ENTOMOLOGICAL Communications

Entomological Communications, 4, 2022: ec04007
doi: 10.37486/2675-1305.ec04007
e-ISSN: 2675-1305
Open Access Full Text Article

Bioassay

Lethal effects of insecticides on adults of *Eriopis connexa* (Germar, 1824) (Coleoptera: Coccinellidae)

Rafael A. Pasini[1], Matheus Rakes[2], Franciele S. De Armas[2], Juliano de B. Pazini[3], Anderson D. Grützmacher[2]

[1]Centro de Ensino Superior Riograndense, Sarandi, Rio Grande do Sul, Brazil. [2]Federal University of Pelotas (UFPel), Pelotas, Rio Grande do Sul, Brazil. [3]University of São Paulo Luiz de Queiroz College of Agriculture, Piracicaba, São Paulo, Brazil.
Corresponding author: rafa.pasini@yahoo.com.br

Edited by: Rafael M. Pitta

Received: December 02, 2021. Accepted: February 23, 2022. Published: March 11, 2022.

Abstract. The predator *Eriopis connexa* (Germar, 1824) (Coleoptera: Coccinelidae), is an important biological control agent in wheat fields. In this work, we evaluated the lethal effects of fifteen insecticides used in the wheat crop on adults of the predator *E. connexa*, using the methodology proposed by International Organization for Biological and Integrated Control (IOBC). Sublethal effects have not been evaluated. The insecticides were sprayed on glass plates that were used to set up the exposure arenas. The chitin synthesis inhibitors diflubenzuron SC, diflubenzuron WP, lufenuron, triflumuron, and the pyrethroid beta-cyfluthrin were considered harmless to adults of the predator and should have their use prioritized. On the other hand, methomyl, lambda-cyhalothrin, thiamethoxam + lambda-cyhalothrin A and B were considered harmful to *E. connexa* and should be avoided when adults of the predator are present in the crop.

Keywords: Ladybug, biological control, chemical control, selectivity, *Triticum aestivum*.

Wheat is an important cereal used worldwide for human and animal nutrition. However, insect pests are among the factors that can reduce crop yield. Wheat is attacked by several insects such as the aphid complex (*Rhopalosiphum padi* Linnaeus, 1758; *Metopolophium dirhodum* Walker, 1849; *Sitobion avenae* Fabricius, 1775; and *Schizaphis graminum* Rondani, 1852 - Hemiptera: Aphididae), stink bugs (*Diceraeus furcatus* Fabricius, 1775 and *Diceraeus melacanthus* Dallas, 1851 - Hemiptera: Pentatomidae), and some larvae (*Mythimna adultera* Schaus, 1894; *Mythimna sequax* Franclemont, 1951 and *Spodoptera frugiperda* Smith, 1797 - Lepidoptera: Noctuidae) (Kuhnem et al. 2020).

Chemical control with broad-spectrum insecticides is the most used method to control these insect pests. Despite the proven efficiency of this method, the use of these products is associated with several negative effects in the agroecosystem, such as selection of resistant individuals and the resurgence of secondary pests. Also, the indiscriminate use of insecticides can impair the biological control of natural enemies (Pasini et al. 2021).

The predator *Eriopis connexa* (Germar, 1824) (Coleoptera: Coccinellidae) is an important biological control agent in wheat fields, that can feed on a large number of insect pests, especially the aphid complex that occurs in this crop. This ladybug has high biotic potential and polyphagia. Both larvae and adults can feed on aphids, aleyrodids, mealybugs, mites, insect eggs, and larvae of lepidopterans and coleopterans (Santos et al. 2020).

To achieve success in an Integrated Pest Management (IPM) program, it is essential to integrate chemical and biological control through the use of selective pesticides. Thus, our study aimed to evaluate the lethal effects of insecticides registered to control insect pests in the wheat crop on adults of *E. connexa*, using the standards proposed by the International Organization for Biological and Integrated Control (IOBC).

