A study of the effect of the fungus *Beauveria bassiana* on killing the cotton aphid Glover Aphis gossypii on okra (*Abelmoschus esculentus*).

Tabark Shaker Saeed[1*] , Abdul Nabi Abdul Amir Matroud[2] and Muhammed Alwan Salman[3]

[1,2,3] Department of Plant Protection, College of Agriculture, Basrah University, Basra, Iraq.
*Corresponding author's email: tabark.shaker@uobasrah.edu.iq ,
abdul_nabi.matrwod@uobasrah.edu.iq , mohammed.salman@uobasrah.edu.iq

Abstract

Okra is affected by several pests, including the aphid (*Aphis gossypii Glover*). Nymphs are 0.5-1.2 mm in size, pale green to yellow, and sometimes gray. Infestations with this insect cause significant economic losses to their hosts due to direct or indirect damage. The results showed a significant ($P \leq 0.05$) superiority of the 10^6 CFU/ml *B. bassiana* suspension in killing cotton aphids compared to other treatments, with the highest insect killing rate reaching 69% after 72 hours. Meanwhile, the 104 concentration recorded a weak effectiveness, not exceeding 30% after 72 hours of testing. While the 20% concentration of *B. bassiana* fungal filtrate significantly ($P \leq 0.05$) killed the cotton aphid, *A. gossypii*, compared to the other treatments, achieving a 54% insect kill rate after 72 hours. The lowest insect kill rate after 24 hours of testing was achieved with the 10% fungal filtrate concentration compared to the other treatments. The insect kill rate increased with increasing time, rising with the length of exposure, regardless of the concentration used. The highest kill rate was achieved after 72 hours compared to 24 hours for all treatments. However, the optimal concentration is 20% for use in biological control programs.

Keywords

Beauveria bassiana, *Aphis gossypii* Glover, *Abelmoschus esculentus*.

Introduction

Global okra production is estimated at 7.83 million tons annually [14]. Canned, dried, and frozen okra are consumed off-season. Okra seeds contain between 15 and 26% protein and more than 14% edible oil [23, 18].

The cotton aphid typically infects the underside of okra leaves, causing curling and distortion of young leaves and stems. In severe cases, it also occurs on stems, fruits, and the upper surface of leaves. It causes significant economic losses through feeding, through bark secretions and honeydew secretion, which

ISSN 2072-3857

leads to the growth of black sooty mold, which inhibits photosynthesis, or through the transmission of viruses to the plant, leads to reduced plant vigor, reduced fruit quality, and decreased productivity [22, 16 , 8].

Beauveria bassiana is characterized by its high ability to infect a wide range of economically harmful insects, which has made it a major focus of integrated biological control (IPM) programs [33, 10].

B. bassiana grows naturally in soil and on various insect species. Infection with this fungus begins when spores (conidia) attach to the surface of the insect's body. After entering the host, they grow and penetrate the exoskeleton using chitin- and protein-degrading enzymes such as chitinase and protease. The fungus spreads internally, releasing toxins such as beauvericin and bassianolide, which lead to insect death within several days. The fungus then emerges to the surface to produce more spores, causing white muscardine disease [35,26].

The use of *B. bassiana* is a more environmentally friendly control method, as it is harmless to human health [27, 21].

Therefore, the study aimed to find effective and environmentally friendly natural alternatives, such as the fungus *B. bassiana*, to combat the cotton aphid on okra as part of Integrated Pest Management (IPM) programs. This alternative, instead of using chemical control methods such as pesticides, is due to their harmful effects on health and the environment in general.

Materials and Methods:

Preparation of Potato Dextrose Agar (PDA) media:

The media was prepared according to the manufacturer's instructions. Dissolving 40 gm of prepared PDA powder in 1 liter of distilled water in a 1000 ml flask, the flask was then filled with 250 mg of the antibiotic chloramphenicol. The mouth of the volumetric flask was sealed with cotton and then placed in an autoclave at 121°C and 15 psi for 15 minutes. After sterilization, the flask was allowed to cool to 55°C. Before the medium solidified, it was poured into sterile Petri dishes for laboratory experiments [28].

***Beauveria bassiana* Fungus Cultivation:**

The identified and prepared *Beauveria bassiana* fungus was obtained from the laboratories of the Plant Protection Department, College of Agriculture, University of Basrah, Iraq. It was grown on sterile PDA culture media. A 0.5 cm disc was taken using a sterile cork piercer and the fungus disc was placed in the center of the dish. The dishes were incubated at 28°C in an incubator for 7 days, after which they were refrigerated at 4°C until use [24].

