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Selectivity and pathogenicity of Beauveria bassiana and Cordyceps javanica in arthropod pest management systems

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Dissertation submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

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ABSTRACT

CUESTA, Larissa Lucas Coca, M.Sc., Universidade Federal de Viçosa, February, 2026. **Selectivity and pathogenicity of Beauveria bassiana and Cordyceps javanica in arthropod pest management systems**. Adviser: Angelo Pallini Filho.

Integrated pest management (IPM) may benefit from the combined use of biological control agents to reduce pesticide dependence and contribute to the sustainability of agricultural systems. However, the integration of multiple natural enemies requires prior evaluation of their compatibility, particularly when microbial agents are applied in systems where predatory arthropods are also present. This study evaluated the selectivity and pathogenicity of commercial isolates of the entomopathogenic fungi *Beauveria bassiana* and *Cordyceps javanica* through laboratory bioassays with arthropods that commonly coexist in agroecosystems, including the whitefly *Bemisia tabaci*, the two-spotted spider mite *Tetranychus urticae*, and two predatory mites of the genus *Amblyseius*. Bioassays were conducted using the field-recommended concentration for the control of *Bemisia tabaci*. At this concentration, the effects of fungal exposure on survival, development, and reproduction of the predatory mites *A. herbicolus* and *A. tamatavensis* were assessed following direct exposure by immersion. In parallel, the pathogenicity and efficacy of the fungi against the target pest *B. tabaci* were evaluated, as well as their effects on *T. urticae*. Exposure to both fungal species, at the tested concentration, did not significantly affect survival of adult or juvenile stages of either predator species. Oviposition of *A. tamatavensis* was not affected, whereas *A. herbicolus* exhibited only transient, time-dependent variation in oviposition following exposure to *C. javanica*, without persistent reductions. Against the pests, the fungal isolates exhibited pathogenic effects on *B. tabaci* and influenced the performance of *T. urticae*. Overall, the results indicate that *B. bassiana* and *C. javanica*, at the evaluated concentration, can be used in combination with predatory mites of the genus *Amblyseius* without pronounced negative effects on key predator life-history traits. These findings support the feasibility of combining these agents within IPM programs and contribute to the development of integrated strategies for managing arthropod pest complexes.

Keywords: biological control; predatory mites; entomopathogenic fungi.

RESUMO

CUESTA, Larissa Lucas Coca, M.Sc., Universidade Federal de Viçosa, fevereiro de 2026. **Seletividade e patogenicidade de Beauveria bassiana e Cordyceps javanica em sistemas de manejo de pragas artrópodes**. Orientador: Angelo Pallini Filho.

O manejo integrado de pragas (MIP) pode se beneficiar do uso combinado de agentes de controle biológico para reduzir a dependência de pesticidas e contribuir para a sustentabilidade dos sistemas agrícolas. No entanto, a integração de múltiplos inimigos naturais requer avaliação prévia de sua compatibilidade, especialmente quando agentes microbianos são aplicados em sistemas nos quais artrópodes predadores também estão presentes. Este estudo avaliou a seletividade e a patogenicidade de isolados comerciais dos fungos entomopatogênicos *Beauveria bassiana* e *Cordyceps javanica* por meio de bioensaios laboratoriais com artrópodes que comumente coexistem em agroecossistemas, incluindo a mosca-branca *Bemisia tabaci*, o ácaro-rajado *Tetranychus urticae* e ácaros predadores do gênero *Amblyseius*. Os bioensaios foram conduzidos utilizando a concentração de campo recomendada para o controle de *Bemisia tabaci*. Nessa concentração, foram avaliados os efeitos da exposição fúngica sobre a sobrevivência, o desenvolvimento e a reprodução dos ácaros predadores *Amblyseius herbicolus* e *Amblyseius tamatavensis*, após exposição direta por imersão. Em paralelo, foram avaliadas a patogenicidade e a eficácia dos fungos contra a praga-alvo *B. tabaci*, bem como seus efeitos sobre *T. urticae*. A exposição a ambas as espécies fúngicas, na concentração testada, não afetou significativamente a sobrevivência dos estágios adulto ou juvenil de nenhuma das espécies de predadores. A oviposição de *A. tamatavensis* não foi afetada, enquanto *A. herbicolus* apresentou apenas variação transitória e dependente do tempo na oviposição após a exposição a *C. javanica*, sem reduções persistentes. Em relação às pragas, os isolados fúngicos exibiram efeitos patogênicos sobre *B. tabaci* e influenciaram o desempenho de *T. urticae*. De modo geral, os resultados indicam que *B. bassiana* e *C. javanica*, na concentração avaliada, podem ser utilizados em combinação com ácaros predadores do gênero *Amblyseius* sem efeitos negativos pronunciados sobre traços-chave do histórico de vida dos predadores. Esses achados sustentam a viabilidade da combinação desses agentes em programas de MIP e contribuem para o desenvolvimento de estratégias integradas para o manejo de complexos de artrópodes-praga.

Palavras-chave: controle biológico; ácaros predadores; fungos

entomopatogênicos

SUMMARY

General Introduction

Pest infestations and the consequent reduction in crop productivity stand out as some of the main challenges faced by modern agriculture, intensified by land-use intensification and ongoing climate changes of the Anthropocene (Marshman et al., 2019; Dror et al., 2021). Pests and pathogens are responsible for a 17% to 30% reduction in global agricultural production (Savary et al., 2019). However, these losses are not exclusively driven by pest pressure, but are also strongly influenced by inadequate management practices, including the improper use of control strategies within agricultural systems (Li et al., 2011; Leskovac & Petrović, 2023). Among the principal contributors to these losses are phytophagous arthropods, which cause direct and indirect damage by impairing plant vigor, development, and physiological performance (Hauser et al., 2013; Showler, 2013; Kumar et al., 2024). These impacts are intensified by the high reproductive capacity, rapid development, and high adaptability of these organisms to different environmental conditions (Fox & Czesak, 2000; Shrestha, 2019). As a result, many phytophagous species complete multiple generations within a single cropping cycle, and this high rate of population turnover can promote demographic outbreaks (Carey & Krainacker, 1988; Paredes et al., 2022), which favor the development of pesticide resistance under high selective pressures (Gul et al., 2023; Naggar et al., 2025). In this context, Integrated Pest Management (IPM) emerges as an essential approach, especially in intensive production systems, where high host availability and favourable environmental conditions often promote rapid pest population growth, thereby challenging effective infestation control.

Among the most economically important agricultural pests worldwide, the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) stands out as a key challenge for modern crop production and integrated pest management programs (Oliveira et al., 2001; Perring et al., 2018). This species is considered a major pest in a wide range of agricultural systems, including vegetables (e.g., tomato, pepper, and cucumber), fiber crops such as cotton, and several ornamental and industrial crops worldwide (Cock, 1986; Horowitz et al., 2011). This phloem-feeding insect lives predominantly on herbaceous plants (Sani et al., 2020) and causes both direct and indirect damage to crops, directly by extracting plant sap and indirectly through honeydew excretion, which favors sooty mold development (Byrne & Bellows, 1991; Byrne & Miller, 1990). Moreover, *B. tabaci* can act as a vector for phytopathogenic viruses, including those belonging to the genera *Begomovirus*, *Ipomovirus*, *Crinivirus*, *Torradovirus*, and *Carlavirus* (Gilbertson et al., 2015). Its wide geographic distribution, broad host range, and high ecological plasticity contribute to its ability to cause significant economic losses, reinforcing its relevance as a key target within integrated pest management strategies (Naranjo et al., 2009; Horowitz et al., 2020; Cremonez et al., 2023).

Similarly to the whitefly *Bemisia tabaci*, the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a cosmopolitan and highly polyphagous pest that poses a major challenge to agricultural systems worldwide (Duso et al., 2010; Domingos et al., 2014; Lagziri et al., 2015; Jakubowska et al., 2022). This herbivore causes damage to more than 1,100 cultivated plant species and results in significant losses both in Brazil and in several other regions worldwide (Jeppson et al., 1975; Oliveira et al., 2007; Adesanya, 2019). This species causes direct damage by feeding on leaf cellular contents, leading to reduced photosynthetic rates, defoliation, and loss of plant vigor (Parmagnani et al., 2023). At high population densities, these effects are intensified and may result in tissue necrosis and severe defoliation, significantly compromising crop development and productivity (Helle & Sabelis, 1985; Gorman et al., 2002; Kumari et al., 2017). *T. urticae* is characterized by a high reproductive rate and short life cycle, traits that favor rapid population growth and frequent outbreaks, particularly in warm, dry environments (Mitchell, 1973; Gallo et al., 2002; Riahi et al., 2013; Teodoro-Paulo et al., 2024). In addition to its wide geographic distribution and high adaptability across different niches, *T. urticae* exhibits rapid proliferation, intensifying its impact on horticultural, ornamental, and fruit crops, underscoring the need for highly efficient management strategies.

Even today, pest control relies heavily on the frequent use of chemical products, frequently applied outside an integrated management framework (Deguine et al., 2021; Araújo et al., 2023). However, the continuous application of these compounds exerts strong selective pressure on pest populations, favoring the emergence of resistance, reducing control efficacy, and increasing production costs (Sparks & Nauen, 2015; Galli et al., 2024). In the case of whiteflies, resistance to more than 50 chemical compounds has already been reported (Sparks & Nauen, 2015; Horowitz et al., 2020; Victor et al., 2022). Similarly, populations of the two-spotted spider mite have also shown reduced sensitivity to pesticides (Van Leeuwen et al., 2015), and the species has been reported to rank first among those that develop resistance most rapidly worldwide (APRD, 2025). In light of this scenario, an emphasis on Integrated Pest Management (IPM) tactics focused on biological control emerges as an environmentally safe, sustainable, and economically viable alternative to chemical control (Van Lenteren, 2012; Lahlali et al., 2022).

Biological control uses living organisms to reduce pest populations or limit the damage they cause in cropping systems (Van Lenteren, 2012; Stenberg et al., 2021). Among these organisms, predatory mites have received increasing attention over recent decades, particularly those belonging to the family Phytoseiidae, which is recognized as one of the most relevant groups for the natural control of phytophagous arthropods (Knapp et al., 2018). These predators prey on different groups of phytophagous arthropods, including pest mites and economically important small insects, making them widely applicable in management programs (McMurtry & Croft, 1997; Gerson et al., 2003). Within this group, species of the genus *Amblyseius* stand out for their adaptability and effectiveness in managing key pests, including whiteflies, thrips, tarsonemid mites, and tetranychid mites, reinforcing their potential for biological control in productive contexts (Calvo et al., 2010; Cavalcante et al., 2017; Barbosa et al., 2019; Pio, 2023; Cardoso et al., 2025).

Predators of the genus *Amblyseius* have a generalist feeding habit, being able to develop and reproduce on multiple prey types (McMurtry et al., 2013). The predatory mite *Amblyseius herbicolus* (Chant, 1959) (Acari: Phytoseiidae) feeds on small phytophagous arthropods, including *B. tabaci*, and is also able to exploit alternative food resources such as pollen and nectar (Rodríguez-Cruz et al., 2013; Duarte et al., 2015; Marcossi et al., 2020; Kalile et al., 2023; Iasczczaki et al., 2024). Another relevant species within the genus is *Amblyseius tamatavensis* Blommers (Acari: Phytoseiidae), which is known to consume tetranychid mites, astigmatid mites, nematodes, pollen, and the eggs of small hexapods (Cavalcante et al., 2015; Massaro et al., 2016). In Brazil, *A. tamatavensis* has stood out particularly for its performance in managing *B. tabaci*, leading to its production and commercialization as a biological control agent against this pest (Cavalcante et al., 2017; Promip, 2023). In general, the generalist habit is positively correlated with these predators' ability to establish and persist on plants (Tylianakis et al., 2004; Généau et al., 2012), although this persistence does not necessarily translate into consistent performance across cropping systems, as it may be modulated by plant traits, prey density, and management practices, which impose ecological trade-offs and limit predator effectiveness under certain conditions (Ibrahim & Rahman, 1997; Buitenhuis et al., 2014; Elmoghazy et al., 2024).

In addition to predators, biological control strategies may also include the use of microorganisms (Fontes & Valadares-Inglis, 2020; Kumar et al., 2024). Among the microbial agents used in this context, entomopathogenic fungi stand out, as they have well-described application methodologies, a wide variety of strains available on the market, and infect target pests through contact with the cuticle, without relying on ingestion by the host (Hajek & St. Leger, 1994; Vega et al., 2012). Entomopathogenic fungi are organisms capable of causing disease or leading to the death of insect hosts (Fontes & Valadares-Inglis, 2020). After the death of the insect, the fungus continues its development and reproduction, completing its life cycle through the production and release of spores capable of infecting new susceptible individuals (Wolfart et al., 2022). Demonstrating the ability to infect different groups of arthropods, including both sap-sucking and chewing pests, fungal bioinsecticides have gained space in the market as a possible alternative to chemical control (Shah & Pell, 2003; De Faria & Wright, 2007; Panwar & Szczepaniec, 2024).

Among the entomopathogenic fungi used commercially, *Beauveria bassiana* (Bals.-Criv.) Vuill., 1912, and *Cordyceps javanica* (Frieder. & Bally) Kepler, B. Shrestha & Spatafora (formerly known as *Isaria javanica*) stand out, as they are formulated as biopesticides and registered for the management of several agricultural pests (Zimmermann, 2007; Mascarin et al., 2016; Behle et al., 2022; Xing et al., 2024). In Brazil, products derived from these fungi are regulated and authorized for agricultural use by the Ministry of Agriculture and Livestock (MAPA) and are registered as biological control agents in the official AGROFIT system, allowing their application across different crops and production systems. Both *B. bassiana* and *C. javanica* are recognized for their broad spectrum of action and their ability to infect hosts through the production of conidia that germinate and penetrate the insect cuticle, leading to internal colonization and host death, and are effective against a wide range of insects and mites (Shah & Pell, 2003; De Faria & Wraight, 2007; Zimmermann, 2007; Zimmermann, 2008; Mascarin et al., 2016; Xing et al., 2023). Both fungi show remarkable efficacy against sap-sucking insects such as whiteflies (Ou et al., 2019; Shah et al., 2020; Wari et al., 2020; Gebremariam et al., 2022). The availability of multiple commercial strains of these fungi, adapted to different environmental conditions and cropping systems, consolidates them as central tools in integrated management programs aimed at reducing dependence on chemical insecticides (De Faria & Wraight, 2007; Fontes & Valadares-Inglis, 2020).

The integration of different biological control agents, such as entomopathogenic fungi and predatory mites, may enhance pest management resilience and effectiveness (Van Lenteren, 2012; Dent & Binks, 2020). This approach is based on manipulating food webs to create trophic cascades that can impact pest populations (Janssen et al., 2006). However, in agricultural systems, these induced cascades may be disrupted by intraguild interactions or by the actions of natural enemies of the control agents themselves (Rosenheim et al., 1995; Ulug et al., 2014), leading to management failure (Rosenheim et al., 1995). Importantly, an integration strategy is not limited to the application of agents alone. The introduction of multiple natural enemies into a crop creates an artificial food web in which direct and indirect interactions among species determine pest population dynamics and control efficacy (Ehler, 1996; Janssen et al., 1998). In this context, the effects on pest populations go beyond the additive mortality caused by each agent in isolation and are instead modulated by interactions such as apparent competition, intraguild predation, and behavioral interference (Holt & Polis, 1997; Janssen et al., 1998). Therefore, it is essential to assess how these agents will coexist and interact within this novel community.

Thus, the combined application of different biological control agents requires a prior assessment of compatibility among candidate organisms, since entomopathogenic fungi, although selective toward target pests, may potentially affect natural enemies such as predatory mites (Roy & Pell, 2000; Quesada-Moraga et al., 2022). This issue is particularly relevant in intensive cropping systems, such as vegetable crops grown under protected or open-field conditions (e.g., cucumber, bean, pepper, and cotton), where *B. tabaci* and *T. urticae* frequently co-occur and are often managed simultaneously using multiple biological control agents (Schoonhoven & Cardona, 1980; Zhang et al., 2003; Aslan et al., 2004; Abou Jawdah et al.; 2024;). In these systems, entomopathogenic fungi such as *B. bassiana* and *C. javanica* are commonly applied for whitefly suppression, but their application may influence the entire assemblage of organisms present within the local food web (Meyling & Hajek, 2010; Quesada-Moraga et al., 2022). Otherwise, generalist predatory mites of the genus *Amblyseius* are released to target whiteflies and associated secondary pests.

Therefore, investigating the compatibility between commercial isolates of *B. bassiana* and *C. javanica* and key predators of the genus *Amblyseius* is essential for developing combined release protocols aimed at controlling the target pest, in this case *B. tabaci*, as well as the indirect control of *T. urticae*, a pest frequently associated with agroecosystems impacted by *B. tabaci* (Aslan et al., 2004; Soleymani et al., 2016). The success of an integrated pest management program combining these agents will depend on how these interactions shape the structure and dynamics of the artificial food web within the agroecosystem.

In various agricultural systems, the whitefly *Bemisia tabaci* and the two-spotted spider mite *Tetranychus urticae* often occur simultaneously, sharing host plants and causing significant economic damage. For the integrated management of these pests, two biological control tactics are frequently employed: the use of predatory mites, such as mites from the genus *Amblyseius*, and the application of entomopathogenic fungi, such as *Beauveria bassiana* and *Cordyceps javanica*. Both of these control agents can act against *B. tabaci*, and in some cases, against *T. urticae* as well, making them promising tools for simultaneous pest suppression. However, to effectively integrate these strategies, it is essential to understand the potential interactions between them.

Therefore, the present study was designed to evaluate both the selectivity of these entomopathogenic fungi to the predatory mites and their direct pathogenicity against the target pests. Specifically, in Chapter 1, we assessed the compatibility of commercial isolates of B. bassiana and C. javanica with the predators *Amblyseius herbicolus* and *Amblyseius tamatavensis*, testing the hypothesis that exposure to field-recommended concentrations does not negatively affect key predator performance parameters. In Chapter 2, we evaluated the pathogenicity and efficacy of the same fungal isolates against the target pest *B. tabaci,* as well as their effects on the co-occurring pest *T. urticae*. Together, these chapters provide an evaluation of the potential for combining entomopathogenic fungi and predatory mites within sustainable pest management programs, establishing a foundation for their combined application in agroecosystems.

References

ABOU JAWDAH, Y. *et al.* Biological control of three major cucumber and pepper pests: whiteflies, thrips, and spider mites, in high plastic tunnels using two local phytoseiid mites. *Plants*, Basel, v. 13, n. 6, art. 889, 2024. DOI: 10.3390/plants13060889.

ADESANYA, A. W. *et al.* Physiological resistance alters behavioral response of *Tetranychus urticae* to acaricides. *Scientific Reports*, v. 9, art. 19308, 2019. DOI: 10.1038/s41598-019-55856-7.

ARTHROPOD PESTICIDE RESISTANCE DATABASE (APRD). Arthropod Pesticide Resistance Database. Responsáveis: MOTA-SANCHEZ, D.; WISE, J. C. East Lansing: Michigan State University, 2025.

ARAÚJO, M. F.; CASTANHEIRA, E. M. S.; SOUSA, S. F. The buzz on insecticides: a review of uses, molecular structures, targets, adverse effects, and alternatives. *Molecules*, v. 28, n. 8, p. 3641, 2023. DOI: 10.3390/molecules28083641.

ASLAN, İ.; ÖZBEK, H.; ÇALMAŞUR, Ö.; ŞAHİN, F. Toxicity of essential oil vapours to two greenhouse pests, *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. *Industrial Crops and Products*, v. 19, n. 2, p. 167–173, 2004. DOI: 10.1016/j.indcrop.2003.09.003.

BARBOSA, M. F. C.; POLETTI, M.; POLETTI, E. C. Functional response of *Amblyseius tamatavensis* Blommers (Mesostigmata: Phytoseiidae) to eggs of *Bemisia tabaci*. *Biological Control*, v. 138, p. 104030, 2019.

BEHLE, R. W. *et al.* Comparing production and efficacy of *Cordyceps javanica* with *Cordyceps fumosorosea*. *Journal of Economic Entomology*, v. 115, n. 2, p. 455–461, 2022. DOI: 10.1093/jee/toac002.

BUITENHUIS, R.; SHIPP, L.; SCOTT-DUPREE, C. et al. Host plant effects on the behaviour and performance of *Amblyseius swirskii* (Acari: Phytoseiidae). *Experimental and Applied Acarology*, Dordrecht, v. 62, p. 171–180, 2014. DOI: 10.1007/s10493-013-9735-1.

BYRNE, D. N.; BELLOWS, T. S. Whitefly biology. *Annual Review of Entomology*, v. 36, n. 1, p. 431–457, 1991.

BYRNE, D. N.; MILLER, W. B. Carbohydrate and amino acid composition of phloem sap and honeydew produced by *Bemisia tabaci*. *Journal of Insect Physiology*, v. 34, n. 5, p. 433–437, 1990. DOI: 10.1016/0022-1910(88)90106-6.

CALVO, F. N.; BELDA, J. E. Control of *Bemisia tabaci* and *Frankliniella occidentalis* in cucumber by *Amblyseius swirskii*. *Biological Control*, v. 56, n. 2, p. 185–192, 2010.

