



Pathogenicity of *Beauveria Bassiana* Against the Whitefly (*Bemisia Tabaci*) on Tomato Plants Under Laboratory Conditions

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Abstract. The pathogenic efficacy of *Beauveria bassiana* against *Bemisia tabaci* adults and second-instar nymphs on tomato plants was evaluated under laboratory conditions and the effect of exposure time and application rate of conidia on the adults and second-instar nymphs of *B. tabaci* was investigated. The three concentrations of conidia (1×10^6 , 1×10^7 and 1×10^8 conidia/mL) and the control treatment were tested. Mortality was observed at day 3, 5, 7 and 10 following treatments. The higher the concentration of conidia, the higher the number of deaths in both developmental stages, and the longer the exposure time. In adults, the 1×10^8 conidia/mL treatment produced the highest mortality (86.6%) after 10 days, followed by 65.7% and 53.4% at 1×10^7 and 1×10^6 conidia/mL, respectively. The reported LT50 values decreased from 8.72 days at 1×10^6 conidia/mL to 6.76 and 4.93 days at 1×10^7 and 1×10^8 conidia/mL, respectively. There was no mortality in the control treatment. In nymphs, the highest mortality was 78.7% at 1×10^8 conidia/mL after 10 days, compared with 62.0% and 51.0% at 1×10^7 and 1×10^6 conidia/mL, respectively. The reported LT50 values were 9.34, 8.15, and 6.22 days at 1×10^6 , 1×10^7 , and 1×10^8 conidia/mL, respectively. The results showed concentration and time-dependent pathogenic activity of *B. bassiana* against adults and nymphs of *B. tabaci*, which confirmed its suitability for biological control in IPM of tomato whitefly.

Keywords: *Beauveria bassiana*; *Bemisia tabaci*; whitefly; biological control; entomopathogenic fungus.

1. Introduction

Tomato (*Solanum lycopersicum*) is an economically valuable crop and is highly grown for food production and export. The nutritional value of tomatoes is attributed to the presence of vitamins, minerals, dietary fiber, proteins, essential amino acids, monounsaturated fatty acids as well as carotenoids (Chaudhary et al., 2018; Vats et al., 2020). The production of tomatoes has further grown in the world indicating its significance in agricultural markets (Rudrapur & Hiremath, 2024). The tomato is also an important model crop for plant and agricultural research (Makhadmeh et al., 2022).

Bemisia tabaci Gennadius (Hemiptera: Aleyrodidae), whitefly is one of the most important of tomato plants in Iraq. It is very polyphagous and has been reported from over 600 plant species. Its persistence may be favored and spread under favorable environmental conditions by continuous crop production (Haj Hasan et al., 2015).

Whiteflies cause damage to the plants by inserting proboscis into the phloem and sucking plant sap, causing leaf yellowing, drying and early leaf drop. Honeydew excretion also favors sooty mold growth which decreases plant marketability and photosynthetic activity of affected plants (Horowitz et al., 2011).

Bemisia tabaci is also a vector of many plant viruses leading to losses. It has the ability to develop resistance against insecticides which makes chemical control more challenging, and requires alternative biological control measures to be found (Gerling, 1990; Kareem et al., 2020).

Beauveria bassiana, an entomopathogenic fungus, has been shown to be pathogenic against *B. tabaci* and other economically important insect pests. Some of its merits are that it can be used in a biological control program and it may be effective in insects upon contact with fungal conidia (Faria & Wraight, 2001; Sani et al., 2020). *B. bassiana* has been recently studied as a part of sustainable pest management, such as *B. bassiana* application to tomato pests (Matrood & Fotouhifar, 2023; Keerthi et al., 2023). Accordingly, the pathogenicity of *B. bassiana* was assessed against adult and nymphal *B. tabaci* in laboratory conditions in the present study.

