In vitro compatibility of *Isaria fumosorosea* (Wize) Brown y Smith (Hypocreales: Clavicipitaceae) with commercial plaguicides

Compatibilidad *in vitro* de *Isaria fumosorosea* (Wize) Brown y Smith (Hypocreales: Clavicipitaceae) con plaguicidas comerciales

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Abstract

Colombian farmers use pesticides with toxicological category I, II and III to control the whitefly *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) in cotton and eggplant crops; although, its indiscriminate use offers a risk for the environment and the people. The use of biological control with entomopathogenic fungi can be an alternative to replace this is the use of biological control as the entomopathogenic fungi. However, the successful use of a biopesticide in an integrated pest management (IPM) would depend largely on the effect of pesticides on the entomopathogenic microorganism. The aim of this study was to determine the *in vitro* compatibility of a biopesticide based on the fungi *Isaria fumosorosea* formulated as a wettable powder (WP) to control the whitefly *Bemisia tabaci* with four fungicides and five insecticides currently used in cotton and eggplant crops. For this, the effect of the pesticides was evaluated at three concentrations (FR = Field recommendation; 0.5 x FR and 0.25 x FR). Effects of these products on conidia germination and the number of colony forming units (CFU)/g were compared. The four fungicides (Benlate, Carboxin Captan, Metalaxyl Mancozeb and Mancozeb) at the three doses evaluated were incompatible with the biopesticide, while one insecticide (Thiamethoxam) was compatible with the biopesticide when 0.25 x FR of its dose was tested.

Key words: Biopesticide, entomopathogenic fungi, *Isaria fumosorosea,* pesticides synergists,whitefly.

Resumen

Los agricultores colombianos utilizan productos químicos con categorías toxicológicas I, II y III para el control de insectos como la mosca blanca *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae); pero su uso indiscriminado genera un riesgo para la salud y el medio ambiente. El control microbiológico con hongos entomopatógenos surge como alternativa de control ambientalmente sostenible. No obstante, su empleo exitoso dentro de una estrategia de manejo integrado de plagas (MIP) depende, en gran parte, del efecto de los productos químicos sobre el bioplaguicida. El objetivo del presente trabajo fue determinar la compatibilidad *in vitro* de un bioplaguicida con base en el hongo *Isaria fumosorosea* formulado como polvo mojable con cuatro fungicidas y cinco insecticidas comerciales. Para esto, se evaluó el efecto de tres concentraciones de los productos químicos: FR = dosis recomendada en campo, 0.5 x FR y 0.25 x FR sobre la germinación de los conidios y el número de unidades formadoras de colonia UFC/g de *I. fumosorosea*. Los cuatro fungicidas (Benlate, Carboxin Captan, Metalaxil-Mancozeb y Mancozeb) en las tres dosis evaluadas fueron incompatibles con el bioplaguicida, mientras que el insecticida Thiametoxam fue compatible cuando se utilizó la dosis de 0.25 x FR.

Palabras clave: Bioplaguicida, hongos entomopatógenos, *Isaria fumosorosea,* mosca blanca, sinergismo de los plaguicidas.

Introduction

The whitefly *Bemisia tabaci* (Genandius) (Homoptera: Aleyrodidae) is one of the major pests worldwide because it is widely distributed in various field crops (Ahmed *et al.*, 2009). In Colombia, its control mainly depends on the use of insecticides of chemical origin belonging to the group of organophosphates, carbamates, pyrethroids and neonicotinoids with toxicological category of types I, II or III, which are harmful to human and animal health.

Studies by Roditakis *et al.* (2005) and Yuan *et al.* (2012) show that whitefly is resistant to neonicotinoids such as imidacloprid, organophosphates such as methyl pirimiphos, carbamates as carbosulfan and pyrethroids such as cypermethrin and bifenthrin. This situation demonstrates the importance of seeking environmentally sustainable alternatives with less impact on health and that doesn't economically affect farmers, being the Integrated Pest Management (IPM) schemes an option that combines chemical, cultural and biological strategies –within which are biopesticides- to reduce the frequency of application of chemical products and maintain populations considered pests within a range that does not economically affect crop yields. Among the prospects in the industry of biopesticides, are presented as challenges increasing productivity and yield per unit area, lower pesticide residues in food and promote new alternatives for environmentally sustainable control; challenges that can only be met if this tool is set within IPM programs (Marcus, 2009).

