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RESEARCH ARTICLE

Harnessing biological control agents to mitigate red palm weevil infestations

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ABSTRACT

The red palm weevil (*Rhynchophorus ferrugineus*) is the primary insect pest causing damage to date palms (*Phoenix dactylifera* L.) in production areas. In the pursuit of safe control methods, this study aims to evaluate the efficacy of the entomopathogenic fungi *Trichoderma virens* and *Metarhizium anisopliae* against red palm weevil larvae and adults under laboratory conditions, as well as their impact on palm recovery in field conditions. The results indicated that *T. virens* achieved the highest mortality rates in both larvae and adults. Both fungi also compromise the weevil's defense mechanisms by altering its chemical composition, including total lipid, protein, and carbohydrate levels, in addition to the activities of superoxide dismutase and glutathione peroxidase. Additionally, *T. virens* demonstrated superior palm recovery compared to *M. anisopliae* and untreated control trees in the field, facilitating the recovery of approximately 70% of the treated palm trees under experimental conditions. These findings suggest *T. virens* as a promising biocontrol agent for managing red palm weevil infestations and enhancing palm health.

Key words: Date palm, entomopathogenic fungi, *Metarhizium*, *Rhynchophorus ferrugineus*, *Trichoderma*.

INTRODUCTION

Agricultural pests pose a significant threat to all aspects of agricultural production. Among these, the red palm weevil (RPW) is particularly notable as a key pest affecting date palms in their cultivation regions. The red palm weevil, *Rhynchophorus ferrugineus* (*Coleoptera: Dryophthoridae*), is recognized as a serious pest in date palm production areas (Al-Dosary et al., 2016).

Numerous biocontrol fungi are not only effective in managing insect pests and plant diseases but also interact directly with plants to promote healthy growth. *Metarhizium anisopliae*, a widely used insect-pathogenic fungus, is significant in controlling various agricultural and forest pests, including *Locusta migratoria manilensis*, *Dendrolimus punctatus*, *Frankliniella occidentalis*, *Spodoptera frugiperda*, and the soil-dwelling *Holotrichia diomphalia* (Mwamburi, 2021; González-Pérez et al., 2022).

Trichoderma harzianum has been shown to parasitize adult hemipterans (Sánchez-García et al., 2017), including the silverleaf whitefly (*Bemisia tabaci*) and the tropical bed bug (*Cimex hemipterus*), resulting in mortality rates of 40% within 5 d (Anwar et al., 2016) and 90% within 14 d (Zahran et al., 2017), respectively. Additionally, different species of *Trichoderma* can cause nearly 100% mortality in adult coleopterans after 15 d,

such as the coconut palm rhinoceros beetle (*Oryctes rhinoceros*) (Nasution et al., 2018) and the bean weevil (*Acanthoscelides obtectus*) (Rodríguez-González et al., 2017).

Trichoderma harzianum acts as an antagonist against a range of plant pathogens, such as those affecting *Vitis vinifera*. It is commonly employed as a biocontrol agent to reduce the impact of seedling blight and wilt caused by *Fusarium* spp., *Rhizoctonia solani*, and *Pythium* spp. (Liu et al., 2022). Currently, these two biocontrol fungi are recognized for their effectiveness in promoting plant growth as rhizosphere-competent organisms and endophytes.

Under management, weevils undergo various chemical changes, with antioxidant defense mechanisms involving both non-enzymatic and enzymatic systems (Gonçalves et al., 2023). Enzymatic antioxidant defense includes a range of enzymes such as superoxide dismutase, peroxidase, glutathione reductase, glutathione peroxidase, glutathione *S*-transferase, catalase, and phenol oxidase (Khedr et al., 2023). Superoxide dismutase (SOD; EC 1.15.1.1) plays a crucial role in protecting living cells from oxidative damage by catalyzing the conversion of superoxide radicals (O_2^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) (Dmochowska-Ślęzak et al., 2015; El-Beltagi et al., 2022; Khedr and Khedr, 2023), which can be potentially toxic. Similarly, glutathione peroxidase (GPO; EC 1.11.1.9) is an essential antioxidant enzyme that shields cells from oxidative damage by catalyzing the reduction and inactivation of harmful organic hydroperoxides (Ibrahim et al., 2016). The defense mechanisms of insects can be compromised by the inhibition of their antioxidant enzymes through the use of insecticidal plants (Demir et al., 2012). Hence, targeting the oxidative damage response of *R. ferrugineus* by inhibiting its defense mechanisms presents a potential strategy for pest management. However, there is currently limited information available on the antioxidant enzymes of the RPW.

The primary objective of this study is to assess the efficacy of *T. virens* and *M. anisopliae* as biological control agents in mitigating infestations of the red palm weevil. The study aims to investigate these agents' activity under laboratory conditions, examine any resulting changes in specific chemical compositions, and evaluate their effectiveness in field conditions, including the health recovery of affected plants.