Adults of *E. connexa* used in the bioassays came from a colony kept in the Integrated Pest Management Laboratory of the Federal University of Pelotas (LabMIP/UFPel, RS, Brazil). The coccinellid population was collected in wheat fields from the municipality of Rondinha, RS (27°48'S and 52°54'W) and was reared in laboratory conditions (temperature 25 ± 1 °C, RH 70 ± 10%, photophase 14 h). Larvae of *E. connexa* were reared with *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) eggs *ad libitum* until the adult stage. Adults were separated into couples and kept in disposable plastic pots (9 cm high × 12 cm in diameter) and fed with *E. kuehniella* eggs and honey *ad libitum*.

Fifteen insecticides registered for wheat (Brasil 2021) were selected for the bioassays, as follows; insecticide [chemical group/active ingredient (maximum dosage recorded for wheat in L ha^{-1})]: Actara 250 WG [neonicotinoid/thiamethoxam (0.075)]: Certero [benzoylurea/triflumuron (0.030)]; Karate Zeon 50 CS [pyrethroid/lambda-cyhalothrin (0.10)]; Connect [organophosphate+pyrethroid/imidacloprid+beta-cyfluthrin (0.75)]; Difluchen 240 SC [benzoylurea/diflubenzuron (0.10)]; Dimilin [benzoylurea/diflubenzuron (0.10)]; Eforia [neonicotinoid+pyrethroid/ thiamethoxam+lambda-cyhalothrin A (0.15)]; Engeo Pleno [neonicotinoid+pyrethroid/thiamethoxam+lambda-cyhalothrin B (0.15)]; Lannate BR [carbamate/methomyl (0.50)]; Match EC [benzoylurea/lufenuron (0.10)]; Mustang 350 EC [pyrethroid/zeta-Cypermethrin (0.15)]; Nexide [pyrethroid/gamma-cyhalothrin (0.15)]; Piredan [pyrethroid/permethrin (0.065)]; Safety [pyrethroid/etofenprox (0.50)]; Turbo [pyrethroid/beta-Cyfluthrin (0.10)] (Tab. 1). The selection was based on the widespread use of these products in the crop by farmers. The insecticides were tested in the maximum field recommended concentration (MFRC), adjusted to correspond to a spray volume of 200 $L.ha^{-1}$, using a CO_2 pressurized spray, with a uniform flat spray nozzle (Teejet XR110015EVS). The insecticides were sprayed on glass plates. The working pressure used in the spraying was approximately 50 psi, which corresponded to a spray deposit of 2 ± 0.2 $mg.cm^{-2}$ (Schmuck et al. 2000).

The bioassays were conducted in the laboratory using the methodology proposed by IOBC (Schmuck et al. 2000). Adults (males and females) of *E. connexa* were exposed to insecticide residues applied on glass plates. The glass plates served as a background and cover to produce the exposure cages. Each exposure cage was composed of a methacrylate ring (10 cm in diameter x 3 cm in height), with five holes of 1.3 cm in diameter closed with "voile" type fabric to allow ventilation. One hole was connected to a suction pump for the elimination of toxic gases. In another hole measuring 0.8 cm, we supplied water to the insects. Eggs of *E. kuehniella* were provided in strips of paper in sufficient quantity to perform the bioassay. We evaluated male and female mortality as well as total mortality and IOBC selectivity classification at 24, 72 and 120 hours after exposure to dry residues of insecticide.

For this purpose, adults previously separated by sex, with approximately one week old, were added to the exposure cages. We used a completely randomized experimental design. Each treatment consisted of four cages each containing five couples, each cage being considered a repetition in the completely randomized design. In addition to the insecticides tested, the control treatment consisted of the absence of insecticides. Accumulated mortality (number of dead insects) of males and females, as well as overall mortality, were assessed at 24, 72 and 120 hours after exposure to the insecticides.

Adult mortality was corrected by the Schneider-Orelli formula (Püntener 1981). The insecticides were classified according to their harmfulness effect on the adult mortality according to the scale proposed by IOBC, in 1) harmless (mortality <30%); 2) slightly harmful (mortality between 30-79%); 3) moderately harmful (mortality between 80-99%) and 4) harmful (mortality > 99%) (Sterk et al. 1999).

