



## Research Article

# Efficacy of *Beauveria bassiana* and *Trichoderma viride* against *Bemisia tabaci* (Hemiptera: Aleyrodidae) on tomato plants

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**ABSTRACT:** *Bemisia tabaci* poses a major threat to tomato crops, causing significant yield losses and economic damage to farmers. This study investigates the conidia filtration method as a potential management strategy for *B. tabaci* under greenhouse conditions. Evaluating the efficacy of this approach in controlling the whitefly population while potentially promoting plant growth is crucial for developing sustainable solutions for tomato production. Both *Trichoderma viride* and *Beauveria bassiana* fungi effectively killed adult whiteflies in this study, significantly exceeding the untreated control. *Beauveria bassiana* showed slightly higher mortality rates at all time points, achieving a maximum of 69.47% compared to *T. viride*'s 62.22% after 14 days. *Trichoderma viride* and *Beauveria bassiana* significantly stimulated plant growth compared to the untreated control. However, *T. viride* emerged as the superior growth promoter across all assessed parameters: plant height, fresh weight, and dry weight. It achieved the tallest plants (27.31 cm), exceeding both the control and *B. bassiana*. Similarly, its impact on fresh and dry weight surpassed both the control and *B. bassiana*, reaching respective values of 14.21 and 2.83 g. These findings suggest that *T. viride* holds greater potential as a plant growth promoter under the examined conditions.

**KEYWORDS:** *Bemisia tabaci*, *Beauveria bassiana*, *Trichoderma viride*, *Solanum lycopersicum*

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## INTRODUCTION

Tomato (*Solanum lycopersicum* L., 1753), second only to potato in global vegetable production, boasts a remarkable journey from its South American origin to widespread cultivation (Kumar *et al.*, 2022). Today, it transcends local consumption, serving as a critical export commodity with increasing production and consumption trends (Pan *et al.*, 2023). Beyond its economic importance, tomato holds nutritional value, containing abundant vitamin C and the phytochemical lycopene (Kumar *et al.*, 2022).

Tomato's versatility extends beyond a staple crop, finding diverse culinary applications as raw additions to salads, dehydrated crisps, or foundational ingredients in countless dishes. Its processed forms include ubiquitous staples like canned tomatoes, juice, ketchup, puree, and sun-dried varieties (Kumar *et al.*, 2022; Włodarczyk *et al.*, 2023). Additionally, beyond its culinary importance, the tomato serves as a critical model fruit-bearing crop for essential research, solidifying its significance in both agriculture and scientific exploration (Makhadmeh *et al.*, 2022).

*Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), commonly known as the whitefly, poses a significant threat to tomato production in Iraq. This highly polyphagous pest feeds on over 600 plant species, including tomatoes, and its presence throughout the year due to year-round crop production likely contributes to its adaptation and spread (Haj Hasan *et al.*, 2015). Whiteflies damage plants by piercing and sucking sap from phloem tissue, causing leaves to yellow, dry, or fall. Additionally, their sugary honeydew excretion promotes the growth of sooty mold, hindering plant respiration and photosynthesis, and rendering fruits commercially unviable (Horowitz *et al.*, 2011). Furthermore, *B. tabaci* acts as a vector for multiple plant viruses, exacerbating yield losses for farmers (Kareem *et al.*, 2020). This emphasizes the need for effective control strategies to minimize their impact on tomato production and farmer income (Saady, 2022).

Effective whitefly (*B. tabaci*) management requires strategies that suppress population size while minimizing environmental and human health risks. While traditional approaches rely heavily on insecticides, their negative

impacts have driven the search for sustainable alternatives. Enter biological control, the utilization of natural enemies like predatory organisms to manage pest populations (Matrood *et al.*, 2023; Rhouma *et al.*, 2023).

