# Effects of the combination of phytosanitary products and entomopathogenic fungi for the control of insect pests

## Efectos de la combinación de productos fitosanitarios y hongos entomopatógenos para el control de insectos plagas

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## Abstract

The combination of phytosanitary products (PPs) and entomopathogenic fungi (EF) can be used in integrated pest management (IPM) programs. The aim of the present work was to study the effects of PPs, commonly used in Argentina and Brazil, combined with *Beauveria bassiana*, *Metarhizium anisopliae* and *Cordyceps (Isaria) fumosorosea*, for the control of insect pests in the post-harvest of wheat, under laboratory and semi-field conditions. *In vitro* compatibility was assessed using the biological index (BI) based on the percentages of fungal vegetative growth, sporulation, and germination. The number of colony-forming units (CFUs) was evaluated under semi-field conditions. In all cases, PPs evaluated reduced the mean germination percentages as compared with the control. According to the BI, none of the treatments evaluated were compatible with any of the three isolates, except for the treatment L7 of Lambdacialotrine, which was moderately toxic on *B. bassiana*. Evaluated PPs did not affect the number of CFUs of *B. bassiana* or *C. fumosorosea* with respect to the control. In contrast, PPs affected the number of CFUs of *M. anisopliae*, independently of the persistence time of fungi on the grains. PPs and EF should be applied separately under semi-field conditions, however, further research under field conditions should be conducted to confirm the compatibility within an IPM strategy.

**Keywords:** entomopathogenic fungi, integrated pest management, phytosanitary products, pirimiphos methyl, postharvest treatment.

## Resumen

La combinación de productos fitosanitarios (PFs) y hongos entomopatógenos (HE) puede ser utilizada en programas de manejo integrado de plagas (MIP). El objetivo del presente trabajo fue estudiar los efectos de los PFs comúnmente utilizados en Argentina y Brasil combinados con *Beauveria bassiana*, *Metarhizium anisopliae* y *Cordyceps (Isaria) fumosorosea* para el control de plagas de insectos en la poscosecha de trigo, bajo condiciones de laboratorio y semi-campo. La compatibilidad entre los diferentes tratamientos se evaluó *in vitro* utilizando el índice biológico (IB) basado en los porcentajes de crecimiento vegetativo, esporulación y germinación del hongo. El número de unidades formadoras de colonias (UFC) se evaluó en condiciones de semicampo. En todos los casos, los PFs evaluados redujeron los porcentajes medios de germinación, en comparación con el control. Según el IB, ninguno de los tratamientos evaluados fue compatible con los tres aislamientos, excepto el tratamiento L7 de Lambdacialotrina, que resultó moderadamente tóxico sobre *B. bassiana*. Los PFs evaluados no afectaron el número de UFC de *B. bassiana* o *C. fumosorosea* con respecto al control. Por el contrario, los PFs afectaron el número de UFC de *M. anisopliae*, independientemente del tiempo de persistencia de los hongos sobre los granos. Los PFs y los HE deben aplicarse por separado en condiciones de semicampo; sin embargo, se deben realizar más investigaciones en condiciones de campo para confirmar la compatibilidad dentro de una estrategia de MIP.

**Palabras clave:** hongos entomopatógenos, manejo integrado de plagas, pirimifos metil, productos fitosanitarios, tratamiento poscosecha.

## Introduction

The excessive use of phytosanitary products (PPs) in agriculture has led to the development of insecticide resistance, the generation of chemical residues, and the elimination of beneficial insects, as well as to environmental pollution and human toxicity. However, the demand for healthy and contamination-free food has promoted the development of alternative control measures for pest and disease control (Lacey *et al.*, 2015; Nguyen *et al.*, 2015; Potrich *et al.*, 2018).

Among these alternative control measures, entomopathogenic fungi (EF) are considered important to control and reduce pest populations in the post-harvest of cereal Fcrops. However, they cannot totally replace synthetic chemical insecticides. Thus, several studies have evaluated the combination of EF and chemical insecticides to be used in integrated pest management (IPM) programs (Lacey *et al.*, 2015; Dal Bello *et al.*, 2018; Fregonesi *et al.*, 2016). This alternative would allow the reduction of insecticide application due to the presence of substances contained in PPs, which act as insect stressors that stimulate fungal infection and thus increase the efficiency of pest control. It may also minimize the dangers for human and animal health and environmental contamination because it would imply applying low doses or subdoses of insecticides (Moino and Alves, 1998).

However, PPs can also act deleteriously on microorganisms. In particular, the toxicity of these products on EF may vary with the fungal species and strains, the chemical nature of the active ingredient, the action mode, and the product formulation and doses, as well as with the environmental conditions (Pessoa *et al.*, 2020). These factors can inhibit fungal vegetative growth and conidial survival, prevent the occurrence of genetic mutations, and alter the virulence of the EF (Alves *et al.*, 2008; Oliveira *et al.*, 2018). Thus, before recommending a specific PP to be used in combination with an EF, the action of these products on the microorganisms requires the knowledge and evaluation of the compatibility between them (Lacey *et al.*, 2015; Fregonesi *et al.*, 2016).

