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Original Article

Tactics of applying entomopathogenic fungi in the control of *Plutella xylostella* and *Brevicoryne brassicae*

Táticas de aplicação de fungos entomopatogênicos no controle de *Plutella xylostella* e *Brevicoryne brassicae*

M. O. Campagnani[a], W. G. Campos[b], R. M. Maurício[b], A. P. Madureira[b], A. C. R. Silva[b], N. B. M. Silva[b], M. J. F. Morenz[a] and A. M. Auad[a]*

[a]Empresa Brasileira de Pesquisa Agropecuária – Embrapa, Laboratório de Entomologia, Juiz de Fora, MG, Brasil
[b]Universidade Federal de São João del-Rei – UFSJ, Departamento de Biotecnologia, São João del-Rei, MG, Brasil

Abstract

Plutella xylostella L., 1758 (Lepidoptera: Plutellidae) and *Brevicoryne brassicae* L., 1758 (Hemiptera: Aphididae) are important pests of Brassicaceae, causing significant damage that makes human consumption of these vegetables impossible. As an alternative to the use of chemical insecticides, this study aimed to (i) evaluate the endophytic capacity of the native fungi *Fusarium multiceps* (Hypocreales: Nectriaceae) and *Metarhizium anisopliae* (Metschn.) Sorokīn (Hypocreales: Clavicipitaceae) and a commercial strain of *M. anisopliae* on cabbage plants (*Brassica oleracea* L. var. *acephala*) through inoculations via seed treatments, foliar spraying or root applications and (ii) evaluate the mortalities of *P. xylostella* and *B. brassicae* fed on plants treated with these fungi. Ten *P. xylostella* caterpillars or ten *B. brassicae* nymphs were used per repetition, and the infestations were quantified daily (for *P. xylostella*) or after 15 days (for *B. brassicae*). The control treatment consisted of water. The fungi *M. anisopliae* and *F. multiceps* demonstrated endophytic colonization capacities in *B. oleracea* through different inoculation techniques, including application via seeds, leaves and roots. This colonization resulted in the infection and consequent significant mortality of *P. xylostella* and *B. brassicae*, reinforcing the potential of these microorganisms as promising agents for biological control.

Keywords: insect, entomopathogenic fungi, endophytes.

Resumo

Plutella xylostella L., 1758 (Lepidoptera: Plutellidae) e *Brevicoryne brassicae* L., 1758 (Hemiptera: Aphididae) são importantes pragas de Brassicaceae, causando danos significativos que impossibilitam o consumo humano dessas hortaliças. Como alternativa ao uso de inseticidas químicos, este estudo teve como objetivos (i) avaliar a capacidade endofítica dos fungos nativos *Fusarium multiceps* (Hypocreales: Nectriaceae) e *Metarhizium anisopliae* (Metschn.) Sorokīn (Hypocreales: Clavicipitaceae) e de uma estirpe comercial de *M. anisopliae* em plantas de couve (*Brassica oleracea* var. *acephala*) por meio de inoculações via tratamento de sementes, pulverização foliar ou aplicação radicular e (ii) avaliar a mortalidade de *P. xylostella* e *B. brassicae* alimentadas em plantas tratadas com esses fungos. Dez lagartas de *P. xylostella* ou dez ninfas de *B. brassicae* foram utilizadas por repetição, e as infestações foram quantificadas diariamente (*P. xylostella*) ou após 15 dias (*B. brassicae*). O tratamento controle consistiu em água. Os fungos *M. anisopliae* e *F. multiceps* demonstraram capacidade de colonização endofítica em *B. oleracea* por meio de diferentes técnicas de inoculação, incluindo aplicação via sementes, folhas e raízes. Essa colonização resultou na infecção e consequente mortalidade significativa de *P. xylostella* e *B. brassicae*, reforçando o potencial desses microrganismos como agentes promissores para o controle biológico.

Palavras-chave: inseto, fungos entomopatogênicos, endófitos.

