



Biocontrol of Leafhopper *Amrasca Biguttula* Infesting Eggplants Using Entomopathogenic Fungi and Plant Extracts

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Abstract: The efficiency of entomopathogenic fungi and plant extracts were evaluated against the leafhopper *Amrasca biguttula* infesting eggplant; the trials were conducted at the Agricultural station and the lab of College of Agriculture - University of Basrah during the growing season of 2023. At the lab, the treatments included fungal suspensions and filtrates of *Beauveria bassiana* and *Trichoderma harzianum*, as well as the plant extracts of neem leaves (*Azadirachta indica*) and castor leaves and seeds (*Ricinus communis*). The results of fungal suspensions showed no significant differences in adult mortality between *B. bassiana* and *T. harzianum*, with rates of 55.9% and 59.0%, respectively. Mortality increased over time, reaching 83.5% at the highest concentration (1×10^8 spores/ml) after 72 hours. The lowest rate of 28.4% was observed at a lower concentration (1×10^6 spores/ml) after 24 hours. Also, the fungal filtrates indicated that the highest mortality was 64.7% for *B. bassiana* at a 100% filtrate concentration after 72 hours. However, *B. bassiana* at 75% concentration recorded the lowest rate of 17.71% after 24 hours. No significant differences were noticed in the mortality of the insect among the treatments of neem, castor leaves, and castor seeds; the rates were 82.97%, 82.76%, and 85.45%, respectively. All plant extracts at concentrations of 1% and 0.5% achieved 90% mortality after 24, 48, and 72 hours. Under the greenhouse conditions, the treatments included fungal suspensions, and plant extracts (neem and castor leaves), with their mixtures compared to the positive control, pesticide Sivanto. Neem extract caused the highest mortality rate of 80.8%. However, castor leaf extract recorded the lowest mortality rate of 66.8%, compared to Sivanto, reaching 100% mortality after 21 days.

Keywords: *Amrasca biguttula*, *Beauveria bassiana*, *Trichoderma harzianum*, leafhopper, plant extracts.

Introduction

Eggplant (*Solanum melongena*), which belongs to the Solanaceae family, is one of the important vegetable crops; this crop is widely cultivated in tropical and subtropical regions. Asia produces approximately 94% of the global eggplant cultivation area and about 92% of the world's production (Ghosh, 2023). In Iraq, the estimated cultivated area of eggplant was 51,100 dunams, producing 215,700 tons in 2021 (CSO, 2022). This crop is attacked by different insect pests, including the eggplant fruit and shoot borer (*Leucinodes orbonalis*), whitefly (*Bemisia tabaci*), leafhopper (*Amrasca biguttula*), aphid (*Aphis gossypii*), and thrips (*Thrips palmi*) (Prashanth *et al.*, 2024)

The leafhopper *A. biguttula* (Cicadellidae: Hemiptera), is one of the most dangerous pests affecting eggplant. Nymphs and adults suck sap from leaves, causing crop losses ranging from 40-60% (Madhu *et al.*, 2024). They cause severe damage to vegetables and ornamental plants; the insects feed primarily on the underside of leaves, which leads to yellow spots and curling of the affected leaves (Ghosh and Karmakar, 2021).

Entomopathogenic fungi are used in the biocontrol of insect pests; the application of these fungi is safe for the human, environment, and non-target organisms (Bugti *et al.*, 2018). These fungi attack the insect's cuticle, invading the body, and causing the death of the insects (Copping, 2004). *Beauveria bassiana* is one of the important bioagents, which has been used against hemiptera insects such as aphids, whiteflies, and leafhoppers (Upamanya, *et al.*, 2023). Recently, *Trichoderma* spp. has been employed as a biocontrol against insect pests, via direct and indirect mechanisms. Some species of the fungus can directly affect insects

by producing toxic secondary compounds, antifeedant compounds, and repellents. However, the indirect mechanisms enhance plant defense responses or attract natural predators or parasitoids of the insects (Poveda, 2021).

Plant extracts are an eco-friendly method (Majeed *et al.*, 2018; Salah *et al.*, 2023). These plant-derived insecticides are less toxic to a wide range of organisms because they are biodegradable in the environment. Also, their applications are cheaper than chemical alternatives, helping to preserve biodiversity (Souto *et al.*, 2021). Moreover, using extract materials from aromatic and medical plants prevents the development of resistance in insects against active compounds. These plant extracts are generally less stable in the environment and more affordable, especially for small-scale farmers (Arshad *et al.*, 2019). Neem (*Azadirachta indica*) tree is considered an important source of natural insecticides for various insect pest species of Lepidoptera, Diptera, Hemiptera, and Coleoptera. Neem oil is applied externally on the insects' bodies, and blocks the respiratory openings, leading to death (Chaudhary *et al.*, 2017). Also, Azadirachtin is the main active compound found in many neem-based insecticides; this compound disrupts the growth and reproduction of insects; it interacts with their hormones, affecting molting, development, and feeding behaviors (Mordue, 2004; Muhammad & Kashere, 2020; Kilani-Morakchi *et al.*, 2021).

