

Efficacy of entomopathogenic fungi against major piercing-sucking insect pests infesting sunflower under laboratory conditions

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ABSTRACT

Piercing-sucking insects are among the most destructive pests affecting sunflower crops, causing significant losses and affecting the seed quality worldwide. The present study evaluated the pathogenicity of fungi *Baeuveria bassiana*, *Metarhizium acridum*, *Lecanicillium muscarium*, and *Isaria fumosorosea* against three major piercing-sucking pests of sunflower, which were the lace bug *Galeatus scrophicus*, the cotton aphid *Aphis gossypii*, and the whitefly *Bemisia tabaci*. Laboratory bioassays were conducted under controlled environmental conditions at $25 \pm 2^\circ\text{C}$ and 65–70% relative humidity. Fungal suspensions were prepared at a concentration of 1×10^7 conidia mL^{-1} and applied using a leaf spray bioassay technique. Each treatment consisted of five replicates with ten insects per replicate. The results indicated significant differences in the pathogenicity of fungal species against targeted pests. The highest mortality was recorded with *B. bassiana* and *M. acridum*, reaching 100% on *G. scrophicus* and *A. gossypii* within 7 days post-treatment, while causing 92.2 and 88.6% mortality on *B. tabaci*, respectively. There was a significant difference between the treatments of *L. muscarium* and *I. fumosorosea*, with lace bug control of 77.8% and 86.2%, respectively, after 7 days of post-treatment. In comparison, the effect of *L. muscarium* and *I. fumosorosea* on *A. gossypii* was 85.5 and 92.6%, respectively. The impact of *L. muscarium* and *I. fumosorosea* on *B. tabaci* was 75.5 and 85.4%, respectively. The mortality rate increased gradually over time and reached 100% across all targeted insects by day 8 due to fungal infection and colonization of insect hosts.

Keywords: sunflower pests, biological control, entomopathogenic fungi, biopesticides, sucking pests

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INTRODUCTION

Sunflower (*Helianthus annuus*) is one of the most important oilseed crops cultivated worldwide due to its high oil content and economic value. It is considered the second most important oilseed crop after soybean. The total annual production of sunflowers in Iraq is about 96 tons of seed produced from 193 Donum (COSIT, 2025). However, sunflower production is frequently threatened by various insect pests that cause considerable production losses and affect the seed quality. Among the most damaging insects are piercing-sucking pests such as aphids, whiteflies, and lace bugs. They cause up to 35-40% production loss because they

feed on plant sap and lead to nutrient depletion, reduced photosynthetic capacity (Nazrin *et al.*, 2022; Nayak *et al.*, 2022). In addition to the secretion of honeydew that covers the surface of the leaf, causing indirect damage through encouraging the growth of sooty mould fungi, the multiplicity of egg-laying holes and the accumulation of excrement cause the plant to weaken, and the yellowing of leaves and fall off, negatively affecting the quantity and quality of production (Kıvan and Aysal, 2011; Altae and AL-Dahwi, 2023).

These potential risks require that growers follow integrated pest management (IPM) practices.

IPM is a sustainable approach to pest management that combines biological, cultural, physical, and chemical controls to minimize economic, health, and environmental risks and maintain pest populations below economic thresholds that cause unacceptable losses in crop quality or yield (Charlet and Gary, 2004). Chemical insecticides are commonly used to control insect pests, but their overuse has led to environmental contamination, pesticide resistance, and adverse effects on beneficial organisms. Therefore, sustainable pest management strategies are increasingly required. Entomopathogenic fungi are considered promising biological control agents because they infect insects through direct contact with the cuticle and subsequently proliferate within the host body. These fungi produce several enzymes, such as chitinases, proteases, and lipases, that enable them to penetrate the insect cuticle and colonize host tissues, ultimately leading to insect death (Shahid *et al.*, 2012; Islam *et al.*, 2021; Sharma *et al.*, 2025). Among the most important of these types successfully employed to eliminate various insect pests across different ecosystems were *Beauveria bassiana*, *Lecanicillium muscarium*, *Metarhizium acridum*, and *Isaria fumosorosea* (Ambethgar, 2009; Mseddi *et al.*, 2022; Alhadidi, 2023; Sain *et al.*, 2024).

