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Selectivity of phytosanitary products used in organic farming on adult of *Cryptolaemus montrouzieri* (Coleoptera, Coccinellidae) under laboratory conditions

Seletividade de produtos fitossanitários, usados no sistema de produção orgânica, sobre adultos de *Cryptolaemus montrouzieri* (Coleoptera, Coccinellidae), em laboratório

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Abstract

The association of pesticides with biological control is possible only if they provide some selectivity to natural enemies. In the organic production system the effect of insecticides on beneficial insects has not been extensively studied. Thus, this study aimed to evaluate in laboratory conditions the effect of pesticides used in this system on the adults of the predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera, Coccinellidae). We used four treatments, which corresponded to four multiple values (0.25x, 0.5x, 2x and 1x) of the concentration recommended by manufacturers of the following products: Rotenat CE®, Pironat®, Biopirol 7M®, Organic Neem®, Natuneem® and lime sulfur, which were tested in *C. montrouzieri* by topical application and residual exposure. None of the products, at any concentrations tested, had a significant effect on the survival of adults of *C. montrouzieri*, what did not differ from the distilled water control, showing that the products tested are selective to the adults of this species. However, other forms of exposure and evaluation methods must be studied.
Key words: Predator, beneficial insect, rotenone, lime sulfur, neem, pyroligneous extract

Resumo

A associação do controle biológico com produtos fitossanitários só é possível se estes apresentarem alguma seletividade aos inimigos naturais. No sistema de produção orgânica, o efeito de inseticidas sobre insetos benéficos não tem sido extensivamente estudado. Assim, este trabalho objetivou avaliar em laboratório o efeito de produtos fitossanitários utilizados neste sistema, sobre adultos do predador *Cryptolaemus montrouzieri* Mulsant (Coleoptera, Coccinellidae). Foram utilizados quatro tratamentos, os quais corresponderam quatro múltiplos (0,25x, 0,5x, 1x e 2x) da concentração recomendada pelos fabricantes dos seguintes produtos comerciais: Rotenat CE®, Pironat®, Biopirol 7M®, Organic neem®, Natuneem® e calda sulfocálcica, que foram testados sobre *C. montrouzieri* por aplicação tópica e exposição a resíduos. Nenhum dos produtos, em qualquer das concentrações testadas, apresentou efeito significativo sobre a sobrevivência de adultos de *C. montrouzieri,* não diferindo da testemunha água destilada, demonstrando

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que os produtos testados são seletivos aos adultos dessa espécie.
Palavras-chave: Predador, inseto benéfico, rotenona, calda sulfocálcica, nim, extrato pirolenhoso

Introduction

In organic production systems, although it is recognized the increase of the family income and the reduction in the amount of negative impacts to the environment (MAGANO et al., 2003), there is still a lack of appropriate technologies for the management of pests and diseases. Phytosanitary treatments usually applied to pests control have a side effects on beneficial insects such as pollinators and natural enemies, mainly due to the most common form of contamination, which is the contact with residual products. Generally, these insects have a larger contact surface in relation to their body volume, are quite mobile, depend on their hosts and have enzyme systems that are less complex (CROFT, 1990).

Thus, the use of phytosanitary products should be made only if these products present some selectivity for beneficial fauna, such as natural enemies (RIPPER; GREENSLADE; HARTLEY, 1951; CROFT, 1990; RIGITANO; CARVALHO, 2001). However, very little has been done in order to study the effects of such chemicals on beneficial organisms (TEDESCHI; ALMA; TAVELLA, 2001; ULRICHS; MEWIS; SCHNITZLER, 2001; ROCHA, 2008).

Cryptolaemus montrouzieri Mulsant (Coleoptera, Coccinellidae), a native species from Australia, has been used commercially in many programs of classical biological control of several species of scale insects and aphids in the world (SANCHES; SILVA; CARVALHO, 2000). According to Babu and Azam (1987a), this ladybug is a generalist predator that feeds on a wide range of insect species. It is considered the most important agent to control the citrus mealybug *Planoccocus citri* (Risso) (Hemiptera, Pseudococcidae), particularly in citrus (BARTLETT; LLOYD, 1958). In Brazil, in 1973, there was the first introduction of *C. montrouzieri* to control *Dysmicoccus* sp. (Hemiptera: Pseudococcidae) in pineapple, but with no successes in the establishment in the new habitat (BERTI FILHO; MENEZES; MORAES, 1973). In 1998, it was introduced again, with the goal of establishing this predator in commercial citrus orchards and other tropical fruit to control scale insects and aphids, with success particularly in control of *Planococcus citri* (Hemiptera: Pseudococcidae) (NARDO et al., 1999).