MATERIALS AND METHODS

Entomopathogenic fungal production

For conidiospores production of *Trichoderma virens* and *Metarhizium anisopliae*, isolates of both fungi from a naturally infected palm weevil adults (López-Luján et al., 2022) were cultured on potato dextrose agar (PDA) medium poured into sterilized Petri-dishes (12 cm in diameter). Inoculated Petri-dishes with a spore suspension of *Trichoderma virens* and *Metarhizium anisopliae* were incubated for 16 d at 26 °C; conidial suspensions were made using a sterile 0.1% Tween-80 aqueous solution (Sánchez-García et al., 2017). Spore counts were determined using a hemocytometer under a microscope, and the final concentration was adjusted to 1×10^7 spores mL^{-1}.

Larvae and adults of RPW

Larvae of the RPW were collected from highly infested date palm trees (*Phoenix dactylifera* L.) that suddenly fell on the ground at date palm field Al Wahat, Giza Governorate, Egypt. Cultivated with cv. Barhi. A large number of adult weevils were present inside the fallen palm trees associated with many larvae in different instars and cocoons having pupae inside. Adult weevils and larvae were collected by hand and transferred in plastic boxes to the laboratory. Sugarcane slices (diameter of 2 cm and length of 5.0 cm) placed on wet absorbent paper were used to feed the insects and as an oviposition site for the weevils. The sugarcane and wet absorbent paper were renewed once a week, and emergent larvae were collected weekly during the experimental period.

Effectiveness of *M. anisopliae* and *T. harzianum* against RPW larvae

A preliminary study was conducted using various concentrations of spores, ranging from 5×10^2 to 5×10^8 spores mL^{-1}. These concentrations were prepared in distilled water from the stock suspension, which contained Tween 80, through successive dilutions. The optimal concentrations were determined to be 5×10^7 spores mL^{-1} for *T. virens* and 5×10^8 spores mL^{-1} for *M. anisopliae*.

A small atomizer was used to apply the tested spore concentrations directly onto larvae of various larval instars. The larvae were treated in six replicates, each consisting of 12 larvae. After the sprayed spore

suspension dried, the treated larvae were gently transferred into plastic boxes (20 × 20 × 30 cm) with perforated covers, using soft forceps. The boxes, containing food for the larvae, were kept under laboratory conditions at 26 °C and 60%-70% relative humidity (RH). A control group of untreated larvae was set up, sprayed only with water containing 0.01% Tween 80. The larvae were monitored daily for mortality over a 15-d period, with food provided regularly. To confirm death caused by *M. anisopliae* and *T. harzianum*, dead larvae were surface-sterilized for 4-5 s in formaldehyde and rinsed with sterilized distilled water under aseptic conditions. They were then placed in sterilized Petri dishes lined with wet, sterilized filter paper (Yan et al., 2022). The Petri dishes were kept inside self-sealing polyethylene bags to maintain high humidity and incubated at room temperature (26 °C and 60%-70% RH) to promote fungal growth.

Effectiveness of *M. anisopliae* and *T. harzianum* against adult red palm weevils

The conidial suspensions were applied directly to the adult RPW in Petri dishes, similar to the larvae treatment. Once the sprayed suspension had dried, the weevils were transferred to plastic boxes and monitored daily over a 10 d period for mortality, with food provided as needed. To confirm death caused by *M. anisopliae* and *T. harzianum*, dead adults were surface-sterilized for 4-5 s in formaldehyde and then rinsed with sterilized distilled water under aseptic conditions. They were subsequently placed in sterilized Petri dishes lined with wet, sterilized filter paper (Yan et al., 2022). The Petri dishes were enclosed in self-sealing polyethylene bags to maintain high humidity and incubated at room temperature (26 °C and 60%-70% RH) to facilitate fungal development. To find the alterations in the infected RPW, light microscopy was used. The B-350 light microscope (OPTIKA, Ponteranica, Italy) was used for the examination, and the HD-DV 1080P (AIPTEK, Hsinchu, Taiwan) was used to take pictures.

Determination of chitinolytic activity

Chitinolytic activity was assessed using the dinitro salicylic acid (DNS) method (Malviya et al., 2009). To this end, 0.5 mL cell-free supernatant was combined with 0.5 mL 0.5% colloidal chitin in phosphate buffer (pH 5. 6) and 1.0 mL sterile distilled water in test tubes. Controls were prepared as follows: One with 0.5 mL 0.5% colloidal chitin in phosphate buffer and 1.5 mL sterile distilled water (substrate control), and another with 0.5 mL cell-free supernatant and 1.5 mL sterile distilled water (enzyme control). Absorbance was measured at 540 nm using 300 µL aliquots. Chitinolytic activity was defined as the amount of enzyme needed to release 1.0 µg reducing sugars per millimeter per minute from a 0.5% colloidal chitin solution under the specified conditions.

