

Biological effects of *Beauveria bassiana* and *Akanthomyces attenuatus* isolates on *Aphis gossypii* Glover (Hemiptera: Aphididae)

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Abstract

The biological effects of entomopathogenic species, *Beauveria bassiana* (Bals.-Criv.) Vuill and *Akanthomyces attenuatus* Zare & Gams on cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) nymphs at 1×10^8 conidia concentration (ml^{-1}) were investigated at the laboratory conditions. The experiments were conducted at 25°C, 65% relative humidity, and 16:8 hours of lighting in a climatic cabinets. Alive nymphs were recorded on the 1st, 3rd, 5th, 7th, and 9th days of incubation. *B. bassiana* caused higher mortality of the nymphs than *A. attenuatus* did. A statistical difference was determined between the isolates on the 7th and 9th days of the experiment. The highest mortality rates were determined in the isolate of *B. bassiana* with 72% and the isolate of *A. attenuatus* with 54% on the 9th day. The LT_{50} value for the isolates of *B. bassiana* and *A. attenuatus* was 6.02 days and 8.33 days, respectively.

Keywords: *Aphis gossypii*, *Beauveria bassiana*, *Akanthomyces attenuatus*, Entomopathogenic fungus, Biological control

INTRODUCTION

Aphis gossypii Glover (Hemiptera: Aphididae) is widely distributed in tropical, subtropical and temperate regions. It is a polyphagous species that disrupts plant growth by feeding on phloem sap and is act as a virus vector (Martin et al., 2003). Among its hosts, there are more than 50 plant families, including Asteraceae, Cucurbitaceae, Rosaceae, and Solanaceae (Guldmond et al., 1994; Basu and Patro, 2007). *Aphis gossypii* is the vector of potato virus, citrus tristeza virus, cucumber mosaic virus, and turnip mosaic virus (Kennedy et al., 1962; Blackman and Eastop 2000; Mnari-Hattab et al., 2008; Behi et al., 2019). Pesticides with active ingredients, such as, bifenthrin, deltamethrin, imidacloprid and malathion are used to control this pest. With the long-term use of these chemicals, changes occur in the genetic structure of the pest and chemical resistance occurs. (Herron et al., 2000; Herron et al., 2001; Wang et al., 2002). It is reported that this pest was resistant to 50 active substances worldwide (Hollingsworth et al., 1994; Nauen and Elbert, 2003; Seyedbrahimi et al., 2016). As a result of the intensive application of these insecticides, the natural ecosystem (including the air, non-target organisms and humans) is affected and therefore, there is a tendency towards alternative control methods (Revathi et al., 2014). In recent decades, entomopathogenic fungi have been widely used as biocontrol agents for against harmful insects as they are pathogenic to insects (Akbari et al., 2013). These fungi are important in pest management with their metabolite production and biosecurity levels (Wang et al., 2019). Of these, *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea*, and several *Lecanicillium* species have attracted to great interest with their use in controlling aphids, and

a few of them have been used commercially (de Faria and Wraight, 2007; Lacey et al., 2015; Kumar et al., 2019). Well known entomopathogenic fungi such as *Beauveria* has been found to be effective on pests of Lepidoptera (Soetopo, 2004), Coleoptera (Lord, 2001; Wraight and Ramos, 2002) and Homoptera (Wraight et al., 1998). However, *Akanthomyces attenuatus* Zare & Gams is a well-known pathogen of whitefly, aphid and thrips, and some isolates of this species have been developed as commercial biopesticides (Wang et al., 2007; Ainsworth et al., 2008; Gottel et al., 2008; Lu et al. al., 2015). In this study, the effectiveness of *B. bassiana* and *A. attenuatus* isolates isolated from *Ips sexdentatus* (Boerner, 1776) (Coleoptera: Curculionidae) adults, on the second instar nymphs of *A. gossypii* was investigated.

MATERIALS AND METHODS

Rearing of plants and aphids

For the cotton plant (*Gossypium hirsutum* L.) to be used in the experiments, cotton seeds of variety Ergüven was planted in plastic pots with an equal ratio of soil:peat. The growth and development of these sown seeds was ensured through regular irrigations. When the cotton plants reached the height (10-15 cm) to be used in the experiments, adult individuals of *A. gossypii* were transferred to these plants and aphids were reared. For the continuity of this mass production, clean cotton plants were added to the medium regularly at weekly intervals. The production of both clean-healthy cotton plants and aphids was carried out in the climatic cabinets with 25°C temperature, 60±5% relative humidity, 16:8 (light:dark) hours conditions.

