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# The Integrated Use of a Commercial Formulation of the Entomopathogenic Fungus *Beauveria bassiana* with two Insecticides Against Cotton Whitefly, *Bemisia tabaci* (Genn.) on Tomato Plants

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# ABSTRACT

Tomato is an economically important crop that is attacked by several pests, including the cotton whitefly, Bemisia tabaci (Genn.). B. tabaci causes direct and indirect damage to crops by piercing and sucking the plant foliage and transmitting viruses. In this study, we investigated the efficacy of individual and sequential treatments with a commercial formulation of the *Beauveria bassiana*, azadirachtin and imidacloprid against both eggs and nymphs of B. tabaci on tomato plants under laboratory and greenhouse conditions. Results of the leaf-dip bioassay, comparing the individual treatments, revealed that imidacloprid exhibited the highest toxicity ( $LC_{50}$ = 151 and 153 mg/L) against the eggs and the  $2^{nd}$  nymphal instar, respectively, followed by azadirachtin and *B. bassiana*. The leaf spray bioassay results indicated that, B. bassiana alone caused 66.5% and 70 % mortality of eggs and the  $2^{nd}$  nymphal instar, respectively, while its toxicity was elevated in either sequential treatments [B. bassiana/imidacloprid] to 85.7% and 88.2%, or [imidacloprid/B. bassiana] to 90% and 80%, respectively. Concerning the greenhouse experiment, all treatments resulted in a significant reduction in the nymph and egg populations of B. tabaci at all period intervals after treatment. With the prolongation time, the residual effect of *B. bassiana* on eggs and nymph populations in the individual treatment decreased gradually to reach 8.5 and 2.52% mortality, respectively, day 15 after treatment, while it significantly increased when it was applied in sequences with both imidacloprid and azadirachtin treatments. These results emphasize the importance of integration between chemical and biological agents to improve control efficacy and achieve environmentally friendly requirements.

Keywords: Beauveria bassiana, azadirachtin, imidacloprid, Bemisia tabaci, tomato, sequential treatments

## Introduction

Tomato, *Solanum lycopersicum*, is one of the most important economic vegetable crops in Egypt and worldwide (Mahmoud *et al.*, 2020; Abd-Elgawad, 2020). Tomato plants are attacked by several pests, including the cotton whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), a global serious and destructive pest of many vegetables, horticultural, field and ornamental in many countries (Srinivasan *et al.*, 2012; Mahmoud *et al.*,2020 and Khatun *et al.*, 2023). It causes damage by piercing and sucking the plant foliage and transmitting the viruses (Alegbejo, 2000 and Nomikou *et al.*, 2004).

Insecticides are an essential control option for reducing *B. tabaci* infestation and are involved in any management programs (Khalifa and Bedair, 2023). Imidacolprid, a neonicotinoid insecticide, possesses a broad spectrum of activity against various pests, including sucking insects (aphids, whiteflies, thrips and bugs), and also many Coleopterans and selected Lepidopteran pests. According to its high insecticidal potency and relatively low mammalian toxicity (Sheets, 2010 and Awasthi *et al.*, 2013), it is widely used to control sucking insects (Malekan *et al.*, 2012 and El-Naggar and Zidan, 2013). However, using chemical insecticides faces major challenges, including the development of insect resistance (Pavan *et al.*, 2020; Horowitz *et al.*, 2020) and negative environmental impacts

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(Shalaby et al., 2012; Shalaby and Abdou, 2020; Abdelgaleil et al., 2022). Consequently, developing and utilizing environmentally friendly controlling measures is in high demand (Horowitz and Ishaaya, 2004). Botanical insecticides include azadirachtin, which controls a wide range of agricultural and medical insect pests attributable to its broad-spectrum mode of action (Dua et al., 2009; Muthusamy, et al., 2024). Azadirachtin performs as an antifeedant, a repellent, delays molting, and decreases growth development and oviposition all resulting in considerable mortality of many insects including B. tabaci (Mitchell et al., 2004; Kumar et al., 2005 and Kumar and Poehling, 2014). In addition, entomopathogenic fungi are known as an effective bio-agents against many insect pests (Ahmed et al.2022) particularly B. bassiana (Al-Deghairi, 2008 and Gebremariam et al., 2021)). This fungus is described as an environmentally friendly for plants and humans as well as the non-targeted organisms (Khetan, 2001 and Wu et al., 2014). The combination of chemical or biorational agents and entomopathogenic fungi against various insects was investigated by many researchers (Halder et al., 2021, Suarez-Lopez et al., 2022, Li et al., 2023 and Dearlove et al., 2024). In particular, the combination of certain biologically compatible insecticides such as imidacloprid with fungi may enhance fungal effects on insect pests, Feng et al. (2004 a and b) reported a synergism between the entomopathogenic fungus B. bassiana, and imidacloprid against Empoasca vitis and Trialeurodes vaporariorum Moreover, Halder et al. (2021) found that, a combination of Lecanicillium lecanii and neem oil in half of their recommended rats showed a higher reduction of the jassid and whitefly populations compared with the individual treatments. Consequently, utilizing such a combination in IPM programs provides an environmentally friendly measure that is essential to achieving the goals of pest control. Therefore, the purpose of this study was to evaluate the efficacy of individual and sequential treatments of a commercial formulation of entomopathogenic fungus B. bassiana, a bio-rational insecticide (azadirachtin) and a chemical insecticide imidacloprid against both eggs and nymphs of B. tabaci attacking tomato plants under laboratory and greenhouse conditions.