Assessment of biochemical changes in RPW

The enzyme crude extracts were made by grinding samples in a chilled mortar and pestle with 50 mM ice-cold potassium phosphate buffer at moderate pH. The resulting homogenates were then centrifuged at 14 000×g for 15 min at 4 °C. The two resulting supernatants were combined, labeled as enzyme crude extract, and stored at -20 °C until needed for enzyme analysis.

Superoxide dismutase (SOD) activity was assessed using the method described by McCord and Fridovich (1969). The reaction mixture had a total volume of 1.0 mL, consisting of 50 mM potassium phosphate buffer at pH 7, 20 mM cytochrome C, 10 mM xanthine disodium salt, and enzyme crude extract in 20 µL. The reaction was initiated by the addition of 30 mU xanthine oxidase. The change in absorbance was measured over 3 min at 560 nm. One unit of SOD is defined as the enzyme amount that inhibits cytochrome C reduction by 50%.

Glutathione peroxidase (GPO) activity was determined following the method outlined by Hamed et al. (2019). The reaction mixture, with a total volume of 2.0 mL, included 100 mM potassium phosphate buffer at pH 7.0, 10 mM EDTA, 10.0 mM reduced glutathione, 0.15 mM H_2O_2, 0.3 mM NADPH, 1.0 IU glutathione reductase, and 50 µL enzyme crude. The decrease in absorbance was measured at 340 nm. One unit of GPO corresponds to the oxidation of 1.0 µmol NADPH per minute under standard assay conditions.

Total proteins were quantified using the Bradford (1976) method, which involves mixing the sample with Bradford reagent and measuring the absorbance at 595 nm. Total lipids were measured using the technique described by Knight et al. (1972), which involves extracting lipids from the sample using a solvent system, typically a mixture of chloroform and methanol. Total carbohydrates were determined using the phenol-sulfuric acid method, as described by Nielsen (2010). In this method, the sample's extract is reacted with phenol and

sulfuric acid, resulting in a color change that is measured spectrophotometrically at 490 nm. The intensity of the color is proportional to the carbohydrate concentration in the sample.

Lethality effects of tested fungi on red palm weevil (*Rhynchophorus ferrugineus*)

Mortality rates were recorded at intervals ranging from 24 h to several days post-treatment. To ensure accuracy, bioassay data were adjusted for control mortality and analyzed using probit analysis as described by Finney (1952). The median lethal concentration (LC_{50}), as well as the lethal concentration for 90% mortality (LC_{90}), and the regression slope were determined for both larval and adult stages of the RPW.

Field experiment and date palm injection protocols

For the preparation of the fungal solutions used in date palm injections, fungal concentrations were assessed using a hemocytometer under a microscope. The concentrations for the experiment were set at 5×10^7 spores mL^{-1} for *T. virens* and 5×10^8 spores mL^{-1} for *M. anisopliae*, consistent with previous laboratory conditions. Six liters of each fungal solution were prepared and stored in plastic containers, with distilled water used for the control treatment.

For entomopathogenic fungus treatments optimization, a central composite rotatable design (CCRD) with a two-factor mixed-level experimental design, utilizing response surface methodology (RSM), was employed to optimize fungus concentration and treatment time for enhance controlling RPW. Data analysis was applied using De-sign Expert software version 11 (Stat-Ease Inc., Minneapolis, Minnesota, USA) and the RSM was constructed after 13 runs of process parameter optimization and experiments conducted in accordance with the real experimental design matrix.

For the injection procedure, each fungal solution was drawn into a 20 mL syringe and used to fill a balloon injector. A total of 54 moderately naturally infested date palm trees (18 per treatment group) were selected for injection. The treatment was administered using a balloon injector at multiple points on each tree. To execute the injections; drill four holes around the trunk of each date palm in a clockwise spiral pattern. Each hole was drilled at a 45° downward angle, 30, 60, 90, and 120 cm above the ground, using a brad point drill bit (8 mm diameter). Immediately after drilling, inject the fungal solution into each hole. Each hole received 100 mL solution, resulting in a total injection volume of 500 mL per tree. For control trees, sterile distilled water was used instead of the fungal solution. Clay and fiber were used to fill the injection holes. All treatments were repeated after 7 d. The post-treatment number of dry and wet galleries within the trunk was recorded. Assess signs of tree recovery by checking for the absence of fresh sap and a pungent odor on the trunk. Insert a thin twig into the drilled holes to confirm the dryness of the internal fluids. The effectiveness of field treatments was assessed over several months by evaluating the reduction in the percentage of infested palm trees, the percentage decrease in captured weevils, and the incidence of RPW infection.

Statistical analysis

The lab experiment was conducted using a randomized complete design with 12 replicates per treatment, while the field experiment employed a randomized complete block design. ANOVAs were performed with MSTAT-C (Michigan State University, East Lansing, Michigan, USA) to compare mean differences in temperatures measured outside versus inside each date palm, as well as mean rates of impulse bursts for various treatments. Means were separated using the Duncan test, with a significance level of $p < 0.05$.