The data were submitted to the Shapiro-Wilk normality test and homogeneity of variances of Bartlett. When these assumptions were not met, Kruskal-Wallis non-parametric analysis of variance (ANOVA) was performed and the means were compared using the Dunn test, with the Bonferroni correction (p<0.05). The statistical software R 4.0.0 (R Development Core Team 2021) was used to perform the analyzes.

The chitin synthesis inhibitors diflubenzuron SC, diflubenzuron WP, lufenuron, and triflumuron showed mortality ranging from 0.00 to 0.50 (Tab. 1) to males and females; the pyrethroids beta-cyfluthrin and zeta-cypermethrin exhibited mortality rates of 0.00 to 2.25 (Tab. 1) of *E. connexa*. Due to these mortality values obtained, these insecticides can contribute to the maintenance of the population of the predator in wheat fields.

The insecticides diflubenzuron SC, diflubenzuron WP, lufenuron, and triflumuron were considered harmless (class 1) (Tab. 2) to adults of *E. connexa*. The selectivity of diflubenzuron SC and WP, lufenuron, and triflumuron is possibly associated with the mode of action of chitin biosynthesis inhibitors. Growth regulating insecticides affect immature stages of insects throughout the molting process and, as a result, adults of non-target species, such as predatory insects, are rarely affected (De Armas et al. 2020). However, despite their reduced lethal effect on adult mortality, these insecticides can negatively other life stages of the predator as eggs, pupae (Pasini et al. 2018) and larvae (Pasini et al. 2021).

The carbamate methomyl showed 100% of mortality after 24 hours of exposure (Tab. 1) and was considered harmful (class 4), while the neonicotinoid thiamethoxam had 95% mortality and was classified moderately harmful (class 3) for adults of *E. connexa* (Tab. 2).

The insecticide imidacloprid + beta-cyfluthrin showed a 95% mortality and was classified as moderately harmful (class 3), while thiamethoxam + lambda-cyhalothrin A and thiamethoxam + lambda-cyhalothrin B killed 100% of the ladybird adults and were classified as harmful (class 4) (Tab. 2).

The pyrethroid beta-cyfluthrin with 27.50% of accumulated adult mortality was considered harmless (class 1). Etofenprox and zeta-cypermethrin were considered slightly harmful (class 2) showing 52.50 and 35% of mortality, respectively. Permethrin and gamma-cyhalothrin presented, both, adult mortality of 92.50%, and were classified as moderately harmful (class 3). The insecticide lambda-cyhalothrin was classified as harmful (class 4) to adults of *E. connexa* with 100% of accumulated mortality (Tab. 2). The observed survival in beta-cyfluthrin, etofenprox, and zeta-cypermethrin (Tab. 2) is probably due to enzymatic detoxification or lack of sensitivity of the active site present in the organism of the predator because 24 hours after contact with the insecticide part of the insects were still alive. Pyrethroids contain ester bonds that are susceptible to inactivation by enzymes such as esterases and cytochrome P450 monooxygenases (Yu 2004).

Neurotoxic insecticides, such as pyrethroids, carbamates, and neonicotinoids, are generally classified as compounds that are less selective to predators because of the similarity in the mode

Table 1. Accumulated mortality (nº ± SE) of males and females of *Eriopis connexa* in 24, 72 and 120 hours of exposure to insecticides.