# 2. Materials and Methods

# 2.1. Insecticides used:

**Entomopathogenic fungus**, *Beauveria bassiana*, (Bio Power) a commercial formulation containing 8  $\times 10^8$  conidia /ml, produced by Stanes company, India. The recommended rate is 5ml / L of water according to the manufacturer's recommendations.

**Azadirachtin:** a botanical insecticide named Nimbecidine 0.03% EC. was obtained from, Stanes Company, India. The recommended rate is 5 ml / L according to the company directions.

**Imidacloprid**: a commercial sample named "Imida-max" 70%WG was obtained from the Agri cure company. Egypt. The recommended rate is 30 g /100 L water, according to APC, (2022).

## 2.2. Treatments:

Eight treatments were tested in the laboratory and greenhouse experiments as mentioned in (Table 1). In Sequential treatments the second treatments were applied 24 h next to the first one (Cuthbertson *et al.*, 2005).

# **2.3.** Laboratory Experiments

# 2.3.1. Leaf -dip Bioassay:

A leaf-dip bioassay method described by Moores *et al.* (1996) was used to evaluate the toxicity of *B. bassiana*, azadirachtin and imidacloprid against egg and 2<sup>nd</sup> nymph stages *of B. tabaci*. A serial concentration of each tested insecticide was prepared in distilled water. The alive eggs and 2<sup>nd</sup> nymph stages were counted on each leaf before treatment, then dipped in the desirable insecticide solution for 10 seconds and transferred to petri dishes laid on an agar layer (0.2%). The treatments were kept at (28 °C ± 2, 60% RH ± 10 and 12:12 D/L photoperiod). The data were recorded 72 h after treatment, using a binocular microscope.

Treatments	Treatments detail*
Beauveria bassiana	Beauveria bassiana (recommended rate)
Azadirachtin	Azadirachtin (recommended rate)
Imidacloprid	Imidacloprid (recommended rate)
<i>B.bassiana</i> / azadirachtin	<i>Beauveria bassiana</i> (recommended rate) following by Azadirachtin ( <sup>1</sup> / <sub>2</sub> recommended rate)
B.bassiana / imidacloprid	<i>Beauveria bassiana</i> (recommended rate) following by Imidacloprid ( <sup>1</sup> / <sub>2</sub> recommended rate)
Azadirachtin / B. bassaina	Azadirachtin ( <sup>1</sup> / <sub>2</sub> recommended rate) following by <i>Beauveria Bassiana</i> (recommended rate)
Imidacloprid / B. bassaina	Imidacloprid ( <sup>1</sup> / <sub>2</sub> recommended rate) following by <i>Beauveria Bassiana</i> (recommended rate)
Control	Water

**Table 1:** Sequential and individual treatments for leaf spray bioassay and greenhouse experiments

\*In the sequential treatments, the second spray was applied 24h after the first spray.

### 2.3.2. Leaf spray bioassay:

This technique was used to evaluate the toxicity of all treatments against egg and  $2^{nd}$  nymph instar of *B. tabaci*. The alive eggs and the  $2^{nd}$  nymphal instar were counted on tomato seedlings and the other instars were eliminated, then sprayed by a fine nozzle hand sprayer with the insecticide solution as shown in (Table 1), then incubated as described above. The data was recorded 7 days after treatment, using a binocular microscope.

## 2.4. Greenhouse experiment:

The experiment was conducted in a greenhouse at the National Research Centre, Cairo. The experiment was arranged as a completely randomized design with eight treatments and three replicates for each. Tomato plants were planted in plastic pots three seedlings/pot (two weeks old). Pots were prepared by adding usual fertile soil. The 1<sup>st</sup> spray was applied three weeks after plantation and the 2<sup>nd</sup> spray was applied 24 h after the first one (Table 1). Data were recorded as egg and nymph numbers found on the lower surface of 10 leaflets per replicate using a binocular microscope before treatments and then (3, 5, 7, 10 and 15 days after treatments. The reduction in egg and nymph populations was calculated using Henderson and Tilton equation (1955).

## 2.5. Statistical analysis:

The leaf dip bioassay data were subjected to probit analysis according to Finney (1971) using Ldpline program to estimate the  $LC_{50}$ , confidence limits and slope values. The greenhouse experiment data were implemented to an analysis of variance (ANOVA) followed by least significant difference (L.S.D.) at 5% using Costat Statistical Software (1990).

# 3. Results and Discussion

## 3.1. Leaf -dip Bioassay:

The susceptibility of *B. tabaci* eggs and the  $2^{nd}$  instar nymphs to *B. bassiana*, azadirachtin and imidacloprid is presented in Tables 2 and 3. The results indicate that, the susceptibility of the  $2^{nd}$  instar nymphs of *B. tabaci* to all tested treatments were higher than the eggs susceptibility. Imidacloprid showed the highest toxicity against eggs and the  $2^{nd}$  instar nymphs followed by azadirachtin and *B. bassaina*, with LC<sub>50</sub> values of (151 and 153), (2006 and 1824) and (3404 and 3356) ppm, respectively. Imidacloprid was 13.3 and 22.5 times more toxic to eggs than azadirachtin and *B. bassaina*, respectively, while, azadirachtin was 1.7 times more toxic than *B. bassaina*. Moreover, imidacloprid was 11.9 times more toxic to the  $2^{nd}$  instar nymphs than azadirachtin and 21.9 times more than *B. bassaina*.