2. Materials and Methods

2.1 Rearing of the Insect

A colony of *B. tabaci* was started from specimens that were collected from infested tomato leaves in Wasit Governorate, Iraq. The insects were reared under tomato plants (*Solanum lycopersicum*) in net cages 60 cm high and 60 cm diameter, at the temperature of 25 ± 2 °C, relative humidity $65 \pm 5\%$ and photoperiod of 16 h light and 8 h dark. The rearing protocol was as described by Tan et al. (2014).

2.2 Fungal Culture

The *B. bassiana* isolate was collected from Agricultural Research Center, Ministry of science and technology, Iraq. The isolate was grown on Potato Dextrose Agar (PDA) with chloramphenicol to keep bacteria to a minimum. Plates were incubated for 4-7 days at a temperature of 27 ± 2 °C and $65 \pm 5\%$ RH. The isolate was identified morphologically, following standard taxonomic procedures (Rehner et al., 2011), by characteristics of the colonies and the conidial morphology. Conidial suspension was prepared for each culture by adding 10 mL of sterile distilled water with 0.05% Tween 80 to the culture. Conidia were gently scraped, vortexed for 2 min and passed through sterile muslin cloth to remove mycelial fragments and debris. The conidial concentrations were adjusted to 1×10^6 , 1×10^7 , and 1×10^8 conidia/mL using a hemocytometer (Lacey, 2012).

2.3 Culture Medium Used for Fungal Cultivation

2.3.1 Potato Dextrose Agar (PDA)

PDA was used for cultivation of *B. bassiana*. Commercial PDA powder was prepared according to manufacturer's instructions at 39 g/L distilled water and then it was sterilized before pouring into sterile Petri dishes.

2.4 Laboratory Experiments

2.4.1 Activation of the Fungal Isolate

PDA was used for activation of the fungal isolate. Commercial PDA powder was dissolved in 1 L of distilled water and chloramphenicol was added at 250 mg/L to inhibit bacterial contamination, thus preparing the medium. The medium was poured into sterile Petri dishes and kept in a refrigerator at 4 °C until used. The *B. bassiana* isolate was reactivated by transferring a 0.5 cm diameter agar plug from the stored culture to PDA and growing it for one week at 25 °C for fungal growth and sporulation. The 0.5-cm diameter agar plugs were transferred to the sterile tube filled with PDA slant, cultured at 25 ± 2 °C for 1 week, and stored at refrigerated temperature (Wang et al., 2010).

2.4.2 Laboratory Bioassay of *B. bassiana* Against *B. tabaci*

2.4.2.1 Effect of *B. bassiana* on Adult Mortality of *B. tabaci*

The effect of *B. bassiana* on the mortality of adult *B. tabaci* was evaluated.

Adult *B. tabaci* (2–3 days old) were used in the study of the effect of *B. bassiana* on this species. Three conidial concentrations (1×10^6 , 1×10^7 and 1×10^8 conidia/mL) and a distilled-water control were tested, each with three

replications. Adult whiteflies were placed in each replicate (10 per replicate). Appropriate suspension was applied to each replicate with one milliliter of the suspension per replicate on a hand sprayer in small plastic cages. The treated insects were then placed in 9-cm-diameter Petri dishes. Distilled water was given to the control insects. Mortality was noted daily and dead insects were examined for symptoms of fungal infection.

Corrected mortality (%) was calculated using Abbott's formula:

$$\text{Corrected mortality (\%)} = [(T - C) / (100 - C)] \times 100$$

Where T is the mortality percentage in the treated group and C is the mortality percentage in the control group (Abbott, 1925). Because control mortality was 0% at all assessment times in the present experiment, the Abbott-corrected mortality values were numerically identical to the observed treatment mortality values.