Based on this, the aim of the present work was to study the effects of two PPs commonly used in Argentina and Brazil combined with three EF (*Beauveria bassiana*, *Metarhizium anisopliae* and *Cordyceps (Isaria) fumosorosea*), for the control of insect pests in the post-harvest of wheat, under laboratory and semi-field conditions.

## Materials and methods

The study was carried out at the Reference Laboratory Unit (RLU) for Biological Control of the Advanced Center for Research in Plant Protection and Animal Health of the Instituto Biológico, located in Campinas, São Paulo, Brazil.

## Entomopathogenic fungi (EF)

EF used in this study were obtained from the collection of entomopathogenic fungi "Oldemar Cardim de Abreu" from the RLU for Biological Control: *Beauveria bassiana* isolate IBCB 66, *Metarhizium anisopliae* isolate IBCB 425, and *Cordyceps (Isaria) fumosorosea* isolate IBCB 130 (Table 1). The isolates were cultured on potato dextrose agar (PDA) and incubated at $25 \pm 2$ °C and a photoperiod of 12:12 h (light: darkness) for 14 days until obtaining a matrix plate.

## Phytosanitary products (PPs)

The PPs used in this study are commonly recommended for the control of insect pests in post-harvest in both Argentina and Brazil (Table 2). The following five PP treatments were evaluated: Pirimiphos methyl 6 mL $L^{-1}$ (PM6), Pirimiphos methyl 10 mL $L^{-1}$ (PM10), Pirimiphos methyl 16 mL $L^{-1}$ (PM16), Pirimiphos methyl 8 mL $L^{-1}$ + Lambdacialotrine 2 mL $L^{-1}$ (PM8+L2), and Lambdacialotrine 7 mL $L^{-1}$ (L7).

## Bioassay 1: Assessment under *in vitro* laboratory conditions

### Broth test: Germination bioassay (conidia + PPs)

In the first *in vitro* experiment, each PP was dissolved in 0.01 % (v/v) Tween 80 (sodium polysorbate) in sterile distilled water and then combined with conidia of *B. bassiana*, *M. anisopliae* or *C. fumosorosea*, mixed separately. The control had conidia without PPs. Then, this mixture (conidia + PPs) was vortexed for 2 min to homogenize the suspension. One hour later, in absolute rest at room temperature, a 0.1 mL aliquot from each mixture was spread with a Drigalsky loop onto the PDA contained in five sterile Petri dishes (90 mm in diameter). The experiment was repeated twice (n = 10 replicates). The dishes were incubated at $25 \pm 2$ °C and a photoperiod of 12:12 h (light: dark). After 18 h of incubation, the germination percentage was calculated (adapted from Rossi-Zalaf *et al.*, 2008).

**Table 1.** Hosts and origin of the fungal isolates used

| Isolate | Species | Original host | Original place |
| --- | --- | --- | --- |
| IBCB 66 | *Beauveria bassiana* | *Hypothenemus hampei* | São José do Rio Pardo – SP |
| IBCB 425 | *Metarhizium anisopliae* | Soil sample | Iporanga – SP |
| IBCB 130 | *Cordyceps fumosorosea* | Soil sample | Florínia – SP |

## Compatibility calculation of the biological index (BI): Vegetative growth (colony diameter), sporulation and germination percentage bioassays

The compatibility between the different PP treatments and the EF chosen was determined *in vitro*. To this end, PPs were incorporated into the autoclaved PDA before it solidified, at approximately 40 – 45 °C. The mixture was poured into five disposable sterilized Petri dishes. After solidification, the *B. bassiana*, *M. anisopliae* and *C. fumosorosea* isolates were inoculated with a platinum loop at three equidistant points per dish on the surface of the medium. The control had PDA without PPs. The experiment was repeated twice. The dishes were incubated at 25 ± 2 °C and a photoperiod of 12: h (light:dark). After 14 days of incubation, vegetative growth was evaluated by measuring the diameter in two perpendicular senses in each colony to obtain the mean diameter.

The fungal colonies described in the previous bioassay were cut with a scalpel and transferred individually to a sterile glass tube containing 10 mL of 0.01 % (v/v) Tween 80 (sodium polysorbate) in sterile distilled water. The conidia of each colony were dislodged from the PDA by vortexing for 2 min. Successive dilutions were performed until the desirable suspension was obtained to quantify sporulation using a hemocytometer (Neubauer chamber), and germination percentages were calculated using the same methodology described above (Adapted from Rossi-Zalaf *et al.*, 2008).

