Native Isolates of Entomopathogenic Fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, as Potential Biological Control Agents Against Pistachio Stink Bug, *Acrosternum arabicum* (Hemiptera: Pentatomidae)

Abbas Esmaeili Sardary (PhD Student)[1,2] , Fateme Ranjbara (PhD)[2], Mandana Motesaddi Zarandi (BSc)[2], Yazdan Nasiri (BSc)[2], Sepehr Salari Rafsanjanipoor (BSc)[2], Maryam Rashki (PhD)[3*], Mohammad Amin Jalali (PhD)[1,2]

[1] *Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran*

[2] *Department of Crop Protection, College of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran*

[3] *Department of Biodiversity, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran*

Information

Article Type:
Original Article

Article History:

Received: 10.06.2024
Accepted: 15.09.2024

Doi: 10.22123/PHJ.2025.489611.1176

Keywords:
Myco-entomopathogens
Humidity
Virulence
Mortality
Integrated Pest Management

Corresponding Author:
Maryam Rashki

Email: ma_rashkigh@yahoo.com

Tel: +34-33776611

Abstract

Background*:* Entomopathogens such as *Metarhizium anisopliae* and *Beauvaria bassiana* have undergone extensive study for their pathogenic impact on various insects and have been globally employed as myco-biocontrol agents for the ecological regulation of agricultural pests.

Materials and Methods: This study assesses the biocontrol efficacy of three isolates of *B. bassiana* (GHA, DEBI008, and Z1) and one isolate of *M. anisopliae* against *Acrosternum arabicum,* a primary pest of pistachio nuts in Iran, and explores the impact of humidity on their virulence under controlled laboratory conditions (25±2 ºC, 75-95% RH, and a 16L:8D photoperiod).

Results: The research confirmed the susceptibility of *A. arabicum* to all four fungal isolates, with higher humidity levels intensifying their virulence. Specifically, *B. bassiana* isolates DEBI008 and Z1 displayed the shortest median lethal times (LT50) at 75% relative humidity, inducing 100% mortality within 16 days. In contrast, *M. anisopliae* exhibited a heightened response to humidity, leading to a rapid 50% mortality rate at 95% relative humidity within 4 days. While none of the isolates achieved complete mortality at a low concentration of 1×10^5 conidia/mL, the findings suggest promising avenues for future field investigations.

Conclusions: This study underscores the potential of *B. bassiana* and *M. anisopliae* as effective biological control agents, potentially integrating them into sustainable pest management strategies for pistachio orchards. Further field trials are recommended to validate laboratory results and promote the adoption of these biocontrol agents in Integrated Pest Management (IPM) programs.

►Please cite this article as follows:
Esmaeili Sardary A, Ranjbar F, Motesaddi Zarandi M, Nasiri Y, Salari Rafsanjanipoor S, Rashki M, Jalali MA. Native isolates of entomopathogenic fungi, *Beauvaria bassiana* and *Metarhizium anisopliae* as potential biological control agents against pistachio stink bug, *Acrosternum arabicum* (Hemiptera: Pentatomidae). Pistachio and Health Journal. 2024;7(1-2):82-91.

1. Introduction

Pistachio (*Pistachio vera* L.) is an economically important agricultural crop, known for its nutritional value and health benefits [1]. The global demand for pistachios has increased, significantly influencing the economies of major producing countries such as Iran and the United States [2]. This valuable tree is consistently targeted by different pests such as green stink bugs (Hemiptera: Pentatomidae). *Acrosternum arabicum*, *Brachynema germari*, and *Chroanta ornatula* are the most abundant pentatomid species found in Iranian pistachio orchards. Their damage begins at the pistachio hardening stage and continues until storage.

The feeding activities of stink bugs can cause pistachio deformation or lead to pathogen infections, resulting in significant economic losses. As a result, the primary management strategy for stink bugs in pistachio cultivation has been the application of synthetic chemical insecticides [3]. However, the development of insecticide resistance within stink bug populations has become a major challenge [4], reducing the efficacy of pesticides and often necessitating increased application rates. This has raised concerns about the environmental and health impacts of chemical pesticides, leading to a growing global emphasis on sustainable pest management practices. In response, Integrated Pest Management (IPM) strategies have been promoted, and alternative approaches have been explored as potential substitutes for chemical pesticides. These alternatives include the use of botanical insecticides [5], parasitoids [6- 8], and entomopathogenic fungi (EPF) [9], which offer promising solutions for managing stink bug populations in a more environmentally friendly and sustainable manner.