2.4.2.2 Effect of *B. bassiana* on Mortality of *B. tabaci* Nymphs

Using a soft brush, 2nd instar nymphs of *B. tabaci* were transferred to 30-day-old tomato plants. Using a hand sprayer, the nymphs were treated with 1 mL of a *B. bassiana* suspension at a concentration of 1×10^6 , 1×10^7 or 1×10^8 conidia/mL. Distilled water was used for the control plants. Plastic cages 60 cm high and 60 cm in diameter covered with gauze were used for individual plant cultivation in tomato plants. Each treatment was replicated 3 times, and consisted of 10 nymphs per experimental unit. The daily mortality of the nymphs was recorded until 10 days after treatment (d.a.t.) (Flowerina et al., 2021).

2.5 Statistical Analysis

The experiments were designed as a Completely Randomized Design (CRD). Data analysis was performed using SPSS version 20. Treatment means were compared using Duncan's Multiple Range Test (DMRT), and differences were considered significant at $P \leq 0.05$. The letter groupings in Tables 1 and 2 represent the comparisons reported for each assessment time. The manuscript did not contain replicate-level mortality data; therefore, the reported LT50 estimates were retained as originally reported and were not independently recalculated during the present revision. No new LT50 values were introduced.

3. Results and Discussion

3.1 Efficacy of *Beauveria bassiana* Against Adult *Bemisia tabaci*

The mortality of *B. tabaci* adults increased with the concentration and time upon exposure to *B. bassiana* (Table 1). After 10 days, mortality reached 53.4%, 65.7%, and 86.6% at 1×10^6 , 1×10^7 , and 1×10^8 conidia/mL, respectively, whereas no mortality was recorded in the control. At 1×10^8 conidia/mL, mortality increased from 23.3% after 3 days to 46.7%, 73.3%, and 86.6% after 5, 7, and 10 days, respectively. The reported LT50 values were 8.72, 6.76, and 4.93 days for 1×10^6 , 1×10^7 , and 1×10^8 conidia/mL, respectively. Therefore, the highest conidial concentration resulted in the highest mortality and shortest reported LT50.

Table (1): Effect of different concentrations of *B. bassiana* on the mortality of *B. tabaci* adults

Spore concentrations (ml ⁻¹)	Percentage mortality % at different exposure periods				
	3days	5days	7days	10days	LT ₅₀
1×10^6	6.7% (a)	24.3% (a)	43.4% (a)	53.4% (a)	8.72
1×10^7	16.7% (ab)	33.2% (a)	56.7% (b)	65.7% (b)	6.76
1×10^8	23.3% (b)	46.7% (b)	73.3% (c)	86.6% (c)	4.93
Control	0.00	0.00	0.00	0.00	-

Note: Values are means of three replicates. Different letters within a column indicate significant differences at $P \leq 0.05$ (DMRT).

The present findings demonstrated a clear concentration- and time-dependent response. Adult mortality at 10 days increased from 53.4% at 1×10^6 conidia/mL to 65.7% at 1×10^7 conidia/mL and 86.6% at 1×10^8 conidia/mL, while the corresponding reported LT50 values decreased from 8.72 to 6.76 and 4.93 days. The same directional pattern was

observed in second-instar nymphs, supporting the conclusion that greater conidial exposure was associated with higher mortality and a shorter time to reach 50% mortality.

The increase in mortality with increasing conidial concentration was consistent with the pathogenic activity reported for entomopathogenic fungi. *B. bassiana* can infect the host after the conidia attach to the surface, germinate and penetrate the cuticle, and then grow inside the host. Secondary metabolites that are generated during infection can also cause impairment and mortality in the host (Pedrini, 2022). Based on the findings of the present study, the tested *B. bassiana* isolate has activity dependence on the concentration against the adult *B. tabaci*. The same biological-control ability has been observed in the recent reviews and experiments (Keerthi et al., 2023; Kumar et al., 2024).