Castor (*Ricinus communis*) plant extracts are used in the management of various insect pests. The aqueous extracts of the leaves have lethal and repellent effects on stored grain pests such as *Callosobruchus chinensis* (Upasani *et al.*, 2003), and *Lasioderma*

serricorne and *Tribolium castaneum*, respectively (Salem *et al.*, 2017). Moreover, Castor oil negatively affected insect pests such as *Zabrotes subfasciatus* (Mushobozy *et al.*, 2009).

Due to the increasing eggplant cultivation areas in Basrah province, and pesticide application against agricultural pests, particularly the leafhopper insect. This study aimed to find alternatives to chemical pesticides and evaluate integrated control methods for this pest in Basra.

Materials & Methods

Lab trials:

1- Leafhopper (*Amrasca biguttula*) colony

In 2022, the colony of the leafhoppers was reared on Eggplant plants (variety Nasar) in the cage; the lab condition was controlled at a temperature of $25\pm 2^{\circ}\text{C}$, relative humidity of $65\pm 5\%$, and photoperiod of 16:8 hours (light: dark). The plants were infested with adult leafhoppers by moving infested leaves to uninfested plants at the stage of 3-4 leaves.

2- Effect of Entomopathogenic fungi on the mortality of adult leafhoppers (*A. biguttula*)

• Preparation of Fungal Spore Suspensions

Beauveria bassiana and *Trichoderma harzianum* isolates were obtained from the Biocontrol Laboratory at Department of Plant Protection at the College of Agriculture, University of Basrah. The isolates were cultured on potato dextrose agar (PDA); the petri dishes were incubated at $28\pm 2^{\circ}\text{C}$ for one week. The spore suspensions of the isolates were prepared by transferring a disc from the culture in 9 ml of sterile water in sterile test tubes. For releasing the spores, the tube was

shaken for 3 minutes. The spore concentration was calculated using a hemocytometer under a light microscope. The 1×10^6 , 1×10^7 , and 1×10^8 spores/ml concentrations were prepared for each isolate.

• Application of spore suspensions of the entomopathogenic fungi against Leafhoppers (*A. biguttula*)

Part of leaves (4x4 cm) of eggplant were placed in 9 cm plastic Petri dishes including moistened cotton. 10 adult leafhoppers were transferred to each dish. The fungal spore suspensions of *B. bassiana* and *T. harzianum* were separately sprayed at the tree concentrations. Each dish was sprayed with 1 ml of the spore suspension, with 0.02% Tween 80, and incubated at $25\pm 2^{\circ}\text{C}$, $65\pm 5\%$ relative humidity, and a 16:8 h (light: dark) photoperiod. The control treatment was sprayed with sterile distilled water, with 0.02% Tween 80. The trial was replicated three times. The mortality of the insects was recorded after 24, 48, and 72 hours of treatment. Mortality rates were corrected using the Schneider-Orell formula (Puntener, 1981).

$$\text{Corrected mortality (\%)} = \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

• Preparation of Fungal culture Filtrates

The fungal culture filtrates were prepared using liquid potato dextrose (PD) medium; 150 ml of PD was transferred into 250 ml sterilized flasks. Each flask was inoculated separately by transferring a 1 cm agar disc from the isolates of *B. bassiana* and *T. harzianum*. The flasks were incubated for 28 days at 25°C . After that, the flasks were filtered using Whatman No. 1 filter paper. Then, the culture filtrates passed through a $0.45 \mu\text{m}$ filter using a vacuum pump.

Finally, the fungal culture filtrates were collected (Huxham & Lackie, 1988).

• **Application of fungal culture filtrates against adult leafhoppers (*A. biguttula*)**

Five concentrations (0, 25, 50, 75, and 100%) of fungal culture filtrates from *B. bassiana* and *T. harzianum* were applied. Ten adult leafhoppers were placed on eggplant leaves (4x4 cm) in Petri dishes. Each dish was sprayed with 1 ml of the filtrate, separately for each fungus and each concentration. The experiment was replicated three times. The mortality rates were recorded and corrected following the same method used to apply spore suspensions.

3- Effect of plant extracts on the mortality of leafhoppers (*A. biguttula*)

• **Collection of Plant Samples**

Plant samples of neem (leaves) and castor (leaves and seeds) were collected from Abu Al-Khasib, Basrah province. The samples were washed and dried at room temperature. Then, the dried samples were ground using an electric grinder.

• **Preparation of Plant Extracts**

The dried powder of the samples was extracted by n-hexane as a non-polar solvent using a Soxhlet extractor at 40°C for 24 hours (Harborne, 1973). 20 g of the sample was dissolved with 200 ml of the solvent. The sample extracts were filtered and concentrated using a rotary evaporator at 60 °C. One gram of deposit from each plant was taken separately, dissolved in 1 ml of n-Hexane, and diluted to 99 ml with distilled water. One milliliter of paraffin and two drops of Tween 80 were added to create a stock solution of 1%.

This helped to prepare concentrations of 0.25%, 0.5%, and 1%. The control treatment contained 100 ml of distilled water with one drop of paraffin and 2 drops of Tween.

