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Enhanced Biocontrol of Red Palm Weevil Using Insect Pathogens in Date Palm Cultivation: A Sustainable Alternative to Pesticides

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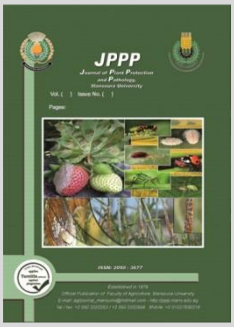

ABSTRACT

The red palm weevil (RPW) (*Rhynchophorus ferrugineus*) is a significant pest threatening date palm cultivation globally, including in Egypt. Traditional chemical control methods have proven inadequate due to resistance development and environmental concerns, underscoring the need for sustainable alternatives. This study evaluated the efficacy of various biological control agents, including the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, *Trichoderma harzianum*, *Aspergillus flavus*, and the bacterium *Serratia nematodiphila*, against RPW eggs and larvae under controlled laboratory conditions. The lethal concentrations (LC_{50}) and lethal times (LT_{50}) for each pathogen were determined. Results revealed that *B. bassiana* and *S. nematodiphila* were the most effective, achieving up to 90% and 88% larval mortality, respectively, at concentrations of 1.5×10^7 (spore/ml, c/ml). *B. bassiana* exhibited an LC_{50} of 6.7×10^5 spores/ml for larvae, while *S. nematodiphila* had a slightly higher LC_{50} of 8.3×10^5 c/ml. Both agents demonstrated rapid efficacy, with LT_{50} values of 7.33 days and 8.33 days for larvae, respectively. Other pathogens, such as *T. harzianum* and *A. flavus*, showed moderate efficacy with lower mortality rates (75% and 80%) and higher LC_{50} values, indicating the need for higher concentrations to achieve significant mortality.This study is the first to compare these biocontrol agents systematically in the RPW management in Egypt, providing critical insights into their potential integration into integrated pest management (IPM) programs. The findings suggest that *B. bassiana* and *S. nematodiphila* are promising candidates for sustainable RPW control, warranting further field trials to validate their effectiveness under diverse environmental conditions.

Keywords: Red Palm Weevil, RPW biocontrol, Entomopathogenic fungi, *Serratia nematodiphila*, *Beauveria bassiana* , RPW mortality rates

INTRODUCTION

Date palm (*Phoenix dactylifera*) is a critical agricultural crop, especially in regions like Egypt, where it contributes approximately 19% of the world's date production and 24% of the Arab region's production. With over 16 million palm trees and an annual production of 1.8 million tons, the date palm sector is crucial to Egypt's economy (FAO, 2023). The date palm in Egypt and globally offers diverse benefits: fruit for nutrition, trunk for furniture, leaves for crafts, pulp for alcohol and gel, seed for oil, and ornamental value (withurfwqn, 2023). Date processing waste can be transformed into high-value products, including biofuels and pharmaceuticals, promoting sustainability (Shefali *et al*. 2023).

The date palm sector in Egypt faces several significant challenges that hinder its growth and development. One of the primary obstacles is the lack of technical know-how among farmers, which affects the quality and quantity of date production like palm diseases Khaled (2024), and insect pests Ali *et al*., (2020). All these challenges lead to inefficient farming practices and reduced yields. Additionally, there is a notable deficiency in post-harvest and processing facilities, limiting the ability to add value to the harvested dates and thereby reducing their competitiveness in international markets. Consequently, Egypt's date export volumes remain relatively low despite being one of the largest producers globally (FAO, 2024).

The red palm weevil (*Rhynchophorus ferrugineus*) is the latest insect pest that threatens palm cultivation, especially date palms, globally and in Egypt in particular, due to its widespread distribution and highly destructive nature. This invasive species originated in Southeast Asia and has now spread to the Middle East, North Africa, southern Europe and parts of the Americas, causing significant economic losses in date palm, coconut and oil palm plantations (Faleiro *et al*., 2016). Since its detection in Egypt in 1992 (Saleh *et al*., 1992), it has become a major threat to palm cultivation in Egypt. After laying eggs, the larvae burrow into the trunks of the palm trees, causing severe damage that disrupts the flow of nutrients within the plant, often leading to plant death (Hussain *et al*., 2020). In order to control this pest and reduce its damage, a number of strategies have been used, including traditional pest control methods, which rely primarily on chemical insecticides, that alone are no longer sufficient to mitigate the spread of this pest due to the development of resistance, off-target effects, and environmental concerns (El-Sayed *et al*., 2023). In addition to plant extracts and essential oils (Ali *et al*., 2019 and 2020), which have been used recently.

In response to these challenges, there has been a growing interest in exploring biological control methods that

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utilize natural predators, parasitoids, and entomopathogens, such as fungi and bacteria, to manage pest populations more sustainably (Wakil *et al*., 2017). Among these biological control agents, entomopathogenic fungi (EPFs) like *Beauveria bassiana*, *Metarhizium anisopliae*, *Trichoderma harzianum*, and *Aspergillus flavus*, as well as the entomopathogenic bacterium *Serratia nematodiphila*, have gained prominence due to their broad host range and effectiveness against various insect pests (García *et al*., 2018, Ali *et al* 2024). These pathogens act by infecting the insect host, proliferating within its body, and eventually causing death through mechanical damage and toxin production (Toledo *et al*., 2019). Biopesticides derived from natural sources are also being developed to disrupt the weevil's life cycle effectively. These biological control strategies offer a safer, environmentally friendly alternative, contributing to sustainable agriculture by reducing reliance on chemical pesticides and enhancing the resilience of date palm cultivation against RPW infestations.

Globally, *B. bassiana* and *S. nematodiphila* have been shown to exhibit high virulence against RPW. For example, studies by Dembilio *et al*. (2010) and Manzoor *et al*. (2020) found that *B. bassiana* could achieve significant mortality in adult RPW populations. Similarly, Wakil *et al*. (2017) demonstrated the effectiveness of *S. nematodiphila* under laboratory conditions. However, few studies have evaluated these agents in combination or tested their effectiveness against both eggs and larvae under controlled conditions, as we do here. Additionally, research on the integration of these agents into broader IPM programs remains limited, particularly in Egypt, where pest management approaches tend to rely heavily on chemical controls.