The *Beauveria bassiana* (Bals.-Criv.) Vuill. strain used in the study

The *B. bassiana* strain used in this study were isolated on Malt Extract Agar (MEA) media from surface of the bark beetle, *I. sexdentatus* distributed in *Pinus nigra* Arn. stands in the Western Mediterranean Region of Turkey (Karaceylan, 2023). After performing the morphological characterization studies of the isolate, the sequence information of the ITS gene regions of the rDNA was used for molecular characterizations and ITS1-ITS4 primer pairs were used for the amplification of the ITS region. The isolate was consistent with *B. bassiana* isolates (100% sequence similarity to *B. bassiana* KP862996.1 and OK094889.1; NCBI GenBank). Based on abundant conidia production, this isolate was selected for laboratory and field testing. The *B. bassiana* solution used in the study was prepared at a concentration of 1×10^8 conidia/ml.

The *Akanthomyces attenuatus* Zare & Gams strain used in the study

The *A. attenuatus* strain used in this study were isolated on MEA media from surface of the *I. sexdentatus* bark

beetle distributed over *P. nigra* stands in the Western Mediterranean Region of Turkey (Karaceylan, 2023). After performing the morphological characterization studies of the isolate, the sequence information of the ITS gene regions of the rDNA was used for molecular characterizations and ITS1-ITS4 primer pairs were used for the amplification of the ITS region. The isolate was consistent with *A. attenuatus* isolates (>98.90% sequence similarity to *A. attenuatus* MN908945.1 and MH231313.1; NCBI GenBank). Based on abundant conidia production, this isolate was selected for laboratory and field testing. The *A. attenuatus* solution used in the study was prepared at a concentration of 1×10^8 conidia/ml.

Application of entomopathogenic fungi against *Aphis gossypii*

Petri dishes with a diameter of 6 cm were used in the experiments. A thinly cut sponge was placed at the bottom of these Petri dishes to retain water. A layer of blotting paper was placed on this sponge, and a leaf of a cotton plant was placed on it. In each Petri dish prepared in this way, five 2nd nymph stage cotton aphid transfers were carried out. The isolates prepared at a conidia density of 1×10^8 (ml⁻¹) on aphids, were sprayed 3 times (2 ± 0.5 mg/1cm²) with a hand sprayer from a distance of 10-15 cm. Pure water was used for control application. The experiments were designed with 10 replications, allocating five nymphs in each Petri dish. The experiments were carried out in climatic cabinets with 25°C temperature, 60±5% relative humidity, 16:8 hours lighting conditions. Alive individuals were counted and recorded on the 1st, 3rd, 5th, 7th and 9th days after the applications.

Analysis of the data

Mortality rates (%) were calculated by applying the Abbott formula (Abbott, 1925). Probit analysis program was used to determine the LT₅₀ (50% time to death) value. By applying one-way analysis of variance to the obtained data, the difference between the mean numbers of alive nymphs was compared by use Tukey multiple comparison test at P<0.05 importance level. Statistical analyzes SPSS® 20.0 package program was used to analyze data obtained.

RESULTS AND DISCUSSION

The percent mortality rates (%) that occurred as a result of the application of entomopathogenic fungus isolates used in this study on *A. gossypii* nymphs are given in Figure 1. As a result of the applications, the percent mortality rate in the *B. bassiana* isolate was recorded at a higher rate than the *A. attenuatus* isolate on all counted days. Statistical differences were recorded between *B. bassiana* and *A. attenuatus* isolates only on the 7th and 9th days of the counts. While the percent mortality rates in *B. bassiana* isolate were 13, 25, 38, 62 and 72%, respectively, the percent mortality rates in *A. attenuatus* isolates were

recorded as 6, 17, 32, 41 and 54%, respectively.

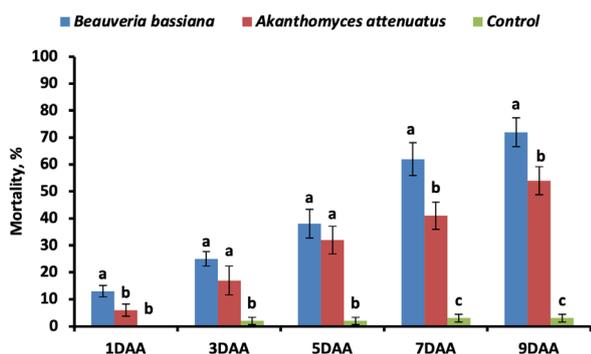


Figure 1. Percent mortality rates resulting from the application of entomopathogenic fungus isolates to *Aphis gossypii* nymphs. (The differences between the means (\pm standard error) of the columns indicated with different letters for each day are statistically significant (Tukey's HSD test $P < 0.05$)). DAA: Days after application.

As a result of probit analysis, the death time% (LT_{50}) was recorded as 6.02 days in *B. bassiana* isolate and 8.33 days in *A. attenuatus* isolate (Figure 2.3).