RESULTS

The susceptibility of RPW to *T. virens* and *M. anisopliae*

The results indicated that young larvae of the RPW were highly susceptible to the fungi *T. virens* and *M. anisopliae*, with 100% mortality observed by the 10^{th} day post-treatment (Figure 1). Within the first 2 d of infection, white hyphae began to form around the larvae's integuments (Figure 2A). By the sixth day, green spores had spread across the entire body of the larvae (Figure 2B). Additionally, dead RPW displayed hyphal growth and sporulation. Hyphae typically emerge 2 to 3 d post-mortem, followed by the appearance of green spores (Figures 2C, 2D).

When assessing the relative toxicity at LC_{50} and LC_{90} levels, *T. virens* demonstrated greater effectiveness than *M. anisopliae*. The LC_{50} values for *T. virens* were determined to be 3.7×10^2 spores mL^{-1} for 6th instar larvae and 5.6×10^1 spores mL^{-1} for 11th instar larvae. The corresponding LC_{90} values were 3.5×10^2 spores mL^{-1} and 4.6×10^3 spores mL^{-1}, respectively (Table 1). *Trichoderma virens* exhibited superior entomopathogenic activity compared to *M. anisopliae*, with an LT_{50} of 4.3 d for larvae. In adult palm weevils, *T. virens* also showed better efficacy, achieving an LT_{50} of 11.7 d (Figure 3).

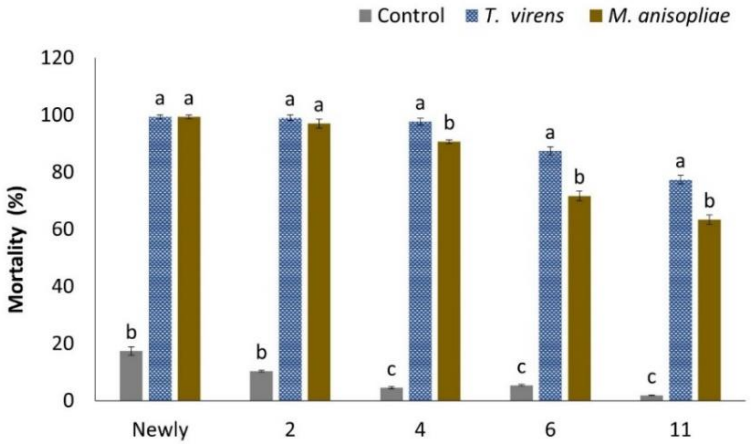

Figure 1. Percentage mortality of various instar larvae stages of the red palm weevil (*Rhynchophorus ferrugineus*) treated with *Trichoderma virens* and *Metarhizium anisopliae*. The vertical bars represent the standard error of the mean. Different letters indicate significant differences ($P \leq 0.05$) between the means, as determined by Duncan's multiple range test.

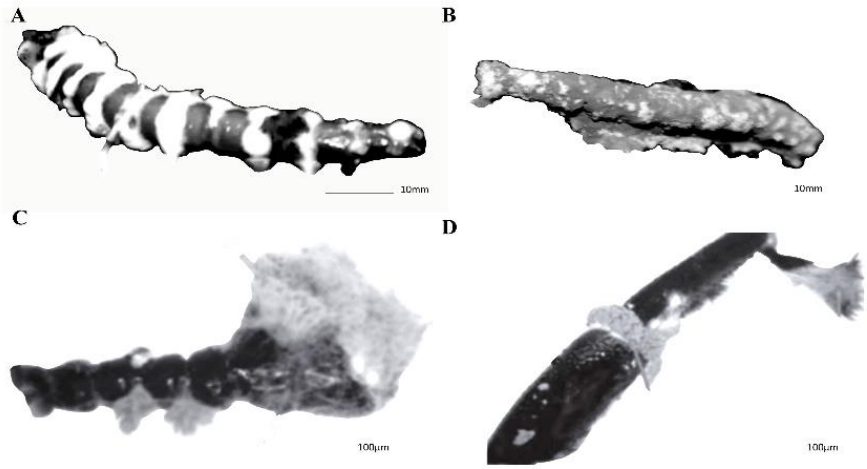

Figure 2. The infected larvae after two days of treatment (A) and 6 d later (B), the hyphae growth and sporulation of *Trichoderma virens* on antenna of the dead body of a RPW (C), and hind leg (D).

Table 1. Lethal concentrations (LC_{50}, LC_{90}) of *Trichoderma virens* and *Metarhizium anisopliae* against red palm weevil (*Rhynchophorus ferrugineus*) at 25 ± 1 °C and 65 ± 2% relative humidity (RH).