Beauveria spp. (Ascomycota: Hypocreales) and *Metarhizium* spp. (Ascomycota: Hypocreales) are among the most widely used entomopathogenic fungi for microbial control [10]. Several studies have examined the effects of *B. bassiana* isolates on various stink bug species in controlled environments [11]. The relationship between humidity and the viability of B. bassiana and M. anisopliae conidia is an active area of research. Several research works suggest that elevated relative humidity (RH) levels, typically ranging from 70% to 90%, are optimal for spore germination and the ensuing fungal development [12]. For instance, one study demonstrated that prolonged exposure to high humidity significantly increased germination rates of both fungi compared to cultures maintained at low humidity levels. These findings highlight the critical role of environmental moisture in sustaining the life cycle and virulence of entomopathogenic fungi [13]. In contrast, low humidity conditions can significantly reduce spore longevity and compromise the fungi's overall infectious capacity. Conidia are highly susceptible to desiccation, which can cause the collapse of the protective layers surrounding the spores, ultimately leading to reduced viability [12]. This highlights the importance of considering environmental conditions when developing strategies that utilize these fungi for pest control, as variations in humidity can substantially limit control efficacy. Despite the widespread use of *Beauveria* spp. and *Metarhizium* spp., the specific responses of these fungal isolates to varying humidity levels and their differential effects on the mortality of *Acrosternum arabicum*, a primary pest of pistachio nuts in Iran, remain relatively unexplored. The objective of this study was to evaluate the biological control potential of three isolates of *B. bassiana* and one isolate of *M. anisopliae* against *A. arabicum*, and to investigate the impact of humidity on their virulence under laboratory conditions. The findings of this study are expected to enhance our understanding of the pathogenicity of these fungi against pistachio green stink bugs and may have implications for the practical application of myco-entomopathogens in augmentative biological control strategies against these pests.

2. Materials and methods

2.1. Rearing *A. arabicum*

Adults of *A. arabicum* (420 individuals, sex ratio 1:1) were collected from a pistachio orchard in Ahmad Abad Dafee (N= 30º 27' 34.807, E= 55º 40' 27.2172), Rafsanjan, Kerman. The insects were distributed into six plastic boxes (28x11x21 cm) with perforated lids for aeration and maintained in a constant temperature room at 25±2 ºC, 50%±10 RH, and a 16L:8D photoperiod as described by [14]. The insects were fed a diet of fresh green beans, sunflower seeds, and peanuts, which was replaced every 4 days [15].

2.2. Fungi Isolates

Three isolates of *B. bassiana* (GHA, the active ingredient of BotaniGard® (Certis USA, Columbia, MD), DEBI008, and Z1) and one isolate of *M. anisopliae* (GeneBank Number: ITS GENE: (NCBI): KP213288) were used in this experiment. The isolates were kindly provided by Maryam Rashki (Department of Biodiversity, Institute of Science and High Technology and Environmental Sciences, Kerman, Iran), Javad Karimi, (Department of Plant Protection, Faculty of Agriculture, University of Ferdowsi, Mashhad, Iran), and Department of Plant Protection, Urmia university, Urmia, Iran. The isolates were first cultivated on PDA (Potato Dextrose Agar) media, and after approximately 14 days, during which the isolates had reached maximum growth, they were harvested and subsequently utilized to inoculate adult A. arabicum individuals. The dead insects were then sterilized by dipping them in 1.5% sodium hypochlorite for 3 minutes, followed by 2 minutes in 70% ethanol, and then rinsed three times in sterile distilled water [16]. After 4 days, the dead insects were incubated in a growth chamber at 25±1 ºC and 95%±5 RH in complete darkness, allowing the isolates to emerge from the insect bodies. The isolates were then harvested and cultivated on PDA media again. After three passages, the experiment began.