• **Application of Plant Extracts on the mortality of adult leafhoppers (*A. biguttula*)**

Ten adults were placed in each dish, with three replicates per stage. One ml of plant extract per dish was sprayed individually at four concentrations (0, 0.25%, 0.5%, and 1%). The mortality of the insects was recorded after 24, 48, and 72 hours of application. The experimental conditions and mortality rates were conducted following the same method used to apply spore suspensions.

Greenhouse Trial:

Effect of the fungi and plant extracts on the field population of the leafhopper *A. biguttula*

Plant extracts, entomopathogenic fungi, and their interactions were evaluated against the leafhoppers infesting eggplant crop (Nasar variety) under greenhouse conditions; The experiment was conducted at the Agricultural Research Station, University of Basrah, during the growing season of the 2022/2023. The treatments included aqueous extracts of neem and castor leaves (Harborne, 1973), fungal spore suspensions of *T. harzianum* and *B. bassiana*, insecticide Sivanto (positive control), and control treatment (Table 1), with four replicates per treatment. The application of treatments was carried out on March 7, 2024, using a 2-liter sprayer. The pest population was collected 1 day before the application and 1,3,7,14,21 days post-application; the treatment efficacy was assessed using Henderson and Tilton formula

(Henderson and Tilton 1955), (Puntener, 1981).

Corrected mortality (%)

$$= 1 - \frac{T_a \times C_b}{T_b \times C_a} \times 100$$

T_b = Number of leafhoppers in the treatment observed before the application

T_a = Number of leafhoppers in the treatment observed after the application

C_b = Number of leafhoppers in the control observed before the application

C_a = Number of leafhoppers in the control observed after the application

Table (1): Treatments used in the experiment

Treatment	Dose
1. <i>Beauveria bassiana</i>	1×10 ⁸ spores/liter
2. <i>Trichoderma harzianum</i>	1×10 ⁸ spores/liter
3. Neem leaves	5 ml/liter
4. Castor leaves	5 ml/liter
5. Neem + <i>B. bassiana</i>	5 ml + 1×10 ⁸ spores/liter
6. Neem + <i>T. harzianum</i>	5 ml + 1×10 ⁸ spores/liter
7. Castor + <i>B. bassiana</i>	5 ml + 1×10 ⁸ spores/liter
8. Castor + <i>T. harzianum</i>	5 ml + 1×10 ⁸ spores/liter
9. SIVANTO	0.25 ml/liter
10. Control (water)	-

Data Analysis

The field experiment was analyzed using a Randomized Complete Design (R.C.D.) at a 0.01 probability level. The Data of the lab were analyzed using randomized complete block design (RCBD) at a 0.05 probability level (Al-Rawi, 1980) by using Statistical (GenStat). Treatment means showing significant differences ($p < 0.05$) were compared using L.S.D.

Results

1- Effect of Fungal spore suspensions against adults of Leafhoppers (*A. biguttula*) under laboratory condition

The results of Table (2) indicated no significant differences in the effect of spore suspensions of *B. bassiana* and *T. harzianum* on the mortality percentages of adult leafhoppers. The mortality rates were 55.9% and 59.0%, respectively. The mortality rates increased over time, reaching a maximum of 68.9% after 72 hours of treatment, while the lowest mortality rate was 44.9% after 24 hours of treatment. The results of the concentrations of the spore suspension over time showed the highest mortality rate was recorded for both fungi (*B. bassiana* and *T. harzianum*); the mortality rates were 83.5% at a concentration of 1×10⁸ spores/ml after 72 hours of the application. However, the lowest mortality rate was observed for *B. bassiana* at a concentration of 1×10⁶ spores/ml; the mortality rate was 28.4% after 24 hours of the application.

The results of Figure (1) showed significant differences among the spore concentrations on adult leafhoppers' mortality. *T. harzianum* caused the highest mortality rate at a concentration of 1×10⁸ spores/ml, with a rate of 69.2%. In contrast, *B. bassiana* resulted in the lowest mortality rate at a concentration of 1×10⁶ spores/ml; the mortality rate was 46.6%. Figure (2) demonstrated highly significant differences in the interaction between the fungal species over time after treatment. *B. bassiana* caused the highest mortality rate after 72 hours of treatment, reaching 70.2%, with a significant difference compared to other treatments. Additionally, *B. bassiana* had the

lowest mortality rate after 24 hours, with a mortality of 41.1%.

Table (2): Effect of fungal spore suspensions on adult leafhopper *A. biguttula* under laboratory condition

Fungi	Con. (spores /ml)	Mortality (%) After Hours			Mean
		24	48	72	
<i>B. bassiana</i>	1×10^8	55.1	68.1	83.5	55.9
	1×10^7	39.8	52.5	64.3	
	1×10^6	28.4	48.9	62.3	
<i>T. harzianum</i>	1×10^8	58.7	65.2	83.5	59
	1×10^7	52	63	64.7	
	1×10^6	35.5	53.2	54.7	
Mean		44.9	58.6	68.9	
L.S.D (0.01)		7.12			5.82

