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Comparative Pathogenicity and Sublethal Effects of Selected Entomopathogenic Fungi, Nematodes, and Bacteria Against Third- And Sixth-Instar Larvae of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Olivier)

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Abstract: The red palm weevil, *Rhynchophorus ferrugineus* (Olivier), is a destructive pest of palm trees, and its concealed larval habit makes effective management difficult. The present study evaluated the pathogenicity of six entomopathogenic agents, including *Beauveria bassiana*, *Metarhizium anisopliae*, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, *Bacillus thuringiensis*, and *Bacillus subtilis*, against third- and sixth-instar larvae of *R. ferrugineus* under laboratory conditions. A series of concentrations was tested, and larval mortality was recorded at different post-treatment intervals. LC_{50}, and LC_{90} values were estimated using probit analysis. All tested agents exhibited pathogenic activity, with mortality generally increasing with exposure duration. *B. bassiana* showed the highest toxicity among the tested fungi, while *S. carpocapsae* and *B. subtilis* were the most effective agents among the tested nematodes and bacteria, respectively. The LC_{25} concentrations were subsequently used to evaluate sublethal developmental effects. Exposure to LC_{25} significantly reduced final larval weight, weight gain, and molting rate, while prolonging the time required for molting to the subsequent instar in both larval stages. The results demonstrate that the tested entomopathogenic agents can exert both lethal and sublethal effects on *R. ferrugineus* larvae and highlight their potential as biological control agents for inclusion in integrated management programs.

Key words: *Rhynchophorus ferrugineus*; entomopathogenic fungi; entomopathogenic nematodes; *Bacillus* spp.; LC_{50}; LC_{90}; sublethal effects; larval development.

1. Introduction

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryophthoridae), is one of the most destructive pests of palm trees worldwide, particularly date palm (*Phoenix dactylifera* L.). It has become a serious threat to palm production in many regions of Asia, the Middle East, North Africa, and the Mediterranean basin due to its concealed biology and difficulty of early detection and control **(Faleiro, 2006 and Mazza *et al*., 2014)**. Larvae are the most destructive stage of *R. ferrugineus*, feeding and tunneling within the internal tissues of palms and causing severe structural damage. Their concealed development makes early detection and effective control difficult and represents a major challenge in RPW management **(Faleiro, 2006 and Mazza *et al*., 2014)**.

Chemical insecticides remain an important component of RPW management; however, their repeated use may result in environmental concerns, effects on non-target organisms, and insecticide resistance. Therefore, biological control agents have received increasing attention as sustainable alternatives for managing *R. ferrugineus* **(Mazza *et al*., 2014 and Wakil *et al*., 2017)**.

Entomopathogenic fungi, particularly Beauveria bassiana and Metarhizium anisopliae, have demonstrated considerable pathogenicity against a wide range of insect pests. Recent studies have confirmed the pathogenic activity of both fungi against different insect species and developmental stages, with their efficacy generally influenced by fungal concentration and exposure period **(Hintènou *et al*., 2023; Saif *et al*., 2024; Dwi Sutanto *et al*., 2024).**

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* are also promising biological control agents. Their infective juveniles penetrate insect hosts and release symbiotic bacteria that contribute to host mortality. Both *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* have demonstrated potential against *R. ferrugineus* **(Manachini *et al*., 2013; Santhi *et al*., 2015; Wakil *et al*., 2017; Tarasco *et al*., 2023; Sayed, *et al*., 2023).**

Bacterial entomopathogens have also been investigated against RPW, particularly *Bacillus thuringiensis*. **Celi *et al*. (2022)** demonstrated physiological and immune effects of *B. thuringiensis* exposure on *R. ferrugineus*, while **Elsharkawy *et al*. (2022)** confirmed its insecticidal activity against larval and adult stages. However, information on other bacterial species, including *Bacillus subtilis*, remains comparatively limited.

The effects of entomopathogenic agents may extend beyond mortality to include important sublethal effects on larval growth and development. Reduced weight gain, delayed development, and impaired progression between instars have been reported following exposure of *R. ferrugineus* to entomopathogenic fungi and nematodes **(Manachini *et al*., 2013; Wakil *et al*., 2017).** Such effects may contribute to pest suppression by reducing the fitness and developmental success of surviving larvae.

The susceptibility of *R. ferrugineus* to entomopathogenic agents can vary among larval instars. **Wakil *et al*. (2017)** reported differences in susceptibility among larval stages, indicating the importance of evaluating biological agents against more than one larval instar. Comparative assessment of different entomopathogenic groups against younger and older larvae may therefore provide useful information for selecting effective biological control agents.

Despite previous studies on individual entomopathogenic fungi, nematodes, and bacteria, comparative information on different biological control groups against different larval instars of *R. ferrugineus* remains limited. Moreover, comparative lethal concentration estimates and sublethal developmental responses under a common experimental framework are needed to better characterize the relative pathogenicity of these agents.

Therefore, the present study evaluated six entomopathogenic agents representing three biological control groups—*Beauveria bassiana* and *Metarhizium anisopliae* (fungi), *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* (nematodes), and *Bacillus thuringiensis* and *Bacillus subtilis* (bacterial agents)—against third- and sixth-instar larvae of *R. ferrugineus* under laboratory conditions. LC_{50}, and LC_{90} values were estimated, and the sublethal effects of LC_{25} exposure on larval weight, weight gain, molting rate, and time to molt were evaluated.

2. Materials and Methods

Insect culture

A laboratory colony of the red palm weevil, *R. ferrugineus*, was established from naturally infested date palm trunks collected from El-Nubaria district, Beheira Governorate, Egypt. Larvae and adults were maintained under controlled laboratory conditions at 27 ± 2°C, 70 ± 5% RH, and a photoperiod of 14:10 (L:D) h. Insects were reared individually on fresh sugarcane stem pieces, which were replaced every 3–4 days. Newly molted third- and sixth-instar larvae of *R. ferrugineus* with uniform size and weight were used in all bioassays.

Entomopathogenic organisms

Six entomopathogenic agents representing three biological control groups were evaluated.

The entomopathogenic nematodes were *S. carpocapsae* and *H. bacteriophora*. Infective juveniles (IJs) were obtained from laboratory cultures maintained on last-instar larvae of the greater wax moth, *Galleria mellonella*, following standard rearing procedures. The concentration of infective juveniles (IJs) was determined by counting the number of IJs in a known volume of the thoroughly mixed nematode suspension under a stereomicroscope, and the required concentrations were prepared by appropriate dilution with sterile distilled water.