This study aims to fill the gap in existing research by systematically evaluating the efficacy of multiple entomopathogenic fungi and *S. nematodiphila* against RPW eggs and larvae in Egypt, with a specific focus on lethal concentrations (LC_{50}) and lethal times (LT_{50}) across varying dosages. While previous studies have focused on individual agents, this research is the first to comprehensively compare these biocontrol agents within the same experimental framework. In addition, our study investigates the potential for these pathogens to be used in combination to enhance overall pest control effectiveness.

The novelty of this research lies in its approach to evaluating multiple biological control agents simultaneously while providing critical insights into their potential synergistic effects and adaptability to local environmental conditions. By addressing key gaps in dosage optimization and agent combination strategies, our findings aim to support the development of more sustainable, environmentally friendly pest management practices for date palm cultivation.

MATERIALS AND METHODS

Collection of Red Palm Weevil Adults

Adults of red palm weevils (*Rhynchophorus ferrugineus*) were collected from infested date palm trees (*Phoenix dactylifera*) located in Faculty of Agriculture, South Valley University, Qena, Egypt. Infestation sites were identified based on visible symptoms such as wilting fronds, oozing sap, boreholes at the base of fronds, and the presence of frass.

Upon arrival at the laboratory, the adult weevils were transferred to a controlled environment room maintained at 27 ± 2°C temperature, 70 ± 5% relative humidity, and a 12:12 h light photoperiod. Adults were placed in a rearing cage made of transparent plastic (20 cm × 15 cm × 15 cm) with mesh-covered ventilation holes to allow air circulation while preventing escape.

Fresh sugar cane pieces, approximately 10 cm in length and 2 cm in diameter, were provided as both a food source and an oviposition substrate. Food was replaced every 48 hours to ensure freshness and prevent mold growth. Deposited eggs were collected and incubated until hatching. The larvae until the fourth instar were reared on shredded sugar cane, then the later instars transferred to sugar cane pieces until pupation.

Experimental Design

A randomized complete block design (RCBD) was employed to evaluate the effectiveness of various entomopathogenic fungi and *S. nematodiphila* on red palm weevil first instar larvae and eggs. The experimental layout included 5 treatments, each replicated 10 times. Control groups, where no biological treatments were applied, were included for comparative purposes.

Pathogen Preparation

The entomopathogenic fungi (*B. bassiana*, *M. anisopliae*, *T. harzianum*, and *A. flavus*) and *S. nematodiphila* were obtained from infected red palm weevil stages which collected from infested palm trees. Fungal spores were cultured on potato dextrose agar (PDA) at 28° C for three days, while bacterial cultures were grown on nutrient agar under similar controlled conditions. Spores and bacterial cells were harvested and suspended in sterile distilled water to prepare concentrations of $1,1.5\times10^6$ and $1,1.5\times10^7$ spores/mL or cells/mL for application.

Harvesting Spores and Bacterial Cells

Spores from fungal cultures were harvested by adding sterile distilled water containing 0.1% Tween 80 and gently scraping the surface with a sterile loop. The suspension was filtered through sterile cheesecloth to remove mycelial fragments. Bacterial cells were harvested by washing the culture plates with sterile distilled water and gently scraping with a sterile loop. Concentrations were verified using a hemocytometer for fungal spores and a spectrophotometer for bacterial cells (OD600).

Application Methods

Red palm weevil eggs and larvae were carefully collected from mass rearing and placed in sterile petri dishes. Each group of eggs and larvae (n = 30 per treatment group) was dipped individually into 10 mL of the prepared pathogen suspension using sterilized forceps. The dipping duration was standardized to 10 seconds for each specimen to ensure consistent exposure. Control groups were dipped in sterile distilled water containing 0.1% Tween 80 to account for the effect of the dipping process itself.

After dipping, the specimens were placed on sterile filter paper to remove excess liquid and then transferred to separate sterile petri dishes lined with moistened filter paper to maintain humidity. The treated and control groups were maintained in an environmental chamber set to 27 ± 2°C and 70 ± 5% relative humidity under a 12-hour light/dark cycle.

Monitoring and Data Collection

Specimens were observed daily for 7 days post-treatment. Mortality was recorded at 24-hour intervals, noting the number of dead larvae or non-viable eggs in each treatment group. Larvae were considered dead if they showed no movement when prodded gently with a sterile needle. Eggs were considered non-viable if they did not hatch within the observation period or showed visible signs of fungal or bacterial infection.

Mortality data were analyzed using probit analysis to determine the lethal concentration (LC_{50}) and lethal time (LT_{50}) for each pathogen. An Analysis of Variance (ANOVA) was performed to compare the effectiveness of different pathogens and concentrations using SPSS, with significance determined at the $P < 0.05$ level. Post-hoc Tukey's tests were used to identify significant differences between treatment means. All experiments were conducted under the same environmental conditions to minimize variability. Randomization was used to assign treatments to specimens to avoid bias, and replicates were included to ensure statistical robustness.

Ethical Considerations

All experiments were conducted in accordance with the ethical guidelines of the Faculty of Agriculture, South Valley University, ensuring minimal harm to the insects and adherence to environmental safety standards.

RESULTS AND DISCUSSION

The study evaluated the effectiveness of different five insect pathogens against the egg and larval stages of the red palm weevil. This included various concentrations of the tested pathogens, *S. Nematodiphila*, *T. harzianum*, *B. bassiana*, *M. anisopliae*, and *A. flavus*.

During this study, four different concentrations of each pathogen were used. The results of the experiments showed that *S. Nematodiphila* 1.5×10^7 showed the highest effectiveness with a mortality rate of 9.67±0.58% ranging from 8.32 to 10.11 larva Table (1). This was followed directly by *T. harzianum* $1.5 \times 10^7$and *M. anisopliae* 1.5×10^7, with an average mortality rates of 9 .00±1.00, and 8.67 ± 0.58%, respectively. These previous treatments showed statistically significant mortality rates compared to the other treatments (F value = 8.772, P<0.0001). However, with the decrease in concentrations, the treatments effectiveness began to gradually decrease, for example *S. Nematodiphila* at a concentration of 1×10^6 had an average mortality rate reached 6.33 ±0.58%, which is much lower than the higher concentrations. Also, *A. flavus* at the lowest concentrations of 1×10^6 had the least mortality rate of 4.67±0.58%.