Treatment	Stage	LC_{50}	LC_{90}	Slope
		Spores mL^{-1}	Spores mL^{-1}	
T. virens	6^{th}	3.7×10^2	3.5×10^2	0.294
	11^{th}	5.6×10^1	4.6×10^3	0.503
M. anisopliae	6^{th}	3.9×10^2	3.7×10^2	0.361
	11^{th}	5.9×10^1	4.8×10^3	0.532

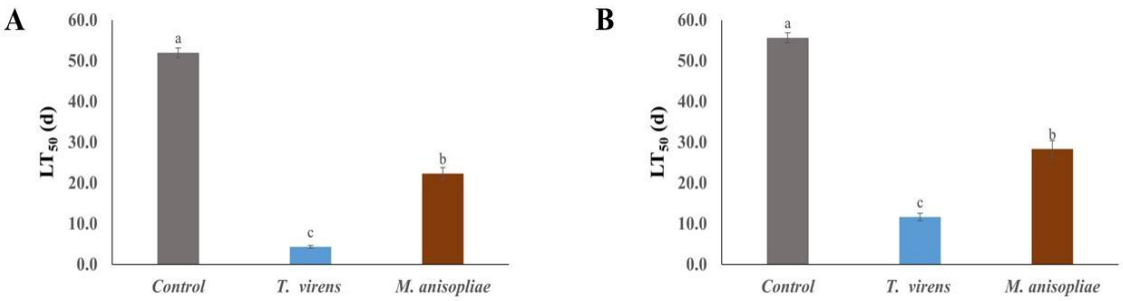

Figure 3. Lethal time (LT_{50}) for larvae (a) and adults (b) of *Trichoderma virens* and *Metarhizium anisopliae* on the palm weevil (*Rhynchophorus palmarum*). Bars with different letters indicate significant differences according to Duncan's multiple range test at the 5% probability level.

Chitinase activity and biochemical alterations in RPW after exposure to *T. virens* and *M. anisopliae*.

Figure 4A depicts the variation in chitinase activity in RPW following treatment with different agents. At 2 d post-treatment, *T. virens* exhibited the highest chitinolytic activity (8.9 U). This elevated enzyme activity suggests a robust degradation of chitin, a key component of the insect exoskeleton, which may compromise larval integrity and survival.

As the experiment progressed, *T. virens* continued to elicit strong chitinolytic responses. At later time points, it maintained significantly higher chitinase activity levels, recording 4.07 U compared to the control (1.30 U). This sustained enzyme activity indicates a prolonged impact of *T. virens* on the larvae's chitin degradation processes. The control group, in contrast, exhibited minimal changes in chitinase activity, highlighting the potent effect of *T. virens* as a biocontrol agent.

Results in Figure 4 showed that exposure of RWP to *T. virens* and *M. anisopliae* had a substantial impact. Total lipids and carbohydrate levels dropped, when compared with those obtained in the control. As for total lipid and total carbohydrate content, it showed a detectable decrease in treated RWP, while total protein showed a slight or no decrease.

The data in Figure 4 revealed that the total lipids had a significant decrease by *T. virens* and *M. anisopliae* compared with the control, with the highest value for the control. In comparison, on the contrary, total proteins had a significant increase by increasing *T. virens* and *M. anisopliae* used compared with control. In addition, total proteins had a significant decrease by *T. virens* and *M. anisopliae*.

Both fungi, *T. virens* and *M. anisopliae*, significantly reduced superoxide dismutase (SOD) and glutathione peroxidase (GPO) activity in *R. ferrugineus* larvae compared to untreated individuals (Figure 5). Figure 5A demonstrates that SOD activity in the fat body of RPW larvae was markedly reduced following fungal treatment. Treated larvae exhibited only about half the SOD activity of untreated larvae, indicating a significant impairment in their antioxidant defense mechanisms.

Figure 5B reveals that GPO activity was also notably lower in larvae treated with both fungi. Specifically, *T. virens*-treated larvae displayed a GPO activity of 20.2 U, while those treated with *M. anisopliae* had a GPO

activity of 30.1 U. Both values are significantly lower compared to the levels found in untreated larvae. These findings underscore the effectiveness of *T. virens* and *M. anisopliae* in disrupting key antioxidant enzyme activities, thereby compromising the larvae's ability to manage oxidative stress. This reduction in enzymatic activity contributes to the overall effectiveness of these fungi as biocontrol agents against the RPW.

