





Entomopathogenic fungi: an *in vitro* control alternative for *Frankliniella schultzei* (Thysanoptera: Thripidae)

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ABSTRACT

Lettuce (*Lactuca sativa* L., Asteraceae) is the world's most consumed leafy vegetable and is widely cultivated throughout Brazil. The crop is highly susceptible to insect herbivory and phytopathogenic attacks; among the most damaging pests are thrips (order Thysanoptera), which act as vectors of a tospovirus complex locally termed "vira-cabeça." Infections caused by diverse Tospoviridae species induce mosaic, necrosis, deformation, mottling, and bronzing, collectively reducing lettuce productivity. The present study evaluated the efficacy of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, both individually and as a combined suspension, against thrips, and compared their performance with the neonicotinoid insecticide imidacloprid. Experiments were conducted under controlled-environment conditions using leaf-disc assays; mortality was recorded at 12-hour intervals for 72 h and analyzed with Kruskal-Wallis, Fisher's LSD, and Kaplan-Meier survival methods. Both fungal treatments achieved mean control efficacies exceeding 50 %, which were not statistically different from the 67 % efficacy observed for imidacloprid ($p > 0.05$). Survival-analysis curves indicated comparable time-to-mortality among the fungal suspensions and the chemical control. These findings demonstrate that *B. bassiana* and *M. anisopliae* can provide biological control performance comparable to a conventional insecticide, supporting their integration into lettuce Integrated Pest Management (IPM) programs as either complementary agents or viable alternatives to chemical control.

RESUMO

Hortaliça folhosa mais consumida no mundo a espécie *Lactuca sativa* L. (alface) é cultivada em todas as regiões do Brasil e caracteriza-se por sua susceptibilidade ao ataque de insetos e fitopatógenos. Dentre os insetos praga de interesse econômico para a cultura destacam-se os do gênero Thysanoptera (tripes), pois são vetores de um complexo de viroses popularmente denominada de vira-cabeça, que tem por agente várias espécies do gênero tospovírus (família Bunyaviridae) causando sintomas como mosaico, necrose, deformação, mosqueado e bronzeamento nas plantas, sendo estes fatores de influência a redução em produtividade para cultivos de alface. Tendo em vista a problemática em questão, este estudo buscou comparar os níveis de controle estabelecido por meio da aplicação de suspensões de *Beauveria bassiana*, *Metarhizium anisopliae*, mistura binária destes fungos e do inseticida imidacloprido em ação sobre tripses. Foram comparadas populações médias após cada tratamento, bem como eficiência de controle dos mesmos, além do tempo de ação de cada tratamento sobre os insetos. Observou-se que as suspensões de fungos entomopatogênicos apresentaram eficiência de controle acima de 50%, estatisticamente semelhante a estabelecida pelo produto químico comercial (67%). Portanto, os fungos entomopatogênicos *Beauveria bassiana* e *Metarhizium anisopliae* apresentam eficiência de controle biológico comparável ao inseticida imidacloprido, tendo potencial para serem utilizados em auxílio ou em substituição a este produto químico.

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Introduction

Lettuce (*Lactuca sativa* L., Asteraceae) a species belonging to the Asteraceae family is widely cultivated across Brazil. Yet, in many production systems, inadequate phytosanitary monitoring and limited farmer awareness foster the emergence of pests and diseases that inflict substantial yield losses (Lima et al., 2016; Freitas, 2018).

The persistent shortage of phytosanitary monitoring is particularly worrisome in protected-culture environments, where high humidity and stable temperatures foster rapid pathogen proliferation. Among the protected-culture technologies, one of the most widely adopted for *L. sativa* is hydroponic systems, which enable lettuce to be produced across diverse climatic zones (e.g., temperate, tropical), while shielding the crop from adverse weather events (Carvalho, 2017).