Treatment	**C.a.i.(%)[1]**	**M[2] [24 hours]**		**M[2] [72 hours]**		**M[2] [120 hours]**	
		♀	♂	♀	♂	♀	♂
control	---	0,00±0,00bA	0,00±0,00eA	0,00±0,00bA	0,00±0,00bA	0,00±0,00cdA	0,00±0,00eA
beta-cyfluthrin	0,002	0,00±0,00bB	0,25±0,25eB	0,50±0,28bB	1,00±0,40bA	1,25±0,25cA	1,50±0,28cdeA
diflubenzuron SC	0,012	0,25±0,25bA	0,50±0,50eA	0,25±0,25bA	0,50±0,50bA	0,25±0,25cdA	0,50±0,50deA
diflubenzuron WP	0,012	0,00±0,00bA	0,00±0,00eA	0,00±0,00bA	0,00±0,00bA	0,00±0,00dA	0,00±0,00eA
etofenprox	0,075	0,50±0,28bB	0,75±0,47deB	1,50±0,28bB	1,50±0,28bB	2,50±0,28bA	2,75±0,47bcA
gamma-cyhalothrin	0,001	4,25±0,25aA	4,75±0,25abA	4,50±0,50aA	4,75±0,25aA	4,50±0,50aA	4,75±0,25abA
imidacloprid + beta-cyfluthrin	0,037+0,004	3,25±0,75aA	3,50±0,64abA	4,25±0,25aA	4,75±0,25aA	4,50±0,28aA	5,00±0,00aA
lambda-cyhalothrin	0,002	5,00±0,00aA	5,00±0,00aA	5,00±0,00aA	5,00±0,00aA	5,00±0,00aA	5,00±0,00aA
lufenuron	0,002	0,00±0,00bA	0,00±0,00eA	0,00±0,00bA	0,25±0,25bA	0,00±0,00dA	0,25±0,25eA
methomyl	0,139	5,00±0,00aA	5,00±0,00aA	5,00±0,00aA	5,00±0,00aA	5,00±0,00aA	5,00±0,00aA
permethrin	0,012	3,00±0,40aB	3,25±0,25abcB	4,25±0,25aA	3,75±0,25aB	4,50±0,28aA	4,75±0,25aA
thiamethoxam	0,009	3,50±0,50aB	2,75±0,47bcdB	4,50±0,50aA	4,50±0,28aA	5,00±0,00aA	4,50±0,28abA
thiamethoxam + lambda-cyhalothrin A	0,010+ 0,007	3,75±0,25aA	4,00±0,70abA	5,00±0,00aA	5,00±0,00aA	5,00±0,00aA	5,00±0,00aA
thiamethoxam + lambda-cyhalothrin B	0,010+ 0,007	3,5±00,95aA	3,25±0,62abcA	4,00±0,57aA	4,25±0,47aA	5,00±0,00aA	5,00±0,00aA
triflumuron	0,007	0,00±0,00bA	0,00±0,00eA	0,00±0,00bA	0,00±0,00bA	0,00±0,00dA	0,00±0,00eA
zeta-cypermethrin	0,026	0,75±0,47bA	1,25±0,75cdeA	1,00±0,57bA	1,25±0,75aA	1,25±0,47cA	2,25±1,03cdA
X^2		**55.27**	**55.98**	**56.47**	**57.06**	**59.01**	**56.82**
df		**15, 48**	**15, 48**	**15, 48**	**15, 48**	**15, 48**	**15, 48**
p-value		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

[1]C.a.i. (%) = Concentration of active ingredient in the mixture; [2]Average values obtained from four repetitions with five couples each; Means followed by the same lowercase letter in the columns and uppercase in the lines for each evaluation period do not differ significantly from each other by the Dunn test with Bonferroni correction (p> 0.05).

of transmission of nerve impulses between the different orders of insects (Amarasekare et al. 2016). These insecticides can affect not only Coccinellids but also other important orders of predators such as Neuroptera (De Armas et al. 2020; Pasini et al. 2021) and Hemiptera (Machado et al. 2019; Morales et al. 2019).

Laboratory tests expose natural enemies to the worst-case scenario of insecticide residues. In our study, the growth regulators diflubenzuron SC, diflubenzuron WP, lufenuron, and triflumuron and the pyrethroid beta-cyfluthrin were considered harmless and should whenever possible, have their use prioritized when adults of *E. connexa* are present in the crop. Insecticides considered to be slightly harmful, moderately harmful, and harmful to adults of *E. connexa* should be avoided since show high levels of predator mortality. However, more research is needed to understand the lethal and also, sublethal effects of these pesticides mainly under field conditions, given the importance of this coccinellid for IPM in wheat.

Table 2. Accumulated mortality (nº ± SE) of *Eriopis connexa* adults exposed to insecticides and classification by the International Organization for Biological and Integrated Control (IOBC).

