pyraclostrobin was classified harmless to the parasitoid (Class 1) with 17% reduction in parasitism (Table 2, Bioassay I). These results arecorroborating with Manzoni et al. (2006a), that confirmed Class 1 to the same commercial productfor those applied in apple orchards, but is important verify that the dose is different and technology

The difenoconazolewas classified as harmless to the parasitoids with a decrease in parasitism by 29.79%. In studies on the selectivity of the fungicide in apple orchards on the species *T. pretiosum* and *Trichogramma atopovirila* (Oatman and Platner, 1993) (*Hymenoptera: Trichogrammatidae*), such results were classified the same, harmless (Class 1) (Manzoni et al., 2007).

It was observed in the following: Carbendazim (SC), carbendazim + thiram, tetraconazole (CE), tetraconazole + azoxystrobin (SC), epoxyconazol + pyraclostrobin, tebuconazole (CE), prothioconazole + trifloxystrobin, tebuconazole + trifloxystrobin, azoxystrobin, azoxystrobin + cyproconazole, and thiophanate methyl(WG), presented themselves as slightly harmful (Class 2), for a total of 47.83% of the fungicides tested (Figure 1). The largest portion of the fungicides tested met Class 2, revealsa result not expected, due biological targetwas achieved by these products are pathogenic organisms that damaged the crops, presented a specific actionmode (Reis et al., 2007), and we thought that these products made any effect by natural enemies.

Moreover, azoxystrobin, that belongs to the group strobilurin, which is similar to Domark XL, and thatPriori

Table 2. Average number of females per cage and effect of fungicides on soybeans on the number (± SE) of eggs parasitized by females, reduction (%) in adults parasitism capacity of *T. pretiosum* and classification of toxicity depending on conditions IOBC laboratory.