The compatibility between the evaluated PPs and EF was calculated based on the BI, according to Rossi-Zalaf *et al.* (2008), as follows: BI = [47(VG) + 43(ESP) + 10(GER)]/100, where BI = biological index, based on the percentage of vegetative growth (colony diameter) (VG), the percentage of sporulation (ESP), and the germination percentage (GER) of fungal colonies in relation to the respective control. The limits established were: Toxic: 0 – 41; moderately toxic: 42 – 66; and compatible: > 66.

## Bioassay 2: Assessment under semi-field conditions

### Colony forming units (CFUs)

Conidia were inoculated on the surface of 15 g of wheat grains onto Petri dishes by spraying them with 1 mL of *B. bassiana*, *M. anisopliae* or *C. fumosorosea* at a concentration of 2 x $10^8$ conidia mL$^{-1}$ using a Potter tower (Burkard Manufacturing Ltd., Mod. 1, England). After 1 h, all the PP treatments were sprayed in the same way. The control was sprayed with each fungal suspension and 0.01% (v/v) Tween 80 (sodium polysorbate) in sterile distilled water. After application, Petri dishes were incubated at 25 ± 2 °C and a photoperiod of 12:12 h (light: dark) (the same conditions as described above), and then evaluated for 24, 48 and 72 h (times of persistence of EF on grains). Then, 1 g of wheat grains was collected at random from each Petri dish, diluted with 10 mL 0.01 % (v/v) Tween 80 (sodium polysorbate) in sterile distilled water and vortexed for 2 min for homogenization. Then, 0.1 mL of each dilution was inoculated with a Drigalsky loop on the surface of Petri dishes containing PDA supplemented with 0.5 mg L$^{-1}$ of pentabiotic. Five replicates were used, and the experiment was repeated twice. The plates were incubated for 4 days at the same conditions described in the previous assay. After this period, colonies were counted and the number of CFUs was quantified.

### Statistical analysis

For each isolate, germination percentage, colony diameter, sporulation, and number of CFUs were analyzed. Differences between PP treatments were verified assessing whether the data met the assumptions of normality and homogeneity of variance with the Shapiro-Wilk and Levene's tests, respectively. In the case of reaching the assumptions, an ANOVA F-test was used, whereas in the case of not reaching the assumptions, the Welch's t-test (with

**Table 2.** List of phytosanitary products used for the control of insect pests in post-harvest in Argentina and Brazil

| Origin | Active ingredient (a. i.) | Chemical group | Trade name | Formulation type | Recommended dose[a] |
|---|---|---|---|---|---|
| **Argentina** | Pirimiphos methyl | Phosphorous | ACTELLIC 50® | EC[b] | 6-10 mL L$^{-1}$ |
| | Pirimiphos methyl + Lambdacialotrine | Phosphorous + Pyrethroid | ACTELLIC PLUS® | EC + SC | Part 1: 8 cm$^3$ L$^{-1}$ + Part 2: 2cm$^3$ L$^{-1}$ |
| **Brazil** | Pirimiphos methyl | Phosphorous | ACTELLIC 500® | EC | 8-16 mL L$^{-1}$ |
| | Lambdacialotrine | Pyrethroid | ACTELLICLAMBDA | SC | mL L$^{-1}$ |

**Note.** [a] Information provided by the manufacturers. [b] EC = emulsifiable concentrate, SC = suspension concentrate.

normality and heterogeneity of variance), the Kruskal-Wallis test (with non-normality and homogeneity of variance), or the Fisher-Pitman permutation test (with non-normality and heterogeneity of variance) were used. The post-hoc analyses used for each situation were the Tukey's HSD test, the t-test for difference of pairs, the Wilcoxon test and the test of difference of pairs with permutations. The global tests were considered significant when the $p$ value < 0.05. In the case of multiple comparisons, a significance of 5 % was considered for the Tukey's HSD test, while for the remaining tests to achieve a false discovery rate of 5 %, the $p$ values were adjusted using the Benjamini-Hochberg method. All analyses were carried out with the R software version 4.0.3.

# Results

## Bioassay 1: Assessment under *in vitro* laboratory conditions

### Broth test: Germination bioassay (conidia + PPs)

In each of the isolates studied, the mean germination percentages were reduced by the effect of the PPs, with differences between the treatments and the control (Table 3). Particularly, the PPs with the active ingredient pirimiphos methyl led to a gradual reduction in the germination percentage as the dose increased. PM16 caused the greatest reductions in the germination percentage of *B. bassiana* and *C. fumosorosea*, followed by PM10. Differently, in *M. anisopliae*, PM10 showed no differences in relation to PM16. PM8+L2 caused the lowest reductions in the three isolates (Table 3).

### Compatibility

All isolates showed differences in their colony diameter, sporulation and germination percentage between the treatments and the control (Table 4). In all the fungal isolates, PPs caused a decrease in colony diameter (Figure 1). The greatest decrease was observed with the application of PM16 and PM8+L2, followed by L7 with great variability, and then by PM10 and PM6, both of which led to the lowest decrease. In *B. bassiana* and *M. anisopliae*, no differences between PM10 and PM6 were found.