1. Introduction

One of the main pests affecting crops in the Brassicaceae L. (Cruciferae) family is the cruciferous moth *Plutella xylostella* L., 1758 (Lepidoptera: Plutellidae) (Kuchár et al., 2019). This pest is responsible for losses of more than 80% of the annual agricultural production worldwide, resulting in estimated losses of between 4 and 5 billion dollars (Zalucki et al., 2012). Throughout its life cycle, *P. xylostella* remains predominantly on the abaxial side of host plant leaves (Sarfraz et al., 2005). During the larval stage, which lasts approximately 15 days, severe damage is caused by the formation of surface mines, visible as whitish spots, which compromise photosynthesis and reduce

*e-mail: alexander.auad@embrapa.br
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the size and quality of leaves intended for consumption (Chávez and Hurtado, 2010).

Another pest that compromises Brassicaceae production is the aphid *Brevicoryne brassicae* L., 1758 (Hemiptera: Aphididae). Nymphs and adults suck sap from leaves, young shoots and inflorescences, introducing toxins into the vascular system, which can lead to the death of the plant (Dias de Almeida et al., 2007). In addition, these insects are vectors of viral diseases, causing leaf deformation and damaging plant development (Rando et al., 2023). Their high reproductive capacity and short life cycle favor population outbreaks, which require intensive applications of insecticides (Shah and Chunduri, 2023).

The abovementioned insect pests are controlled mainly by the continuous use of phytosanitary products. In crop cycles lasting up to 99 days, three to five applications of insecticides are common for controlling *P. xylostella* and *B. brassicae* (Tabashnik et al., 1987; Chen et al., 1996; Silva et al., 2003), which can result in the presence of toxic residues in food and environmental contamination. This makes it necessary to develop more sustainable control strategies that are less harmful to human health and the environment. One promising alternative is the use of entomopathogenic fungi. Fungi are important biocontrol agents for various pests of cultivated plants (Silva et al., 2003). Some commercial formulations of fungi are already used in protected crops, reducing aphid populations, especially on ornamental plants (Loureiro and Moino Junior, 2006). The use of these microorganisms to control *P. xylostella* has not been extensively explored. The fungi *Beauveria bassiana* (Balsamo) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorok stand out worldwide as biocontrol agents for various orders of insects, including Hemiptera (Aphididae) and Lepidoptera (Plutellidae) (Alves and Faria, 2010; Butt et al., 2016; Kuchár et al., 2019).

The application method for these fungi generally involves spraying the conidia on the plants, either by air or ground, depending on the scale of production and growing conditions. To ensure effectiveness, it is essential that applications take place at times of low solar radiation and in conditions of high humidity to favor the germination and dissemination of the conidia, since suitable abiotic factors are essential for the successful use of these fungi. In addition, the use of precise formulations and doses are factors for maximizing results. Research has shown that when used correctly, these fungi can significantly reduce pest infestations, promoting healthier vegetable production with less dependence on chemical pesticides (Ahsan et al., 2024).

Abiotic factors are directly associated with the efficacy and stability of fungi in the field, which reduce the viability of the conidia (Vega et al., 2012). In this context, the potential of endophytic entomopathogenic fungi, which colonize plant tissues without causing damage, establish a mutualistic relationship with the host and act as insect pathogens, stands out (Wilson, 1995; Branine et al., 2019). In addition, these fungi can increase plant tolerance to biotic and abiotic stresses (Sasan and Bidochka, 2012), providing greater stability and persistence in the field.

Thus, the objectives of this study were (i) to evaluate the endophytic colonization capacities of the native fungi, *Fusarium multiceps* and *Metarhizium anisopliae*, and a commercial strain of *M. anisopliae* on cabbage (*Brassica oleracea* L. var. *acephala*) by inoculation via roots, leaves and seeds and (ii) to evaluate the mortality of *P. xylostella* and *B. brassicae* fed on plants previously treated with these entomopathogenic fungi.

2. Material and Methods

The experiments were conducted at the Federal University of São João del Rei (UFSJ) in the CTAN Field, located in São João del Rei (MG), Brazil. The experimental activities were carried out in a greenhouse with no climate control. During the experimental period, the average temperature inside the greenhouse was 28 ± 4°C, and the relative humidity was 65 ± 30%.

2.1. Obtaining the host plant and insect pests

B. oleracea seeds were purchased from HI-CROP (Takii Seed) lot 41 VK and were not chemically treated. In the first stage of the experiments, the *B. oleracea* seeds were subjected to surface disinfestation by immersion in 2% sodium hypochlorite for two minutes, followed by immersion in 70% alcohol for one minute. After these steps, the seeds were washed with sterile distilled water according to the methodology adapted from Carvalho et al. (2012) and Ferreira et al. (2017).