CAREY, J. R.; KRAINACKER, D. A. Demographic analysis of mite populations: extensions of stable theory. *Experimental & Applied Acarology*, v. 4, n. 3, p. 191–210, 1988. DOI: 10.1007/BF01193853.

CARDOSO, A. C. *et al.* Potential of *Amblyseius herbicolus* and *Amblyseius tamatavensis* to control *Frankliniella occidentalis*. *Journal of Pest Science*, v. 98, p. 2117–2127, 2025. DOI: 10.1007/s10340-025-01928-9.

CAVALCANTE, A. C. C.; DOS SANTOS, V. L. V.; ROSSI, L. C.; MORAES, G. J. D. Potential of five Brazilian populations of Phytoseiidae (Acari) for the biological control of *Bemisia tabaci*. *Journal of Economic Entomology*, v. 108, n. 1, p. 29–33, 2015.

CAVALCANTE, A. C. C. *et al. Amblyseius tamatavensis* Blommers (Acari: Phytoseiidae) as a candidate for biological control of *Bemisia tabaci*. *International Journal of Acarology*, v. 43, n. 1, p. 10–15, 2017. DOI: 10.1080/01647954.2016.1225816.

CREMONEZ, P. S. G. *et al.* Precision and accuracy of field versus laboratory bioassay insecticide efficacy for the control of immature *Bemisia tabaci*. *Insects*, v. 14, n. 7, p. 645, jul. 2023. DOI: 10.3390/insects14070645.

COCK, M. J. N. *Bemisia tabaci: a literature survey on the cotton whitefly with an annotated bibliography*. Ascot: FAO/CAB International Institute of Biological Control, 1986. 21 p.

DE FARIA, M. R.; WRAIGHT, S. P. Mycoinsecticides and mycoacaricides. *Biological Control*, v. 43, n. 3, p. 237–256, 2007.

DEGUINE, J.-P. *et al.* Integrated pest management: good intentions, hard realities. *Agronomy for Sustainable Development*, v. 41, n. 3, 2021.

DENT, D. R.; BINKS, R. H. *Insect pest management*. Wallingford: CABI, 2020.

DROR, I.; YARON, B.; BERKOWITZ, B. The human impact on all soil-forming factors during the Anthropocene. *ACS Environmental* Au, v. 2, n. 1, 2021.

DUARTE, M. V. A. *et al.* Alternative food promotes broad mite control on chilli pepper plants. *BioControl*, v. 60, p. 817–825, 2015. DOI: 10.1007/s10526-015-9688-x.

EHLER, L. E. Structure and impact of natural enemy guilds. In: POLIS, G. A.; WINEMILLER, K. O. (org.). *Food webs*. New York: Chapman & Hall, 1996. p. 337–342.

ELMOGHAZY, M. M. E. *et al.*. Implications of temperature and prey density on predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae) functional responses. *Insects*, Basel, v. 15, n. 6, art. 444, 2024. DOI: 10.3390/insects15060444.

FOX, C. W.; CZESAK, M. E. Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*, v. 45, p. 341–369, 2000.

FONTES, E. M. G.; VALADARES-INGLIS, M. C. *Controle biológico de pragas da agricultura*. Brasília: Embrapa, 2020.

GALLI, M. *et al.* Can biocontrol be the game-changer in integrated pest management? *Journal of Plant Diseases and Protection*, v. 131, 2024.

GALLO, D. et al. *Entomologia agrícola*. Piracicaba: FEALQ, 2002.

GEBREMARIAM, A.; CHEKOL, Y.; ASSEFA, F. Extracellular enzyme activity of entomopathogenic fungi. *BMC Research Notes*, v. 15, n. 1, 2022.

GÉNEAU, C. E.; WÄCKERS, F. L.; LUKA, H.; DANIEL, C.; BALMER, O. Selective flowers to enhance biological control of cabbage pests by parasitoids. *Basic and Applied Ecology*, v. 13, p. 85–93, 2012.

GILBERTSON, R. L. *et al.* Role of insect supervectors in virus spread. *Annual Review of Virology*, v. 2, p. 67–93, 2015.

GERSON, U.; SMILEY, R. L.; OCHOA, R. *Mites (Acari) for pest control*. Oxford: Blackwell Publishing, 2003.

GORMAN, K. *et al.* New developments in insecticide resistance. *Pest Management Science*, v. 58, n. 2, p. 123–130, 2002.

GUL, H. *et al.* Fitness costs of resistance to insecticides. *Frontiers in Physiology*, v. 14, 2023.

HAJEK, A. E.; ST. LEGER, R. J. Interactions between fungal pathogens and insect hosts. *Annual Review of Entomology*, v. 39, p. 293–322, 1994.

HAUSER, T. P. *et al.* Combined effects of arthropods and phytopathogens. *Functional Ecology*, v. 27, n. 3, p. 623–632, 2013.

HELLE, H.; SABELIS, M. W. *Spider mites: their biology, natural enemies and control*. Amsterdam: Elsevier, 1985. v. 1A.

HOLT, R. D.; POLIS, G. A. A theoretical framework for intraguild predation. *The American Naturalist*, v. 149, n. 4, p. 745–764, 1997.

HOROWITZ, A. R.; ANTIGNUS, Y.; GERLING, D. Management of *Bemisia tabaci* whiteflies. In: THOMPSON, W. (ed.). *The whitefly, Bemisia tabaci (Homoptera: Aleyrodidae): interaction with geminivirus-infected host plants*. Dordrecht: Springer, 2011. cap. 11. DOI: 10.1007/978-94-007-1524-0_11.

HOROWITZ, A. R. *et al.* Insecticide resistance in *Bemisia tabaci*. *Journal of Pest Science*, v. 93, p. 893–910, 2020.

IASCZCZAKI, R. S. *et al.* Extrafloral nectar from coffee-associated trees as alternative food for a predatory mite. *Experimental and Applied Acarology*, v. 94, n. 1, 5 dez. 2024.

IBRAHIM, Y. B.; RAHMAN, R. B. A. Influence of prey density, species and developmental stages on the predatory behaviour of *Amblyseius longispinosus*

(Acari: Phytoseiidae). *BioControl*, Dordrecht, v. 42, p. 319–327, 1997. DOI: 10.1007/BF02769824.

JANSSEN, A. *et al.* Behaviour and indirect interactions in food webs. *Experimental and Applied Acarology*, v. 22, n. 8, p. 497–521, 1998.

JANSSEN, A. *et al.* Intraguild predation usually does not disrupt biological control. In: BRODEUR, J.; BOIVIN, G. (org.). *Trophic interactions in biological control*. Dordrecht: Springer, 2006.

JEPPSON, L. R.; KEIFER, H. H.; BAKER, E. W. *Mites injurious to economic plants*. Berkeley: University of California Press, 1975.

KALILE, M. O. *et al.* A predatory mite suppresses *Diaphorina citri*. *Entomologia Experimentalis et Applicata*, v. 171, p. 592–602, 2023.

KNAPP, M. *et al.* Use of predatory mites in commercial biocontrol. *Acarologia,* v. 58, p. 72–82, 2018.

KUMAR, S.; SHARMA, A.; SAMAL, R. R. Management of agricultural pests: strategies and constraints. *Agricultural sciences.* Londres: IntechOpen, 2024. DOI: 10.5772/intechopen.1006075

KUMARI, S. *et al.* Comparative toxicities of acaricides. *Journal of the Saudi Society of Agricultural Sciences*, v. 16, n. 2, p. 191–196, 2017.

LAHLALI, R. *et al.* Biological control of plant pathogens. *Microorganisms*, v. 10, p. 596, 2022.

LESKOVAC, A.; PETROVIĆ, S. Pesticide use and degradation strategies: food safety, challenges and perspectives. *Foods*, Basel, v. 12, n. 14, p. 2709, 2023. DOI: 10.3390/foods12142709.

LI, An et al. *Persistent organic pollutants in Asia: sources, distributions, transport and fate*. v. 7. Amsterdam: Elsevier, 2011.

MARCOSSI, Í.; FONSECA, M. M.; CARBAJAL, P. A.; CARDOSO, A.; PALLINI, A.; JANSSEN, A. *High-quality alternative food reduces cannibalism in the predatory mite* Amblyseius herbicolus *(Acari: Phytoseiidae)*. *Experimental and Applied Acarology*, v. 81, p. 189–200, 2020. DOI: 10.1007/s10493-020-00500-7.

MARSHMAN, J.; BLAY-PALMER, A.; LANDMAN, K. Anthropocene crisis. *Environments*, v. 6, n. 2, p. 22, 2019.

MASCARIN, G. M.; JARONSKI, S. T. Production and uses of *Beauveria bassiana*. *World Journal of Microbiology and Biotechnology*, v. 32, p. 177, 2016.

MASSARO, M., MARTIN, J. P. I.; de MORAES, G. J. (2016). Factitious food for mass production of predaceous phytoseiid mites (Acari: Phytoseiidae) commonly found in Brazil. *Experimental and Applied Acarology*, 70, 411-420.

MCMURTRY, J. A.; CROFT, B. A. Life-styles of phytoseiid mites and their roles in biological control. *Annual Review of Entomology*, v. 42, n. 1, p. 291–321, 1997.

McMURTRY, J. A.; MORAES, G. J.; SOURASSOU, N. F. Revision of the lifestyles of phytoseiid mites and implications for biological control strategies. *Systematic and Applied Acarology*, v. 18, p. 297–320, 2013.

MEYLING, N. V.; HAJEK, A. E. Principles from community and metapopulation ecology: application to fungal entomopathogens. *BioControl*, Dordrecht, v. 55, p. 39–54, 2010. DOI: 10.1007/s10526-009-9246-5.

MITCHELL, R. Growth and population dynamics of *Tetranychus urticae*. *Ecology*, v. 54, n. 6, p. 1349–1355, 1973.

NAGGAR, A. Y. *et al.* Mechanisms and genetic drivers of resistance of insect pests to insecticides and approaches to its control. *Toxics*, v. 13, n. 8, p. 681, 2025. DOI: 10.3390/toxics13080681.

OLIVEIRA, H. *et al.* A phytoseiid predator from the tropics as potential biological control agent for the spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *Biological Control*, [S. l.], v. 42, n. 2, p. 105–109, ago. 2007. DOI: 10.1016/j.biocontrol.2007.04.011.

OU, D. *et al.* Compatibility of parasitoid and *Cordyceps javanica*. *Insects*, v. 10, n. 12, p. 425, 2019.

PANWAR, N.; SZCZEPANIEC, A. Endophytic entomopathogenic fungi. *Pest Management Science*, v. 80, p. 6033–6040, 2024.

PAREDES, D.; ROSENHEIM, J. A.; KARP, D. S. Pest population variability. *Ecological Applications*, v. 32, n. 5, p. e2607, 2022.

PARMAGNANI, A. S. *et al.* Biology of two-spotted spider mite (*Tetranychus urticae*): ultrastructure, photosynthesis, guanine transcriptomics, carotenoids and chlorophylls metabolism, and decoyinine as a potential acaricide. *International Journal of Molecular Sciences*, [S. l.], v. 24, n. 2, art. 1715, jan. 2023. DOI: 10.3390/ijms24021715.

PIO, G. H. Potencial de *Amblyseius tamatavensis* no controle de *Tetranychus urticae*. 2023. Dissertação (Mestrado) – UFV, Viçosa, 2023.

Promip (2023) https://promip.agr.br/amblymip/. Acessado em 20 de novembro de 2025.

QUESADA-MORAGA, E. *et al.* Multitrophic interactions of entomopathogenic fungi. *BioControl*, v. 67, p. 457–472, 2022.

RIAHI, E. *et al.* Temperature effects on *Tetranychus urticae*. *Journal of Agricultural Science and Technology*, v. 15, n. 4, p. 661–672, 2013.

RODRÍGUEZ-CRUZ, F. A.; VENZON, M.; PINTO, C. M. F. Performance of *Amblyseius herbicolus* on broad mites and on pollen. *Experimental and Applied Acarology*, v. 60, p. 497–507, 2013.

ROSENHEIM, J. A. et al. Intraguild predation among biological-control agents: theory and evidence. *Biological Control*, v. 5, n. 3, p. 303–335, 1995.

ROY, H. E.; PELL, J. K. Interactions between entomopathogenic fungi and other natural enemies. *Biocontrol Science and Technology*, v. 10, n. 6, p. 737–752, 2000.

SANI, I. *et al.* Biology and control of *Bemisia tabaci*. *Insects*, v. 11, n. 9, p. 619, 2020.

SAVARY, S. *et al.* The global burden of pathogens and pests. *Nature Ecology & Evolution*, v. 3, p. 430–439, 2019.

SCHOONHOVEN, A. van; CARDONA, C. Insects and other bean pests in Latin America. In: SCHWARTZ, H. F.; GÁLVEZ E., G. E. (eds.). *Bean production problems: disease, insect, soil and climatic constraints of Phaseolus vulgaris*. Cali: Centro Internacional de Agricultura Tropical (CIAT), 1980. p. 363–412.

SHAH, P. A.; PELL, J. K. Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology*, v. 61, p. 413–423, 2003.

SHAH, R. et al. Efficacy of an Omani strain of *Cordyceps javanica* and its culture filtrate against whitefly (*Bemisia tabaci*) under laboratory conditions. *All Life*, v. 13, n. 1, p. 615–622, 2020.

SHRESTHA, Saroj. Effects of climate change in agricultural insect pest. *Acta Scientific Agriculture*, v. 3, n. 12, p. 74–80, 2019.

SHOWLER, A. Plant-arthropod interactions affected by water deficit stress through association with changes in plant free amino acid accumulations. In: *Molecular approaches in plant abiotic stress*. Boca Raton: CRC Press, 2013. p. 339–352. DOI: 10.1201/b15538-21.

SOLEYMANI, S.; HAKIMITABAR, M.; SEIEDY, M. Prey preference of predatory mite *Amblyseius swirskii* (Acari: Phytoseiidae) on *Tetranychus urticae* (Acari: Tetranychidae) and *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Biocontrol Science and Technology*, v. 26, n. 4, p. 562–569, 2016. DOI: 10.1080/09583157.2015.1133808.

SPARKS, T. C.; NAUEN, R. IRAC classification and resistance management. *Pesticide Biochemistry and Physiology*, v. 121, p. 122–128, 2015.

STENBERG, J. A. *et al.* When is it biological control? *Journal of Pest Science*, v. 94, n. 3, p. 665–676, 2021.

TEODORO-PAULO, J. *et al.* Rising temperatures favour herbivores. *Journal of Pest Science*, v. 98, n. 1, p. 535–548, 2024.

TYLIANAKIS, J. M.; DIDHAM, R. K.; WRATTEN, S. D. Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology*, v. 85, p. 658–666, 2004

ULUG, D. *et al.* Natural enemies of natural enemies. *Ecological Entomology*, v. 39, p. 462–469, 2014.

VAN LENTEREN, J. C. Commercial augmentative biological control. *BioControl*, v. 57, p. 1–20, 2012.

VAN LEEUWEN, T. *et al.* Economic importance of acaricides. *Pesticide Biochemistry and Physiology*, v. 121, p. 12–21, 2015.

VEGA, F. E. *et al.* Fungal entomopathogens. In: VEGA, F. E.; KAYA, H. K. (org.). *Insect pathology*. 2. ed. Amsterdam: Academic Press, 2012. p. 171–220.

VICTOR, M. *et al.* Efeito inseticida do extrato de *Deguelia amazonica*. Revista, 2022.

XING, P. *et al.* Identification and pathogenicity of *Cordyceps javanica*. *Journal of Economic Entomology*, v. 116, n. 1, p. 98–107, 2023.

XING, P. *et al.* Secondary metabolites in *Cordyceps javanica*. *Pesticide Biochemistry and Physiology*, v. 204, p. 106076, 2024.

WARI, D. et al. Augmentation and compatibility of *Beauveria bassiana* with pesticides against different growth stages of *Bemisia tabaci* (Gennadius): an *in vitro* and field approach. *Pest Management Science*, v. 76, n. 9, p. 3236–3252, 2020.

WOLFART, C. F.; VOGT, A.; GABRIEL, V. J. Agentes de controle biológico de pragas. *Revista Conexão*, 2022.

ZIMMERMANN, G. Review on safety of *Beauveria bassiana*. *Biocontrol Science and Technology*, v. 17, n. 6, p. 553–596, 2007.

ZIMMERMANN, G. The entomopathogenic fungi *Isaria* spp. *Biocontrol Science and Technology*, v. 18, n. 9, p. 865–901, 2008.

ZHANG, Zhi-Qiang. *Mites of greenhouses: identification, biology and control*. Wallingford: CABI, 2003.

Chapter 1

Compatibility of *Beauveria bassiana* and *Cordyceps javanica* with two predatory mites of the genus *Amblyseius*

Abstract

The integration of entomopathogenic fungi and predatory mites represents a promising strategy within Integrated Pest Management (IPM) programs; however, its success depends on the compatibility and selectivity of microbial agents toward beneficial arthropods. In several agricultural systems, including protected crops and open-field production, the whitefly *Bemisia tabaci* and the two-spotted spider mite *Tetranychus urticae* frequently co-occur and are often managed simultaneously. In these systems, both entomopathogenic fungi and predatory mites of the genus *Amblyseius* may be employed against whiteflies and spider mites, either individually or in combination, which makes it essential to assess whether fungal applications interfere with predator performance. This chapter evaluated the compatibility of the entomopathogenic fungi *Beauveria bassiana* and *Cordyceps javanica*, applied at field-recommended concentrations for the management of *Bemisia tabaci*, with two predatory mite species of the genus *Amblyseius*: *Amblyseius herbicolus* and *Amblyseius tamatavensis*. Adult females and immature stages of both predators were directly exposed to fungal suspensions by immersion, and the effects on survival, developmental time, and oviposition were assessed under controlled laboratory conditions. Adult survival and juvenile survival did not differ significantly among fungal treatments and the control for either species, and no effects on developmental duration from larva to adult were detected. Oviposition responses were species-specific: *A. herbicolus* exhibited transient, time-dependent variation in egg production in fungal treatments, particularly with *C. javanica*, whereas *A. tamatavensis* showed no treatment-related effects on oviposition. Overall, the absence of lethal and persistent sublethal effects indicates a high degree of selectivity of the tested fungal products toward both predatory mite species. These results support the tactical compatibility of *B. bassiana* and *C. javanica* with *Amblyseius* spp. and reinforce their potential for combined use in IPM programs targeting whiteflies, contributing to the development of biologically based strategies that reduce reliance on chemical insecticides.

Keywords: biological control; entomopathogenic fungi; predatory mites; selectivity;

Introduction

The conventional approach to pest control, often focused on isolated and reactive actions, may fail to achieve long-term suppression. Measures such as recurrent pesticide applications can select for resistant populations (Georghiou, 1983; Tabashnik et al., 2013) and destabilize agroecosystems by eliminating natural enemies, thereby favoring pest resurgence and outbreaks of secondary pests (Gutierrez et al., 2013). To overcome these limitations, the Integrated Pest Management (IPM) paradigm proposes the coordinated combination of different control methods, aiming at synergy, sustainability, and effectiveness (Van Lenteren, 2012; Dent & Binks, 2020). The success of IPM is therefore closely linked to the ability to integrate complementary tactics (Casula et al., 2006; Tylianakis & Romo, 2010; Stenberg, 2017).

In this context, biological control, using both macrobials and microbial agents, has assumed a central role, particularly in tropical systems prone to population outbreaks (Van Lenteren, 2012; Stenberg et al., 2021). Augmentative control programs frequently involve the release of multiple biological control agents (BCAs) against one or more pests (Midthassel et al., 2016; Messelink et al., 2011; Calvo et al., 2009). This multifaceted approach aims to create a comprehensive management program, including the combination with bioinputs, to reduce dependence on broad-spectrum pesticides (Cock et al., 2010). However, the simultaneous combination of multiple natural enemies introduces an additional layer of ecological complexity: interactions among different BCAs may be antagonistic, neutral, or synergistic, and one agent may potentially impair the effectiveness of another (Van Lenteren, 2012; Janssen & Sabelis, 2015), highlighting the need for knowledge regarding compatibility and selectivity among the agents used.

One way to structure the integration of different BCAs is by employing strategies that differ in nature and timing, such as preventive and curative control (Flint & Van den Bosch, 1981). Preventive control aims to establish a line of defense before pest population outbreaks occur, often by maintaining generalist natural enemies within the agroecosystem (Pedigo & Rice, 2021). In contrast, curative control is implemented in response to established infestations, aiming to rapidly reduce the target pest's population density (Roberts & Hajek, 1992; Pedigo & Rice, 2021). The juxtaposition of these temporal strategies creates an expanded control window, in which agents can act in a complementary and synergistic manner (Midthassel et al., 2016; Castillo-Ramírez et al., 2020).

