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Toxicity of phytosanitary products to *Coccidophilus citricola* (Coleoptera: Coccinellidae)

Toxicidad de productos fitosanitarios sobre *Coccidophilus citricola* (Coleoptera: Coccinellidae)

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Abstract: *Coccidophilus citricola* is an important predator of *Diaspis echinocacti*, a scale insect that is the main pest of the forage cactus *Opuntia ficus-indica* cultivated in northeastern Brazil. The efficiency of a management program involving the natural enemy of an agricultural pest can be increased by adopting conservation techniques. The aim of the present study was to evaluate the selectivity of querobão, mineral oil, Natuneem™, aqueous extract of neem leaves, and conidia of *Beauveria bassiana* and *Metarhizium anisopliae* towards the predator. Aliquots of phytosanitary products were sprayed on sections (5 x 5 cm) of cactus rackets infested with *D. echinocacti* obtained from forage cactus grown under greenhouse conditions. Sections were dried and offered to second-generation *C. citricola* adults that had been reared under laboratory conditions. Predator mortalities were assessed at 24, 48 and 72 h after application of the products or water control. Querobão and Natuneem™ were most toxic to the predator, with high levels of mortality recorded at 24 and 48 h. Conidia of *B. bassiana* and *M. anisopliae* (1 x 10^8 conidia mL^{-1}) were less toxic and slower acting, with highest mortalities occurring between 48 and 72 h. The toxicities of mineral oil, neem leaf extract and *M. anisopliae* (1 x 10^7 conidia mL^{-1}) towards *C. citricola* were not significantly different from the control. It is concluded that these agents may be selective to the natural enemy of *D. echinocacti* and could be used in combination with the predator to control this pest in forage cactus cultures.

Key words: Ladybird beetle. Natural predator. *Diaspis echinocacti*. Forage cactus. Selectivity.

Resumen: *Coccidophilus citricola* es un depredador importante de *Diaspis echinocacti*, un insecto escama, la principal plaga del nopal forrajero, *Opuntia ficus-indica*, cultivado en el noreste de Brasil. La eficacia de un programa de manejo que implica el enemigo natural de una plaga agrícola se puede aumentar mediante la adopción de técnicas de conservación. El objetivo del presente estudio fue evaluar la selectividad de cinco productos fitosanitarios: querobão, aceite mineral, Natuneem™, extracto acuoso de hojas de neem y conidios de *Beauveria bassiana* y *Metarhizium anisopliae* hacia el depredador. Las alícuotas de los productos se pulverizaron en secciones (5 x 5 cm) de raquetas de cactos infestados con *D. echinocacti* obtenidos a partir de cactus forrajero cultivado bajo condiciones de invernadero. Las secciones se secaron y se ofrecieron a la segunda generación de adultos *C. citricola* que habían sido criados en laboratorio. Fue evaluada la mortalidad del depredador a las 24, 48 y 72 h después de la aplicación de los productos o el control (agua). Querobão y Natuneem™ fueron más tóxicos para el depredador, con altos niveles de mortalidad registrados a las 24 y 48 h. Los conidios de *B. bassiana* y *M. anisopliae* (1 x 10^8 conidios mL^{-1}) fueron menos tóxicos y de más lenta acción, alcanzando sus más altas mortalidades entre 48 y 72 h. Las toxicidades de aceite mineral, extracto de hoja de neem y *M. anisopliae* (1 x 10^7 conidios mL^{-1}) hacia *C. citricola* no fueron significativamente diferentes al control. Se concluye que estos agentes pueden ser selectivos para el enemigo natural de *D. echinocacti* y podrían utilizarse en combinación con el depredador para controlar esta plaga en cultivos de nopal forrajero.

Palabras clave: Mariquita. Depredador natural. *Diaspis echinocacti*. Nopal forrajero. Selectividad.

Introduction

The forage cactus *Opuntia ficus-indica* (L.) Mill. (Cactaceae) exhibits remarkable resistance to water deficiency, elevated temperatures and poor soils. The organs of the plant are adapted for the efficient use of water, most notably the cladode in which the photosynthetic apparatus is located and where crassulacean acid metabolism occurs with the capture of solar energy during the day and the fixation of carbon dioxide during the night (Sampaio 2005). Along with its ability to withstand extreme conditions, the plant is relatively easy to cultivate and can be used as the main feed for horses, cows and goats during extended periods of drought (Dos Santos *et al*. 2006). For these reasons, cultivation of the species extends to an area of approximately 500,000 ha in the semi-arid regions of northeastern Brazil, particularly in the states of Pernambuco, Alagoas and Paraíba (Cordeiro dos Santos and Gonzaga de Albuquerque 2001).