Although hydroponic cultivation shields lettuce from climate-related abiotic stresses, the crop remains vulnerable to insect-borne damage. Thrips (Thysanoptera: Thripidae), particularly *Frankliniella occidentalis* (Pergande, 1895) and *Frankliniella schultzei* (Trybom, 1910) (Thysanoptera: Thripidae), inflict both mechanical injury and virus transmission. These vectors disseminate the tospovirus complex, that causes the disease locally referred to as “vira-cabeça,” which elicits chlorosis, leathery leaf tissue, leaf breakage, and subsequent leaf abscission (Cavalcanti, 2006).

Given that the tospovirus complex is transmitted by thrips, its control must be primarily preventative, focusing on vector management. Regrettably, the repertoire of insecticides authorized by the Ministry of Agriculture, Livestock and Supply (MAPA) for thrips is limited, compelling growers to rely repeatedly on a narrow set of chemistries, mostly neonicotinoids (e.g., imidacloprid, thiacloprid) and carbamates such as phenyl-methylcarbamate (Agrofit, 2026).

On that matter it is important to acknowledge that intensive reliance on these insecticides can foster resistance in thrips populations and may drive growers toward non-selective or unregistered compounds. Such practices increase the risk of pesticide residues in harvested lettuce, contribute to environmental contamination, and generate additional agronomic and public-health concerns (Lopes, Alves & Tamai, 2000).

Consequently, given the drawbacks associated with the repeated application of a single chemical active ingredient (Junior et al., 2018), entomopathogenic fungi emerge as a viable alternative for thrips management. These microorganisms exhibit high host specificity, negligible toxicity to humans, and can be integrated with complementary control measures, rendering them suitable for deployment across extensive production areas. Accordingly, the present study was designed to evaluate and compare in vitro insecticidal efficacy of selected

fungal suspensions with that of a commercially registered chemical formulation against members of the order Thysanoptera.

Materials and Methods

Collection site and experimental setup

Adult thrips were harvested from a commercial hydroponic lettuce operation located in Ananindeua, Pará State, within the Belém Metropolitan Region. The specimens were subsequently transported to the Animal Nutrition Laboratory of the Federal Institute of Education, Science and Technology of Pará (Castanhal Campus), located in the Northeast Pará State mesoregion along the BR-316 highway (km 62, Castanhal, Pará State). All experimental procedures were conducted at this facility during the period spanning November to December 2019.

Tools for capture and species identification

Thrips were collected manually from the hydroponic benches using a customized suction apparatus and portable plastic cages. The suction device consisted of a silicone hose terminating in a 1 mL pipette tip fitted with a fine-mesh voile fabric, allowing the insects to be drawn into the hose while excluding debris. The containment cages were fashioned from 400 mL plastic containers whose lids were modified with a cut-out that was sealed with a composite of polypropylene and non-woven viscose (TNT) fabric. This fabric permits adequate airflow inside the container yet prevents the captured insects from escaping, thereby facilitating subsequent morphological identification.

Immediately after capture, fifteen individuals were dispatched for taxonomic identification and organized into five groups of three specimens each. Identification was carried out under a light microscope at $40\times$ magnification, following the morphological keys of Monteiro, Mound & Zucchi (2001) and Cavalleri & Mound (2012), as incorporated in the “Thrips of Brazil” database (Lima, 2018).

Control-efficacy test

Five treatments were evaluated: (i) a control solution (distilled water); (ii) a *Beauveria bassiana* suspension derived from the commercial product Beauveria Oligos WP (isolate IBCB 66; Oligos Biotec, São Paulo, Brazil); (iii) a *Metarhizium anisopliae* suspension obtained from Metarhizium Oligos WP (isolate IBCB 425; Oligos Biotec, São Paulo, Brazil); (iv) a combined fungal suspension containing equal parts of the two wettable powders (2.5 g of each product per litre of distilled water); and (v) the neonicotinoid insecticide imidacloprid (Evidence 700 WG; Bayer S.A., São Paulo, Brazil). The fungal products were diluted at 5 g L^{-1} , producing suspensions of 5×10^8 conidia mL^{-1} , whereas the imidacloprid formulation was applied at 0.20 g L^{-1} , corresponding to the label-recommended rate of $0.14\text{ g a.i. L}^{-1}$.