Table 1. Descriptive Statistics for Red Palm Weevil Larvae Under Various treatments

Pathogens	Concentrations (spore/ml, c/ml)	Mean ± SD	95% Confidence Interval for Mean	Minimum	Maximum	F value	P value
A. flavus	1×10^6	4.67 ± 0.58^{a}	3.23 - 6.10	4.00	5.00	8.772	0.000
	1.5×10^6	5.33 ± 0.58^{a}	3.90 - 6.77	5.00	6.00		
	1.5×10^7	7.33 ± 0.58^{ab}	5.90 - 8.77	7.00	8.00		
	1× 10 7	6.67 ± 1.53^{ab}	2.87 - 10.46	5.00	8.00		
B. bassiana	1×10^6	5.67 ± 0.58^{a}	4.23-7.10	5.00	6.00		
	1×10^7	7.33 ± 0.58^{ab}	5.90 - 8.77	7.00	8.00		
	1.5×10^6	6.67 ± 0.58^{a}	5.23 - 8.10	6.00	7.00		
	1.5×10^7	8.00 ± 1.00^{abc}	5.52 - 10.48	7.00	9.00		
M. anisopliae	1×10^7	7.33 ± 0.58^{ab}	5.90 - 8.77	7.00	8.00		
	1×10^6	5.33 ± 0.58^{a}	3.90 - 6.77	5.00	6.00		
	1.5×10^6	6.33 ± 0.58^{a}	4.90 - 7.77	6.00	7.00		
	1.5×10^7	8.67 ± 0.58^{abc}	7.23 - 10.10	8.00	9.00		
S. Nematodiphila	1×10^6	6.33 ± 0.58^{a}	4.90 - 7.77	6.00	7.00		
	1×10^7	8.33 ± 0.58^{abc}	6.90 - 9.77	8.00	9.00		
	1.5×10^6	7.33 ± 0.58^{ab}	5.90 - 8.77	7.00	8.00		
	1.5×10^7	9.67 ± 0.58^{abcd}	8.23 - 11.10	9.00	10.00		
T. harzianum	1×10^6	5.67 ± 0.58^{a}	4.23 - 7.10	5.00	6.00		
	1×10^7	7.67 ± 1.53^{ab}	3.87 - 11.46	6.00	9.00		
	1.5×10^6	6.67 ± 0.58^{a}	5.23 - 8.10	6.00	7.00		
	1.5×10^7	9.00 ± 1.00^{abc}	6.52 - 11.48	8.00	10.00		

Results shown in Table (2) indicate that there are differences comparing to the larvae, *S. Nematodiphila* showed the highest effectiveness with an average mortality rate 8.33 ± 0.58%, at a concentration of 1.5×10^7, followed directly by *B. bassiana* at concentration of 1.5×10^7, with an average mortality rate of 8.00 ± 1.00%. The results showed a convergence in the averages with decreasing concentrations. Despite this, there were statistically significant differences between the treatments and each other. The results showed that, *A. flavus* at concentration of 1×10^6 had the lowest mortality rate of 2.33 ± 0.58%.

As shown in Table 3, which shows the cumulative percentages of RPW larval mortality after applying different insect pathogen treatments over five days, a clear variation and variability can be observed between treatments. *B. bassiana* showed the highest efficacy (Fig. 1), especially at a concentration of 1.5×10^7 spores/ml, where larval mortality gradually increased from 50% after three days of treatment to a peak of 90% on day seven. Similarly, *T. harzianum* at a concentration of 1.5×10^7 spores/ml showed significant larval killing activity, with mortality rates increasing from 45% on day three to 85% by day seven. Lower concentrations of these pathogens also showed lethal effects on larvae, but with low mortality rates, confirming a concentration-dependent as well as time-dependent response after treatment. Both *Metarhizium anisopliae* and *Aspergillus flavus* showed moderate efficacy compared to the previous one, with mortality rates reaching 78% and 80%, respectively, at the highest concentrations (1.5×10^7 bacteria/ml) by day 7. *Serratia nematodiphila* showed promising results, with maximum mortality reaching 88% at 1.5×10^7 c/ml by day 7 (Fig. 1), indicating its potential as a bio-agent for pest control.

Table 2. Descriptive Statistics for Red Palm Weevil Eggs Under Various treatments

Pathogens	Concentrations (spore/ml, c/ml)	Mean ± Sd	95% Confidence Interval for Mean (Lower - Upper)	Minimum	Maximum	F value	P value
A. flavus	1 × 10^6	2.33 ± 0.58^{a}	0.90 - 3.77	2.0	3.0		
	1 × 10^7	5.00 ± 1.00^{ab}	2.52 - 7.48	4.0	6.0		
	1.5 × 10^6	3.67 ± 0.58^{a}	2.23 - 5.10	3.0	4.0		
	1.5 × 10^7	6.67 ± 0.58^{ab}	5.23 - 8.10	6.0	7.0		
B. bassiana	1 × 10^6	3.00± 1.00^{a}	0.51-5.48	2.00	4.00		
	1 × 10^7	6.33 ± 0.58^{ab}	4.90 - 7.77	6.0	7.0		
	1.5 × 10^6	4.67 ± 0.58^{a}	3.23 - 6.10	4.0	5.0		
	1.5 × 10^7	8.00 ± 1.00^{abc}	5.52 - 10.48	7.0	9.0		
M. anisopliae	1 × 10^6	3.67 ± 0.58^{a}	2.23 - 5.10	3.0	4.0		
	1 × 10^7	6.67 ± 0.58^{ab}	5.23 - 8.10	6.0	7.0		
	1.5 × 10^6	4.67 ± 1.15^{ab}	1.80 - 7.54	4.0	6.0	17.559	0.0
	1.5 × 10^7	7.67 ± 0.58^{abc}	6.23 - 9.10	7.0	8.0		
S. Nematodiphila	1.5 × 10^6	5.67 ± 0.58^{ab}	4.23 - 7.10	5.0	6.0		
	1.5 × 10^7	8.33 ± 0.58^{abcd}	6.90 - 9.77	8.0	9.0		
	1 × 10^6	3.33 ± 0.58^{a}	1.90 - 4.77	3.0	4.0		
	1 × 10^7	7.67 ± 0.58^{abc}	6.23 - 9.10	7.0	8.0		
T. harzianum	1 × 10^6	3.00 ± 1.00^{a}	0.52 - 5.48	2.0	4.0		
	1 × 10^7	5.67 ± 0.58^{ab}	4.23 - 7.10	5.0	6.0		
	1.5 × 10^6	4.33 ± 1.15^{ab}	1.46 - 7.20	3.0	5.0		
	1.5 × 10^7	7.67 ± 1.15^{abc}	4.80 - 10.54	7.0	9.0		