One of the main limitations of biological products when used in the field is the incompatibility posing with supplies applied within traditional farming practices; therefore, if the marketing of organic products and their integration into IPM programs is sought, it is necessary to determine the compatibility of isolates that constitute the active ingredient of these biopesticides with agrochemicals. These assessments should be performed for each isolate individually, since even differences arise between various isolates of the same genus, as a result of genetic variation (Cantone and Vandenberg, 1998) and experimental protocols.

In research conducted by CORPOICA (Colombian Corporation for Agricultural Research) (Espinel *et al.*, 2006) it was found that the formulations based on the entomopathogenic fungus *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) used to control *Bemisia tabaci* resulted in greater efficiency and profitability of the tomato crop, compared with those obtained with the traditional handling by the farmer. This product was also evaluated under field conditions on cotton in Tolima and Córdoba and eggplant in Huila and Córdoba. The biopesticide in both cases and for both crops showed a benefit ratio: cost > 1, which indicates that it is an economically possible option to manage whiteflies.

Considering the prospects and potential of the biopesticide based on *I. fumosorosea*, this study proposes as an objective to determine its viability in vitro, expressed as the germination percentage and the number of colony forming units (CFU), compared to full dose (100%), medium (50%) and a quarter (25%) of the recommended for five and four insecticides and four fungicides commonly used in cotton and eggplant crops.

Materials and methods

Tested agrochemicals

The selected chemicals are often used by farmers in the departments of Tolima, Huila and Córdoba, for conventional pest and diseases in commercial crops (Table 1).

Table 1. Agrochemicals evaluated for compatibility with the *I. fumosorosea*-based biopesticide.

Product (commercial name)	Active ingredient	Chemical class	Action mode	100% dose (ppm)	50% dose (ppm)	25% dose (ppm)
Fungicides						
Benomil	Benlate	Benzimidazol	Systemic	500	250	125
Vitavax	CarboxinCaptan	Carboxanilides & dithiocarbamates	Systemic	4000	2000	1000
Ridomil	Metalaxil - Mancozeb	Acylalanine	Systemic & contact	8500	4250	2125
Manzate	Mancozeb	Dithiocarbamate	Contact	8000	4000	2000
Insecticides						
Confidor	Imidacloprid	Chloronicotinyl	Contact	380	190	95
Oportune	Buprofezin	Thiodiazine	Contact	750	375	187.5
Actara	Tiametoxam	Neonicotinoid	Systemic & translaminar	1875	937.5	468.75
Malathion	Malathion	Organophosphate	Contact	5700	2850	1425
Match	Lufenuron	Acylureas	Ingestion	125	62.5	31.25

Biological product evaluated

The biopesticide employed presented the characteristic of a wettable powder based on conidia of the entomopathogenic fungus *I. fumosorosea* (isolation Pc 013), with a concentration of 3.62 x 10^9 conidia/g, 80% germination after 24 h incubation at a temperature of 25 ± 2 °C, and 6.02 x 10^9 CFU/g after 8 days of incubation under the same conditions of temperature. The particle size of this powder was equal to or less than 100 microns and humidity < 5%. Inert ingredients of the formulation corresponded to a sunscreen (Uv01), a drying protector (Ps01) and a diluent (Di03).

Viability of the biopesticide active ingredient in agrochemicals

To determine the viability in solution, the biopesticide was suspended in a solution of Tween 80 and inoculated on the culture medium containing agrochemicals. As experimental unit a Petri dish with agar medium and the agrochemical Saboureaud was used, which was previously reconstituted in water at a concentration of 100,000 ppm and added in the middle before it solidified. The concentration of the agrochemical in the medium was adjusted to 100%, 50% and 25% of the dose recommended by the manufacturer (Table 1). As control treatment the germination of the biopesticide in Agar Saboureaud, without the presence of the agrochemical was included.