PPs also caused a decrease in sporulation of all the isolates. In *B. bassiana*, the lowest mean sporulation was observed with the application of PM8+L2, followed by PM16. PM6 and PM10 caused lower mean decreases than the previous treatments, but greater than L7. In contrast, in *M. anisopliae*, the lowest mean sporulation was observed with the application of L7. This treatment had great variability, so that it was like PM8+L2 and PM16, as well as with PM6 and PM10. Finally, in *C. fumosorosea*, the lowest

mean sporulation was observed with the application of PM16, PM10 and PM8+L2 (without significant differences between them), followed by PM6 and L7.

PP also caused a decrease in the germination percentage compared to the control in all the isolates, except for L7 in *M. anisopliae* and *C. fumosorosea*. In *B. bassiana*, the lowest mean germination was observed with the application of PM16, PM10 (without significant differences between them) and PM6. L7 caused lower mean decreases than the previous treatments and did not show differences with PM8+L2. The latter treatment had great variability, so that it was similar to PM16, PM10 and PM. In contrast, in *M. anisopliae*, the lowest mean germination was observed with the application of PM16, followed by PM10 and PM6. PM8+L2 caused lower mean decreases than the previous treatments. Finally, in *C. fumosorosea*, the lowest mean germination was observed with the application of PM16 and PM10 (without significant differences between them), followed by PM6. PM8+L2 caused lower mean decreases than the previous treatments.

The BI showed that the PP treatments were not compatible with any of the three isolates, ranging from 13.91 to 39.42 (i.e. toxic), except for the L7 treatment in *B. bassiana*, which was moderately toxic (45.08) (Table 4).

## Bioassay 2: Assessment under semi-field conditions

### Colony-forming units (CFUs)

The PPs evaluated did not affect the number of CFUs of *B. bassiana* or *C. fumosorosea* with respect to the control, independently of the persistence time of EF

**Table 3.** Germination percentage (mean ± SE) of *B. bassiana* (IBCB 66), *M. anisopliae* (IBCB 425), and *C. fumosorosea* (IBCB 130) with different PP treatments

| Treatments [a] | IBCB 66 | IBCB 425 | IBCB 130 |
|---|---|---|---|
| Control | 91.4 ± 2.6 a[b] | 87.5 ± 2.1 a | 93.0 ± 2.0 a |
| PM6 | 54.9 ± 1.6 b | 13.2 ± 2.1 c | 45.8 ± 3.3 cd |
| PM10 | 22.7 ± 3.0 c | 7.9 ± 1.3 c | 38.1 ± 2.4 d |
| PM16 | 4.2 ± 0.5 d | 5.6 ± 1.1 c | 22.9 ± 2.4 e |
| PM8+L2 | 51.5 ± 2.5 b | 37.4 ± 3.7 b | 50.6 ± 3.6 bc |
| L7 | 54.0 ± 1.3 b | 44.5 ± 4.1 b | 61.0 ± 2.0 b |
| p value | < 2.2 e-16 | < 2.2 e-16 | < 2.0 e-16 |

**Note.** [a] Control = control treatment; PM6 = Pirimiphos methyl 6 mL L^{-1}; PM10 = Pirimiphos methyl 10 mL L^{-1}; PM16 = Pirimiphos methyl 16 mL L^{-1}; PM8+L2 = Pirimiphos methyl 8 mL L^{-1} + Lambdacialotrine 2 mL L^{-1}; L7 = Lambdacialotrine 7 mL L^{-1}.

[b] Means followed by different letters in the column for each isolate tested are significantly different (p value < 0.05).

**Table 4.** Biological Index (BI): Colony diameter (mean ± SE), sporulation (mean ± SE) and germination percentage (mean ± SE) of *B. bassiana* (IBCB 66), *M. anisopliae* (IBCB 425), and *C. fumosorosea* (IBCB 130) with different PP treatments