Treatment	C.a.i.(%)*	M[1] [24 hours]			M[1] [72 hours]			M[1] [120 hours]		
		nº ± EP[2]	%**	C***	nº ± EP[2]	%**	C***	nº ± EP[2]	%**	C***
control	---	0,25±0,25c	---	---	0,25±0,25bc	---	---	0,25±0,25cd	---	---
beta-cyfluthrin	0,002	0,25±0,25c	2,50	1	1,50±0,64bc	15,00	1	2,75±0,47bc	27,50	1
diflubenzuron SC	0,012	0,75±0,47c	7,50	1	0,75±0,47bc	7,50	1	0,75±0,47cd	7,50	1
diflubenzuron WP	0,012	0,00±0,00c	0,00	1	0,00±0,00c	0,00	1	0,00±0,00d	0,00	1
etofenprox	0,075	1,25±0,75c	12,50	1	3,00±0,57b	30,00	2	5,25±0,75b	52,50	2
gamma-cyhalothrin	0,001	9,00±1,00ab	90,00	3	9,25±0,75a	92,50	3	9,25±0,75a	92,50	3
imidacloprid + beta-cyfluthrin	0,037+ 0,004	6,75±0,47ab	67,50	2	9,00±0,40a	90,00	3	9,50±0,28a	95,00	3
lambda-cyhalothrin	0,002	10,00±0,00a	100,00	4	10,00±0,00a	100,00	4	10,00±0,00a	100,00	4
lufenuron	0,002	0,00±0,00c	0,00	1	0,25±0,25bc	2,50	1	0,25±0,25cd	2,50	1
methomyl	0,139	10,00±0,00a	100,00	4	10,00±0,00a	100,00	4	10,00±0,00a	100,00	4
permethrin	0,012	6,25±0,62ab	62,50	2	8,00±0,40a	80,00	3	9,25±0,25a	92,50	3
thiamethoxam	0,009	6,50±0,25ab	65,00	2	9,00±0,70a	90,00	3	9,50±0,28a	95,00	3
thiamethoxam + lambda-cyhalothrin A	0,010+ 0,007	7,75±0,62ab	77,50	2	10,00±0,00a	100,00	4	10,00±0,00a	100,00	4
thiamethoxam + lambda-cyhalothrin B	0,010+ 0,007	6,75±1,49ab	67,50	2	8,25±1,03a	82,50	3	10,00±0,00a	100,00	4
triflumuron	0,007	0,00±0,00c	0,00	1	0,00±0,00bc	0,00	1	0,00±0,00d	0,00	1
zeta-cypermethrin	0,026	2,00±1,15c	20,00	1	2,25±1,31bc	22,50	1	3,50±1,44b	35,00	2
X^2		**55.90**			**56.40**			**58.03**		
df		**15, 48**			**15, 48**			**15, 48**		
p-value		**<0.0001**			**<0.0001**			**<0.0001**		

*C.a.i. (%) = Concentration of active ingredient in the mixture; ** Mortality corrected by Schneider-Orelli; *** C = IOBC Classes, 1 = harmless (<30%), 2 = slightly harmful (30-79%), 3 = moderately harmful (80-99%), 4 = harmful (> 99%); [1]Average value obtained from four repetitions with five couples each; [2]Means followed by the same letter in the columns, do not differ significantly from each other by the Dunn test with Bonferroni correction (p>0,05).

Acknowledgments

This research was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Authors' Contributions

RAP planned, designed, executed experimental work, conducted data analyses, and wrote the manuscript. FSA, JBP, MR executed experimental work and helped with data analyses. ADG wrote the manuscript and secured funding. All authors read and approved the manuscript.

Conflict of Interest Statement

The authors declare no potential conflict of interest.

References

Amarasekare, K. G.; Shearer, P. W.; Mills, N. J. (2016) Testing the selectivity of pesticides effects on natural enemies in laboratory biossays. *Biological Control*, 102: 7-16. doi: 10.1016/j.biocontrol.2015.10.015

Brasil (2021) Ministério da Agricultura, Pecuária e Abastecimento. Sistema de Agrotóxicos Fitossanitários (Agrofit). Brasília: Ministério da Agricultura, Pecuária e Abastecimento. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Access on: 2.x.2021.