Pluella xylostella and *B. brassica* were obtained from the Entomology Laboratory and the greenhouse, respectively, of the Biotechnology Department of the Federal University of São João del Rei on *B. oleracea* host plants.

2.2. Obtaining the fungi

The isolates of the native fungi *M. anisopliae* and *F. multiceps* and the commercial *M. anisopliae* strain were reactivated and prepared separately to produce conidia. The fungi were subsequently grown in Petri dishes (9.0 cm × 1.5 cm) containing Sabouraund dextrose agar culture medium (4% dextrose, 1% casein and 1.5% agar; KASVI®, Spain), which was poured into a previously sterilized vertical laminar flow chamber. These plates were incubated in germination chambers (BOD, biochemical oxygen demand; EletroLab®, Campinas, Brazil) at 25 ± 2°C and 70 ± 10% RH with a 12-h photoperiod to promote vegetative growth and conidiogenesis.

After seven days of incubation, the conidia produced were removed from the surface of the culture medium with a sterile metal spatula and inoculated separately to prepare suspensions containing sterile distilled water and an ionic surfactant (Tween 80 [0.001%], Sigma–Aldrich®, Steinheim, Germany) at a concentration of 10^8 conidia/mL. The suspensions were homogenized using a magnetic stirrer, and the conidia concentrations were estimated using a hemocytometer.

The viability of the conidia was tested before the experiments began, according to the methodology described by Lopes et al. (2013).

2.3. Inoculation of the fungi via the seeds, roots or leaves of Brassica oleracea

For inoculation via the seeds, a fungal suspension was prepared containing 100 mL of sterile distilled water, 10^8 conidia/mL and 0.001% Tween 80. The seeds were immersed in this suspension for 60 min, according to the methodology adapted from Keyser et al. (2014). A suspension containing only sterile distilled water with 0.001% Tween 80 and no conidia was used as a control. After treatment, the seeds were sown in the seedbeds. Thirty days after sowing, the seedlings were transferred to 10-L plastic pots containing a mixture of 60% soil fertilized with NPK (4:14:8), 20% manure and 20% plant substrate. One seedling was planted per pot, and irrigation was applied every other day. Every 30 days, the plants were fertilized with NPK (4:14:8).

For inoculation via roots or leaves, plants from untreated seeds were subjected to fungal application 30 days after transplanting. Suspensions containing 50 mL of each fungus (native fungus *M. anisopliae* or *F. multiceps* or the commercial *M. anisopliae* strain) at a concentration of 10^8 conidia/mL, plus 0.001% Tween 80, were applied directly to the soil (via roots) or sprayed onto the leaves (via leaves). The negative control consisted of 50 mL of sterile distilled water without fungi, which was applied in the same way.

2.4. Plutella xylostella bioassay

The experiment was conducted in a randomized block design with seven treatments: *B. oleracea* plants were inoculated with the native fungus *M. anisopliae* or *F. multiceps* and the commercial strain of *M. anisopliae* and were applied via roots and seeds; also a control treatment without fungal inoculation (3 fungi × 2 inoculation routes + 1 control) was also included. Five replicates were used, each containing four pots of plants per treatment, totaling 140 plants (7 treatments × 4 pots × 5 blocks). The pots inside a greenhouse, with the treatments organized in isolated groups, were separated from each other by a minimum distance of one meter to avoid cross-contamination.

Sixty days after the seedlings were transplanted, all the plants were artificially infested with *P. xylostella* caterpillars at the 3rd or 4th stage. Each plant received two clip-cages containing five caterpillars each, totaling ten caterpillars per plant. Insect mortality was monitored daily for 10 days.

2.5. Brevicoryne brassicae bioassay

The experiment was conducted in a completely randomized design (DIC), with ten treatments: *B. oleracea* plants inoculated with the native fungus *Metarhizium anisopliae* or *Fusarium multiceps* or the commercial strain of *M. anisopliae*, which were applied via roots, seeds or leaves; and a control treatment without fungal inoculation (3 fungi × 3 inoculation routes + 1 control) was used.

Each treatment consisted of 20 plant pots, resulting in a total of 200 pots. The pots were located inside a greenhouse, with the treatments organized in isolated groups, and were separated from each other by a minimum distance of one meter to avoid cross-contamination.