Cactaceous plants are subject to attack by various pests, although scale insects are probably the most important in terms of the damage caused and the effect on productivity (De Vasconcelos *et al*. 2009). The principal pest of forage cactus in northeastern Brazil is the armored scale *Diaspis echinocacti* (Bouché, 1833) (Hemiptera: Sternorrhyncha: Diaspididae), also known as the cactus scale or the prickly-pear-scale (Arruda-Filho and Arruda 2002). The natural enemies of the scale insects include beetles of the family Coccinellidae, and in South America the most important

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predator of *D. echinocacti* is the ladybird beetle *Coccidophilus citricola* (Brèthes, 1905) (Coleoptera: Coccinellidae) (Silva *et al*. 2003). Various studies have focused on increasing the predation efficiency of the coccinellids for biological control through, for example, successive releases or the adoption of conservation techniques (Dos Santos and Gravena 1997). In this context, Souza Born *et al.* (2009) evaluated the possibility of controlling *D. echinocacti* using alternative insecticides and entomopathogenic fungi, and found that a 3% hexane fraction from neem [*Azadirachta indica* A. Juss. (Meliaceae)] seeds caused 78.68% mortality of the pest. However, no information regarding the effects of the biopesticide on the natural enemies of the armored scale was provided.

Interest in the use of selective phytosanitary products alongside biological control has been growing over the last few years, but little information is available concerning the sensitivity of *C. citricola* to such agents. The aim of the present study was, therefore, to evaluate the effects of querobão, mineral oil, neem seed oil, aqueous extract of neem leaves, and the conidia of *Beauveria bassiana* (Balsamo) (Vuilleimin, 1912) (Ascomycota: Clavicipitaceae) and *Metarhizium anisopliae* (Metschnikoff, 1879) Sorokin, 1883 (Ascomycota: Nectriaceae) on the predator *C. citricola*.

Materials and methods

Predators and prey. A population of *C. citricola* was maintained in the laboratory at a mean temperature of 25.5 ± 2 ºC with relative humidity (RH) of 60.5 ± 10% and a photophase of 14 h, and adults from the second generation were employed as predators. The prey, *D. echinocacti*, was cultivated on forage cactus grown in the green house under similar conditions.

Preparation of phytosanitary products. Querobão was prepared using 100 g of soap, 100 g of tobacco, 10 mL of kerosene and 10 L of water. Neem leaves were collected from a two year-old plant located at the Centro de Ciências Agrárias (CECA), Universidade Federal de Alagoas (UFAL), Rio Largo, AL, Brazil (9º27'S 35º27'W; altitude 127 m). Plant material was identified by Maria Noêmia Rodrigues [Instituto do Meio Ambiente do Estado de Alagoas (IMA), Maceió, AL, Brazil] and a voucher specimen was deposited in the herbarium at IMA with the identification number 34904. Freshly collected leaves (500 g) were washed with distilled water, dried for 48 h at 40-45 °C in an air-recirculating oven, and triturated in a knife mill to yield a powder with small particle size. The material was stored in sealed amber glass bottles until required for experimentation. An aqueous extract was prepared by shaking the powdered material (100 g) with distilled water (900 mL) for 24 h at room temperature followed by filtration.

The fungal isolates used in the study were from the mycological collection of the Laboratory Fitossan - Assistência Fitossanitária e Controle Biológico Ltda (Maceió, AL, Brazil). Isolate *B. bassiana* Fitossan 1 was collected from *Orthezia praelonga* (Douglas, 1891) (Hemiptera: Ortheziidae) while isolate *M. anisopliae* PL43 was collected from *Mahanarva posticata* (Stal, 1855) (Hemiptera: Cercopidae). Fungi were cultured at 26 ± 1 ºC and 70 ± 10% RH under a 14 h photophase in Petri dishes containing autoclaved (120 °C for 20 min) potato-dextrose-agar (PDA) medium supplemented with streptomycin sulfate and Nujol™ oil (PDA+A). After 7 days incubation, the isolates were subcultured onto fresh PDA medium for a further 7 days and subsequently multiplied in assay tubes containing the same medium and maintained under the same conditions for 14 days (Alves 1986; 1998). Suspensions of conidia were prepared by diluting the contents of the assay tubes with 100 mL of sterilized distilled water containing 0.01% (*v/v*) of spreader-sticker Tween™ 80 (SDW+T). Conidial concentrations were determined using an optical microscope and a Neubauer chamber, and values were adjusted to 1 x 10^7 or 1 x 10^8 conidia mL^1 by addition of SDW+T. In order to evaluate the viabilities of isolates, 0.1 mL aliquots of suspensions were spread evenly over PDA+A plates using a Drigalsky spreader, and 100 conidia per plate were examined under the optical microscope after 24 h of incubation. Duplicate assessments of percentage germination were performed for each concentration of conidia. In all cases, conidial viability was established to be > 95%.

