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PATHOGENIC ACTIVITY OF *ISARIA* SPP. FOR CONTROL OF *DACTYLOPIUS OPUNTIAE* (HEMIPTERA: DACTYLOPIIDEAE) AND THE EFFECTS OF SELECTED INSECTICIDES

ACTIVIDAD PATOGÉNICA DE *ISARIA* SPP. PARA EL CONTROL DE *DACTYLOPIUS OPUNTIAE* (HEMIPTERA: DACTYLOPIIDEAE) Y LOS EFECTOS DE ALGUNOS INSECTICIDAS

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ABSTRACT. We analyzed the activity of the entomopathogenic fungi *Isaria farinosa*, *I. fumosorosea* and *I. javanica* against the cochineal *Dactylopius opuntiae*, as well as the effects of the insecticides clorpirifos, acetamiprid, thiamethoxam and lambda-cyhalothrin on the conidia germination, mycelium growth and a sporulation of the fungi. These fungi were not pathogenic to cochineal. This the first report of the efficiency of *Isaria* spp. against this the insect. The bioassays of the compatibility of the insecticides acetamiprid and thiametoxam with species of *Isaria* indicate the possibility of the joint use of these products in studies on the control of *D. opuntiae*.

Key words: Biological control; cochineal; entomopathogenic fungi; insecticide

Lopes, R. S., Oliveira, L. G., Lima, G., Correia, M. T. S., Costa, A. F., Luna-Alves Lima, E. A., Lima, V. L. M. (2019) Acción patogénica de *Isaria* spp., para el control de *Dactylopius opuntiae* (Hemiptera: Dactylopiideae) y los efectos de algunos insecticidas. *Acta Zoológica Mexicana (nueva serie)*, 35, 1–5. https://doi.org/10.21829/azm.2019.3502249

RESUMEN. El trabajo analizó las acciones de los hongos entomopatógenos *Isaria farinosa*, *I. fumosorosea* y *I. javanica* contra la cochonilla *Dactylopius opuntiae*, así como el efecto de los insecticidas clorpirifos, acetamiprid, tiametoxam y lambda-cialotrina sobre la germinación, el crecimiento micelial y esporulación del mismo. Los hongos no fueron patógenos a *D. opuntiae*, siendo éste el primer reporte de la eficiencia de *Isaria* spp., sobre este insecto. Los bioensayos de la compatibilidad de los insecticidas acetamiprid y thiametoxam con especies de *Isaria* indican la posibilidad de uso conjunto de estos productos en estudios para el control de *D. opuntiae*.

Palabras clave: Control biológico; cochinilla-del-carmín; hongo entomopatógeno; insecticida

The cochineal scale insect, *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiideae) is an insect used in countries such as Peru, the Canary Islands, and Mexico to produce the natural food coloring carmine. It has also been used as a biological control agent against invasive cactus species in South Africa. In Brazil and Mexico, this insect attacks crops of *Opuntia ficus-indica* (L.) cactus, causing severe loss of forage and fruit production, resulting in economic losses for farmers (Nobel, 2001; Paterson *et al*., 2011; Tiago *et al*., 2016).

The control of insect pests is often achieved using chemical products, which it used excessively can cause the contamination of the environment and harm to animal health. However, the use of entomopathogenic fungi can contribute to insect pest control (Alves, 1998; Avery *et al*., 2013). The genus *Isaria* comprises important pathogens that can be used to control insect pests. *Isaria farinosa* (Holm: Fries) Fries and *Isaria fumosorosea* (Wize) Brown & Smith were efficient against the mealy bugs *Rhizoecus kondonis* Kuwana and *Planococcus citri* (Risso) (Zimmermann, 2008; Demirci *et al*., 2011). The combined application of entomopathogenic agents and insecticides can amplify the lethality of natural pathogens (Avery *et al*., 2013; Silva *et al*., 2015). This study analyze the potential of the *I. farinosa*, *I. javanica*, and *I. fumosorosea* in controlling *D. opuntiae,* and evaluated the effects of the insecticides chlorpyrifos, acetamiprid, thiamethoxam, and lambda-cyhalothrin on these fungi in terms of conidia germination, mycelium growth, and spore formation.