2.3. Experimental setup and bioassay

A total of 255 pairs (510 individuals, sex ratio 1:1, 40-day-old adults) of *A. arabicum* were individually dipped for 8 seconds in 40 ml of each of 17 treatments (Table 1) following the method described by [17]. To evaluate the effect of humidity on isolate virulence, each treatment was inoculated into bugs (15 pairs, 30 individuals) and separated into 3 parts. Each part, containing 10 bugs, was placed in a sterile Petri dish (12.5 cm) with an autoclaved moistened filter paper, sterile diet, and a lid with at least 20 pores for aeration. Each of 10 Petri dishes was then placed in a large plastic box (50x20x30 cm) with a completely closed lid. To maintain a 75% RH condition in the large box, 1071g of NaCl was dissolved in 3 liters of distilled water at the bottom of the box. Sterile cups filled with saturated distilled water and NaCl were placed in the box, and the Petri dishes were placed on top of the cups. Similarly, for the

80% RH condition, 1020g KCl was dissolved in 3 liters of distilled water, and for the 95% RH condition, sterile distilled water was used. All large boxes were placed in a growth chamber at 25±1 ºC, 16L, 8D photoperiod, and observed daily for 17 days to evaluate stink bug mortality.

Table 1. List of treatments and concentration rates used in different experiments.

No	Treatments	Application concentration
1	Sterile distilled water + 0.02% tween 80 (control)	-
2	*Beauveria bassiana* GHA + 0.02% tween 80	1×10^5 conidia/ml
3	*Beauveria bassiana* GHA + 0.02% tween 80	1×10^6 conidia/ml
4	*Beauveria bassiana* GHA + 0.02% tween 80	1×10^7 conidia/ml
5	*Beauveria bassiana* GHA + 0.02% tween 80	1×10^8 conidia/ml
6	*Beauveria bassiana* DEBI008 + 0.02% tween 80	1×10^5 conidia/ml
7	*Beauveria bassiana* DEBI008 + 0.02% tween 80	1×10^6 conidia/ml
8	*Beauveria bassiana* DEBI008 + 0.02% tween 80	1×10^7 conidia/ml
9	*Beauveria bassiana* DEBI008 + 0.02% tween 80	1×10^8 conidia/ml
10	*Beauveria bassiana* Z1 + 0.02% tween 80	1×10^5 conidia/ml
11	*Beauveria bassiana* Z1 + 0.02% tween 80	1×10^6 conidia/ml
12	*Beauveria bassiana* Z1 + 0.02% tween 80	1×10^7 conidia/ml
13	*Beauveria bassiana* Z1 + 0.02% tween 80	1×10^8 conidia/ml
14	*Metarhizium anisopilae* + 0.02% tween 80	1×10^5 conidia/ml
15	*Metarhizium anisopilae* + 0.02% tween 80	1×10^6 conidia/ml
16	*Metarhizium anisopilae* + 0.02% tween 80	1×10^7 conidia/ml
17	*Metarhizium anisopilae* + 0.02% tween 80	1×10^8 conidia/ml

3. Results

The fungal isolates showed varying mortality rates under different relative humidity conditions, as illustrated in Figures 1 through 3. Specifically, at 75% relative humidity, the *B. bassiana* isolates DEBI008 and Z1 had the shortest median lethal time (LT50), recorded at 6 ± 0.5 days. Additionally, these two isolates achieved the highest mortality rates among all tested, with 100% mortality within 16 ± 0.5 days. Conversely, probit analysis revealed that the highest median lethal concentration (LC50) ten days post-treatment was for *B. bassiana* isolate Z1 at 1.06 × 10^8 conidia mL^{-1}, while isolate DEBI008 had the lowest LC50 value of 3.1 × 10^6 (Table 2).