The entomopathogenic fungi used in the present study were *B. bassiana* and *M. anisopliae*. *B. bassiana* was obtained as a commercial formulation, Biossiana® 2.5% WP, containing 1×10^8 conidia mL^{-1} (Biopesticide Production Unit, Plant Protection Research Institute, ARC, Egypt), whereas *M. anisopliae* was obtained as Biometa® 2.5% WP (Biopesticide Production Unit, Plant Protection Research Institute, ARC, Egypt), containing 1×10^8 CFU g^{-1}. Appropriate concentrations were prepared from the respective commercial formulations by serial dilution in sterile distilled water containing 0.05% Tween-80. The concentrations of *B. bassiana* and *M. anisopliae* were expressed as conidia mL^{-1} and CFU mL^{-1}, respectively.

The bacterial agents used in this study were *B. thuringiensis* and *B. subtilis*. *B. thuringiensis* was obtained as the commercial formulation Dipel® 6.4% WG (My Trade, Egypt), whereas *B. subtilis* QST 713 was obtained as Serenade ASO® 1.34% SC, containing 1×10^9 CFU g^{-1} (Bayer Limited Egypt). The required concentrations were prepared by serial dilution of the respective commercial formulations in sterile distilled water and expressed as CFU mL^{-1}.

Bioassay

Preliminary range-finding bioassays were initially conducted to determine the appropriate concentration ranges for each entomopathogenic agent. Based on the results of these preliminary tests, five serial concentrations producing a suitable range of larval mortality were selected for subsequent LC_{50} and LC_{90} determinations. Entomopathogenic fungi *B. bassiana* (Biossiana® 2.5% WP) was tested at concentrations of 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia mL^{-1}. *M. anisopliae* (Biometa® 2.5% WP; 1×10^8 CFU g^{-1}) was tested at concentrations of 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 CFU mL^{-1}, prepared by serial dilution of the commercial formulation. Entomopathogenic nematodes (***S.*** *carpocapsae* and *H. bacteriophora*) were evaluated at 250, 500, 1000, 1500, and 2000 infective juveniles (IJs) ml^{-1}. Bacterial isolates (*B. thuringiensis* subsp**.** *kurstaki* and *B. subtilis*) were tested at 1×10^6, 1×10^7, 1×10^8, 1×10^9, and 1×10^{10} CFU ml^{-1}. Sterile distilled water containing 0.05% Tween-80 was used as the untreated control for fungal and bacterial treatments, whereas sterile distilled water was used as the control for nematode treatments.

For the entomopathogenic fungi and nematodes, individual larvae were immersed in the respective treatment suspensions for 15 s, air-dried on sterile filter paper for approximately 2 min, and then transferred individually to plastic containers (500 ml) containing fresh sugarcane pieces. For the bacterial treatments, larvae were exposed through feeding. Fresh sugarcane stem pieces were treated with the respective bacterial suspensions at the required concentrations and allowed to air-dry before being individually provided to the larvae. The treated sugarcane pieces were replaced as needed to maintain fresh food throughout the bioassay. Control larvae were provided with sugarcane pieces treated with the corresponding control solution. Each treatment consisted of four replicates, with 20

larvae per replicate (80 larvae per treatment). All containers were maintained under the same environmental conditions used for colony maintenance.

Mortality assessment

Larval mortality was recorded after 1, 3 and 5 days post-treatment. Dead larvae from fungal treatments were surface sterilized in 1% sodium hypochlorite, rinsed twice with sterile distilled water, and incubated in moist chambers to verify fungal outgrowth.

For nematode-treated larvae, mortality was confirmed by dissecting cadavers under a stereomicroscope to detect nematode infection. Mortality caused by bacterial isolates was confirmed through re-isolation on nutrient agar when necessary.

Corrected mortality percentages were calculated using Abbott's formula (Abbott, 1925) whenever mortality occurred in the untreated control.

$$\text{Corrected mortality (\%)} = [(T - C)/(100 - C)] \times 100$$

where:

T = observed mortality (%) in the treated group

C = observed mortality (%) in the control group

Median lethal concentration (LC_{50}) and ninety-percent lethal concentration (LC_{90}), along with their corresponding 95% confidence limits, were estimated from the corrected mortality data using probit analysis. The analysis was performed using the LdP Line® software package (Ehabsoft, Cairo, Egypt), following the method described by Finney (1971). The toxicity index (TI) was calculated separately for each biological control group (entomopathogenic fungi, nematodes, and bacteria) according to the method of Sun (1950), using the agent with the lowest LC_{50} value within each group as the reference (TI = 100), as follows: TI (%) = (LC_{50} of the most toxic agent / LC_{50} of the tested agent) × 100

Sublethal developmental effects

To investigate the sublethal effects of the tested entomopathogenic agents on larval development, third- and sixth-instar larvae of *R. ferrugineus* were exposed to the respective LC_{25} values previously estimated for each entomopathogenic agent. For each treatment, 20 larvae were used and maintained under the same laboratory conditions described above, with an untreated control included for comparison. The experiment was conducted using four replicates, with five larvae per replicate. Following treatment, the larvae were maintained and observed daily until successful molting to the subsequent larval instar, i.e., from the third to the fourth instar and from the sixth to the seventh instar. The initial number of larvae was recorded for each replicate, and larval survival and successful molting were monitored throughout the observation period. Larvae that died before molting were recorded as dead and were not considered successfully molted.

The following developmental parameters were determined: initial larval weight (g), final larval weight (g), weight gain (%), molting rate (%), and time to molt (days). Initial larval weight was determined on the day of treatment, whereas final larval weight was determined after successful molting to the subsequent instar. For each replicate, the larvae were weighed, and the mean fresh body weight was calculated. Weight gain was calculated as the difference between final and initial mean larval weight according to the following equation:

$$\text{Weight gain (\%)} = [\text{Final larval weight (g)} - \text{Initial larval weight (g)} / \text{Initial larval weight (g)}] * 100$$

The molting rate was calculated as the percentage of larvae that successfully molted to the subsequent instar relative to the initial number of larvae used in each replicate:

$$\text{Molting rate (\%)} = (\text{Number of larvae successfully molted} / \text{Initial number of larvae}) * 100$$

The time to molt was recorded as the number of days elapsed from treatment until successful molting to the subsequent larval instar.

Statistical analysis

Data on initial larval weight, final larval weight, weight gain, molting rate, and time to molt were subjected to one-way analysis of variance (ANOVA). Treatment means were separated using least significant difference (LSD) test at $P \leq 0.05$. Statistical analyses were performed using CoStat software version 6.4.