Insect Rearing:

Pupae and adult Aphis gossypii were collected from a field infested with this insect, were placed in special collection boxes. Then, they were transported to the laboratory after being placed in a special insect rearing box. The insect was kept at a temperature of 25°C, humidity of 50-75%, and 16 hours of light and 8 hours of darkness. Insect rearing was monitored daily or every few days. The offspring count was checked, the leaves were observed, and any predatory or dead insects were removed [19].

Insect Diagnosis:

ISSN 2072-3857

Aphid-infected leaves were collected from okra fields infected with this insect. The infected leaves were placed in nylon bags. These bags were labeled with information (plant host name and sample collection date). They were transported to the laboratory at the University of Basra, College of Agriculture, for identification. The insect was identified using taxonomic keys by Prof. Dr. Muslim Ashour Abdul Wahid at the Natural History Museum of the University of Basra [5].

The effect of different concentrations of *B. bassiana* fungal filtrate on killing the cotton aphid, *A. gossypii*:

Three concentrations (10, 20, and 30%) of *B. bassiana* fungal filtrate were used with a control treatment (distilled water) and sprayed onto the insect. Killer readings were taken from 25 insects within 24, 48, and 72 hours [13].

The effect of different concentrations of *B. bassiana* fungus suspension on killing the cotton aphid, *A. gossypii*:

Three dilutions (10^4, 10^5, and 10^6) of B. bassiana suspension were used with a control treatment (distilled water) and sprayed onto the insect. Killing readings were taken from 25 insects at 24, 48, and 72 hours [1].

Statistical Analysis:

SPSS version 12 was used to analyze the data, using a completely randomized design (CRD). Test data were analyzed using the least significant difference (LSD) test at a probability level of ($P \leq 0.01$) [7].

Results and Discussion:

Diagnosing the cotton aphid *A. gossypii*:

Figure (1) shows the diagnosis of the cotton aphid on okra plants based on its morphological characteristics. The nymphs are 0.5-1.2 mm in size. The color is pale green to yellow, sometimes gray. The body is oval and flat, and the antennae are short, often consisting of five segments.

The wingless adult (apterous adult) measures 1.0–1.8 mm. The color varies depending on the plant host (green, yellow, brown, gray, black). The body is small and oval with a smooth surface. The antennae are usually six-segmented, and the tail is short, conical, or triangular in shape, and light in color. The caudal horns (cornicles) are short, thick, dark, and cylindrical [6].

As for the winged adult, the head and thorax are dark (shiny black), and the wings are transparent with distinct veins that cover the body at rest. The abdomen is variable in color (green, gray), with dark spots, and the antennae are relatively longer than those of the wingless adult [2,3].

A. gossypii is a polyphagous insect, it infects more than 700 plant species, most notably cotton, melon, cantaloupe, cucumber, okra, tomato, pepper, and other crops. It causes general plant weakness by sucking sap. It secretes honeydew that leads to the growth of sooty mold, transmits several plant viruses, including cucumber mosaic virus (CMV) and watermelon yellow dwarf virus (WMV, ZYMV), causes damage by sucking sap from leaves and growing tips, leading to leaf curl, stunted growth, yellowing, or, in severe cases, plant death [4,29].

ISSN 2072-3857

Figure (1) *A. gossypii* cotton borer at 40x magnification.

Effect of *B. bassiana* fungus suspension on the cotton aphid, *A. gossypii*:

Results of the effect of *B. bassiana* on the cotton aphid (Table 1) showed that the fungus' biological effectiveness varied significantly between the tested concentrations (10^4, 10^5, and 10^6). 10^6 CFU/ml diluted was significantly superior ($P \leq 0.05$) to the other treatments. The highest insect mortality rate reached 69% after 72 hours at the 106 concentration. This is attributed to the fact that high concentrations produce a sufficient number of spores to initiate a successful infection, starting by attaching to the insect's body, then penetrating the cuticle and spreading within the body, ultimately leading to its death. Meanwhile, the 10^4 concentration recorded a weak effectiveness, not exceeding 30% after 72 hours of testing. These results are consistent with de Faria and Wraight. [11], where they indicated that the effectiveness of entomopathogenic fungi depends directly on the concentrations used to achieve effective infection.