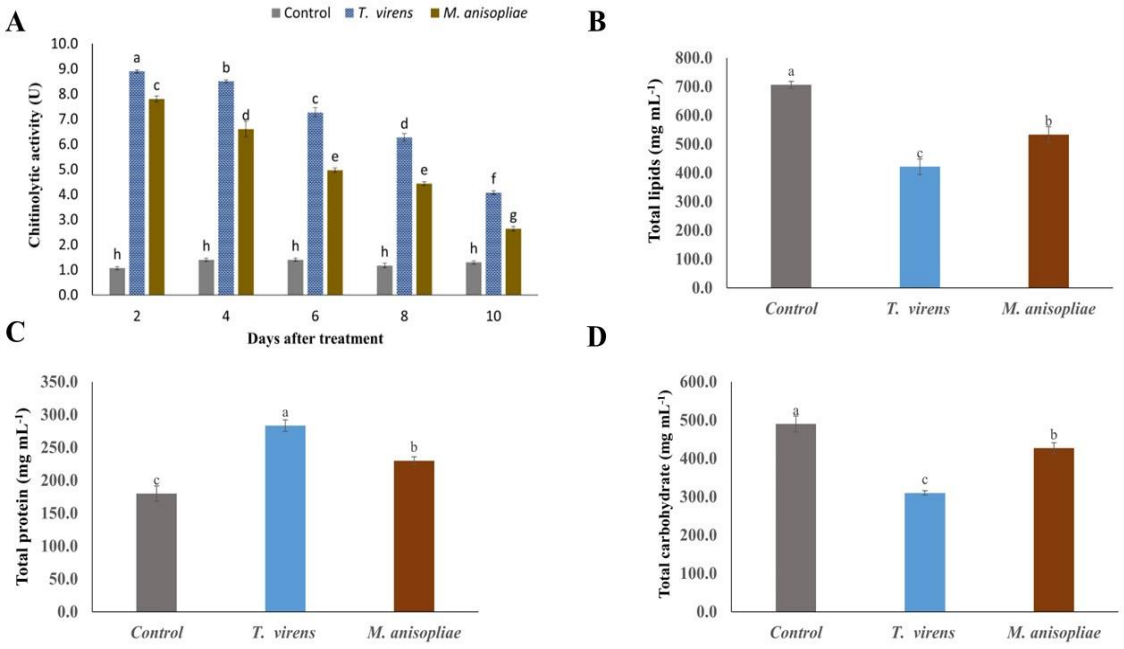

Figure 4. Efficiency of *Trichoderma virens* and *Metarhizium anisopliae* on chemical changes of *R. ferrugineus* infestation. Vertical bars indicate the standard error of the means with different letters indicating significant variance ($P \leq 0.05$) between means, as determined by Duncan's multiple range test.

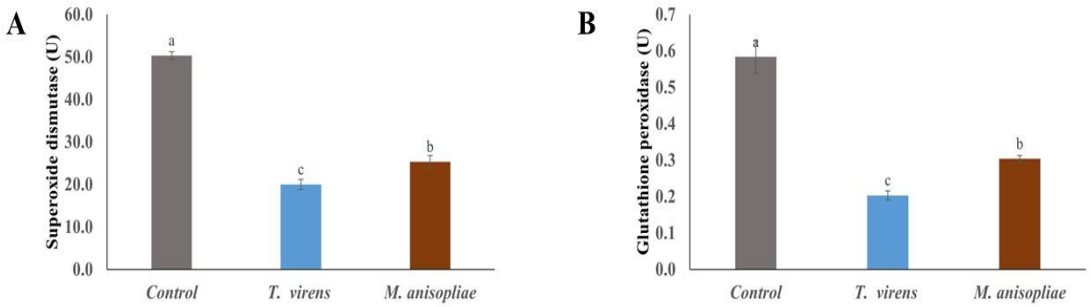

Figure 5: Superoxide dismutase (SOD) (A) and glutathione peroxidase (GPO) (B) activity in *Rhynchophorus ferrugineus* ± SE. Bars marked with the same letters are not significantly different ($p > 0.05$), while those with different letters indicate significant differences ($p < 0.05$).

Field evaluations of biological control agents for the recovery of palm trees.

Figure 6 illustrates a 3D response surface that depicts the efficacy of *M. anisopliae* and *T. virens* in controlling *R. ferrugineus* infestation in date palm trees. The results showed that using spore concentrations of 5×10^7 spores mL^{-1} for *T. virens* and 5×10^8 spores mL^{-1} for *M. anisopliae*, reflecting the optimized conditions established in the laboratory. The 3D surface clearly demonstrates that the most effective treatment periods were from March to May, during which the highest recovery rates from RPW infestation were observed. This period corresponds with peak seasonal activity, indicating that the biocontrol agents are most potent when applied during these months.

In the field, the damage is primarily caused by the larvae, which bore tunnels and create large cavities within the palm. These larvae can be found throughout the palm, feeding on the growing tissue in the crown, often destroying the apical growth region and ultimately leading to the palm's death. Early-stage infestations are challenging to detect, and the pest is usually discovered only after significant damage has occurred. However, several indicators may signal the presence of the pest: Holes in the crown or trunk, from which chewed-up fibers are expelled, sometimes accompanied by oozing brown viscous liquid; a distinct crunching sound from feeding larvae, which can be heard by placing an ear against the trunk; and in some cases, a withered bud (Figure 7).