De Armas, F. S.; Grützmacher, A. D.; Nava, D. E.; Pasini, R. A.; Rakes, M., Pazini, J. B. (2020) Non-target toxicity of nine agrochemicals toward larvae and adults of two generalist predators active in peach orchards. *Ecotoxicology*, 29: 327-339. doi: 10.1007/s10646-020-02177-5

Kuhnem, P., Rosa, A. C., Wagner, F., Rosa, A. T. S. (2020) *Informações técnicas para trigo e triticale - safra 2020*. Passo Fundo: Biotrigo Genética.

Machado, A. V. A.; Potin, D. M.; Torres, J. B.; Silva Torres, C. S. A. (2019)

Selective insecticides secure natural enemies action in cotton pest management. *Ecotoxicology and Environmental Safety*, 184: 109669. doi: 10.1016/j.ecoenv.2019.109669

Morales, S. I.; Martínez, A. M.; Figueroa, J. I.; Campos-García, J.; Gómez-Tagle, A.; Lobit, P.; Smagghe, G.; Pineda, S. (2019) Foliar persistence and residual activity of four insecticides of different mode of action on the predator *Engytatus varians* (Hemiptera: Miridae). *Chemosphere*, 235: 76-83. doi: 10.1016/j.chemosphere.2019.06.163

Pasini, R. A.; Grützmacher, A. D.; Pazini, J. B.; Armas, F. S.; Bueno, F. A.; Pires, S. N. (2018) Side effects of insecticides used in wheat crop on eggs and pupae of *Chrysoperla externa* and *Eriopis connexa*. *Phytoparasitica*, 46: 115-125. doi: 10.1007/s12600-018-0639-9

Pasini, R. A.; Rakes, M.; Castilhos, R. V.; Armas, F. S.; Pazini, J. B.; Grützmacher, A. D. (2021) Residual action of five insecticides on larvae and adults of the neotropical predators *Chrysoperla externa* (Neuroptera: Chrysopidae) and *Eriopis connexa* (Coleoptera: Coccinellidae). *Ecotoxicology*, 30: 44-56. doi: 10.1007/s10646-020-02314-0

Püntener, W. (1981) *Manual for field trials in plant protection*. Basle, Ciba-Geigy.

R Development Core Team (2021) R - A language and environment for statistical computing. Vienna, Austria. http://www.r-project.org/ Access on: 10.i.2021.

Santos, D. S.; Trindade, R. C. P.; Torres, J. B.; Lima, M. S.; Santos, L.; Batista, F. C. (2020) Predation of *Brevicoryne brassicae* and *Aphis craccivora* by *Eriopis connexa* depending on availability. *Acta Biológica Colombiana*, 6(1): 99-104. doi: 10.15446/abc.v26n1.83303

Schmuck, R.; Candolfi, M. P.; Kleiner, R.; Mead-Briggs, M.; Moll, M.; Kemmeter F.; Jans, D.; Waltersdorfer A.; Wilhelmy, H. A. (2000) Laboratory test system for assessing effects of plant protection products on the plant dwelling insect *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). In: Candolfi, M.P.; Blumel, S.; Forster, R.; Bakker, F. M.; Grimm, C.; Hassan, S. A.; Heimbach, U.; Mead-Briggs, M.A.; Reber, B.; Schmuck, R.; Vogt, H. (Eds.), *Guidelines to evaluate side-effects of plant protection products to non-target arthropods*. pp. 45-56. Reinheim: IOBC/ WPRS.

Sterk, G.; Hassan, S. A.; Baillod, M.; Bakker, F.; Bigler, F.; Blume, S.; Bogenschutz, H.; Boller, E.; Bromand. B.; Brun, J., et al. (1999) Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms'. *Biocontrol*, 44: 99-117. doi: 10.1023/A:1009959009802

Yu, S. J. (2004) Induction of detoxification enzymes by triazine herbicides in the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). *Pesticide Biochemistry and Physiology*, 80(2): 113-122. doi: 10.1016/j.pestbp.2004.06.005