Sixty days after the seedlings were transplanted, all the plants were artificially infested with *B. brassicae* nymphs. Each plant received 10 nymphs, totaling 2000 insects (10 nymphs × 200 pots). After 15 days of infestation, the total number of individuals per plant was evaluated.

2.6. Confirmation of the presence of fungi in plant tissues

To verify the endophytic establishment of the fungi, leaf samples were collected from the plants used in the bioassays with the insects. For the *P. xylostella* bioassay, five leaves were taken at random from each treatment ($n = 5 \times 7$), totaling 35 samples. For the *B. brassicae* bioassay, three leaves were collected per treatment ($n = 3 \times 10$), totaling 30 samples. The leaves were packed in sterile plastic bags, stored in a cooler and transported to the laboratory for analysis.

In the laboratory, each leaf was disinfected on its surface by immersion in 70% ethanol (1 min), washed in sterile distilled water (2 min) and then immersed in 2% sodium hypochlorite (1 min), followed by washing with sterile distilled water (2 min) (Carvalho et al., 2012; Ferreira et al., 2017). After disinfestation, three fragments from different points on each leaf were individually placed in sterile Petri dishes containing Sabouraud Dextrose Agar, (Kasvi®).

The plates were incubated at 26° ± 2°C for approximately 15 days in an air-conditioned chamber (BOD). To verify the presence of the inoculated fungi, the micro- and macromorphological characteristics of the fungi present on the fragments, such as colony color, surface texture, edge aspect and growth time, were compared with those of the native fungi *M. anisopliae* and *F. multiceps* and the commercial *M. anisopliae* strain according to methodology adapted from Klieber and Reineke (2016). To confirm the fungal identities, microcultivations were carried out on the isolated colonies, allowing the reproductive structures to be observed. Taxonomic identifications were based on morphological identification keys, according to Alves (1998). Endophytic establishment was considered positive when more than 60% of the analyzed samples showed characteristics compatible with those of the inoculated fungi.

2.7. Statistical analysis

The normality of residuals and homogeneity of variance were examined for each continuous dependent variable analyzed after model fitting. To study the effects of fungi and inoculation, all the data were analyzed according to a completely randomized design in a 3 × 3 factorial scheme using the MIXED procedure of SAS. The statistical model considered the fixed effects of fungi, fungal inoculations (via root, leaves or seeds) and their interactions, and the random effects of errors. The means were compared by Fischer's minimum significant difference, using the PDIFF option of the LSMEANS. To study the effect of inoculation on each fungus, all the data were analyzed as follows:

The mortality of *P. xylostella* for each fungus (on the roots and seeds) as a function of time was studied using the MIXED procedure of SAS, with repeated measurements over time (day). In all analyses, the control was included as a reference. Fungi, time and their interactions were considered fixed effects, and experimental error was considered a random effect. The covariance matrix was

chosen on the basis of the Akaike information criterion (AIC) (Wolfinger, 1993). The means of the fungal effects were determined by the Tukey test, using the PDIFF option of the LSMEANS. For all analyses, a critical probability level of 0.05 for a type I error was adopted.

Responses regarding the presence/absence of endophytes were analyzed using a binomial logistic regression model with a logit link function, considering treatment as a fixed factor. The problem of complete separation caused by the absence of positive cases in the control treatment was solved using Firth's bias-reduced penalized likelihood. Adjusted means were obtained on the original scale using the ILINK option. Multiple comparisons between treatments were performed using Tukey's test ($P < 0.05$).

All analyses were performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Confirmation of the presence of fungi in plant tissues

The percentages of endophytic colonization by the native fungi *M. anisopliae* and *F. multiceps* and the commercial *M. anisopliae* strain in *B. oleracea* tissues did not significantly differ between the different inoculation methods after 70 or 110 days of treatment (Table 1). Regardless of the application sites (e.g., seeds, roots or leaves), all the fungi showed an endophytic colonization capacity, with rates greater than 60%. These results indicate that most of the plant samples evaluated developed colonies with macro- and micromorphological characteristics that were compatible with those of the inoculated fungi, confirming effective endophytic establishment under the different conditions tested.