Insecticides	LC50 (ppm)	Confidence Limits	LC <sub>90</sub> (ppm)	Confidence Limits	Slope ± SE
B. bassiana	3404	2190 - 3864	9099	7936 - 12568	2.93±0.29
Azadirachtin	2006	1815-2197	5326	4538-6661	3.02±0.29
Imidacloprid	151	138 – 165	421	351 - 543	2.88±0.27

Table 2: Toxicity of *B. bassiana*, azadirachtin and imidacloprid against *B. tabaci* eggs

**Table 3:** Toxicity of *B. bassiana*, azadirachtin and imidacloprid against the  $2^{nd}$  nymphal instar of *B. tabaci* 

Insecticides	LC50 (ppm )	Confidence Limits	LC90 (ppm)	Confidence Limits	Slope ± SE
B. bassiana	3356	3076 - 3666	9401	7839 - 12146	$2.87 \pm 0.27$
Azadirachtin	1824	1658 -1983	4672	4094 - 5572	3.14±0.27
imidacloprid	153	141 – 165	403	351 - 483	3.05±0.24

#### 3.2. Leaf spray bioassay:

The data in Figs. 1 and 2 illustrate the efficiency of the individual and the sequential treatments of either imidacloprid or azadirachtin with *B. bassiana* against eggs and the  $2^{nd}$  nymphal instar of *B. tabaci*. The data revealed that, the treatment of *B. bassiana* alone caused 66% mortality of eggs, while its toxicity was elevated in the sequential treatments with the other tested insecticides. Moreover, the highest egg mortality rate of *Bemisia tabaci* was observed with individual imidacloprid treatment (93.3%), followed by the sequential treatment of [imidacloprid /*B. basaina*] (90%) and [*B. bassiana* /imidacloprid] (85.7%) (Fig.1.).

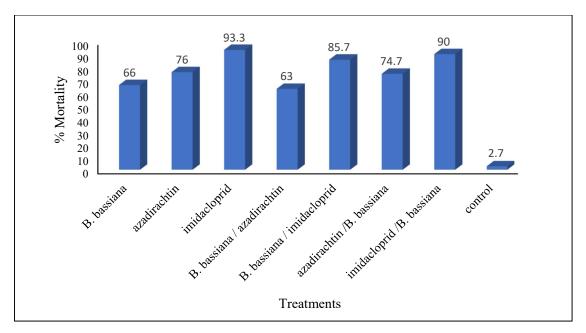
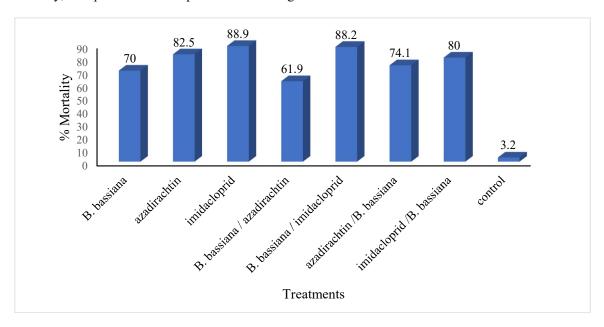


Fig. 1: Mortality percent of *B. tabaci* eggs on tomato seedling treated with individual and sequential treatment of *B. bassiana*, azadirachtin and Imidacloprid under laboratory condition

A similar trend was obtained for the  $2^{nd}$  nymphal instar as *B.bassiana* alone caused 70% mortality which increased to 88.2% in the sequential treatment with imidacloprid [*B.bassiana* /imidacloprid], which approximately was equal to the highest mortality (88.9%) that achieved by imidacloprid alone at

its recommended rate. However, the sequential treatment of [*B.bassiana* / azadirachtin] caused 61.9% mortality lower than the individual treatments of azadirachtin (82.5%) or *B.bassiana* (70%) mortality (Fig. 2).

These results agree with Al-Deghairi (2008), who demonstrated that, nymphs of *B. tabaci* were more susceptible to *B. bassaina* compared with eggs. Also, Cuthbertson *et al.* (2005 and 2010) reported that the  $2^{nd}$  instar nymphs of *B. tabaci* were the most susceptible life stage to fungal infection of *B.bassiana*. Malekan *et al.* (2012) found that, the combination of imidacloprid with *B. bassiana* or *L. muscarium* in the laboratory bioassays were more effective on both young and old nymphal stages of whitefly, *T. vaporariorum* compared with the fungus alone.