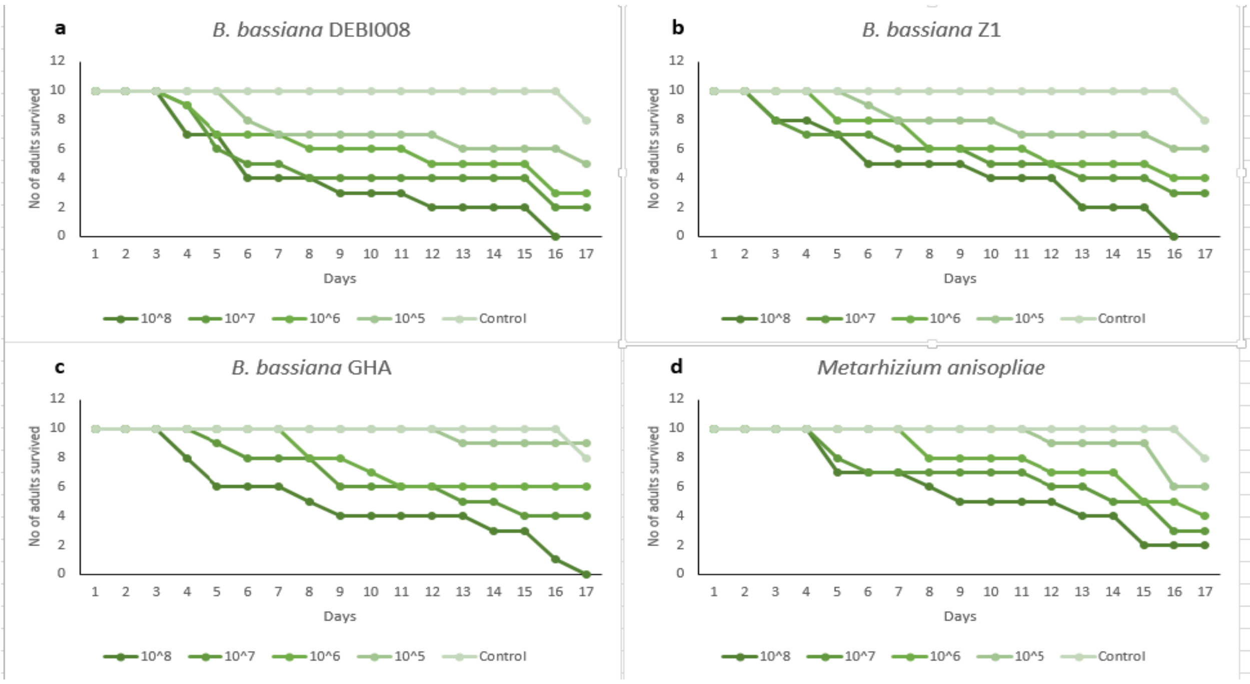
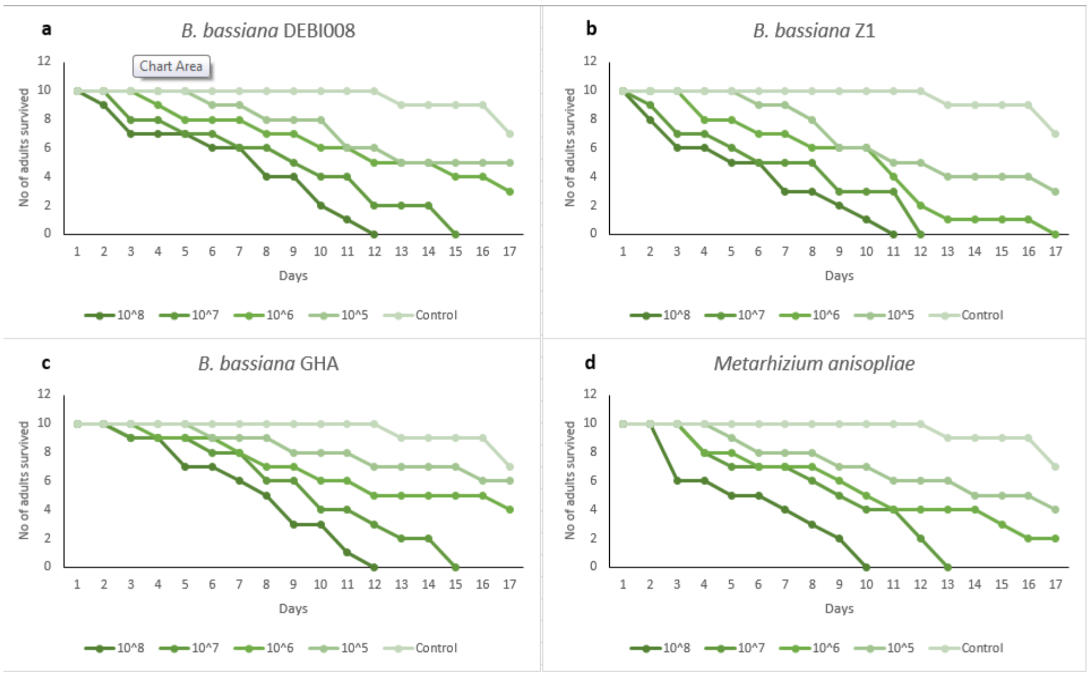