By analyzing the time-course data, it is observed that the fungal efficacy gradually increases over time. At the highest concentration, the killing rate increased from 56% after 24 hours to 69% after 72 hours, a gradual progression consistent with the physiological stages of fungal growth within the host. Hajek *et al*. [17] indicated that most *Beauveria* species require at least 48 hours after attachment for germination to begin, followed by penetration, enzyme secretion, and reproduction within the insect's body. The longer the time, the greater the chance of the fungus spreading and infecting. This is also confirmed by Gottel *et al.* [15], who believed that infection requires sufficient time to ensure internal spread and suppress the insect's immune system.

The results of this study are largely consistent with previous research. El-Husseini [9] confirmed that *B. bassiana* was effective against aphids when applied at a concentration of 106, achieving a 70% kill rate within 72 hours. Wraight *et al.* [30] also noted that low concentrations may be insufficient to induce infection, especially in species with relatively thick cuticles or strong immune defenses. Yar *et al.* [32] reported that high temperatures or low humidity may reduce conidia germination and, consequently, fungal activity.

ISSN 2072-3857

Table (1) The killing rate of the cotton aphid *A. gossypii* per 100 ml of *B. bassiana* fungus suspension.

Time (Hours)	Dilution			Control
	10^4	10^5	10^6	
24	16	28	56	12
48	28	36	58	16
72	30	40	69	28
L.S.D$_{0.05}$	4.32	4.75	4.98	N.S

Effect of *B. bassiana* fungal filtrate on the cotton aphid *A. gossypii*:

The results of the *B. bassiana* fungal filtrate on killing the cotton aphid *A. gossypii* (Table 2) showed that the 20% concentration significantly (P≤0.05) killed the aphid compared to the other treatments, with a 54% insect kill rate after 72 hours. The lowest insect kill rate was 20% after 24 hours of testing with the 10% fungal filtrate concentration compared to the other treatments. These results demonstrated a clear progressive effect with increasing time in the effectiveness of *B. bassiana* in killing the insect. It increased with the length of exposure, regardless of the concentration used. The mortality rates after 72 hours were higher than those recorded at 24 hours for all treatments. However, the 20% concentration was the most efficient and effective in controlling *A. gossypii*. It may be the optimal concentration for biological use in control programs. These results were consistent with Lnglis *et al*. [20], who suggested that very high concentrations of the fungus may negatively affect its biological effectiveness, due to interference between conidia or toxic effects on the microenvironment surrounding the insect's body. These results were also consistent with Quesada Moraga *et al*. [25], who suggested that medium concentrations of *B. bassiana* were more effective in killing aphids than high and low concentrations.

Faria *et al.* [12] reported that using moderate concentrations of fungi with appropriate exposure periods yielded significantly better results than using high concentrations, which may cause decreased effectiveness due to conidia clumping or fungal self-inactivation. Wraight *et al.* [31] confirmed that the biological effectiveness of the fungus is directly related to its growth period on the insect's body, which fully supports our results, which showed increased lethality over time.

Table (1) The killing rate of the cotton aphid *A. gossypii* per 100 ml of *B. bassiana* fungus filtrate.

Time (Hours)	Concentrate (%)			Control
	10	**20**	**30**	
24	20	24	20	22
48	30	42	28	26
72	35	54	32	29
L.S.D$_{0.05}$	4.52	4.75	N.S	N.S

ISSN 2072-3857

Conclusions:

The results of the *B. bassiana* fungus demonstrated high efficacy in killing cotton aphids in the laboratory. 20% *B. bassiana* filtrate was the most efficient at killing aphids, achieving a 54% mortality rate after 72 hours. *B. bassiana* suspension was the most effective, achieving a 69% mortality rate after 72 hours, proving its biological effectiveness as a pest control agent.

Recommendations:

The possibility of using the fungus *B. bassiana* in integrated pest management (IPM) programs to increase the effectiveness of cotton aphid control. Conduct additional field studies to confirm laboratory results, particularly in different agricultural environments and diverse climatic conditions, to ensure the effectiveness of the results on a large scale. Expand the study of the side effects of *B. bassiana* fungi on other beneficial natural enemies (predators and parasitoids) to avoid harming the ecological balance. Encourage farmers to partially replace chemical pesticides with plant and fungal extracts to reduce environmental pollution and protect human health. Developing commercial bioproducts from *B. bassiana* for use as environmentally friendly alternatives in sustainable agriculture.

References:

1- **Baki, D., & Erler, F. (2024).** Evaluation of indigenous isolates of Beauveria bassiana (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) against the cotton *Aphis gossypii* Glover. Journal of Plant Diseases and Protection, 131, 1583–1595.