Table 3. Efficacy of Entomopathogenic Fungi and *Serratia nematodiphila* in Increasing Red Palm Weevil Larval Mortality Across Different Concentrations and Treatment Durations

pathogen	Concentrations (spore/ml, c/ml)	Days after treatment 3	4	5	6	7
Control	0	0	0	0	0	0
B. bassiana	1 × 10^6	20	28	37	45	52
	1.5 × 10^6	25	32	42	50	58
	1 × 10^7	40	52	65	75	82
	1.5 × 10^7	50	60	73	83	90
M. anisopliae	1 × 10^6	0	0	0	0	0
	1.5 × 10^6	15	23	30	38	45
	1 × 10^7	20	28	37	46	54
	1.5 × 10^7	35	47	60	70	78
T. harzianum	1 × 10^6	18	24	32	40	48
	1.5 × 10^6	24	32	40	50	58
	1 × 10^7	38	48	58	68	75
	1.5 × 10^7	45	55	65	75	85
A. flavus	1 × 10^6	12	18	25	33	40
	1.5 × 10^6	18	25	33	42	50
	1 × 10^7	30	40	50	60	70
	1.5 × 10^7	38	48	58	70	80
S. Nematodiphila	1 × 10^6	15	20	28	35	42
	1.5 × 10^6	22	28	35	45	52
	1 × 10^7	35	45	55	65	75
	1.5 × 10^7	42	55	68	78	88

Table 4 shows the mortality rates of Red Palm Weevil (RPW) larvae and eggs at a high concentration of 1.5 × 10^7 spores/ml or cells/ml for various biocontrol agents. *B. bassiana* demonstrated the highest mortality for both larvae (90%) and eggs (88%) (fig.1).

Table 4. Mortality Rates of RPW Developmental Stages by Each Biocontrol Agent

Biocontrol Agent	Concentration (spores/ml or cells/ml)	Larval Mortality (%)	Egg Mortality (%)
A. flavus	1.5 × 10^7	80	73
B. bassiana	1.5 × 10^7	90	88
M. anisopliae	1.5 × 10^7	78	75
S. nematodiphila	1.5 × 10^7	88	85
T. harzianum	1.5 × 10^7	75	70

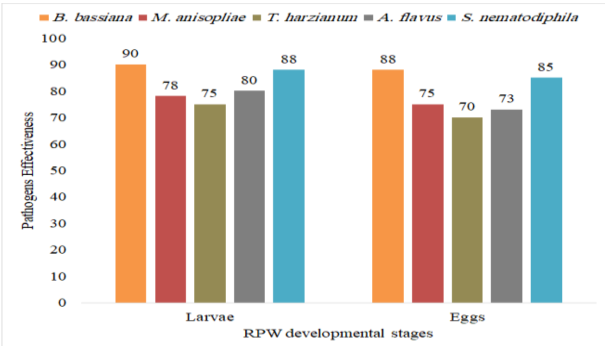

Figure 1. Comparative Efficacy of Entomopathogenic Fungi and *Serratia nematodiphila* Against Red Palm Weevil Larvae and Eggs.

Conversely, *Trichoderma harzianum* exhibited the lowest mortality rates for both stages, while *Serratia nematodiphila* and *Aspergillus flavus* had moderate efficacy.

Table 5 presents the LC_{50} values of the insect pathogens. The results showed the effectiveness of the different pathogens against both red palm weevil eggs and larvae. *B. bassiana* and *A. flavus* were the most effective, with low LC_{50} values of 5.5×10^5 and 4.7×10^5 spores/ml for eggs, respectively, and 6.7×10^5 and 6.0×10^5 spores/ml for larvae (fig. 2). These low LC_{50} values indicate that these pathogens require lower concentrations to achieve 50% mortality, making them highly effective.

Whereas *M. anisopliae* and *T. harzianum* showed moderate significant control agents with LC_{50} values ranging from 5.8×10^5 to 9.0×10^5 spores/ml across eggs and larvae by the second one. *Serratia nematodiphila* showed slightly higher LC_{50} values of 7.0×10^5 for eggs and 8.3×10^5 for larvae, indicating acceptable efficacy. Regression equations for each pathogen confirmed a strong dose-response relationship, supporting their use in integrated pest management strategies.

Overall, *B. bassiana* and *A. flavus* showed the highest efficacy, making them the most effective biocontrol agents for managing redpalm weevil populations at lower concentrations.

Table 5. Percentages of Red Palm Weevil Larvae Treated with 4 Concentrations of Entomopathogenic Fungi and *Serratia Nematodiphila*

Pathogens	Insect Stage	LC_{50}	Lower Limit	Upper Limit	Regression Equation	Chi-square
A. flavus	Eggs	4.7×10^{5}	2.0×10^{5}	6.6×10^{6}	Y = 1.080 + 0.197X	2.91
A. flavus	Larvae	6.0×10^{5}	4.2×10^{5}	8.1×10^{6}	Y = 1.220 + 0.211X	3.75
B. bassiana	Eggs	5.5×10^{5}	3.2×10^{4}	7.7×10^{6}	Y = 1.168 + 0.203X	3.65
B. bassiana	Larvae	6.7×10^{5}	4.5×10^{5}	9.2×10^{6}	Y = 1.320 + 0.215X	4.88
M. anisopliae	Eggs	6.0×10^{5}	4.0×10^{5}	8.0×10^{6}	Y = 1.198 + 0.208X	3.87
M. anisopliae	Larvae	7.7×10^{5}	5.2×10^{5}	1.0×10^{7}	Y = 1.395 + 0.221X	4.12
S. Nematodiphila	Eggs	7.0×10^{5}	5.0×10^{4}	9.0×10^{6}	Y = 1.230 + 0.215X	4.15
S. Nematodiphila	Larvae	8.3×10^{5}	6.9×10^{5}	1.0×10^{7}	Y = 1.452 + 0.244X	5.03
T. harzianum	Eggs	5.8×10^{5}	3.9×10^{5}	7.9×10^{6}	Y = 1.172 + 0.209X	3.92
T. harzianum	Larvae	9.0×10^{5}	6.5×10^{5}	1.2×10^{7}	Y = 1.480 + 0.237X	4.88

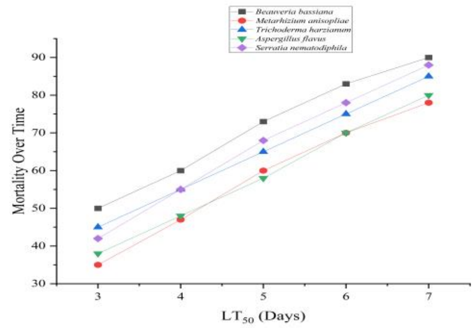

Figure 2. Mortality of Red Palm Weevil Over Time by Pathogen and LT_{50} Values.

