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Notes and Comments

Nicosulfuron's selectivity on Trichogrammatidae (Hymenoptera) in free-choice tests

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Chemical control is the most used weed control practice due to its high efficiency and usability (Monteiro and Santos, 2022). Several herbicides, such as nicosulfuron, are licensed for maize crops in Brazil. Nicosulfuron is recommended for post-emergence control and belongs to the sulfonylurea group, inhibiting the acetolactate synthase (ALS) and suppressing plant growth.

Pests also reduce maize production by damaging plants and seeds in the field and seeds in storage facilities (Leite et al., 2017). Cost-effective, efficient, and sustainable control tactics, such as biological control using parasitoids are essential to manage pests and reduce pesticide use (Huang et al., 2020; Santos et al., 2022). *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) are the most used parasitoids in biological control programs worldwide due to their efficiency in controlling Lepidoptera, wide geographical distribution, and low production cost.

Herbicide use may reduce the efficiency, survival, and development of parasitoids. Thus, selective products must be used to mitigate adverse effects on non-target organisms and natural enemies used in integrated pest management programs (Leite et al., 2021). The aim was to check the impacts of nicosulfuron on the parasitism rate, the emergence of females, and the sex ratio of ten Trichogrammatidae species, in free-choice tests.

Ten species of Trichogrammatidae were evaluated: *Trichogramma acacioi* Brun, Moraes & Soares, *T. atopovirilia* Oatman & Platner, *T. bennetti* Nagaraja & Nagarkatti, *T. brasiliensis* Ashmead, *T. brunni* Nagaraja, *T. demoraesi* Nagaraja, *T. galloi* Zucchi, *T. pretiosum* Riley, *T. soaresi* Nagaraja, and *Trichogrammatoidea annulata* de Santis. They were obtained from the George W. G. de Moraes insectary at the campus of the "Universidade Federal de Minas Gerais" (ICA/UFMG), Montes Claros, Brazil. The insects were reared on eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) at 25 ± 4°C, and 12:12 h (L:D).

Fresh UV-sterilized *A. kuehniella* eggs were collected, washed, and glued onto paper strips to make egg cards. The egg cards (0.5 x 5 cm, about 45 eggs) were sprayed with nicosulfuron (Sanson® 40 SC, ISK Biosciences), a systemic selective herbicide for post-emergence application, at a dose of 1.5 L ha^{-1}, at a concentration of 0.60 kg a.i. ha^{-1}, with the volume corresponding to 200 L ha^{-1} from the portable spray (0.06 mL/cm^2 commercial product or 0.03 mg/cm^2 a.i. per carton) (treatment) or with distilled water (control) (Leite et al., 2017). The cards were air dried at room temperature for 1 h after spraying. Then, two egg cards (with and without herbicide) was placed into glass tubes (9.0 x 1.0 cm), into which a young mated female parasitoid (<24 h) was allowed to parasitize until it died. The tubes were covered with plastic film and kept in a room under the same lab conditions used for rearing the Trichogrammatidae.

The parasitism rate (dark eggs were considered parasitized), the number of emerged males and females, and the sex ratio were evaluated. The toxicity of nicosulfuron was classified based on the percentage reduction of parasitized eggs and emerged females, and the sex ratio, being: 1 = harmless (<30%); 2 = slightly harmful (30-79%); 3 = moderately harmful (80-99%); and 4 = harmful (> 99% reduction), according to the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) classification of side effects to beneficial organisms. Parasitism rate and the reduction in the emergence of females were calculated with the equation: % reduction = 100 - mean [(% mean of the treatment / % mean of the control) x 100] (Manzoni et al., 2007). A factorial design of 2×10 (herbicide × *Trichogramma* species) with ten replicates was used, with 10 cards without and 10 with herbicide per replicate. Data were submitted to analysis of variance (ANOVA) and Tukey's test, both at $P \leq 0.05$ using SAEG Version 9.1 software.

Parasitism rate of *T. acacioi*, *T. annulata*, *T. bennetti*, *T. demoraesi*, and *T. pretiosum* was reduced in eggs treated with nicosulfuron. However, no effect ($P > 0.05$) was observed on the parasitism of *T. atopovirilia*, *T. brunni*, *T. brasiliensis*, *T. galloi*, and *T. soaresi*. Nicosulfuron was harmless (class I) to parasitism rates of *T. atopovirilia*, *T. brunni*, *T. brasiliensis*, and *T. galloi*; slightly harmful (class II) to *T. acacioi*, *T. annulata*, *T. demoraesi*, and

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T. soaresi; and moderately harmful (class III) to *T. bennetti* and *T. pretiosum* (Table 1).