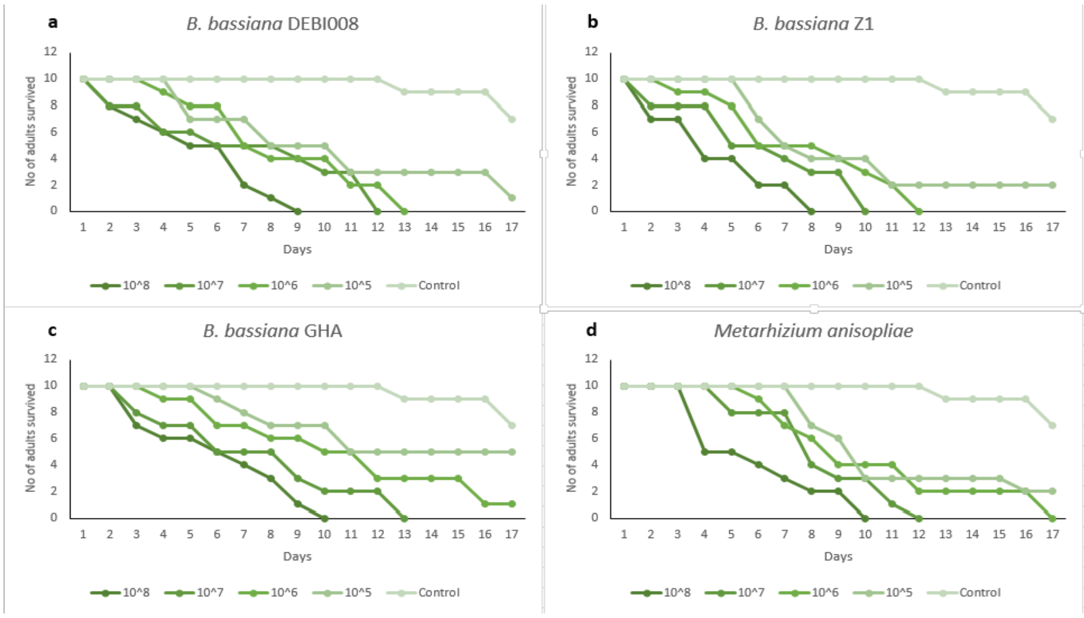
Table 2. Probit analysis results for different concentrations of fungal isolates on *Acrosternum arabicum* adults following a 10-day exposure.

Isolates	Slope±SE[a]	Intercept±SE	χ2(df)b	LC50(95% CLs[c])	LT50(1×10^8 conidia)	100% Mortality (1×10^8 conidia)
B. bassiana DEBI008				75% RH		
	0.37±0.19	-2.38±1.23	0.07(2)	3.1×10^6 (0-3.8×10^{17})	6±0.5 (days)	16±0.5 (days)
				80% RH		
	0.55±0.20	-3.6±1.3	0.01(2)	3.1×10^6 (2.7×10^5-3.5×10^7)	8±0.5 (days)	12±0.5 (days)
				95% RH		
	0.53±0.22	-2.84±1.34	1.96(2)	2.1×10^5 (19.4-1.3×10^6)	5±0.5 (days)	9±0.5 (days)
B. bassiana Z1				75% RH		
	0.32±0.20	-2.53	0.29(2)	1.06×10^8 (--)	6±0.5 (days)	16±0.5 (days)
				80% RH		
	0.58±0.21	-4.01±1.39	0.08	1.07×10^7 (1.8×10^6-3.1×10^8)	6±0.5 (days)	11±0.5 (days)
				95% RH		
	0.27±0.19	-1.47±1.21	0.51(2)	3.2×10^5	4±0.5 (days)	8±0.5 (days)