L.S.D (0.01) (interaction: fungi x concentration x time): 17.45

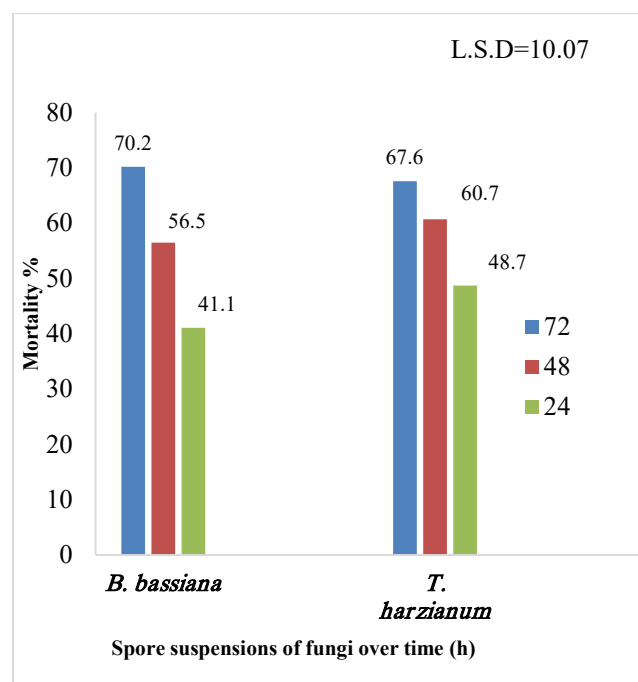


Fig. (2): The effect of the spore suspensions of fungi over time after treatment on the mortality rates of adult leafhopper *A. biguttula* under laboratory conditions.

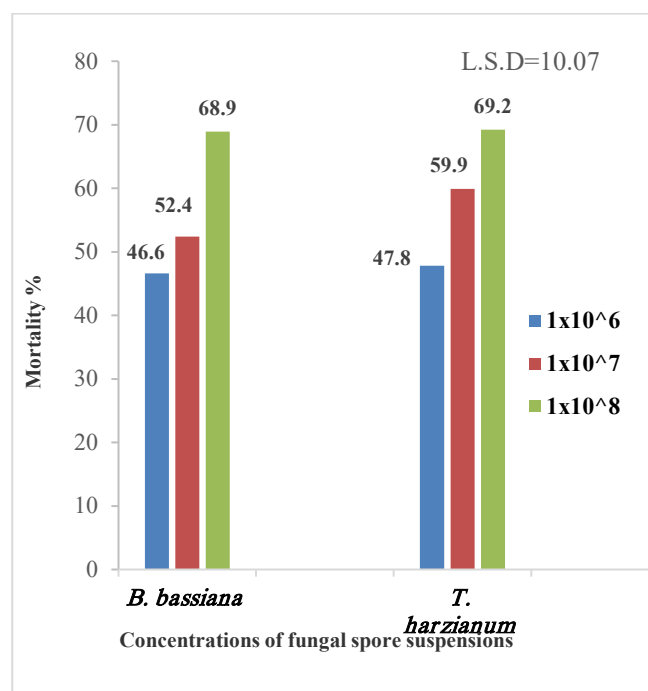


Fig. (1): Effect of the concentration of fungal spore suspensions on the mortality percentages of adult leafhopper *A. biguttula* under laboratory conditions

2- Effect of fungal culture filtrates on adult leafhoppers (*A. biguttula*) under laboratory condition

The results in Table 3 indicated that there were no significant differences ($P < 0.01$) in the effect of the culture filtrates of the fungi *B. bassiana* and *T. harzianum* on the mortality rates of adult leafhoppers; the mortality rates were 34.27% and 31.36%, respectively. Generally, the mortality rate increased over time, reaching its highest at 42.53% after 72 hours after the application; while the lowest mortality rate was 24.20% after 24 hours. However, the highest mortality rate was achieved using the culture filtrates of *B. bassiana*, with an average of 64.70% at a 100% concentration after 72 hours. Conversely, the lowest mortality rate of 17.71% was observed with the culture filtrates of *B. bassiana* at a 75% concentration after 24 hours.

Results of Figure 3 revealed significant differences ($P < 0.01$) in the effect of concentrations on the mortality rates of adults. *B. bassiana* recorded the highest mortality rate at a 100% concentration, reaching 51.92%, with highly significant differences compared to other treatments, while the lowest mortality rate recorded by *B. bassiana* was 21.83% at a 25% concentration. Figure 4 showed significant differences in the effect of the culture filtrates from the fungi over time on the mortality; *B. bassiana* caused the highest mortality rate of 45.01% after 72 hours post-treatment, which was significantly different from other treatments, while the culture filtrate of *T. harzianum* achieved the lowest mortality rate of 23.74% after 24 hours.