There are few studies related to the effect of phytosanitary products on *C. montrouzieri*. However, in these studies it was clear the tolerance of different species to distinct products (BELLOWS et al. 1985; BABU; AZAM, 1987b; SIMMONDS et al., 2000; SMITH; KRISCHIK, 2000; ROSSINI et al., 2003; BOYERO et al., 2005; ROCHA, 2008). In Brazil, the study of Rocha (2008) is a pioneer in evaluating the selectivity of pesticides used in coffee culture on *C. montrouzieri.*

Considering that the effect of insecticides on beneficial insects in organic production system has not been extensively studied the aim of this work was to evaluate the effects on adult *C. montrouzieri* of topical and residual exposure of six commercial formulations allowed in organic farming under laboratory conditions, classifying them according to the criteria of the IOBC (International Organization of Biological Control) (HASSAN et al., 1992).

Material and Methods

The bioassays to assess the effects of the products on *Cryptolaemus montrouzieri* were conducted in laboratory (25 ± 2 º C, R.H. 70 ± 10% and photoperiod of 12 hours). The adults of *C. montrouzieri* were purchased from the company Gravena Manecol Ltda. The experiments were performed two hours after the insects arrival.

Topical exposure

The adults were treated individually with a dose of 0.5 ml of pesticide solution (Table 1) applied topically on the dorsal surface of the thorax, using 1 ml Microsyringe, coupled with a manual micro-applicator (Burkard Manufacturing Co. Ltd.).

Table 1. Products and concentrations evaluated on *Cryptolaemus montrouzieri* (Coleoptera, Coccinellidae) adults mortality in the laboratory.

Product	**Trade name**	**Product Information**	**Concentrations used (ml 100 l^{-1} of water)***	**Manufacturer**
Neem Oil	Organic neem®	80% neem oil	125, 250, **500**, 1000	Dalquim
Neem Oil	Natuneem®	1500 ppm azadirachtin	125, 250, **500**, 1000	Natural Rural
Rotenone	Rotenat ®	extract of *Derris* spp. with 5% rotenone	150, 300, **600**, 1200	Natural Rural
Lime sulfur	---	20% S + 9% Ca	1250, 2500, **5000**, 10000	Sul Fertilizantes
Pyroligneous extract	Pironat®	-	62,5, 125, **250**, 500	Natural Rural
Pyroligneous extract	Biopirol 7M®	-	50, 100, **200**, 400	Biocarbo
Carbaryl	Sevin 480 SC®	480 ppm a.i.	**225**	Bayer Cropscience

* The concentration recommended by the manufacturers in bold.

The pattern of control used was the product Sevin 480 SC ® (225 ml 100 l^{-1} water), as used by Smith and Krischik (2000).

After application, the insects were placed in plastic pots (550 ml), covered with voile fabric to allow gas exchange, and kept in an environmental room, fed with an aqueous solution of honey at 15% through a strip of Spontex Resist® inserted into a glass tube of 5 ml, fixed with parafilm, and a solid diet consisting of sucrose, brewer's yeast, wheat germ and corn gluten, in the ratio 3:1:1:1, respectively. The bioassay was conducted in a randomized design that took into account products and concentrations (treatments), using three replicates per treatment. Each experimental unit consisted of ten adults of *C. montrouzieri*.

Residual exposure

The bioassay of residual effect used a methodology based on Jacas and Viñuela (1994). Fifteen adult of *C. montrouzieri* in each concentration/treatment, with three replicates, were placed in cages whose detachable parts, upper and lower, were square plates of glass (12 cm x 12 cm), and the sidewall was an acrylic cylinder (4 cm high x 9 cm diameter) with seven holes to allow gas exchange side (connected to an air pump to force the flow) and food (the same of the previous experiment) and water were provided. The treatments consisted on four concentrations of commercial formulations of products (Table 1). The products were applied using hand sprayers (500 ml) on the internal faces of the glass plates in a volume of 1.5 ± 0.25 mg/cm ², measured on an electronic scale (Indústria e Comércio Eletro Eletrônica Gehaka Ltda.). The cages were assembled again, after the plates were dried out at room temperature, and then the insects were introduced. The experiment was kept in an environmental room.