**Fig. 2:** Percent mortality of *B. tabaci* 2<sup>nd</sup> nymph instar on tomato plant treated with individual and sequential treatment of *B. bassiana*, azadirachtin and imidacloprid under laboratory condition

Similarly, Sarhozaki *et al.* (2021) reported that the toxicity of *B. bassiana* and NeemAzal-T/S combination against eggs, nymphs and pupae of the *B.tabaci* was higher than each of them alone. In addition, Amjad *et al.* (2012) mentioned that imidacloprid, diafenthuron and buprofezin, were most compatible with fungi, *Isaria fumosorosea* and *L. muscarium*, among nine commercial pesticides evaluated. Feng *et al.* (2004a) reported also a synergistic effect of imidacloprid with *Paecilomyces fumosoroseus* and *B bassiana* against greenhouse whitefly *T. vaporariorum* than using a single application

## 3.3. Greenhouse experiment:

The percent reduction of *B. tabaci* eggs on tomato plants at different time intervals after application is presented in Table 4 and Fig. 3. All treatments caused significant reduction percentages in the egg number of *B. tabaci* at all periods after treatment. After three days of treatment, imidacloprid alone achieved the highest percent reduction of the egg count (81.44 %), followed by [imidacloprid / *B.bassiana*] (78.88%), single treatments *B. bassaina* (69.04%) and azadirachtin (65.87%). After 5 days, the percent reduction in egg count was increased in most treatments. By prolonging the time after application, the residual effect of the single treatment [*B. bassaina*] decreased gradually; as the percent reductions were 44.54%, 21.41% and 8.50% at 7, 10 and 15 days after application, respectively.

<b>Table 4:</b> Efficacy of individual and sequential treatments of <i>B. bassaina</i> , azadirachtin and imidacloprid	
against B. tabaci eggs on tomato at different intervals	

Treatment	% Reduction relative to control of <i>Bemisia tabaci</i> eggs per 10 leaflets at different intervals (days)					
	3	5	7	10	15	
B. bassiana	$69.04^{b} \pm 4.25$	$79.45^{\text{c}}\pm0.16$	$44.54^{e}\!\pm2.37$	21.41 <sup>e</sup> ± 3.15	$8.50^{e}\pm 3.64$	
Azadirachtin	$65.87^b\pm5.85$	$75.40^{\text{e}}\pm0.60$	$40.11^{\rm f}{\pm}~0.81$	$39.71^d \pm 4.51$	$86.24^d\pm2.11$	
Imidacloprid	$81.44^{a}\pm2.49$	$77.84^{d}\pm0.57$	$80.87^b\pm0.35$	$95.67^{\mathrm{a}}\pm0.37$	$91.33^{\text{a}}\pm0.19$	
<i>B. bassiana /</i> azadirachtin	$67.76^b\pm3.66$	$54.29^{\rm f}\pm0.29$	$59.73^{d}\pm0.50$	$41.14^{d} \!\pm 0.43$	$78.19 \ ^{d}\pm 2.76$	
B. bassiana / imidacloprid	$47.52^{\rm c}\pm5.72$	$83.30^b\pm0.31$	$65.93^{\text{c}}\pm0.65$	$65.96^{\text{b}}\pm1.80$	$86.94^{\text{b}}\pm3.22$	
Azadirachtin / <i>B. bassiana</i>	$48.13^{\text{c}}\pm8.91$	$83.82^b\pm0.67$	$39.67^{\rm f}\pm3.00$	$55.96^{\text{c}} \pm 1.23$	$48.65^d \!\pm 1.12$	
Imidacloprid / <i>B. bassiana</i>	$78.88^{a}\pm3.37$	$86.83^{\text{a}}\pm0.43$	$88.46^{\text{a}}\pm0.17$	$94.18^{\mathrm{a}}\pm0.84$	$88.72^{a}\pm1.27$	
LSD 0.05	5.56	0.60	1.55	2.57	3.56	

The reduction percentages followed by the same letter(s) in the column are not significantly different (P < 0.05).

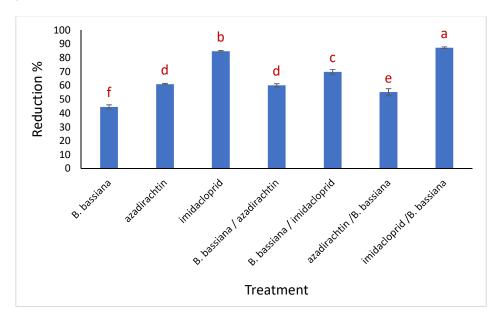


Fig. 3: The overall mean of the percent reduction in count eggs of B. tabaci/ 10 tomato leaflets

However, the residual effect of *B. bassiana* increased when it was applied in sequence with imidacloprid at the half-recommended rate Additionally, *B. bassiana* became ineffective 15 days post-treatment, whereas the other treatments continued to demonstrate prolonged effects. The general mean of the percent reduction of eggs is illustrated in Fig.3. The [imidacloprid /*B. bassiana*] treatment showed significant elevation in eggs reduction (87.42%) compared to the reduction achieved by imidacloprid or *B. bassiana* alone, which were 84.85% and 44.59%, respectively. The efficacy against nymph populations, as presented in Table 5 and illustrated in Fig. 4, revealed that after three days of treatment (74.10%) and the sequential treatments, [*B.bassiana* / imidacloprid] (76.94%), [*B.bassiana* / azadirachtin] (74.72%) and [imidacloprid / *B. bassiana*] (72.62%). After 5 days of treatment, the bioactivity of all single and sequential treatments increased whereas the sequential treatment