After preparing the suspensions, 50-mm-diameter leaf discs of lettuce cultivar ‘Americana’ (sixth phenological stage) were immersed in each treatment for 30 seconds. The treated discs were then transferred to Petri dishes, and two thrips individuals were introduced onto each disc.

To preclude insect egress, each Petri dish was sealed with a polyvinyl-chloride (PVC) film pierced with a No. 12 hand-needle, allowing adequate air circulation while retaining the thrips. Mortality was evaluated by counting the number of live individuals in each dish. To verify viability at the moment of assessment, the film was gently removed and a soft brush was used to stimulate movement; only insects that responded were recorded as alive.

Experimental design and evaluations

The experiment was arranged according to a completely randomized design, allocating ten independent replicates to each of the five treatments. Each replicate comprised two thrips, yielding a total of twenty individuals per treatment. Mortality assessments were performed at twelve-hour intervals—specifically at 12, 24, 36, 48, 60 and 72 hours (i.e., over a three-day period).

Data Analysis

Mortality data were subjected to a non-parametric Kruskal-Wallis test ($\alpha = 0.05$) to detect treatment effects at each observation time. Whenever the omnibus test indicated significant differences, pairwise comparisons were performed using Fisher’s Least Significant Difference (LSD) test at the same 5 % significance level.

Finally, to compare the temporal efficacy of the treatments on insect mortality, survival curves were estimated with the non-parametric Kaplan-Meier (product-limit) estimator. This approach accommodates censored observations, here representing individuals that had not yet died at a given assessment, and generates step-function plots whose jumps correspond to observed mortality events. The resulting survival functions enable a direct comparison of mortality as a function of treatment over time (Miranda, 2012).

All statistical computations were carried out in R 3.6.1 (R Core Team, 2019) using the RStudio 1.2.5001 interface (RStudio Team, 2019), with analytical routines from the agricolae package (De Mendiburu, 2019).

Results and Discussion

Species Identification

The collected specimens were taxonomically identified as *F. schultzei*. Identification relied on several diagnostic characters: an eight-segmented antenna with the distal two segments markedly tapered; the presence of two well-developed intercalary setae and a prominent post-ocular seta; a conspicuous pair of marginal setae on each quadrant of the

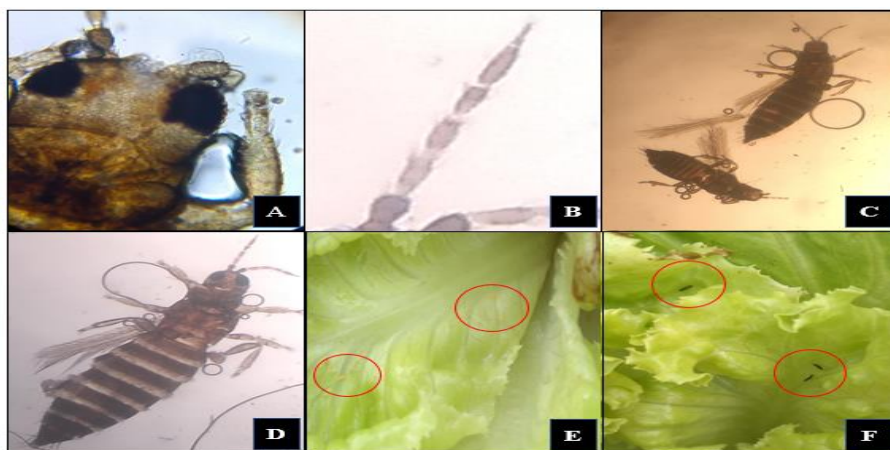
prothorax; and two longitudinal veins of the forewing bearing evenly spaced setae along their entire length (Cavalleri & Mound, 2012; Bertino et al., 2015).