Numerous studies highlight the promising potential of entomopathogenic fungi for whitefly control. Species like *B. bassiana* (Bals.-Criv.) Vuill. (James *et al.*, 2003), *Metarhizium anisopliae* (Metschn.) Sorokin (Anwar *et al.*, 2019), *Akanthomyces lecanii* (Zimm.) Spatafora, Kepler and B. Shrestha (= *Verticillium lecanii* (Zimmermann) Viégas (Saady, 2022), *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones and Samson (Dong *et al.*, 2016), *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha and Spatafora (= *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. and G. Sm. (Zimmerman, 2008), *Trichoderma* spp. Persoon (Batoool *et al.*, 2020) and even *Mucor* sp. (Anwar *et al.*, 2019) have demonstrated high efficacy against *B. tabaci*. These fungi offer crucial advantages: minimal risk to human health, compatibility with other natural enemies, and the ability to both directly reduce whitefly populations and induce plant resistance (Faria & Wraight, 2001; Vidal *et al.*, 2003). Therefore, integrating entomopathogenic fungi into whitefly management strategies holds immense potential for Iraqi farmers (Matrood *et al.*, 2023). This approach aligns with the principles of sustainability and environmental responsibility, offering a promising solution to protect tomato crops and ensure farmer livelihoods (James *et al.*, 2003; Zimmerman, 2008; Anwar *et al.*, 2019; Batoool *et al.*, 2020).

Entomopathogenic fungi hold significant promise for managing *B. tabaci* populations, with numerous studies exploring their efficacy against various *B. tabaci* life stages in both greenhouse and field settings (James *et al.*, 2003; Zimmerman, 2008; Dong *et al.*, 2016; Anwar *et al.*, 2019; Batoool *et al.*, 2020; Saady, 2022). Popular fungal choices include *T. viride* and *B. bassiana*, known for effectively controlling whitefly populations. Upon spore germination, these fungi rapidly develop hyphae that penetrate the whitefly body, leading to death within a day (Batoool *et al.*, 2020). This study aimed to evaluate the efficacy of *T. viride* and *B. bassiana* as control agents against *B. tabaci* while also assessing their potential to promote plant growth under greenhouse conditions.

## MATERIALS AND METHODS

### *Beauveria tabaci* adults' bioassays using conidia filtration method

This study investigated the efficacy of two entomopathogenic fungi isolated from infected whitefly adults: *T. viride* and *B. bassiana*. For routine use, these fungi were cultured separately on Sabouraud dextrose yeast agar under controlled conditions (25°C ± 1°C, 16-hour photoperiod). After seven days, conidia

were harvested using sterile distilled water supplemented with 0.02% Tween-80 (10 mL per culture). The resulting suspension was filtered through sterile muslin and collected in a sterile container. Standardized conidial suspensions were obtained by shaking for 5 minutes on a horizontal shaker (40 mm horizontal movement, 300 oscillations per minute). The initial concentration was adjusted to 100% (equivalent to 10<sup>9</sup> conidia/mL), followed by dilution with sterile distilled water to achieve the desired concentrations (10<sup>6</sup> conidia/mL). To ensure efficacy, each batch underwent viability testing, and only suspensions with over 95% viable conidia were utilized (Matrood *et al.*, 2023).

*Beauveria tabaci* whiteflies were sourced in February 2022 from tomato leaves grown in Basrah-Al-Zubayr, Iraq. They were subsequently maintained on the same host plant species within a controlled greenhouse environment. For fungal virulence bioassays, *B. tabaci* adults were reared and prepared following the established protocol described by Huang *et al.* (2010).

Tomato seeds were initially sown in nursery seed trays with 20 plants per treatment replicated three times. These seedlings were then transplanted into individual plastic pots and grown in a controlled environment with an artificial climate (8 h/16 h night/day photoperiod at 20-22°C). At the six- to eight-leaf stage (approximately 12-18 cm height), individual plants were selected and transferred to identical cages measuring 100 cm x 100 cm x 100 cm located within a greenhouse. These intact plants, maintained under standardized greenhouse conditions, were then utilized for the subsequent experiment.