Studies have shown that pathogenic fungi cause lethargy, reduced movement, and reduced feeding in the first few days of treatment in both nymphs and adults. Then the lethal effect becomes apparent in the following days, in direct proportion to time and in varying degrees. This indicates that its effectiveness in causing death varies from one fungus to another, and that the low mortality rates in the first days of treatment may be attributed to the fact that it needs sufficient time to secrete enzymes that break down the insect's body wall, such as chitinase, protease, and lipase, thus allowing the fungus to germinate, penetrate, and spread through its body cavity, in addition to producing lethal toxins (Lacey and Mercadier, 1998; Ali *et al.*, 2010; Smagghe *et al.*, 2023).

Among the most widely studied entomopathogenic fungi are *B. bassiana* and *Metarhizium* sp., which have demonstrated high

pathogenicity against a wide range of insect pests. Despite their potential, information regarding the effectiveness of these fungi against piercing-sucking pests of sunflower remains limited (Elkhateeb *et al.*, 2021). Studies have shown that fungal spores begin to germinate when suitable conditions are present, most importantly temperature and humidity, and that they are efficient in secreting enzymes that break down the insect body wall, such as the enzyme exocellule chitinase, to digest chitin (Ali *et al.*, 2010; Islam *et al.*, 2021). Therefore, the present study aimed to evaluate the pathogenicity of four entomopathogenic fungi against three economically important sunflower pests: *G. scrophicus*, *A. gossypii*, and *B. tabaci* under laboratory conditions.

MATERIALS AND METHODS

Sources of the entomopathogenic fungi isolates

The laboratory experiment was conducted using four types of entomopathogenic fungi: *B. bassiana*, *L. muscarium*, *M. acridum*, and *I. fumosorosea*, obtained from the Biopesticide Production Laboratory, Department of Crop Protection, Director, Ministry of Agriculture, Iraq.

Preparation of fungal suspensions

Entomopathogenic fungi were produced as a dry product formulation from local isolates. They were used in the recommended concentrations are A local preparation of *Baeuveria bassiana* (1×10^7 conidia mL⁻¹), used at a concentration of 5 g/ L. (T1), a local preparation of *Metarhizium acridum* (1×10^7 conidia mL⁻¹), was used at a concentration of 5 g/ L (T2), A local preparation of *Lecanicillum muscarium* (1×10^7 conidia mL⁻¹), used at a concentration of 5 g/ L (T3), the local preparation of *Isaria fumosorosea* (1×10^7 conidia mL⁻¹), used at a concentration of 5 g/ L. Fungal suspensions were prepared by dissolving the dry formulation in sterile distilled water containing 0.05% Tween-80 to ensure uniform spore dispersion. The concentration was adjusted to 1×10^7 conidia mL⁻¹ using a hemocytometer.

Insect collection and maintenance

Individuals of *G. scrophicus*, *A. gossypii*, and *B. tabaci* were collected from sunflower fields and maintained in the laboratory on sunflower plants

at $25 \pm 2^\circ\text{C}$ and 65–70% relative humidity.

Bioassay of the entomopathogenic fungi on target insects

A leaf-spray bioassay technique was used to evaluate fungal pathogenicity. Sunflower leaves were placed upside down in Petri dishes containing moist cotton, with the dishes positioned at a height of 9 cm and a width of 2 cm to maintain leaf freshness. Ten insects, comprising a mix of nymphs and adults, were introduced into each dish, and the leaves were sprayed with 2 mL of the fungal suspension. Control treatments received distilled water containing Tween-80. Five replicates per treatment for each entomopathogenic fungus. All experimental units were incubated at $25 \pm 2^\circ\text{C}$, 65–70% relative humidity, and a 16 h light: 8 h dark photoperiod after sealing the petri dishes with adhesive tape to prevent the insects. Mortality was recorded daily for eight days. The cumulative observed mortality of insects was calculated using Orelli and Schneider's (1947) equation to correct for mortality.