F. schultzei is a small thrips species, with adult females ranging from 1.0 to 1.5 mm in length and exhibiting modest intraspecific variation in body proportions (Fig. 4:C). Colouration is polymorphic: individuals may appear uniformly dark brown, or display a bicolored pattern in which a pale, yellowish-tinged morph co-occurs with the darker form. Both colour morphs were recovered in the present survey (Fig. 4:E,F). Despite these external differences, the two morphs are anatomically indistinguishable, a phenomenon that has been reported across the species' global distribution (Kakkar, Seal & Jha, 2010).

The specimens displayed a dorsally viewed head that was markedly broader than long, with convex genae and ellipsoidal compound eyes that are only minimally protrusive. Their antennae conform to the diagnostic criteria of Group III, exhibiting a modestly fungiform pedicel, while the pronotum is largely smooth but bears transverse striations that become more pronounced toward the posterior margin. The tarsi display the characteristic segmentation typical of the genus (Fig. 4A). These morphological traits are fully consistent with the descriptions provided by Johansen (2002).

Figure 4.

Morphological characteristics of *Frankliniella schultzei* observed in this study. (A) – Tarsal segmentation; (B) – Antenna, composed of eight segments; (C) – Intraspecific size variation among adults; (D) – Brown adult mounted on a slide; (E) – light-yellow nymphs; (F) – Brown adults.



Note: Authors (2019).

Concerning its ontogeny, the female *F. schultzei* oviposits within plant tissue. Upon hatching, the offspring pass through two actively feeding larval instars followed by two quiescent, non-feeding stages (prepupa and pupa). Under a constant temperature of 24.5 °C (conditions representative of Brazilian greenhouse environments) a complete generation requires approximately 12.6 days. The embryonic period averages 4 days; the first and second

larval instars each last about 2.5 days, the prepupal stage 1.2 days, and the pupal stage 2.1 days. Adult longevity averages 13 days for both females and males, although reports in the literature document a broader range of 14–30 days (Kakkar, Seal & Jha, 2010; Bertino et al., 2015).

Control efficacy

At 60 hours after exposure to the respective treatments, mortality began to diverge markedly among the groups. The entomopathogenic fungal suspensions caused a significant decline in the number of surviving thrips, while the imidacloprid formulation did not differ statistically from either the single-fungal applications or the combined-fungal mixture (Table 1).

Table 1.
Mean number of *Frankliniella schultzei* individuals on lettuce leaf discs, var. 'americana', treated with five different treatments. Means followed by the same letters did not differ significantly by Fisher's LSD test at the 5% significance level.

Treatment	Exposure time					
	12h ^{NS}	24h ^{NS}	36h ^{NS}	48h ^{NS}	60h*	72h*
Imidacloprid	1,9	1,8	1,8	1,8	1,1 b	0,6 b
<i>Beauveria bassiana</i>	2,0	2,0	1,8	1,7	1,3 b	0,9 b
<i>Metarhizium anisopliae</i>	1,9	1,9	1,8	1,8	1,5 b	0,9 b
<i>B. bassiana</i> + <i>M. anisopliae</i>	2,0	2,0	1,9	1,8	1,5 ab	1,0 b
Control	2,0	2,0	2,0	2,0	2,0 a	1,8 a

NS – Treatments did not differ according to the Kruskal-Wallis test, at a 5% significance level.

* - Significant difference according to the Kruskal-Wallis test at a 5% significance level.

The efficacy of *Beauveria bassiana* in controlling thrips has been also corroborated by Wu et al. (2014), who reported mortality patterns comparable to those observed in the present study. In their laboratory bioassays, 28 isolates of *B. bassiana* were evaluated against *F. occidentalis*; at a concentration of 1×10^7 conidia mL⁻¹, a pronounced mortality of adult insects was recorded four days after treatment.