Isaria farinosa (URM5016 and URM5060) and *I. javanica* (URM4993 and URM4995) were obtained from the URM Culture Collection (WDCM604) of Federal University of Pernambuco (UFPE); *I. farinosa* (ESALQ1205 and ESALQ1355) and *I. fumosorosea* (ESALQ1296 and ESALQ1297) were obtained from the ESALQ Collection of Microorganisms - ESALQ of the University of São Paulo (USP). The strains were cultivated in Sabouraud Agar Medium (SAD) for 12 days, and then suspensions (1×10^8 conidia/mL) were made in 10 ml of Tween 80 (0.1%) solution.

The cochineal was collected in *O. ficus-indica* plantations, and the insects were raised in culture chambers at 28 ± 1ºC, on healthy cactus cladodes. The experiments were performed with three replicates and consisted of nine treatments (eight strains and one control), totaling 150 females and 150 nymphs per treatment. The cactus cladodes infested with nymphs second instar and adult females were sprayed with 10 mL of 1×10^8 conidia/mL of each strain, and for control with Tween 80 (0.1%) solution using a manual sprayer. The cladodes were placed on rectangular plastic trays (30 ×15 × 8 cm) and maintained at 26 ± 1°C, for 10 days; after which 50 adult females and 50 nymphs were collected from each cladode. Confirmation of insect mortality was observed after fungal sporulation by the fungal infection according to Alves (1998).

The chemical insecticides chlorpyrifos, acetamiprid, thiamethoxam and lambda-cyhalothrin were tested at three different concentrations based on the recommendations of the manufacturers, with the median concentration (CMed) corresponding to that recommended for field use; the minimum concentrations (CMed/2) and maximum concentrations (CMedx2) (Table 1), according to (Lopes *et al*., 2018). To evaluate the germination, 0.1 mL of each suspensions with 1×10^8 conidia/mL in Tween 80 (0.1%) solution +

insecticide were mixed and after one hour, were inoculated into Petri dishes containing SDA and incubated (26 ± 1°C; 80 ± 10% RH); the control was the fungal suspension without the insecticide. The germination percentage was determined after 16 hours, counting 500 conidia, between germinated and non-germinated by plates, and then the results were given in the formula G = N × 100/500, where N is germinated spore according to Alves and Pereira (1998). To evaluate mycelial growth, a filter paper disc (0.3 mm diameter) was soaked with 0.01 mL of 1×10^8 conidia/mL in a Petri dish with SDA + insecticides (as well as control plates without pesticide), and incubated (26 ± 1°C; 80 ± 10% RH) for 12 days. The mycelial growth was determined by measuring the diameter of each colony. To analyze sporulation, colony fragments were transferred to test tubes containing 10 mL of Tween 80 (0.1%) solution, and the number of spores in each suspension was counted. Statistical analyses of the data were performed by analysis of variance (ANOVA) and the means compared using the Tukey test at a 5% probability (SAS Institute, 1999-2001).

Table 1. Chemical pesticides utilized for controlling cochineal (order Hemiptera). Font: Lopes *et al*. (2018)

Product's name	**Type of formulation**	**Chemical Type**	**Constituent Active**	**Concentration recommended by the manufacturer (L)**		
				Minimum	Median	Maximum
Lorsban 480 BR	Emulsifiable concentrate	Organophosphate	Chlorpyrifos	0.75 mL/L	1.5mL/L	3.0 mL/L
Mospilan	Soluble powder	Neonicotinoid	Acetamiprid	0.125 g/L	0.25 g/L	0.5 g/L
Actara 250 WG	Dispersible granules	Neonicotinoid	Thiamethoxam	0.10 g/L	0.20 g/L	0.40 g/L
Karatê ZEON 250CS	Encapsulated suspension	Pyrethroid	Lambda-cyhalothrin	0.50 mL/L	1.0 mL/L	2.0 mL/L

