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Biological and Microbial Control

Potential of Entomopathogenic Fungi as Biological Control Agents of Diamondback Moth (Lepidoptera: Plutellidae) and Compatibility With Chemical Insecticides

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Abstract

The objectives were to evaluate the efficiency of entomopathogenic fungi against *Plutella xylostella* (L.) and the compatibility of the most virulent isolates with some of the insecticides registered for use on cabbage crops. Pathogenicity tests used isolates of *Beauveria bassiana*, *Metarhizium rileyi*, *Isaria fumosorosea*, *Isaria sinclairii*, and *Lecanicillium muscarium* standardized at a concentration of 10^7 conidia/ml. Cabbage leaf discs were immersed in these suspensions, and after evaporation of the excess water, were placed 10 second-instar larvae of *P. xylostella*, totaling 10 leaf discs per treatment. Mortality was assessed 7 d after treatment, and the isolates that caused mortality >80% were used to estimate LC_{50} and LT_{50}. The compatibilities of the most virulent isolates and the insecticides were tested from the mixture of these into the culture medium, and after solidifying, the medium was inoculated with an aliquot of the isolated suspension. The following parameters were evaluated: growth of the colony, number and viability of conidia after 7 d. The isolated IBCB01, IBCB18, IBCB66, and IBCB87 of *B. bassiana*, LCMAP101 of *M. rileyi*, and ARSEF7973 of *I. sinclairii* caused mortality between 80 and 100%, with LC_{50} and LT_{50} between 2.504 to 6.775 $\times$ 10^4 conidia/ml and 52.22 to 112.13 h, respectively. The active ingredients thiamethoxam and azadirachtin were compatible with the entomopathogenic fungi. The results suggest that the use of these isolates is an important alternative in the pesticidal management of *P. xylostella*, with the possible exception of the associated use of chemical controls using the active ingredients thiamethoxam or azadirachtin.

Key words: *Plutella xylostella*, biological control, chemical control, interaction

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a major pest of plants in the family Brassicaceae in many parts of the world. The impacts of this pest are magnified by its great capacity to disperse, by its adaptability to adverse weather conditions, and is intensified by its short life cycle and high reproductive rates (Honda et al. 1992, Chapman et al. 2002, Coulson et al. 2002, Ulmer et al. 2002, Attique et al. 2006, Sarfraz et al. 2006, Grzywacz et al. 2010). In Brazil, this species is important in most areas of brassica cultivation (Marchioro and Foerster 2014), and it distributed from the Amazon to the state of Rio Grande do Sul (Ferronatto 1984, Castelo Branco and Guimarães 1990, Barros et al. 1993, Melo et al. 1994, Cardoso 1999, Cardoso et al. 2012).

Among commercial species, cabbage (*Brassica oleracea* var. *capitata*) is one of the main hosts of this microlepidopteran. Diamondback moth larvae feed on leaf tissue, and cause quantitative and qualitative damage ranging from yield losses to the death of the plant, and may infest as much as 100% of the plants in individual fields (Ooi and Kelderman 1979, Shelton et al. 1982, Barros et al. 1993, Castelo Branco et al. 1997, Ohsawa 2001, Monnerat et al. 2004). To reduce the population densities of this pest, chemical control methods using highly toxic, broad-spectrum insecticides have been the main pest control strategy, with particular dependence on pyrethroid and organophosphate active ingredients (Castelo Branco and Medeiros 2001, Castelo Branco et al. 2003, Filgueira 2007). However, the high selection pressure induced by excessive applications is one of crucial factors for increasing incidence of resistance of populations of *P. xylostella* to many classes of chemical insecticides (Castelo Branco et al. 2003, Sarfraz and Keddie 2005, Sayyed et al. 2005, Zhao et al. 2006, Thuler et al. 2007, Endersby et al. 2008, Oliveira et al. 2011).

To minimize this problem, different biological control agents can contribute to the control of *P. xylostella*, especially using entomopathogenic microorganisms, such as fungi complex, composed by *Beauveria bassiana* (Bals.-Criv.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorok., *Metarhizium rileyi* (Farlow) Kepler, S.A. Rehner & Humber, *Isaria* sp., and *Lecanicillium* sp. (Gopalakrishnan 1989, Silva et al. 2003, Godonou et al. 2009, Xu et al. 2011, Méndez 2012). One of the barriers to using more efficient and also the conservation of these microbial agents in the field is related to the compatibility with the pesticides used to control other pests or agricultural diseases (Batista Filho et al. 2001, Almeida et al. 2003, Batista Filho et al. 2003, Cuthbertson et al. 2005, Wenzel et al. 2008, Botelho and Monteiro 2011, Niassy et al. 2012, Schumacher and Poehling 2012).