Evaluation of experiments of contact and residual in Cryptolaemus mountrozieri

In both experiments the action of the products was evaluated by the number of individuals surviving 15 min, 30 min, 1, 4, 12, 24, 48, 72 and 96 hours after treatment with insecticides. The survival values were corrected by Abbott's formula (ABBOTT, 1925) and the variation in the number of surviving insects per treatment was transformed to arcsine of √(x/100) and subjected to repeated measures analysis of variance, using SPSS 15 software, comparing the means by Tukey test at 5% probability, when in the presence of significance. The results were classified according to criteria of the IOBC (International Organization for Biological Control) for laboratory tests on beneficial organisms (HASSAN et al., 1992), and classified into: 1) harmless (mortality < 30%), 2) slightly harmful (> 30% and <79%), 3) moderately harmful (> 80% and <99%), and 4) harmful (> 99%).

Results and Discussion

None of the products, at any tested concentrations, had a significant effect on the survival of adult *C. montrouzieri* by either topical application (Table 2) and residual exposure (Table 3), except for the control Carbaryl (225ml 100 l^{-1}), where mortality was only observed 24 hours after treatment, without significant differences between 24, 48, 72 and 96 hours (P=0.857). Thus, only observations at 24 and 96 hours are presented in Tables 2 and 3. The mortality values always remained below 4% during all observations, for all treatments and concentrations, and in the two application methods tested.

The absence of significant differences among the experiment repetitions indicated that the commercial lots of *C. montrouzieri* used in the experiment responded similarly to treatment (topical, P = 0.863; residual exposure, P = 0.894). With respect to products based on neem oil, the results are similar to those reported by Smith and Krischik (2000) and Rossini et al. (2003).

In both studies the authors found no significant differences on mortality of *C. montrouzieri* adults when they were sprayed with a commercial formulation of neem. In other species of coccinellid, *Coccinella septempunctata* L., Banken and Stark (1998) did not observed adults mortality when sprayed with commercial products containing azadirachtin. The authors, however, found a reduction in oviposition and a delay on the larval developmental, factors that were not evaluated in this study.

Testing neem oil on larvae of *Cycloneda sanguinea* (L.), Silva and Martinez (2004) found that the product caused larval mortality (60% mean survival at 5 ml l^{-1}). However, the adults emerged from treated larvae showed no changes in sex ratio, fecundity, fertility and longevity.

Table 2. Average number of live insects (± SD) and corrected mortality (Mc in %) of *Cryptolaemus montrouzieri* (Coleoptera, Coccinellidae) adults 24 and 96 hours after treatment via topical application (0.5 µl/insect) (25 ± 2 ºC, 70 ± 10% RH, photoperiod: 12 hours).

Treatments		Conc.[1]	Time (hours)			
			24	Mc	96	Mc
Product	trade name					
Neem Oil	Organic neem®	125	10.0±0.00 Aa2	0	10.0±0.00 Aa	0
		250	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		500	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		1000	10.0±0.00 Aa	0	10.0±0.00 Aa	0
Neem Oil	Natuneem®	125	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		250	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		500	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		1000	9.7±0.57 Aa	3.33	9.7±0.57 Aa	3.33
Rotenone	Rotenat®	150	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		300	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		600	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		1200	9.7±0.57 Aa	3.33	9.7±0.57 Aa	3.33
Lime sulfur	---	1250	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		2500	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		5000	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		10000	10.0±0.00 Aa	0	10.0±0.00 Aa	0
Pyroligneous extract	Pironat®	62,5	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		125	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		250	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		500	10.0±0.00 Aa	0	10.0±0.00 Aa	0
Pyroligneous extract	Biopirol 7M®	50	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		100	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		200	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		400	9.7±0.57 Aa	3.33	9.7±0.57 Aa	3.33
Carbaryl	Sevin 480 SC®	**225**	2.0±1.00 Ab	90	0±0.00 Bb	100
Control	-	-	20±0.00 Aa	-	20±0.00 Aa	-

[1] Conc. = Concentration. ml of commercial product per 100 l of water. The concentration recommended by the manufacturers in bold

[2] Averages followed by different uppercase letters in rows and lowercase letters in columns differ significantly by Tukey's test (P<0.05).