| Treatments [a] | Colony diameter | | Sporulation | | Germination | | Values of BI [d] |
|---|---|---|---|---|---|---|---|
| | (mm) | Reduction (%)[c] | N x 10^6 | Reduction (%) | (%) | Reduction (%) | Classification |
| **IBCB 66** | | | | | | | |
| Control | 28.2 ± 0.9 a [b] | | 4762.6 ± 815.1 a | | 92.8 ± 1.0 a | | |
| PM6 | 8.0 ± 0.2 d | (-71.8) | 8.5 ± 1.7 d | (-99.8) | 70.7 ± 1.9 c | (-23.7) | 21.0 T[(1)] |
| PM10 | 7.9 ± 0.2 d | (-72.0) | 10.1 ± 2.0 d | (-99.8) | 69.8 ± 2.5 c | (-24.7) | 20.7 T |
| PM16 | 6.0 ± 0.3 c | (-78.6) | 4.3 ± 0.5 c | (-99.9) | 66.6 ± 2.5 c | (-28.2) | 17.3 T |
| PM8+L2 | 6.0 ± 0.9 c | (-78.7) | 6.6 ± 3.5 bcd | (-99.9) | 71.8 ± 6.2 bc | (-22.6) | 17.8 T |
| L7 | 20.1 ± 1.4 b | (-28.6) | 299.8 ± 103.5 b | (-93.7) | 81.8 ± 1.1 b | (-11.8) | 45.1 MT |
| *p* value | < 2.0 e-04 | | < 2.0 e-04 | | 5.0 e-14 | | |
| **IBCB 425** | | | | | | | |
| Control | 29.3 ± 1.2 a | | 1710.8 ± 201.1 10^2 a | | 93.3 ± 0.8 a | | |
| PM6 | 11.6 ± 0.4 c | (-60.4) | 10.0 ± 1.6 c | (-99.4) | 64.3 ± 3.4 c | (-31.0) | 25.8 T |
| PM10 | 11.3 ± 0.5 cd | (-61.6) | 13.3 ± 3.3 c | (-99.2) | 61.3 ± 2.7 c | (-34.2) | 24.9 T |
| PM16 | 9.7 ± 0.6 d | (-66.8) | 4.1 ± 0.7 b | (-99.8) | 47.1 ± 3.4 d | (-49.5) | 20.8 T |
| PM8+L2 | 11.4 ± 1.0 cd | (-61.0) | 3.2 ± 1.5 b | (-99.8) | 80.7 ± 1.4 b | (-13.4) | 27.1 T |
| L7 | 17.0 ± 1.9 b | (-41.9) | 108.1 ± 89.9 bc | (-93.7) | 87.7 ± 1.6 ab | (-5.9) | 39.4 T |
| *p* value | < 2.0 e-04 | | < 2.0 e-04 | | < 2.2 e-16 | | |
| **IBCB 130** | | | | | | | |
| Control | 34.8 ± 1.7 a | | 1162.6 ± 250.5 a | | 92.0 ± 1.0 b | | |
| PM6 | 12.5 ± 0.4 c | (-64.2) | 5.8 ± 0.9 c | (-99.5) | 44.8 ± 3.1 c | (-51.3) | 21.9 T |
| PM10 | 9.7 ± 0.4 e | (-72.1) | 2.0 ± 0.4 d | (-99.8) | 31.5 ± 2.7 d | (-65.8) | 16.6 T |
| PM16 | 7.6 ± 0.5 d | (-78.2) | 1.8 ± 0.6 d | (-99.8) | 33.2 ± 3.1 d | (-63.9) | 13.9 T |
| PM8+L2 | 7.1 ± 0.7 d | (-79.6) | 1.0 ± 1.6 bd | (-99.9) | 73.1 ± 1.4 a | (-20.5) | 17.6 T |
| L7 | 16.2 ± 1.8 b | (-53.4) | 12.0 ± 3.5 bc | (-99.0) | 91.3 ± 1.8 b | (-0.8) | 32.7 T |
| *p* value | < 2.0 e-04 | | < 2.0 e-04 | | < 2.0 e-04 | | |

**Note.** [a] See Table 3. [b] See Table 3. [c] Equation = Reduction (%): [Treatments mean/control mean) x 100]-100. [d] Values of BI (Rossi- Zalaf *et al.*, 2008). Toxic = 0 – 41 (T); moderately toxic = 42 –66 (MT); and compatible = > 66 (C).

on the grains. In *B. bassiana*, the variation percentage ranged from 2 to 50 % between the different times evaluated, whereas in *C. fumosorosea*, it ranged from 0 to 25 %. In *M. anisopliae*, the number of CFUs varied, independently of the persistence time of EF on the grains. The greatest decrease in the number of CFUs was observed with the application of L7, followed by PM16, PM10, PM6 and PM8+L2. The control showed a mean compatible with all PPs (Table 5).

# Discussion

The combination of PPs and EF could be a new alternative in IPM programs for improved control and regulation of insect pest populations in the post-harvest of cereal crops (Moino and Alves, 1998; Lacey *et al.*, 2015). In this study, both positive and negative interactions were observed depending on the different active ingredients of the PPs, the entomopathogenic fungal species and the application conditions.

The tests performed under laboratory conditions and in the direct culture medium showed that the PPs evaluated had a toxic effect on EF and affected negatively their vegetative growth, germination percentage and sporulation. Oliveira *et al.* (2018) and Pessoa *et al.* (2020) showed that *in vitro* studies have the advantage of exposing the microorganism as much as possible to the action of PPs, which could explain the results obtained in this study.