3.2. Bioassay with Brevicoryne brassicae

Compared with the control treatment, the fungal treatment resulted in a significant reduction (90%) in the number of *B. brassicae* ($F = 516.44$; $P < 0.0001$), with an average of 318.25 individuals 15 days after infestation.

There was a significant interaction between the species of fungus used and the mode of application ($F = 20.25$; $P < 0.0001$). There were significant differences in the mean numbers of aphids depending on the inoculation method (e.g., roots, leaves or seeds) ($F = 58.79$; $P < 0.0001$). In addition, there were significant differences in the mean numbers of aphids among the different fungal species ($F = 175.02$; $P < 0.0001$) (Table 2).

Inoculation of the fungi via seeds promoted the greatest reductions in the aphid populations ($F = 58.79$; $P < 0.0001$), followed by inoculation via the leaves. Inoculation via roots was the least effective for commercial and native fungal *M. anisopliae* strains. No significant difference was observed between inoculations when the fungus *F. multiceps* was applied (Table 2).

When the performance of the entomopathogenic fungi was analyzed within each inoculation, *F. multiceps* performed significantly better when it was applied via roots and leaves ($F = 175.02$; $P < 0.0001$), resulting in greater efficacy in controlling aphids after 15 days of exposure (Table 2). On the other hand, the native *M. anisopliae* strain had the smallest effect when inoculated via leaves and seeds.

Table 1. Mean persistence (%) of the fungi on *Brassica oleracea* var *acephala* 70 days after the inoculation of the fungi on the plants via the seeds, leaves and roots (bioassay with *Brevicoryne brassicae*) and 110 days after foliar and root inoculations (bioassay with *Plutella xylostella).*

Days After Treatment	Method Inoculation	*M. anisopliae nativa strain*	*M. anisopliae commerical strain*	*F. multiceps native strain*	Control	X^2	*P*
	Seed	70 ± 10.51[a]	65 ± 10.94[a]	60 ± 11.23 [a]	0 [b]	*25.8*	<0.0001
70 days	Leaves	65 ± 10.94 [a]	60 ± 11.24 [a]	70 ± 10.51 [a]	0 [b]	25.8	<0.0001
	Root	60 ± 11.24 [a]	60 ± 11.24 [a]	60 ± 11.23 [a]	0 [b]	21.8	<0.0001
110 days	Seed	70 ± 10.51 [a]	55 ± 10.41 [a]	60 ± 11.24 [a]	0 [b]	*23.8*	<0.0001
	Root	65 ± 10.94 [a]	65 ± 10.94 [a]	70 ± 10.51 [a]	0 [b]	26.8	<0.0001

Means with distinct letters in the lines for each day after treatment with the fungi indicate significant differences between the percentages of fungal presence according to the Tukey test ($P = 0.005$).

Table 2. Mean numbers of *Brevicoryne brassicae* on cabbage plants (*Brassica oleracea var acephala*) 15 days after being infested with 10 nymphs. The fungi were inoculated via the seeds, leaves and roots

Fungus|	Root	Leaf	Seed	
Native strain *M. anisopliae*	86.1± 5.83 cC	64.95 ± 6.00 bC	26.25 ± 2.97 aB	59.10 ± 4.34 C
commercial strain M. anisopliae	33.7 ± 3.48 cB	22.90± 3.03 bB	2.70 ± 0.60 aA	19.77 ± 2.26 B
Native strain *F. multiceps*	11.2 ± 3.33 aA	5.20 ± 0.70 aA	10.90 ± 0.74 aA	9.10 ± 1.20 A
	43.67 ± 4.78 c	31.01 ± 3.94 b	13.28 ± 1.63 a	

Different lower-case letters in the lines indicate significant differences between the methods; uppercase columns indicate significant differences between the fungi according to the Fischer test ($P < 0.05$).