For the preparation of solutions, the biological product was suspended in a solution of 0.1% Tween 80 and from it serial dilutions were performed 10^{-1}, 10^{-3} and 10^{-4} ml which were applied over the Petri dishes. Each Petri dish with the respective treatment was incubated at a temperature of 25 ± 2 °C for 24 h; after this time a sample of agar of 1 cm^2 was removed and a drop of lactophenol blue was added to perform the reading of the germinated and non-germinated conidia in ten optical fields or until a total of 100 conidia per experimental unit to calculate the percentage of germination (Santos *et al.*, 2012). The experimental design was randomized with three replicates per treatment and three experimental units.

To determine the viability of the biopesticide in agrochemicals by the formation of colony forming units (CFU), a Petri dish with agar-potato-sucrose medium with the selected agrochemical was used as experimental unit and as in the previous assay, three concentrations were assessed for each product (Table 1). From the serial dilutions, 0.1 ml of the dilution

corresponding to 10^{-8} was inoculated in Petri dishes. Each experimental unit was incubated at 25 ± 2 °C for eight days, time after which CFU count was performed on each box. The experimental design used was a complete randomized with three replicates per treatment.

Statistical analysis

The data of the results obtained for the viability expressed as CFU were transformed with the logarithmic function base 10. The results were normally distributed (Shapiro Wilk, α = 0.05) and presented homogeneous variances (Barlett, α = 0.05), therefore were subjected to an Anova test and Tukey (α = 0.05) comparison of means using the Statistix program version 7.0 -2000 (Analitycal Software, Florida, USA).

To determine the effect of the agrochemicals on the two evaluated variables, germination and vegetative growth expressed as CFU, Alves classification (1998) cited by Neves *et al.* (2001) was followed, which is based on the mean values of the percentage of germination and the percentage of vegetative growth (CFU) for each treatment compared to the control, using the following formula:

$$T = \frac{20(VG) + 80(SP)}{100}$$

where *T* is the corrected value for vegetative growth and reproductive growth for the classification, VG is the percentage of vegetative growth (CFU) compared to the control and SP is the sporulation percentage compared to the control.

Results and discussion

With the fungicides Benlate and Carboxin-Captan the fungus germination percentage varied according to the dose applied (Table 2), however with the three doses, significantly lower values were obtained compared to the control treatment. Germination results obtained in this study were higher than those reported by Garcia *et al.* (2007), who evaluated Benlate in the same doses as in this report, on unformulated conidia of *Trichoderma koningii* Oudem and after 24 h found that germination of conidia was < 10% at all doses tested. Moreover, the results obtained with the fungicide Captan-Carboxin differ from those found by Bruck (2009), who when evaluating the effect of this fungicide on conidia of *Metarhizium anisopliae* (Metchnikoff) Sorokin found 100% inhibition of germination. Kubilay and Gökce (2004) evaluated the effect of Benlate and Captan at concentrations of 500 and

Table 2. Effect of the dose of agrochemicals on the germination and CFU formation of *I. fumosorosea.*

Agrochemicals	**Dose**							
	Control	**100%**	**50%**	**25%**	**Control**	**100%**	**50%**	**25%**
	Germination (%)				**CFU/g**			
Imidacloprid	80	23.46 b*	25.27 b	26.46 b	9.78	9.83 a	9.77 a	9.78 a
Buprofezin		16.15 c	19.39 bc	26.51 b		9.55 b	9.67 a	9,68 a
Malathion		25.61 b	39.50 ab	43.45 ab		0 b	0 b	0 b
Tiametoxam		20.88 c	28.61 c	51.38 b		9.66 b	9.72 a	9.70 a
Lufenuron		7.64 b	8.00 b	11.38 b		9.79 a	9.71 a	9.78 a
Benlate		14.16 c	17.74 b	20.46 b		0 b	0 b	0 b
Carboxin Captan		18.36 b	25.26 b	41.04 ab		0 b	0 b	0 b
Mancozeb		0 b	0 b	0b		0 b	0 b	0 b
MetalaxilMancozeb		0 b	0 b	0b		0 b	0 b	0 b

*Values in the same row with different letters indicate differences with respect to the control (P <0.05), according to the Tukey test.