When evaluating the combination between PPs and EF, the germination percentage is considered as the most important factor because pathogens infect insects through conidia germination by ingestion or contact. In this study, the PP treatments evaluated caused a reduction in the fungal germination percentage with respect to the control. Thus, the use of the mixtures with EF is not recommended. This agrees with the results observed by Mamprim *et al.* (2014), who found inhibition of *B. bassiana* isolates when combined with several insecticide formulations, and thus proposed separate applications of *B. bassiana* and insecticide to prevent severe interaction.
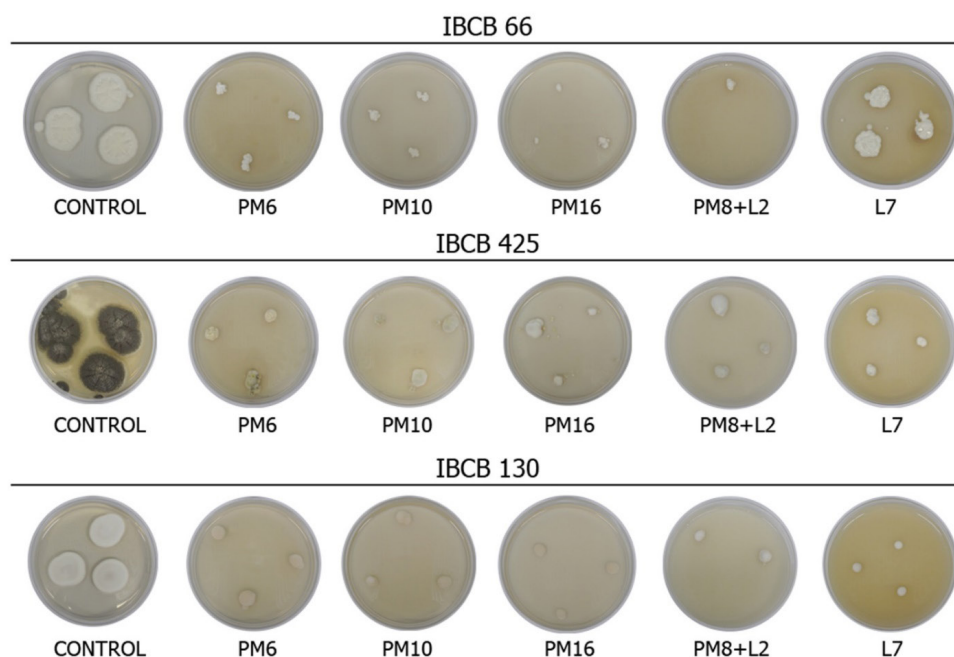
**Figure 1**. Vegetative growth (colony diameter) of *B. bassiana* (IBCB 66), *M. anisopliae* (IBCB 425), and *C. fumosorosea* (IBCB 130) with different PP treatments. Note. Control = control treatment; PM6 = Pirimiphos methyl 6 mL L$^{-1}$; PM10 = Pirimiphos methyl 10 mL L$^{-1}$; PM16 = Pirimiphos methyl 16 mL L$^{-1}$; PM8+L2 = Pirimiphos methyl 8 mL L$^{-1}$ + Lambdacialotrine 2 mL L$^{-1}$; L7 = Lambdacialotrine 7 mL L$^{-1}$.

**Table 5**. Number of colony forming units (mean ± SE) of *B. bassiana* (IBCB 66), *M. anisopliae* (IBCB 425), and *C. fumosorosea* (IBCB 130) with different PP treatments evaluated for 24, 48 and 72 h (times of persistence on grains)