Table 6 showed that the pathogen LT_{50} values ranged in three levels: rapid, moderate, and slow. *A. flavus* demonstrated the fastest action with LT_{50} values of 4.67 days for eggs and 6.67 days for larvae (Fig.3), indicating rapid mortality. *B. bassiana* and *M. anisopliae* had moderate LT_{50} values, requiring more time to reach 50% mortality. *S. nematodiphila* and *T. harzianum* had the highest LT_{50} values, reflecting a slower action suitable for sustained pest management. This stratification suggests a strategic use of these pathogens for both immediate and long-term control of red palm weevil populations in integrated pest management programs. The regression equations provided for each pathogen offer insight into the time-response relationship. For example, *T. harzianum* for larvae (Y = 1.480 + 0.237X) suggests a positive linear relationship between time and cumulative mortality.

Figure 3. Infection symptoms of the tested pathogens.

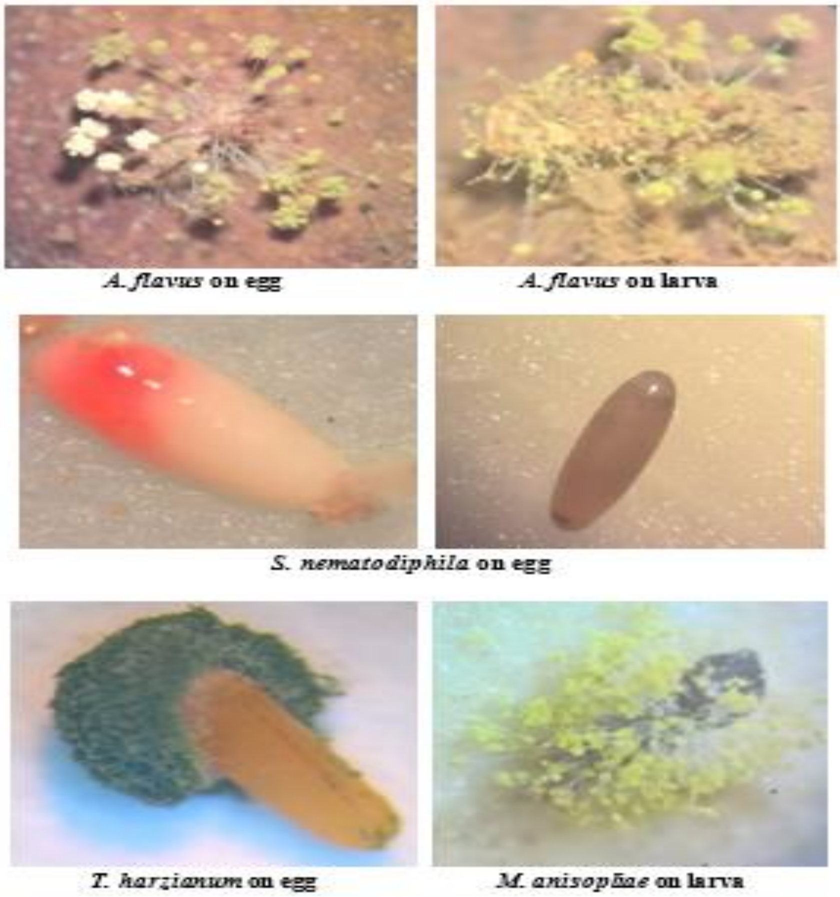

Figure 3. Cont. Infection symptoms of the tested pathogens.

Table 6. LT_{50}, Regression Equations, and Chi-square Values for Pathogens on Red Palm Weevil (RPW) Eggs and Larvae

Pathogen	Insect Stage	LT_{50} (Days)	Lower Limit (Days)	Upper Limit (Days)	Regression Equation	Chi-square
A. flavus	Eggs	4.67	4.33	3.23 - 6.05	Y = 1.080 + 0.197X	2.91
	Larvae	6.67	6.33	5.90 - 8.10	Y = 1.220 + 0.211X	3.75
B. bassiana	Eggs	6.00	6.67	3.23 - 7.77	Y = 1.168 + 0.203X	3.65
	Larvae	7.33	6.67	5.90 - 8.77	Y = 1.320 + 0.215X	4.88
M. anisopliae	Eggs	5.67	5.33	4.23 - 7.10	Y = 1.198 + 0.208X	3.87
	Larvae	8.67	7.67	6.90 - 9.77	Y = 1.395 + 0.221X	4.12
S. Nematodiphila	Eggs	6.3333	5.66	4.90 - 7.77	Y = 1.230 + 0.215X	4.15
	Larvae	8.33	7.33	6.90 - 9.77	Y = 1.452 + 0.244X	5.03
T. harzianum	Eggs	5.66	5.00	4.23 - 7.10	Y = 1.172 + 0.209X	3.92
	Larvae	7.67	7.33	6.23 - 9.10	Y = 1.480 + 0.237X	4.88

The coefficients in these equations (e.g., 1.480 for intercept and 0.237 for slope) help to quantify the rate of mortality over time.

Discussion

The present study evaluated the efficacy of multiple entomopathogenic agents, including *Beauveria bassiana*, *Metarhizium anisopliae*, *Trichoderma harzianum*, *Aspergillus flavus*, and *Serratia nematodiphila*, against the red palm weevil (RPW) (*Rhynchophorus ferrugineus*), a major pest threatening date palm cultivation in Egypt and globally. The results demonstrate a concentration-dependent efficacy, with higher concentrations of these pathogens achieving greater mortality rates in both eggs and larvae stages of RPW.

Among the tested pathogens, *B. bassiana* at a concentration of 1.5×10^7 spores/ml exhibited the highest larval mortality rate (90%), closely followed by *S. nematodiphila* and *A. flavus*, which showed mortality rates of up to 88% and 80%, respectively, at the same concentration. These findings, to a large extent, align with previous research by Ali *et al* (2024), which highlighted the effectiveness of *S. nematodiphila* against red palm weevil under controlled conditions, and *B. bassiana* against insect pests (Hussain *et al*., 2020; Wakil *et al*., 2017).