Table (3): Effect of fungal culture filtrates on adult Leafhoppers *A. biguttula* in the laboratory

Fungus	Cultural filtrates (%)	Mortality (%) After Hours			Mean
		24	48	72	
<i>B. bassiana</i>	100	37.8	53.3	64.7	34.27
	75	17.7	36.1	54.9	
	50	24.4	24.0	32.9	
	25	18.8	19.1	27.6	
<i>T. harzianum</i>	100	35.9	46.9	62.3	31.36
	75	18.8	24.7	32.9	
	50	21.5	24.7	37.4	
	25	18.8	24.9	27.6	
Mean		24.2	31.7	42.5	
L.S.D 0.01		5.07			4.14

L.S.D 0.01 Concentrations = 5.861

L.S.D (0.01) (interaction: fungi X concentration X time): 14.356

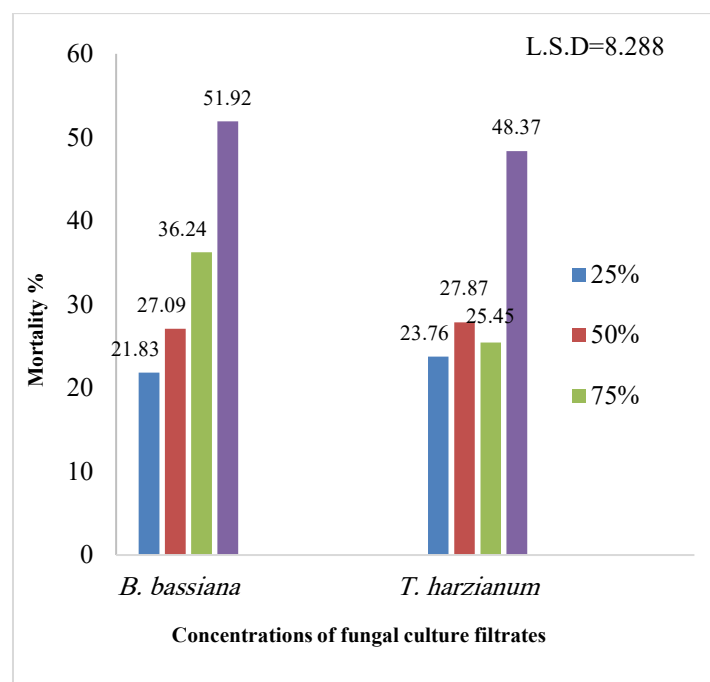


Fig. (3): The effect of the concentration of fungal culture filtrates on the percentage mortality rates of adult leafhoppers *A. biguttula* under laboratory conditions.

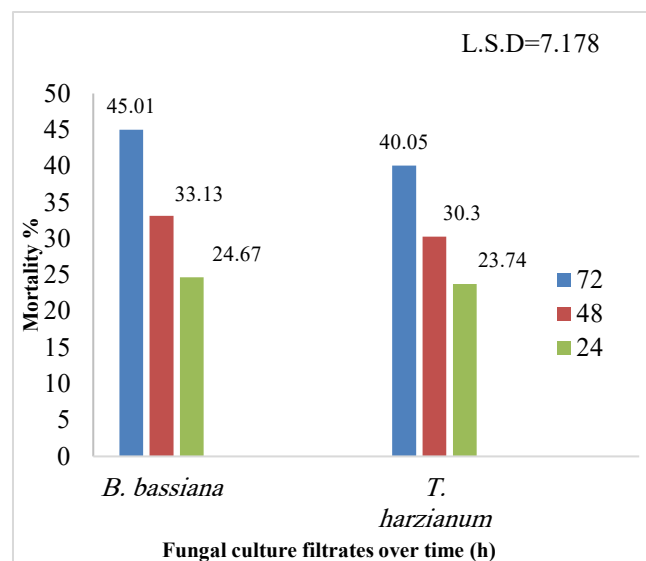


Fig. (4): The effect of fungal culture filtrates over time after treatment on the percentage mortality rates of adult leafhoppers *A. biguttula* under laboratory conditions.

3- Effect of Plant Extracts on the mortality of adult leafhoppers (*A. biguttula*) under laboratory condition

The results in Table 4 indicated that there were no significant differences in the effects of the

plant extracts (neem leaves, castor leaves, and castor seeds) on the mortality rates of adult leafhoppers; the mortality rates were 82.97%, 82.76%, and 85.45%, respectively. However, the lowest mortality rate was recorded at 77.30% after 24 hours of treatment, while the highest mortality rate was 87.72% after 72 hours after the application. Also, it was found that the 1% and 0.5% concentrations of neem leaves, castor leaves, and castor seeds caused 90% mortality at various times (24, 48, and 72 hours). However, the neem leaf extract only reached a mortality rate of 83.48% after 24 hours. This suggests that using these extracts at 0.5% could be the effective concentration due to their quick and strong effect on insect mortality.

The results of Figure 5 recorded highly significant differences in the effect of plant extracts at different concentrations on the mortality rates of adult leafhoppers. All plant extracts caused the highest mortality rate of 90.00% at a 1% concentration. In contrast, the castor leaf extract provided the lowest mortality rate at a concentration of 0.25%, reaching 68.28%. Figure 6 showed highly significant differences in the interaction between plant extract types and time. The neem leaf and castor leaf extracts resulted in the highest mortality rates after 72 hours of treatment, reaching 90.00%. However, the neem leaf extract caused the lowest mortality rate after 24 hours of treatment, with a rate of 71.08%.