Nicosulfuron reduced the female emergence of *T. annulata*, *T. atopovirilia*, *T. bennetti*, *T. demoraesi*, *T. pretiosum*, and *T. soaresi*. But it did not affect the emergence of *T. acacioi*, *T. brunni*, *T. brasiliensis*, and *T. galloi* females (*P*> 0.05). Nicosulfuron effect on female emergence was harmless to *T. acacioi*, *T. brunni*, *T. brasiliensis*, and *T. galloi*; slightly harmful to *T. annulata*, *T. atopovirilia*, *T. bennetti*, *T. demoraesi*, and *T. soaresi*; and moderately harmful to *T. pretiosum* (Table 1).

The number of females on the sex ratio of *T. annulata*, *T. bennetti*, *T. demoraesi*, *T. pretiosum*, and *T. soaresi* decreased on eggs treated with nicossulfuron. The sex ratio of *T. acacioi*, *T. atopovirilia*, *T. brunni*, *T. brasiliensis*, and *T. galloi* was not affected (*P*> 0.05). Nicosulfuron effect on sex ratio was harmless to *T. acacioi*, *T. atopovirilia*, *T. brunni*, *T. brasiliensis*, and *T. galloi*; slightly harmful to *T. annulata*, *T. demoraesi*, and *T. soaresi*; and moderately harmful to *T. bennetti* and *T. pretiosum* (Table 1).

The herbicide nicosulfuron reduced the parasitism rates, the emergence of females, and the sex ratio of some Trichogrammatidae species. In no-choice tests with nicosulfuron, the emergence of *T. brunni* females, and *T. galloi*, *T. bennetti*, and *T. pretiosum* sex ratios were also reduced (Leite et al., 2017).

Nicosulfuron reduced the parasitism rates of *T. acacioi*, *T. annulata*, *T. bennetti*, *T. demoraesi*, and *T. pretiosum*.

Table 1. Parasitism rates, female emergence, sex ratio, reduction (%), and IOBC classification of side effects to beneficial organisms to *Trichogrammatoidea annulata* (Hymenoptera: Trichogrammatidae), and nine *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) treated with nicosulfuron and distilled water (control).

Species	Nicosulfuron		Control		Reduc.	Class.	ANOVA (*df* = 9)	
	Mean	SE	Mean	SE			*F*	*P*
Parasitism								
*T. acacioi**	29.11b	5.54	69.63a	4.70	58.2	II	29.637	0.00041
*Tr. annulata**	17.33b	8.64	58.27a	3.24	70.3	II	22.176	0.00111
T. atopovirilia n.s.	61.33a	11.52	39.51a	2.33	-55.2	I	3.092	0.11252
*T. bennetti**	4.19b	2.87	55.56a	2.45	92.5	III	169.651	0.00000
T. brunni n.s.	42.22a	9.53	59.68a	5.05	29.3	I	2.717	0.13365
T. brasiliensis n.s.	58.89a	7.53	67.50a	3.62	12.8	I	0.930	***
*T. demoraesi***	21.11b	7.98	48.40a	8.24	56.4	II	6.199	0.03444
T. galloi n.s.	40.22a	10.06	39.85a	5.13	-0.9	I	0.001	***
*T. pretiosum**	6.89b	4.59	38.52a	2.74	82.1	III	23.112	0.00096
T. soaresi n.s.	26.22a	11.47	44.44a	3.19	41.0	II	2.171	0.17474
Female Emergence								
T. acacioi n.s.	63.83a	11.60	81.95a	2.29	22.1	I	2.146	0.17695
*Tr. annulata**	23.36b	9.63	82.54a	3.84	71.7	II	28.450	0.00047
*T. atopovirilia***	43.47b	8.63	72.84a	5.27	40.3	II	6.783	0.02853
*T. bennetti**	19.17b	12.79	92.49a	1.63	79.3	II	29.063	0.00044
T. brunni n.s.	70.00a	15.27	98.33a	0.78	28.8	I	3.398	0.09839
T. brasiliensis n.s.	80.90a	9.75	90.33a	1.01	10.4	I	1.014	0.34025
*T. demoraesi**	27.75b	9.33	93.24a	2.53	70.2	II	48.867	0.00006
T. galloi n.s.	67.06a	14.67	62.22a	4.81	-7.8	I	0.094	h.ns
*T. pretiosum**	20.00b	13.33	100.00a	0.00	80.0	III	36.000	0.00020
*T. soaresi**	18.48b	10.41	83.17a	3.01	77.8	II	56.122	0.00004
Sex Ratio								
T. acacioi n.s.	0.63a	0.10	0.85a	0.02	25.9	I	3.720	0.08584
*Tr. annulata**	0.40b	0.16	1.00a	0.00	60.0	II	13.500	0.00512
*T. atopovirilia*n.s.	0.74a	0.12	0.80a	0.04	7.5	I	0.176	h.ns
*T. bennetti**	0.20b	0.13	1.00a	0.00	80.0	III	36.000	0.00020
T. brunni n.s.	0.70a	0.15	0.99a	0.01	29.3	I	3.463	0.09569
T. brasiliensis n.s.	0.90a	0.10	1.00a	0.00	10.0	I	1.000	h.ns
*T. demoraesi***	0.50b	0.16	1.00a	0.00	50.0	II	9.000	0.01496
T. galloi n.s.	0.70a	0.15	0.91a	0.02	23.1	I	1.797	0.21298
*T. pretiosum**	0.20b	0.13	1.00a	0.00	80.0	III	36.000	0.00020
*T. soaresi**	0.30b	0.15	1.00a	0.00	70.0	II	21.000	0.00132