Bioassays. The bioassays were carried out at the Entomology Laboratory of CECA, UFAL. The completely randomized experiment involved eight treatments (plus distilled water control) with ten repetitions, each comprising ten *C. citricola* adults. The phytosanitary products evaluated were: i) querobão; ii) 1% (*v/v*) aqueous mineral oil; iii) 3% (*v/v*) water emulsion of Natuneem™ (Natural Rural Indústria e Comércio de Produtos Orgânicos e Biológicos, Araraquara, SP, Brazil); iv) 2% (*w/v*) aqueous extract of neem leaves; v) *B. bassiana* (1 x 10^8 conidia mL^{-1}); vi) *B. bassiana* (1 x 10^7 conidia mL^{-1}); vii) *M. anisopliae* (1 x 10^8 conidia mL^{-1}); and viii) *M. anisopliae* (1 x 10^7 conidia mL^{-1}). In each of the assays, 1 mL aliquots of phytosanitary product (or distilled water control) were sprayed under sections (5 x 5 cm) of cactus rackets infested with *D. echinocacti*. After drying, the sections were placed into plastic containers and offered to the predator, *C. citricola.* The mortality rates of the predator were evaluated at 24, 48, and 78 h after application of the treatment.

Statistical analysis. Analyses were carried out using SPSS™ Statistics for Windows software package version 17.0 (IBM Corp., Armonk, NY, USA). Data relating to the percentage mortality of *C. citricola* at 24, 48, and 72 h were analyzed using the general linear model and submitted to univariate analysis of variance according to the full factorial model. The averages of pairs of treatments, as well the evaluated averages, were compared using the Tukey post-hoc test with an alpha value of 0.05 (Box *et al*. 2008).

Results and discussion

The percentage mortalities of *C. citricola* adults recorded after treatment with various phytosanitary agents are presented in Table 1. Comparison of mortalities recorded within each of the 24 h observational periods revealed significant negative effects of the treatments with respect to exposure times of 24 h ($F = 3.280$; $df_1 = 8$; $df_2 = 81$; $P = 0.003$), 48 h ($F = 16.608$; $df_1 = 8$; $df_2 = 81$; $P = 0.001$) and 72 h ($F = 11.577$; $df_1 = 8$; $df_2 = 81$; $P = 0.001$).

Within individual treatments, no statistically significant differences in percentage mortalities were recorded between the 0 - 24 and 24 - 48 h observational periods. Moreover,

Table 1. Percentage mortality of *Coccidophilus citricola* adults following exposure to different phytosanitary products.

Treatment	Percentage mortality[1,2] 24 h	48 h	72 h
Querobão	82.00 ± 4.67 [aA]	70.00 ± 15.28 [aA]	0.00 ± 0.00 [bB]
1% (v/v) aqueous mineral oil	10.00 ± 4.47 [aB]	4.00 ± 4.00 [aB]	4.50 ± 3.02 [aB]
3% (v/v) Natuneem™ emulsion	62.00 ± 7.57 [aA]	63.33 ± 15.28 [aA]	3.33 ± 2.34 [bB]
2% (w/v) aqueous extract of neem leaves	4.00 ± 2.67 [aB]	8.50 ± 4.60 [aB]	2.50 ± 1.25 [aB]
Beauveria bassiana (1 x 10^8 conidia mL^{-1})	12.00 ± 4.42 [bB]	16.83 ± 7.53 [abB]	41.50 ± 9.86 [aA]
B. bassiana (1 x 10^7 conidia mL^{-1})	8.00 ± 4.42 [abB]	23.33 ± 6.21 [aB]	4.50 ± 3.02 [bB]
Metarhizium anisopliae (1 x 10^8 conidia mL^{-1})	12.00 ± 6.80 [bB]	22.333 ± 7.17 [abB]	45.66 ± 12.08 [aA]
M. anisopliae (1 x 10^7 conidia mL^{-1})	2.00 ± 2.00 [aB]	2.00 ± 2.00 [aB]	4.00 ± 2.67 [aB]
Control (distilled water)	4.00 ± 2.67 [aB]	2.00 ± 2.00 [aB]	10.50 ± 3.53 [aB]

[1] The mean values (± standard error) are not cumulative but refer to the mortality of predators during the previous 24 h observational period.
[2] Within each row (column), values bearing different lowercase (uppercase) letters are significantly different (P < 0.05).

for treatments involving mineral oil, neem leaf extract, *M. anisopliae* (1 x 10^7 conidia mL^{-1}) and the control, there were no statistically significant differences between mortalities recorded in the 48 - 72 h period compared with the other periods. In contrast, *B. bassiana* (1 x 10^8 conidia mL^{-1}) and *M. anisopliae* (1 x 10^8 conidia mL^{-1}) showed the highest percentages of mortality between 48 and 72 h, while querobão and Natuneem™ presented the lowest mortalities during this period. The most likely explanation for the latter finding is that those predators remaining viable during this period showed some resistance to the phytosanitary agents, thus reflecting normal biological diversity within a population. In the experiment involving treatment with *B. bassiana* (1 x 10^7 conidia mL^{-1}), the highest percentage mortality was observed during the period 24 - 48 h.