Isaria species did not cause efficient mortality averages on the cochineal. *Isaria javanica* URM4995 and *I. farinosa* ESALQ1355 caused only 14 and 16% mortality of the nymphs, compared to 5% mortality in the control ($p = 0.05$) (Table 2). *Isaria farinosa* ESALQ1205 and *I. javanica* URM4993 caused the death of 6 and 7% of *D. opuntiae* nymphs, respectively, while the other strains did not cause nymph mortality (Table 2). None of the *Isaria* strains caused adult female mortality. This is the first study to report on the pathogenicity of *Isaria* spp. on *D. opuntiae*. *Dactylopius* have protective layers of fatty and waxy substances covering their bodies that repel aqueous solutions (Nobel, 2001; Demirci *et al*., 2011). This waxy barrier probably impedes the contact of fungal conidia with the insects' tegument, thus stifling fungal germination. The association of entomopathogens with adjuvants (such as oils, plant extracts, and/or insecticides) could represent viable options for increasing the prevalence of infection with fungal pathogens in this insect (Silva *et al*., 2015).

Table 2. Mean percentage of mortality (± SD) of the nymphs of *Dactylopius opuntiae* when treated with different strains of *Isaria*. Means followed by the same letters do not differ significantly at a 5% probability level the Tukey Test.

Strains	**Mortality (%)**
Control	5.00 ± 2.37 bc
Isaria farinosa ESALQ1205	6.00 ± 0.94 bc
Isaria farinosa ESALQ1355	16.00 ± 1.09 a
Isaria farinosa URM5016	0.00 ± 0.00 c
Isaria farinosa URM5060	0.00 ± 0.00 c
Isaria javanica URM4995	14.00 ± 0.94 a
Isaria javanica URM4993	7.00 ± 1.44 b
Isaria fumosorosea ESALQ1296	0.00 ± 0.00 c
Isaria fumosorosea ESALQ1297	0.00 ± 0.00 c
Coefficient of Variation (%)	17.28

The insecticides chlorpyrifos and lambda-cyhalothrin inhibited conidia germination, mycelium growth and a sporulation, principally when used at the maximum concentrations (Table 3). Asi *et al*. (2010), verified that 13 insecticides significantly inhibited the germination and mycelial growth of *M. anisopliae* and *I. fumosorosea*, with chlorpyrifos being the most toxic in insecticide. Avery *et al*. (2013), observed that the adjuvant oils significantly inhibited germination and mycelial growth of *I. fumosorosea*. The insecticides thiamethoxam and acetamiprid caused no negative effects on conidia germination, mycelium growth, or sporulation of the strains (p = 0.05), although the maximum concentrations of the insecticides tested were detrimental to *I. fumosorosea* ESALQ1297 (Table 3); those insecticides can therefore be considered for combined applications. Similar results were reported by Amjad *et al*. (2012), who found that acetamiprid inhibited germination in *I. fumosorosea* only at the highest concentration tested, having no effect at lower concentrations. Entomopathogenic fungi and selective insecticides can act synergistically, reducing the of insecticide dosages, which contributes to the preservation of natural enemies and, may reduce the environmental pollution and the appearance of resistant insects (Silva *et al*., 2015).

Table 3. Effects of different pesticides on the germination (%), growth (cm) and sporulation (10^7 conidia/mL) of *Isaria* species. Means followed by the same letters do not differ significantly at a 5% probability level (Tukey test).