3.2 Efficacy of *Beauveria bassiana* Against *Bemisia tabaci* Nymphs

As seen in Table 2, *B. bassiana* also caused mortality in *B. tabaci* nymphs and exposure duration and conidial concentration had a significant influence on the nymph mortality. At 1×10^8 conidia/mL, mortality increased from 20.0% after 3 days to 40.4%, 61.3%, and 78.7% after 5, 7, and 10 days, respectively. At 10 days, mortality was 51.0% at 1×10^6 conidia/mL and 62.0% at 1×10^7 conidia/mL, with no mortality in the control. The reported LT50 values were 9.34, 8.15, and 6.22 days at 1×10^6 , 1×10^7 , and 1×10^8 conidia/mL, respectively.

This pattern could be explained by the concentration-dependent efficacy of entomopathogenic fungi (Figure 2) which may be attributable to the variety of mechanisms used by the fungi to infect and kill the insect hosts. The broad range of biologically active secondary metabolites with antimicrobial properties that entomopathogenic fungi are known for, as reported by Wadhwa et al. (2024), also appeals to their pathogenicity, overcoming host defense mechanisms, and supports their use in biological control programs.

The rising death rate in nymphs due to the duration of exposure was consistent with the infection process of entomopathogenic fungi. After the contact with the insect surface, conidia stick, germinate, penetrate the insect cuticle and multiply inside the insect. The time needed for these stages coincides with the progressive mortality obtained in the present experiment. The greatest mortality was associated with the highest conidial concentration, and the shortest reported LT50, suggesting a greater pathogenic activity with increased conidial density. Concentration- and time-dependent mortality of *B. bassiana*-treated specimens has also been reported in previous studies (Aş et al., 2024; Aly et al., 2025).

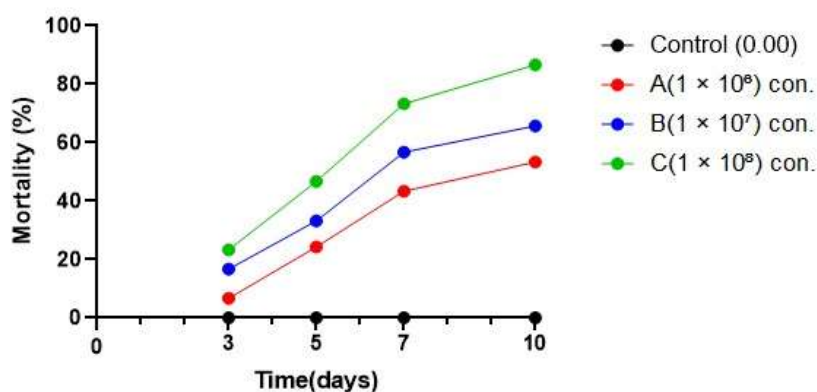


Figure 1: Adult mortality of *B. tabaci* treated with different concentrations of *B. bassiana* at different exposure periods.

The results of the current study are similar to others which have demonstrated higher mortality rates of insects as a result of increasing entomopathogenic fungal conidia. Aly et al. (2025) found that *B. bassiana* led to a greater number of dead larvae of *S. frugiperda* as the concentration of the fungus increased. Similarly, Aş et al. (2024) found that as the concentration and exposure duration of *Zophobas morio* larvae increased, so did the rate of mortality. The results

indicate that the conidial concentration and exposure period are important factors to consider in testing *B. bassiana* pathogenicity against insect hosts.

Table (2): Effect of different concentrations of *B. bassiana* on the mortality of *B. tabaci* nymphs

Concentrations) spores	ML ⁻¹ /(	Percentage mortality % at different exposure periods				
		3days	5days	7days	10days	LT ₅₀
1×10 ⁶		3.3% (a)	26.7% (a)	36.7% (a)	51.0% (a)	9.34
1×10 ⁷		13.3% (b)	30.0% (a)	46.7% (b)	62.0% (b)	8.15
1×10 ⁸		20.0% (b)	40.4% (b)	61.3% (c)	78.7% (c)	6.22
Control		0.00	0.00	0.00	0.00	–

Note: Values are means of three replicates. Different letters within a column indicate significant differences at $P \leq 0.05$ (DMRT).

