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Effect of Three Insecticides Used in Mexico on Whiteflies (Homoptera: Aleyrodidae) and a Fungicide on the Viability and Morphology *In Vitro* of *Verticillium lecanii* (Zimm.) Viégas

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ABSTRACT. Three formulated chemical insecticides (lambda-cyhalotrin, permethrin, methamidophos) and one fungicide (benomyl) were tested *in vitro* on two strains of *Verticillium lecanii*, one isolated in Cuba (A) from scales, and one in Mexico (C) from whiteflies. Viability was assessed by colony forming units using three different concentration, and colonial morphology was tested in pesticide-impregnated agar plates. All tested chemicals caused viability inhibition on the strains assayed. Lambda-cyhalotrin and benomyl caused the greatest inhibition (86.0-100%) on both fungal isolates (P<0.05). Strain A did not show significant differences (P<0.05) between viability inhibition by permethrin (44.8%) and metamidophos (47.6%) using the recommended field concentration, whereas strain C exhibited significant differences (P<0.05) with these insecticides, 78.5% and 29.7%, respectively. Viability inhibition by pesticides showed a significant (P<0.05) dose-response with permethrin for both strains tested. All four formulated pesticides induced an atypical morphology change, from a typical white color to cinnamon, and from cottony to leather-like colonies with anomalous conidiation and hyphal organization that was reverted when grown in pesticide free culture medium.
Key Words: Insecticides, Fungicides, *Verticillium lecanii*.

RESUMEN. Tres insecticidas (lambda-cialotrina, permetrina y metamidofos) y un fungicida (benomilo) fueron probados *in vitro* en dos cepas de *Verticillium lecanii*, una aislada de insectos escama en Cuba (A) y otra de la mosquita blanca en México (C). Se determinó la viabilidad por unidades formadoras de colonias (UFC) y se observó la morfología colonial. Los plaguicidas inhibieron la viabilidad en las cepas estudiadas. Lambda-cialotrina y benomilo provocaron la inhibición mayor (86.0-100%) en ambas cepas (P<0.05). La cepa A no mostró diferencias significativas (P<0.05) entre la inhibición de la viabilidad por permetrina (44.8%) y metamidofos (47.6%) a la concentración recomendada para su uso en campo; la cepa C sí exhibió diferencias significativas (P<0.05) con esos insecticidas, 78.5% y 29.7%, respectivamente. Para ambas cepas se encontraron diferencias significativas (P<0.05) dosis-respuesta respecto a la inhibición de la viabilidad por permetrina. Se observaron cambios morfológicos atípicos en las colonias, el color blanco típico cambió a canela, la apariencia algodonosa tomó un aspecto similar al cuero, con anomalías en la conidiación y en la organización hifal que revertieron en medio de cultivo libre de plaguicidas.
Palabras Clave: Insecticides, Fungicides, *Verticillium lecanii*.

INTRODUCTION

The entomopathogenic *Verticillium lecanii* (Zimm.) Viégas controls several insects in field and glasshouse conditions by its capabilities as a natural bioregulator of pests. This fungus is an important parasite of scales (Homoptera: Coccidae),[28] coffee rust basidiomycete (Uredinales: Pucciniaceae),[5] aphids (Homoptera: Aphididae), and whiteflies (Homoptera: Aleyrodidae).[7] This last insect is considered a major pest of economically important crops such as beans, tomatoes, peppers, cucumbers, lettuce, cotton, ornamentals and garden plants grown in Latin America,[9,24] in the Caribbean,[17] and worldwide.[12]

Due to the increase in chemical pesticide resistant insect strains and the need to minimize side-effects of these products on the environment, there is now a continuous demand in selecting and introducing alternative methods to control insect pests.[19] Although microbial products currently constitute only 2% of the world pesticide market, this percentage is expected to increase sharply in the future.[22]

The compatibility of entomopathogenic fungi with selective pesticides is needed in modern integrated pest management control programs to minimize their negative side effects on important natural enemies.[25] Anderson & Roberts[2] have shown that in *Beauveria bassiana*-insecticide tank mixes, most inhibitory effects occurred within the first few hours, and by separate applications of each control agent the inhibition was minimized. Furthermore, Anderson *et al.*[3] demonstrated that combinations of sublethal concentration of an insecticide were clearly compatible with *B. bassiana,* hence use of mixtures might have advantages by minimizing the danger of pesticide contamination on non-target sites and organisms, as well as by delaying the expression of insecticide resistance in insects.