Commercial product (Active ingredient)	DC[1]	Cia[2]	Females per cage[3]	Parasited egg's per female[3]	RP[4]	Class IOBC[5]
Bioassay I						
Distilled water (testemunha negativa)	-	-	132.41±14.69^{ns}	17.13±1.19^{a}	-	-
Brio (epoxiconazole+ kresoxim-methyl)	0.600	0.0375+0.0375	153.76±18.81	14.65±1.53^{a}	14.48	1
Caramba 90 (Metconazole)	0.600	0.0270	112.30±7.18	13.07±3.85^{a}	23.70	1
Comet (pyraclostrobin)	0.300	0.0375	135.00±17.52	14.20±3.91^{a}	17.00	1
Envoy (epoxiconazole + pyraclostrobin)	0.700	0.0218+0.0297	123.86±8.49	11.97±1.39^{a}	30.12	2
Lannate BR (methomyl)	1.000	0.1075	126.90±6.13	0.00±0.00^{b}	100.0	4
Bioassay II						
Distilled water (negative control)	-	-	207.44±6.07^{ns}	21.45±0.70^{a}	-	-
Cercobin 500 SC (thiophanate - methyl)	0.800	0.2000	217.16±11.43	16.28±3.54^{ab}	24.10	1
Domark XL (tetraconazol + azoxystrobin) *	0.600	0.0240+0.0300	240.30±15.77	10.85±3.67^{b}	49.42	2
Score (difenoconazole)	0.300	0.0375	251.26±2.52	15.06±0.83^{ab}	29.79	1
Support (thiophanate - methyl)	0.900	0.2250	232.00±11.29	18.40±2.15^{ab}	14.22	1
Lannate BR (methomyl)	1.000	0.1075	244.31±18.12	0.00±0.00^{c}	100.0	4
Bioassay III						
Distilled water (negative control)	-	-	203.44±2.77^{ns}	25.45±1.38^{a}	-	-
Alto 100 (ciproconazol)	0.300	0.0150	196.16±10.43	21.56±0.54^{a}	15.28	1
Priori (azoxystrobin)	0.200	0.0250	199.56±15.77	9.85±3.67^{b}	61.30	2
Priori Xtra (azoxystrobin + ciproconazol)	0.300	0.0300+0.0120	201.26±2.52	8.06±0.83^{b}	68.33	2
Metiltiofan (thiophanate - methyl)	0.600	0.2100	201.09±1.29	3.04±1.57^{c}	88.06	3
Lannate BR (methomyl)	1.000	0.1075	194.31±6.82	0.00±0.00d	100.0	4
Bioassay IV						
Distilled water (negative control)	-	-	113.75±15.24^{ns}	28.80±2.01^{a}	-	-
Derosal 500 SC (carbedazim)	0.500	0.1250	115.03±23.56	14.61±2.40^{b}	49.27	2
Derosal Plus (+ carbedazim take)	0.200	0.0150+0.0350	94.91±20.56	12.16±1.51^{b}	57.78	2
Fox (trifloxystrobin + prothioconazole)	0.400	0.0350+0.0300	111.35±37.02	17.18±1.03^{b}	40.35	2
Native (tebuconazo l + trifloxystrobin)	0.600	0.0600+0.0300	126.55±34.63	11.31±3.55^{b}	60.73	2
Lannate BR (methomyl)	1.000	0.1075	90.70±38.25	0.00±0.00^{c}	100.0	4
Biossay V						
Distilled water (negative control)	-	-	188.49±12.20^{ns}	22.89±1.95^{a}	-	-
Folicur 200 EC (tebuconazole)	0.750	0.0750	180.93±18.12	5.97±0.74^{b}	77.77	2
Impact 125 SC (flutriafole)	1.000	0.0625	213.71±6.79	2.77±0.65^{bc}	89.71	3
Opera (pyraclostrobin + epoxiconazole)	0.600	0.0150+0.0399	184.19±17.23	2.75±0.65^{bc}	89.75	3
Sphere Max (ciproconazole + trifloxystrobin)	0.200	0.0160+0.0375	191.49±13.71	3.71±0.79^{bc}	86.18	3
Lannate BR (methomyl)	1.000	0.1075	188.49±12.20	0.00±0.00^{c}	100.0	4
Bioassay VI						
Distilled water (negative control)	-	-	247.11±1.04^{ns}	26.45±0.70^{a}	-	-
Domark XL (tetraconazol + azoxystrobin) *	0.600	0.0240+0.0300	245.16±11.43	15.34±3.54^{ab}	42.00	2
Domark 100 EC (tetraconazol)	0.500	0.0250	244.30±15.77	16.85±0.67^{b}	36.29	2
Kummulus DF (sulfur)	2.500	1.0000	244.26±2.52	0.00±0.00^{c}	100.0	4
Support WG (thiophanate - methyl)	0.700	0.2250	238.00±7.29	13.40±6.15^{ab}	49.34	2
Lannate BR (methomyl)	1.000	0.1075	244.31±16.12	0.00±0.00^{c}	100.0	4

[1]Commercial dosage (L ou kg.ha^{-1}) to soybean crop; [2]Concentration (%) of active ingredient in the syrup used in the bioassays; [3]Means followed by identical letters do not differ significantly (p> 0.05) by the Tukey test, [4]RP = Reduction parasitism compared with the negative control (distilled water) used in the bioassays; [5]IOBC classes, 1-Harmless (PR <30%), 2-Slightly harmful (30 <PR <79 %), 3-Moderately harmful (80 <PR <99%),4-Harmful (RP > 99%).*This fungicide was used in two bioassays, due to the ability of the operating system installed in the lab (6Tx4R).