| Treatments [a] | Colony forming unit | | | | | |
|---|---|---|---|---|---|---|
| | 24 h [c] | 48 h | 72 h | [d] Variation 24 h – 48 h (%) | Variation 24 h – 72 h (%) | Variation 48 h – 72 h (%) |
| **IBCB 66** | | | | | | |
| **Control** | 138.9 ± 20.7 a[b] | 131.1 ± 22.3 a | 84.0 ± 32.6 a | 5.6 | 39.5 | 35.9 |
| **PM6** | 121.1 ± 29.7 a | 118.7 ± 31.9 a | 68.8 ± 14.9 a | 2.0 | 43.2 | 42.0 |
| **PM10** | 147.1 ± 33.8 a | 73.9 ± 21.34 a | 121.8 ± 32.6 a | 49.8 | 17.2 | (-64.8) |
| **PM16** | 153.0 ± 33.4 a | 116.8 ± 32.3 a | 117.4 ± 26.4 a | 23.7 | 23.3 | (-0.5) |
| **PM8+L2** | 144.2 ± 24.9 a | 131.7 ± 13.2 a | 99.4 ± 4.5 a | 8.7 | 31.1 | 24.5 |
| **L7** | 132.5 ± 18.9 a | 139.7 ± 12.4 a | 120.0 ± 9.4 a | (-5.4) | 9.4 | 14.1 |
| ***p* value** | 1.0 | 0.5 | 0.1 | | | |
| **IBCB 425** | | | | | | |
| Control | 48.5 ± 10.1 ab[(2)] | 30.1 ± 4.5 b | 20.9 ± 2.0 b | 38.1 | 57.0 | 30.4 |
| PM6 | 66.1 ± 9.1 a | 34.8 ± 7.1 ab | 31.3 ± 3.6 a | 47.4 | 52.6 | 10.1 |
| **PM10** | 48.9 ± 7.4 a | 54.7 ± 6.0 a | 22.6 ± 2.2 ab | (-11.9) | 53.8 | 58.7 |
| **PM16** | 42.1 ± 4.1 a | 32.4 ± 4.6 b | 24.2 ± 1.8 ab | 23.0 | 42.5 | 25.3 |
| **PM8+L2** | 16.4 ± 2.2 b | 9.8 ± 1.1 c | 12.4 ± 1.8 c | 40.2 | 24.4 | (-26.5) |
| **L7** | 19.0 ± 2.9 b | 13.3 ± 2.0 c | 11.2 ± 1.8 c | 30.0 | 41.1 | 15.8 |
| ***p* value** | 0.0 | 2.5 - 07 | 4.6 - 06 | | | |
| **IBCB 130** | | | | | | |
| **Control** | 132.0 ± 11.9 a[(2)] | 109.0 ± 12.3 a | 125.0 ± 13.1 a | 17.4 | 5.3 | (-14.6) |
| **PM6** | 141.0 ± 22.9 a | 125.0 ± 27.1 a | 129.4 ± 28.8 a | 11.6 | 8.2 | (-3.9) |
| **PM10** | 146.0 ± 18.2 a | 127.0 ± 31.6 a | 137.8 ± 23.8 a | 12.9 | 5.3 | (-8.8) |
| **PM16** | 129.0 ± 15.6 a | 110.0 ± 29.7 a | 103.7 ± 19.5 a | 14.8 | 19.7 | 5.8 |
| **PM8+L2** | 120.0 ± 10.2 a | 113.0 ± 10.1 a | 113.0 ± 10.1 a | 5.8 | 5.8 | 0.0 |
| **L7** | 144.0 ± 14.3 a | 108.0 ± 7.7 a | 154.0 ± 11.1 a | 25.1 | (-6.7) | (-42.5) |
| ***p* value** | 0.1 | 1.0 | 0.2 | | | |

**Note.** [a] See Table 3. [b] See Table 3. [c] x10$^3$. [d] Equations: Variation 24 h – 48 h % = [48 h mean/24 h mean) x 100]-100; Variation 24 h – 72 h % = [72 h mean/24 h mean) x 100]-100; Variation 48 h – 72 h % = [72 h mean/48 h mean) x 100]-100.

In the compatibility calculation, the vegetative growth (colony diameter) represents the growth potential of the fungus (Potrich *et al.*, 2018). In the present study, the PPs used reduced this parameter in the three fungi evaluated. In contrast, Pessoa *et al.* (2020) observed that the vegetative growth of *B. bassiana* increased when it was applied together with PPs. These authors considered that, when microorganisms are in the presence of toxic compounds that alter the culture medium and impair their development, they use all their reproductive effort, thus resulting in greater vegetative growth.

The sporulation of an isolate is important for the maintenance of the pathogen-host cycle, since the increase of this parameter results in greater dispersal of propagules in the environment (Potrich *et al.*, 2018). In the present work, this parameter was reduced by the PPs evaluated as compared to control conditions. In contrast, Alves *et al.* (2008) and Fregonesi *et al.* (2016) found that PPs do not inhibit the sporulation of EF. Moino and Alves (1998) explained that vegetative growth and sporulation may be promoted by a mechanism of physiological resistance of microorganisms, which can metabolize the toxic compounds of the active ingredient, or, by using the molecules resulting from this process, release them in the culture medium as secondary nutrients.

In this study, the treatments with organophosphates, particularly PM16, had toxic effects on almost all the isolates tested, with the exception of the *B. bassiana* isolate tested with the L7 pyrethroid treatment, whose effect was moderately compatible. Fregonesi *et al.* (2016) observed differences in the response to different PPs in isolates of *B. bassiana*. Moreover, as pointed out by Oliveira *et al.* (2018), the commercial formulations prepared with the same active ingredient by different manufacturers can present different biological effects. Also, the addition of other products in the preparation, for example, emulsifiers and additives, can contribute to having varied biological effects on vegetative growth and sporulation.

The results observed here showed that, when PPs were applied under semi-field conditions in which the exposure was conditioned by several factors, the number of CFUs of the fungi was not affected by the PPs. Large productions of conidia on the substrate are fundamental for pathogen control. This coincides with that reported by Dal Bello *et al.* (2018), who showed the highest survival of EF. This demonstrates that the incompatibility *in vitro* does not always indicate that the same occurs under field conditions (Alves *et al.*, 1998).