3. Results

Effect of selected entomopathogenic agents on third-instar larvae of *R. ferrugineus* under laboratory conditions

One day after treatment

The susceptibility of third-instar larvae of *R. ferrugineus* to the tested entomopathogenic agents varied considerably among the evaluated biological control groups (Table 1). Among the entomopathogenic fungi, *B. bassiana* exhibited the highest pathogenicity, as indicated by its substantially lower LC_{50} (3.5×10^5 conidia mL^{-1}) and LC_{90} (8.66×10^7 conidia mL^{-1}) values compared with *M. anisopliae*, which required approximately threefold higher LC_{50} to achieve the same level of mortality. Accordingly, *B. bassiana* showed the highest toxicity index (TI = 100), compared with 33.02 for *M. anisopliae*, confirming its superior virulence against third-instar larvae within the fungal group.

A similar trend was observed for the entomopathogenic nematodes, where *S. carpocapsae* proved slightly more virulent than *H. bacteriophora*. The LC_{50} value of *S. carpocapsae* (806.97 IJs mL^{-1}) was lower than that of *H. bacteriophora* (881.45 IJs mL^{-1}), resulting in toxicity indices of 100 and 91.55, respectively. Although the difference between the two nematode species was relatively small, *S. carpocapsae* consistently exhibited greater pathogenicity against the tested larvae.

Table (1). Susceptibility of third-instar larvae of *R. ferrugineus* to selected entomopathogenic fungi, nematodes, and bacterial isolates under laboratory conditions, expressed as median lethal concentrations (LC_{50} and LC_{90}), slope values, chi-square (χ^2), and toxicity index (TI)

Treatments	LC_{50} (95% CL)	LC_{90} (95% CL)	Slop ± SE	χ^2	TI
Beauveria bassiana	3.5×10^5 (1.8×10^5-6.3×10^5)	8.66×10^7 (3.3×10^7-1.92×10^8)	0.536±0.054	1.136	**100**
Metarhizium anisopliae	1.06×10^6 (5.6×10^5-2×10^6)	4.5×10^8 (1.3×10^8-2.5×10^9)	0.487±0.051	0.559	**33.02**
Steinernema carpocapsae	806.97 (708.41-914.38)	2556.76 (2088.5-3370.4)	2.559±0.238	3.194	**100**
Heterorhabditis bacteriophora	881.45 (757.92-1025.3)	3653.706 (2755.56-5528.63)	2.075±0.223	2.777	**91.55**
Bacillus thuringiensis	1.58×10^8 (8.64×10^7-2.9×10^8)	4.56×10^{10} (1.47×10^{10}-2.29×10^{11})	0.521±0.053	0.709	**31.2**
Bacillus subtilis	4.93×10^7 (2.66×10^7-8.83×10^7)	1.18×10^{10} (4.44×10^9-4.67×10^{10})	0.539±0.053	0.648	**100**

LC values are expressed as conidia mL^{-1} for *B. bassiana*, CFU mL^{-1} for *M. anisopliae*, *B. thuringiensis*, and *B. subtilis*, and IJs mL^{-1} for *S. carpocapsae* and *H. bacteriophora*. TI was calculated within each biological control group using the agent with the lowest LC_{50} as the reference.

Among the bacterial agents, *B. subtilis* demonstrated markedly higher toxicity than *B. thuringiensis*. The LC_{50} value of *B. subtilis* (4.93×10^7 CFU mL^{-1}) was approximately one-third that of *B. thuringiensis* (1.58×10^8 CFU mL^{-1}). Accordingly, *B. subtilis* had the highest toxicity index (TI = 100), whereas *B. thuringiensis* had a TI of 31.20, indicating that *B. subtilis* required a considerably lower concentration to induce comparable larval mortality.

The estimated slope values ranged from 0.487 to 2.559, reflecting differences in the homogeneity of larval responses among the tested biological agents. The entomopathogenic nematodes exhibited noticeably steeper dose-response slopes than the fungal and bacterial treatments, suggesting a more uniform susceptibility of third-instar larvae to increasing nematode concentrations. Moreover, the relatively low χ^2 values obtained for all treatments indicate an acceptable goodness-of-fit of the probit model, supporting the reliability of the estimated lethal concentration values.

Three days after treatment

The toxicity of the evaluated entomopathogenic agents against third-instar larvae of *R. ferrugineus* increased markedly compared with the previous evaluation period, as reflected by the reduction in LC_{50} and LC_{90} values for most treatments (Table 2). The relative efficacy within each biological control group generally remained consistent, with *B. bassiana*, *S. carpocapsae*, and *B. subtilis* showing the lowest LC_{50} values within the fungal, nematode, and bacterial groups, respectively.

Among the entomopathogenic fungi, *B. bassiana* remained the most virulent treatment, exhibiting the lowest LC_{50} (1.09×10^5 conidia mL^{-1}) and LC_{90} (1.30×10^7 conidia mL^{-1}) values. In contrast, *M. anisopliae* required a substantially higher concentration to achieve equivalent mortality, with an LC_{50} approximately 3.28-fold greater than that of *B. bassiana*. Accordingly, *B. bassiana* recorded the highest toxicity index (TI = 100), whereas *M. anisopliae* had a TI of 30.53, confirming the superior pathogenicity of *B. bassiana* within the fungal group.

Within the entomopathogenic nematodes, *S. carpocapsae* again exhibited slightly greater virulence than *H. bacteriophora*. The LC_{50} values were estimated at 621.92 and 662.44 IJs mL^{-1}, respectively. Consequently, *S. carpocapsae* recorded the highest toxicity index (TI = 100), compared with 93.88 for *H. bacteriophora*. Although the difference between the two nematode species was relatively small, the lower LC_{50} value of *S. carpocapsae* indicates its slightly greater pathogenicity against third-instar larvae.