An illustrative example of this integration is the preventive release of generalist predatory mites, such as *Amblyseius swirskii*, which are able to establish in crops by exploiting alternative food sources like pollen and nectar (Van Rijn, 2002; Nomikou et al., 2001), thereby suppressing early populations of key pests including thrips and whiteflies (Calvo et al., 2015; Midthassel et al., 2016). This strategy can be combined with curative applications of entomopathogenic fungi, such as *Beauveria bassiana* and *Cordyceps javanica*, applied upon pest detection to reduce ongoing infestations (Roberts & Hajek, 1992; Lacey et al., 2015). Combined biological control strategies (predators plus microbial agents) have also shown synergistic potential in systems affected by the two-spotted spider mite, *Tetranychus urticae* (Hernández-Valencia et al., 2024). In addition to acting at complementary times, recent studies suggest that phytoseiid mites may transport conidia adhered to their bodies, potentially contributing to the dissemination of the pathogen within the plant structure, thereby allowing the microbial agent to reach pest individuals that are not directly exposed during spraying (Baverstock et al., 2009; Lin et al., 2019; Castillo-Ramírez et al., 2020).

However, this interaction is not risk-free. Entomopathogenic fungi may affect natural enemies in multiple ways, including reducing survival, altering foraging behavior, or impairing reproduction and development (Furtado et al., 1996; Wu et al., 2015; Liu et al., 2019). The selectivity of entomopathogenic fungi toward natural enemies is a major concern in programs that integrate predators and microorganisms (Cardoso et al., 2008; Maddalena et al., 2024; Hernández-Valencia et al., 2024). In this context, selectivity refers to the ability of these fungi to suppress target pest populations while causing minimal or no adverse effects on non-target beneficial organisms (Sharma et al., 2022; Albana et al., 2025). Therefore, before implementing integrated strategies involving predators and pathogens, it is indispensable to experimentally evaluate the compatibility between these agents, ensuring that their combined use does not compromise the efficiency of biological control (Mesquita et al., 1997; Alma et al., 2007; Labbé et al., 2009; Avery et al., 2020). Although *Beauveria bassiana* and *Cordyceps javanica* are considered relatively safe for many beneficial organisms (Midthassel et al., 2016; Ou et al., 2019; Seiedy, 2015), sublethal effects on reproductive, behavioral, and developmental parameters may impair predatory efficacy and reduce the population regulation potential exerted by phytoseiid mites (Gómez-Marco et al., 2016).

Compatibility assessment is particularly relevant for joint applications involving generalist predators, such as phytoseiid mites of the genus *Amblyseius*, whose persistence and performance on plants strongly depend on their ability to feed, reproduce, and maintain population stability under variable cropping conditions, factors that may be affected by susceptibility to pathogens (McMurtry et al., 2013). Among species of the genus *Amblyseius*, at the global level, *Amblyseius swirskii* is among the most important commercially used phytoseiid predator and became widely adopted in protected cultivation worldwide after its introduction to the market (Calvo et al., 2015). In the Neotropical context, *A. herbicolus* and *A. tamatavensis* have been highlighted as efficient suppressors of key pests such as whiteflies, thrips, tetranychid mites, and tarsonemid mites (Franco et al., 2007; Reis et al., 2007; Duarte et al., 2015; Cavalcante et al., 2017; Toledo et al., 2018; Barbosa et al., 2019; Cardoso et al., 2025; Yang et al., 2025), and they can also exploit alternative food sources such as pollen and nectar (Rodríguez-Cruz et al., 2013; Duarte et al., 2015; Marcossi et al., 2020; Kalile et al., 2023, Iasczczaki et al., 2025). The dietary versatility and colonization capacity of *Amblyseius* spp., typical features of type III generalist predators (McMurtry et al., 2013), position them as ideal candidates for integrated programs combining predatory and microbial control. Nevertheless, the literature still lacks detailed studies assessing their biological parameters following direct exposure to commercial fungal products.

In intensive cropping systems (e.g., cucumber, bean, pepper, and cotton), *Bemisia tabaci* is often managed using selected strains of entomopathogenic fungi (e.g., *B. bassiana* and *C. javanica*), while predatory mites of the genus *Amblyseius* are released as biological control agents against whiteflies and to associated secondary pests. This planned joint use implies co-presence of both agents in the crop and may create a bottleneck for IPM: fungal applications targeting whiteflies can directly expose predatory mites, making compatibility and selectivity critical factors to verify under field-relevant doses. Because detailed information on the predator's performance following direct exposure to commercial fungal products remains limited, the objective of this chapter was to evaluate the selectivity of commercial *B. bassiana* and *C. javanica* isolates toward *A. herbicolus* and *A. tamatavensis*.

To achieve this objective, the following specific objectives were established:

1. To evaluate the survival of adults and immature stages of *A. herbicolus* and *A. tamatavensis* after direct exposure by immersion in fungal suspensions at the concentration recommended for the field management of *B. tabaci*.
2. To quantify the impact of fungal exposure on the developmental rate of immature stages of both predatory mite species.
3. To determine the effects of exposure on the oviposition capacity of adult females of the predators.

Assessing these effects at a concentration relevant to the management of the target pest is essential for determining the tactical compatibility of these agents. The results will support inferences regarding the safety of joint use and provide a scientific basis for developing IPM protocols that reduce reliance on chemical insecticides in agroecosystems.

Materials and Methods

Pollen

The pollen used for rearing and conducting experiments with predatory mites was collected from plants of the genus *Typha* (*Typha* spp.) located in rural areas of the municipality of Viçosa, Minas Gerais, Brazil. This pollen allows for development and reproduction of both predator species (Cavalcante et al., 2015; Duarte et al., 2015; Marcossi et al., 2020).

After harvesting, the pollen was dried in a forced-air oven at 60 °C for 48 h to remove moisture and ensure preservation during storage. The material was then placed in airtight containers and kept in a freezer at approximately −18 °C until use. For routine use, small amounts of pollen were removed from the main stock, transferred to 1.5 mL microtubes (Eppendorf), and dried again at 60 °C for an additional 48 h. Subsequently, the pollen was stored at 8 °C and used during subsequent weeks for bioassays and predator rearing.

Rearing of *Amblyseius herbicolus*

The line of the predatory mite *A. herbicolus* was collected from tomato plants in the municipality of Prados, Minas Gerais, Brazil (latitude: 21°03′00″ S; longitude: 44°04′47″ W), and was maintained at the Laboratory of Acarology of the Federal

University of Viçosa since 2014 (Cardoso et al., 2025). The mites were reared on arenas consisting of a black plastic sheet (10 × 14 cm) surrounded by a moistened cotton wool barrier, supported on a wet sponge placed inside a plastic tray containing water, following the methodology described by McMurtry and Scriven (1965). The arenas were kept at 25 ± 2 °C, 70 ± 10% relative humidity, and a 12:12 h (light:dark) photoperiod. Mites were fed *Typha* pollen twice per week. Cotton threads were provided as oviposition sites placed beneath a piece of folded black plastic (1 × 1 cm), serving as refuge.

To obtain individuals of similar age, cotton threads were placed in the rearing arenas for oviposition and removed the following day, resulting in eggs with a maximum age difference of one day. The threads containing eggs were then transferred to new arenas and supplied with ample pollen. For the developmental experiment, newly hatched larvae were collected immediately after egg eclosion. For the oviposition experiment, the phytoseiids used were 7 ± 1 days old from egg to adult.

Rearing of *Amblyseius tamatavensis*

The rearing of *A. tamatavensis* used in the experiments was established from individuals collected from *Tetranychus urticae* cultures maintained at the Laboratory of Acarology of the Federal University of Viçosa (Cardoso et al., 2025). They were reared in plastic containers (15 cm diameter, 9 cm height) placed on a foam pad (15 cm diameter, 3 cm height). The pad was positioned inside a tray (length x width x hight: 30.3 x 22.1 x 7.5 cm) containing a 2 cm layer of water and covered with another tray to maintain humidity. This tray was placed inside a larger tray (length x width x hight: 34.9 x 28.9 x 7.5), filled with a water–detergent solution to prevent predator escapes and contamination by other arthropods. Rearing arenas were kept in a climate-controlled room at 25 ± 2 °C, 70 ± 10% relative humidity, and a 12:12 h (light:dark) photoperiod. Predators were fed *T. cracentiseta weekly*.

The mite *T. cracentiseta* was reared in transparent plastic containers placed in trays, as described above for *A. tamatavensis*, and fed wheat germ previously sterilized in an autoclave (120 °C at 1.4 atm for 20 min).

To standardize the age of adult predators used in the experiments, adult *A. tamatavensis* females were transferred to an arena with *Typha* sp. pollen. After 24 h, the adults were removed, and their eggs were maintained with pollen provided twice per week. Age-synchronized larvae and adults were obtained as above.

Preparation of fungal suspensions

The conidial suspensions used in the experiments were prepared from commercial wettable powder formulations of *Beauveria bassiana* (isolate IBCB 66; commercial product GRANADA®, Lallemand Soluções Agrobiológicas Ltda., containing 1 × 10^{10} CFU g^{-1} of product) and *Cordyceps javanica* (isolate BRM 27666; commercial product LALGUARD JAVA®, Lallemand Soluções Agrobiológicas Ltda.; containing 1 × 10^{10} viable conidia g^{-1}). The concentrations tested corresponded to the field-recommended application rates for the control of *Bemisia tabaci* and were selected to reflect application levels commonly used in pest management programs. For *B. bassiana*, 0.0375 g of the product was diluted in 100 mL of distilled water containing 0.01% (v/v) Tween 80®, resulting in a final concentration of approximately 5.75 × 10^7 conidia/mL. For *C. javanica*, the suspension was prepared by diluting 0.1 g of the commercial formulation with the same volume of surfactant solution, yielding an estimated concentration of 8.5 × 10^8 conidia/mL. Both suspensions were kept on a magnetic stirrer for 30 min to ensure adequate conidial dilution. A solution containing only distilled water and Tween 80® was used as control. Once prepared, 3 mL of each solution was transferred to a Petri dish for topical application. Before topical application, all solutions were manually homogenized to prevent sedimentation.

Adult survival and oviposition

To evaluate effects of the fungi on reproduction and survival, adult females of *Amblyseius herbicolus* and *A. tamatavensis* were immersed in one of three suspensions: (1) the *Beauveria bassiana* suspension, (2) the *Cordyceps javanica* suspension, and (3) the control solution containing 0.01% Tween 80®. Each treatment included 20 females from the same cohort, each individually immersed using a fine brush for 30 s in 3 mL from the suspensions described below. Subsequently, the mites were transferred to Petri dishes lined with filter paper, where they remained until dry; resumption of movement was then verified as an indicator of survival. The experimental procedures were adapted from Midthassel et al. (2016). Predators were subsequently transferred to Petri dishes containing ample *Typha* sp.

pollen and moistened cotton wool, used as food and water source, respectively. The Petri dishes were maintained under controlled conditions (25 ± 2 °C; 70 ± 10% RH; 12 h photophase) and the number of eggs laid and survival of the females was evaluated daily for 10 days to check for infection signs. The cotton and pollen were replaced daily to ensure adequate moisture and feeding conditions.

Juvenile development and survival

The larval development assay followed the same procedure as used for adult females, with newly hatched larvae immersed in similar suspensions and for the same duration as above. They were subsequently allowed to dry as above and movement was assessed as an indicator of survival. Subsequently, individuals were incubated as above. Evaluations were conducted daily until individuals reached adulthood, and survival and developmental stage were recorded. As in the adult experiment, pollen and cotton wool were renewed daily.

Statistical analyses

Oviposition (number of eggs per female per day) was analyzed using linear mixed-effects models with the ‘nlme’ package. The identity of individual mites was included as a random factor to account for repeated measures, and treatment and time and their interaction were included as fixed factors. Significance of interactions and factors were assessed through model simplifications and comparing models using likelihood ratio tests. Survival of adults and juveniles were analyzed using the Cox proportional hazards model (coxph). Multiple comparisons among treatments were performed using pairwise contrasts with Tukey adjustment with the ‘emmeans’ package. All statistical analyses were done using R (v. 4.5.2).

Results

Survival and oviposition of *Amblyseius herbicolus* and *A. tamatavensis* adults

For *A. herbicolus*, there was a significant treatment × day interaction (Likelihood ratio = 34.1; d.f. = 2; $p < 0.0001$), indicating that treatment effects varied over time (Fig. 1A). Oviposition on day 1 was significantly higher in the *Cordyceps javanica* treatment than in the *Beauveria bassiana* and control treatments. The *C. javanica* treatment showed significantly higher oviposition than *B. bassiana* on days 2 and 8, and lower oviposition than the control on day 7.

For *A. tamatavensis*, oviposition was not affected by the treatments (L. ratio = 1.40; d.f. = 2; p = 0.497), and there was no significant treatment × day interaction (L. ratio = 1.86; d.f. = 2, p = 0.395). However, there was a significant effect of time (L. ratio = 12.23; d.f. = 1; p = 0.0005), indicating that oviposition increased over the observation period regardless of treatment (Figure 1B).

The survival of *A. herbicolus* adults (Figure 2A) was not affected by exposure to either of the two entomopathogenic fungi tested (Cox proportional hazards: L. ratio = 1.55; d.f. = 2; p = 0.5). Similarly, for *A. tamatavensis* (Figure 2B), adult survival did not differ significantly among treatments (L. ratio = 0.91; d.f. = 2; p = 0.6).

Survival and development of *Amblyseius herbicolus* and *A. tamatavensis* juveniles

Juvenile survival of *A. herbicolus* (Figure 3A) showed no significant effect of treatment (Cox proportional hazards: Likelihood ratio = 5.51; d.f. = 2; p = 0.06). For *A. tamatavensis* (Figure 3B), juvenile survival was also not significantly affected by the treatments (L. ratio = 2.28; d.f. = 2; p = 0.3).

Development from larva to adult was not significantly affected by the treatments for either species. (*A. herbicolus*, Figure 4A, L. ratio = 1.31; d.f. = 2; p = 0.5; *A. tamatavensis*, Figure 4B, χ^2 = 0.20; d.f. = 2; p = 0.9).

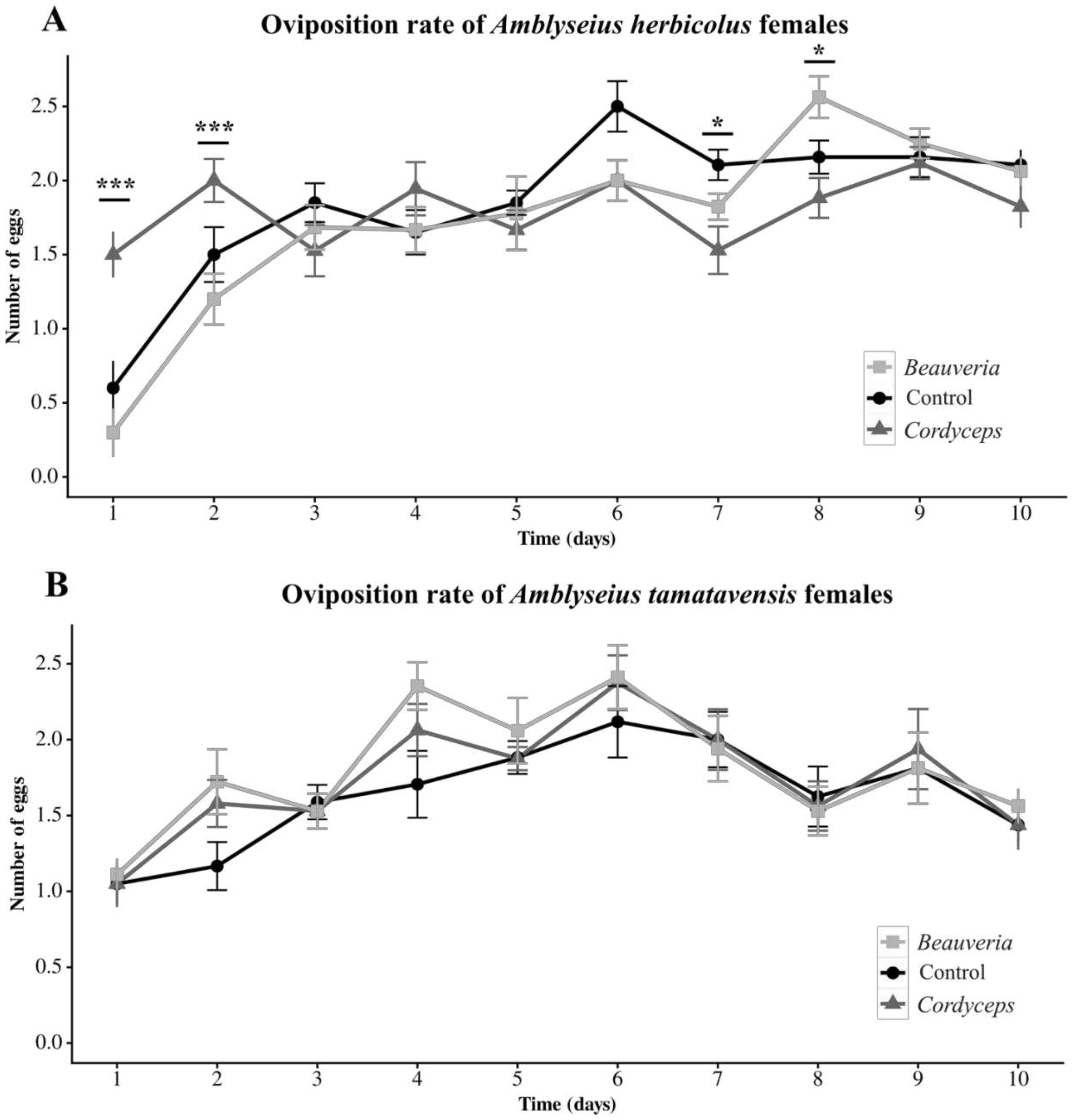

Figure 1. Daily oviposition rate of predatory mite females after exposure to entomopathogenic fungi *Beauveria bassiana* and *Cordyceps javanica* by immersion in conidial suspensions. (A) *Amblyseius herbicolus* egg production per female (mean ± SE). Significant treatment × day interaction. Asterisks above bars indicate significant pairwise differences among treatments within each day (* = $p < 0.05$; *** = $p < 0.001$). Day 1: *Cordyceps* > *Beauveria* (***) and *Cordyceps* > Control (***); Day 2: *Cordyceps* > *Beauveria* (***); Day 7 - Control > *Cordyceps*; Day 8 - *Cordyceps* > *Beauveria*. (B) *A. tamatavensis* egg production per female (mean ± SE), which significantly increased over time.

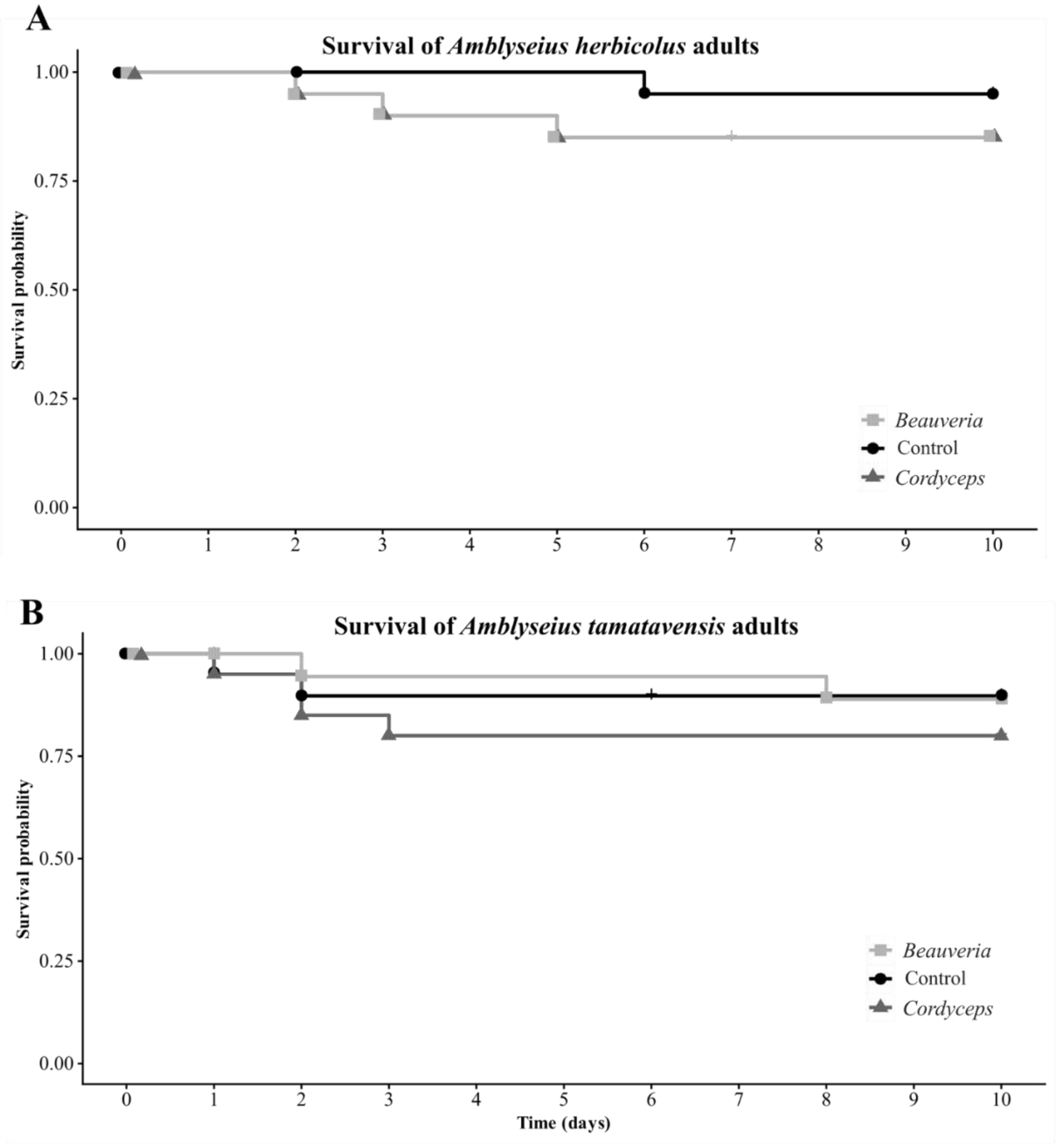

Figure 2. Kaplan-Meier survival curves for (A) *Amblyseius herbicolus* and (B) *A. tamatavensis* adults immersed in conidial suspensions of *Beauveria bassiana*, *Cordyceps javanica*, or Tween 80% solution (Control).