However, few studies relate the potential of different species of entomopathogenic fungi in the biological control of *P. xylostella* and the possible compatibility of these with the chemical insecticides (Tian and Feng 2006), which requires better understanding of possible interactions between these microbiological and chemical agents involved with the aim of improve control strategies. The objectives of this research were to analyze the efficiency of entomopathogenic fungi to control second-instar larvae of *P. xylostella* and to evaluate the compatibility of the most virulent isolates with the active ingredients of insecticides registered for the control of cabbage crop pests.

Materials and Methods

P. xylostella Population

Bioassays were conducted using a population of *P. xylostella* originating from a conventionally farmed commercial cabbage field in the municipality of Recife (Pernambuco), from a collection of 143 adults on 15 January 2007. This population was maintained at the Laboratory of Biology and Insects Rearing (LBIR) and kept reproductively isolated using a rearing methodology described by De Bortoli et al. (2011).

For laboratory rearings, the larval stage of *P. xylostella* were fed leaves (45–60 d old) of cabbage (*B. oleracea* var. *capitata* cv. Chato de Quintal); this cultivar is known to be susceptible to diamondback moth (Boiça Júnior et al. 2013). Plants ~20 d old were obtained from the company Agrimonte Produtos Agrícolas Ltda. (Monte Alto São Paulo, Brazil), and transplanted individually in polyethylene pots with a capacity of 5 liters, containing a homogeneous mixture of soil (red latosol), coarse sand, and cattle manure sifted together in a proportion of 2:1:1 (Boiça Júnior et al. 2013). These pots were placed in the greenhouse. Cabbage seedlings were transplanted on a monthly basis to maintain constant leaf production to feed the diamondback moth larvae.

The rearing of adults was conducted in transparent plastic cylindrical containers (15 cm diameter by 25 cm height), containing a cabbage leaf disc 8 cm in diameter in a filter paper disc of the same diameter that was slightly moistened with distilled water. This paper disc was placed in a transparent plastic cup (5 cm diameter by 8 cm height) with the opening turned down, containing the elevated cabbage leaf disc inside the cage, where oviposition occurred. The top of the container included a 3-cm-diameter opening over which was placed a sponge soaked with a 10% aqueous solution of honey. In each cage also included a lateral opening (10 cm length by 10 cm width), covered with tissue type "voile," to allow gas exchange. These leaf discs were replaced daily; discs with the presence of the diamondback moth eggs were transferred to Petri dishes (9 cm diameter) until the outbreak of larvae.

Entomopathogenic Fungal Isolates

The fungal isolates used in bioassays belong to species *B. bassiana*, *Lecanicillium muscarium* (Petch.) Zare & W. Gams, *Isaria fumosorosea* Wize, *Isaria sinclairii* (Berk.) Lloyd, and *M. rileyi* (Table 1). These isolates were grown on potato dextrose agar (PDA) and stored in test tubes or Petri dishes (9 cm diameter) at a temperature of −4°C (freezer) at the Laboratory of Microbial Control of Pests (LMCP) bank. For their use, the isolates were transferred to Petri dishes (9 cm diameter) containing PDA, and incubated at a temperature of 28 ± 2°C, 70 ± 10% relative humidity (RH), and a photoperiod of 12:12 (L:D) h for 15 d. Thereafter, conidial suspensions were prepared in 10 ml autoclaved water + 0.05% Tween 20 (as an adhesive spreader) + fungal conidia. From this suspension, two serial dilutions were made to quantify the number of conidia/ml, with the help of a Neubauer chamber, and standardized at a final concentration of 10^7 conidia/ml.

Pathogenicity of Entomopathogenic Fungal Isolates

Ten second-instar larvae of *P. xylostella* were placed on each of 10 leaf cabbage leaf discs (8 cm diameter) for each treatment (with a total of 10 replicates per experiment). The *B. oleracea* var. *capitata* cv. Chato de Quintal leaf discs for these bioassays were immersed in 10 ml of the conidial suspensions of each fungus for 1 min, and then dried before adding the diamondback moth larvae.