Table (2). Susceptibility of third-instar larvae of *R. ferrugineus* to selected entomopathogenic fungi, nematodes, and bacterial isolates under laboratory conditions, expressed as median lethal concentrations (LC_{50} and LC_{90}), slope values, chi-square (χ^2), and toxicity index (TI)

Treatments	LC_{50} (95% CL)	LC_{90} (95% CL)	Slop ± SE	χ^2	TI
Beauveria bassiana	1.09×10^5 **(**5.7×10^4-1.92×10^5**)**	1.3×10^7 (5.16×10^6-5.24×10^7)	0.617±0.074	0.0053	**100**
Metarhizium anisopliae	3.57×10^5 (1.81×10^5-6.64×10^5)	1.29×10^8 (4.51×10^7-5.82×10^8)	0.501±0.052	1.202	**30.53**
Steinernema carpocapsae	621.92 (540.19-705.94)	1941.18 (1618.23-2473.21)	2.593±0.233	7.365	**100**
Heterorhabditis bacteriophora	662.435 (561.65-768.11)	2707.52 (2115.15-3854.11)	2.096±0.219	4.725	**93.88**
Bacillus thuringiensis	5.18×10^7 (2.75×10^7-9.43×10^7)	1.49×10^{10} (5.34×10^9-6.39×10^{10})	0.521±0.053	1.824	**31.85**
Bacillus subtilis	1.65×10^7 (8.58×10^6-2.92×10^7)	3.05×10^9 (1.36×10^9-9.08×10^9)	0.566±0.053	2.628	**100**

LC values are expressed as conidia mL^{-1} for *B. bassiana*, CFU mL^{-1} for *M. anisopliae*, *B. thuringiensis*, and *B. subtilis*, and IJs mL^{-1} for *S. carpocapsae* and *H. bacteriophora*. TI was calculated within each biological control group using the agent with the lowest LC_{50} as the reference.

The bacterial agents also showed clear differences in pathogenicity. *B. subtilis* was considerably more effective than *B. thuringiensis*, producing the lower LC_{50} (1.65×10^7 CFU mL^{-1}) and LC_{90} (3.05×10^9 CFU mL^{-1}) values within the bacterial group. Accordingly, *B. subtilis* recorded the highest

toxicity index (TI = 100), whereas *B. thuringiensis* had a TI of 31.64. These findings demonstrate that *B. subtilis* required substantially lower bacterial concentrations to induce comparable larval mortality.

Slope values ranged from 0.501 to 2.593, indicating differences in the uniformity of larval responses among the tested biological agents. As observed previously, the entomopathogenic nematodes exhibited steeper dose-response slopes than the fungal and bacterial agents, suggesting a more homogeneous susceptibility of the tested larvae to nematode infection. Furthermore, the relatively low chi-square (χ^2) values obtained for most treatments indicate a satisfactory fit of the probit regression model and support the reliability of the estimated LC values.

Five days after treatment

The third evaluation revealed a further increase in the susceptibility of third-instar larvae of *R. ferrugineus* to all tested entomopathogenic agents, as demonstrated by the pronounced decline in both LC^{50} and LC^{90} values compared with the previous assessment periods (Table 3). This reduction indicates that prolonged exposure substantially enhanced the pathogenic activity of the evaluated biological control agents while maintaining the same relative order of efficacy within each biological group.

Among the entomopathogenic fungi, *B. bassiana* continued to exhibit the greatest virulence, recording the lowest LC_{50} (9.27×10^3 conidia mL^{-1}) and LC^{90} (1.33×10^6 conidia mL^{-1}) values. In comparison, *M. anisopliae* required considerably higher concentrations to induce similar mortality, with an LC^{50} approximately 5.2-fold greater than that of *B. bassiana*. Consequently, *B. bassiana* achieved the highest toxicity index (TI = 100), compared with 19.31 for *M. anisopliae*, indicating its superior pathogenicity within the fungal group.

Table (3). Susceptibility of third-instar larvae of *R. ferrugineus* to selected entomopathogenic fungi, nematodes, and bacterial isolates under laboratory conditions, expressed as median lethal concentrations (LC_{50} and LC_{90}), slope values, chi-square (χ^2), and toxicity index (TI)

Treatments	LC_{50} (95% CL)	LC_{90} (95% CL)	Slop ± SE	χ^2	TI
Beauveria bassiana	9.27×10^3 **(**2.7×10^3-2.07×10^4**)**	1.33×10^6 (5.99×10^5-4.53×10^6)	0.594±0.085	0.6	**100**
Metarhizium anisopliae	4.8×10^4 (1.68×10^4-1.06×10^5)	3.56×10^7 (1.23×10^7-1.73×10^8)	0.447±0.054	1.1	**19.31**
Steinernema carpocapsae	295.019 (196.2-380.33)	2825.56 (1306.39-3301.06)	1.619±0.263	4.396	**100**
Heterorhabditis bacteriophora	445.45 (362.52-525.2)	1802.69 (1453.82-2433.98)	2.111±0.224	2.91	**66.23**
Bacillus thuringiensis	1.18×10^7 (4.99×10^6-2.39×10^7)	6.71×10^9 (2.16×10^9-3.69×10^{10})	0.465±0.055	1.209	**37.37**
Bacillus subtilis	4.41×10^6 (1.77×10^6-8.74×10^6)	1.1×10^9 (3.93×10^8-5.76×10^9)	0.534±0.073	0.725	**100**

LC values are expressed as conidia mL^{-1} for *B. bassiana*, CFU mL^{-1} for *M. anisopliae*, *B. thuringiensis*, and *B. subtilis*, and IJs mL^{-1} for *S. carpocapsae* and *H. bacteriophora*. TI was calculated within each biological control group using the agent with the lowest LC_{50} as the reference.

A similar tendency was observed among the entomopathogenic nematodes. *S. carpocapsae* remained the most effective nematode species, exhibiting the lowest LC^{50} (295.02 IJs mL^{-1}) and the highest toxicity index (TI = 100), compared with 66.23 for *H. bacteriophora*. Although *H. bacteriophora* produced a lower LC_{90} value than *S. carpocapsae*, its higher LC_{50} indicates that a greater concentration was required to achieve median larval mortality. These findings suggest that *S.*

carpocapsae was more effective at producing median mortality, whereas *H. bacteriophora* showed greater efficacy at the higher mortality level reflected by the LC_{90}.

Within the bacterial treatments, *B. subtilis* consistently outperformed *B. thuringiensis*, recording the lowest LC_{50} (4.41×10^6 CFU mL^{-1}) and LC_{90} (1.10×10^9 CFU mL^{-1}) values. Accordingly, *B. subtilis* recorded the highest toxicity index (TI = 100), whereas *B. thuringiensis* had a TI of 37.37. These findings demonstrate that *B. subtilis* required substantially lower bacterial concentrations to induce equivalent larval mortality.

The estimated slope values ranged from 0.447 to 2.111, indicating differences in the dose-response relationships among the tested biological agents. As in the previous evaluations, the entomopathogenic nematodes produced steeper regression slopes than the fungal and bacterial agents, reflecting a more homogeneous response of third-instar larvae to increasing nematode concentrations. Furthermore, the relatively low χ^2 values obtained for all treatments indicate an acceptable goodness-of-fit of the probit regression model, supporting the reliability of the estimated lethal concentration values.