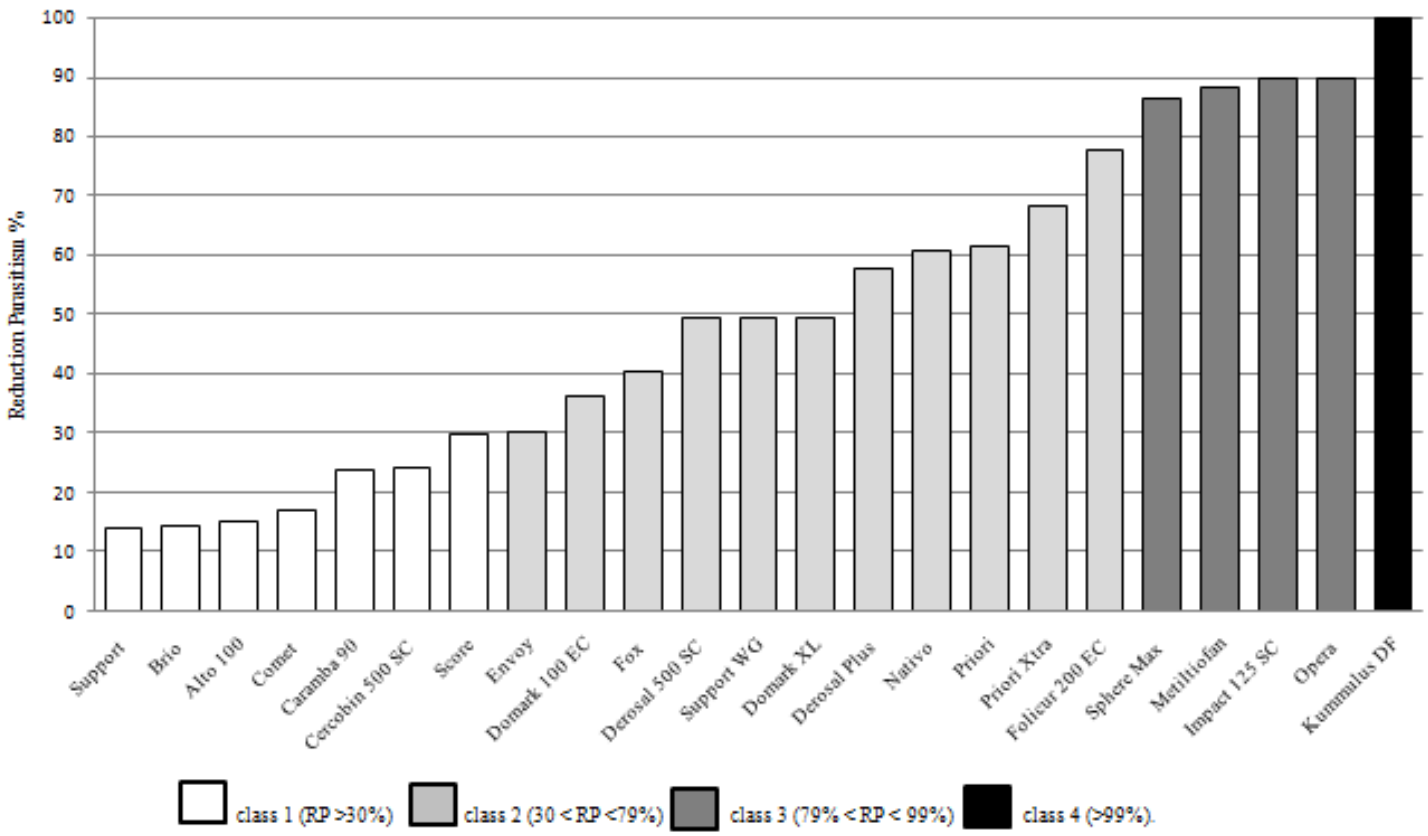

Figure 1. Classification of fungicides tested in bioassays selectivity for the soybean crop to *Trichogrammapretiosum* in accordance with the parameters of IOBC.

Xtra is associated with triazoles (Table 1). Abdelgadder and Hassan (2002) evaluating the product Amistar 250 SC (azoxystrobin) was identified by the same selectivity class (Class 2) for *T. cacoeciea* adults, using 6.4 µcm^{-2} of the commercial product. These results show that sometimes the association between two active products not prevent on selectivity class. With fungicides flutriafol, thiophanate methyl (WP), epoxyconazole + pyraclostrobin, and cyproconazole + trifloxystrobin, they were classified as moderately harmful (Class 3) to the egg parasitoids of *T. pretiosum* (Table 2), for what was used in the tests accounted for 17.39% of the fungicides tested (Figure 1).