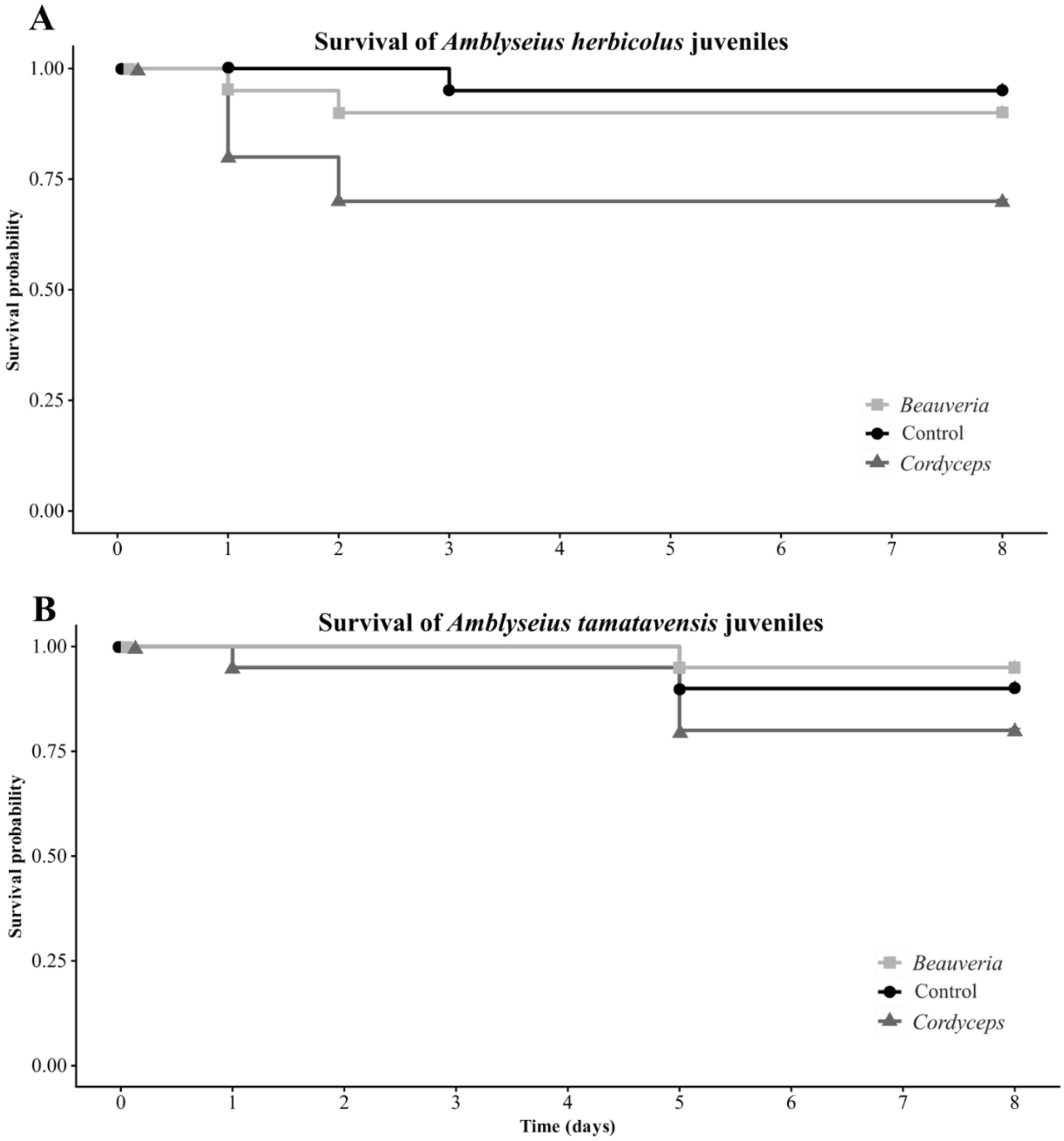

Figure 3. Kaplan-Meier survival curves for (A) *Amblyseius herbicolus* and (B) *A. tamatavensis* juveniles immersed in conidial suspensions of *Beauveria bassiana*, *Cordyceps javanica*, or Tween 80% solution (Control).

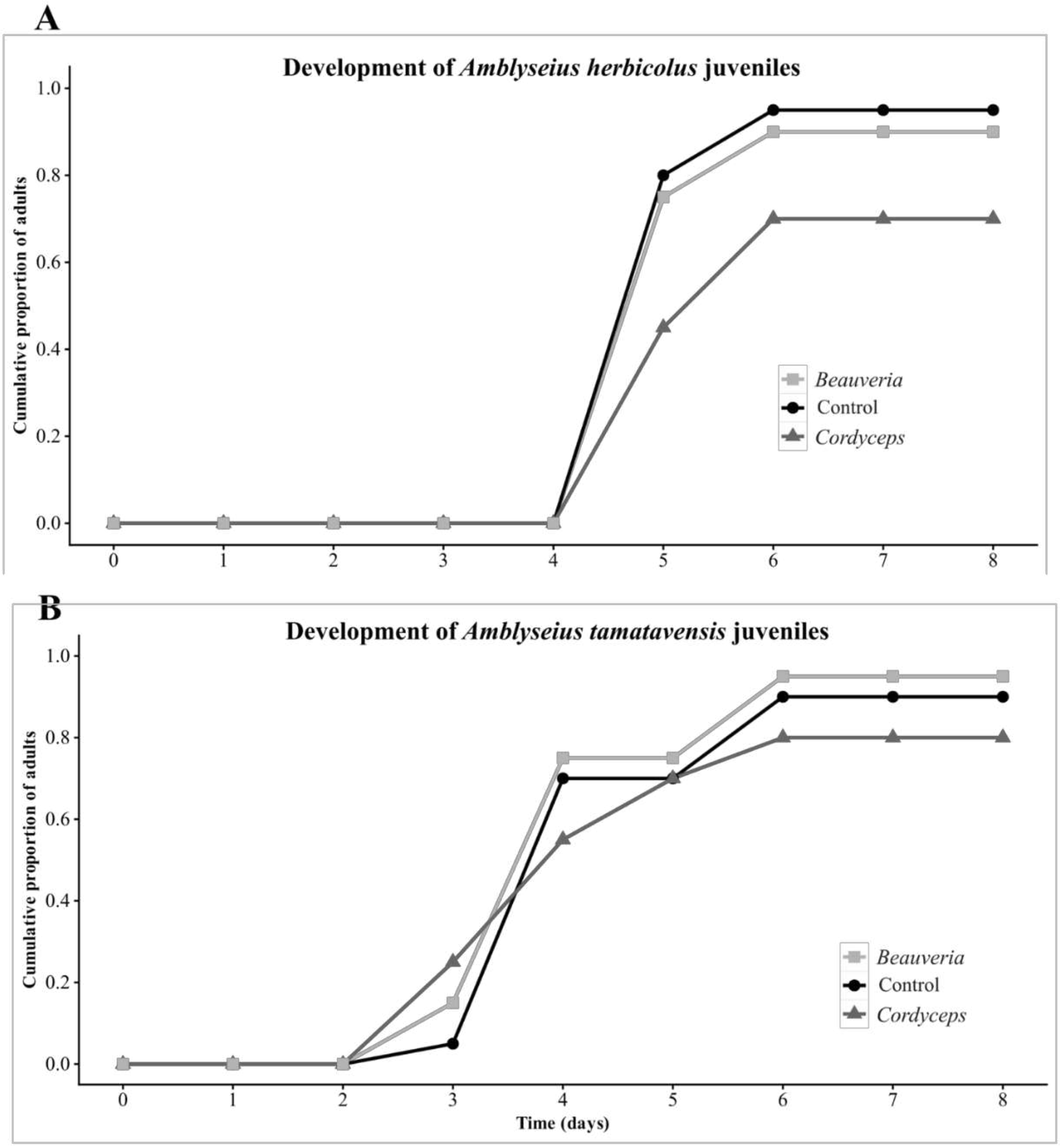

Figure 4. Cumulative development curves showing time to adulthood for (A) *Amblyseius herbicolus* and (B) *A. tamatavensis* larvae exposed to entomopathogenic fungi.

Discussion

The results obtained in this chapter demonstrate that the entomopathogenic fungi *Beauveria bassiana* and *Cordyceps javanica*, when applied at concentrations recommended for *Bemisia tabaci* management, do not negatively affect the survival or key biological parameters of the predatory mites *Amblyseius herbicolus* and *Amblyseius tamatavensis*. This absence of lethal effects on different developmental stages of the predators suggests a favorable selectivity profile of these fungal isolates, acting against the target pest while sparing these non-target natural enemies (Albana et al., 2025; Seiedy et al., 2015; Roy & Pell, 2000). These findings are consistent with studies reporting minimal impacts of entomopathogenic fungi on various beneficial arthropods (Huang et al., 2012; Ullah et al., 2019; Castillo-Ramírez et al., 2020; Rizwan et al., 2021) and reinforce the potential for integrating predatory mites and entomopathogenic fungi within IPM programs, especially considering that, under field conditions, the intensity and frequency of predator exposure to pathogens tend to be lower than those simulated in laboratory bioassays (Butt & Goettel, 2000; Lacey et al., 2015; Sain et al., 2021).

Adult survival of both *Amblyseius* species was not affected by direct exposure to the fungal suspensions, a result that corroborates previous studies indicating high selectivity of entomopathogenic fungi toward phytoseiid mites (Wekesa et al., 2007; Cavalcanti et al., 2008; Wu et al., 2016a; Lin et al., 2017; Castillo-Ramírez et al., 2020). This tolerance has been associated with various factors, including morphological and behavioral characteristics of predators, such as cuticle structure and the ability to mechanically remove conidia through grooming behavior (Wu et al., 2016a; Wu et al., 2016b; Hassan et al., 2017; Shang et al., 2018).

Similarly, the survival of immature stages was also not affected by the fungi tested, indicating that the initial exposure by immersion did not result in lethal infection during predator development. The high survival of immature stages of both *Amblyseius* species can be explained by biological processes inherent to their development. During molting, the cuticle is renewed, which helps remove conidia adhered to the body and reduces the likelihood of infection (Vey & Fargues, 1977; Wekesa et al., 2006). As *A. herbicolus* and *A. tamatavensis* undergo several molts over a short period before reaching adulthood, this process occurs frequently, hindering fungal establishment.

Although no significant effects on survival or development were detected for *A. herbicolus* or *A. tamatavensis* under the conditions evaluated in this study, responses to entomopathogenic fungi may vary among predatory mite species, including species within the same genus. Such interspecific variation has been documented in experiments using *Beauveria bassiana* isolates (Devi et al., 2008), including negative effects on the survival of *Amblyseius swirskii* (Seiedy et al., 2015; Midthassel et al., 2016), in contrast to the absence of negative effects observed in *Amblyseius cucumeris* (Jacobson et al., 2001). These differences may be associated with physiological and behavioral traits among species (Hajek & St. Leger, 1994; Eilenberg et al., 2019), leading to high levels of pathogen–host specificity, or may be linked to genetic differences that result in genotype-level specificity, in which particular fungal genotypes exhibit greater virulence toward more susceptible host genotypes (Edwards et al., 2025).

Pathogen–host specificity is also evident at the pathogen species level, as no negative effects of another species within the genus *Cordyceps*, *C. fumorosea*, were observed on *A. herbicolus* in previous studies (Beghelli, 2024). Therefore, compatibility between biological control agents should be evaluated on a species-by-species basis. Entomopathogenic fungi have been reported to induce species-specific sublethal effects on predatory mites under certain conditions, even in the absence of lethal outcomes (Dogan et al., 2017; Ullah & Lim, 2017; Liu et al., 2019). Developmental delay is a possible response to biotic stress and may arise from physiological costs associated with immune activation, transient metabolic alterations, or indirect effects of metabolites produced by the biotic agent (Schmid-Hempel, 2003; Li & Zhang, 2019). Similar responses have already been documented in other mite species, including compensatory effects on longevity, as observed in *Tyrophagus putrescentiae* under predation stress (Wei et al., 2022).

Regarding oviposition, responses to fungal exposure differed between the two predatory mite species. For *Amblyseius herbicolus*, a significant treatment × time interaction indicates that fungal exposure influenced oviposition dynamics during the experiment, with treatment-related differences occurring on specific days rather than as a uniform effect across the evaluation period. Oviposition was higher in the first 2 days of the experiment and lower on days 7 and 8 in the *Cordyceps* treatment compared with the *Beauveria* and control treatments. These temporal variations were not cumulative and did not result in a sustained reduction in egg production over time. Similar effects in oviposition have been reported for other predatory mites, such as *Neoseiulus californicus*, in which exposure to *Cordyceps fumosorosea* led to decreased egg production (Vergel et al., 2011). Fecundity is a central parameter for the persistence of predator populations used in preventive biological control strategies (Stiling & Cornelissen, 2005; Ouattara et al., 2024), and that sublethal effects on oviposition can compromise the efficiency of natural enemies even in the absence of direct mortality (Desneux et al., 2007; Gentz et al., 2010), but, given our results for other life trait parameters, this pattern may suggest a time-dependent modulation of reproductive output rather than persistent reproductive impairment.

In contrast, oviposition of *Amblyseius tamatavensis* was not affected by fungal treatments, and no treatment × time interaction was detected, although egg production increased over time irrespective of treatment. This pattern is consistent with previous studies involving other *Cordyceps* species, in which no sustained or long-term changes in oviposition rates were reported for *A. herbicolus* females exposed to *C. fumorosea* (Beghelli, 2024). Overall, these results indicate species-specific responses to fungal exposure, with *A. herbicolus* showing transient, time-dependent variation in oviposition in fungal treatments and *A. tamatavensis* showing no significant treatment-related effects.

The compatibility observed, together with studies demonstrating synergistic effects in pest control when entomopathogenic fungi such as *B. bassiana* are combined with phytoseiid mites (Chandler et al., 2005; Ullah & Lim, 2017; Hernández-Valencia et al., 2024), reinforces the potential of this integrated strategy for IPM. Thus, there is scope to further explore the potential for positive interactions between agents, such as the passive dissemination of conidia by predators, a mechanism already suggested to enhance the efficacy of entomopathogenic pathogens within agroecosystems (Zhang et al., 2015; Castillo-Ramírez et al., 2020).

Although the direct effects of the fungi on predators were minimal, indirect interactions via infected prey may occur (Roy & Pell, 2010; Quesada-Moraga, 2022). Entomopathogenic pathogens can alter the nutritional quality of prey, thereby influencing predator performance, as already demonstrated for other predatory mites feeding on prey treated with *B. bassiana* (Seiedy et al., 2012; Wu et al., 2015; Zhang et al., 2025). Therefore, future assessments should consider not only direct exposure of predators to fungi, but also the effects of consuming infected prey on survival, development, and reproduction.

Finally, it is important to consider that the bioassays were conducted under controlled laboratory conditions, with a single exposure to the fungi and using specific isolates. In cropping systems, factors such as microclimate, plant architecture, application frequency, and prey availability can modify interactions between predators and pathogens (Jaronski, 2010). In addition, different isolates of the same fungal species may exhibit distinct levels of pathogenicity toward predatory mites (Ferron, 1978; Goettel et al., 1990; Brodeur, 2012; Seiedy et al., 2015). Therefore, future studies evaluating repeated exposures, behavioral responses (such as predation and foraging), and effects across generations may contribute to a more realistic understanding of the results of integrating these agents into pest management.

References

ALBANA, H. et al. Effects of entomopathogenic fungi on natural enemies: a systematic review of their use in biological control. *Malaysian Applied Biology*, [S. l.], v. 54, n. 4, p. 25–39, 2025. DOI: 10.55230/mabjournal.v54i4.3566.

ALMA, C. R.; GOETTEL, M. S.; ROITBERG, B. D.; GILLESPIE, D. R. Combined effects of the entomopathogenic fungus *Paecilomyces fumosoroseus* Apopka-97 and the generalist predator *Dicyphus hesperus* on whitefly populations. *BioControl*, v. 52, p. 669–681, 2007.

AVERY, P. B.; KUMAR, V.; FRANCIS, A.; McKENZIE, C. L.; OSBORNE, L. S. Compatibility of the predatory beetle *Delphastus catalinae* with an entomopathogenic fungus, *Cordyceps fumosorosea*, for biocontrol of invasive pepper whitefly, *Aleurothrixus trachoides*, in Florida. *Insects*, v. 11, p. 590, 2020. DOI: 10.3390/insects11090590.

BARBOSA, M. F. C.; POLETTI, M.; POLETTI, E. C. Functional response of *Amblyseius tamatavensis* Blommers (Mesostigmata: Phytoseiidae) to eggs of *Bemisia tabaci*. *Biological Control*, v. 138, p. 104030, 2019.

BAVERSTOCK, J.; ROY, H. E.; PELL, J. K. Entomopathogenic fungi and insect behaviour: from unsuspecting hosts to targeted vectors. *BioControl*, v. 55, p. 89–102, 2009. DOI: 10.1007/s10526-009-9238-5.

BEGHELLI, Gabriel Modesto. Compatibility of a predator and an entomopathogenic fungus of Asian citrus psyllid. 2024. 65 f. Dissertação (Mestrado em Entomologia) – Universidade Federal de Viçosa, Viçosa, 2024.

BRODEUR, J. Host specificity in biological control: insights from opportunistic pathogens. *Evolutionary Applications*, v. 5, n. 5, p. 470–480, 2012. DOI: 10.1111/j.1752-4571.2012.00273.x.

BUTT, T. M.; GOETTEL, M. S. Bioassays of entomogenous fungi. In: NAVON, A.; ASCHER, K. R. S. (ed.). *Microbes and nematodes*. Wallingford: CAB International, 2000. v. 4, p. 141–195.

CALVO, F. J.; BOLCKMANS, K.; BELDA, J. E. Development of a biological control-based integrated pest management method for *Bemisia tabaci* for protected sweet pepper crops. *Entomologia Experimentalis et Applicata*, v. 133, n. 1, p. 9–18, 2009.

CARDOSO, E. R. et al. Seletividade de *Lecanicillium lecanii* e *Metarhizium anisopliae* para larvas de primeiro ínstar de *Ceraeochrysa cincta* (Neuroptera: Chrysopidae) em laboratório. *Neotropical Entomology*, Londrina, v. 39, n. 2, p. 243–249, 2008. DOI: 10.1590/S1519-566X2010000200015.

CARDOSO, A. C. *et al.* Potential of *Amblyseius herbicolus* and *Amblyseius tamatavensis* to control *Frankliniella occidentalis*. *Journal of Pest Science*, v. 98, p. 2117–2127, 2025. DOI: 10.1007/s10340-025-01928-9.

CARDOSO, A. C. et al. A predatory mite as potential biological control agent of *Bemisia tabaci* on tomato plants. *Journal of Pest Science*, v. 98, n. 1, p. 277–289, 2025. DOI: 10.1007/s10340-024-01809-7

CASTILLO-RAMÍREZ, O.; GUZMÁN-FRANCO, A. W.; SANTILLÁN-GALICIA, M. T.; TAMAYO-MEJÍA, F. Interaction between predatory mites (Acari: Phytoseiidae) and entomopathogenic fungi in *Tetranychus urticae* populations. *BioControl*, v. 65, p. 433–445, 2020. DOI: 10.1007/s10526-020-10004-3.

CASULA, P.; WILBY, A.; THOMAS, M. B. Understanding biodiversity effects on prey in multi-enemy systems. *Ecology Letters*, v. 9, n. 9, p. 995–1004, 2006.

CAVALCANTE, A. C. C.; DOS SANTOS, V. L. V.; ROSSI, L. C.; MORAES, G. J. D. Potential of five Brazilian populations of Phytoseiidae (Acari) for the biological control of *Bemisia tabaci*. *Journal of Economic Entomology*, v. 108, n. 1, p. 29–33, 2015.

CAVALCANTE, A. C. C.; MANDRO, M. E. A.; PAES, E. R.; MORAES, G. J. Amblyseius tamatavensis Blommers (Acari: Phytoseiidae) a candidate for biological control of *Bemisia tabaci* biotype B in Brazil. *International Journal of Acarology*, v. 43, n. 1, p. 10–15, 2017. DOI: 10.1080/01647954.2016.1225816.