After this procedure, leaf disks were transferred individually to Petri dishes containing distilled water-moistened filter paper (8 cm diameter). A soft brush was used to transfer 10 second-instar larvae of *P. xylostella* to each leaf disc, and the dishes were placed in a growth chamber at 25 ± 2°C, 70 ± 10% RH, and a photoperiod of 12:12 (L:D) h. The control treatments followed the same methodology described earlier without adding any fungus to the autoclaved Tween solutions.

Mortality was assessed on the 7th day after treatment application, when dead insects were placed in a humid chamber to confirm whether the larvae were killed by the entomopathogen. The humid chamber consisted of filter paper disc (8 cm diameter) and a foam, both sterilized and moistened with distilled water, placed on Petri dishes (9 cm diameter) and maintained in growth chamber at 28 ± 2°C, 70 ± 10% RH, and a photoperiod of 12:12 (L:D) h. Confirmation of mortality was performed after 10 d of incubation in the humid chamber by observing the conidial structures of the fungus with a stereoscopic microscope model Stemi 2000-C, Carl Zeiss Corporation, Germany.

Total mortality was corrected in relation to the mortality observed in control (Abbott 1925). The experiment was conducted in a completely randomized design, and the data were submitted to analysis of variance. Means were compared by the Tukey's test ($P < 0.05$; PROC GLM, SAS Institute 2002). The median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were determined for isolates that caused > 80% mortality, using probit analysis ($P > 0.05$) by Minitab 15 (Minitab 2007) statistical software.

Estimate of LC_{50} and LT_{50}

The estimate of LC_{50} was performed for conidial suspensions of 10^4, 10^5, 10^6, 10^7, and 10^8 conidia/ml, using the mortality recorded on the 7th day after treatment application. The estimate of LT_{50} were prepared from 10 replicates for each isolate used and treated as indicated earlier. The fungus-induced mortality of larvae was

Table 1. Isolates of the entomopathogenic fungi used in bioassays

Entomopathogen	Isolates	Origin
B. bassiana	IBCB01	Biological Control Laboratory of Experimental Center of the Biological Institute, Campinas (SP)
	IBCB17	
	IBCB18	
	IBCB33	
	IBCB35	
	IBCB63	
	IBCB66	
	IBCB87	
	JAB01	Microbiology Laboratory of the Department of Plant Protection (Unesp), Jaboticabal (SP)
	JAB09	
	JAB48	
	JAB63	
L. muscarium	ARSEF5128	United States Department of Agriculture (USDA)–Collection of Entomopathogenic Fungal Cultures, Ithaca (USA)
I. fumosorosea	ARSEF7050	
I. sinclairii	ARSEF6925	
	ARSEF7973	
M. rileyi	LCMAP101	Laboratory of Microbial Control of Pests (Unesp), Jaboticabal (SP)

checked every 24 h for 7 d. The conduct of bioassays related to LC_{50} and LT_{50} was identical to the methodology described earlier to assess pathogenicity.

Confirmation of the causes of mortality was performed 10 d after the transfer of dead larvae into the humid chamber by monitoring for the presence of conidial structures as described earlier. The mortality results were subjected to probit analysis ($P > 0.05$) using Minitab 15 (Minitab 2007) statistical software.

Compatibility Between Entomopathogenic Fungi and Pesticides

The most virulent isolates of fungal pathogens against *P. xylostella* were used in compatibility tests with active ingredients of pesticides routinely used on cabbage crops: azadirachtin (AzaMax; 200 ml × 100 liter^{-1}), deltamethrin (Decis; 30 ml × 100 liter^{-1}), methomyl (Lannate BR; 100 ml × 100 liter^{-1}), thiamethoxam (Actara; 20 g × 100 liter^{-1}), and acephate (Orthene; 100 g × 100 liter^{-1}; Mapa 2013).

The toxic effect of insecticides on the entomopathogenic fungi was determined by adding the chemical products to PDA culture medium, prepared from the dissolution of 39 g of the formulated medium, Potato Dextrose Agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India), in 1 liter of distilled water, and subsequently autoclaved at 1 atm for 20 min. After autoclaving, when the medium had cooled to 45°C but not yet solidified yet was cool enough not to affect the activities of the pesticides (Botelho and Monteiro 2011), each pesticide was added and homogenized into the culture medium, and then poured onto Petri dishes to solidify. For each test, pesticides were prepared and inoculated in 10 Petri dishes from a single batch of fungal inoculum and the experiment was replicated 10 times (ten replicates).