Bioassays for *T. viride* and *B. bassiana* followed established protocols from Al-Juburi and Awad (1999), and Huang *et al.* (2010). Each fungal species was separately applied to infested tomato leaves in a concentration of 10<sup>6</sup> conidia/mL using a spray method. At least ten leaves with over 50 adult whiteflies per leaf were included in each treatment. The entire experiment was replicated three times using fresh insect batches and new conidial suspensions. Randomization was ensured by treating groups of *B. tabaci* from the same batch at the same time within each replicate. Treated plants were maintained in a greenhouse under controlled conditions. Following a randomized complete block design, whitefly mortality was recorded in 1, 3, 4, 7, and 14 days after inoculation. Deceased whiteflies were promptly isolated in individual Petri dishes to monitor fungal sporulation on their cadavers. The presence of *T. viride* or *B. bassiana* sporulation confirmed mortality caused by the respective fungus. The experiment included an untreated control group (T0) where tomato plants were sprayed solely with water. Plant height and fresh/dry weights were also measured 14 days after inoculation (Matrood *et al.*, 2023).

In a time-course experiment, the mortality of *B. tabaci* adults was assessed at five distinct time points: 1, 3, 4, 7, and 14 days after inoculation (DAI). The mortality rate was calculated for each time point using the following formula: Mortality Rate (%) = (Number of dead *B. tabaci* / Initial Number of *B. tabaci*) x 100 (Matrood *et al.*, 2023). At 14 DAI, tomato plants were carefully harvested. Plant height (cm) was measured, and fresh weight and dry weight (g) were determined following the methodology outlined by Hajji-Hedfi *et al.* (2023).

### Statistical analysis

Statistical analysis was conducted on mean values of replicates using ANOVA in SPSS version 20.0 software. Homogeneity of variances and normality were assessed with the Levene's test and Shapiro-Wilk test, respectively. The Least Significant Difference (LSD) test was employed to identify significant differences between treatment groups at a 5% significance level ( $P \leq 0.05$ ).

### RESULTS

Table 1 shows the comparative effectiveness of *T. viride* and *B. bassiana* fungi in bringing mortality of adult whiteflies using the conidia filtration method. Mortality rates were measured at five time points: 1, 3, 4, 7, and 14 days after inoculation (DAI). Both *B. bassiana* and *T. viride* significantly increased whitefly mortality compared to the untreated control at all time points ( $P < 0.01$ ). *B. bassiana* was slightly more effective than *T. viride* in killing whiteflies, with higher mortality rates at all time points (Table 1).

Mortality rates increased over time for all treatments except the untreated control. This suggests that both fungi

have a delayed effect on whiteflies, with most deaths occurring several days after exposure. By 14 DAI, *B. bassiana* achieved the highest mortality rate (69.47%), while *T. viride* reached 62.22%. Both fungi significantly reduced whitefly populations compared to the control (9.44%) (Table 1). Overall, Table 1 demonstrates that both *B. bassiana* and *T. viride* can be effective biological control agents against whiteflies. Although *B. bassiana* was slightly more effective in this study, both fungi offer promising alternatives to chemical insecticides for managing whitefly populations (Table 1).

Table 2 compares the effects of *T. viride* and *B. bassiana* fungi on the plant height, fresh weight, and dry weight of treated plants compared to an untreated control. Both *B. bassiana* and *T. viride* significantly increased plant height and fresh weight compared to the untreated control ( $P < 0.01$ ). *T. viride* resulted in the highest plant height (27.31 cm), exceeding both the control and *B. bassiana* (23.91 cm) treatments. Both fungal treatments led to significantly higher fresh weights compared to the control (9.65 g). *T. viride* again showed the highest value (14.21 g), followed by *B. bassiana* (13.76 g). All treatments had significantly higher dry weights than the control (1.87 g), with *T. viride* exhibiting the greatest increase (2.83 g) (Table 2).

Overall, both *B. bassiana* and *T. viride* positively impacted plant growth parameters compared to the untreated control. However, *T. viride* demonstrated significantly greater effects on plant height, fresh weight, and dry weight, suggesting it may be a more effective plant growth promoter in this experiment (Table 2).