Insecticide/	***Isaria farinosa* ESALQ1355**			***Isaria javanica* URM4995**			***Isaria fumosorosea* ESALQ1297**		
Concentration	Germination	Growth	Sporulation	Germination	Growth	Sporulation	Germination	Growth	Sporulation
Chlorpyrifos									
Control	99±0.46a	4.00±0.27a	8.00±1.04a	99±0.00a	3.00±0.10a	2.00±0.1a	99±0.26a	3.40±0.0a	3.00±0.0a
Minimum (0.75 mL/L)	55±3.23b	1.40±0.01b	1.90±0.33b	84±0.26 b	1.70±0.04b	0.90±0.3ab	87±2.78b	1.20±0.0bc	1.20±0.0b
Median (1.5 mL/L)	29±5.81c	0.70±0.10c	0.70±0.03c	83±1.62b	1.20b±0.07b	0.60±0.04b	87±0.70b	0.00±0.00c	0.00±0.0b
Maximum (3.0 mL/L)	0.00±0.00d	0.00±0.00d	0.00±0.00c	63±4.66c	0.50±0.00c	0.40±0.06b	51±2.80b	0.00±00c	0.00±0.0b
Acetamiprid									
Control	99±0.53a	3.60±0.00a	6.30±0.60a	99±0.80a	3.20±0.00a	1.60±0.00a	99±1.4a	3.40±0.06a	3.00±0.80a
Minimum (0.125 mL/L)	98±1.16a	3.5±0.00a	5.90±0.21a	94±1.92a	3.20±0.15a	1.80±0.13a	92±0.8a	3.20±0.08ab	2.90±1.20a
Median (0.25 mL/L)	96±0.46a	3.5±0.02a	4.30±0.03a	92±1.33a	2.80±0.02a	1.50±0.59a	90±2.4a	3.00±0.03ab	2.70±0.03a
Maximum (0.5 mL/L)	95±0.26a	2.80±0.06a	3.60±0.07b	91±1.41a	2.70±0.07a	0.90±0.15b	75±11.6b	2.70±0.01b	2.60±0.38a
Thiamethoxam									
Control	96±0.53a	3.30±0.03a	7.80±0.34a	98±0.53a	3.60±0.06a	2.80±0.04 a	99±0.2a	3.70±0.02a	3.00±0.19a
Minimum (0.10 mL/L)	94±0.96a	3.30±0.07a	7.30±0.38a	97±0.26a	3.30±0.04a	2.70±0.25a	98±0.5a	3.40±0.04ab	2.30±0.15ab
Median (0.20 mL/L)	93±0.46a	3.20±0.08a	6.20±0.40a	96±0.00a	3.20±0.10a	2.50±0.21a	98±0.8a	3.30±0.06ab	2.20±0.16ab
Maximum (0.5 mL/L)	92±1.48a	3.20±0.07a	5.40±0.60a	96±0.53a	3.00± 0.03a	1.80±0.14ab	98±0.4a	3.00±0.01b	1.50±0.02b
Lambda-cyhalothrin									
Control	99±0.26a	3.30±0.03a	7.30±0.34a	99±1.4a	3.50±0.18a	2.80±0.44a	99±0.5a	3.40±0.03a	1.30±0.14a
Minimum (0.50 mL/L)	86±1.22b	2.00±0.01b	4.30 ±0.15 b	87±0.5b	3.00±0.10 ab	2.30±0.21ab	86±5.2b	3.40±0.05a	0.800±0.04b
Median (1.0 mL/L)	86±0.02b	1.90 ± 0.11b	3.10±0.17bc	86±1.0b	2.80±1.12ab	1.80±0.11ab	85±0.2b	3.30±0.08a	0.80±0.11b
Maximum (2.0 mL/L)	80±1.86b	2.30±0.08b	2.00±0.52c	80±1.0b	2.70±0.06b	1.50±0.06b	75±3.3b	2.20±0.08b	0.00±0.02c

Our results indicate that the species of *Isaria* were not pathogenic to adult females and nymphs of *D. opuntiae*. Additionally, the insecticides thiamethoxam and acetamiprid demonstrated compatibility with all the *Isaria* fungal strains tested, suggesting that their use in mixtures could enhance the effectiveness of *Isaria* in the control of insect pests.

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