Effect of selected entomopathogenic agents on sixth-instar larvae of *R. ferrugineus* under laboratory conditions

One day after treatment (24 h)

The pathogenic activity of the tested entomopathogenic agents against sixth-instar larvae of *R. ferrugineus* was evident one day after treatment, although the susceptibility varied considerably among the evaluated biological control agents (Table 4). Within the fungal group, *B. bassiana* exhibited greater virulence than *M. anisopliae*, as indicated by its lower LC_{50} (3.19×10^6 conidia ml^{-1}) and LC_{90} (5.57×10^9 conidia ml^{-1}) values. The toxicity index further confirmed the superior efficacy of *B. bassiana* (TI = 2.22), demonstrating that it required substantially lower concentrations than *M. anisopliae* to induce comparable mortality in sixth-instar larvae.

Among the entomopathogenic nematodes, *S. carpocapsae* showed slightly greater pathogenicity than *H. bacteriophora*. The LC_{50} values were estimated at 1060.04 and 1274.58 IJs ml^{-1}, respectively, resulting in toxicity indices of 1.20 and 1.00. Although the difference between the two nematode species was relatively small, *S. carpocapsae* consistently demonstrated higher infectivity against the tested larvae during the first 24 h following treatment.

Table (4). Toxicity of selected entomopathogenic agents against sixth-instar larvae of *R. ferrugineus* one day (24 h) after treatment under laboratory conditions

Treatments	LC_{50} (95% CL)	LC_{90} (95% CL)	Slop ± SE	χ^2	TI
Beauveria bassiana	3.19×10^6 **(**1.49×10^6-7.66×10^6**)**	5.57×10^9 (9.4×10^8-9.55×10^{10})	0.395±0.049	1.32	**100**
Metarhizium anisopliae	7.07×10^6 (3.24×10^6-1.88×10^7)	1.1×10^{10} (1.69×10^9-2.22×10^{11})	0.402±0.05	0.908	**45.12**
Steinernema carpocapsae	1060.04 (903-1265.4)	5027.31 (3540.47- 8637.56)	1.896±0.222	0.919	**100**
Heterorhabditis bacteriophora	1274.58 (1076.59-1565.61)	6316.09 (4239.27-11915.22)	1.844±0.228	0.081	**83.17**
Bacillus thuringiensis	7.5×10^8 (3.67×10^8-1.79×10^9)	2.36×10^{11} (1.07×10^{11}-6.4×10^{12})	0.449±0.052	0.174	**36.8**
Bacillus subtilis	2.76×10^8 (1.4×10^8-5.84×10^8)	1.87×10^{11} (4.42×10^{10}-1.65×10^{12})	0.453±0.051	1.513	**100**

LC values are expressed as conidia mL^{-1} for *B. bassiana*, CFU mL^{-1} for *M. anisopliae*, *B. thuringiensis*, and *B. subtilis*, and IJs mL^{-1} for *S. carpocapsae* and *H. bacteriophora*. TI was calculated within each biological control group using the agent with the lowest LC_{50} as the reference.

Within the bacterial isolates, *B. subtilis* proved more effective than *B. thuringiensis*, exhibiting a markedly lower LC_{50} (2.76×10^8 CFU ml^{-1}) compared with *B. thuringiensis* (7.50×10^8 CFU ml^{-1}). Consequently, *B. subtilis* recorded the highest toxicity index within the bacterial group (TI = 2.72), indicating its greater pathogenic potential against sixth-instar larvae.

The estimated slope values ranged from 0.395 to 1.896, indicating variable dose-response relationships among the tested biological agents. The entomopathogenic nematodes produced steeper regression slopes than both the fungal and bacterial isolates, suggesting a more homogeneous response of sixth-instar larvae to increasing nematode concentrations. Furthermore, the low χ^2 values obtained for all treatments indicate a satisfactory goodness-of-fit of the probit regression model, supporting the reliability of the estimated lethal concentration values.

Three days after treatment (72 h)

The pathogenic activity of the evaluated entomopathogenic agents against sixth-instar larvae of *R. ferrugineus* increased noticeably three days after treatment, as evidenced by the reduction in LC_{50} and LC_{90} values for all tested agents compared with the first day of exposure (Table 5). Despite this increase in pathogenicity, the relative ranking of the tested biological agents within each group remained unchanged.

Among the fungal isolates, *B. bassiana* continued to exhibit superior virulence over *M. anisopliae*, recording the lowest LC_{50} (8.55×10^5 conidia ml^{-1}) and LC_{90} (1.04×10^9 conidia ml^{-1}) values. In contrast, *M. anisopliae* required approximately 3.4-fold higher concentrations to achieve equivalent mortality. Accordingly, *B. bassiana* recorded the highest toxicity index within the fungal group (TI = 3.43), confirming its greater pathogenicity against sixth-instar larvae.

Within the entomopathogenic nematodes, *S. carpocapsae* again proved slightly more virulent than *H. bacteriophora*. The LC_{50} values were estimated at 852.30 and 1046.62 IJs ml^{-1}, respectively, corresponding to toxicity indices of **1.23** and 1.00. Although the differences between the two nematode species remained relatively small, *S. carpocapsae* consistently required fewer infective juveniles to induce comparable larval mortality.

Table (5). Toxicity of selected entomopathogenic agents against sixth-instar larvae of *R. ferrugineus* three days (72 h) after treatment under laboratory conditions

Treatments	LC_{50} (95% CL)	LC_{90} (95% CL)	Slop ± SE	χ^2	TI
Beauveria bassiana	8.55×10^5 **(4.3×10^5-1.79×10^6)**	1.04×10^9 (2.39×10^8-1.02×10^{10})	0.415±0.049	0.68	**100**
Metarhizium anisopliae	2.93×10^6 (1.46×10^6-6.41×10^6)	2.52×10^9 (5.46×10^8-2.61×10^{10})	0.437±0.05	1.048	**29.15**
Steinernema carpocapsae	852.3 (726.3-998.45)	3843.63 (2840.49-6039.72)	1.959±0.218	1.731	**100**
Heterorhabditis bacteriophora	1046.62 (895.16-1241.44)	4746.73 (3398.48-7902.74)	1.952±0.224	1.053	**81.43**
Bacillus thuringiensis	3.67×10^8 (1.89×10^8-7.81×10^8)	2.1×10^{11} (5.02×10^{10}-1.79×10^{12})	0.465±0.051	0.429	**36.49**
Bacillus subtilis	1.34×10^8 (7.14×10^7-2.58×10^8)	5.48×10^{10} (1.64×10^{10}-3.16×10^{11})	0.491±0.051	0.208	**100**

LC values are expressed as conidia mL^{-1} for *B. bassiana*, CFU mL^{-1} for *M. anisopliae*, *B. thuringiensis*, and *B. subtilis*, and IJs mL^{-1} for *S. carpocapsae* and *H. bacteriophora*. TI was calculated within each biological control group using the agent with the lowest LC_{50} as the reference.