CALVALCANTI, R. S. *et al.* Patogenicidade de fungos entomopatogênicos a três espécies de ácaros em cafeeiro. *Coffee Science*, Lavras, v. 3, n. 1, p. 68–75, jan./jun. 2008.

CHANDLER, D.; DAVIDSON, G.; JAKOBSON, R. J. Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. *Biocontrol Science and Technology*, v. 15, p. 37–54, 2005.

COCK, M. J. W. et al. Do new Access and Benefit Sharing procedures under the Convention on Biological Diversity threaten the future of biological control? *BioControl*, v. 55, p. 199–218, 2010. DOI: 10.1007/s10526-009-9234-9.

DENT, D. R.; BINKS, R. H. *Insect pest management*. Wallingford: CABI, 2020.

DESNEUX, N.; DECOURTYE, A.; DELPUECH, J.-M. The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, v. 52, n. 1, p. 81–106, 2007.

DEVI, K. Uma *et al.* A study of host specificity in the entomopathogenic fungus *Beauveria bassiana* (Hypocreales, Clavicipitaceae). *Biocontrol Science and Technology*, [S. l.], v. 18, n. 10, p. 975–989, 2008. DOI: 10.1080/09583150802450451.

DOGAN, Y. O. *et al.* Evaluation of entomopathogenic fungi for the control of *Tetranychus urticae* (Acari: Tetranychidae) and the effect of *Metarhizium brunneum* on the predatory mites (Acari: Phytoseiidae). *Biological Control*, v. 111, p. 6–12, 2017.

DUARTE, M. V. A. et al. Alternative food promotes broad mite control on chilli pepper plants. *BioControl*, v. 60, p. 817–825, 2015. DOI: 10.1007/s10526-015-9688-x.

EDWARDS, S. *et al.* Patterns of genotype-specific interactions in an obligate host-specific insect pathogenic fungus. *Journal of Evolutionary Biology*, [S. l.], v. 38, n. 2, p. 225–239, 2025. DOI: 10.1093/jeb/voae149.

EILENBERG, J. *et al.* Factors driving susceptibility and resistance in aphids that share specialist fungal pathogens. *Current Opinion in Insect Science*, v. 33, p. 91–98, 2019.

ENT, D. R. (Ed.). *Integrated pest management*. London: Chapman & Hall, 1995.

FERRON, P. Biological control of insect pests by entomogenous fungi. *Annual Review of Entomology*, v.23, p.409-442, 1978

FLINT, M. L.; VAN DEN BOSCH, R. A history of pest control. In: FLINT, M. L.; VAN DEN BOSCH, R. *Introduction to integrated pest management*. Boston: Springer, 1981.

FRANCO, R. A.; REIS, P. R.; ZACARIAS, M. S.; ALTOÉ, B. F. Predation potential of three species of phytoseiids on *Oligonychus ilicis* (McGregor, 1917) (Acari: Tetranychidae). *Coffee Science*, v. 2, p. 175–182, 2007.

FURTADO, I. P.; MORAES, G. J.; KELLER, S. Infection of *Euseius citrifolius* (Acari: Phytoseiidae) by an entomophthoralean fungus in Brazil. *Ecossistema*, v. 21, p. 85–86, 1996.

GENTZ, M.; MURDOCH, C. G.; KING, G. F. Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. *Biological Control*, v. 52, p. 208–215, 2010.

GEORGHIOU, G. P. Management of resistance in arthropods. In: GEORGHIOU, G. P.; SAITŌ, T. (Ed.). *Pest resistance to pesticides*. New York: Plenum Press, 1983. p. 769–792.

GOETTEL, M. S.; POPRAWSKI, T. J.; VANDENBERG, J. D.; LI, Z.; ROBERTS, W. D. Safety to nontarget invertebrates of fungal biocontrol agents. In: LAIRD, M.; LACEY, L. A.; DAVIDSON, E. W. (ed.). *Safety of microbial insecticides*. Boca Raton: CRC Press, 1990. p. 209–232.

GÓMEZ-MARCO, F.; URBANEJA, A.; JACAS, J. A.; TENA, A. Keep walking: soybean plants do not avoid induced resistance after being exposed to an herbivore. *Pest Management Science*, v. 72, n. 11, p. 2157–2163, 2016.

GURR, G. M. et al. Multi-country evidence that crop diversification promotes ecological intensification of agriculture. *Nature Plants*, v. 2, art. 16014, 2016. DOI: 10.1038/nplants.2016.14.

GUTIERREZ, A. P.; MESSENGER, P. S. *An introduction to biological control*. New York: Springer, 2013.

HAJEK, A. E.; ST. LEGER, R. J. Interactions between fungal pathogens and insect hosts. *Annual Review of Entomology*, v. 39, p. 293–322, 1994. DOI: 10.1146/annurev.en.39.010194.001453.

HASSAN, D. *et al.* Virulent entomopathogenic fungi against the two-spotted spider mite *Tetranychus urticae* and some associated predator mites as non-target organisms. *Egyptian Academic Journal of Biological Sciences*, v. 10, n. 6, p. 37–56, 2017.

HERNÁNDEZ-VALENCIA, V. et al. Combined application of entomopathogenic fungi and predatory mites for the biological control of *Tetranychus urticae* on chrysanthemum. *Pest Management Science*, v. 80, n. 9, p. 4199–4206, 2024. DOI: 10.1002/ps.8123.

HUANG, Z.; ALI, S.; REN, S.; WU, J.; ZHANG, Y. Influence of the entomopathogenic fungus *Beauveria bassiana* on *Prynocaria congener* (Billberg) (Coleoptera: Coccinellidae) under laboratory conditions. *Pakistan Journal of Zoology*, v. 44, n. 1, p. 209–216, 2012.

IASCZCZAKI, R. S. et al. Extrafloral nectar from coffee-associated trees as alternative food for a predatory mite. *Experimental and Applied Acarology*, v. 94, n. 1, p. 2, 2025. DOI: 10.1007/s10493-024-00967-8.

JACOBSON, R. J.; CHANDLER, D.; FENLON, J.; RUSSELL, K. M. Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on cucumber plants. *Biocontrol Science and Technology*, v. 11, p. 391–400, 2001. DOI: 10.1080/09583150120055808.

JANSSEN, A.; SABELIS, M. W. Alternative food and biological control by generalist predatory mites: the case of *Amblyseius swirskii*. *Experimental and Applied Acarology*, v. 65, p. 413–418, 2015. DOI: 10.1007/s10493-015-9901-8.

JARONSKI, S. T. Ecological factors in the inundative use of fungal entomopathogens. *BioControl*, v. 55, n. 1, p. 159–185, 2010. DOI: 10.1007/s10526-009-9248-3.

KALILE, M. O. et al. A predatory mite that suppresses *Diaphorina citri* populations on plants with pollen and oviposition sites. *Entomologia Experimentalis et Applicata*, v. 171, p. 592–602, 2023. DOI: 10.1111/eea.13326.

LABBÉ, R. M.; GILLESPIE, D. R.; CLOUTIER, C.; BRODEUR, J. Compatibility of an entomopathogenic fungus with a predator and a parasitoid in the biological control of greenhouse whitefly. *Biocontrol Science and Technology*, v. 19, p. 429–446, 2009.

LACEY, L. A. et al. Insect pathogens as biological control agents: back to the future. *Journal of Invertebrate Pathology*, v. 132, p. 1–41, 2015. DOI: 10.1016/j.jip.2015.07.009.

LI, Guang-Yun; ZHANG, Zhi-Qiang. Development, lifespan and reproduction of spider mites exposed to predator-induced stress across generations. *Experimental and Applied Acarology,* [S. l.], v. 79, p. 335–348, 2019. DOI: 10.1007/s10493-019-00431-y.

LIN, G. *et al.* A new method for loading predatory mites with entomopathogenic fungi for biological control of their prey. *Biological Control*, v. 115, p. 105–111, 2017.

LIN, G. et al. Phytoseiid predatory mites can disperse entomopathogenic fungi to prey patches. *Scientific Reports*, v. 9, p. 19435, 2019. DOI: 10.1038/s41598-019-55499-8.

LIU, J.-F.; ZHANG, Z.-Q.; BEGGS, J. R.; WEI, X.-Y. Influence of pathogenic fungi on the life history and predation rate of mites attacking a psyllid pest. *Ecotoxicology and Environmental Safety*, v. 183, p. 109585, 2019. DOI: 10.1016/j.ecoenv.2019.109585.

MADDALENA, A. et al. Seletividade de fungos entomopatogênicos sobre o predador *Marava arachidis*. *Journal of Environmental Analysis and Progress*, v. 9, n. 1, p. 050–059, 2024.

MARCOSSI, Í.; FONSECA, M. M.; CARBAJAL, P. A.; CARDOSO, A.; PALLINI, A.; JANSSEN, A. *High-quality alternative food reduces cannibalism in the predatory mite* Amblyseius herbicolus *(Acari: Phytoseiidae)*. *Experimental and Applied Acarology*, v. 81, p. 189–200, 2020. DOI: 10.1007/s10493-020-00500-7.

McMURTRY, J. A.; SCRIVEN, G. T. Insectary production of phytoseiid mites. *Journal of Economic Entomology*, v. 58, n. 2, p. 282–284, 1965. DOI: 10.1093/jee/58.2.282.

McMURTRY, J. A.; MORAES, G. J.; SOURASSOU, N. F. Revision of the lifestyles of phytoseiid mites and implications for biological control strategies. *Systematic and Applied Acarology*, v. 18, p. 297–320, 2013.

MESSELINK, G. J. et al. Hyperpredation by generalist predatory mites disrupts biological control of aphids by the aphidophagous gall midge *Aphidoletes aphidimyza*. *Biological Control*, v. 57, n. 3, p. 246–252, jun. 2011.

MESQUITA, A. L. M.; LACEY, L. A.; LECLANT, F. Individual and combined effects of the fungus *Paecilomyces fumosoroseus* and parasitoid *Aphelinus asychis* on *Diuraphis noxia*. *Journal of Applied Entomology*, v. 121, p. 155–163, 1997.

MIDTHASSEL, A.; LEATHER, S. R.; WRIGHT, D. J. Compatibility of *Amblyseius swirskii* with *Beauveria bassiana*: two potentially complementary biocontrol agents. *BioControl*, v. 61, p. 437–447, 2016. DOI: 10.1007/s10526-016-9718-3.

NOMIKOU, M.; JANSSEN, A.; SCHRAAG, R.; SABELIS, M. W. Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. *Experimental and Applied Acarology*, v. 25, n. 4, p. 271–291, 2001.

OU, D. et al. Compatibility and efficacy of the parasitoid *Eretmocerus hayati* and the entomopathogenic fungus *Cordyceps javanica* for biological control of *Bemisia tabaci*. *Insects*, v. 10, p. 425, 2019. DOI: 10.3390/insects10120425.

OUATTARA, Téné Yacine *et al.* Reproductive parameters of a new biocontrol agent, *Eupeodes americanus* (Diptera: Syrphidae) and comparison with the commercialized *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae). *Journal of Economic Entomology*, [S. l.], v. 117, n. 5, p. 1760–1768, 2024. DOI: 10.1093/jee/toae133.

PEDIGO, L. P.; RICE, M. E.; KRELL, R. K. *Entomology and pest management*. Long Grove: Waveland Press, 2021.

QUESADA-MORAGA, E.; GARRIDO-JURADO, I.; YOUSEF-YOUSEF, M. *et al.* Multitrophic interactions of entomopathogenic fungi in biological control. BioControl, v. 67, p. 457–472, 2022. DOI: 10.1007/s10526-022-10163-5.

REIS, P. R. et al. Life history of *Amblyseius herbicolus* on coffee plants. *Neotropical Entomology*, v. 36, p. 282–287, 2007.

RIZWAN, M.; ATTA, B.; ARSHAD, M. *et al.* Nondetrimental impact of two concomitant entomopathogenic fungi on life history parameters of a generalist predator, *Coccinella septempunctata* (Coleoptera: Coccinellidae). *Scientific Reports*, v. 11, art. 20699, 2021. DOI: 10.1038/s41598-021-00037-8.

ROBERTS, D. W.; HAJEK, A. E. Entomopathogenic fungi as bioinsecticides. In: *Frontiers in industrial mycology*. Boston: Springer, 1992. p. 144–159.

RODRÍGUEZ-CRUZ, F. A.; VENZON, M.; PINTO, C. M. F. Performance of *Amblyseius herbicolus* on broad mites and on pollen. *Experimental and Applied Acarology*, v. 60, p. 497–507, 2013.

ROY, H.; PELL, J. Interactions between entomopathogenic fungi and other natural enemies: implications for biological control. *Biocontrol Science and Technology*, v. 10, p. 737–752, 2000. DOI: 10.1080/09583150020011708.

SAIN, S. K. *et al.* Evaluation of bioefficacy potential of entomopathogenic fungi against the whitefly (*Bemisia tabaci* Genn.) on cotton under polyhouse and field conditions. *Journal of Invertebrate Pathology*, [S. l.], v. 183, p. 107618, 2021. DOI: 10.1016/j.jip.2021.107618.

SEIEDY, M.; SABOORI, A.; ALLAHYARI, H. Interactions of two natural enemies of *Tetranychus urticae*, the fungal entomopathogen *Beauveria bassiana* and the predatory mite *Phytoseiulus persimilis*. *Biocontrol Science and Technology*, v. 22, p. 873–882, 2012.

SEIEDY, M. Compatibility of *Amblyseius swirskii* and *Beauveria bassiana* for biological control of *Trialeurodes vaporariorum*. *Systematic and Applied Acarology*, v. 20, n. 7, p. 731–738, 2015.

SHANG, S.-Q.; CHEN, Y.-N.; BAI, Y.-L. The pathogenicity of entomopathogenic fungus *Acremonium hansfordii* to two-spotted spider mite, *Tetranychus urticae*, and predatory mite *Neoseiulus barkeri*. *Systematic and Applied Acarology*, v. 23, n. 11, p. 2173–2185, 2018.

SHARMA, A.; SHARMA, S.; YADAV, P. K. Entomopathogenic fungi and their relevance in sustainable agriculture: a review. *Cogent Food & Agriculture*, Abingdon, v. 9, n. 1, 2023. DOI: 10.1080/23311932.2023.2180857.

SCHMID-HEMPEL, P. Variation in immune defence as a question of evolutionary ecology. *Proceedings of the Royal Society B: Biological Sciences*, [S. l.], v. 270, n. 1513, p. 357–366, 2003. DOI: 10.1098/rspb.2002.2265.

STENBERG, J. A. A conceptual framework for integrated pest management. *Trends in Plant Science*, v. 22, n. 9, p. 759–769, 2017.

STENBERG, J. A. et al. When is it biological control? *Journal of Pest Science*, v. 94, n. 3, p. 665–676, 2021.

STILING, Peter; CORNELISSEN, Tatiana. What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Biological Control*, [S. l.], v. 34, n. 3, p. 236–246, 2005. DOI: 10.1016/j.biocontrol.2005.02.017.

TABASHNIK, B.; BRÉVAULT, T.; CARRIÈRE, Y. Insect resistance to Bt crops: lessons from the first billion acres. *Nature Biotechnology*, v. 31, p. 510–521, 2013. DOI: 10.1038/nbt.2597.

TOLEDO, M. A.; REIS, P. R.; LISKA, G. R.; CIRILLO, M. Â. Biological control of southern red mite, *Oligonychus ilicis* (Acari: Tetranychidae), in coffee plants. *Advances in Entomology*, v. 6, p. 74, 2018. DOI: 10.4236/ae.2018.62007.

TYLIANAKIS, J. M.; ROMO, C. M. Natural enemy diversity and biological control: making sense of context dependency. *Basic and Applied Ecology*, v. 11, n. 8, p. 657–668, 2010.

ULLAH, M. S.; LIM, U. T. Laboratory evaluation of the effect of *Beauveria bassiana* on the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Journal of Invertebrate Pathology*, v. 148, p. 102–109, 2017. DOI: 10.1016/j.jip.2017.06.006.

ULLAH, M. I. *et al.* Effects of entomopathogenic fungi on the biology of *Spodoptera litura* (Lepidoptera: Noctuidae) and its reduviid predator, *Rhynocoris marginatus* (Heteroptera: Reduviidae). *International Journal of Insect Science*, v. 11, 2019. DOI: 10.1177/1179543319867116.

VAN LENTEREN, J. C. The state of commercial augmentative biological control. *BioControl*, v. 57, p. 1–20, 2012.

VAN RIJN, P. C. J.; VAN HOUTEN, Y. M.; SABELIS, M. W. How plants benefit from providing food to predators. *Ecology*, v. 83, n. 10, p. 2664–2679, 2002.

VERGEL, S. J. N. *et al*. Laboratory and greenhouse evaluation of the entomopathogenic fungi and garlic–pepper extract on the predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* and their effect on the spider mite *Tetranychus urticae*. *Biological Control*, [S. l.], v. 57, n. 2, p. 143–149, 2011.

VEY, A.; FARGUES, J. Histological and ultrastructural studies of *Beauveria bassiana* infection in *Leptinotarsa decemlineta* larvae during ecdysis. *Journal of Invertebrate Pathology*, v. 30, p. 207–215, 1977. DOI: 10.1016/0022-2011(77)90221-X.

WEI, Xiaoying; LIU, Jianfeng; ZHANG, Zhi-Qiang. Predation stress experienced as immature mites extends their lifespan. *Experimental and Applied Acarology*, [S. l.], v. 88, p. 143–154, 2022. DOI: 10.1007/s10493-022-00745-4.

WEKESA, V.; KNAPP, M.; MANIANIA, N.; BOGA, H. Effects of *Beauveria bassiana* and *Metarhizium anisopliae* on mortality, fecundity and egg fertility of *Tetranychus evansi*. *Journal of Applied Entomology*, v. 130, p. 155–159, 2006. DOI: 10.1111/j.1439-0418.2006.01043.x.

WEKESA, V. W. *et al.* Interactions of two natural enemies of *Tetranychus evansi*, the fungal pathogen *Neozygites floridana* (Zygomycetes: Entomophthorales) and the predatory mite, *Phytoseiulus longipes* (Acari: Phytoseiidae). *Biological Control*, v. 41, n. 3, p. 408–414, 2007.

WRAIGHT, S. P. Synergism between insect pathogens and entomophagous insects. In: KOUL, O.; DHALIWAL, G. S. (Ed.). *Advances in biopesticides research*. London: Taylor & Francis, 2003. p. 139–161.

WU, S. et al. Feeding on *Beauveria bassiana*-treated prey causes negative effects on the predatory mite *Neoseiulus barkeri*. *Scientific Reports*, v. 5, p. 12033, 2015.

WU, S.; XIE, H.; LI, M. *et al.* Highly virulent *Beauveria bassiana* strains against the two-spotted spider mite, *Tetranychus urticae*, show no pathogenicity against five phytoseiid mite species. *Experimental and Applied Acarology*, v. 70, p. 421–435, (2016a). DOI: 10.1007/s10493-016-0090-x.

WU, S. Y.; GAO, Y. L.; SMAGGHE, G.; XU, X. N.; LEI, Z. R. Interactions between the entomopathogenic fungus *Beauveria bassiana* and the predatory mite *Neoseiulus barkeri* and biological control of their shared prey/host *Frankliniella occidentalis*. *Biological Control*, v. 98, p. 43–51, (2016b).

YANG, Y.; ZHANG, K.; ZHANG, Z. Q. Predatory mites as potential biocontrol agents in avocado. *Journal of Economic Entomology*, v. 118, n. 3, p. 1335–1343, 2025.

ZHANG, Y.-X. *et al.* A novel use of predatory mites for dissemination of fungal pathogen for insect biocontrol: the case of *Amblyseius swirskii* and *Neoseiulus* *cucumeris* (Phytoseiidae) as vectors of *Beauveria bassiana* against *Diaphorina citri* (Psyllidae). *Systematic and Applied Acarology*, v. 20, n. 2, p. 177–177, 2015.

ZHANG, Xingrui *et al.* Feeding on *Beauveria bassiana*-treated *Frankliniella occidentalis* causes negative effects on the predatory mite *Stratiolaelaps scimitus*. *Journal of Economic Entomology*, [S. l.], v. 118, n. 5, p. 2209–2217, 2025. DOI: 10.1093/jee/toaf208.