2- **Blackman, R. L., & Eastop, V. F. (2008).** *Aphids on the world's herbaceous plants and shrubs, 2 volume set*. John Wiley & Sons.

3- **Bouker, S., Mansour, R., Selmi, G., & Kamel Ben Halima, M. (2025).** Biological parameters of *Aphis gossypii* (Hemiptera: Aphididae) on three Armenian cucumber varieties grown in Tunisia. *Phytoparasitica*, *53*(5), 64.

4- **Capinera, J. L. (2001).** *Vegetable Leafminer, Liriomyza sativae Blanchard (Insecta: Diptera: Agromyzidae)*. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS.

5- **Choi, H., Favret, C., & Lee, S. (2025).** First record of the tropical aphid Schoutedeniaralumensis (Hemiptera, Aphididae) from Cambodia, with a re-description of the oviparous females and DNA barcoding. *Biodiversity Data Journal*, *13*, e159374.

6- **Cocuzza, G. (2022).** *Aphis gossypii* (cotton aphid). *Pest, natural enemy, invasive species, vector of plant pest (CABI Compendium)*.

7- **Dean, A. M., & Voss, D. (1999).** Design and Analysis of Experiments. Springer (Springer Texts in Statistics).

ISSN 2072-3857

8- **Delancy, D. D., Hall, T. A., & Garraway, E. (2024).** An Assessment of Host Preference of *Aphis gossypii* Glover, 1877 (Hemiptera) in Jamaica. *Caribbean Journal of Science*, *54*(1), 1-10.

9- **El Husseini, M. M. (2019).** Effect of the fungus, *Beauveria bassiana* (Balsamo) Vuillemin, on the beet armyworm, Spodoptera exigua (Hübner) larvae (Lepidoptera: Noctuidae), under laboratory and open field conditions. *Egyptian Journal of Biological Pest Control*, *29*(1), 52.

10- **El-Dydamony, M. K., Soltan, E., & Said, S. M. (2021).** The Effect of an Entomopathogenic Fungus, *Beauveria bassiana* on Flight Muscles of the Desert Locust, Schistocerca gregaria Forskal. *Egyptian Academic Journal of Biological Sciences. A, Entomology*, *14*(1).

11- **Faria, M. R., & Wraight, S. P. (2007).** Mycoinsecticides and mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Control, 43(3), 237–256.

12- **Faria, M., Hotchkiss, J. H., Hajek, A. E., & Wraight, S. P. (2010).** Debilitation in conidia of the entomopathogenic fungi *Beauveria bassiana* and Metarhizium anisopliae and implication with respect to viability determinations and mycopesticide quality assessments. *Journal of invertebrate pathology*, *105*(1), 74-83.

13- **Ferreira, J. M., et al. (2024).** The combination of enzymes and conidia of entomopathogenic fungi against *Aphis gossypii* nymphs and Spodoptera frugiperda larvae. Journal of Fungi, 10(4), 292.

14- **Food and Agriculture Organization of the United Nations. (2023).** Okra (Abelmoschus esculentus) — Production — FAOSTAT Statistical Database. Retrieved from FAOSTAT via Wikipedia.

15- **Goettel, M. S., Douglas Inglis, G., Duke, G. M., Lord, J. C., & Jaronski, S. T. (2021).** Measurement of internal *Beauveria bassiana* to ascertain non-target impacts on arthropods in field environments. *Biocontrol Science and Technology*, *31*(8), 834-849.

16- **Guo, P., Wang, L., Miao, P., Niu, Y., Li, C., An, J., ... & Zhang, T. (2025).** A combination of predator and selective pesticide improves control of resistant *Aphis gossypii* (Hemiptera: Aphididae). *Journal of Economic Entomology*, *118*(3), 1083-1092.

17- **Hajek, A. E., Everest, T. A., & Jaronski, S. T. (2025).** Application of *Beauveria bassiana* conidia to spotted lanternfly forewings causes fewer infections than abdominal applications. *Journal of Invertebrate Pathology*, *211*, 108335.

18- **Harinkhere, S. (2014).** Studies on insect pest complex of okra (Abelmoschus esculentus (L.) Moench) and their chemical control. *M. Sc.(Ag.) thesis, JNKVV, Jabalpur, Madhya Pradesh*, 20-56.

19- **Hosseini, M., Ashouri, A., Enkegaard, A., Goldansaz, S. H., Nassiri Mahalati, M., & Hosseininaveh, V. (2010).** Performance and population growth rate of the cotton aphid, and associated yield losses in cucumber, under different nitrogen fertilization regimes. *International Journal of Pest Management*, *56*(2), 127-135.