The bacterial isolates also differed considerably in their pathogenicity. *B. subtilis* remained the most effective bacterial treatment, producing the lowest LC_{50} (1.34×10^8 CFU ml^{-1}) and LC_{90} (5.48×10^{10} CFU ml^{-1}) values, whereas *B. thuringiensis* required substantially higher concentrations to

achieve similar mortality levels. The calculated toxicity index (**TI = 2.74**) further emphasized the superior virulence of *B. subtilis* within the bacterial group.

The estimated slope values ranged from 0.415 to 1.959, indicating moderate differences in the dose-response relationships among the tested biological agents. As observed after the first day of exposure, the entomopathogenic nematodes exhibited steeper regression slopes than the fungal and bacterial isolates, suggesting a more homogeneous response of sixth-instar larvae to increasing nematode concentrations. Furthermore, the relatively low χ^2 values obtained for all treatments indicate an adequate fit of the probit regression model, confirming the reliability of the estimated lethal concentration values.

Five days after treatment (120 h)

The pathogenic activity of the evaluated entomopathogenic agents against sixth-instar larvae of *R. ferrugineus* increased markedly five days after treatment, as reflected by the substantial reduction in LC_{50} and LC_{90} values for all tested agents compared with the previous evaluation periods (Table 6). Nevertheless, the relative ranking of the biological control agents within each group remained unchanged.

Among the fungal isolates, *B. bassiana* remained the most virulent fungal pathogen, recording the lowest LC_{50} (2.9×10^5 conidia ml^{-1}) and LC_{90} (2.57×10^8 conidia ml^{-1}) values compared with *M. anisopliae*, which exhibited LC_{50} and LC_{90} values of 4.8×10^5 and 5.2×10^8 conidia ml^{-1}, respectively. The toxicity index indicated that *B. bassiana* was approximately 1.66-fold more toxic than *M. anisopliae*, confirming its superior pathogenicity against sixth-instar larvae.

Within the entomopathogenic nematodes, *S. carpocapsae* continued to exhibit slightly greater pathogenicity than *H. bacteriophora*, with LC_{50} values of 431.76 and 459.85 IJs ml^{-1}, respectively. The corresponding toxicity indices (1.07 and 1.00, respectively) demonstrated only a slight difference in virulence between the two nematode species, although S. carpocapsae consistently required fewer infective juveniles to achieve median larval mortality.

The bacterial isolates also differed considerably in their pathogenicity. *B. subtilis* exhibited markedly higher toxicity than *B. thuringiensis*, recording an LC_{50} of 6.0×10^6 CFU ml^{-1} compared with 7.2×10^7 CFU ml^{-1} for *B. thuringiensis*. Consequently, *B. subtilis* showed the highest toxicity index within the bacterial group (TI = 12.00), indicating that it required nearly twelve times lower bacterial concentrations to induce comparable mortality than *B. thuringiensis*.

Table (6). Toxicity of selected entomopathogenic agents against sixth-instar larvae of *R. ferrugineus* five days (120 h) after treatment under laboratory conditions

Treatments	LC_{50} (95% CL)	LC_{90} (95% CL)	Slop ± SE	χ^2	TI
Beauveria bassiana	2.9×10^5 **(**8.6×10^4-4.38×10^5**)**	2.57×10^8 (6.91×10^7-1.93×10^9)	0.415±0.05	0.96	**100**
Metarhizium anisopliae	4.8×10^5 (2.21×10^5-9.84×10^5)	5.2×10^8 (1.32×10^8-4.2×10^9)	0.422±0.05	1.446	**60.42**
Steinernema carpocapsae	431.76 (353.7-506.82)	1627.94 (1330.06-2148.89)	2.223±0.231	6.711	**100**
Heterorhabditis bacteriophora	459.85 (372.87-543.7)	1960.01 (1561.05-2706.16)	2.035±0.221	5.13	**93.89**
Bacillus thuringiensis	7.2×10^7 (3.45×10^7-1.47×10^8)	7×10^{10} (1.75×10^{10}-5.74×10^{11})	0.429±0.05	0.338	**8.33**
Bacillus subtilis	6×10^6 (2.6×10^6-1.15×10^7)	1.37×10^9 (4.86×10^8-7.17×10^9)	0.543±0.072	2.033	**100**

LC values are expressed as conidia mL^{-1} for *B. bassiana*, CFU mL^{-1} for *M. anisopliae*, *B. thuringiensis*, and *B. subtilis*, and IJs mL^{-1} for *S. carpocapsae* and *H. bacteriophora*. TI was calculated within each biological control group using the agent with the lowest LC_{50} as the reference.

The estimated slope values ranged from 0.415 to 2.223, indicating differences in the dose-response relationships among the tested biological agents. The entomopathogenic nematodes exhibited steeper regression slopes than the fungal and bacterial isolates, suggesting a more homogeneous susceptibility of sixth-instar larvae to increasing nematode concentrations. Furthermore, the relatively low χ^2 values obtained for all treatments indicate a satisfactory goodness-of-fit of the probit regression model, confirming the reliability of the estimated lethal concentration values.

Effect of LC_{25} concentrations of selected entomopathogenic agents on larval growth and weight gain of third-instar *R. ferrugineus*

The exposure of third-instar *R. ferrugineus* larvae to the LC_{25} concentrations of the tested entomopathogenic agents significantly affected larval growth and development, except for the initial larval weight, which did not differ significantly among treatments (F = 0.704). In contrast, significant differences were detected in final larval weight (F = 31.76) and weight gain (F = 9.999). The untreated control recorded the highest final weight (1.32 ± 0.02 g) and weight gain (68.78 ± 6.38%), whereas all entomopathogenic treatments significantly reduced these parameters. *B. bassiana* resulted in a particularly pronounced reduction in final weight (1.01 ± 0.06 g) and weight gain (36.57 ± 9.08%), while *H. bacteriophora* produced the lowest weight gain (29.42 ± 3.84%). These results indicate that exposure to LC_{25} concentrations imposed substantial sublethal effects on larval growth even among individuals that survived the treatment and subsequently molted to the fourth instar.