The groups of benzimidazole fungicides all have the active ingredient methyl thiophanate, where reductions observed in parasitism ranged from 14.22 to 88.06% (Table 2). The products Support and Cercobim 500 SC (Table 2, Bioassay II) are considered harmless (Class 1) to the parasitoid and both have suspension concentrate (SC) formulationwhich differentiates them from fungicide Support WG (granules dispersed in water), (Table 2, Bioassay VI), in which classified them as slightly harmful (Class 2), a 49.34% decrease in parasitism. For fungicide Metiltiofan (Tab. 2, Bioassay III), it presented a wet table powder formulation with a parasitism reduction of 88.06% (Class 3). Since the active ingredient(s), inert ingredient(s), dosage, and other factors change, they can also influence the behavior and activity of the parasitoid, even to tolerable substances (Carmo et al., 2009).

The commercial product Kumulus DF was considered harmful (Class 4) to *T. pretiosum*, corresponding to 4.35% of the tested fungicide and providing 100% reduction in parasitism (Table 2, Bioassay VI). Manzoni et al. (2006b) and Grützmacher et al. (2004) observed the same results for Class 4 products on *T. pretiosum*and *T. cacoeciae*adults. Previously Reis et al. (2007) reported the toxic action of the fungicideKumulus DF was due to its interference in many biochemical processes, since sulfur can form chelates with heavy metals that inhibit breathing through the respiratory tract.

Formerly,Kissmann (1998) reported that each company develops its own formulations in ways more convenient for them, and therefore, commercial products with the same type of formulation from two different companies, may differ in their physical characteristics and inert ingredients. This reinforcedthe importance ofinformation of commercial products used in the bioassay when dealing with selectivity studies as proposed by Hassan et al. (2000).

According to the guidelines of IOBC, fungicides

classified as non-safe (Class 2,3, and/or 4) should be tested in their immature stage, where the parasitoid finds lower susceptibility to pesticide exposure due to the protection provided by the egg chorion (Orr et al., 1989), which was carried out in soybeans by Bueno et al. (2008) and Carmo et al. (2009).

Toxicity tests for the insects in the laboratory were subjected to maximum exposure to fungicide residues and constituted the first step of the test sequence recommended by IOBC (Hassan, 1998; Hassan et al., 2000; Hassan and Abdelgader, 2001). The fungicides in Class 1 are no longer to be tested as selective for parasitoids, however, the results obtained in Class 2, 3, and 4 (non-safe) are not to be extrapolated to field conditions. Therefore, the fungicides will be required to be tested in a laboratory greenhouse, with immature forms of the parasitoids eggs, larvas, and pupas, to evaluate the persistence of biological activity necessary to determine the impact against natural enemies in the field.

Conclusions

The results showed that fungicides may affect negatively the parasitoid *T. pretiosum.* Fungicides cyproconazole, epoxyconazole + kresoxim-methyl, metconazole, thiophanatemethyl (CS), pyraclostrobin, difenoconazole, were classified as harmless (Class 1); carbendazim, carbendazim + thiram, tetraconazole, tetraconazole+ azoxystrobin, epoxyconazol + pyraclostrobin, tebuconazole, prothioconazole + trifloxystrobin, tebuconazole + trifloxystrobin, azoxystrobin, azoxystrobin + cyproconazole, and thiophanate methyl (WG) were slightly harmful (Class 2); flutriafol, thiophanate methyl (WP), epoxyconazole + pyraclostrobin, and cyproconazole + trifloxystrobin were moderately harmful (Class 3); and Kumulus DF was harmful (Class 4) to the adulthood parasitoids.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The Coordination for the Improvement of Higher Education (CAPES) by Masters Scholarship, granted to the first author; the Financer of Studies and Projects (FINEP); the Research Foundation of the State of Rio Grande do Sul (FAPERGS); and the National Council for Scientific and Technological Development (CNPq), for the financial resources granted for the implementation of this research.

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