**Table 1.** Comparative effect of *T. viride* and *B. bassiana* on mortality rate (%) of *B. tabaci* adults using conidia filtration method

Treatments	Mortality rates (%)				
	1 DAI	3 DAI	4 DAI	7 DAI	14 DAI
Untreated control	1.21b <sup>a</sup>	2.06b	2.22b	6.13b	9.44b
<i>B. bassiana</i>	6.51a	31.95a	43.17a	61.52a	69.47a
<i>T. viride</i>	6.03a	27.83a	41.51a	52.13a	62.22a
<b><i>P-value</i><sup>b</sup></b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>

DAI: days after inoculation.

<sup>a</sup> Least Significant Difference test is for comparison of mortality rates means of different treatments.

<sup>b</sup> Probabilities associated with individual F tests.

**Table 2.** Comparative effect of *T. viride* and *B. bassiana* on plant height and fresh/dry weights

Treatments	Plant height (cm)	Fresh weight (g)	Dry weight (g)
Untreated control	19.89 <sup>b</sup>	9.65 <sup>b</sup>	1.87 <sup>b</sup>
<i>B. bassiana</i>	23.9 <sup>a</sup>	13.76 <sup>a</sup>	2.01 <sup>a</sup>
<i>T. viride</i>	27.3 <sup>a</sup>	14.21 <sup>a</sup>	2.83 <sup>a</sup>
<b><i>P-value</i><sup>b</sup></b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>

<sup>a</sup> Least Significant Difference test is for comparison of mortality rates means of different treatments.

<sup>b</sup> Probabilities associated with individual F tests.

## DISCUSSION

Our findings align with those of Batool *et al.* (2020), demonstrating the potential of *Trichoderma* spp. and *Beauveria* spp. as biocontrol agents against *B. tabaci* through their ability to induce mortality in whitefly populations. Although not directly investigated in this study, previous research by Gabarty *et al.* (2014) and Nazir *et al.* (2019) suggests potential advantages of *B. bassiana* strains, reporting aphid mortality rates as high as 95%, exceeding those achieved by *Lecanicillium lecanii* and *Metarhizium* spp. Li *et al.* (2012) explored the potential of a transgenic *Trichoderma* strain harboring the chit42 gene from *Metarhizium anisopliae* CY1 for insect pest control. This modified strain exhibited significant insecticidal activity against Asian corn borer larvae, exceeding the efficacy of the wild-type *Trichoderma* (Li *et al.*, 2012). Additionally, the study explored the impact of both species on silkworm (*Bombyx mori*) larvae growth (Banerjee *et al.*, 2016). Interestingly, the transgenic *Trichoderma* demonstrated a greater ability to hinder silkworm growth and development compared to the wild-type strain. Furthermore, transcriptomic analysis of Asian corn borer larvae mid-guts revealed that feeding on transgenic biomass resulted in more significant inhibition of three development and stress-response genes compared to the wild-type *Trichoderma* (Li *et al.*, 2012).

Building upon the findings of Anwar *et al.* (2019), this section explores the potential of entomopathogenic fungi for whitefly control. Studies suggest that fungi like *B. bassiana* and *Trichoderma* spp. employ chitinases to degrade the chitin exoskeleton of *B. tabaci*, facilitating infection and ultimately leading to whitefly mortality (Li *et al.*, 2012; Gabarty *et al.*, 2014). This aligns with the opportunistic nature of these fungi, known for infecting weakened or injured insects (Hajek, 1997). Interestingly, their diverse secondary metabolites hold various biotechnological applications (Siddhardha *et al.*, 2009). These metabolites exhibit wide-ranging activities, including antibiotic, cytotoxic, and insecticidal properties, offering the potential for developing transgenic plants with enhanced resistance to *B. tabaci* (Boldo *et al.*, 2009) and furthering pest control strategies (Demain, 1999; Vey *et al.*, 2001).