Table 3. Average number of live insects (± SD) and corrected mortality (Mc in %) of *Cryptolaemus montrouzieri* (Coleoptera, Coccinellidae) adults, 24 and 96 hours after treatment via residual exposure (25 ± 2 ºC, 70 ± 10% RH, photoperiod: 12 hours).

Treatments		**Conc.**[1]	**Time (hours)**			
			24	**Mc**	**96**	**Mc**
Product	**Trade name**					
Neem Oil	Organic neem®	125	10.0±0.00 Aa2	0	10.0±0.00 Aa	0
		250	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		500	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		1000	10.0±0.00 Aa	0	10.0±0.00 Aa	0
Neem Oil	Natuneem®	125	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		250	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		500	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		1000	10.0±0.00 Aa	0	10.0±0.00 Aa	0
Rotenone	Rotenat®	150	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		300	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		600	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		1200	9.7±0.57 Aa	3.33	9.7±0.57 Aa	3.33
Lime sulfur	---	1250	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		2500	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		5000	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		10000	10.0±0.00 Aa	0	9.7±0.57 Aa	3.33
Pyroligneous extract	Pironat®	62,5	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		125	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		250	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		500	10.0±0.00 Aa	0	10.0±0.00 Aa	0
Pyroligneous extract	Biopirol 7M®	50	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		100	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		200	10.0±0.00 Aa	0	10.0±0.00 Aa	0
		400	10.0±0.00 Aa	0	10.0±0.00 Aa	0
Carbaryl	Sevin 480 SC®	225	0.0±0.00 Ab	100	0±0.00 Ab	100
Control	-	-	20±0.00 Aa	-	20±0.00 Aa	-

[1] Conc. = Concentration. ml of commercial product per 100 l of water.
[2] Averages followed by different uppercase letters in rows and lowercase letters in columns differ significantly by Tukey's test (P<0.05).

Neem-based products action on natural enemies is considered highly variable (ISMAN, 2006). Despite that no mortality has been recorded in this study, it must be taken into account the effects that this product may have on the insects' behavior. Simmonds et al. (2000) found that the searching behavior of larvae and adult *C. montrouzieri* on *P. citri* was affected after application of neem seed extract and a commercial formulation of azadirachtin (Azatin®), with the predator remaining less time in the treated leaves and decreasing the rate of encounters with the prey.

Regarding pyroligneous extract, the results are similar to those of Busoli, Bissoli and Pereira (2003) who found no significant mortality of larvae of *Hippodamia convergens* Guérin-Meneville (Coleoptera: Coccinellidae) using four concentrations (0.5 to 2.5%) of pyroligneous extract (Biopirol 7M®), which led the authors to consider the product selective to the species.

In this paper toxicity of rotenone on *C. montrouzieri* was not observed. However and Aguiar-Menezes (2005) argues that this extract is toxic to fish, ladybugs and mites.Although other studies have described toxic effects of lime sulfur on predators such as *Iphiseiodes zuluagai* (Denmark and Muma) (Acari: Phytoseiidae) (AMARAL; VENZON; PALLINI, 2003), this effect was not found for *C. montrouzieri* adult. However, Venzon et al. (2006) studding products selectivity to spider mites, warn that, before the use of lime sulfur, it is necessary to assess the possible adverse effects on beneficial organisms present in the agroecosystem.

The insecticide carbaryl (Sevin® 480 SC - 225 ml 100 l^{-1}) used as a control, in the topical application, resulted in 100% of insect mortality, only 48 hours after application. Its application through residual exposure, caused a fourfold faster mortality, since in this case 100% of the insects dead within the first 12 hours. This insecticide is recommended to control coleopteran pests, and it has been used as reference in tests on *C. montrouzieri* (SMITH; KRISCHIK, 2000).

Classifying the products tested in this experiment, according to IOBC criteria (BAKKER et al., 1992; HASSAN et al., 1992), all can be considered harmless in relation to mortality by contact our residual action on *C. montrouzieri* adults, since the mortality was lower than 30% (Tables 2 and 3), being, therefore, selective. However, other side effects the insecticides to *C. montrouzieri*, such as ingestion of contaminated prey, changes in predator searching behavior, as well as sublethal effects, mainly on larvae, must be studies.

Conclusions

None of the products had a significant effect on the mortality of *C. montrouzieri* adults either by topical application or residual exposure.

According to IOBC criteria the tested products can be considered harmless by contact and residual action to *C. montrouzieri* adults.

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