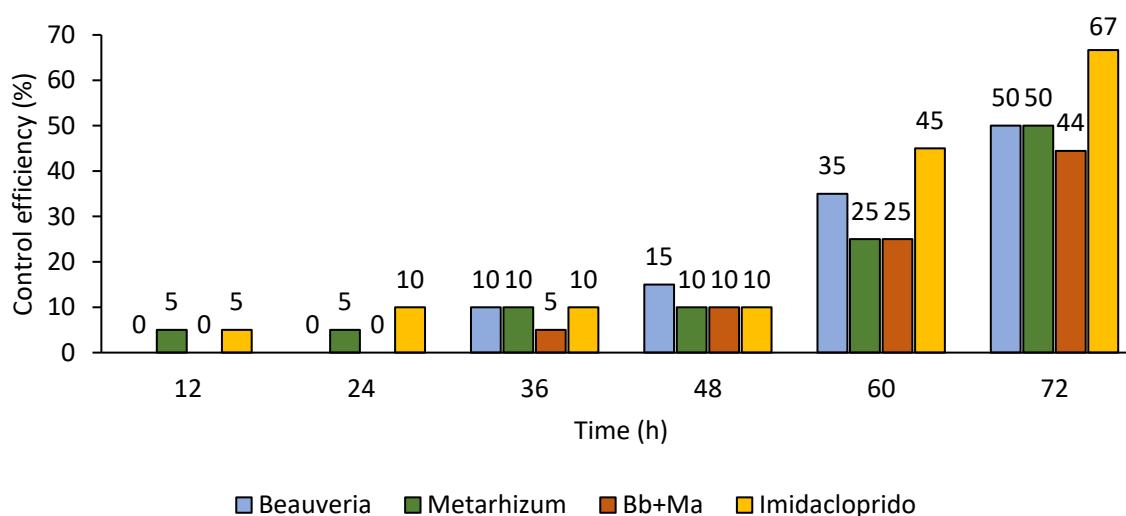
Regarding the control efficacy of *Metarhizium anisopliae* against *F. occidentalis*, Lopes et al. (2000) identified, in bioassays, the concentration of 1×10^8 conidia mL⁻¹ as the one that provided the highest mortality, capable of reducing the number of live adults on hydroponic lettuce plants; however, the authors did not measure the level of control efficacy established by this treatment because their experiments were conducted in the field.

The insecticidal performance of *Metarhizium anisopliae* against *F. occidentalis* has also been documented in bioassays conducted by Lopes et al. (2000), in which a suspension delivering 1×10^8 conidia mL⁻¹ achieved the highest mortality and markedly reduced the number of surviving adults on hydroponic lettuce. Because those experiments were carried out under field conditions, the authors did not quantify the precise level of control efficacy achieved by this treatment.

Concerning control efficacy as calculated with Abbott's correction (1925), the spore suspensions achieved approximately 50 % mortality, whereas the imidacloprid treatment reached roughly 67 % (Figure 1). Although the chemical treatment displayed higher efficacy, its performance remains below the values reported in the literature. For instance, Fernandes et al. (2017) obtained control efficiencies exceeding 80 % for *F. schultzei* on mango (*Mangifera indica*) using the same active ingredient, and Pires (2003) documented 87–98 % mortality of *Thrips tabaci* (Lindeman, 1889) on garlic (*Allium sativum*) merely two days after direct application.

Graph 1.

Control efficacy of *Beauveria bassiana*, *Metarhizium anisopliae*, and Imidacloprid in the *in vitro* control of *Frankliniella schultzei* on lettuce, at different exposure times of the insects to the treatments.



In contrast to earlier reports, our analysis revealed only 10 % mortality two days after imidacloprid application, a modest efficacy that may reflect the development of resistance in the target thrips, as documented by Junior et al. (2018). Although the imidacloprid treatment achieved higher overall control efficacy than the fungal preparations, the mean mortality rates for the chemical and biological treatments did not differ significantly. This lack of a statistical distinction supports the consideration of entomopathogenic fungi as viable alternatives to conventional insecticides.