Investigations into the potential of *S. nematodiphila* as a biocontrol agent against the red palm weevil RPW, suggest a promising avenue for sustainable pest management. This entomopathogenic bacterium operates through multiple mechanisms, including the production of extracellular enzymes and secondary metabolites that disrupt the physiological processes of the RPW. (Ali *et al*.,2024). thereby offering a broader spectrum of biological agents for potential use in IPM programs.

Recent studies underscore the promising role of *B. bassiana* in managing RPW. *B. bassiana* can infect and kill all developmental stages of RPW, with high mortality rates observed in both larvae and adults, The fungus shows varying levels of effectiveness depending on the concentration of spores used, with higher concentrations leading to higher mortality rates (Manzoor *et al*.2020, Husseini, M. 2019, Qayyum, *et al*., 2019, Hajjar *et al*., 2021, Dembilio *et al*., 2010 , Kichaoui *et al*., 2017, Ahmed *et al*., 2021., Jalinas *et al*., 2022, and Abdel-Baky *et al*., 2021). Previous experimental applications have demonstrated that *B. bassiana* not only increased RPW mortality rates significantly reaching up to 88% in female weevils, but also inhibits egg hatching, effectively curtailing future generations (D Ment *et al*., 2023). Furthermore, the protective effects of this entomopathogenic fungus extend to the overall health of treated palms, as evidenced by a direct correlation between the colony-forming units of *B. bassiana* in the soil and the number of healthy palms observed in various trials (Zer-Aviv *et al*., 2024). These findings accentuate the potential of *B. bassiana* as a sustainable alternative to synthetic pesticides, aligning with public demands for environmentally safe agricultural practices. Thus, leveraging *B. bassiana* could pave the way for integrated pest management strategies focused on ecological balance and reduced chemical reliance.

The high mortality rates observed in this study can be attributed to the mechanisms of action employed by these pathogens. *S. nematodiphila*, a bacterium, is known to produce toxins that disrupt the physiological processes of the host, leading to rapid mortality. In contrast, *B. bassiana* and *T. harzianum*, both fungi, utilize a different mode of action, primarily through the production of enzymes that degrade the host's cuticle, facilitating infection.

In contrast, *A. flavus* showed moderate efficacy, with a maximum mortality rate of 80% at a concentration of 1.5×10^7 spores/ml. This level of effectiveness is somewhat lower than that observed for *B. bassiana* and *S. nematodiphila* but is consistent with earlier studies that reported moderate pathogenicity of *A. flavus* against insect pests under field conditions (Kassab *et al*., 2020). The relatively lower efficacy of *A. flavus* could be attributed to its slower infection rate and the requirement for specific environmental conditions to enhance its pathogenicity.

A. flavus fungus exhibits distinct antifungal and insecticidal properties that can be leveraged against the RPW, targeting both its eggs and larvae forms. The pathogenicity of *A. flavus* stems from its ability to produce a variety of secondary metabolites, which can disrupt the physiological processes of the pest (Trinh LL *et al*., 2024). Furthermore, the utilization of entomopathogenic fungi, such as *A. flavus*, can be advantageous due to their environmentally safe profile and specificity to target pests, minimizing impact on non-target organisms (Gouda R *et al*., 2023). Laboratory studies have demonstrated that *A. flavus* not only induces mortality in RPW populations but also disrupts their development and reproductive capabilities, thereby representing a sustainable approach for managing this invasive pest within palm plantations.

The potential of *T. harzianum* as a biocontrol agent against the red palm weevil (RPW) is underscored by its robustness and versatility in various environmental conditions. Field studies have increasingly highlighted the efficacy of entomopathogenic fungi, such as Trichoderma species, in disrupting pest life cycles while showing minimal environmental impact Future research should evaluate optimal application methods, timing, and the potential synergistic effects with other biological control agents to maximize its utility in date palm management strategies (Ara Vújo Dalbon *et al*., 2024), (D Ment *et al*., 2023).

M. anisopliae is an entomopathogenic fungus that has shown significant promise as a biological control agent against the red palm weevil. Several studies have demonstrated the high pathogenicity of *M. anisopliae* against red palm weevil. For example, an indigenous strain called MET-GRA4 achieved 100% mortality in adult weevils 21 days post-infection, with mycosis observed in 92% of cases at high spore viability (Ishak *et al*., 2019). Additionally, a study using an oil-emulsion formulation of *M. anisopliae* improved the fungus's stability, shelf life, and resistance to environmental stress, achieving up to 56.67% mortality in red palm weevil through contact transmission (Lei *et al*., 2023).The efficacy of *M. anisopliae* is influenced by environmental conditions such as humidity. In laboratory bioassays, an isolate showed the highest efficacy at 90% relative humidity, with a median lethal time (LT50) of around 6 days (Cheong & Azmi, 2020) This indicates that maintaining optimal humidity levels could enhance the fungus's effectiveness in field applications.

CONCLUSION

The findings of this study underscore the potential of using entomopathogenic fungi and bacteria as viable alternatives to chemical pesticides for managing red palm weevil infestations. The data suggests that *B. bassiana* and *S. nematodiphila* are particularly promising candidates for further development and field trials. Future research should focus on optimizing application strategies, including exploring synergistic combinations of these pathogens and integrating them into existing IPM programs. Additionally, assessing the environmental impact and non-target effects of these biocontrol agents will be critical to ensuring their sustainable use in agricultural ecosystems.

REFERENCES

Abdel-Baky, N. F., Hamed, K. E., Al-Otaibi, N. D., & Aldeghairi, M. A. (2021). Bioassay of some indigenous entomopathogens for controlling *Rhynchophorus ferrugineus*, Olivier in Saudi Arabia. Pakistan Journal of Biological Sciences, 24(9), 944-952. https://doi.org/10.3923/pjbs.2021.944.952

Ahmed, R., & Freed, S. (2021). Virulence of *Beauveria bassiana* Balsamo to red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). *Egyptian Journal of Biological Pest Control*, 31, 1-4. https://doi.org/ 10.1186/ s41938-021-00422-5.

Ali, M., Mohanny, K., Mohamed, G., & Allam, R. (2019). Efficacy of some promising plant essential oils to control the red palm weevil *Rhynchophorus ferrugineus* olivier (coleoptera: curculionidae) under laboratory conditions. SVU-International Journal of Agricultural Sciences, 1(2), 12-45. doi: 10.21608/svuijas.2019.67092

Ali, M. A., Mohanny, K. M., Mohamed, G. S., & Allam, R. O. H. (2020). Insecticidal potential of some plant extracts in nano and normal form on immatures stages of red palm weevil *Rhynchophorus ferrugineus*. *SVU-International Journal of Agricultural Sciences*, *2*(2), 306-315.