Table (4): Effect of plant extracts on the mortality of adult Leafhopper *A. biguttula* under the laboratory conditions

Plant Extract	Concent. (%)	Mortality (%) After (Hours)			Mean
		24	48	72	
Neem Leaves	1	90.00	90.00	90.00	82.97
	0.5	83.48	90.00	90.00	
	0.25	39.76	83.48	90.00	
Castor Leaves	1	90.00	90.00	90.00	82.76
	0.5	90.00	90.00	90.00	
	0.25	52.89	61.94	90.00	
Castor Seeds	1	90.00	90.00	90.00	85.45
	0.5	90.00	90.00	90.00	
	0.25	69.54	90.00	90.00	
Mean		77.3	86.16	90.00	
L.S.D 0.01		4.328			4.328

L.S.D 0.01 Concentrations = 4.328

L.S.D (0.01) (interaction: plant extract X concentration X time): 12.985

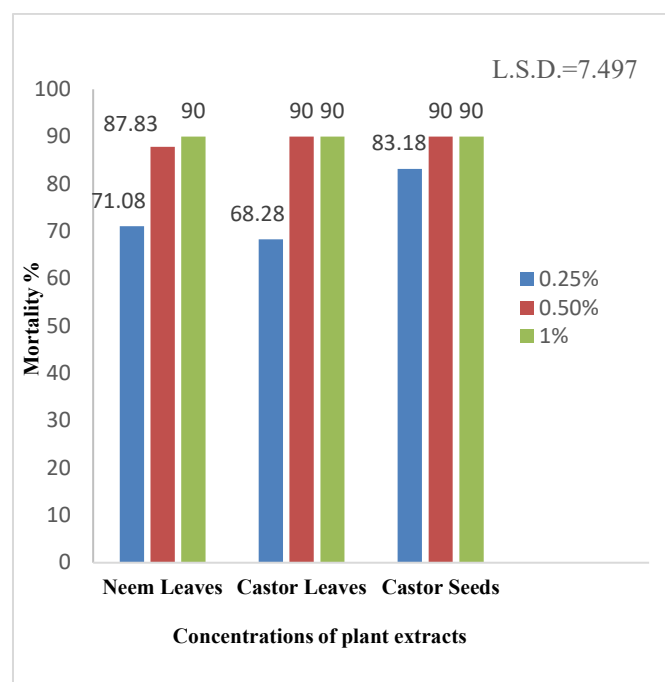


Fig.(5): The effect of the concentration of plant extracts on the percentage mortality rates of adult leafhoppers *A. biguttula* under laboratory conditions.

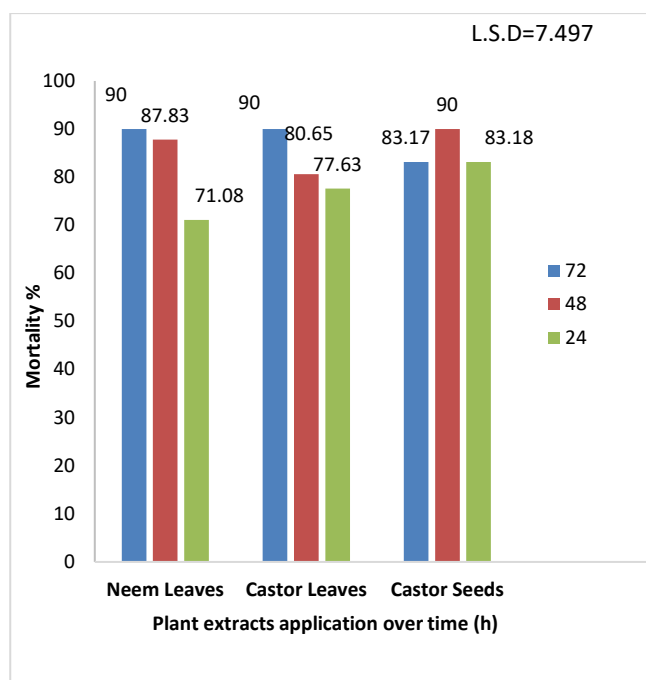


Fig.(6): The effect of the plant extracts over time after treatment on the percentage mortality rates of adult leafhoppers *A. biguttula* under laboratory conditions.

4- The relative efficiency of the fungi and plant extracts against the leafhopper *A. biguttula* under the field conditions

The results of Table 5 showed the relative efficiency of the fungi *B. bassiana* and *T. harzianum*, neem, and castor leaf extracts compared to the positive control treatment (insecticide, Fungi Sivanto) against the leafhopper. Fungi *B. bassiana* and *T. harzianum* recorded a mean mortality rate of 76.6% and 76.8% respectively. *B. bassiana* showed the highest rate of 82.8% after 14 days. *T. harzianum* reached the highest mortality on Day 7 (85.8%).

The extract of neem leaf caused a high initial mortality of 96.6% one day after the application, but its effectiveness dropped to 64.6% after Day 28. This rapid, and early effect made neem extract an excellent short-term solution. However, the extract of Castor leaf was less effective, with a mean mortality of 66.8%. it caused a mortality of 85.8% one

day after the application; its effectiveness declined reaching 59% on day 14 after the application.