The combined entomopathogenic suspension displayed a modestly lower efficacy than each fungus applied alone. Therefore, a mixture of *Beauveria bassiana* and *Metarhizium anisopliae* cannot be dismissed as a viable thrips-management option, because interactions between entomopathogens may be synergistic or antagonistic (Cavalcanti, 2006). The outcome of such fungus-host relationships is strongly influenced by environmental variables (e.g., temperature, light, humidity, solar radiation, nutritional status) and host susceptibility (Picanço, 2010).

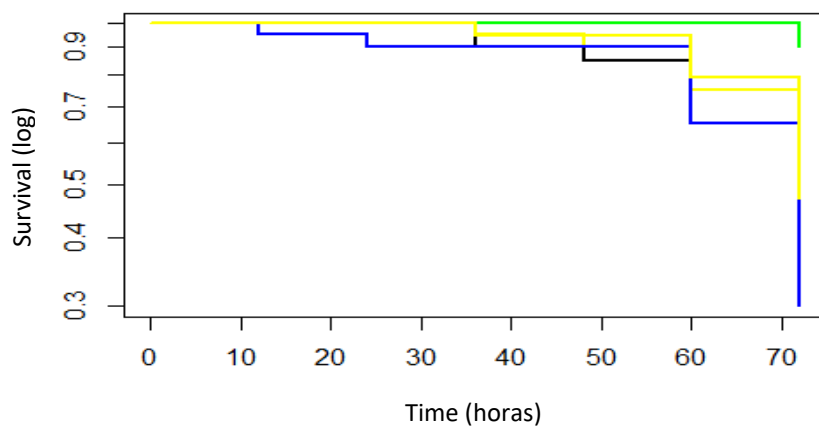
Considering the entomopathogens evaluated by this approach, Junior et al. (2018), state that future research could explore an integrated-management strategy that combines the entomopathogenic fungi with seven-day-interval applications of the commercial Azadirachtin® formulation and the periodic release of predatory mites. Such a synergistic approach may accelerate the onset of control provided by the biological agents.

For this study, survival-analysis results revealed statistically significant differences among the treatments. Pairwise comparisons, however, revealed that only the control differed significantly from each active treatment; the time to mortality induced by the entomopathogenic fungi, whether *B. bassiana*, *M. anisopliae*, or their combination, did not differ from one another nor from the imidacloprid treatment.

Consequently, the fungal formulations are expected to achieve mortality at a rate comparable to that of the chemical insecticide for the examined thrips population. The temporal pattern of insect death for each treatment is illustrated in Figure 2.

Graph 2.

Kaplan-Meier non-parametric method for the second test, presented as a step function with steps at the observed times of insect death. (Green) – Control solution; (Black) – *B. bassiana* + *M. anisopliae*; (Yellow 1) – *M. anisopliae*; (Yellow 2) – *B. bassiana*; (Blue) – Imidacloprid



The insights provided from this study may guide the hydroponic lettuce production chain, enabling a refinement of insect-management practices that is more fully aligned with Integrated Pest Management (IPM) principles. It is important to acknowledge, however, that the trials were conducted under controlled-environment conditions with constant temperature, an environment that optimizes the efficacy of the evaluated products. Under these stable conditions, four of the treatments (the two fungal suspensions, their combination, and imidacloprid) did not differ statistically from one another in terms of control efficacy.

The observed mortality patterns are partly attributable to the confined nature of the Petri-dish assay, which limited thrips movement and thereby prolonged their contact with the applied treatments. In contrast, field conditions afford unrestricted mobility, allowing insects to disperse away from treated surfaces and consequently diminishing their exposure.

Moreover, in the present study the insecticides were administered via leaf-disc immersion rather than direct spray onto the insects, a delivery method that differs from typical field applications and could underestimate the treatments' true efficacy.

Conclusion

The entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* demonstrated biological-control efficacy comparable to that of the neonicotinoid imidacloprid, indicating their suitability either as complementary agents or as direct replacements for the chemical insecticide. Nonetheless, subsequent investigations should assess their performance under field conditions, examine persistence and formulation stability, and explore their integration within comprehensive integrated-pest-management (IPM) programs.

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