Ali, M. A., Mahmoud, M. A. B., Shoaib, M., Bhutta, Z. A., Ali, N. M., Ali, N., ... & Eletmany, M. R. (2024). Isolation and Molecular Identification of *Serratia Nematodiphila* associated with Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) as bio-insecticide in Egypt. *Asian Journal of Agriculture and Biology*, *2023352*.

Ara Vújo Dalbon, J. Acevedo, Karlos Antônio Lisboa Ribeiro Júnior, João Manoel da Silva, M. Ferro, A. N. Negrisoli Junior, Henrique Goulart Fonseca, Antônio Euzébio Goulart Santana, Francesco Porcelli (2024). Native Entomopathogenic Fungi Isolated from Rhynchophorus palmarum (Linnaeus, 1758) in Northeast Brazil. Volume (15). Insects. https:// www. semanticscholar. org/ paper/ df584647697f62587557e5c83494ee8a8b1f0d7a

Cheong, J., & Azmi, W. (2020). Dataset on the influence of relative humidity on the pathogenicity of *Metarhizium anisopliae* isolates from Thailand and Malaysia against red palm weevil (*Rhynchophorus ferrugineus*, Olivier) adult. Data in Brief, 30. https://doi. org/ 10.1016/j.dib.2020.105482.

D. Ment, Noa Levy, Arnon Allouche, Michael Davidovitz, Gal Yaacobi (2023). Efficacy of Entomopathogenic Fungi as Prevention against Early Life Stages of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in Laboratory and Greenhouse Trials. Volume (14). Insects. https://www. semanticscholar.org/ paper/e842855b93f214909da9bce4d4ffb85f5d47538b

Dembilio, Ó., Quesada-Moraga, E., Santiago-Álvarez, C., & Jacas, J. (2010). Potential of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana* as a biological control agent against the Red Palm Weevil, *Rhynchophorus ferrugineus*.. *Journal of invertebrate pathology*, 104 3, 214-21. https:// doi. org/10.1016/j.jip.2010.04.006.

El-Sayed, W. S., El-Khawass, K. A. M., & Abdalla, M. E. (2023). Impact of Entomopathogenic Fungi on Date Palm Pests under Greenhouse and Field Conditions. Journal of Plant Protection Research, 63(1), 105-116. https://doi.org/10.24425/jppr.2023.140421

Faleiro, J. R., *Be*n Abdallah, A., El-Bellaj, M., Al Ajlan, A. M., & Oihabi, A. (2016). Overview of the Red Palm Weevil, *Rhynchophorus ferrugineus*, in the Middle-East and North Africa: Recent Advances in Its Management. Journal of Agricultural and Urban Entomology, 32(1), 1-24. https://doi.org/ 10.3954/ 1523-5475-32.1.1

FAO. (2023). Egypt charts strategies for a sustainable date palm sector under the OCOP initiative. Food and Agriculture Organization of the United Nations. Retrieved from https://www.fao.org/egypt/news/ detail-events/en/c/1675045/.

FAO. (2024). Dates Value Chain Development in Egypt. Food and Agriculture Organization of the United Nations. Retrieved from https://www.fao.org/ egypt/programmes-and-projects/dates-palm/en/.

García, M. A., Moraga, E. Q., Maranhao, E. A., Ortiz-Urquiza, A., & Santiago-Álvarez, C. (2018). Molecular mechanisms involved in the interactions between entomopathogenic fungi and their insect-hosts. Advances in Entomopathogenic Fungal Research, 1-23. https://doi.org/10.1007/978-3-030-12382-3_1

Gouda, M. R., & Ashwini, L. M. B. (2023). Emerging Role of Entomopathogenic Fungi in Nanoparticle Synthesis and their Application in Pest Control. http:// meddocsonline. org/

Hajjar, M., Ajlan, A., & Al-Ahmad, M. (2021). Integration of Repellency Effect of Neem-Based Insecticide and Pheromone Bio-Trap® with *Beauveria bassiana* (Hypocreales: Cordycipitaceae) to Control the Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae). *African Entomology*, 29, 611 - 619. https://doi.org/10.4001/003.029.0611.

Hussain, A., Rizwan-ul-Haq, M., Al-Jabr, A. M., & Al-Ayedh, H. (2020). Managing Invasive Populations of Red Palm Weevil: A Worldwide Perspective. Current Biology, 30(22),R1218-R1220.https://doi.org/10.1016/j.cub. 2020.09.040

Husseini, M. (2019). Efficacy of the fungus *Beauveria bassiana* (Balsamo) Vuillemin on the red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) larvae and adults under laboratory conditions. *Egyptian Journal of Biological Pest Control*, 29, 1-4. https://doi.org/10.1186/s41938-019-0155-3.

Ishak, I., Ng, L., Haris-Hussain, M., Jalinas, J., Idris, A., Azlina, Z., Samsudin, A., & Wahizatul, A. (2019). Pathogenicity of an Indigenous Strain of the Entomopathogenic Fungus *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) (MET-GRA4 Strain) as a Potential Biological Control Agent Against the Red Palm Weevil (Coleoptera: Dryophthoridae). *Journal of Economic Entomology*, 113, 43 - 49. https://doi.org/ 10.1093/ jee/toz233.

Jalinas, J., Lopez-Moya, F., Marhuenda-Egea, F., & López-Llorca, L. (2022). *Beauveria bassiana* (Hypocreales: Clavicipitaceae) Volatile Organic Compounds (VOCs) Repel *Rhynchophorus ferrugineus* (Coleoptera: Dryophthoridae). *Journal of Fungi*, 8. https://doi.org/10.3390/jof8080843.

Kassab, A., El-Borollosy, F. M., & Nasser, M. (2020). Efficacy of Different Formulations of Entomopathogenic Fungi for the Management of Red Palm Weevil. Journal of Pest Science, 93, 215-224. https://doi.org/10.1007/s10340-019-01167-5

Khaled, Arafat., Esraa, Hussein. (2024). (2) Novel Fungal Pathogens Associated with Date Palm Leaf Spot in Egypt. Al-Mağallah Al-ʿilmiyyaẗ Li ʿulūm Al-Rīyāḍaẗ Bi Gamiʿāẗ Al-Minūfiyyaẗ, https://doi:10.21608/ nvjas. 2024.239013.1253

Kichaoui, A., Asaker, B., & El-Hindi, M. (2017). Isolation, Molecular Identification and under Lab Evaluation of the Entomopathogenic Fungi *M. anisopliae* and B. bassiana against the Red Palm Weevil R. ferrugineus in Gaza Strip. *Ai Magazine*, 07, 109-124. https://doi.org/10.4236/AIM.2017.71009.