The lifecycle of entomopathogenic fungi on *B. tabaci* involves a precise cascade of events. Spores germinate and penetrate the insect's cuticle, followed by vigorous fungal hyphae proliferation within the host. As the fungus thrives, the insect succumbs and dries out, ultimately serving as a platform for new spore production (Mondal *et al.*, 2016). Notably, these fungi produce a diverse arsenal of extracellular enzymes, including lipases, proteases, chitinases, and catalases, potentially contributing to their pathogenicity and antagonistic activity against *B. tabaci* (Fernandes *et al.*, 2012; Dhawan and Joshi, 2017). These enzymes play a crucial role

in breaching the insect's defenses, facilitating penetration, and ultimately leading to its demise (Gebremariam *et al.*, 2022). Moreover, research by Umaru *et al.* (2022) suggests that successful penetration and infection rely on a synergistic interplay between enzymatic degradation and mechanical pressure exerted by the fungus. Abdel-Baky *et al.* (2005) identified spore germination and penetration as critical steps for entomopathogenic fungi to infect *B. tabaci*. The fungi employ two main strategies: 1) developing penetration pegs from appressoria, specialized structures that pierce the insect's exoskeleton, and 2) direct hyphal penetration, bypassing appressoria altogether. This knowledge of infection mechanisms underpins the development of effective biocontrol strategies against *B. tabaci* (Abdel-Baky *et al.*, 2005).

The insect cuticle, a barrier with hydrophobic properties, serves as the first line of defense for *B. tabaci*. To overcome this challenge, entomopathogenic fungi deploy a specialized arsenal of extracellular enzymes. Lipase initiates the attack by hydrolyzing waxes, fats, and ester bonds within the insect's integument, facilitating deep penetration and unlocking precious nutrients (Ali *et al.*, 2009). Following epicuticle degradation, proteases (both exopeptidases and amino peptidases), further degrade proteins into readily usable amino acids, fueling the fungus's growth (Wang *et al.*, 2002; Silva *et al.*, 2010; Wang *et al.*, 2018). Chitinases (1,4-b-chitobiosidases, endochitinases, and N-acetyl-b-D-glucosaminidases) specialize in demolishing the chitinous exoskeleton of *B. tabaci*, paving the way for further invasion (Bidochka & Khachatourians, 1991; Hegedus & Khachatourians, 1996). Catalase neutralizes reactive oxygen species produced by the insect as a defense mechanism, safeguarding the fungus and enhancing the effectiveness of chitinases and proteases (Duriya *et al.*, 2013). This coordinated enzymatic assault dismantles the *B. tabaci* defenses, ultimately leading to successful fungal colonization and insect demise (Matrood *et al.*, 2023).

## CONCLUSION

While the utilization of entomopathogenic fungi for *B. tabaci* control is established, the exciting prospect lies in using fungi isolated from the same ecological niche. Greenhouse environments, with their optimal temperature and humidity, favour fungal efficacy. However, recognizing that entomopathogenic fungi alone are not a silver bullet, their integration with other methods is crucial for maximized impact. This comprehensive approach involves: (1) Early detection and removal: Prompt identification and elimination of infested tomato plants nip population growth in the bud; (2) Weed management: Eradicating alternative weed hosts for *B. tabaci* curbs its population size; (3) Optimized cultivation practices: Regulating fertilization and irrigation minimizes plant stress, creating an unfavorable environment

for the whitefly; (4) Planting and harvest timing: Strategic planning disrupts pest life cycles through well-timed planting and harvesting; (5) Physical barriers: Soil mulches, insect-proof netting in greenhouses, and yellow sticky traps act as physical barriers and attractants; (6) Population control techniques: Mass trapping, mating disruption techniques, and crop rotation strategically suppress *B. tabaci* populations; (7) Resistant varieties: Utilizing resistant or tolerant tomato varieties offers inherent defense mechanisms; (8) Predatory and parasitic biocontrol: Introducing natural enemies like *Amblyseius swirskii*, *Macrolophus caliginosus*, or *Encarsia formosa* leverages natural biocontrol. By harmoniously combining these strategies, including native entomopathogenic fungi, tomato growers can effectively manage *B. tabaci* populations, ensuring healthier crops and sustainable agricultural practices.

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