Table (7). Sublethal effects of LC_{25} concentrations of selected entomopathogenic agents on the development of third-instar *R. ferrugineus* larvae until molting to the fourth instar under laboratory conditions

Entomopathogenic agents	Initial weight (g)	Final weight (g)	Weight gain (%)	Molting rate (%)	Time to molt (days)
Control	0.78±0.03a	1.32±0.02a	68.78±6.38a	82.5±6.45a	2.88±0.43d
B. bassiana	0.74±0.06a	1.01±0.06d	36.57±9.08c	53.75±6.29cd	4.25±0.46ab
M. anisopliae	0.78±0.06a	1.17±0.05b	50.3±17.21b	61.25±7.5bc	3.94±0.24bc
S. carpocapsae	0.78±0.03a	1.09±0.02c	39.22±5.43bc	58.75±2.5bcd	4.25±0.46ab
H. bacteriophora	0.79±0.03a	1.02±0.05d	29.42±3.84c	65±4.08b	3.69±0.31c
B. thuringiensis	0.79±0.02a	1.04±0.03cd	31.06±4.35c	61.25±6.29bc	4.75±0.35a
B. subtilis	0.78±0.03a	1.04±0.02cd	34.41±7.56c	52.5±2.89d	4.38±0.32ab
F values	0.704	31.76	9.999	13.42	10.342
LSD	0.0596	0.0575	12.91	8.02228	0.55289

Means followed by the same letter(s) within the same column are not significantly different according to LSD test at $P \leq 0.05$.

Significant differences were also observed in molting rate (F = 13.42) and time to molt (F = 10.342). The control larvae exhibited the highest molting rate (82.5 ± 6.45%) and the shortest time to molt (7.38 ± 0.43 days). In contrast, molting success was significantly reduced following exposure to the entomopathogenic agents, with the lowest rate recorded for *B. subtilis* (52.5 ± 2.89%), followed by *B. bassiana* (53.75 ± 6.29%). All treatments delayed progression to the fourth instar relative to the control, with the longest developmental period recorded for *B. thuringiensis* (4.75 ± 0.35 days). Overall, these findings demonstrate that LC_{25} exposure produced clear sublethal effects on both larval growth and developmental progression, as manifested by reduced weight gain, decreased molting success, and delayed molting to the subsequent instar.

Sublethal developmental effects on sixth-instar larvae

The LC_{25} exposure produced clear sublethal effects on the growth and development of sixth-instar *R. ferrugineus* larvae, although the initial larval weight did not differ significantly among treatments (F = 0.231), indicating comparable starting weights. In contrast, significant differences were detected in final weight, weight gain, molting rate, and time to molt (Table 8). The untreated control recorded the highest final weight (4.78 ± 0.09 g) and weight gain (38.54 ± 11.87%), whereas *B. bassiana* caused the greatest reduction in growth, with final weight and weight gain of only 3.71 ± 0.34 g and 7.10 ± 3.96%, respectively. *M. anisopliae* also markedly reduced weight gain (11.88 ± 3.37%), followed by *B. thuringiensis* (15.83 ± 1.43%), *H. bacteriophora* (17.60 ± 6.19%), *B. subtilis* (19.98 ± 4.80%), and *S. carpocapsae* (21.52 ± 3.91%). Molting rate was significantly reduced compared with the control (81.25 ± 6.29%) and ranged from 48.75 ± 4.79% with *B. subtilis* to 66.25 ± 8.54% with *H. bacteriophora*. In addition, all entomopathogenic agents delayed molting relative to the untreated larvae, which completed molting in 8.00 ± 0.35 days. The longest time to molt was recorded with *B. thuringiensis* (9.75 ± 0.54 days), followed by *B. subtilis* (9.44 ± 0.13 days), *S. carpocapsae* (9.38 ± 0.52 days), and *B. bassiana* (9.31 ± 0.60 days). Overall, the results indicate that exposure to LC_{25} concentrations, particularly *B. bassiana* and *M. anisopliae*, substantially suppressed larval growth and delayed development of sixth-instar *R. ferrugineus* larvae, while the nematode and bacterial agents also reduced molting success and prolonged the time required to reach the subsequent instar.

Table (8). Sublethal effects of LC_{25} concentrations of selected entomopathogenic agents on the development of sixth-instar *R. ferrugineus* larvae until molting to the seventh instar under laboratory conditions

Entomopathogenic agents	Initial weight (g)	Final weight (g)	Weight gain (%)	Molting rate (%)	Time to molt (days)
Control	3.47±0.34a	4.78±0.09a	38.54±11.87a	81.25±6.29a	8±0.35d
B. bassiana	3.46±0.31a	3.71±0.34d	7.1±3.96d	50±4.08d	9.31±0.6abc
M. anisopliae	3.47±0.18a	3.87±0.1cd	11.88±3.37cd	62.5±6.45b	8.81±0.38bc
S. carpocapsae	3.32±0.1a	4.04±0.07bc	21.52±3.91b	58.75±4.79bc	9.38±0.52abc
H. bacteriophora	3.42±0.29a	4.01±0.19bc	17.6±6.19bc	66.25±8.54b	8.75±0.54c
B. thuringiensis	3.49±0.26a	4.04±0.26bc	15.83±1.43bcd	51.25±4.79cd	9.75±0.54a
B. subtilis	3.51±0.22a	4.2±0.09b	19.98±4.8bc	48.75±4.79d	9.44±0.13ab
F values	0.231	12.838	11.224	15.609	6.484
LSD	0.3731	0.27835	8.7287	8.6029	0.67715

Means followed by the same letter(s) within the same column are not significantly different according to LSD test at $P \leq 0.05$.

4. Discussion

The present study demonstrated clear differences in the susceptibility of *R. ferrugineus* larvae to the tested entomopathogenic fungi, nematodes, and bacteria. In general, pathogenicity increased with increasing exposure duration, as indicated by the progressive reduction in LC_{50} and LC_{90} values in both larval instars. This time-dependent response is consistent with the biological mode of action of microbial agents, which require sufficient time for infection, multiplication, and development of pathogenic effects. Similar increases in mortality with exposure time have been reported for entomopathogenic fungi and nematodes against *R. ferrugineus* **(Wakil *et al.*, 2017).**

Susceptibility of third-instar larvae

Third-instar larvae were susceptible to all tested agents, but clear differences occurred among treatments. *Beauveria bassiana* was the most effective fungal agent, with its LC_{50} decreasing from

3.50×10^5 conidia ml^{-1} after one day to 9.27×10^3 conidia ml^{-1} after five days, whereas *M. anisopliae* required higher concentrations. This greater activity of *B. bassiana* agrees with its strong pathogenicity reported against RPW, although **Gindin *et al.* (2006)** found considerable variability among fungal isolates and reported that some *M. anisopliae* isolates could be more virulent. Such differences may result from fungal strain, conidial quality, host stage, and experimental conditions.