				(--)		
B. bassiana GHA	75% RH					
	0.63±0.23	-4.65±1.55	1.61(2)	$2.6×10^{7}$ ($5.1×10^{6}$-$1.9×10^{9}$)	8±0.5 (days)	17±0.5 (days)
	80% RH					
	0.45±0.19	-3.05±1.27	0.15(2)	$4.5×10^{6}$ ($2.1×10^{5}$-$3.1×10^{8}$)	8±0.5 (days)	12±0.5 (days)
	95% RH					
	0.83±0.25	-4.85±1.55	0.76(2)	$6.5×10^{5}$ ($8.2×10^{4}$-$2.4×10^{6}$)	6±0.5 (days)	10±0.5 (days)
Metarhizium anisopliae	75% RH					
	0.60±0.24	-4.76±1.68	0.92(2)	$7.5×10^{7}$ ($1.2×10^{7}$-$3.8×10^{12}$)	9±0.5 (days)	In 17 days, 80%
	80% RH					
	0.68±0.22	-4.07±1.40	2.33(2)	$9.9×10^{5}$ ($8.4×10^{4}$-$4.8×10^{6}$)	5±0.5 (days)	10±0.5 (days)
	95% RH					
	0.35±0.21	-1.56±1.35	3.27(2)	$2.6×10^{4}$ (--)	4±0.5 (days)	10±0.5 (days)

Under conditions of 80% relative humidity (RH), the *M. anisopliae* isolate had the lowest LT50 of 5 ± 0.5 days, with 100% mortality observed within 10 ± 0.5 days. These findings suggest that *M. anisopliae* germination is highly dependent on humidity levels (Fig. 2). Tests at 95% further support this, showing rapid mortality of 50% of *A. arabicum* within 4 ± 0.5 days under optimal humidity conditions (Fig. 3).





Moreover, *B. bassiana* isolate Z1 was also prominent under both 80% and 95% RH, with LT50 values of 6 ± 0.5 days and 4 ± 0.5 days, respectively. Although *M. anisopliae* and *B. bassiana* Z1 had similar LT50 values, their median lethal concentration values differ significantly. The LC50 for *M. anisopliae* at 10 days under 80% and 95% RH is 9.9×10^5 and 2.6×10^4 conidia mL^{-1}, respectively. In contrast, the LC50 for *B. bassiana* isolate Z1 under the same humidity conditions is significantly higher, at 1.07×10^7 and 3.2×10^5 conidia mL^{-1}, respectively.

4. Discussion

Although the results of this study demonstrate that *A. arabicum* is susceptible to all four tested isolates under laboratory conditions, it was observed that, at the lowest relative humidity condition evaluated, *B. bassiana* isolate DEBI008 exhibited the highest virulence among the isolates. Furthermore, a related study investigating the virulence of multiple isolates reported that, among seven tested *B. bassiana* isolates and four *M. anisopliae* isolates, the B. bassiana strains exhibited significantly higher efficacy at relative humidity levels below 70%, achieving 100% mortality of various pentatomid bug species within 7 to 9 days [18]. Additionally, multiple studies [19-22], have indicated that the performance of *B. bassiana* isolate GHA was at least comparable to, if not superior to, some *M. anisopliae* isolates when targeting pentatomid bugs. These findings suggest that *B. bassiana* isolates, particularly under lower humidity conditions, may serve as effective biocontrol agents for managing *A. arabicum* and related pests, reinforcing the potential of utilizing entomopathogenic fungi in integrated pest management strategies.

Research conducted from the 1940s through 2013 on various crops, including almond, tomato, cucumber, and walnut, has consistently shown that the average relative humidity of leaf surfaces is approximately 70%. Although there is a lack of specific measurements for the leaf surface relative humidity (RH) of pistachio, related studies on almond, a species closely related to pistachio, provide valuable insights. Measurements indicate that the leaf surface RH of almond during nighttime is around 50%, while during the daytime and peak photosynthesis periods, it reaches approximately 80% [23, 24]These findings suggest that deploying *B. bassiana* isolate DEBI008 in field conditions may be viable, given that the environmental conditions are likely conducive to its effectiveness. Nonetheless, further research is necessary to fully evaluate the performance and efficacy of this isolate specifically on pistachio plants in natural settings.

In a related study, laboratory research evaluated the efficacy of the *B. bassiana* strain GHA, sourced from BotaniGard®, which was utilized in the present investigation too, at a concentration of 5 × 10^7 conidia/mL. This application resulted in 87% and 88% mortality of the stink bug species *Nezara viridula* and *Piezodorus guildingi*, respectively, over 14 days [25]. Additionally, subsequent studies performed under field conditions with the same isolate and stink bug species confirmed the laboratory findings, revealing over 70% mortality in both species [26]. These results suggest promising potential for field or semi-field trials to further validate our laboratory results, thereby offering a viable alternative strategy for the control of pistachio stink bugs, which may be effectively used in an integrated pest management (IPM) program.