The best combination of extract and fungus was *B. bassiana* + neem, which resulted in a 95.0% mortality rate after one day of treatment. The mortality dropped to 60.4% after day 28. Its mean mortality (78.1%) indicated that the combination may lose effectiveness over time. The combination of *T. harzianum* + neem reached its highest mortality on Day 7 (89.8%); the mortality remained relatively effective throughout the experiment; it was 82.8% after day 28. The lowest mortality rate was for castor leaf extract, which achieved 66.8% compared to Sivanto (95.2%).

Table(5): The relative efficiency of the fungi and plant extracts against the leafhopper *A. biguttula* infesting eggplant crop under the field conditions, 2022.

Treatment	Mortality (%) After (day)					Mean
	1	3	7	14	28	
<i>Beauveria bassiana</i>	76.2	67.8	75	82.8	81.4	76.6
<i>Trichoderma harzianum</i>	72.2	69.8	85.8	80.6	75.8	76.8
Neem Leaf Extract	96.6	93	68.4	81.4	64.6	80.8
Castor Leaf Extract	85.8	68.6	51.8	59	68.6	66.8
<i>B. bassiana</i> + Neem	95	93.6	64.4	77	60.4	78.1
<i>B. bassiana</i> + Castor	79.2	68.8	73.6	84.6	68.2	74.9
<i>T. harzianum</i> + Neem	81.8	69.2	89.8	73.4	82.8	79.4
<i>T. harzianum</i> + Castor	80.8	66.6	63	83.4	72.4	73.2
Sivanto (Insecticide)	96.2	96.8	84.8	98	100	95.2
Mean	84.8	77.1	73	80	74.9	
L.S.D 0.05	9.56					12.83

L.S.D (0.05) (interaction: treatments X time): 28.69.

Discussion

The study showed that entomopathogenic fungi and plant extracts can effectively control *A. biguttula* infesting eggplant crops. The fungal strains *B. bassiana* and *T. harzianum* showed significant efficacy. Three concentrations (1×10^6 , 1×10^7 , and 1×10^8 spores/mL) of spore suspensions were tested against the leafhopper. The concentration of 1×10^8 spores/mL for both fungi achieved the highest mortality rates. These results agreed with previous findings indicating the increase in mortality with higher spore concentrations, particularly the 1×10^8 spores/mL. Nazir *et al.* (2018) tested three concentrations (1×10^4 , 1×10^6 , and 1×10^8 spores/mL) of the commercial fungus *B. bassiana* against the green peach aphid *Myzus persicae*; they found that the highest mortality rate occurred at 1×10^8 spores/mL.

Numerous studies indicated the effectiveness of fungi, *B. bassiana* and *T. harzianum*, in controlling various insects in the order Hemiptera, including leafhoppers. For example, *Metarhizium brunneum* (EAMa 01/58-Su) and *B. bassiana* (EABb 01/33-Su) were used as part of an Integrated Pest Management (IPM) program to control the spread of *Xylella fastidiosa* by targeting the insect *Philaenus spumarius* (Hemiptera: Aphrophoridae); higher mortality rates were observed in insects feeding on plants treated with these fungi (Yousef-Yousef *et al.*, 2023). Similarly, Wangkeeree *et al.* (2024) successfully applied *B. bassiana* SUTTU01 to control the leafhopper *Matsumuratettix hiroglyphicus*, a vector of phytoplasma causing white leaf disease in sugarcane; this biocontrol program reduced phytoplasma transmission. Moreover, *B. bassiana* has been employed against the green leafhopper

Nephotettix virescens (Distant) (Homoptera: Cicadellidae) on rice crops.

Additionally, *T. harzianum* has demonstrated effectiveness against various pests, such as cotton aphid *Ceratovacuna lanigera* (Hemiptera: Aphididae) (Islam *et al.*, 2021). Mahdi *et al.* (2017) reported the effectiveness of the spore suspensions of both fungi, *B. bassiana* and *T. harzianum*, in the mortality of adult *Tetranychus urticae*; the mortality rates recorded at 90% for both fungi after 72 hours of treatment. The effectiveness of these fungi against insects is due to their producing enzymes breaking down the insect cuticle and releasing toxic metabolites that interfere with the insect's growth and development (Wattanalai *et al.*, 2004; Boucias and pendland, 2012).

Plant extracts are utilized in pest control, as repellents, attractants, feeding inhibitors, assistive agents, sterilizing agents, and insect growth regulators (Ngegba, 2022). The toxicity of neem against insect pests is attributed to various chemicals, such as nimbin, gedunin, salannin, meliantriol, nimbidin, mahmoodin, nimbolin, sodium nimbin, 22,23-dihydronimocinol, gallic acid, and quercetin, that disrupt the reproduction and growth of the insect (Adusei & Azupio, 2022). The extraction of phytochemicals from neem employs different organic solvents like hexane; these extracts showed lethal activity against insects. Hexane is used as a solvent to extract neem oil, which contains insecticidal components, such as azadirachtin and salanin. Both components work by inhibiting insect feeding and disrupting their molting process. That makes them effective pest control tools (Chaudhary *et al.*, 2017; Isman, 2006; Sharma *et al.*, 2012). Various studies showed the effectiveness of the plant extracts of neem against leafhoppers,

such as Green Leafhopper, *Empoasca decipiens* Paoli (Aziz *et al.*, 2018), and other sap-sucking pests like aphids (Brotodjojo & Arbiwati, 2016).