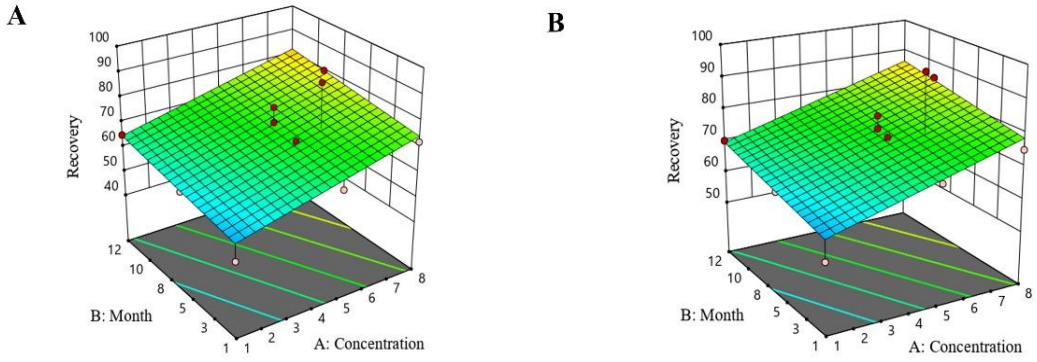

Figure 6. 3d Response surface represents effectiveness of *Metarhizium anisopliae* (A) *Trichoderma virens* (B) in controlling *Rhynchophorus ferrugineus* infestation on date palm trees.

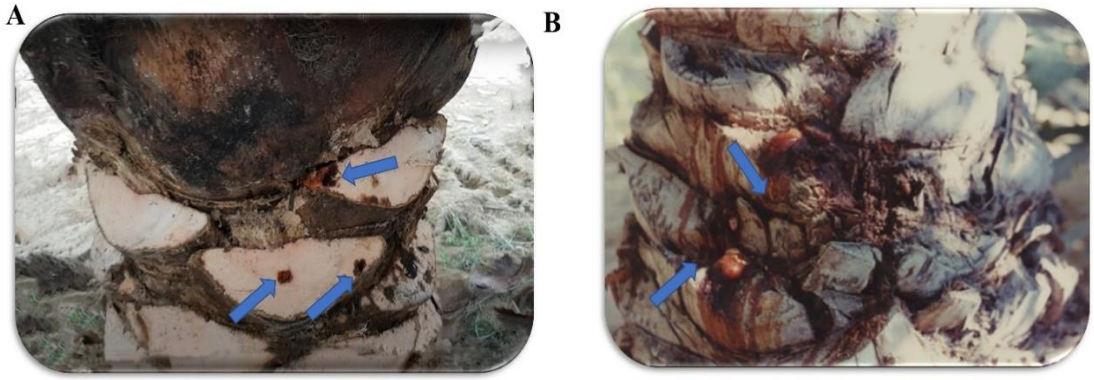

Figure 7. Date palm damage caused by the red palm weevil. (A) Trunk holes brought on by red palm weevil larvae feeding. (B) Viscous fluid, releasing excrement over the surface of the trunk.

The effectiveness of both fungi on *R. ferrugineus* was evaluated under field conditions 3 wk after treatment, with results depicted in Figure 8. *Trichoderma virens* was notably effective, facilitating the recovery of approximately 70% of the treated palm trees. This significant recovery rate underscores the potential of *T. virens* as a promising biological control agent for managing red palm weevil infestations.

Figures 9A and 9B indicate that both fungi *T. virens* and *M. anisopliae* showed increasing effectiveness over time. This increase is reflected in the high efficiency of these fungi, as evidenced by the reduction in the percentage of infested palm trees, the decrease in captured weevils, and the incidence of RPW infection, which were all evaluated across different months to study the efficiency of field treatments.

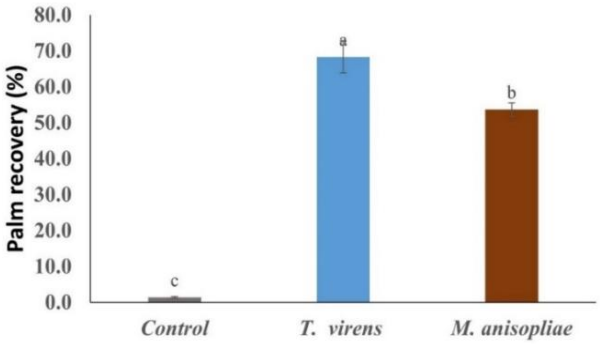

Figure 8. Effectiveness of *Trichoderma virens* and *Metarhizium anisopliae* in controlling *Rhynchophorus ferrugineus* infestation on date palm trees. Vertical bars represent the standard error of the mean. Bars with different letters denote significant differences ($P \leq 0.05$) between means, as determined by Duncan's multiple range test.