The working group on Pesticides and Beneficial Organisms of the International Organization for Biological Control (IOBC) has recently claimed that selective pesticides suitable for use in integrated control programs and the development of standard methods to test the side effects of pesticides on most natural enemies are urgently needed.[8] *V. lecanii* effectiveness as a mycoinsecticide for whiteflies has been demonstrated In Mexico, this fungus has been isolated from whiteflies[14] and coffee rust,[5] and its compatibility tested against two chemical pesticides used for coffee rust.[4] In most compatibility studies, the effects of pesticides have been examined *in vitro* by germination and sporulation inhibition on agar plates.[1,16,18,20,28] In this study, two strains, one isolated in Cuba and another in México, were tested *in vitro* to determine if lambda-cyhalotrin, permethrin, and methamidophos, commonly used in this country for whiteflies chemical control, and benomyl used in vegetable crops to control fungal diseases caused by phytopathogenic fungi, are harmful to *V. lecanii.*

MATERIALS AND METHODS

Fungi. Two strains of *V. lecanii* were used. Strain A was isolated in Cuba from *Coccus viridis* (Green) (Homoptera: *Coccidae*) in grapefruit (Cabrera, personal communication). Strain C was isolated in México from *Trialeurodes vaporariorum* (West.) (Homoptera: *Aleyrodidae*) by Mier *et al.*[14] from beans. The strains are maintained in Sabouraud agar at 4°C.

Conidia were produced by growing mycelia on mycological agar (Bioxón, México) with 0.25% yeast extract (Bioxón). Plates were incubated at 26°C for 12 days. Conidia were prepared for testing, by suspending them in sterile water and thoroughly mixing with a Vortex mixer (Fisher Scientific, New York). Hemocytometer counts were made to achieve a 1 x 10^6 conidia/ml suspension.

Pesticides. Assays were performed with one fungicide: Benomyl, methyl 1-(butylcarbamoyl) benzimidazol-2-ylcarbamate (Benomilo, WP, 500 g AI/Kg, Promotora Técnica Industrial, México), two piretroid insecticides: lambda-cyhalotrin, (S)-α-cyano-3-phenoxybenzyl-(1S + 1R)-*cis*-3-(Z-2-chloro-=3,3,3-trifluoropropenyl)-2,2-=dimethylcyclopropanecarboxylate (Karate SL, 70 g AI/l, ICI Agroquímicos, México) and permethrin, 3-=phenoxybenzyl (1RS)-*cis-trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (Ambush SL, 50 g AI/l, ICI Agroquímicos), and an organophosphorus insecticide: methamidophos, *O,S*-dimethyl phosphoramidothioate (Tamaron 600 SL, 600 g AI/l, Bayer, Mexico). Each pesticide was tested at three concentrations: the recommended one for field use (II), and two additional ones, a lower (I) and a higher (III). Pesticide concentrations were: 0.05, 0.1, and 0.2 mg/l for benomyl; 0.5, 1.3, and 2.3 ml/l for lambda-cyhalotrin; 0.5, 1.4, and 2.4 ml/l for permethrin, and 3.13, 4.13, and 5.13 ml/l for methamidophos.

Compatibility assay. The inhibition percentage was determined as an inverse measure of fungal viability, using a modification of the Anderson & Roberts[2] method. Briefly, each treatment (strain, pesticide, and concentration) consisted of 1 ml of an aqueous suspension of 1 x 10^6 conidia/ml prepared as mentioned above, mixed with 9 ml of the pesticide concentration. Three replicates per treatment were processed in time, as well as a control for each treatment, without any pesticide. The *V. lecanii*-pesticide combinations were shaken for 15 h at 26°C, as tank mix. The colony forming units (CFU) were determined by dilutions in mycological agar plates, and the average values for each treatment were compared with those obtained for the control. The percent inhibition (%I) was calculated by the following equation:

$$\%I = \frac{\text{CFU control} - \text{CFU treatment}}{\text{CFU control}} \times 100$$

Experimental results were subjected to a factorial variance analysis using a SAS program.[11] The strains, pesticides, and concentrations were taken as the independent variables and the percent inhibition as the dependent variable. When triple interaction in factorial analysis of variance was statistically significant (P<0.05), a Tukey test was applied.

Colonial morphology assay. A sample of 0.3 ml of an aqueous conidial suspension from each strain, prepared as mentioned above, was plated on a mycological agar plate and incubated at 26°C for 7 days. Five to 6 mm mycelial fragments were taken from the middle portion of the colony and transferred to another mycological agar plate previously impregnated with each pesticide concentration. Care was taken to turn the fungal fragments upside down, so that the mycelia would come in contact with the pesticide impregnated medium. The same procedure was performed with the controls in pesticide free agar plates.