%Corrected mortality=

$$\frac{\%mortality\ in\ treatment - \%mortality\ in\ control}{100 - \%mortality\ in\ control} \times 100$$

The same procedure was carried out on all target insect pests.

Statistical Analysis

Data were analyzed using GenStat statistical software, version 12, in a completely randomized design. Treatment means were compared using the least significant difference (LSD) test at the 0.05 level.

RESULTS AND DISCUSSION

Entomopathogenic fungi on the Lace bug *Galeatus scrophicus*

Nymphs and adults of the Lace bug *G. scrophicus* were treated with a suspension of fungal preparations of *B. bassiana*, *L. muscarium*, *M. acridum*, and *I. fumosorosea* at a concentration of 1×10^{10} spores/ mL. The effects of the fungi were clearly observed on the second day of treatment, with lethargy, reduced movement, and refusal to feed. The lethal effect of the fungi was observed on the third day of treatment (Table 1). It was relatively low, as the corrected death percentages for the above preparations after three days of treatment were 29.3, 15.0, 27.4, and 21.7, respectively, and mortality rates gradually increased across all preparations. These rates varied from one preparation to another, reaching 67.4%, 36.5%, 65.8%, and 49.3% on the fifth day. The mortality rate increased over time proportionally for all preparations, reaching 100% on day seven for the *B. bassiana* and *M. acridum* preparations, while the mortality reached 77.8 and 86.2% for the *L. muscarium* and *I. fumosorosea* preparations, respectively. By day eight post-treatment, the adjusted mortality rate was 100% for all treatments. The statistical analysis of the data in Table 1) showed a significant difference between the treatments, as the two preparations *B. bassiana* and *M. acridum* outperformed the other preparations with no difference between them by a clear significant difference, followed by the preparation of the fungus *I. fumosorosea*, which outperformed the preparation of the fungus *L. muscarium*.

Table 1. Efficiency of the entomopathogenic fungi on nymphs and adults of the Lace bug *Galeatus scrophicus*

Treatments	Corrected the mortality percentage (days)							Mean±SE*
	2	3	4	5	6	7	8	
<i>B. bassiana</i>	0	29.3	45.6	67.4	85.7	100	100	71.3±2.8
<i>L. muscarium</i>	0	15.0	28.7	36.5	59.3	77.8	100	52.9±2.2
<i>M. acridum</i>	0	27.4	42.5	65.8	80.0	100	100	69.3±2.6
<i>I. fumosorosea</i>	0	21.7	33.6	49.3	68.5	86.2	100	59.9±2.4
Average	0	23.4	37.6	54.8	73.4	91.0	100	63.4±2.7
LSD _{0.05}	Treatment = 3.3 Days = 4.2 Interaction = 7.6							

* Standard error of mortality means by each treatment

In a previous study, Al-Amry and Al-Dahwi (2022) explained that members of the *Stephanitis pyri*, which infest apple tree leaves, were treated with a suspension of fungal preparations *B. bassiana*, *L. muscarium*, *M. acridum*, and *I. fumosorosea* at a concentration of 1×7^{10} spores/mL and caused 100% mortality after 10 days of nymph treatment and 12 days of adult treatment. Sharma et al. (2023) reported the variation in the mortality rates of the *L. lecanii*, *M. acridum*, *I. fumosorosea*, and *B. bassiana* on the lace bug, which causes a high mortality range between 80 – 100% on nymphs and adults of the *Stephanitis nashi* when using a 10^8 conidia per mL concentration.