[imidacloprid /B. bassiana] showed the highest reduction percent (93.07%), while the lowest reduction percent was recorded with the individual treatment of B. bassiana (78.40%). Over time, the residual effect of *B. bassiana* in the individual treatment decreased gradually, with reduction percentages dropping to 52.00%, 42.41%, and 2.52% at 7, 10, and 15 days post-application, respectively. Moreover, after 15 days of treatment, the residual activity of the single treatment B. bassiana was declined (2.52%), whereas it increased markedly in the sequential treatments: [B. bassiana / imidacloprid] (90.46%), [imidacloprid / B. bassiana] (85.06%), [azadirachtin /B. basaina] (95.82%) and [B. basaina /azadirachtin] (78.38%). The overall mean reduction in *Bemisia tabaci* nymph populations is illustrated in Fig.4. The treatments followed the same trend of eggs reduction as the highest reduction was achieved by [imidacloprid /B. bassiana] and [B. bassiana / imidacloprid] while [B. basaina] showed the lowest reduction. These results are consistent with those reported by Lacey et al. (2008) reported that B. bassiana caused a reduction of approximately 41- 50% in B. tabaci populations compared to untreated plants. Olson and Oetting (1999) mentioned that B. bassiana did not appear to have a residual effect. Moreover, Sarhozaki et al. (2021) stated that the effect of B. bassaia on eggs and second instar nymphs was the lowest at 14 days of treatment which may be referred to the impact of varying humidity, temperature, soil moisture and wind speed. Thuppukonda and kumar (2022) found that imidachloprid showed the highest percent reduction in the chilli thrips population (94.5%) among the tested treatments. El-Naggar and Zidan (2013) noticed that imidacloprid had long residual effect against whitefly after foliar application on cotton seedlings in field.

Aljedani (2018) illustrated that imidacloprid had a stronger effect in the elimination and mortality of black bugs *Coridius viduatus* compared with azadirachtin.

Gill and Chong (2021) noted that among all tested pesticides a drench and spray application of dinotefuran and imidacloprid on poinsettia were the most effective against nymphs and adults of *B. tabaci*. Omprakash and Raju (2013) mentioned that thiamethoxam and imidacloprid showed the highest efficacy in population reduction of *B. tabaci* while azadirachtin caused the lowest efficacy among all tested treatments on brinjal. Khalifa (2021) mentioned that imidacloprid was a highly effective treatment against whitefly adult and nymph stages on tomatoes. Khalifa and Bedair (2023) demonstrated that, among eight tested treatments, imidacloprid showed a significant suppression of the whitefly adult population in cucumber fields.

Treatment	% Reduction relative to control in nymphs population <i>of Bemisia tabaci</i> per10 leaflets at different intervals (days)						
	3	5	7	10	15		
B. bassiana	$67.67^{\rm c} \pm 1.62$	$78.40^{\text{e}}\pm0.6$	$52.00^{\text{e}} \pm 3.50$	$42.41^{f}\pm 2.93$	$2.52^{\text{g}} \pm 1.33$		
Azadirachtin	$48.00^{\text{e}}\pm2.28$	$80.00^{\text{d}} \pm 1.00$	$66.07^d {\pm}\ 3.37$	$94.69^{\mathtt{a}} \pm 1.03$	$47.76^{\rm f}{\pm}3.76$		
Imidacloprid	$74.10^{ab}\pm1.41$	$81.46^{\text{c}}\pm0.2$	$92.35^b\pm0.60$	$88.07^{\rm c}\pm0.42$	54.83 °± 2.56		
<i>B.bassiana /</i> azadirachtin	$74.72^{ab}\pm1.62$	$81.10^{\text{cd}}\pm0.65$	$53.15^{e}\pm4.76$	68.50 °± 2.65	$78.38^{d} \pm 2.53$		
<i>B.bassiana/</i> imidacloprid	$76.94^{a}\pm0.87$	$92.91^{\mathtt{a}}\pm0.15$	$89.64^{b}\pm1.41$	$91.88^{b}\pm0.43$	$90.46^{b} \pm 1.31$		
Azadirachtin / <i>B. bassiana</i>	$59.17^{d}\pm1.21$	$91.62^{\text{b}}\pm0.51$	$75.70^{\rm c}\pm1.63$	$79.57^{\text{d}}\pm1.10$	$95.82^{\rm a}\pm0.61$		
Imidacloprid / <i>B. bassiana</i>	$72.62^b\pm1.36$	$93.07^{a}\pm0.49$	$97.12^{a} \pm 0.55$	$95.49^{\mathtt{a}}\pm0.22$	$85.06^{\circ} \pm 0.31$		
LSD 0.05	1.66	0.82	2.95	1.90	2.95		

 Table 5: Efficacy of individual and sequential treatments of *B. bassaina*, azadirachtin and imidacloprid against *B. tabaci* nymphs population on tomato at different intervals

The reduction percentages followed by the same letter(s) in the column are not significantly different (P < 0.05).