These pesticide-supplemented plates were inoculated with 5 µl each of conidial suspensions (10^7 conidia/ml) for each fungal isolate. Plates were then incubated at 28 ± 2°C, 70 ± 10% RH, and a photoperiod of 12:12 (L:D) h for 7 d. Thereafter, the total area (cm^2) of colonies was measured by cutting blank sheet of paper the same size as the colonies and measuring the areas of this piece of paper using a leaf area meter unit (Model CI-202, CID Bio-Science, WA).

After colony growth was measured, conidial production of each plate was determined by cutting up the colony culture medium, and placing the colony pieces in the tubes with 10 ml of sterile 0.05% Tween-20 solution and agitated vigorously with a Model AP56 agitator (Phoenix, São Paulo, Brazil) for a period of 1 min. Then, a small aliquot of the resultant conidial suspension was counted with a Neubauer hemacytometer to estimate conidial production on each plate.

The viability of conidia was analyzed by microculture technique and direct examination using blade, proposed by Marques et al. (2004). For each test, treatments were prepared and inoculated in 10 microscopy blade from a single batch of fungal inoculum and the experiment was replicated five times (five replicates). The experiment was conducted in a completely randomized design, and the data were submitted to analysis of variance and the average size of the colonies, the number, and viability of conidia for each treatment were compared by Tukey's test at 5% probability (PROC GLM, SAS Institute 2002).

The biological index was standardized by compatibility score developed by Alves et al. (2007), with the help of the following formula (BI = 47 [VG] + 43 [SP] + 10 [GER]/100), in which BI = biological index; VG = percentage of vegetative growth of the colony after 7 d, compared with control; SP = percentage of colony sporulation after 7 d, compared with control; and GER = percentage of spore germination after 24 h. Alves et al. (2007) ranked biological index values as follows: BI > 66 as compatible, 42 ≤ BI ≤ 66 as moderately toxic, and BI < 42 as toxic.

Results and Discussion

All analyzed isolates were pathogenic to *P. xylostella*, with mortality rates ranging between 46 and 100% (Fig. 1). The fungi that caused mortality >80% were *B. bassiana*, *I. sinclairii*, and *M. rileyi*, highlighting the isolates IBCB01, IBCB18, IBCB66 (*B. bassiana*), and LCMAP101 (*M. rileyi*), responsible for mortality between 98 and 100% of second-instar larvae of *P. xylostella*, not differing statistics from each other, followed by ARSEF7973 (*I. sinclairii*) and IBCB87 (*B. bassiana*), with 80 and 94% mortality, respectively (Fig. 1).

The estimated LC_{50} varied from 2.504 and 6.775 × 10^4 conidia/ml, with a significant difference only between *B. bassiana* isolates IBCB01 and IBCB18, with the first of these isolates being characterized as the most virulent (Table 2). *Metarhizium rileyi* LCMAP101