Entomopathogenic fungi on the aphid *Aphis gossypii*

The efficacy of the pathogenic fungi *B. bassiana*, *L. muscarium*, *M. acridum*, and *I. fumosorosea*, used at a concentration of 1×7^{10} spores/mL, varied in affecting nymphs and adults of the aphid *A. gossypii* at different time intervals. Affected aphids were observed 2 days after treatment, as they were lethargic, less mobile, and refused to feed. The lethal effect became apparent on the third day of treatment and was low, as shown in Table 2, where the corrected percentage of death for the above preparations reached 35.2, 22.8, 30.5, and 27.4%, respectively. The mortality rate increased on the fifth day, with the corrected death percentages reaching 72.6%, 53.1%, 70.0%, and 62.9%, respectively. It was also observed that the mortality rate increased gradually and proportionally over time for all preparations, reaching 100% on the seventh day of treatment with the *B. bassiana* and *M. acridum*

preparations, while it reached 85.5% and 92.6% for the *L. muscarium* and *I. fumosorosea* preparations, respectively. On the eighth day after treatment, the corrected mortality rate reached 100% for all treatments. The statistical analysis of the data in Table 2 showed a significant difference between the treatments, as the two preparations *B. bassiana* and *M. acridum* outperformed the other preparations with no difference between them by a clear significant difference, followed by the preparation of the fungus *I. fumosorosea*, which outperformed the preparation of the fungus *L. muscarium*.

Hong and Kim (2007) reported that, when testing the pathogenicity of the fungi *L. muscarium*, *B. bassiana*, *M. acridum*, and *I. fumosorosea* against the cotton aphid *A. gossypii*, *L. muscarium* was most virulent, achieving a 100% mortality rate after 5 days of treatment. Kumari et al. (2024) confirmed that the fungi *V. lecanii* and *I. fumosorosea* have a clear effect on the green peach aphid *Myzus persicae*, with correct mortality rates of 95.5% and 87.5%, respectively. In a study conducted by Hussein and Al-Dahawi (2023) to estimate the relative efficiency of fungal treatments in controlling two aphid species, *A. gossypii* and *A. fabae*, on strawberry plants, the fungus *L. muscarium* was found to be the most efficient of the other fungi used in the study, namely *I. fumosorosea*, *B. bassiana*, and *M. acridum*. The corrected mortality rate after 7 days of treatment for cotton aphid nymphs was 92.8% and for adults 88.78%, while the corrected mortality rate for black broad bean aphid nymphs was 87.32% and for adults 83.65%, respectively.

Table 2. Efficiency of the entomopathogenic fungi on nymphs and adults of the aphid *Aphis gossypii*

Treatments	Corrected the mortality percentage (days)							Mean±SE*
	2	3	4	5	6	7	8	
<i>B. bassiana</i>	0	35.2	51.4	72.6	88.4	100	100	74.6±3.5
<i>L. muscarium</i>	0	22.8	32.7	53.1	67.3	85.5	100	60.2±2.9
<i>M. acridum</i>	0	30.5	48.9	70.0	83.8	100	100	72.2±3.3
<i>I. fumosorosea</i>	0	27.4	42.6	62.9	75.7	92.6	100	66.9±3.1
Average	0	29.0	43.9	64.7	78.8	94.5	100	68.5±3.3
LSD _{0.05}	Treatment = 3.8 Days =4.6 Interaction = 8.2							

* Standard error of mortality means by each treatment

Entomopathogenic fungi on the whitefly *Bemisia tabaci*

The efficacy of the pathogenic fungi *B. bassiana*, *L. muscarium*, *M. acridum*, and *I. fumosorosea*, used at a concentration of 1×710 spores/mL, varied in affecting the nymphs and adults of the whitefly *B. tabaci* at different time intervals. It was observed that they were affected by the fungi after two days of treatment, becoming lethargic, moving little, and refusing to feed. The lethal effect became apparent on the third day of treatment and was low, as shown in Table 3, where the corrected percentage of death for the above preparations reached 31.3, 20.6, 26.8, and 24.5%, respectively. The mortality rate increased on the fifth day, with the corrected death percentages reaching 60.2%, 41.4%, 54.7%, and 44.8%, respectively. It was also observed that the mortality rate increased gradually and proportionally over time for all preparations on the seventh day of treatment, reaching 92.2%, 75.5%, 88.6%, and 85.4%, respectively. On the eighth day after treatment, the adjusted mortality rate reached 100% for all treatments. The statistical analysis of the data in Table 3 showed significant differences among all treatments, with preparation *B.*