Chapter 2

Pathogenicity of *Beauveria bassiana* and *Cordyceps javanica* for *Bemisia tabaci* and *Tetranychus urticae*

Abstract

Entomopathogenic fungi are widely used in biological control programs targeting sap-feeding pests; however, their performance against co-occurring pest species exposed to the same applications remains less explored. In several agricultural systems, including protected horticulture and open-field crops, the whitefly *Bemisia tabaci* and the two-spotted spider mite *Tetranychus urticae* frequently co-occur and are often managed simultaneously using biological control agents. Under these conditions, fungal applications targeting whiteflies may also expose spider mite populations, making it essential to evaluate their effects beyond the primary target pest. This study evaluated the pathogenicity and effects of commercial isolates of *Beauveria bassiana* and *Cordyceps javanica* on the whitefly *Bemisia tabaci* and the two-spotted spider mite *Tetranychus urticae*, two economically important pests. Laboratory bioassays were conducted using the field-recommended concentration for *B. tabaci* control. Whitefly nymphs were exposed through immersion of infested host leaves, whereas *T. urticae* adults and larvae were directly immersed in fungal suspensions. Mortality, sporulation, and confirmation of fungal infection were evaluated for *B. tabaci*. For *T. urticae*, survival, development from larva to adult, oviposition, and visible signs of fungal infection were assessed under controlled conditions. Both fungi caused significantly higher mortality of *B. tabaci* nymphs compared with the control, and pathogenicity was confirmed by sporulation and re-isolation of the pathogens. Exposure to both fungi reduced survival of *T. urticae* adults, while juvenile mortality was significantly increased by *B. bassiana*. Developmental time was not affected by fungal treatments. Oviposition of *T. urticae* females was significantly reduced by both fungal species. These results indicate that entomopathogenic fungi applied at concentrations recommended for whitefly management can also affect co-occurring *T. urticae* populations, highlighting their potential role within integrated pest management strategies targeting multitrophic pest complexes.

Keywords: biological control; entomopathogenic fungi; sap-feeding pests; spider mite

Introduction

Global food production faces increasing pressure from agricultural pests, whose population dynamics and damage potential are being altered by land-use intensification and ongoing climate change (Leblanc et al., 2015; Deutsch et al., 2018; Ma et al., 2025). These changes have contributed to increased incidence and severity of phytophagous arthropods in cropping systems, including sap-sucking insects, leaf-chewing Lepidoptera, and polyphagous mite species (Bottrell & Schoenly, 2018; Kaushik et al., 2023; Mahanta et al., 2023). The resulting crop losses generate substantial economic costs for growers and threaten food supply stability (Mateos Fernández et al., 2021; Irsad et al., 2023).

Historically, growers have relied on synthetic pesticides as the primary strategy for pest suppression. However, their frequent and sometimes inappropriate application has raised concerns regarding human health, environmental contamination, and adverse effects on non-target organisms (dos Santos et al., 2018; Torres & Bueno, 2018; Lopes-Ferreira et al., 2022; Zhou et al., 2024). Among the non-target groups most affected are pollinators, which suffer exposure through direct contact or contaminated resources, compromising essential ecosystem services (dos Santos et al., 2018; Nath et al., 2022; Gebhardt et al., 2025). Furthermore, repeated pesticide applications imply strong selective pressure on pest populations, favoring resistance evolution and demaging the long-term effectiveness of chemical control (Sparks & Nauen, 2015; Galli et al., 2024). These limitations highlight the need for sustainable alternatives, such as biological control, within Integrated Pest Management (IPM) programs.

Agricultural pests such as whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) and the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae) represent major phytosanitary challenges in productive agroecosystems worldwide (Aslan et al., 2004). In the case of whiteflies, resistance to dozens of active ingredients has already been reported worldwide (Sparks & Nauen, 2015; APRD, 2025). In Brazil, the introduction and rapid spread of *Bemisia tabaci* biotype B have resulted in significant challenges to chemical control, with recurrent reports of low insecticide efficacy (Lourenção et al., 2015). In parallel, populations of the two-spotted spider mite have shown reduced sensitivity to pesticides (Van Leeuwen et al., 2015), and the species has been reported to rank first among those that develop resistance most rapidly worldwide (APRD, 2025). Both species are well adapted to tropical and subtropical conditions, as well as to greenhouse cultivation (Aslan et al., 2004; Stansly & Castillo, 2009), and can coexist in a wide range of crops. Consequently, management strategies targeting one of these species, such as spray applications to control *B. tabaci*, often expose *T. urticae* to the same inputs used in the field.

In this context, biological control emerges as a promising and widely recognized alternative to chemical control (Van Lenteren, 2012; Brodeur et al., 2018; Lahlali et al., 2022). Among its tools, entomopathogenic fungi are notable as a particularly promising option (Shehzad et al., 2025; Irsad et al., 2023). Unlike conventional chemical insecticides, these microorganisms act through direct contact, infecting hosts by penetrating the cuticle (Ortiz-Urquiza & Keyhani, 2013; Shehzad et al., 2025). Well-established species such as *Beauveria bassiana* and *Cordyceps javanica* are widely used due to their broad spectrum of action, commercial availability, and infection mechanisms that minimize the risk of cross-resistance development (Dubovskiy et al., 2013; Mascarin et al., 2018; Boaventura et al., 2021; Wu et al., 2021; Gutiérrez et al., 2024; Oliveira et al., 2025).

However, to demonstrate the efficacy of fungi commonly used in biological control strategies, it is necessary to conduct bioassays to confirm their pathogenicity (Sain et al., 2019; Landa et al., 1994; Al-Deghairi, 2008; Zhang et al., 2016). Pathogenicity bioassays that quantify pest mortality and verify infection signs and fungal sporulation ensure confirmed mortality attributable to the applied pathogen, a fundamental principle in insect pathology that establishes the tested microorganism as the causal agent of death rather than abiotic factors or other pathogens (Goettel & Inglis, 1997; FAO, 2017). Furthermore, by monitoring the progression of fungal infection, commonly expressed as mortality rates or the proportion of individuals exhibiting sporulation, these assessments can also provide insights into the potential performance of fungal biological control agents under field conditions (De Faria & Wraight, 2007; Lacey et al., 2015). Although these evaluations are commonly conducted during product development and registration, this chapter investigates how biological differences between *Bemisia tabaci* and *Tetranychus urticae*, as well as the fungal application method used, shape infection outcomes under controlled conditions.

Thus, this chapter aims to evaluate the pathogenicity and viability of commercial strains of *Beauveria bassiana* and *Cordyceps javanica* against *Bemisia* *tabaci* and *Tetranychus urticae*, two economically important pests that frequently coexist within the same agroecosystems, such as protected horticultural crops, and are therefore simultaneously exposed to the same chemical and biological management strategies. Under such conditions, control measures targeting one pest species may inadvertently affect co-occurring pests, highlighting the need to assess fungal performance across multiple targets within a shared system. By investigating these parameters using conidial concentrations commonly applied in field management of *B. tabaci*, this chapter aims to assess the extent to which existing biological control strategies can be extrapolated to co-occurring pest species, thereby contributing to more robust and ecologically realistic integrated pest management approaches. To achieve this goal, the following specific objectives were established:

1. To quantify the mortality of *B. tabaci* nymphs and the manifestation of fungal infection signs after exposure to suspensions of *Beauveria bassiana* and *Cordyceps javanica* at the concentration recommended for field application, using the host leaf immersion method.
2. To evaluate the impact of fungal treatments applied by immersion of *T. urticae* individuals in suspensions of *Beauveria bassiana* and *Cordyceps javanica* at the concentration recommended for field application in systems infested by *B. tabaci*, on survival, development from larva to adult, and oviposition capacity.
3. To confirm the causal relationship between mortality and application of the fungal agent by re-isolating the fungi from cadavers on culture plates and by observing characteristic sporulation indicative of pathogenicity in individuals subjected to the treatments.

Investigating these parameters using conidial concentrations commonly applied in field management of *B. tabaci* may provide a basis for using these agents in pest control and contribute to the development of integrated pest management strategies.

Materials and Methods

Obtaining *Bemisia tabaci*

Individuals of *B. tabaci* were obtained by exposing uninfested kale plants in an open experimental area of the Acarology Laboratory at the Federal University of Viçosa that was previously colonized by the pest. Clean plants were placed in this area for four hours, a period sufficient to allow naturally occurring adult whiteflies to oviposit, the plants were immediately transferred to the laboratory. Adult insects were then removed, and the plants were maintained in a climate-controlled room (25 ± 2 °C; 70 ± 10% RH; 12 h photophase). The infested leaves were subsequently used as a source of nymphs for the mortality experiments and the recording of fungal infection symptoms.

Rearing of *Tetranychus urticae*

The stock population of the two-spotted spider mite, *Tetranychus urticae*, maintained at the Acarology Laboratory of the Federal University of Viçosa, was reared in a greenhouse on jack bean plants (*Canavalia ensiformis*). Plants were cultivated in plastic pots (3 L) containing commercial substrate (Bioplant®, Bioplant Misturadora Agrícola Ltda., Minas Gerais, Brazil). Seeds were sown weekly in ten pots, with five seeds per pot, and plants were irrigated twice daily with 250 mL of water to ensure the continuous replacement of host plants and the maintenance of the stock population, as plants unsuitable for sustaining mite rearing were regularly replaced. For experimental infestation, clean jack bean plants were grown under the same greenhouse conditions and subsequently colonized with *T. urticae* by placing leaves obtained from infested plants belonging to the stock population. They were then transferred to the climate-controlled room (25 ± 2 °C; 70 ± 10% RH; 11L:13D).

Preparation of fungal solutions

The conidial suspensions used in the experiments were prepared from commercial wettable powder formulations of *Beauveria bassiana* (isolate IBCB 66, commercial product GRANADA®, Lallemand Soluções Agrobiológicas Ltda., containing 1×10^{10} CFU g^{-1} of product) and *Cordyceps javanica* (isolate BRM 27666, commercial product LALGUARD JAVA®, Lallemand Soluções Agrobiológicas Ltda., containing 1 × 10^{10} viable conidia g^{-1}). The concentrations tested corresponded to the field-recommended application rates for the control of *Bemisia tabaci* and were selected to reflect application levels commonly used in pest management programs. For *Beauveria bassiana*, 0.0375 g of the commercial product was added to 100 mL of distilled water containing 0.01% (v/v) Tween 80®, resulting in a suspension with an approximate concentration of 5.75 × 10^7 conidia/mL. For *Cordyceps javanica*, 0.1 g of the formulation was mixed with the same volume and composition of solution, yielding approximately 8.5 × 10^8 conidia/mL. Both suspensions were mixed with a magnetic stirrer for 30 min prior to use to ensure homogenization of the suspension. A solution containing only distilled water and 0.01% Tween 80® was used as the control. Once prepared, 3 mL of each solution was transferred to a Petri dish for topical application. For the immersion of leaves infested with *B. tabaci*, 300 mL of the solution described above was prepared.

Exposure of *Bemisia tabaci* to fungal suspensions and biological evaluation

To evaluate the effects of *B. bassiana* and *C. javanica* on *B. tabaci*, five plants were assigned to each of the three treatments, and two leaves per plant were selected for application of the fungal suspensions. Prior to treatment, the number of *B. tabaci* individuals on each selected leaf was recorded. Application consisted of immersing each infested leaf, while still attached to the plant, for 30 s in one of the following solutions: (1) *Beauveria bassiana* suspension, (2) *Cordyceps javanica* suspension, or (3) control solution containing 0.01% Tween 80®. After treatment, excess solution could naturally runoff without rinsing, maintaining plant integrity and ensuring uniform inoculum deposition. The fungal application protocol was adapted from Garrido-Jurado et al. (2017), with adjustments to allow the experimental evaluation to be conducted over the required time period. Plants were placed in individual cages according to treatment and maintained in an area with natural light, protected from rainfall, with temperatures ranging from 14 to 30 °C. Relative humidity was not controlled or continuously monitored, as the cages were kept under semi-natural conditions.

Evaluations were conducted daily for 10 days using a stereomicroscope in the laboratory. For each assessment, plants were taken to the laboratory and leaves were examined individually without being detached from the plant. For each treated leaf, the following were recorded: nymphal mortality, the presence of deformities, and the presence of typical signs of fungal infection, such as changes in coloration, body desiccation, external mycelial growth, or sporulation on cadavers. Individuals were surface-disinfected by sequential immersion for 30 s in 70% ethanol and 1% sodium hypochlorite, with a brief rinse in distilled water between these steps to remove residual ethanol, followed by a final rinse in distilled water for 30 s. Subsequently, individuals were placed on filter paper to dry and then transferred to a humid chamber maintained in the dark at 25 °C in a climate-controlled chamber to favor pathogen development. After the incubation period, individuals showing evident fungal outgrowth were aseptically transferred to Petri dishes containing potato dextrose agar, with separate plates prepared for *Cordyceps* sp. and *Beauveria* sp. Culture plates with isolates were maintained in a climate-controlled chamber, conditions described above. After two weeks of growth, fungal structures were removed from the culture plates, mounted on microscope slides, and examined under a light microscope to verify the presence of morphological characteristics compatible with the evaluated pathogens.

Exposure of adult *Tetranychus urticae* to fungal suspensions and biological assessment

To evaluate the effects of *Beauveria bassiana* and *Cordyceps javanica* on adult *T. urticae*, twenty individuals per treatment were immersed in 3 mL of one of the suspensions for 30 s, and transferred to Petri dishes lined with filter paper to dry. Resumption of movement was assessed as an indicator of survival. Subsequently, mites were individually incubated in Petri dishes containing a leaf disc (2 cm diameter) obtained from jack bean plants (*Canavalia ensiformis*) placed on moistened cotton wool; the latter served as a moisture source and a barrier against escapes. Leaf discs were replaced every three days to maintain suitable feeding conditions. The plates were maintained under controlled conditions (25 ± 2 °C; 70 ± 10% RH; 12 h photophase). Evaluations were conducted daily for 10 days, recording survival, oviposition, and the occurrence of characteristic signs of fungal infection, such as external mycelial growth. The experimental procedures were adapted from Midthassel et al. (2016).

Development of *Tetranychus urticae* larvae exposed to fungal suspensions

The larval development assay followed the same exposure procedure and the same three suspensions as used for the adults. Twenty newly hatched larvae of *Tetranychus urticae* per suspension were immersed, dried and survival of the immersion was assessed as above. Individuals were then placed in Petri dishes containing a leaf disc (2 cm diameter) obtained from jack bean plants (*Canavalia ensiformis*) placed on moistened cotton wool. Leaf discs were replaced every three days to maintain suitable feeding conditions. positioned on moistened cotton. Survival and developmental stage were assessed daily until individuals either died or reached adulthood.

Confirmation of fungal growth in *Tetranychus urticae*

To confirm fungal growth in treated individuals, *Tetranychus urticae* adults and juveniles exhibiting visible signs of infection were selected. These signs included changes in body coloration, reduced body turgidity, and the presence of external fungal structures, such as initial mycelial growth or visible sporulation on the cuticle. Individuals were surface-disinfected by sequential immersion for 30 s in 70% ethanol and 1% sodium hypochlorite, with a brief rinse in distilled water between these steps to remove residual ethanol, followed by a final rinse in distilled water for 30 s. Subsequently, individuals were placed in a humid chamber maintained in a climate-controlled chamber in the dark at 25 °C to favor pathogen development. After the incubation period, confirmation of fungal growth was performed by direct observation of individuals showing fungal signs under a stereomicroscope.

Statistical analyses

Mortality data of *B. tabaci* (number of dead insects out of total insects per replicate) were analyzed using generalized linear models (GLM) with quasibinomial distribution to account for overdispersion. Treatment effects on overall mortality were assessed using analysis of deviance with F-tests. Pairwise comparisons among treatments were performed using contrasts with Tukey adjustment via the 'emmeans' package. To evaluate sporulation (proportion of mycosed insects among total dead insects) for the fungal treatments only (excluding control, which showed no sporulation), a separate GLM with quasibinomial distribution was fitted. The proportion of confirmed mortality of the total mortality and efficacy corrected with Abbott's formula were also calculated for the fungal treatments.

Oviposition of *Tetranychus urticae* females (number of eggs per female per day) was analyzed using linear mixed-effects models with the ‘nlme’ package. The identity of individual mites was included as a random factor to account for repeated measures, and treatment and day and their interaction were included as fixed factors. Significance of interactions and factors was assessed through model simplification and comparison using likelihood ratio tests. Multiple comparisons among treatments were performed using pairwise contrasts with Tukey adjustment with the ‘emmeans’ package. Survival of adults was analyzed using the Cox proportional hazards model (coxph).

Survival of juveniles of *T. urticae*, as well as development from larva to adult, were also analyzed using Cox proportional hazards. The 'emmeans' package (Lenth, 2023) was used to perform pairwise comparisons among treatments through contrasts. All analyses were done using R (v. 4.5.2).

Results

Mortality of *Bemisia tabaci*

Entomopathogenic fungi caused significant mortality in *B. tabaci*. Generalized linear model analysis with quasibinomial distribution revealed significant differences among treatments (Fig. 1, $F(2,27) = 14.1$; $p < 0.0001$). Pairwise comparisons showed that both *Beauveria bassiana* and *Cordyceps javanica* caused significantly higher mortality than the control, with no significant difference in mortality between the two fungal treatments (Figure 1).

Sporulation analysis, considering only the fungal treatments (control excluded), showed no significant difference in the proportion of mycosed insects between *B. bassiana* and *C. javanica*. (Figure 1).

The proportion of mortality with signs of fungal infection relative to total mortality was 83.2 ± 7.98% for *B. bassiana* and 41.5 ± 16.0% for *C. javanica*. Abbott-corrected efficacy was 46.4% for *B. bassiana* and 39.6% for *C. javanica*. Representative signs of fungal infection in treated *B. tabaci* individuals are shown in Figure 2. Juveniles exposed to *B. bassiana* showed characteristic sporulation of the pathogen (Fig. 2A), whereas individuals treated with *C. javanica* exhibited symptoms consistent with fungal infection, such as color changes and the onset of sporulation (Fig. 2B).

The causal relationship between the observed mortality and fungal action was confirmed by re-isolation of the pathogens from infected cadavers. The growth of colonies with typical morphology of *Beauveria bassiana* and *Cordyceps javanica* on PDA medium (Fig. 3), together with the observation of characteristic conidiophores and conidia under microscopy (Fig. 4), confirmed fungal infection as the cause of mortality.

Survival and oviposition of *Tetranychus urticae* adults

Oviposition of *T. urticae* was significantly affected by the entomopathogenic fungi (linear mixed-effects model: df = 2; $p < 0.0001$). No significant interaction between treatment and day was detected (L.ratio test: $\chi^2 = 1.54$; df = 2; p = 0.46), indicating that the oviposition pattern over time was similar among treatments, although at different overall levels (Figure 5).

Multiple comparisons with Tukey's adjustment revealed significant reductions in oviposition for both fungal treatments compared to the control. Both treatments showed lower oviposition than control ($p < 0.0001$). However, there was no significant difference in oviposition between the two fungal treatments (p = 0.834).

The survival of *T. urticae* adults was significantly affected by the treatments (Cox proportional hazards: likelihood ratio = 20.48; d.f. = 2; $p < 0.0001$). *Beauveria bassiana* caused 8.5 times higher mortality than Control (HR = 8.51, 95% CI: 2.84–25.51, p = 0.0004) and 1.7 times higher mortality than *Cordyceps javanica,* although this latter difference was not statistically significant (HR = 1.75, 95% CI: 0.85–3.60, p = 0.285). *Cordyceps javanica* caused 4.9 times higher mortality than Control (HR = 4.87, 95% CI: 1.34–17.68, p = 0.0160) (Figure 6).

During the experiment, visual signs consistent with fungal infection were observed on *T. urticae* cadavers (Figure 7).

Survival and development of *Tetranychus urticae* juveniles

Juvenile survival of *T. urticae* was significantly affected by the entomopathogenic fungi (Figure 8, Cox proportional hazards: likelihood ratio = 9.77; d.f. = 2; p = 0.008). *Beauveria bassiana* caused 5.0 times higher mortality than the control (HR = 5.03, 95% CI: 1.63–15.52, p = 0.0136) and 1.7 times higher mortality than *Cordyceps javanica*, although this latter difference was not statistically significant (HR = 1.67, 95% CI: 0.71–3.91, p = 0.467). *Cordyceps javanica* caused 3.0 times higher mortality than the control, but this difference was not statistically significant either (HR = 3.00, 95% CI: 0.93–9.68, p = 0.158).

Development from larva to adult was not significantly affected by the treatments (Cox proportional hazards: likelihood ratio = 0.08; d.f. = 2; p = 1.000) (Figure 9).