ISSN 2072-3857

20- **Inglis, G. D., Goettel, M. S., Butt, T. M., & Strasser, H. E. R. M. A. N. N. (2001).** Use of hyphomycetous fungi for managing insect pests. In *Fungi as biocontrol agents: progress, problems and potential* (pp. 23-69). Wallingford UK: CABI publishing.

21- **Mishra, S., Kumar, P., & Malik, A. (2016).** Suitability of agricultural by-products as production medium for spore production by *Beauveria bassiana* HQ917687. *International Journal of Recycling of Organic Waste in Agriculture*, *5*(2), 179-184.

22- **Mkhabela, S. S., Shimelis, H., Gerrano, A. S., & Mashilo, J. (2022).** Phenotypic and genotypic divergence in okra [Abelmoschus esculentus (L.) Moench] and implications for drought tolerance breeding: A review. *South African Journal of Botany*, *145*, 56-64.

23- **Neeraja, G., Vijaya, M., Chiranjeevi, C. H., & Gautham, B. (2004).** Screening of okra hybrids against pest and diseases. *Indian Journal of Plant Protection*, *32*(1), 129-131.

24- **Pham, T. A., Kim, J. J., Kim, S. G., & Kim, K. (2009).** Production of blastospore of entomopathogenic *Beauveria bassiana* in a submerged batch culture. Mycobiology, 37(3), 218–224.

25- **Quesada-Moraga, E., Ruiz-García, A., & Santiago-Alvarez, C. (2006).** Laboratory evaluation of entomopathogenic fungi *Beauveria bassiana* and Metarhizium anisopliae against puparia and adults of Ceratitis capitata (Diptera: Tephritidae). *Journal of economic entomology*, *99*(6), 1955-1966.

26- **Raya-Díaz, S., Sánchez-Rodríguez, A. R., Segura-Fernández, J. M., Del Campillo, M. D. C., & Quesada-Moraga, E. (2017).** Entomopathogenic fungi-based mechanisms for improved Fe nutrition in sorghum plants grown on calcareous substrates. *PloS one*, *12*(10), e0185903.

27- **Umaru, F. F., & Simarani, K. (2020).** Evaluation of the potential of fungal biopesticides for the biological control of the seed bug, Elasmolomus pallens (Dallas) (Hemiptera: Rhyparochromidae). *Insects*, *11*(5), 277.

28- **Wang, H., *et al*. (2024).** Ldentification, characterization, and sensitivity to phytochemicals of a novel Curvularia species associated with leaf spot disease on Curcuma kwangsiensis. Scientific Reports, 14, Article 77524.

29- **Wang, W., Zhang, R., Liu, H., Ding, R., Huang, Q., Yao, J., & Liang, G. (2024).** Resistance development, cross-resistance, and fitness costs associated with *Aphis gossypii* resistance towards sulfoxaflor and acetamiprid in different geographical regions. *Journal of Integrative Agriculture*, *23*(7), 2332-2345.

30- **Wraight, S. P., Galaini-Wraight, S., Howes, R. L., Castrillo, L. A., Carruthers, R. I., Smith, R. H., ... & Keith, L. M. (2018).** Prevalence of naturally-occurring strains of *Beauveria bassiana* in populations of coffee berry borer Hypothenemus hampei on Hawai'i Island, with observations on coffee Plant-H. hampei-B. bassiana interactions. *Journal of invertebrate pathology*, *156*, 54-72.

31- **Wraight, S. P., Howes, R. L., Castrillo, L. A., Griggs, M. H., Galaini-Wraight, S., Carruthers, R. I., ... & Keith, L. M. (2022).** Laboratory studies assessing the

ISSN 2072-3857

microbial biocontrol potential of diverse strains of *Beauveria bassiana* isolated from coffee berry borer, with emphasis on strains from Hawai 'I Island and comparisons to commercial strain GHA. *Journal of invertebrate pathology*, *194*, 107819.

32- Yar, A., *et al*. (2024). Unveiling the germination patterns of Alternaria porri (Ellis) Keissl. Scientific Reports, 14, Article 76050.

33- Zimmermann, G. (2007). Review on safety of the entomopathogenic fungus Metarhizium anisopliae. *Biocontrol Science and technology*, *17*(9), 879-920.

34- Mascarin, G. M., & Jaronski, S. T. (2016). The production and uses of Beauveria bassiana as a microbial insecticide. World Journal of Microbiology and Biotechnology, 32(11), 177.

ISSN 2072-3857