Lei, C., Ahmad, R., Halim, N., Asib, N., Zakaria, A., & Azmi, W. (2023). Bioefficacy of an Oil-Emulsion Formulation of Entomopathogenic Fungus, *Metarhizium anisopliae* against Adult Red Palm Weevil, *Rhynchophorus ferrugineus*. Insects, 14. https://doi.org/10.3390/insects14050482.

Manzoor, M., Ahmad, J., Giblin-Davis, R., Javed, N., & Haider, M. (2020). Effects of entomopathogenic nematodes and/or fungus on the red palm weevil, *Rhynchophorus ferrugineus* (Curculionidae: Coleoptera). *Nematology*, 22, 1193-1207. https://doi.org/10.1163/15685411bja10022.

Qayyum, M., Saleem, M., Saeed, S., Wakil, W., Ishtiaq, M., Ashraf, W., Ahmed, N., Ali, M., Ikram, R., Yasin, M., Maqsood, S., Kiran, S., Qaiser, M., Ayaz, R., Nawaz, M., Abid, A., Khan, K., & Alamri, S. (2019). Integration of entomopathogenic fungi and eco-friendly insecticides for management of red palm weevil, *Rhynchophorus ferrugineus* (Olivier). *Saudi Journal of Biological Sciences*, 27, 1811 - 1817. https://doi.org/10.1016/j.sjbs.2019.12.018.

Saleh, M.R.A. (1992) Red palm weevil, *Rhynchophorus ferruginous* (Oliver) in the first record for Egypt and indeed the African continent list No: 10634 Africa collection No', *International Institute of Entomology., 56Queen5 gate. London, Sw., 75 JR.UK.*

Shefali, Patel., Susmita, Sahoo., Vinay, Kumar., Sivarama, Krishna, Lakkaboyana., Ritu, Pasrija. (2023). Use of Date Palm Fruit Processing Wastes to Produce High-Value Products. https://doi.org/10.2174/9789815123074123010009

Toledo, A. V., Franco, M. E. E., Medina, R., Lupo, S., & Balatti, P. A. (2019). Biocontrol strategies of phytopathogenic fungi that cause severe diseases in important crops. Biocontrol Science and Technology, 29(1), 1-22. https://doi.org/ 10.1080/09583157.2018.1502207

Trinh Lai Loi,and Hoai Huong Nguyen (2024). Role of plant-associated microbes in plant health and development: the case of the Serratia genus. Volume(in press). Technology in Agronomy. https://doi.org/ 10.48130/ tia-0024-0025

Wakil, W., Faleiro, J. R., & Miller, T. A. (2017). Sustainable Pest Management in Date Palm: Current Status and Emerging Challenges. Sustainability, 9(9), 1789. https://doi.org/10.3390/su9091789

withurfwqn. (2023). (1) Nonfood Products and Uses of Date Palm. https://doi.org:10.1079/9781800620209.0017

Zer Aviv Omer, Sabina Matveev, Dana Ment (2024). The efficacy of the Entomopathogenic Fungus, *Beauveria bassiana*, in Date Palm Protection against the Red Palm Weevil, *Rhynchophorus ferrugineus*, Under Hot Dessert Climate. bioRxiv. https://www.semanticscholar. org/ paper/854965bb4d569a7eca39dcc3348b58539be69c8a

تحسين المكافحة الحيوية لسوسة النخيل الحمراء باستخدام مسببات الأمراض الحشرية في زراعة نخيل البلح: بديل مستدام للمبيدات الحشرية

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الملخص

تعتبر سوسة النخيل الحمراء (*Rhynchophorus ferrugineus*) آفة خطيرة تهدد زراعة نخيل البلح على مستوى العالم، بما في ذلك مصر. وقد أثبتت المكافحة الكيميائية التقليدية عدم كفايتها بسبب تطور صفة المقاومة والأضرار البيئية، مما يؤكد الحاجة إلى بدائل مستدامة. قامت هذه الدراسة بتقييم فعالية عوامل المكافحة البيولوجية المختلفة، متضمنه الفطريات المسببة للأمراض الحشرية *Beauveria bassiana* و*Metrarhizium anisopliae* و*Trichoderma harzianum* و*Aspergillus flavus* والبكتيريا *Serratia nematodiphila*، ضد بيض ويرقات سوسة النخيل الحمراء تحت ظروف معملية محكمة. تم تحديد التركيز القاتل لنصف التعداد (LC_{50}) والوقت الكافي لقتل نصف التعداد (LT_{50}) لكل مسبب مرض. أظهرت النتائج أن *B. bassiana* و*S. nematodiphila* كانتا الأكثر فعالية، حيث حققتا ما يصل إلى 90٪ و88٪ موت لليرقات، على التوالي، عند تركيزات 1.5×10^7 جرثومة / مل. أعطت *B. bassiana* قيمة للـ LC_{50} لليرقات 6.7×10^5جرثومة / مل، في حين أعطت بكتريا *S. nematodiphila* قيمة أعلى قليلاً للـ LC_{50} لليرقات وهى 8.3×10^5 جراثيم / مل. أظهر كلا العاملين فعالية سريعة للـ LT_{50} هي 7.33 و 8.33 يومًا لليرقات، على التوالي. بينما أظهركلاً من *T. harzianum* و *A. flavus* كفاءة متوسطة بنسب موت أقل (75٪ و 80٪) وقيم أعلى للـ LC_{50} ، مما يشير إلى الحاجة لتركيزات أعلى لتحقيق نسب موت واضحة. تعد هذه الدراسة الأولى في مقارنة هذه العوامل الحيوية في مكافحة سوسة النخيل الحمراء في مصر، معطية رؤية واضحة لإمكانية دمجها في برامج الإدارة المتكاملة للآفات. تقترح النتائج أن *B. bassiana* و *S. nematodiphila* مرشحان واعدان لمكافحة سوسة النخيل الحمراء بشكل مستدام، مما يستدعي إجراء المزيد من التجارب الحقلية للتحقق من فعاليتها تحت ظروف بيئية متنوعة