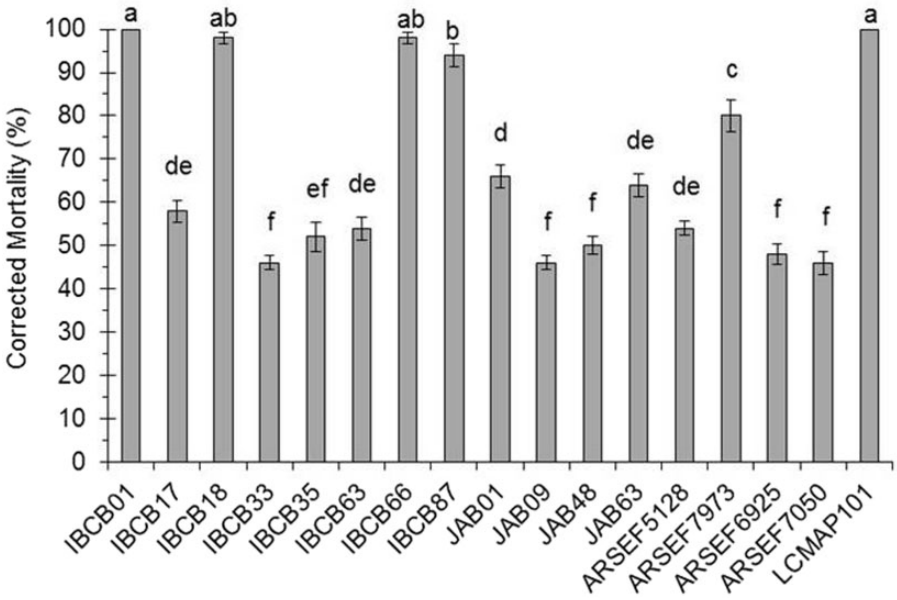

Fig. 1. Corrected mortality of second-instar larvae of *P. xylostella* subjected to treatments using entomopathogenic fungi. Means followed by the same letter do not differ significantly ($P < 0.05$). The error bar represents the standard error (± SE).

Table 2. Estimated LC_{50} of the most virulent entomopathogenic fungi isolates to second-instar larvae of *P. xylostella*

Treatments	*N*	Slope ± SE	LC_{50} (CI_{95}%) × 10^4 conidia/ml	$\chi 2$*
B. bassiana				
IBCB01	500	0.37 ± 0.04	2.504 (1.442–3.923)	5.26
IBCB18	500	0.31 ± 0.03	6.775 (3.975–10.756)	13.31
IBCB66	500	0.36 ± 0.03	2.887 (1.667–4.548)	7.20
IBCB87	500	0.25 ± 0.02	3.796 (1.249–4.822)	8.79
I. sinclairii				
ARSEF7973	500	0.27 ± 0.03	4.600 (2.436–7.774)	28.89
M. rileyi				
LCMAP101	500	0.34 ± 0.03	2.506 (1.377–4.057)	5.07

* Chi-square test ($P > 0.05$).

Table 3. Estimated LT_{50} (h) of the most virulent entomopathogenic fungi isolates to second-instar larvae of *P. xylostella*

Treatments	*N*	Slope ± SE	LT_{50} (CI_{95}%)	$\chi 2$*
B. bassiana				
IBCB01	100	0.03 ± 0.001	69.56 (64.72–74.21)	23.05
IBCB18	100	0.02 ± 0.002	63.48 (58.16–68.41)	10.85
IBCB66	100	0.02 ± 0.001	101.66 (96.77–106.62)	15.43
IBCB87	100	0.02 ± 0.001	84.82 (78.07–91.24)	15.56
I. sinclairii				
ARSEF7973	100	0.02 ± 0.001	112.13 (105.93–118.69)	14.22
M. rileyi				
LCMAP101	100	0.07 ± 0.01	52.22 (49.52–54.88)	4.64

* Chi-square test ($P > 0.05$).

had the lowest LT_{50}, which were statistically significantly different from the other treatments, and represent by the average value of 52.22 h, followed by IBCB18 and IBCB01 with 63.48 and 69.56 h, respectively (Table 3).

The *B. bassiana* isolates IBCB01, IBCB18, IBCB66, and IBCB87 were highly virulent to second-instar larvae of *P. xylostella*, and showed a high potential for management of diamondback moth pest populations. Other studies have also showed promising results for *B. bassiana* to control diamondback moth, but at higher applied conidial concentrations: Silva et al. (2003) reported 78–90% mortality for second-instar diamondback moth larvae treated by *B. bassiana* isolates ESALQ 447, ESALQ 760, ESALQ 900, ESALQ 634, and IPA-205 at concentrations of 10^8 conidia/ml. Batta et al. (2010) and Anaisie et al. (2011) reported 80–100% mortality, respectively, for *P. xylostella* larvae after treatment with *B. bassiana* conidia at a concentration of 10^9 conidia/ml. In this research, the concentration 10^7 conidia/ml was enough to yield mortalities between 94 and 100% for second-instar larvae of *P. xylostella*. The results using much lower conidial concentrations are especially interesting from an economic point of view, especially in relation to the possible development and marketing of such entomopathogenic microbes.

Another result of considerable importance from this study is the complete mortality of *P. xylostella* larvae 7 d after applications of *M. rileyi* (a pathogen best known for its activity against noctuid lepidopteran larvae), in addition to the fact that this fungus yielded the lowest lethal times when compared with other entomopathogens. The results show a surprisingly high virulence for this fungus against diamondback moth. This microorganism is considered pathogenic to *P. xylostella*, but little bit virulent (Jun 2000), in contrast to the results of this study. This virulence variability can be attributed to genetic diversity of this isolates, based on insect host and geographical region, as observed by Suwannakut et al. (2005).