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Three replicates in time were done for each treatment and plates incubated at 26°C for 2 to 3 weeks. Alterations in the colony morphology were noted, microscopic samples stained with 1% acid fuchsin, and observed under a bright field microscope.

RESULTS

The four pesticides assayed showed viability inhibition on both strains of *V. lecanii* as showed in Table 1.

Both strains of *V. lecanii* developed the typical fungal morphology in agar plates without pesticides, forming white colonies of moderate growth, ca. 3 cm in diameter after 10-12 days of incubation at 26°C, turning yellowish with aging, but without diffusible pigment to their reverse (Fig. 1). The somatic hyphae were hyaline and the conidiophores were erect, with verticillate branches along their axes, bearing solitary or clustery phialides, usually divergent, with mucilaginous conidial heads at their tips; conidia were cylindrical or ellipsoidal, with rounded ends, 2.3-10.0 X 1.0-2.5 µm (Fig. 2). In pesticide impregnated agar plates, *V. lecanii* morphology was altered. Benomyl completely inhibited growth of both fungal strains. The three insecticides used, at all concentrations tested, caused a change in colony color, from the characteristic white to a light cinnamon hue, and in colony consistency, from cottony to a more compact, hardened, sclerotioid mass of mycelia, without conidia, and with hyphae united in synnemata-like structures that lacked phialides (Figs. 3-4). After 20-25 days, when these altered cultures were transferred to mycological agar, containing 0.25% yeast extract but no pesticides, morphology reverted to typical *V. lecanii* colonies.

DISCUSSION

The results of this study showed that pesticides commonly used in the field in Mexico for the chemical control of whiteflies and the fungicide are toxic for the natural bioregulator *V. lecanii*. The difference of compatibility shown by the two studied strains leads to consider the relevance and convenience of testing chemical pesticide compatibility with strains of different origins.[13,15,23,27] A point of discussion in pest control management programs should be the selection of fungal strains compatible with chemical pesticides when applied in combination with entomopathogenic fungi.

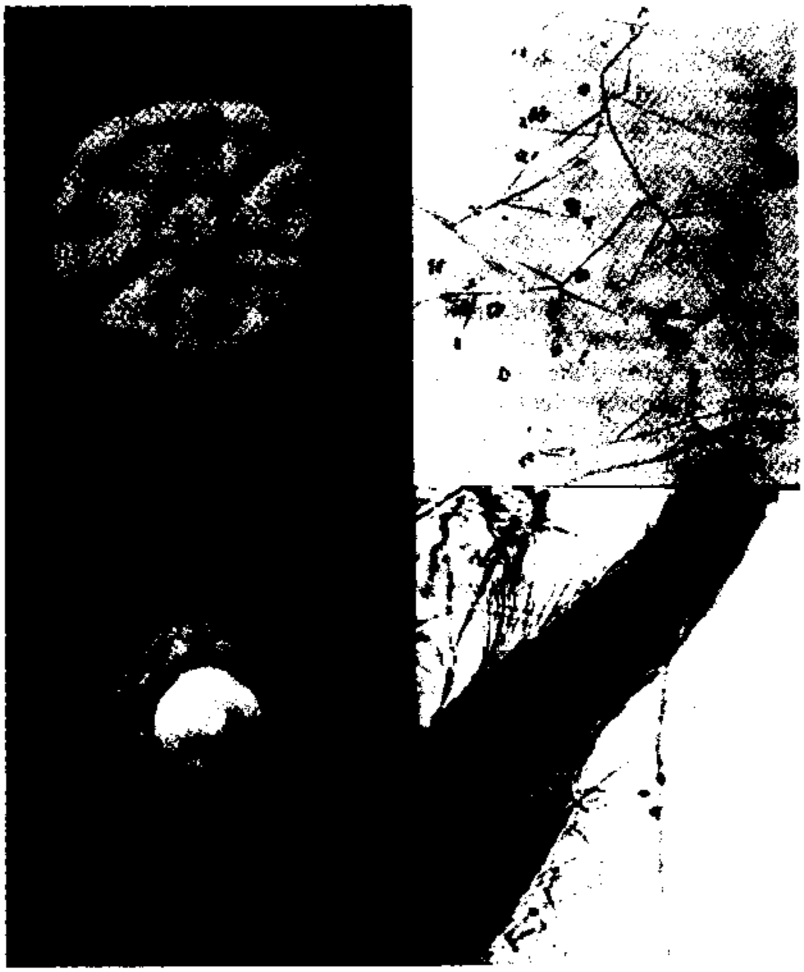
Lambda cyhalotrin and benomyl were the most toxic for both strains of this fungus, even in a lower concentration than that recommended for field use. Olmert & Kenneth[16] also showed 100% growth inhibition of *V. lecanni* isolates from Israel, by a poisoned-bait method, at the fungicides recommended field dose plus two more 1/10 dilutions. Carrión *et al.*[4] showed that two fungicides (triadimephon and copper oxychloride) used for coffee rust control in Mexico were toxic for *V. lecanii*. Benomyl incompatibility with other entomopathogenic fungi has also been demonstrated.[10,21,26] Based on these reports and the results of this study, with two strains of *V. lecanii* isolated in Latin America, carefull surveillance of the applications of this chemical for phytopathogenic fungi control should be performed. Its effect upon both entomopathogenic natural bioregulators and soil fungi is particularly harmful.