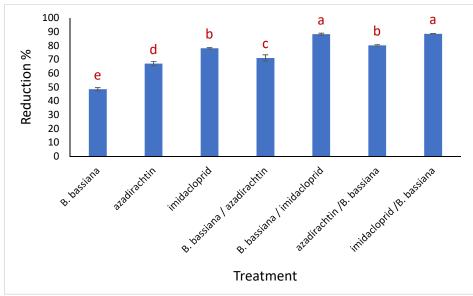


Fig. 4: The overall mean of the percent reduction in infestation of nymph B. tabaci/ 10 tomato leaflets

Our finding about the sequential treatments is similar to that concluded by Islam *et al.*, (2010a and b) as they reported that *B. tabaci* is difficult to control with a single treatment of either neem or *B. bassiana*, but their combined effects have proven successful control of this insect. Also. Cuthbertson *et al.* (2005) summarized the combination treatments of entomopathogenic fungus *L. muscarium* with imidacloprid resulted in high mortality of *B. tabaci* nymphs compared to either teflubenzeron or nicotine on verbena foliage. Also, Cuthbertson *et.al.*, (2008 and 2010) demonstrated that sequential combination of chemicals such as savona, majestic or certis spray oils with fungi *L. muscarium* caused mortality of *B. tabaci* eggs, second instar nymphs and adults that ranged between 90% and 96% under greenhouse condition. Moreover, our data is agrees with Zawrah *et al.* (2020) who reported that, the sequential combinations treatment of imidacloprid and *B. bassiana* caused an equal effect as imidacloprid alone which added more benefits of the combination as we used a low dose of imidacloprid which reported to decrease its effects against the natural enemies.

# 4. Conclusion

We can conclude that the sequential combination of the commercial formulation of the entomopathogenic fungus, *B. bassiana* with half the recommended rate of imidacloprid and azadirachtin is a promising approach for enhancing the benefits and maximizing the efficacy of both chemical and biological control against *B. tabaci*. Moreover, the commercial formulation of *B. bassiana* could be more suitable for combination with imidacloprid than azadirachtin in an integrated pest management program to control *B. tabaci*. This integrated approach not only improves pest control efficacy but also offers a sustainable strategy to reduce reliance on chemical insecticides, thereby mitigating potential resistance development and environmental impact.

# 5. Acknowledgements

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## References

- Abdelgaleil, S. A., N. M. Al-Nagar, H. K. Abou-Taleb and M. S. Shawir, 2022. Effect of monoterpenes, phenylpropenes and sesquiterpenes on development, fecundity and fertility of *Spodoptera littoralis* (Boisduval). International Journal of Tropical Insect Science, 42(1): 245-253.
- Abd-Elgawad, M. M. M., 2020. Optimizing biological control agents for controlling nematodes of tomato in Egypt. Egyptian Journal of Biological Pest Control, 30(1): 58.

- Ahmed, A., Sh. Khalil and A. Sahab, 2022. Identification and evaluation of isolated entomopathogenic fungus from Egyptian soil against the black cutworm larvae of *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 32: 67.
- Al-Deghairi, M. A., 2008. Bioassay evaluation of the entomopathogenic fungi, *Beauveria bassiana* Vaellemin against eggs and nymphs of *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae). Pakistan Journal of Biological Sciences, 11(12): 1551-1560.
- Alegbejo, M. A., 2000. Whitefly transmitted plant viruses in Nigeria. Journal of Sustainable Agriculture, 17(2): 99-109.
- Aljedani, D., 2018. Assessment of effectiveness of the imidacloprid and azadirachtin on the black watermelon bug. International Journal of Zoological Research, 14: 61-70.
- Amjad, M., M. H. Bashir, M. Afzal, M. A. Sabri and N. Javed, 2012. Effects of commercial pesticides against cotton whitefly (*Bemisia tabaci* Genn.) and mites (*Tetranychus urticae* Koch) on growth and conidial germination of two species of entomopathogenic fungi. Pakistan Journal of Life and Social Sciences, 10(1): 22-27.
- APC, 2022. Agriculture pesticide committee. Technical Recommendation for Agriculture Pest Control guidelines, Ministry of Agriculture & Land Reclamation, Cairo, Egypt.
- Awasthi, N. S., U. P. Barkhade, S. R. Patil and G. K. Lande, 2013. Comparative toxicity of some commonly used insecticides to cotton aphid and their safety to predatory coccinellids. The Bioscan, 8(3): 1007-1010.
- Costat Statistical Software, 1990. Microcomputer program analysis Version 4.20. Berkeley, CA: Cohort Software.
- Cuthbertson, A. G. S., K. F. A. Walters and C. Deppe, 2005. Compatibility of the entomopathogenic fungus *Lecanicillium muscarium* and insecticides for eradication of sweetpotato whitefly, *Bemisia tabaci*. Mycopathologia, 160: 35-41.
- Cuthbertson, A. G. S., L. F. Blackburn, P. Northing, W. Luo, R. J. C. Cannon and K. F. A. Walters, 2008. Further compatibility tests of the entomopathogenic fungus *Lecanicillium muscarium* with conventional insecticide products for control of sweet potato whitefly, *Bemisia tabaci* on poinsettia plants. Insect Science, 15: 355-360.
- Cuthbertson, A. G. S., L. F. Blackburn, P. Northing, W. Luo, R. J. C. Cannon and K. F. A. Walters, 2010. Chemical compatibility testing of the entomopathogenic fungus *Lecanicillium muscarium* to control *Bemisia tabaci* in glasshouse environment. International Journal of Environmental Science and Technology, 7(2): 405-409.
- Dearlove, E. L., D. Chandler, S. Edgington, S. D. Berry, G. Martin, C. Svendsen and H. Hesketh, 2024. Improved control of *Trialeurodes vaporariorum* using mixture combinations of entomopathogenic fungi and the chemical insecticide spiromesifen. Scientific Reports, 14(1): 15259.
- Dua, V. K., A. C. Pandey, K. Raghavendra, A. Gupta, T. Sharma and A. P. Dash, 2009. Larvicidal activity of neem oil (*Azadirachta indica*) formulation against mosquitoes. Malaria Journal, 8: 1-6.
- El-Naggar, J. B. and N. A. Zidan, 2013. Field evaluation of imidacloprid and thiamethoxam against sucking insects and their side effects on soil fauna. Journal of Plant Protection Research, 53(4): 375-387.
- Feng, M. G., B. Chen and S. H. Ying, 2004a. Trials of *Beauveria bassiana, Paecilomyces fumosoroseus* and imidacloprid for management of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) on greenhouse grown lettuce. Biocontrol Science and Technology, 14: 531-544.
- Feng, M. G., X. Y. Pu, S. H. Ying and Y. G. Wang, 2004b. Field trials of an oil-based emulsifiable formulation of *Beauveria bassiana* conidia and low application rates of imidacloprid for control of false-eye leafhopper *Empoasca vitis* in southern China. Crop Protection, 23: 489-496.
- Finney, D. J., 1971. Probit analysis. 3rd edition. Cambridge University Press, Cambridge, 314 pp.
- Gebremariam, A., Y. Chekol and F. Assefa, 2021. Phenotypic, molecular, and virulence characterization of entomopathogenic fungi, *Beauveria bassiana* (Balsam) Vuillemin, and *Metarhizium anisopliae* (Metschn.) Sorokin from soil samples of Ethiopia for the development of mycoinsecticide. Heliyon, 7(5):1-12.
- Gill, G. and J. Chong, 2021. Efficacy of selected insecticides as replacement for neonicotinoids in managing sweet potato whitefly on poinsettia. Hort Technology, 31: 1-8.