Lecanicillium muscarium (Hypocreales: Cordycipitaceae) is considered to be an important control agent of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Cuthbertson et al. 2005, 2008). This species proved to be pathogenic to diamondback moth, but did prove to have a lower apparent virulence than the other species entomopathogenic fungi analyzed here (Fig. 1). However, this appears to be the first report about the pathogenicity of *L. muscarium* for *P. xylostella*.

In relation to *Isaria* species (Hypocreales: Cordycipitaceae), it was observed that only one isolate of *I. sinclairii* presented

Table 4. Vegetative growth (cm^2; ± SE) of the most virulent entomopathogenic fungi isolates to *P. xylostella* when in contact with insecticides registered for the control of cabbage crop pests

Treatments	*B. bassiana*				*I. sinclairii*	*M. rileyi*
	IBCB01	IBCB18	IBCB66	IBCB87	ARSEF7973	LCMAP101
Control	38.65 ± 0.52a	37.32 ± 0.50a	34.57 ± 0.63a	34.67 ± 0.58a	26.98 ± 0.66a	29.20 ± 0.62a
Azadirachtin	34.63 ± 0.43a	29.24 ± 0.82c	30.27 ± 0.85b	24.43 ± 1.02b	25.23 ± 0.81a	26.32 ± 1.13ab
Thiamethoxam	35.73 ± 0.91a	33.28 ± 0.61b	31.73 ± 0.75ab	31.56 ± 0.82a	24.90 ± 0.95a	26.38 ± 0.94ab
Deltamethrin	23.04 ± 2.45b	21.83 ± 0.67d	21.83 ± 1.35c	12.39 ± 0.79cd	16.08 ± 0.79b	23.84 ± 0.92b
Acephate	18.47 ± 1.04b	16.68 ± 0.81e	18.97 ± 1.16cd	15.69 ± 0.84c	13.64 ± 0.92bc	14.56 ± 0.81c
Methomyl	13.28 ± 0.91c	10.95 ± 0.66f	16.13 ± 1.13d	9.13 ± 0.96d	11.37 ± 0.94c	11.20 ± 0.69c
F	71.15	218.55	56.49	153.86	63.53	70.46
df	5, 54	5, 54	5, 54	5, 54	5, 54	5, 54
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Means in each column followed by same letter do not differ significantly by the Tukey's test ($P < 0.05$).

promising results against second-instar larvae of diamondback moth (Fig. 1). The few studies of this entomopathogenic genus *Isaria* for the possible control of this lepidopteran pest demonstrated that *I. fumosorosea* may cause diamondback moth mortality (Maketon et al. 2008, Huang et al. 2010) but only at economically unrealistically high conidial concentrations for the isolates test to date. The research presented here, in addition to being the first scientific report in relation to pathogenicity of *I. sinclairii* on *P. xylostella*, also showed the high potential of *I. sinclairii* in the integrated management of diamondback moth, considering the use of experimental low concentration (10^7 conidia/ml).

The difference in results between surveys may be owing to a number of factors, initially characterized by isolates of the entomopathogenic fungi, whose genetic diversity and the origin of collection can determine a response to the virulence on *P. xylostella* larvae (Hajek and St. Leger 1994, Alves 1998). Another issue is related to genetic differences of diamondback moth population used in the research, which may result in different mortality responses to the same isolated from certain species of entomopathogenic fungi. Methodological differences used in conducting pathogenicity and virulence tests can also influence directly on the larvae mortality in relation to the entomopathogenic fungi analyzed.

The compatibility test between the most virulent entomopathogenic fungi with pesticides showed no significant interference with the natural growth of the isolates when in contact with thiamethoxam, save only IBCB18 of *B. bassiana* (Table 4). The active ingredient azadirachtin also did not interfere significantly with vegetative growth IBCB01 of *B. bassiana*, ARSEF7973 of *I. sinclairii*, and LCMAP101of *M. rileyi*. However, other chemical molecules significantly reduced vegetative growth of the colony in all isolates analyzed, especially when in contact with methomyl (Table 4).