Reduc. = reduction; IOBC = International Organization for Biological and Integrated Control of Noxious Animals and Plants Classification; h.ns = highly non-significant. Toxicity index: class I = harmless (<30%), class II = slightly harmful (30-79%), class III = moderately harmful (80-99%) and class IV = harmful (> 99% of reduction). The averages followed by the same lowercase letter in the row do not differ by Tukey's test.
*P <0.01. **P <0.05.
n.s.Not significant by ANOVA (P> 0.05).

Nicosulfuron could be toxic to the adult parasitoid, as observed with *Trichogramma ostriniae* (Pang and Chen) and *Trichogramma dendrolimi* (Matsumura) (Xu et al., 2013). But, nicosulfuron did not change the parasitism rates of *T. atopovirilia*, *T. brunni*, *T. brasiliensis*, *T. galloi*, and *T. soaresi*, possibly, due to a higher tolerance of the adult insects. Instead, nicosulfuron provided *T. atopovirilia* an increase of approximately 55% on parasitism rate. The use of herbicides may improve parasitism rates in some parasitoids, as observed with *Anagrus nilaparvatae* Pang & Wang (Hymenoptera: Mymaridae) on *Chilo suppressalis* Walker (Lepidoptera: Crambidae) eggs treated with 2,4-D (Xin et al., 2012). Nicosulfuron was considered harmless (class I) for *T. atopovirilia*, *T. brunni*, *T. brasiliensis*, and *T. galloi* on the IOBC classification on parasitism rates. The herbicides clomazone and diuron + hexazinone, and the plant growth regulators sulfometuronmethyl and trinexapac-ethyl were also considered harmless to *T. galloi,* in free-choice tests (Antigo et al., 2016). This shows that *T. galloi* is a good candidate for pest management when using herbicide spraying on the crops. Nicosulfuron was moderately harmful (class III) to *T. pretiosum*, but in other evaluations was considered slightly harmful (class II) (Stefanello Júnior et al., 2008). This variation within the same species is due to greater genetic adaptability between strains.

The percentage of emerged females of *T. atopovirilia*, *T. bennetti*, *T. demoraesi*, *T. soaresi*, *T. annulata*, and *T. pretiosum* was reduced with nicosulfuron. But, in no-choice tests, nicosulfuron did not reduce the percentage of emerging females of the first four species cited and increased it in *T. annulata* and *T. pretiosum* (Leite et al., 2017). The bacteria *Wolbachia* sp. on these species may have been suppressed when exposed to nicosulfuron, generating more males than females. These bacteria are responsible for inducing thelytoky, non-fertile eggs that develop into females (Russell et al., 2018). Nicosulfuron did not affect female emergence rates in *T. acacioi*, *T. brunni*, and *T. brasiliensis*. *Trichogramma galloi* increased (≈ 8%) the female emergence, possibly caused by the hormesis phenomenon, a stimulatory effect in an organism caused by exposure to low doses of a chemical (Gowda et al., 2021).

Nicosulfuron did not affect the sex ratios of *T. acacioi*, *T. atopovirilia*, *T. brunni*, *T. brasiliensis*, and *T. galloi*, probably, due to their capacity for detoxification (Leite et al., 2017).

The most negatively affected species by the use of nicosulfuron were *T. annulata*, *T. bennetti*, *T. demoraesi*, and *T. pretiosum*. The parasitism, the emergence of females, and the sex ratio of these species were reduced when exposed to nicosulfuron. Thus, releasing these parasitoids into maize fields when sprayed with nicosulfuron is not recommended. But, nicosulfuron does not affect those parameters of *T. brunni*, *T. brasiliensis* and *T. galloi*. These species could be released simultaneously with the nicosulfuron spraying in maize crops.

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