2500 ppm, respectively, on two isolates of *Paecilomyces fumosoroseus* (Wize); Brown & Smith, finding that Captan completely inhibited germination of conidia, while Benlate reduced germination to 34%. The differences between the germination results obtained in this study and those done by other researchers (Kubilay and Gökce, 2004; Garcia *et al.*, 2007; Bruck, 2009) with other entomopathogenic fungi or even with other isolates of *I. fumosorosea*, show that each isolate behaves differently and results cannot be generalized, therefore it is important to conduct compatibility tests for each strain and formulation developed.

The results of the *in vitro* application of the fungicides Benlate and Carboxin-Captan on CFUs, showed that the mycelial development of *I. fumosorosea* presented a higher sensitivity than the germination of the conidia to the action of these products. These results agree with those obtained by Bruck (2009) who, when evaluating the effect of some fungicides on germination and radial growth of *M. anisopliae*, noted that mycelial growth was more sensitive to the effect of fungicides than germination.

The fungicide Benlate and its major metabolite Carbendazim, interfere with certain processes of the cell as DNA synthesis, mitosis and the mechanism of transmission of genetic messages from DNA to RNA (Liñan, 1997) and the fungicide Carboxin-Captan react with sulfhydryl enzymes to produce thiophosgene, toxic to fungal cells which interferes with the process of cellular respiration (Liñan, 1997). Other fungicides such as Mancozeb and Metalaxyl-Mancozeb applied in similar doses to those of this study completely inhibited germination and formation of CFUs. It is known that the active ingredient Mancozeb alters cellular respiration, breaking the Krebs cycle in several of its stages; while Metalaxil inhibits protein synthesis and prevents the development and growth of the mycelium (Liñan, 1997).

Insecticides tested presented a minor effect on the biopesticide, compared to the fungicides (Table 2), results that coincide with those of Kubilay and Gökce (2004) who, when assessing the effect of five fungicides and six insecticides on two isolates of *P. fumosoroseus* found that these were more sensitive to the application of fungicides, possibly because the insecticides may target specific insect processes that do not directly affect fungi, in contrast to the fungicides that directly affect processes of their metabolism.

In general, it was observed that the effect of insecticides was greater on germination than on CFU. Griffin (1994) considers that many pesticides that do not completely inhibit conidia or hyphae, over time may reduce the effect of these, which may explain some of the findings in this study. This effect was evident with the product Imidacloprid, which interferes with the transmission of stimuli when exciting the nerve cells of insects due to of its union with acetylcholine receptors (Liñan, 1997); this was evident when the formation of CFU was assessed as no differences were observed compared to the control ($P > 0.05$); contrary to the effect that it presented on germination ($P < 0.05$).

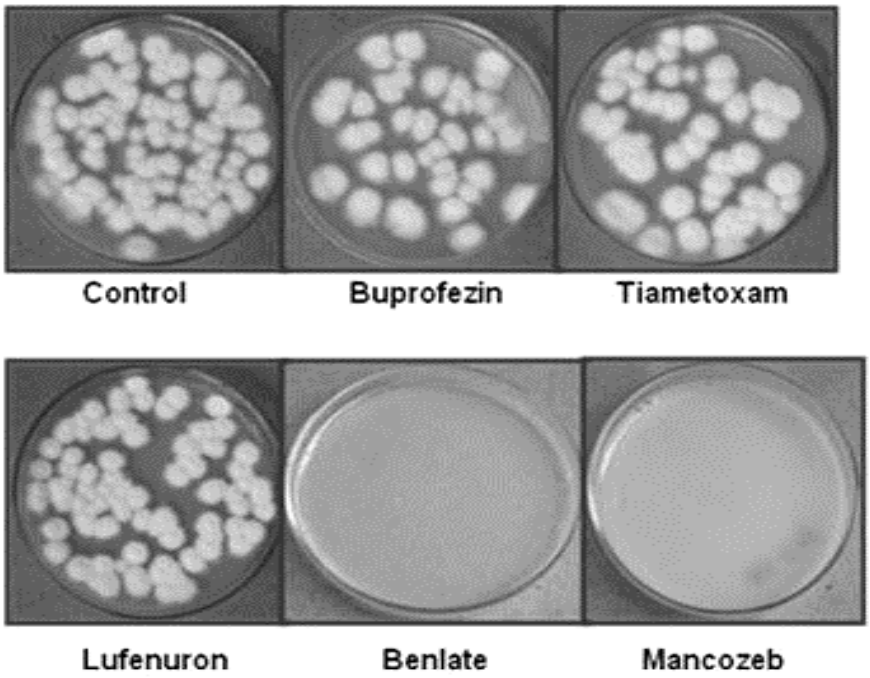

Photo 1. Viability expressed as CFU/g for the *I. fumosorosea*-based biopesticide.