Comparison of the eight treatments applied to *C. citricola* revealed that querobão and Natuneem™ were most toxic to the predator, with high levels of mortality recorded at 24 h ($F = 3.280$; $df_1 = 8$; $df_2 = 81$; $P = 0.003$) and 48 h ($F = 16.608$; $df_1 = 8$; $df_2 = 81$; $P = 0.001$) (Table 1). Conidia of *B. bassiana* (1 x 10^8 conidia mL^{-1}) and *M. anisopliae* (1 x 10^8 conidia mL^{-1}) were somewhat less toxic and slower acting, with highest mortalities occurring between 48 and 72 h ($F = 11.577$; $df_1 = 8$; $df_2 = 81$; $P = 0.001$).

The results obtained in this study demonstrate that querobão, which is often recommended as a treatment for the control of *D. echinocacti,* is not selective and induces high levels of mortality (~80%) in *C. citricola* populations within 24 h. Treatment with Natuneem™ also causes considerable mortality (~60%) within 24 h of application, and this toxicity is almost certainly related to the presence of azadirachtin in the final product. According to Martinez (2008), it is possible extract around 47% of the oil from neem seeds, and this oil may contain up to 10% of the azadirachtin originally present in the fruit. In this context, Cosme *et al.* (2007) studied the action of Nim-I-Go™ (a neem oil-based natural repellant) on eggs and larvae of *Cycloneda sanguinea* (Linnaeus, 1763) (Coleoptera: Coccinellidae) and concluded that, since the product only contained a low level of azadirachtin, it could be applied successfully at a concentration of 10 mg L^{-1} in combination with a predator for pest control in cotton. On the other hand, azadirachtin at 50 and 100 mg L^{-1} showed moderate to high toxicity towards the predator.

In the present study, an aqueous extract of neem leaves was found to be no more toxic to *C. citricola* adults than the control (with both treatments inducing 4% lethality at 24 h), indicating that it may be selective to the natural enemy of *D. echinocacti.* Moreover, mineral oil, which has been suggested as an alternative treatment to control the armored scale insect, showed low toxicity to the predator and induced just 10% mortality at 24 h, a value that was statistically similar to the control. However, according to Yamamoto and Bassanezi (2003), the common practice of mixing mineral oil with other insecticides leads to an increase in the mortality of coccinellids.

Regarding the entomopathogens, moderate levels of mortality of *C. citricola* adults were observed 48 h after treatment with *M. anisopliae* at 1 x 10^8 conidia mL^{-1} and with *B. bassiana* at 1 x 10^7 and 1 x 10^8 conidia mL^{-1}. These results are in agreement with those of Magalhães e*t al.* (1998) and suggest that, since entomopathogens can be harmful to natural predators through modulation of aspects of their biology, treatment with such fungi may be considered unfavorable from a biologic control viewpoint (James and Lighthart 1994). However, treatment of *C. citricola* adults with a suspension of *M. anisopliae* containing 1 x 10^7 conidia mL^{-1} produced percentage mortality values that did not differ significantly from those of the control. According to this finding, *M. anisopliae* conidia can be employed at low concentrations alongside *C. citricola* as part of a program involving biological control of *D. echinocacti.* However, when fungal suspensions are used under field conditions, it is clearly necessary to establish an appropriate concentration that will inflict maximum damage on the pest whilst presenting the lowest toxicity towards the natural enemies.

Alves (1986) reported that the entomopathogenic effect on an insect depends on the isolate employed, while Sosa-Gómez and Moscardi (1998) stressed the importance of studying the effects of several isolates not only on the target pest but also on the natural enemies in order that to select those that that inflicted a lower impact on the beneficial entomofauna. More recently, Thungrabeab and Tongma (2007) have shown that different genera or species of fungi exhibit dissimilar pathogenicity and virulence to natural enemies, a factor that serves to explain the difference in virulence of the fungi employed in the present study.

Conclusions

The present study has revealed that querobão, Natuneem™ and suspensions of *B. bassiana* cause significant mortality to *C. citricola* and cannot be employed in the control of *D. echinocacti* as part of an integrated management program. On the other hand, 1% aqueous mineral oil, 2% aqueous extract of neem leaves and *M. anisopliae* conidia at low concentrations are compatible with the predator *C. citricola* and can be used in combination to control *D. echinocacti* in forage cactus cultures.

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