- Halder, J., P. A. Divekar and A. T. Rani, 2021. Compatibility of entomopathogenic fungi and botanicals against sucking pests of okra: an ecofriendly approach. Egyptian Journal of Biological Pest Control, 31: 30.
- Henderson, C. T. and E. W. Tilton, 1955. Tests with acaricides against the brown wheat mite. Journal of Economic Entomology, 48: 157-161.
- Horowitz, A. R. and I. Ishaaya, 2004. Biorational insecticides—mechanisms, selectivity and importance in pest management. In Insect pest management: field and protected crops (pp. 1-28). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Horowitz, A. R., M. Ghanim, E. Roditakis, R. Nauen and I. Ishaaya, 2020. Insecticide resistance and its management in *Bemisia tabaci* species. Journal of Pest Science, 93(3): 893-910.
- Islam, M. T., S. J. Castle and S. X. Ren, 2010a. Compatibility of the insect pathogenic fungus *Beauveria* bassiana with neem against sweetpotato whitefly, *Bemisia tabaci* on eggplant. Entomologia Experimentalis et Applicata, 134: 28-34.
- Islam, M. T., A. Olleka and S. Ren, 2010b. Influence of neem on susceptibility of *Beauveria bassiana* and investigation of their combined efficacy against sweetpotato whitefly, *Bemisia tabaci* on eggplant. Pesticides Biochemistry and Physiology, 98(1):45-49.
- Khalifa, M. H. and A. F. Bedair, 2023. Field evaluation of some insect growth regulators and plant originated insecticides against sucking-piercing insects on cucumber plant and their side effects on the associated predators. Alexandria Science Exchange Journal, 44(3): 331-338.
- Khalifa, M. H., 2021. Field assessment of some new insecticide classes and their binary mixtures against *Bemisia tabaci* and *Tuta absoluta* and their correlations with tomato productivity. Pakistan Journal of Agricultural Sciences, 58(6): 1689-1698.
- Khatun, M. F., M. Jahan, K. R. Das, K. Y. Lee and E. J. Kil, 2023. Population dynamics and biorational management of sucking insect vectors on chili (*Capsicum annuum* L.) in Bangladesh. Archives of Insect Biochemistry and Physiology, 112(2): e21980.
- Khetan, S.K., 2001. Microbial Pest Control. Journal of Phytopathology, 149: 491-492.
- Kumar, P. and H. M. Poehling, 2014. Effects of azadirachtin, abamectin, and spinosad on sweet potato whitefly (Homoptera: Aleyrodidae) on tomato plants under laboratory and greenhouse conditions in the humid tropics. Journal of Economic Entomology, 100(2): 411-420.
- Kumar, P., H. M. Poehling and C. Borgemeister, 2005. Effects of different application methods of azadirachtin against sweetpotato whitefly *Bemisia tabaci* Gennadius (Hom., Aleyrodidae) on tomato plants. Journal of Applied Entomology, 129(9-10): 489-497.
- Lacey, L. A., S. P. Wraight and A. A. Kirk, 2008. Entomopathogenic fungi for control of *Bemisia tabaci* biotype B: foreign exploration, research and implementation. p. 33–69. In: Classical Biological Control of *Bemisia tabaci* in the United States - A Review of Interagency Research and Implementation (J. Gould, K. Hoelmer, J. Goolsby, eds.). Springer, 343 pp.
- Li, J., X. Zhou, Z. Xiao, S. Wu, H. Cai, K. Teng, et al., 2023. Synergistic interaction of *Beauveria* bassiana (Vuillemin) with Emamectin benzoate improves its pathogenicity against Spodoptera litura (Fabricius). International Journal of Tropical Insect Science, 43(4): 1207-1217.
- Mahmoud, Y. A., I. M. A. Ebadah, W. Attwa, et al., 2020. Susceptibility of different tomato, *Solanum lycopersicum* L., varieties to infestation with some insect pests in Egypt. Bulletin of the National Research Centre, (44:46):1-5
- Malekan, N., B. Hatami, R. Ebadi, A. Akhavan and R. Radjabi, 2012. The singular and combined effects of entomopathogenic fungi, *Beauveria bassiana* (Bals) and *Lecanicillium muscarium* (Petch) with insecticide imidacloprid on different nymphal stages of *Trialeurodes vaporariorum* in laboratory conditions. Advances in Environmental Biology, 6(1): 423-432.
- Mitchell, P. L., R. Gupta, A. K. Singh and P. Kumar, 2004. Behavioral and developmental effects of neem extracts on *Clavigralla scutellaris* (Hemiptera: Heteroptera: Coreidae) and its egg parasitoid, *Gryon fulviventre* (Hymenoptera: Scelionidae). Journal of Economic Entomology, 97(3): 916-923.
- Moores, G. D., X. Gao, W. I. Denholm and A. L. Devonshire, 1996. Characterization of insensitive acetylcholinesterase in insecticide-resistant cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae). Pesticide Biochemistry and Physiology, 56: 102-110.