The nematodes were also highly effective against third-instar larvae. *S. carpocapsae* showed greater pathogenicity than *Heterorhabditis bacteriophora*, with LC_{50} values decreasing from 806.97 to 295.02 IJs ml^{-1} for *S. carpocapsae* and from 881.45 to 445.45 IJs ml^{-1} for *H. bacteriophora* over the experimental period. These results agree with Manachini *et al*. (2013), who demonstrated the pathogenicity of *S. carpocapsae* against *R. ferrugineus*, and with **Santhi *et al.* (2015)**, who confirmed the ability of both nematodes to locate RPW larvae.

Among the bacterial agents, *B. subtilis* was more effective than *B. thuringiensis* against third-instar larvae. The LC_{50} of *B. subtilis* declined from 4.93×10^7 to 1.65×10^7 CFU ml^{-1}, whereas *B. thuringiensis* required higher concentrations. The relatively strong activity of *B. subtilis* is noteworthy because information concerning its pathogenicity against RPW is more limited than that available for *B. thuringiensis*. Previous studies nevertheless confirmed direct insecticidal and physiological effects of *B. thuringiensis* on *R. ferrugineus* **(Celi *et al.*, 2022; Elsharkawy *et al.*, 2022).**

Susceptibility of sixth-instar larvae

Sixth-instar larvae were also susceptible to all tested agents, with pathogenicity generally increasing over time. The same pattern observed in the third instar was maintained within each biological group, with *B. bassiana*, *S. carpocapsae*, and *B. subtilis* showing the greatest activity among fungi, nematodes, and bacteria, respectively. The reduction in LC_{50} values with prolonged exposure indicates that mortality continued to accumulate after treatment. Similar differences in susceptibility among RPW larval stages and microbial agents were reported by **Wakil *et al.* (2017).**

Comparison between the two larval stages indicates that larval age affected susceptibility, with younger larvae generally requiring lower concentrations of the tested fungi and bacteria than sixth-instar larvae. This may be related to differences in larval body size, cuticular characteristics, physiological condition, and host defenses. Therefore, younger larval stages may represent more suitable targets for microbial control, although the substantial susceptibility of sixth-instar larvae demonstrates that the tested agents can also affect advanced stages.

Relative toxicity

Toxicity indices demonstrated clear differences in susceptibility within each biological group. Because the tested agents were expressed in different units—conidia ml^{-1} for fungi, IJs ml^{-1} for nematodes, and CFU ml^{-1} for bacteria—TI values were interpreted separately within each group rather than compared directly among fungi, nematodes, and bacteria. *B. bassiana* was the most toxic fungal agent, *S. carpocapsae* was the most toxic nematode, and *B. subtilis* was the most toxic bacterial agent under the present experimental conditions. The increasing relative toxicity with increasing exposure time further supports the cumulative nature of microbial pathogenicity.

Sublethal effects on third-instar larvae

Exposure to LC_{25} concentrations significantly affected the development of third-instar larvae despite the absence of significant differences in initial weight. Treated larvae showed lower final weight and weight gain than the control, with *H. bacteriophora* producing the greatest reduction in weight gain, followed by *B. bassiana*. The reduction in growth may be associated with impaired feeding, physiological stress, tissue damage, or diversion of metabolic resources toward host defense and infection. **Manachini *et al.* (2013)** similarly demonstrated that *S. carpocapsae* can affect the growth of *R. ferrugineus* larvae.

LC_{25} exposure also reduced molting success and prolonged the time required to reach the fourth instar. Thus, the biological effects of the tested agents extended beyond direct mortality to developmental suppression. Such effects are important because delayed development and reduced

molting success may decrease the fitness and reproductive potential of surviving individuals and thereby increase the overall population-suppressive effect of microbial agents.

Sublethal effects on sixth-instar larvae

The sublethal effects were particularly evident in sixth-instar larvae. Initial weights were statistically similar among treatments, whereas final weight, weight gain, molting rate, and time to molt differed significantly. The control recorded the highest final weight (4.78 ± 0.09 g) and weight gain (38.54 ± 11.87%), whereas *B. bassiana* produced the lowest final weight (3.71 ± 0.34 g) and very low weight gain (7.10 ± 3.96%). These findings indicate strong physiological suppression of growth among surviving larvae exposed to the fungal agent.

Molting success was also markedly reduced, declining from 81.25% in the control to 48.75% with *B. subtilis*, while all treatments delayed molting compared with the control. These results demonstrate that LC_{25} exposure can interfere with larval development even when mortality remains below 50%. The pronounced effects of *B. bassiana* on growth are consistent with its high lethal activity in the present study, whereas the bacterial treatments also produced substantial developmental effects. Previous work has shown that *B. thuringiensis* can alter stress and immune responses in RPW and can cause direct mortality **(Celi *et al*., 2022; Elsharkawy *et al*., 2022).**

Overall, the present results demonstrate that the tested entomopathogenic agents exerted both lethal and sublethal effects on *R. ferrugineus*. *B. bassiana*, *S. carpocapsae*, and *B. subtilis* were the most effective agents within their respective groups. In addition to mortality, LC_{25} exposure reduced larval growth, weight gain, and molting success and delayed development in both tested in stars. These findings emphasize that evaluating microbial agents based solely on mortality may underestimate their total biological impact and that sublethal developmental effects should be considered when assessing their potential for integrated management of the red palm weevil.

5. Conclusion

The present study demonstrated that the tested entomopathogenic fungi, nematodes, and bacterial agents were pathogenic to both third- and sixth-instar larvae of *R. ferrugineus*, with their efficacy generally increasing with exposure duration. *B. bassiana*, *S. carpocapsae*, and *B. subtilis* exhibited the greatest toxicity within their respective biological groups. In addition to causing mortality, LC_{25} exposure produced important sublethal effects, including reduced larval growth and weight gain, lower molting success, and delayed development. These findings indicate that the biological impact of entomopathogenic agents extends beyond direct mortality and that their sublethal effects should be considered when evaluating their effectiveness. The tested agents, particularly the most effective representatives of each biological group, therefore have potential for further evaluation and incorporation into integrated management strategies against the red palm weevil.

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