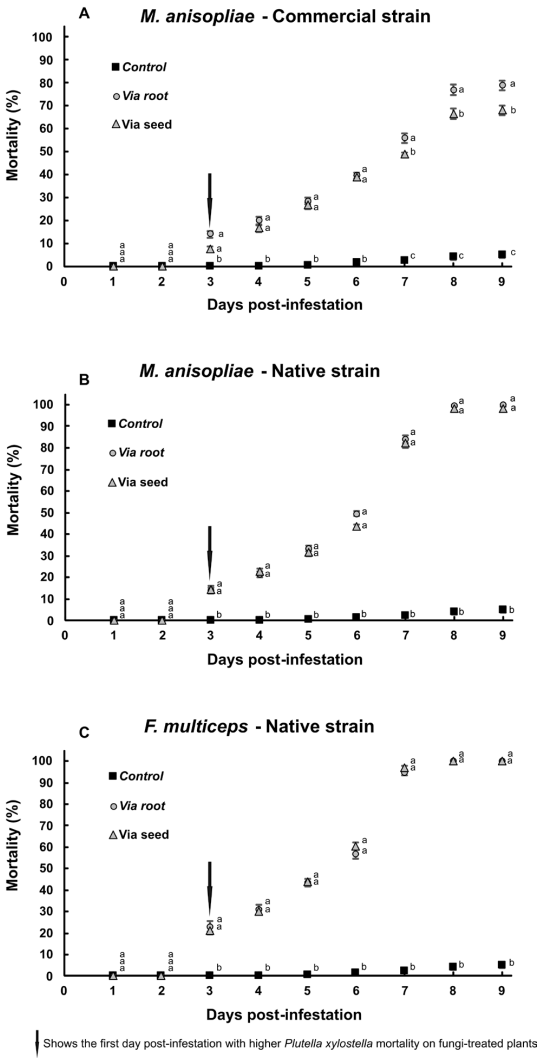

Figure 1. Cumulative mortality of 3rd-stage *Plutella xylostella* larvae in cabbage plants (*Brassica oleracea var acephala*) inoculated at the seed stage or by irrigation of the plant (root inoculation) with entomopathogenic fungi: Commercial *Metarhizium anisopliae* strain (A); native *Metarhizium anisopliae* strain (B); and *Fusarium multiceps* (C) as well as control plants not inoculated with fungi. The points and bars indicate the means ± standard errors of 10 plants, with 10 larvae per plant. Different letters for mortality on the 3rd and 9th days for the same fungus indicate significant differences between inoculation methods according to the Tukey test at 5%.

No significant differences were observed between the native *F. multiceps* strain and the commercial *M. anisopliae* strain when the inoculations were carried out via the seeds (Table 2).

3.3. Bioassay with Plutella xylostella

After three days, there were significant differences in the mortality of *P. xylostella* caterpillars fed *B. oleracea* from seeds treated with the commercial *M. anisopliae* strain ($F = 700.7$; $P = 0.0001$) (Figure 1A) and native strains of *M. anisopliae* ($F = 1398.1$; $P = 0.0001$) (Figure 1B) and *F. multiceps* ($F = 1480.7$; $P = 0.0001$) (Figure 1C) compared with those fed plants that were not inoculated with the fungus. After nine days, the native strains of *M. anisopliae* (Figure 1B) and *F. multiceps* (Figure 1C) caused 100% mortality of the caterpillars, either by inoculation in the seed treatment or by root application. The mortality of the commercial *M. anisopliae* strain was greater than that of the control treatment; however, it was less than 80% for any of the inoculation methods.

It is clear that the different forms of inoculation by the fungi *M. anisopliae* (Figure 1B) and *F. multiceps* (Figure 1C) significantly promoted the same mortality of the insect pest. However, the commercial strain that was inoculated via the roots showed greater mortality of *P. xylostella* from the seventh day onward (Figure 1A).

4. Discussion

This study revealed that the native fungi *M. anisopliae* and *F. multiceps* and the commercial strain of *M. anisopliae* were reisolated from more than 60% of the *B. oleracea* samples, with no significant differences between inoculations via seeds, leaves or roots. This suggests a generalized capacity for endophytic colonization. Similar results were obtained by Tefera and Vidal (2009), who reisolated the entomopathogenic fungus *B. bassiana* from various plant species. It is known that some entomopathogenic fungi can penetrate plant tissues and colonize them systemically (Wagner and Lewis, 2000). In the present study, all inoculations allowed *B. oleracea* to be colonized, which was also observed by Wei et al. (2020), who tested *B. bassiana* on *Solanum lycopersicum* L. and reported the systemic distribution of the fungal colony, especially after inoculation via foliar spraying, which was considered the most effective method because of the greater contact area for spore adhesion. According to Wagner and Lewis (2000), *B. bassiana* spores grow randomly on the surfaces of plant tissues and can penetrate through the stomata and colonize the tissues internally. This behavior has also been shown in the current study with *B. oleracea*.