- Muthusamy, R., G. Vengateswari, S. Kumarasamy, R. Pandi, N. R. Prasannakumar, D. Arul, ... and G. Ramkumar, 2024. Combination effect of azadirachtin and chlorantraniliprole with three synergists against a serious invasive agricultural pest *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Biocatalysis and Agricultural Biotechnology, 55: 102992.
- Nomikou, M., A. R. Schraag and M. W. Sabelis, 2004. Vulnerability of *Bemisia tabaci* immatures to phytoseiid predators: consequences for oviposition and influence of alternative food. Entomologia Experimentalis et Applicata, 110: 95-103.
- Olson, D. L. and R. D. Oetting, 1999. The efficacy of mycoinsecticides *Beauveria bassiana* against silverleaf whitefly (Homoptera: Aleyrodidae) on poinsettia. Journal of Agricultural and Urban Entomology, 16(3): 179-185.
- Omprakash, S. and S. V. S. Raju, 2013. Bioefficacy of some insecticides against *Bemisia tabaci* (Genn.) on brinjal. Indian Journal of Entomology, 75(4): 310-314.
- Pavan, T., S. K. Ghosh and S. C. Bala, 2020. Effect of abiotic factors on seasonal incidence and bioefficacy of some newer insecticides against whitefly (*Bemisia tabaci* G.) on tomato crop (*Solanum lycopersicum* L.) in West Bengal. Plant Archives, 20(2): 2221-2228.
- Sarhozaki, M. T., S. Aramideh, J. Akbarian and S. Pirsa, 2021. Efficacy of *Beauveria bassiana* in combination with NeemAzal-T/S® on the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) and its natural enemies. Tropical Agriculture, 98(2): 160-171.
- Shalaby Sh.E.M., G.Y. Abdou and A.A. Sallam 2012. Pesticide residue relationship and its adverse effects on occupational workers in Dakahlyia, Egypt. Applied Biological Research 14 (1): 24–32.
- Shalaby, S. E. M. and G. Y. Abdou, 2020. Assessment of pesticide residues in blood samples of agricultural workers in Egypt. Journal of Plant Protection Research, 60(4): 369-376.
- Sheets, L. P., 2010. Imidacloprid: a neonicotinoid insecticide. In Hayes' handbook of pesticide toxicology (pp. 2055-2064). Academic Press.
- Srinivasan, R., D. Riley, S. Diffie, A. Sparks and S. Adkins, 2012. Whitefly population dynamics and evaluation of whitefly-transmitted tomato yellow leaf curl virus (TYLCV)-resistant tomato genotypes as whitefly and TYLCV reservoirs. Journal of Economic Entomology, 105(4): 1447-1456.
- Suarez-Lopez, Y. A., H. K. Aldebis, A. E. S. Hatem and E. Vargas-Osuna, 2022. Interactions of entomopathogens with insect growth regulators for the control of *Spodoptera littoralis* (Lepidoptera: Noctuidae). Biological Control, 170: 104910.
- Thuppukonda, M. and A. Kumar, 2022. Efficacy of selected insecticides against chili thrips (*Scirtothrips dorsalis* Hood). The Pharma Innovation Journal, SP-11(5): 591-595.
- Wu, S., Y. Gao, Y. Zhang, E. Wang, X. Xu and Z. Lei, 2014. An entomopathogenic strain of *Beauveria bassiana* against *Frankliniella occidentalis* with no detrimental effect on the predatory mite *Neoseiulus barkeri*: Evidence from laboratory bioassay and scanning electron microscopic observation. PLoS ONE, 9: e84732.
- Zawrah, M. F., A. T. El Masry, L. Noha and A. A. Saleh, 2020. Efficacy of certain insecticides against whitefly *Bemisia tabaci* (Genn.) infesting tomato plants and their associated predators. Plant Archives, 20(2): 2221-2228.