During the experiment, visual signs consistent with fungal infection were observed on *T. urticae* cadavers (Fig. 10)

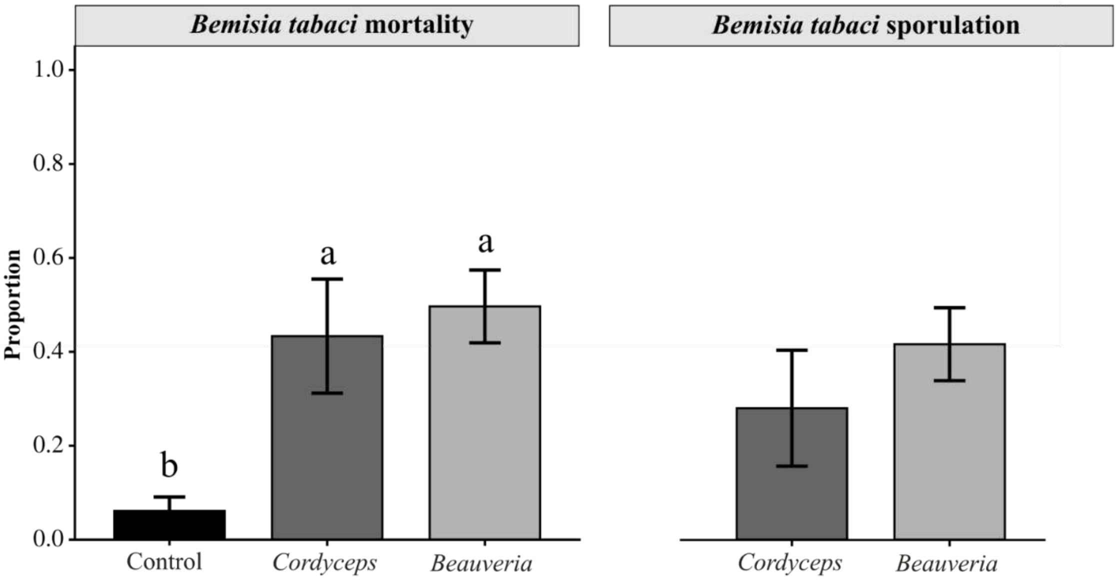

Figure 1. Effects of *Beauveria bassiana* and *Cordyceps javanica* on *Bemisia tabaci* mortality and sporulation. Left: Mortality proportion across all treatments (dead

insects/total insects). Different letters indicate significant differences between treatments. Right: Sporulation proportion for fungal treatments. (mycosed insects/total insects). Sporulation did not differ between fungi (p = 0.846). Bars show mean ± SE.

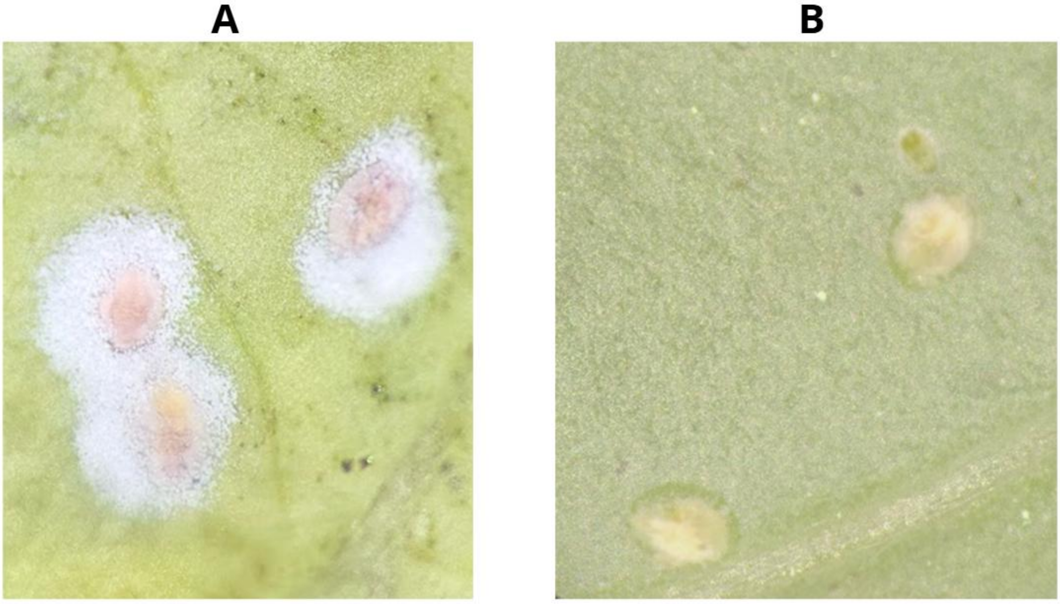

Figure 2. Signs of fungal infection in *B. tabaci* individuals during the experiment. *B. tabaci* juveniles (A) showing sporulation by *B. bassiana* and (B) exhibiting symptoms of infection by *C. javanica*.

Figure 3. Culture plates containing colonies of (A) *Beauveria bassiana* and (B) *Cordyceps javanica* obtained after inoculation of infected *B. tabaci* cadavers on PDA medium.

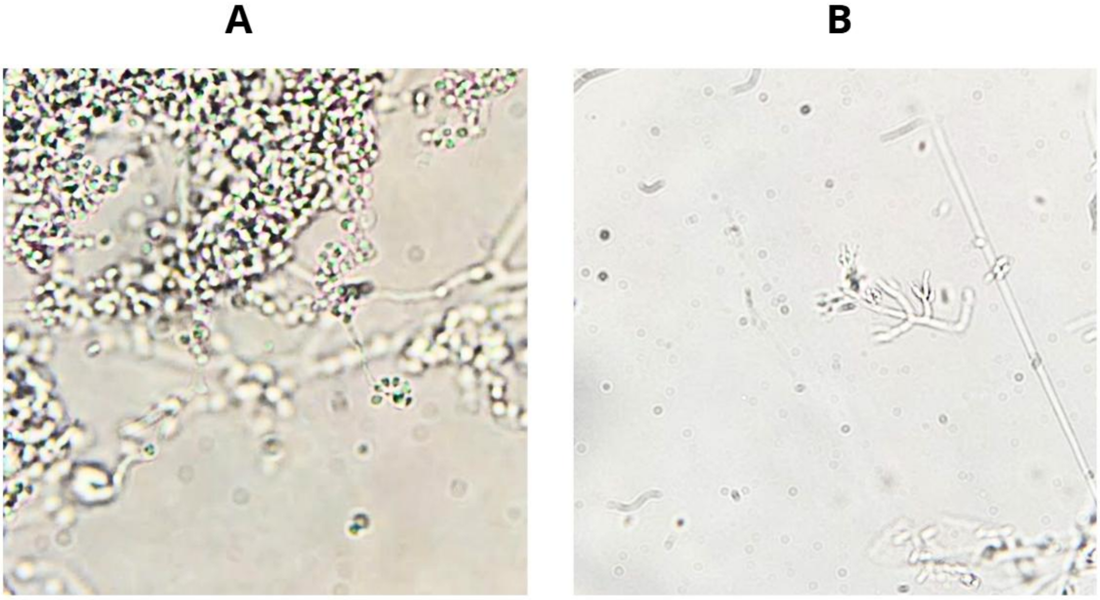

Figure 4. Asexual reproductive structures (conidiophores) and conidia of (A) *Beauveria bassiana* and (B) *Cordyceps javanica*. Images obtained at 400 × magnification under a microscope.

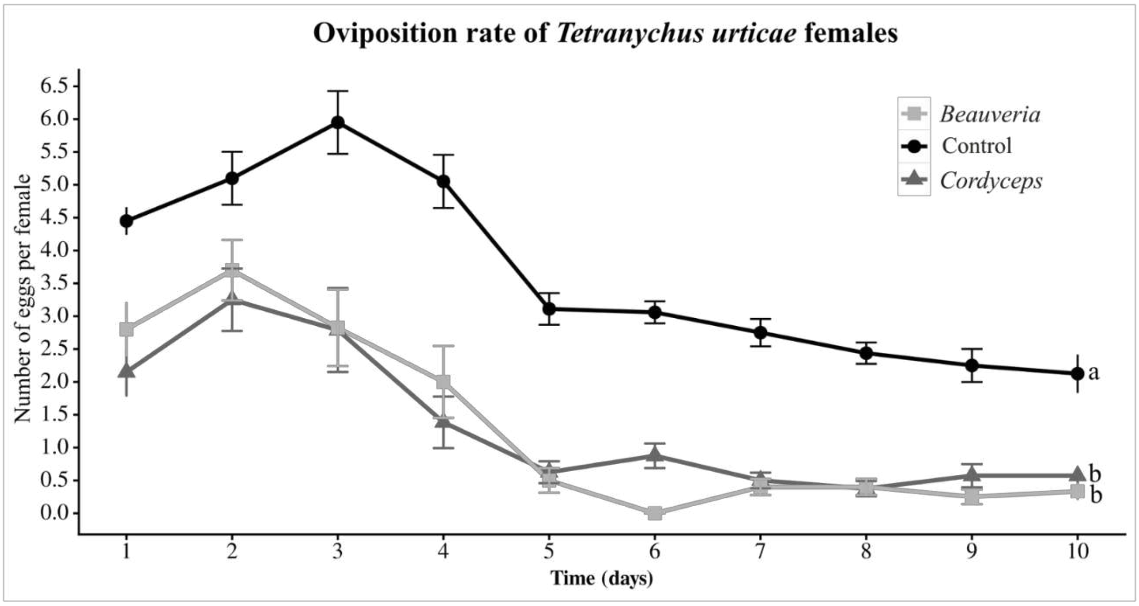

Figure 5. Oviposition of *Tetranychus urticae* exposed to entomopathogenic fungi. Mean number of eggs per female per day (± SE). Different letters indicate significant differences between treatments ($p < 0.0001$)

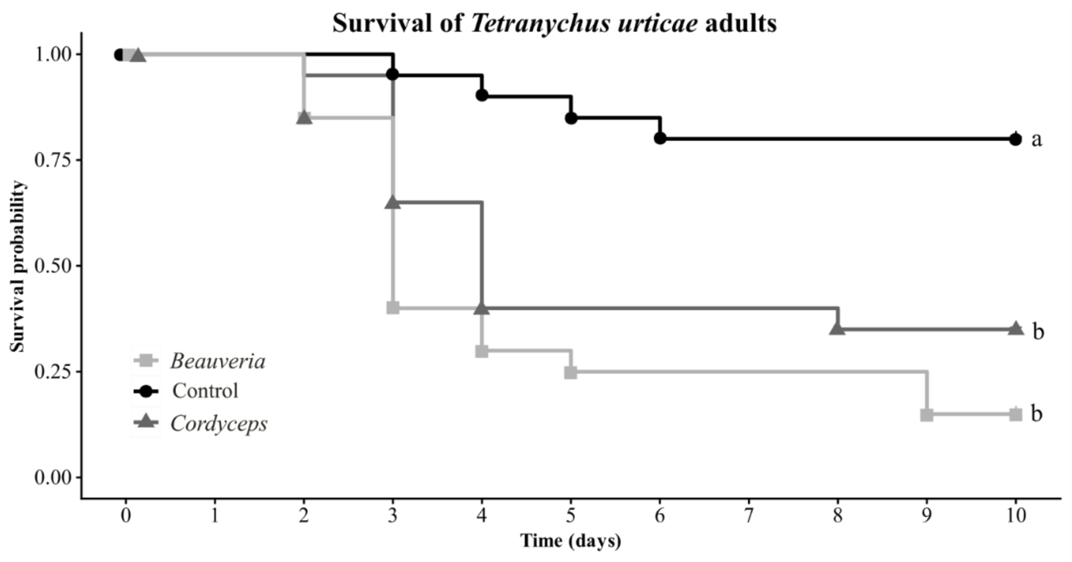

Figure 6. Survival of *Tetranychus urticae* adults exposed to entomopathogenic fungi. Kaplan-Meier curves showing survival probability of mites treated with *Beauveria bassiana, Cordyceps javanica*, or control solution. Different letters indicate significant differences between treatments given by pairwise contrasts after survival analysis ($p < 0.05$).

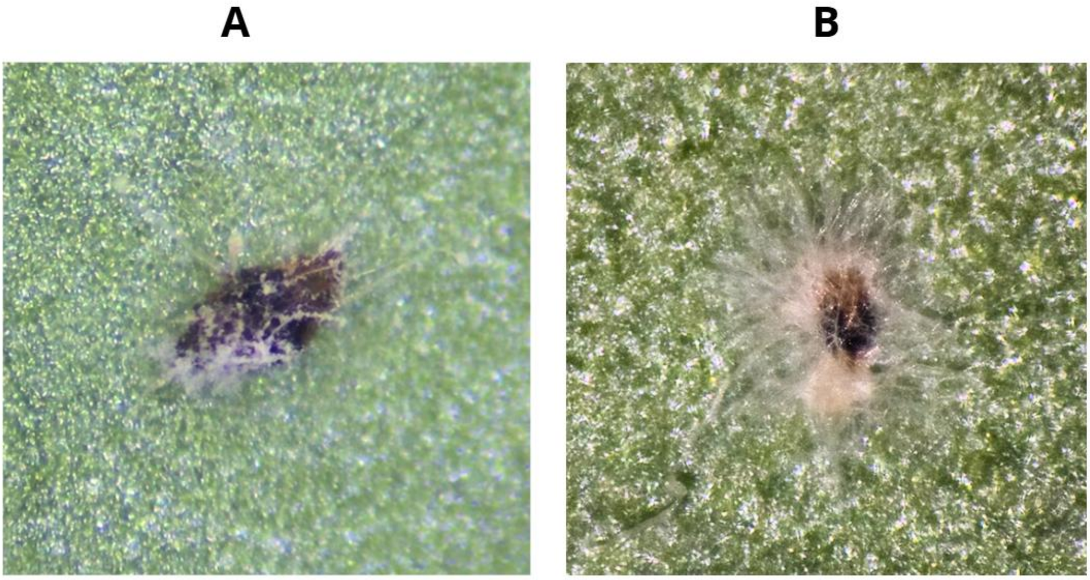

Figure 7. Signs of fungal infection in adult *T. urticae* individuals during the oviposition experiment. Individuals affected by (A) *B. bassiana* and (B) *C. javanica*.

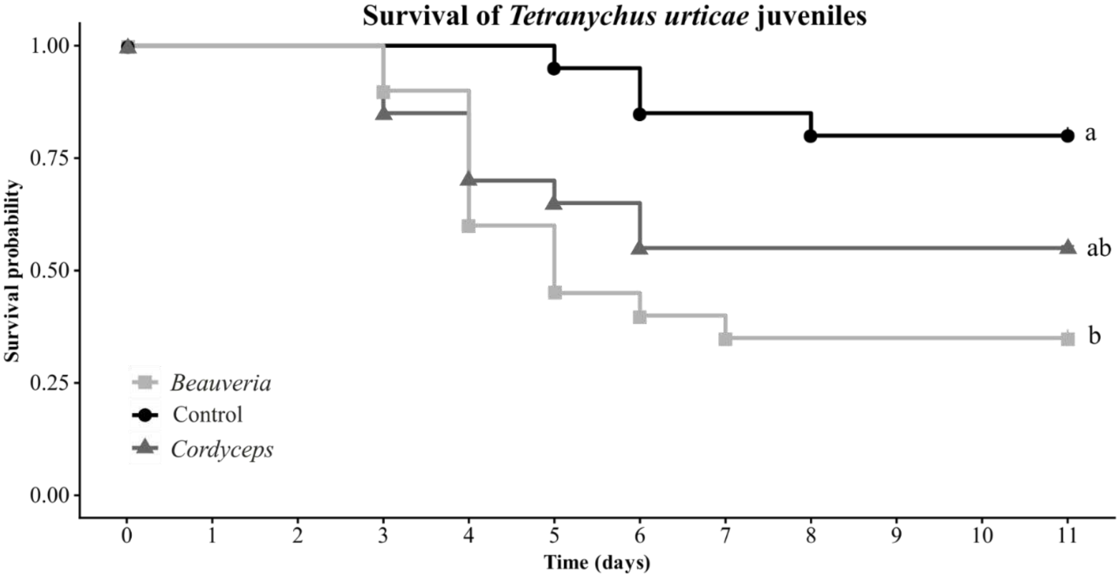

Figure 8. Survival of *Tetranychus urticae* juveniles exposed to entomopathogenic fungi. Kaplan-Meier curves showing survival probability of mites treated with *Beauveria bassiana*, *Cordyceps javanica*, or control solution. Different letters indicate significant differences between treatments given by pairwise contrasts after survival analysis ($p < 0.05$).

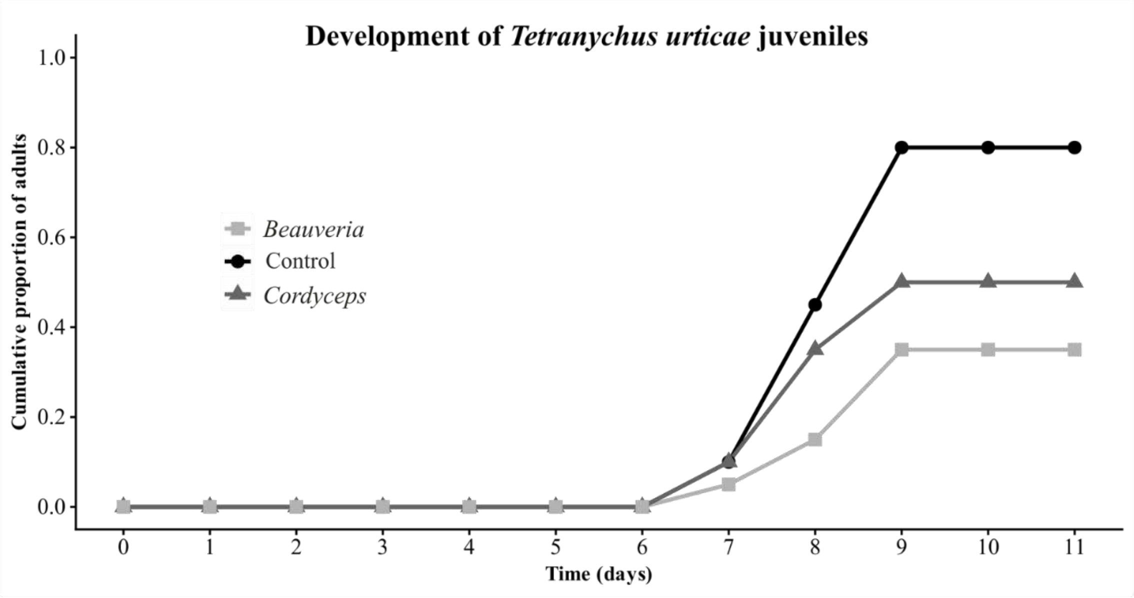

Figure 9. Development of *Tetranychus urticae* exposed to entomopathogenic fungi.

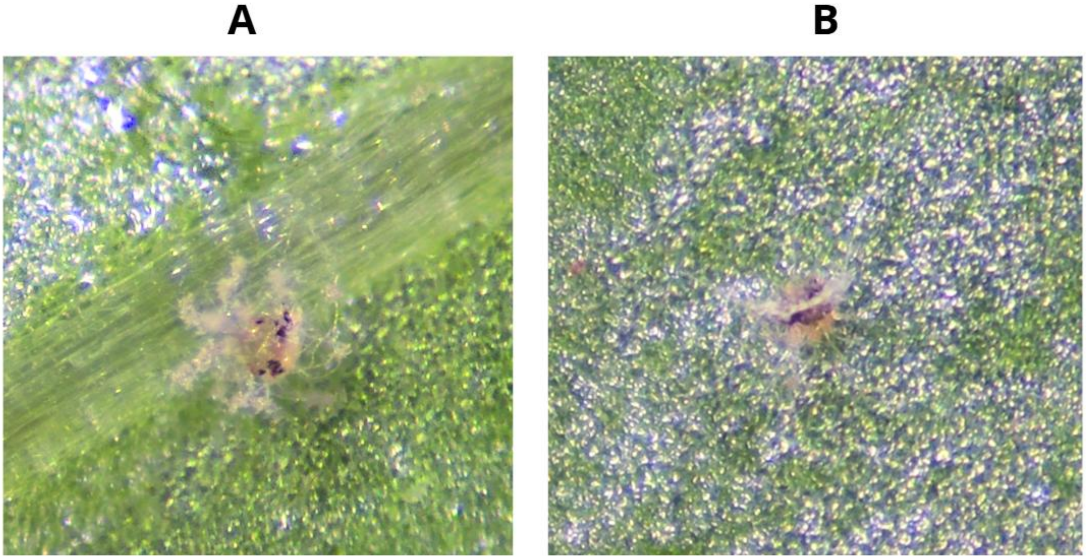

Figure 10. Signs of fungal infection in juvenile *T. urticae* individuals during the developmental experiment. Individuals affected by (A) *B. bassiana* and (B) *C. javanica*.

Discussion

The results obtained in this chapter demonstrate that the entomopathogenic fungi *Beauveria bassiana* and *Cordyceps javanica*, when applied at concentrations recommended for the management of *Bemisia tabaci*, exhibit pathogenicity not only toward the target pest but also toward the two-spotted spider mite *Tetranychus urticae*, a pest commonly co-occurring in cropping systems with whitefly infestations. Overall, *B. bassiana* showed a higher proportion of mycosis in exposed *B. tabaci* nymphs and stronger effects on *T. urticae*, whereas *C. javanica* produced more variable infection expression, though it still caused substantial mortality and showed potential for integrated management. This outcome aligns with *B. bassiana's* reputation as a broad-spectrum, consistently effective agent (Vega et al., 2012; Al-Deghairi, 2008; Mascarin & Jaronski, 2016).

However, the similar corrected efficacy of *C. javanica* (~40%) to that of *B. bassiana* (~46%) indicates that it also possesses considerable virulence against *B. tabaci*. This is consistent with reports of other *Cordyceps* (syn. *Isaria*) species, such as *C. fumosorosea*, showing high efficacy under certain conditions (Corrêa et al., 2020). These findings reinforce that entomopathogenic fungi performance is isolate and context-dependent, and that evaluation should include not only total mortality but also confirmed sporulation, distinguishing between infectious and non-infectious causes of death, as well as sublethal impacts on life history, as recommended in standard protocols for entomopathogen evaluation (Goettel & Inglis, 1997; Hajek & Goettel, 2007; Vega et al., 2012).