The active ingredient thiamethoxam did not affect the number of conidia produced by entomopathogenic microorganisms, while azadirachtin, deltamethrin, acephate, and methomyl were responsible for reducing the number of conidia produced by microorganisms (Table 5).

The viability conidia was higher when in contact with the chemical molecules azadirachtin and thiamethoxam, ranging between 75.20 to 88.80% and 87.40 to 90.80%, respectively, did not differ statistically to the control. The other active ingredients have reduced the viability of the reproductive structures, especially when in contact with acephate and methomyl (Table 6).

Thus, azadirachtin and thiamethoxam, following the pattern of compatibility score developed by Alves et al. (2007), were consistent with the entomopathogenic fungi tested. The deltamethrin chemical molecule was only compatible to *M. rileyi*, being moderately toxic and also toxic to other entomopathogen. The active ingredient acephate was moderately toxic when in contact with most entomopathogenic fungi, while methomyl was classified as toxic to practically every microorganisms, considered the most harmful in relation to the vegetative and reproductive development of these biological control agents (Table 7).

The compatibility of azadirachtin and thiamethoxam, ranked within the tetranortriterpenoide and neonicotinoid chemical groups, respectively, with the isolates of the entomopathogenic fungus *B. bassiana*, *I. sinclairii*, and *M. rileyi* could be related to the low toxicity of these chemical molecules for microorganisms, but also by the possible capacity of entomopathogen to degrade these compounds aiming development and reproduction (Batista Filho et al. 2001, Botelho and Monteiro 2011, Hernández et al. 2012, Niassy et al. 2012, Santos et al. 2013, Silva et al. 2013). Different studies have shown the possibility of using these active ingredients with different species of entomopathogenic fungi, showing positive results on the issues focused on compatibility (Batista Filho et al. 2001, Andaló et al. 2004, Botelho and Monteiro 2011, Hernández et al. 2012, Ribeiro et al. 2012, Rocha et al. 2012, Cintra et al. 2013).

The active ingredient deltamethrin showed different results for the species of entomopathogenic fungi, and it is considered in the most researches toxic or moderately toxic to these microorganisms (Oliveira et al. 2003, Silva et al. 2006, Archana and Ramaswamy 2012, Niassy et al. 2012), fact observed in this study, in contrast with *M. rileyi*, whose chemical molecule was consistent with that biological control agent (Table 7).

Moreover, both methomyl and acephate were harmful to the development of entomopathogens, which demonstrates the high toxicity of these molecules to these microorganisms. Both active ingredients are represented as compatible for some cases, but also moderately toxic or incompatible (Khalil et al. 1985, Batista Filho et al. 2001, Gassen et al. 2008, Wenzel et al. 2008), which may characterize differences forward to the methodology used and the variation in the dosage of pesticides used.

These laboratory results help to guide the selection of candidate fungal isolates for further laboratory and field testing to find new approaches for the microbial control of *P. xylostella*. From this initial process, laboratory tests on the compatibility of pesticides and the most virulent fungal isolates are vital for analyzing their possible impacts on the development and reproduction of these biological control agents, and also to help choices of insecticides that could be

Table 5. Number of produced conidia (±SE) by the most virulent entomopathogenic fungi isolates to *P. xylostella* when in contact with insecticides registered for the control of cabbage crop pests

Treatments	*B. bassiana* ($\times 10^8$ conidia)				*I. sinclairii* ($\times 10^8$ conidia)	*M. rileyi* ($\times 10^8$ conidia)
	IBCB01	IBCB18	IBCB66	IBCB87	ARSEF7973	LCMAP101
Control	1.06 ± 0.07a	1.15 ± 0.06a	1.08 ± 0.05a	1.16 ± 0.06a	1.71 ± 0.12a	1.16 ± 0.07a
Azadirachtin	0.81 ± 0.07bc	0.76 ± 0.06b	0.82 ± 0.04bc	0.77 ± 0.04b	0.82 ± 0.06bc	0.89 ± 0.03bc
Thiamethoxam	1.03 ± 0.06ab	1.00 ± 0.04a	1.03 ± 0.05ab	0.98 ± 0.04a	1.12 ± 0.06ab	1.00 ± 0.06ab
Deltamethrin	0.55 ± 0.04d	0.67 ± 0.04b	0.66 ± 0.06cd	0.69 ± 0.05b	0.35 ± 0.05bc	0.78 ± 0.05c
Acephate	0.64 ± 0.04cd	0.66 ± 0.05b	0.55 ± 0.07de	0.62 ± 0.04b	0.45 ± 0.09bc	0.69 ± 0.04c
Methomyl	0.22 ± 0.05e	0.29 ± 0.04c	0.40 ± 0.06e	0.25 ± 0.04c	0.16 ± 0.03c	0.19 ± 0.03d
F	33.28	36.14	22.21	48.47	6.93	48.59
df	5, 54	5, 54	5, 54	5, 54	5, 54	5, 54
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Means in each column followed by same letter do not differ significantly by the Tukey's test ($P < 0.05$).