According to these results lambda-cyhalotrin had the same deleterious effect on the *V. lecanii* strains tested as benomyl, reaching near 100% inhibition with the three tested concentrations. Therefore, care should be taken to avoid its use when this fungus is naturally present, or if it

Table 1. Viability inhibition percentages by pesticides on strains A and C of *Verticillium lecanii.*

	Viability inhibition percentages		
Pesticides	Conc I X ± SD[a]	Conc II X ± SD	Conc III X ± SD
Strain A			
Benomyl	88.50 ± 4.83ab[b]	95.90 ± 5.68ab	97.90 ± 2.85a
Lambda-cyhalothrin	99.20 ± 0.58a	99.90 ± 0.02a	100a
Permethrin	17.70 ± 8.46d	44.80 ± 2.64cd	76.90 ± 20.56abc
Methamidophos	50.00 ± 23.10bcd	47.60 ± 20.87cd	59.80 ± 20.52bc
Strain C			
Benomyl	86.00 ± 5.05A	96.00 ± 1.57A	99.50 ± 0.64A
Lambda-cyhalothrin	99.00 ± 0.61A	100A	100A
Permethrin	25.40 ± 7.58C	78.50 ± 6.45AB	90.70 ± 9.17A
Methamidophos	28.90 ± 12.23C	29.70 ± 13.15C	58.10 ± 12.06B

[a] X ± SD = Mean ± standard deviation. [b]Means with the same letter are not significantly different (Tukey's, $P<0.05$). Lower-case letters for strain A and upper-case letters for strain C.



Figs. 1-4. Morphological characteristics of *V. lecanii* with and without methamidophos (the least toxic insecticide). 1. Fungal morphology in a mycological agar plate without the insecticide, showing a white cottony colony. 2. Typical fungal microscopic morphology from the agar plate without insecticide, showing the characteristic hyaline hyphae, erect conidiophores with verticillate branches along their axes, bearing phialides with mucilaginous conidial heads and conidia (X 342). 3. The fungus with the insecticide impregnated medium showing a compact, hard colony. 4. Microscopic morphology from the fungus grown in insecticide impregnated medium showing hyphae united in synnemata-like structures without phialides (X 342).

is introduced by spraying on crops or ornamentals attacked by whiteflies. Furthermore, this insecticide's DT_{50} in water-sediment mixtures in sunlight is 20 days, and in soil 22-82 days,[29] which would suggest negative side-effects for naturally-ocurring *V. lecanii* in agroecosystems.

Among the four pesticides tested, permethrin and methamidophos showed the least inhibition effect, on both strains. At the recommended field concentration there is a difference in strain sensitivity to permethrin and methamidophos. This has been also shown by three strains of *V. lecanii* with different fungicides and insecticides.[16] In México, methamidophos is widely used in chemical control of whiteflies. Although its toxicity for the fungus is not as high as lambda-cyhalotrin, its mammalian toxicity according to the acute oral LD_{50} in rats[29] is the highest of the four pesticides assayed.

The morphological alterations observed in the pesticides impregnated agar plates were reverted when the fungus was grown on media without the pesticides, suggesting that the morphological abnormalities observed were physiological adaptations, and that no inherited damage was present, at least in the short term of this study. It is interesting to note that Carrión *et al.*[4] observed that this fungus produced sclerotia, which are fungal structures developed during unfavorable conditions, when *V. lecanii* was grown in fungicide impregnated agar plates.

It is a matter of concern that toxic chemical pesticide users in Latin America are not fully instructed in the correct concentration and management of these chemicals, causing severe damage to humans as well as a negative ecological impact. Pest management programs should include the correct application techniques to all users. Fi-

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nally, the convenience of laboratory and field studies concerning the influence of these chemicals upon soil mycobiota and natural fungal bioregulators is strongly suggested.

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