5. Conclusions

Our findings indicated that none of the tested achieved 100% mortality at a concentration of 1 × 10^5 conidia/mL across all relative humidity conditions, even after 17 days. Conversely, the results demonstrated that the *M. anisopliae* isolate is highly sensitive to environmental relative humidity (RH). Therefore, in potential applications in semi-field or field settings, the use of humidifiers or maintaining elevated RH conditions could enhance its effectiveness, as has been shown in several studies [27]. In comparison, the *B. bassiana* Z1 isolate exhibited superior performance across all three tested RH conditions. Specifically, this isolate induced 50% mortality after 6 days under both 75% and 80% RH conditions, and after just 4 days at 95% RH.

Acknowledgments

This work was funded by Rafsanjan University of Medical Sciences (RUMS), Project No. 97379. We are grateful to the Department of Plant Protection, Vali-e-Asr University of Rafsanjan, Rafsanjan, IRAN, for the use of greenhouse and laboratory facilities. We would like to express our sincere gratitude to Javad Karimi from the Department of Plant Protection, Faculty of Agriculture, University of Ferdowsi, Mashhad, Iran, for generously providing some of the isolates of myco-entomopathogens used in this study. We extend our gratitude to Dr. Mohadeseh Hassanisaadi for her invaluable assistance in the isolation of entomopathogenic fungi from the studied insect bodies and their subsequent culturing.

Funding

This work was funded by Rafsanjan University of Medical Sciences (RUMS), Project No. 97379.

Conflicts of interest

The authors declare that they have no conflict of interest.

References

1- Mandalari G, Barreca D, Gervasi T, Roussell MA, Klein B, Feeney MJ, Carughi A. Pistachio nuts (Pistacia vera L.): Production, nutrients, bioactives and novel health effects. Plants. **2021**;11:*18*.

2- International Nut & Dried Fruit Council. Pistachios Global Statistical Review [Internet]. **2024** [cited 2024 Sep 29]. Available from: https://inc.nutfruit.org/pistachios-global-statistical-review-2/

3- Mehrnejad MR. Arthropod pests of pistachios, their natural enemies and management. Plant Prot Sci. **2020**;56:*231-60*.

4- Ademokoya B, Athey K, Ruberson J. Natural Enemies and Biological Control of Stink Bugs (Hemiptera: Heteroptera) in North America. Insects. **2022**;13:*932*.

5- Werdin González JO, Laumann RA, da Silveira S, Moraes MCB, Borges M, Ferrero AA. Lethal and sublethal effects of four essential oils on the egg parasitoids Trissolcus basalis. Chemosphere. **2013**;92:*608-15*.

6- Ranjbar F, Jalali MJ, Khanamani Falahati-pour S, Malekzadeh Kh, Ziaaddini M. Assessment of three common methods for DNA extraction in trissolcus sp. egg parasitoid wasp of green stink bugs. Pistachio Health J. **2019**;2:*9-16*.

7- Sedigh H, Ranjbar F, Jalali MA, Ziaaddini M. Reproductive biology of Ooencyrtus egeria (Hymenoptera: Encyrtidae) reared on eggs of two economically important pistachio stink bugs: Brachynema germari and Acrosternum arabicum (Hemiptera: Pentatomidae). Pistachio Health J. **2022**;5:*62-69*.

8- Mohammadpour M, Jalali MA, Ziaaddini M, Hashemirad H. Some biological characteristics of Ooencyrtus pityocampae Mercet parasitoid of Brachynema signatum Jakovlev under laboratory conditions. Pistachio Health J. **2018**;1:*20-5*.

9- Oliveira DG, Dudczak AC, Alves LF, Sosa-Gomez DR. Biological parameters of Euschistus heros (F.)(Heteroptera: Pentatomidae) and its susceptibility to entomopathogenic fungi when fed on different diets. Braz Arch Biol Technol. **2016**;59:*e16150141*.