bassiana and *M. acridum* were superior to the other preparations, followed by the preparation of the fungus *I. fumosorosea*, which was superior to that of *L. muscarium*. In a previous study, Sani *et al.* (2020) used isolates of the fungus *B. bassiana* and one isolate of the fungus *V. lecanii* to control the whitefly *B. tabaci*. They noted that the pathogenic fungus *V. lecanii* achieved the highest mortality among insect stages, reaching 72.22%, with a significant difference compared with the two *B. bassiana* isolates after 7 days of treatment. Islam *et al.* (2021) showed that the commercial preparation of entomopathogenic fungi (Mycotal) achieved a high mortality rate on the immature stages of the cotton whitefly *B. tabaci* on vegetable and crops in the field and greenhouses after one week of treatment, in addition to the efficiency of the local isolate of the fungus *B. bassiana*, which achieved a death rate of 75% after 7 days post treatment. Several studies have demonstrated the effectiveness of the pathogenic fungus *L. lecanii* as a biocontrol agent against many insect pests, including the greenhouse whitefly, cotton whitefly, cotton aphid, green peach aphid, and flower thrips (Askary and Yarmand, 2007; Arunthirumeni *et al.*, 2023; Cuthbursten *et al.*, 2010).

Table 3. Efficiency of the entomopathogenic fungi on nymphs and adults of the whitefly *Bemisia tabaci*

Treatments	Corrected the mortality percentage (days)							Mean±SE*
	2	3	4	5	6	7	8	
<i>B. bassiana</i>	0	31.3	37.8	60.2	78.5	92.2	100	66.7±2.9
<i>L. muscarium</i>	0	20.6	26.0	41.4	55.0	75.5	100	53.1±2.3
<i>M. acridum</i>	0	26.8	34.6	54.7	72.3	88.6	100	62.8±2.6
<i>I. fumosorosea</i>	0	24.5	30.4	44.8	62.7	85.4	100	58.0±2.5
Average	0	25.8	32.2	50.3	67.1	85.4	100	60.1±2.7
LSD _{0.05}	Treatment = 3.1 Days = 3.8 Interaction = 6.5							

* Standard error of mortality means by each treatment

Overall, the results demonstrated that entomopathogenic fungi are effective biological control agents against piercing–sucking insect pests of sunflower. *B. bassiana* and *M. acridum* exhibited the highest pathogenicity among the tested fungi, producing high mortality rates against all tested insects. The effectiveness of *B. bassiana* observed in this study agrees with previous research showing its strong virulence against

aphids, whiteflies, and other sap-sucking insects (AL-Amry and AL-Dahwi, 2022; Parajuli *et al.*, 2022). This fungus infects insects through spore adhesion to the cuticle, followed by enzymatic penetration and colonization of the insect body. Similarly, *Metarhizium* sp. produces toxic secondary metabolites that interfere with insect physiological processes and accelerate host mortality (Sain *et al.*, 2020; Hussain *et al.*,

2023). The gradual increase in mortality observed over time reflects the typical infection process of entomopathogenic fungi. The relatively lower efficacy of *L. muscarium* may be related to differences in fungal virulence or host susceptibility. Environmental conditions may also influence the efficiency of fungal infection. Overall, the findings highlight the importance of entomopathogenic fungi as environmentally safe alternatives to chemical pesticides. Their integration into pest management programs could contribute to sustainable sunflower production. Entomopathogenic fungi showed significant potential for controlling major piercing-sucking insect pests of sunflower. *B. bassiana* and *M. acridum* were the most effective fungi against *G. scrophicus*, *A. gossypii*, and *B. tabaci*. These fungi could serve as eco-friendly alternatives to chemical insecticides and may be integrated into sustainable pest management strategies. Further studies under field conditions are necessary to confirm their practical applicability.

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