Salinas-Sánchez *et al.* (2021) noted the efficacy of castor leaf extract using hexane against adult yellow aphids (*Sipha flava*), with a mortality rate of 80%. Also, castor seed extract (*R. communis* L.) was tested against the fall armyworm (*Spodoptera frugiperda*) infesting corn; the larval mortality rates of 46.5%, 60.3%, and 75.8% were recorded at concentrations of 150, 200, and 250 g/L of the plant, respectively (Kombieni *et al.*, 2023). Another study recorded the effectiveness of castor extracts against banana weevil (*Cosmopolites sordidus*), corn leaf aphid (*Rhopalosiphum maidis*), and yellow cane borer (*Sipha flava*) (Sotelo-Leyva *et al.*, 2023).

In the field, biological pesticides, entomopathogenic fungal or plant sources, were effective against leafhoppers infesting eggplants. A previous study evaluated several biological pesticides in the mortality of the pests, aphids, whiteflies, and leafhoppers; the results showed that neem formulations were more effective than *B. bassiana* in reducing leafhopper populations (Naik *et al.*, 2012). Also, the field efficacy of the systemic insecticide Sivanto and neem leaf extract showed the highest mortality rates of 96.2% and 96.6%, respectively, after one day of application. However, neem extracts are applied as an environmentally friendly alternative to the chemical pesticides. Ultimately, the results of this study highlighted the synergistic effects of combining fungal spore suspensions with plant extracts (*B. bassiana* + neem). These combined treatments showed higher mortality rates than individual applications. Thus, biological pesticides could

be used as a key component of integrated pest management.

Conclusion

In conclusion, the fungal strains *B. bassiana* and *T. harzianum* and plant extracts, especially neem can effectively control leafhoppers infesting eggplants. Moreover, the combination treatments of *B. bassiana* + neem showed initial promise control for the pest. Due to the results of this study, the entomopathogenic fungi and plant extracts work as a promising and environmentally friendly alternative to chemical pesticides for pest management.

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Contributions of authors

F.T.L. : Carried out the experiment in the field and collected the data; Data Analysis

A.A.: Constructed the idea and hypothesis for research; planned the methodology; project administration. wrote the manuscript

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Conflicts of Interest

The authors declare no conflicts of interest.

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المكافحة الحيوية لحشرة قفازات الأوراق *Amrasca biguttula* على نباتات الباذنجان باستخدام الفطريات الممرضة للحشرات والمستخلصات النباتية

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المستخلص: تم تقييم كفاءة الفطريات الممرضة للحشرات والمستخلصات النباتية ضد قفازات الأوراق *Amrasca biguttula* الذي يصيب نباتات الباذنجان. أجريت التجارب في محطة البحوث الزراعية ومختبر كلية الزراعة - جامعة البصرة خلال موسم النمو لعام 2023. تضمنت التجارب المختبرية استخدام المعلقات الفطرية ومرشحات لكل من *Trichoderma harzianum*، بالإضافة إلى مستخلصات أوراق النيم (*Azadirachta indica*) وأوراق وبذور الخروع (*Ricinus communis*)، أظهرت نتائج المعلقات الفطرية عدم وجود فروق معنوية في معدل القتل بين البالغات بين *B. bassiana* و *T. harzianum*، إذ بلغت نسب القتل 55.9% و 59.0% على التوالي. وازدادت نسب القتل بمرور الوقت لتصل إلى 83.5% عند أعلى تركيز (1×10^8 جرثومة/مل) بعد 72 ساعة، فيما كانت أدنى نسبة 28.4% عند تركيز أقل (1×10^6 جرثومة/مل) بعد 24 ساعة. كما أظهرت نتائج استخدام الرواشح الفطرية أن أعلى معدل قتل بلغ 64.7% لفطر *B. bassiana* عند تركيز 100% بعد 72 ساعة، فيما سجل *B. bassiana* أدنى معدل قتل بنسبة 17.71% عند تركيز 75% بعد 24 ساعة. ولم تلاحظ فروق معنوية في معدل نسب القتل للحشرات بين معاملات استخدام مستخلصات النيم وأوراق الخروع وبذور الخروع، إذ بلغت النسب 82.97% و 82.76% و 85.45% على التوالي. وحقت جميع المستخلصات النباتية بتركيز 1% و 0.5% نسبة قتل بلغت 90% بعد 24 و 48 و 72 ساعة. تحت ظروف البيت البلاستيكي، وتضمنت معاملات المعلقات الفطرية والمستخلصات النباتية (أوراق النيم والخروع) مع مقارنتها بالسيطرة الإيجابية، مبيد الحشرات سيفانتو. إذ سبب مستخلص النيم أعلى معدل نسب قتل بلغ 80.8%، في حين سجل مستخلص أوراق الخروع أدنى معدل قتل بنسبة 66.8%، مقارنة بمبيد سيفانتو الذي حقق معدل قتل بنسبة 100% بعد 21 يومًا.

الكلمات المفتاحية: *Trichoderma harzianum*، *Beauveria bassiana*، *Amrasca biguttula*، نطاط الأوراق، المستخلصات النباتية