10- Mascarin GM, Jaronski ST. The production and uses of Beauveria bassiana as a microbial insecticide. World J Microbiol Biotechnol. **2016**;32:*177*.

11- Silva-Santana MF, Alves LFA, Ferreira TT, Bonini AK. Selection and characterisation of Beauveria bassiana fungus and their potential to control the brown stink bug. Biocontrol Sci Technol. **2022**;32:*90-102*.

12- Islam W, Adnan M, Shabbir A, Naveed H, Abubakar YS, Qasim M, Tayyab M, Noman A, Nisar MS, Khan KA. Insect-fungal-interactions: A detailed review on entomopathogenic fungi pathogenicity to combat insect pests. Microb Pathog. **2021**;159:*105122*.

13- Quesada-Moraga E, González-Mas N, Yousef-Yousef M, Garrido-Jurado I, Fernández-Bravo M. Key role of environmental competence in successful use of entomopathogenic fungi in microbial pest control. J Pest Sci. **2024**;97:*1-15*.

14- Mohammadpour M, Ziaaddini M, Jalali MA, Hashemirad H, Mohammadi-Khoramabadi A. Egg parasitoids of the pistachio green stink bug, Brachynema germari (Hemiptera: Pentatomidae) in Kerman province, Iran. Zool Ecol. **2016**;26:*28-34*.

15- Pourkhatoon S, Ziaaddini M, Alizadeh A, Amin Jalali M, Ebrahimi M. Biological characteristic of Brachynema germari (Hemiptera: Pentatomidae): comparative study of composite and natural diet. J Econ Entomol. **2016**;109:*1273-82*.

16- Parsa S, Ortiz V, Vega FE. Establishing Fungal Entomopathogens as Endophytes: Towards Endophytic Biological Control. J Vis Exp. **2013**(74):*e50360*.

17- Raafat I, Meshrif WS, Husseiny EME, El-Hariry M, Seif AI. Nezara viridula (Hemiptera: Pentatomidae) Cuticle as a Barrier for Beauveria bassiana and

Paecilomyces sp. Infection. Afr Entomol. **2015**;23:*75-87*.

18- Abate B, Wakgari M, Sori W. The efficacy of entomopathogenic fungi for antestia bugs (Antetiopsis intircata: Pentatomidea, Hemiptera) control. Am J Biol Environ Stat. **2021**;7:*9-18*.

19- Gebremariam A, Chekol Y, Assefa F. 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. **2021**;7:*e07091*.

20- Portilla M, Tertuliano M, Parys K, Glover JP, Reddy GVP. Effects of Beauveria bassiana on the growth and reproductive rates of Nezara viridula. BioControl. **2024**;69:*413-25*.

21- Nada MS. Response of green stinkbug Nezara viridula (Linnaeus), to the activity of entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae. J Plant Prot Pathol. **2015**;6:*1633-44*.

22- Shahbaz M, Palaniveloo K, Tan YS, Palasuberniam P, Ilyas N, Wiart C, Seelan JSS. Entomopathogenic fungi in crops protection with an emphasis on bioactive metabolites and biological activities. World J Microbiol Biotechnol. **2024**;40:*217*.

23- Burkhardt J, Hunsche M. “Breath figures” on leaf surfaces—formation and effects of microscopic leaf wetness. Fron Plant Sci. **2013**;4:*422*.

24- Buckley TN, Sack L. The humidity inside leaves and why you should care: implications of unsaturation of leaf intercellular airspaces. Am J Bot. **2019**;106:*618*.

25- Parys KA, Portilla M. Effectiveness of Beauveria bassiana against Piezodorus guildinii (Hemiptera: Pentatomidae), a key pest of soybeans in the neotropics. Biocontrol Sci Technol. **2020**;30:*451-61*.

26- Ramos Y, Portal O, Meyling NV, Klingen I. Biological control potential of two Beauveria bassiana isolates against the stink bugs Nezara viridula L. and Piezodorus guildinii Westwood (Hemiptera: Pentatomidae) in common bean. Egypt J Biol Pest Control. **2024**;34:*23*.

27- De Oliveira DG, Lopes RB, Rezende JM, Delalibera Jr I. Increased tolerance of Beauveria bassiana and Metarhizium anisopliae conidia to high temperature provided by oil-based formulations. J Invertebr Pathol. **2018**;151:*151-7*.