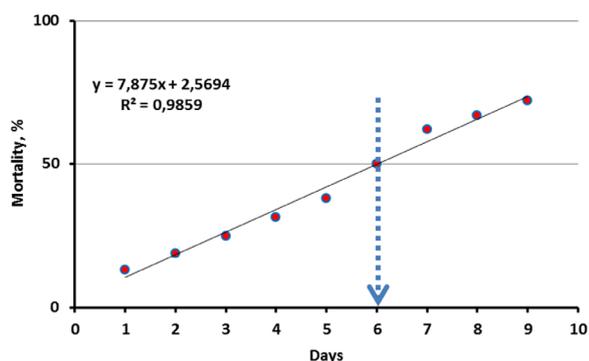


Figure 2. Time dependent mortality rates as a result of application of *Beauveria bassiana* to *Aphis gossypii* nymphs

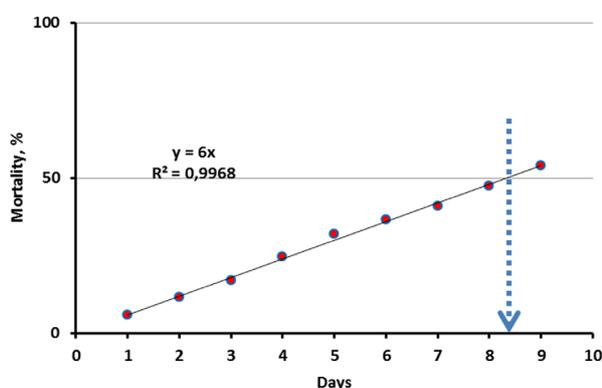


Figure 3. Time dependent mortality rates as a result of application of *Akanthomyces attenuatus* to *Aphis gossypii* nymphs

It was concluded that *B. bassiana* isolate was more effective than *A. attenuatus* isolate on *A. gossypii* nymphs. When we look at the studies, as a result of the application of *B. bassiana* Bb-5a isolate to *A. gossypii* individuals under field conditions, a 75.1% reduction in the pest population was recorded (Ramanujam et al., 2018). As a result of the application of four *Beauveria* isolates and two *Metarhizium* isolates to *A. gossypii* at different temperatures, 73.33-93.33% mortality rates and LT_{50} value 3.83-4.98 days at 25 °C; mortality rates of 82.22-100% at 30 °C and LT_{50} values were recorded as 3.23-4.02 (Tesfaye and Seyoum, 2010). *B. bassiana* (Bals.-Criv.) Vuill IRAN 429C, IRAN 108 and LRC 137 three isolates were administered to adult individuals of *A. gossypii* at a concentration of 10^8 conidia ml^{-1} , resulting in LT_{50} values of 2.90, 3.84, and 4.64 days, respectively (Mousavi et al., 2020). Application of *B. bassiana* isolated from *Hypera postica* Gyllenhal (Coleoptera: Curculionidae) at 10^6 spores/ml concentration to *A. gossypii* adult individuals resulted in LT_{50} value of 5.66 days, and application of *B. bassiana* isolated from *Sphingonotus* sp. (Orthoptera: Acrididae) at the same concentration was recorded as 3.32 days. As a result of the application of *B. bassiana* isolated from *Sphingonotus* sp. to *A. gossypii* at 10^4 , 10^5 and 10^6 concentrations, mortality rates of 33.5-96.7% were recorded (Anonymous, 2023). As a result of the application of *B. bassiana* and *Lecanicillium lecanii* isolates to *A. gossypii* individuals, the reproduction time and reproduction rate of the pest were affected (Gurulingappa et al., 2011). *Akanthomyces attenuatus* Zare & Gams has been reported to be a pathogen of whitefly, aphids, thrips and mites. Some isolates of this species have been developed as commercial biopesticides (Wang et al., 2007; Ainsworth et al., 2008; Gottel et al., 2008; Lu et al., 2015). Considering the studies, it was noted that the net reproductive power of *A. gossypii* decreased as a result of the application of *Lecanicillium attenuatum* Zare & W. Gams CS625 (Kim, 2007). In another study, a mortality rate of 87.7% was recorded as a result of the application of *Akanthomyces muscarius* (DIKA11/1) isolate to sunn pest at a concentration of 1×10^7 conidia/ml (Gül et al., 2022). As a result of the application of *Lecanicillium attenuatum* to *A. gossypii* first-stage nymphs at 1×10^4 and 1×10^8 conidia/ml concentrations, it was noted that the life span of the pest was shortened (10.8 and 8.4 days). Total fecundity was recorded at 1×10^4 (41 ± 7.3 nymph), 1×10^6 (26 ± 0.8 nymph) and 1×10^8 conidia/ml concentrations (22 ± 5.7 nymph) (Kim, 2007). As a result of the application of *Lecanicillium muscarius* (Zare & Gams) at 1×10^7 and 1×10^6 spore/ml concentrations to *A. gossypii*, a 100% mortality rate was determined (Razmjou et al, 2016).

CONCLUSIONS

As a result of this study, it was found that *B. bassiana* isolated from *I. sexdentatus* adults was more effective on *A. gossypii* nymphs than *A. attenuatus* isolate. Considering

the results obtained in this study and previous studies, it was noted that entomopathogenic fungi are a good alternative to chemical pesticides in aphid control. More studies are needed to determine the insecticidal activities of the entomopathogenic fungi used in this study as biological control agents in greenhouse and field conditions.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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