Table 6. Viability of conidia (%; ± SE) of the most virulent entomopathogenic fungi isolates to *P. xylostella* when in contact with insecticides registered for the control of cabbage crop pests

Treatments	*B. bassiana*				*I. sinclairii*	*M. rileyi*
	IBCB01	IBCB18	IBCB66	IBCB87	ARSEF7973	LCMAP101
Control	93.80 ± 1.24a	94.80 ± 1.07a	90.60 ± 0.93a	93.00 ± 1.38a	92.80 ± 1.16a	91.80 ± 0.66a
Azadirachtin	84.80 ± 1.93a	83.20 ± 2.22a	75.20 ± 2.78a	88.80 ± 2.01a	79.00 ± 1.58a	83.40 ± 2.01a
Thiamethoxam	90.80 ± 1.66a	90.80 ± 1.16a	87.40 ± 1.44a	87.80 ± 1.24a	88.20 ± 0.86a	89.00 ± 1.22a
Deltamethrin	59.60 ± 3.11b	54.20 ± 4.89b	47.20 ± 3.06b	56.40 ± 5.28b	51.80 ± 6.62b	43.40 ± 3.04b
Acephate	34.40 ± 5.21c	41.40 ± 4.72bc	50.60 ± 6.92c	38.20 ± 5.31c	34.60 ± 6.56bc	29.20 ± 2.71c
Methomyl	32.60 ± 5.19c	27.00 ± 3.85c	26.00 ± 4.92c	22.00 ± 2.72d	17.40 ± 3.92c	26.60 ± 4.68c
F	67.28	70.19	42.73	76.23	53.43	127.87
df	5. 24	5. 24	5. 24	5. 24	5. 24	5. 24
P	<0.01	<0.01	<0.01	< 0.01	< 0.01	< 0.01

Means in each column followed by same letter do not differ significantly by the Tukey's test ($P < 0.05$).

Table 7. Compatibility score between the most virulent entomopathogenic fungi isolates to *P. xylostella* and pesticides registered for the control of cabbage crop pests

Treatments	*B. bassiana*								*I. sinclairii*		*M. rileyi*	
	IBCB01		IBCB18		IBCB66		IBCB87		ARSEF7973		LCMAP101	
	BI	CC	BI	CC	BI	CC	BI	CC	BI	CC	BI	CC
Azadirachtin	84, 35	C	74, 72	C	82, 86	C	70, 96	C	73, 85	C	84, 54	C
Thiamethoxam	93, 71	C	87, 62	C	91, 67	C	87, 99	C	66, 34	C	91, 37	C
Deltamethrin	59, 41	MT	61, 62	MT	64, 70	MT	51, 15	MT	37, 18	T	78, 91	C
Acephate	54, 38	MT	51, 10	MT	52, 41	MT	49, 89	MT	33, 08	T	55, 77	MT
Methomyl	28, 51	T	28, 77	T	42, 92	MT	25, 46	T	21, 31	T	28, 65	T

BI—Biological index, CC—Compatibility classification (as characterized by Alves et al. 2007).
C—Compatible; MT—Moderately Toxic; T—Toxic

used in combination with these fungi in integrated programs for diamondback moth management (Alves et al. 2008).

Thus, the correct use of virulent fungal entomopathogens for use as biological control agents to reduce populations of *P. xylostella* in combination with compatible chemical control products will enable a significant increase in the adoption of integrated management of this pest, couple with a possible reduction in pesticide use with the correlated benefits of reduced ecological impacts from the indiscriminate use of pesticides. The end result of such advances should be the improved state of the cabbage agroecosystem conditions together with positive impacts for both humans and the overall environment.

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