## Conclusion

It is important to study the effects of PPs in combination with EF to reduce the doses and number of applications of PPs. The treatment with the active ingredient lambdacialotrine would be the most effective compared to the treatments with pirimiphos methyl, which led to a gradual reduction in the germination percentage as the dose increased. On the other hand, PPs caused a decrease in colony diameter, sporulation and germination percentage in all the isolates. In consequence, the BI showed that the treatments were not compatible with any of the three isolates given their toxicity. Based on the results obtained, it was concluded that the application of PPs and EF is recommended separately and under semi-field conditions. However, further research under field conditions should be conducted to confirm the compatibility of PPs and EF within an IPM strategy.

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## References

Alves, S. B.; Moino Júnior, A. and Almeida, J. E. M. (1998). Produtos fitossanitários e entomopatógenos. In *Controle microbiano de insetos* (pp. 217-238), 2nd ed. Piracicaba: FEALQ. https://repositorio.usp.br/item/001006741

Alves, S. B. and Lopes, R. B. (2008). Fungos entomopatogênicos usados no controle de pragas na América Latina. In *Controle microbiano de pragas na América Latina: avanços e desafios* (pp. 69-110), 1st ed. Piracicaba: FEALQ. https://repositorio.usp.br/item/001672395

Dal Bello, G. M.; Fusé, C. B.; Pedrini, N. and Padín, S. B. (2018). Insecticidal efficacy of *Beauveria bassiana*, diatomaceous earth and fenitrothion against *Rhyzopertha dominica* and *Tribolium castaneum* on stored wheat. *International Journal of Pest Management*, 64(3), 279-286. https://doi.org/10.1080/09670874.2017.1397300

Fregonesi, A. F.; Mochi, D. A. and Monteiro, A. C. (2016). Compatibilidade de isolados de *Beauveria bassiana* a inseticidas, herbicidas e maturadores em condições de laboratório. *Arquivos do Instituto Biológico*, 83, e0242014. https://doi.org/10.1590/1808-1657000242014

Lacey, L. A.; Grzywacz, D.; Shapiro-Ilan, D. I.; Frutos, R.; Brownbridge, M. and Goettel, M. S. (2015). Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology*, 132, 1-41. https://doi.org/10.1016/j.jip.2015.07.009

Mamprim, A. P.; Angeli Alves, L. F.; da Silva Pinto, F. G.; Formentini, M. A.; Castilho Martins, C. and Barbosa Pares, R. (2014). Efecto de productos fitosanitarios sobre parámetros biológicos de *Beauveria bassiana* (Bals.) Vuill. (Hypocreales: Cordycipitaceae). *Revista de Protección Vegetal*, 29(2), 128-136. http://scielo.sld.cu/pdf/rpv/v29n2/rpv08214.pdf

Moino, A. Jr. and Alves, S. B. (1998). Efeito de imidacloprid e fipronil sobre *Beauveria bassiana* (Bals.) Vuill. e *Metharhizium anisopliae* (Metsch.) Sorok. e no comportamento de limpeza de *Heterotermes tenuis* (Hagem). *Anais da Sociedade Entomológica do Brasil*, 27(4), 611-619. https://doi.org/10.1590/S0301-80591998000400014

Nguyen, T. T.; Collins, P. J. and Ebert, P. R. (2015). Inheritance and Characterization of Strong Resistance to Phosphine in *Sitophilus oryzae* (L.). *PLoS ONE*, 10(4), e0124335. https://doi.org/10.1371/journal.pone.0124335

Oliveira, R. P.; Pessoa, L. G. A.; de Souza Loureiro, E. and Oliveira, M. P. (2018). Compatibilidade de inseticidas utilizados no controle da mosca branca em soja com *Beauveria bassiana*. *Revista de Agricultura Neotropical*, 5(4), 88-93. https://doi.org/10.32404/rean.v5i4.2416

Pessoa, L. G. A.; Souza, T. M. N. and de Souza Loureiro, E. (2020). Compatibilidade de inseticidas utilizados no manejo de pragas em eucalipto com *Beauveria bassiana* (Cordycipitaceae). *Research, Society and Development*, 9(8), e322985148. https://doi.org/10.33448/rsd-v9i8.5148

Potrich, M.; da Silva, R. T. L.; Maia, F. M. C.; Lozano, E. R.; Rossi, R. M.; Colombo, F. C.; Tedesco, F. G. and Gouvea, A. D. (2018). Effect of entomopathogens on Africanized *Apis mellifera* L. (Hymenoptera: Apidae). *Revista Brasileira de Entomologia*, 62(1), 23-28. https://doi.org/10.1016/j.rbe.2017.12.002

Rossi-Zalaf, L. S.; Alves, S. B.; Lopes, R. B.; Silveira Neto, S. and Tanzini, M. R. (2008). Interação de micro-organismos com outros agentes de controle de pragas e doenças, In *Controle microbiano de Pragas na América Latina—Avanços e Desafios* (pp. 279–302), 1st ed. Piracicaba: FEALQ.