This indicates that increasing the spore concentration not only increased the final mortality rate, but also contributed to reaching of 50% mortality. The fungal concentration and the exposure time of the *B. tabaci* nymphs were shown to be influential in the results, suggesting that *B. bassiana* can be used as a biological control agent for *B. tabaci* nymphs. The increase of mortality of nymphs could be attributed to the sequential process of infection of entomopathogenic fungus (EPF) from the initial spore adhesion to the body surface to the subsequent growth phases of germination and penetration of the cuticle to the growth phase within the host body and the production of compounds that affect the host eventually leading to death. As a result, the mortality effect cannot be seen immediately following treatment, but can gradually increase with length of treatment as the fungal infection works its way through the host (Figure 2).

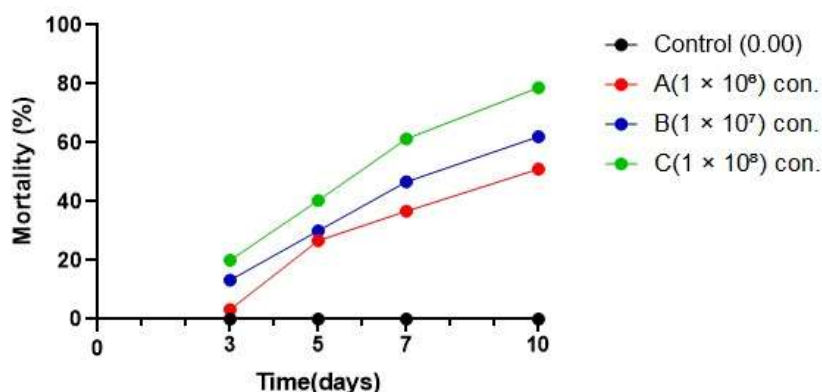


Figure 2: Mortality of second-instar *B. tabaci* nymphs treated with different concentrations of *B. bassiana* at different exposure periods.

The findings that fungal concentration is positively correlated with the mortality rate of treated nymphs were similar to those of several recent studies. As per Aly et al. (2025), as the numbers of spores per mL increased the probability of fungal infection increased, causing more spores to infect the third instar larvae of *S. frugiperda*, and thus increasing the pathogenic effect of the fungus.

The present results are similar to Aş et al. (2024) who showed that the effectiveness of *B. bassiana* against third instar *Zophobas morio* larvae significantly increased as exposure period increased and was continued. The mortality rate reached 50% at a concentration of 1×10^5 conidia/mL, while the mortality increased to 100% at 1×10^7 conidia/mL after six days of treatment. The results of the current study indicated the mortality rate of *B. tabaci* nymphs increased with the increasing number of spores, especially when the spore concentration was 1×10^8 conidia/mL, which is consistent with the results of the above-mentioned study.

3. Conclusion

The high mortality rates seen in *B. bassiana* treated insects could be partly due to its multiple pathogenic mechanisms; *B. bassiana* can produce a number of secondary metabolites with toxic and immunosuppressive effects that are important to the augmentation of its virulence and reduction of insect host defenses, leading to insect host mortality (Pedrini, 2022). The increase in mortality was significant with increasing concentration, and the pathogenic mechanisms were similar to that observed in the present study, which showed that *B. bassiana* isolate is effective against adult *B. tabaci*.

Beauveria bassiana exhibited pathogenic activity on both adult and nymphal stage of *Bemisia tabaci* in the laboratory conditions tested. The higher the concentration of conidia and exposure time, the more deaths were observed. The highest mortality was obtained following the treatment with the 1×10^8 conidia/mL after 10 days with 86.6% and 78.7% mortality in adults and nymphs, respectively. A direct correlation between the reported LT50 values and concentration of conidia was also found. The results here indicate that *B. bassiana* is promising for use as a biological control agent against *B. tabaci* of tomato plants.

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