Reisolation of *M. anisopliae* and *F. multiceps* was possible between 70 and 110 days after inoculation. This persistence time is comparable to that reported by Biswas et al. (2012), who reported the presence of *B. bassiana* on jute (*Corchorus capsularis* L.) for up to 90 days. Posada et al. (2007) observed colonization for 240 days in coffee trees, Brownbridge et al. (2012) reported endophytic fungi for up to 270 days in *Pinus radiata* D. (Don), and Oliveira Netto et al. (2024) reported endophytic fungi in *Urochloa ruziziensis* (Hochst. ex A. Rich.) for up to 360 days. Jaber and Ownley (2018) reported that the extent and persistence of fungal colonization can increase with repeated applications by spraying or irrigation. However, in this study, a single application was sufficient to guarantee colonization, which highlights the potential of the isolates tested. Further studies throughout the plant cycle are needed to assess the durability of colonization and its effects on pests at different phenological stages.

The native fungi *M. anisopliae* and *F. multiceps* and the commercial *M. anisopliae strain* were able to contain an

endophytic *B. brassicae* outbreak on *B. oleracea*. Kim et al. (2007) reported that entomopathogenic fungi, such as *B. bassiana* and *Lecanicillium lecanni*, controlled the aphid population on cotton. Loureiro and Moino Junior (2006) reported 100% mortality of *Aphis gossypii* and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), with *Lecanicillium lecanii* and *Paecilomyces fumosoroseus* (Wise) reaffirming the potential of these fungi in the biological control of aphids. In the current study, seed inoculation was the most effective method for reducing the population density of *B. brassicae*, followed by inoculation via leaves. These findings indicate significant advances, especially in the use of seed treatment as a strategy to protect fungi from abiotic factors while maintaining their viability and virulence within plant tissues.

Plutella xylostella, one of the main pests of Brassicaceae, has been the target for biological control with entomopathogenic fungi, as noted by Correa-Cuadros et al. (2014). Vandenberg et al. (1998) reported mortality rates between 72% and 100% for *P. xylostella* with isolates of *B. bassiana*, whereas Silva et al. (2003) reported efficacy rates between 70% and 96%. Despite these promising results, these microorganisms are still underutilized, which reinforces the importance of the data presented here. In the current study, the native fungal strains of *M. anisopliae* and *F. multiceps* caused 100% mortality of *P. xylostella* larvae after nine days of endophyte infection. The commercial strain of *M. anisopliae,* on the other hand, resulted in a mortality rate between 68% and 79% over the same period, demonstrating less efficacy than the other isolates did.

In a study conducted in Mexico, *M. anisopliae* was identified as the most abundant entomopathogenic fungus on sugarcane leaves (Hernández-Domínguez et al., 2016). In addition to endophytic colonization, recent research has highlighted the potential of secondary metabolites produced by these fungi. In the current study, *F. multiceps* was used, whose strain was also isolated from healthy leaves of *Acanthus ilicifolius* L. (Shah and Chunduri, 2023), demonstrating bioactive characteristics with potential applications in agriculture. This same species has already been used to control spittlebugs in *Urochloa brizantha* (Hochst. ex A. Rich.) (Campagnani et al., 2024) and *Uochloa ruziziensis* (Oliveira Netto et al., 2024), showing good results when applied via seed treatment.

Considering that entomopathogenic fungi are sensitive to environmental factors such as temperature, UV radiation and humidity, endophytic colonization represents a promising strategy. The internalization of microorganisms protects them from environmental adversity and can ensure their persistence and effectiveness in pest control. On the other hand, plants also benefit from the presence of fungi in their tissues, reducing herbivory. This technique has practical advantages for farmers, such as ease of application and affordability. However, further studies are needed to evaluate its application to perennial crops or those with longer cycles, where the persistence of fungal action is a crucial factor.

5. Conclusions

The endophytic colonization capacity of the fungi *M. anisopliae* and *F. multiceps* was demonstrated in *B. oleracea* through different inoculation techniques, including applications via seeds, leaves and roots. This colonization resulted in the infection and consequent mortality of *P. xylostella* and *B. brassicae*, reinforcing the potential of these microorganisms as promising biological control agents.

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Data Availability Statement

The entire data set that supports the results of this study was published in the article itself.