Table 3. *In vitro* compatibility of the agrochemicals with the *I. fumosorosea*-based biopesticide, according to the classification of Alves (1998)a.

Agrochemical	100% dose		50% dose		25% dose	
	***T* Value**	**Classification[b]**	***T* Value**	**Classifiction**	***T* Value**	**Classification**
Imidacloprid	43.46	I	45.25	I	46.46	M T
Buprofezin	35.67	I	39.16	I	46.30	M T
Malathion	25.61	I	39.50	I	43.45	I
Tiametoxam	40.63	I	48.49	MT	71.22	C
Lufenuron	27.64	I	27.85	I	31.38	I
Benlate	14.16	I	17.74	I	20.46	I
Carboxin Captan	18.36	I	25.26	I	41.04	I
Mancozeb	0	I	0	I	0	I
Metalaxil Mancozeb	0	I	0	I	0	I

a. Alves (1998), cited by Neves *et al.* (2001).

b. I = very toxic (0-30) and toxic (31-45), MT = moderately toxic (46-60), C = compatible (> 60).

T value = corrected value of the reproductive and vegetative growth for the classification.

The Buprofezin insecticide, which inhibits the synthesis of chitin in the cuticle of insects (Liñan, 1997) may also interfere in some way in the process of germination of conidia, because when it starts in the endoplasmic reticulum, chitin and glucan are made, which are required for cell wall formation. However, in doses of 100% and 50% CFU formation was not significantly different from control, possibly due to reduced insecticidal effect over time, as mentioned above. Similarly, the *I. fumosorosea*-based product showed greater sensitivity to the insecticide Thiamethoxam, especially during germination; while for CFU only differences ($P < 0.05$) occurred when the total dose (100%) was applied. With the insecticide Malathion, that inhibits cholinesterase by phosphorylation of acetylcholinesterase (Becker *et al.*, 2003),

no colony formation was observed with the three doses tested (Figure 1); however for germination only differences (P <0.05) compared to the control occurred when the recommended commercial dose was evaluated. The results obtained in CFU formation are similar to those achieved by Kubilay and Gökce (2004) who, in addition to this last finding, observed that a dose of 3170 ppm of this insecticide completely inhibited the radial growth of two isolates of *P. fumosoroseus*, currently classified as *Isaria fumosorosea*.

In Table 3, the *T* value or corrected value for the vegetative and reproductive growth, and the compatibility classification of the *I. fumosorosea*-based biopesticide with the agrochemicals evaluated are presented, based on the results obtained with each the agrochemical for germination and the number of CFU/g. It was noted that there was a lower *T* value with the fungicides compared to the calculated values for insecticides, indicating that fungicides are more toxic to the conidia of the *I. fumosorosea*-based biopesticide in comparison to the insecticides evaluated. Thus, the lowest values for *T* were obtained for Benlate, Carboxin-Captan, Mancozeb Metalaxyl-Mancozeb and therefore are incompatible with the biopesticide in the three doses tested.

Regarding the insecticides, it was observed that the product based on *I. fumosorosea* showed the lowest values for *T* with the insecticides Malathion and Lufenuron, followed by Buprofezin and Imidacloprid, to which it was less sensitive. The five insecticides evaluated are incompatible with biopesticide based on *I. fumosorosea* when the full dose was used. Only Thiamethoxam presented a moderately toxic effect when employed at a 50% dose was compatible with the 25% dose. Furthermore, Imidacloprid and Buprofezin were moderately toxic at a 25% dose.

Conclusions

- Under laboratory conditions, the biopesticide based on I. fumosorosea evaluated in this study was incomplatible with the fungicides Benlate, Carboxin-Captan, Mancozeb and MetalaxilMancozeby and with the insecticide Imidacloprid, Buprofezin, Malathion, Thiamethoxam and Lufenuron at the recommended dose.

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