The experimental methodologies employed in this study differed between *B. tabaci* and *T. urticae* due to clear differences in their morphology, biology, and behavior. While *B. tabaci* nymphs are sessile organisms adhered to plant tissue, they were exposed to the fungi through immersion of infested host leaves, a method that preserves pathogen contact within their natural substrate and reflects field spraying conditions, in which infection may occur through both direct contact with spray droplets and subsequent contact with treated leaf surfaces (Negasi et al., 1998; Sain et al., 2019). In contrast, the highly mobile mite *T. urticae* was exposed by individual immersion in fungal suspensions, ensuring homogeneous contact with conidia, as commonly applied in bioassays with phytophagous and predatory mites (Midthassel et al., 2016; Zhang et al., 2018). Consequently, exposure differed in both mode and duration, being potentially prolonged for *B. tabaci* due to its continuous presence on treated tissue, and limited to initial exposure for *T. urticae*. These methodological distinctions represent necessary adaptations to ensure realistic exposure and allow comparison of the effects of entomopathogenic fungi across pests with contrasting ecologies, in accordance with guidelines for pathogen bioassays (Goettel & Inglis, 1997).

The mortality of *B. tabaci* nymphs caused by *Beauveria bassiana* and *Cordyceps javanica* corroborates previous studies highlighting the potential of these entomopathogenic fungi for microbial control of this pest (Vega et al., 2012; Al-Deghairi, 2008; Mascarin & Jaronski, 2016). Both fungi differed significantly from the control in terms of mortality, and fungal infection (mycosis) was confirmed in a proportion of exposed individuals, fulfilling the principle of causal confirmation of pathogenicity recommended in insect pathology and in protocols for evaluating entomopathogenic microorganisms (Goettel & Inglis, 1997; Hajek & Goettel, 2007; Vega et al., 2012).

In addition to the direct effects on *Bemisia tabaci*, the results obtained for *Tetranychus urticae* demonstrate that exposure to fungal suspensions prepared at doses recommended for whitefly management can significantly affect non-target pests coexisting within the same agroecosystem. For adult mites, exposure to both *Beauveria bassiana* and *Cordyceps javanica* resulted in significantly reduced survival compared with the control, indicating that fungal applications targeting whiteflies may also exert lethal effects on adult *T. urticae* populations. In juvenile stages, susceptibility differed between fungal agents: *B. bassiana* caused a marked and significant increase in mortality risk, whereas exposure to *C. javanica* resulted in an increasing, though not statistically significant, trend in juvenile mortality.

These patterns are consistent with previous studies demonstrating that entomopathogenic fungi can induce mortality in phytophagous mites (Chandler et al., 2000), particularly when exposure methods promote efficient conidial deposition on the integument, as occurs with topical immersion (Midthassel et al., 2016; Zhang et al., 2018).

The reduction in oviposition observed in both fungal treatments represents a relevant finding, as fecundity is an important determinant of *Tetranychus urticae* population growth, and sublethal effects on oviposition are known to influence population density in subsequent generations (Helle & Sabelis, 1995). Reductions in oviposition may be associated with physiological costs related to infection stress and immune activation, as well as indirect effects of fungal metabolites, which can affect metabolism and feeding efficiency even in individuals that do not succumb rapidly to infection (Schmid-Hempel, 2003; Li & Zhang, 2019). Sublethal effects of this type have been reported in studies involving entomopathogenic fungi and the target pests evaluated here, including altered development in whiteflies (Sain et al., 2021) and reduced fecundity in the two-spotted spider mite (Shi & Feng, 2009; Al-Zahrani et al., 2023; Liu et al., 2019).

From an applied perspective, such effects may contribute to population regulation over time, even when direct mortality is not maximized (Desneux et al., 2007). Accordingly, these results support the inclusion of parameters beyond mortality in the evaluation of microbial control agents, particularly for mites, whose populations are capable of rapid recovery following population declines (Mitchell, 1973). Changes in oviposition rate, such as those observed in this study, are commonly incorporated into estimates of the intrinsic rate of increase (rm), which is used to interpret population dynamics and inform pest management strategies (Janssen & Sabelis, 1992).

Taken together, the data from this chapter support the conclusion that applications of entomopathogenic fungi to control *B. tabaci* may have a collateral effect on *T. urticae*. This overlap is particularly relevant in intensive production systems and protected crops, where both pests are recurrent and selective pressure from insecticides and acaricides is high, favoring the evolution of resistance (Sparks & Nauen, 2015; Van Leeuwen et al., 2015). Thus, the use of commercial formulations of *B. bassiana* and *C. javanica* may contribute to IPM strategies by reducing reliance on chemical compounds, diversifying modes of action, and possibly reducing selection pressure associated with repeated chemical use, while potentially acting in an integrated manner against multiple pests within the same agroecosystem (Van Lenteren, 2012; Brodeur et al., 2018; Lahlali et al., 2022).

Finally, it is important to consider that the bioassays were conducted under controlled conditions and with a standardized exposure regime (30 s immersion), which ensures comparability among treatments but does not reproduce the full complexity of field conditions. Factors such as microclimate, radiation, canopy cover, application frequency, conidial deposition, and variation among isolates and commercial batches can influence fungal performance and the expression of sporulation on cadavers (De Faria & Wraight, 2007; Lacey et al., 2015; Mascarin et al., 2018). In addition, although this chapter confirmed pathogenicity through re-isolation and observation of fungal signs, future investigations may further elucidate the underlying mechanisms by incorporating dose–response assessments, evaluating different commercial formulations or strains, and conducting analyses across contrasting microclimates, particularly temperature and relative humidity, key factors in infection and sporulation (Devi et al., 2005). Nevertheless, the results obtained provide preliminary evidence that the evaluated commercial strains are viable and capable of significantly affecting both target pests and associated pests, supporting their inclusion as a relevant component in integrated pest management programs.

References

AL-DEGHAIR, M. A. Bioassay evaluation of the entomopathogenic fungus *Beauveria bassiana* Vuillemin against eggs and nymphs of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). *Pakistan Journal of Biological Sciences*, v. 11, n. 12, p. 1551–1560, 2008.

AL-ZAHRANI, Jawaher Khamis *et al.* Entomopathogenic fungi and their biological control of *Tetranychus urticae*: two-spotted spider mites. *Journal of King Saud University – Science*, [S. l.], v. 35, n. 8, art. 102910, 2023. DOI: 10.1016/j.jksus.2023.102910.

APRD – ARTHROPOD PESTICIDE RESISTANCE DATABASE. *Arthropod Pesticide Resistance Database*. East Lansing: Michigan State University, 2025. Available at: https://www.pesticideresistance.org.

ASLAN, İ.; ÖZBEK, H.; ÇALMAŞUR, Ö.; ŞAHİN, F. Toxicity of essential oil vapours to two greenhouse pests, *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. *Industrial Crops and Products*, v. 19, n. 2, p. 167–173, 2004. DOI: 10.1016/j.indcrop.2003.09.003.

BOAVENTURA, H. A. et al. Susceptibility of all nymphal stages of *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) to Brazilian isolates of *Cordyceps* sp. (Hypocreales: Cordycipitaceae). *Neotropical Entomology*, v. 50, p. 100–113, 2021.

BOTTRELL, D. G.; SCHOENLY, K. G. Integrated pest management for resource-limited farmers: challenges for achieving ecological, social and economic sustainability. *The Journal of Agricultural Science*, Cambridge, v. 156, n. 3, p. 408–426, 2018.

BRODEUR, J. et al. Trends in biological control: public interest, international networking and research directions. *BioControl*, v. 63, p. 11–26, 2018. DOI: 10.1007/s10526-017-9850-8.

CHANDLER, D et al. Fungal biocontrol of Acari. *Biocontrol Science and Technology*, v. 10, n. 4, p. 357–384, 2000. DOI: 10.1080/09583150050114972.

CORRÊA, Bianca *et al.* Comparative analysis of blastospore production and virulence of *Beauveria bassiana* and *Cordyceps fumosorosea* against soybean pests. *BioControl*, [S. l.], v. 65, p. 323–337, 2020. DOI: 10.1007/s10526-019-09983-9.

DE FARIA, M. R.; WRAIGHT, S. P. Mycoinsecticides and mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control*, v. 43, n. 3, p. 237–256, 2007.

DESNEUX, N.; DECOURTYE, A.; DELPUECH, J.-M. The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*, v. 52, n. 1, p. 81–106, 2007.

DEUTSCH, C. A. et al. Increase in crop losses to insect pests in a warming climate. *Science*, v. 361, n. 6405, p. 916–919, 2018. DOI: 10.1126/science.aat3466.

DEVI, K. U. et al. Effect of high temperature and water stress on *in vitro* germination and growth in isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin. *Journal of Invertebrate Pathology*, [S. l.], v. 88, n. 3, p. 181–189, 2005.

DOS SANTOS, C. F.; OTESBELGUE, A.; BLOCHTEIN, B. The dilemma of agricultural pollination in Brazil: beekeeping growth and insecticide use. *PLoS ONE*, v. 13, n. 7, e0200286, 2018. DOI: 10.1371/journal.pone.0200286.

DUBOVSKIY, I. M. et al. Can insects develop resistance to insect pathogenic fungi? *PLoS ONE*, v. 8, n. 4, e60248, 2013. DOI: 10.1371/journal.pone.0060248.

FAO – FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. *Technical guidelines for the registration of microbial pest control agents (MPCAs) and semiochemicals*. Roma: FAO, 2017.

GALLI, M.; FELDMANN, F.; VOGLER, U. K.; *et al.* Can biocontrol be the game-changer in integrated pest management? A review of definitions, methods and strategies. *Journal of Plant Diseases and Protection*, v. 131, p. 265–291, 2024. DOI: 10.1007/s41348-024-00878-1.

GARRIDO-JURADO, I.; RESQUÍN-ROMERO, G.; AMARILLA, S. P. *et al.*. Transient endophytic colonization of melon plants by entomopathogenic fungi after foliar application for the control of *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). *Journal of Pest Science*, Heidelberg, v. 90, p. 319–330, 2017. DOI: 10.1007/s10340-016-0767-2.

GEBHARDT, S. et al. Understanding interactive effects between habitat configuration and pesticide use for pollination. *Ecological Processes*, v. 14, n. 1, 2025.

GOETTEL, M. S.; INGLIS, G. D. Fungi: Hyphomycetes. In: LACEY, L. A. (ed.). *Manual of techniques in insect pathology*. London: Academic Press, 1997. p. 213–249.

GUTIÉRREZ, Y. et al. Isolation and characterization of a native strain of *Beauveria bassiana* for control of *Dynamis borassi*. *World Journal of Microbiology and Biotechnology*, v. 40, n. 9, 2024.

HAJEK, Ann E.; GOETTEL, Mark S. Guidelines for evaluating effects of entomopathogens on non-target organisms. In: LACEY, Lawrence A.; KAYA, Harry K. (ed.). *Field manual of techniques in invertebrate pathology*. 2. ed. Dordrecht: Springer, 2007. p. 816–833.

HELLE, H.; SABELIS, M. W. *Spider mites: their biology, natural enemies and control*. Amsterdam: Elsevier, 1985. v. 1A.

IRSAD et al. Entomopathogen-based biopesticides: insights into unraveling their potential in insect pest management. *Frontiers in Microbiology*, v. 14, p. 1208237, 2023.

JANSSEN, A.; SABELIS, M. W. Phytoseiid life-histories, local predator–prey dynamics, and strategies for control of tetranychid mites. *Experimental and Applied Acarology*, v. 14, p. 233–250, 1992. DOI: 10.1007/BF01200566.

JARONSKI, S. T. Ecological factors in the inundative use of fungal entomopathogens. *BioControl*, v. 55, n. 1, p. 159–185, 2010. DOI: 10.1007/s10526-009-9248-3.

KAUSHIK, B. et al. Impact of climate change on crop yield due to pests and crop diseases. *Microscopy and Microanalysis*, v. 29, suppl. 1, p. 56–58, 2023.

LACEY, L. A. et al. Insect pathogens as biological control agents: back to the future. *Journal of Invertebrate Pathology*, v. 132, p. 1–41, 2015. DOI: 10.1016/j.jip.2015.07.009.

LAHLALI, R. *et al.* Biological control of plant pathogens. *Microorganisms*, v. 10, p. 596, 2022.

LANDA, Z.; OSBORNE, L.; LOPEZ, F.; EYAL, J. A bioassay for determining pathogenicity of entomogenous fungi on whiteflies. *Biological Control*, v. 4, n. 4, p. 341–350, 1994.

LEBLANC, L. et al. Declines in biodiversity and pest abundance across land-use gradients in Southeast Asia. *Landscape Ecology*, v. 31, n. 3, p. 505–516, 2015.

LI, Guang-Yun; ZHANG, Zhi-Qiang. Development, lifespan and reproduction of spider mites exposed to predator-induced stress across generations. *Experimental and Applied Acarology*, [S. l.], v. 79, p. 335–348, 2019. DOI: 10.1007/s10493-019-00431-y.

LIU, Jian-Feng *et al.* Influence of pathogenic fungi on the life history and predation rate of mites attacking a psyllid pest. *Ecotoxicology and Environmental Safety*, [S. l.], v. 183, art. 109585, 2019. DOI: 10.1016/j.ecoenv.2019.109585.

LOPES-FERREIRA, M. et al. Impact of pesticides on human health in Brazil. *International Journal of Environmental Research and Public Health*, v. 19, n. 6, p. 3198, 2022.

LOURENÇÃO, A. L.; SAKATE, R. K.; VALLE, G. E. Bemisia tabaci biótipo B. In: VILELA, E.; ZUCCHI, R. A. (org.). *Pragas introduzidas no Brasil: insetos e ácaros*. Piracicaba: FEALQ, 2015. p. 682–707.

MA, C.-S. et al. Crop pest responses to global changes in climate and land management. *Nature Reviews Earth & Environment*, v. 6, n. 4, p. 264–283, 2025.

MAHANTA, D. K. *et al.*. Understanding anthropogenic climate change, its consequences on insect pests, and techniques in forecasting and monitoring pest dynamics: a contemporary scenario. *Climate change and insect biodiversity*. Boca Raton: CRC Press, 2023. p. 44–67.

MASCARIN, G. M.; JARONSKI, S. T. Production and uses of *Beauveria bassiana*. *World Journal of Microbiology and Biotechnology*, v. 32, p. 177, 2016.

MASCARIN, G. M. et al. Phenotype responses to abiotic stresses and virulence among isolates of *Cordyceps javanica*. *Microbiological Research*, v. 216, p. 12–22, 2018.

MATEOS FERNÁNDEZ, R. et al. Insect pest management in the age of synthetic biology. *Plant Biotechnology Journal*, v. 20, n. 1, 2021.

MIDTHASSEL, A.; LEATHER, S. R.; WRIGHT, D. J. Compatibility of *Amblyseius swirskii* with *Beauveria bassiana*: two potentially complementary biocontrol agents. *BioControl*, v. 61, p. 437–447, 2016. DOI: 10.1007/s10526-016-9718-3.

MORA, Margy Alejandra Esparza; CASTILHO, Alzimiro Marcelo Conteiro; FRAGA, Marcelo Elias. Classificação e mecanismo de infecção dos fungos entomopatogênicos. *Arquivos do Instituto Biológico*, Seropédica, v. 84, e0552015, 2017. DOI: 10.1590/1808-1657000552015.

NATH, R.; SINGH, H.; MUKHERJEE, S. Insect pollinator decline in the Anthropocene. *Journal of Apicultural Research*, v. 62, n. 1, p. 1–16, 2022.

NEGASI, A.; PARKER, B. L.; BROWNBRIDGE, M. Screening and bioassay of entomopathogenic fungi for the control of silverleaf whitefly, *Bemisia argentifolii*. *International Journal of Tropical Insect Science*, v. 18, p. 37–44, 1998. DOI: 10.1017/S174275840000744X.

OLIVEIRA, S. C. et al. Physicochemical interactions and viability of *Cordyceps fumosorosea* associated with adjuvants. *Bioscience Journal*, v. 41, e41018, 2025.

ORTIZ-URQUIZA, A.; KEYHANI, N. O. Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects*, v. 4, n. 3, p. 357–374, 2013.

QUESADA-MORAGA, Enrique *et al.* Key role of environmental competence in successful use of entomopathogenic fungi in microbial pest control. *Journal of Pest Science*, [S. l.], v. 97, p. 1–15, 2024. DOI: 10.1007/s10340-023-01622-8.

SAIN, S. K. et al. Comparative effectiveness of bioassay methods for entomopathogenic fungi against *Bemisia tabaci*. *Egyptian Journal of Biological Pest Control*, v. 29, p. 31, 2019.

SAIN, S. K. *et al.* Evaluation of bioefficacy potential of entomopathogenic fungi against the whitefly (*Bemisia tabaci* Genn.) on cotton under polyhouse and field conditions. *Journal of Invertebrate Pathology*, v. 183, p. 107618, 2021.

SCHMID-HEMPEL, P. Variation in immune defence as a question of evolutionary ecology. *Proceedings of the Royal Society B: Biological Sciences*, [S. l.], v. 270, n. 1513, p. 357–366, 2003. DOI: 10.1098/rspb.2002.2265.

SHEHZAD, M.; TARIQ, M.; SIDDIQUI, J. A. Entomopathogenic fungi under climate change. *Acta Tropica*, v. 269, p. 107751, 2025.

SHI, W. B.; FENG, M. G. Effect of fungal infection on reproductive potential and survival time of *Tetranychus urticae* (Acari: Tetranychidae). *Experimental and Applied Acarology*, v. 48, n. 3, p. 229–237, 2009. DOI: 10.1007/s10493-009-9238-2

SPARKS, T. C.; NAUEN, R. IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, v. 121, p. 122–128, 2015.

STANSLY, P. A.; CASTILLO, J. A. Control of mites and whiteflies using predaceous mites. *Proceedings of the Florida State Horticultural Society*, v. 122, p. 253–257, 2009.

VAN LENTEREN, J. C. Commercial augmentative biological control. *BioControl*, v. 57, p. 1–20, 2012.

VAN LEEUWEN, T. et al. Economic importance of acaricides. *Pesticide Biochemistry and Physiology*, v. 121, p. 12–21, 2015.

VEGA, Fernando E. *et al.* Fungal entomopathogens. In: VEGA, Fernando E.; KAYA, Harry K. (ed.). *Insect pathology*. 2. ed. Amsterdam: Academic Press, 2012. p. 171–220. DOI: 10.1016/B978-0-12-384984-7.00006-3.

WU, S. et al. Environmental tolerance of *Cordyceps javanica*. *Insects*, v. 11, n. 10, p. 711, 2020.

ZHANG, X. et al. Laboratory and field evaluation of *Isaria cateniannulata* against *Tetranychus urticae*. *Pest Management Science*, v. 72, n. 5, p. 1059–1066, 2016.

ZHANG, X. *et al.* Pathogenic differences of the entomopathogenic fungus *Isaria cateniannulata* to the spider mite *Tetranychus urticae* (Trombidiformes: Tetranychidae) and its predator *Euseius nicholsi* (Mesostigmata: Phytoseiidae). *Experimental and Applied Acarology,* [S. l.], v. 75, p. 69–84, 2018.

ZHOU, W.; LI, M.; ACHAL, V. Environmental and human health impacts of chemical pesticides. *Emerging Contaminants*, v. 11, n. 1, p. 100410, 2024.

General Conclusion

The results demonstrated that both isolates, *Beauveria bassiana* IBCB 66 and *Cordyceps javanica* BRM 27666, at concentrations recommended for controlling *Bemisia tabaci*, showed a high level of compatibility with the predatory mites *Amblyseius herbicolus* and *Amblyseius tamatavensis* under controlled laboratory conditions. Predator survival was not affected by exposure via immersion in fungal suspensions, and only a developmental delay was observed in *A. herbicolus* exposed to *C. javanica* BRM 27666, without any negative impact on oviposition. These results suggest that the combined use of these fungal isolates and predatory mites may be compatible within integrated biological control programs for whiteflies, although further studies under field or semi-field conditions are necessary to confirm these interactions.

In addition, the pathogenicity of both isolates against *Bemisia tabaci* was confirmed. Beyond the target species, exposure to both isolates at concentrations recommended for whitefly control was associated with detrimental effects on *Tetranychus urticae*, indicating that these applications may also contribute to suppressing non-target pest populations, particularly in treatments involving *B. bassiana*.

Thus, the results indicate that these entomopathogenic fungi can contribute to the suppression of *B. tabaci* populations, are associated with effects on *T. urticae*, and did not cause pronounced or detrimental effects on key life-history traits of the predatory mites *A. herbicolus* and *A. tamatavensis,* under the experimental conditions evaluated. The combination of lethal and sublethal effects observed, together with the limited impact on predators under controlled conditions, suggests that the joint use of the predators and fungi evaluated may be considered within integrated pest management programs, although further studies are required to assess their performance and interactions under field conditions.

Although based on laboratory bioassay conditions, these findings provide preliminary evidence supporting the potential for the combined use of these agents under field conditions. Future field studies are recommended to further validate this integrated strategy, with particular attention to assessing the effectiveness of these agents when applied together for the control of target pests.