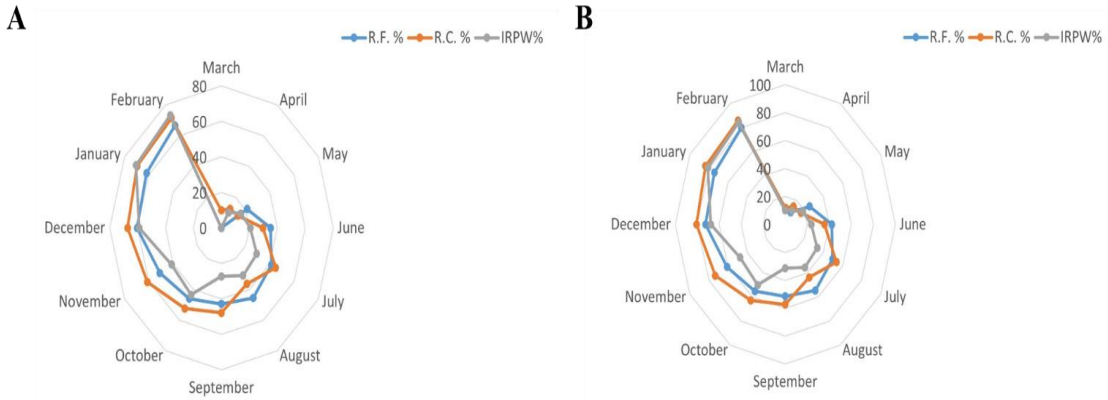

Figure 9. Effectiveness of *Metarhizium anisopliae* (A) and *Trichoderma virens* (B) in controlling *Rhynchophorus ferrugineus* infestation on date palm trees. Reduction of infested palm trees (R.F. %), reduction in captured weevils (R.C.%), and infection of red palm weevil (IRPW%).

DISCUSSION

The study highlights the potent efficacy of *Trichoderma virens* and *Metarhizium anisopliae* in managing *Rhynchophorus ferrugineus* (red palm weevil, RPW), with a particular emphasis on the superior performance of *T. virens*. Both fungal species caused 100% mortality in RPW larvae within 10 d of treatment, demonstrating their high pathogenicity. However, *T. virens* proved to be more effective than *M. anisopliae*. The lower LC_{50} values of *T. virens* (3.7×10^2 spores mL^{-1} for 6^{th} instar and 5.6×10^1 spores mL^{-1} for 11^{th} instar larvae) compared

to *M. anisopliae* indicate a greater potency in causing larval death. Furthermore, *T. virens* achieved faster mortality with an LT_{50} of 4.3 d for larvae and 11.7 d for adults, underscoring its superior efficacy as a biocontrol agent (Figures 1 and 2). Gindin et al. (2006) found similar responses on RPW different stages.

The chitinase activity, which is crucial for the degradation of chitin in the insect exoskeleton, was significantly higher in larvae treated with *T. virens*. At 2 d post-treatment, *T. virens* exhibited the highest chitinolytic activity, and maintained elevated activity levels throughout the experiment, compared to the control. This sustained high activity suggests that *T. virens* effectively disrupts the structural integrity of the larvae, contributing to their high mortality rates. The biochemical analysis revealed significant changes in the lipid and carbohydrate levels of treated larvae. Both *T. virens* and *M. anisopliae* caused notable reductions in total lipids and carbohydrates, whereas total protein levels were either slightly increased or remained unchanged. The decrease in lipid and carbohydrate content indicates a disruption in metabolic processes, which could further impair larval health and development. Ragheb et al. (2018) found similar chemical changes data when using entomopathogenic fungus.

In the current study, *Trichoderma* was used as a reference organism because it is recognized for its ability to produce chitinase with high enzymatic activity (Sandhya et al., 2004). The isolates were tested for their insect control potential, specifically in pathogenicity assays against the bean weevil. The lowest LT_{50} values for RPW were observed with *T. virens*, suggesting its high pathogenicity. This is likely due to the fungus's capacity not only to penetrate the insect cuticle but also to release enzymes and toxins that can compromise the insect's immune defenses (Mondal et al., 2016). Moreover, while Muthukrishnan et al. (2020) reported that adult coleopteran insect cuticles are mainly composed of chitin, Mondal et al. (2016) identified that the outer cuticle of palm weevil larvae contains significant amounts of lipids and proteins. The fungal isolates showed effectiveness against both larvae and adult palm weevils, with specific LT_{50} values being reported. The fungi penetrate the insect cuticle by exploiting the C and N-rich substances in the insect's body (López-Luján et al., 2022).

Additionally, these fungi can bypass the immune response of insects, a process aided by the physical force of hyphal growth and the enzymatic breakdown of the cuticle's components (Lovera et al., 2020). Unlike some other fungi, *Trichoderma* species also exhibit mycoparasitism, produce secondary metabolites with insecticidal properties, and compete with host insects through more complex and gradual processes (Poveda, 2021).

The reduction in superoxide dismutase (SOD) and glutathione peroxidase (GPO) activities in treated larvae reflects significant oxidative stress. *Trichoderma virens*-treated larvae exhibited a reduction in SOD activity to about half of that seen in untreated larvae (Figure 4A). Similarly, GPO activity was considerably lower in both *T. virens* and *M. anisopliae* treatments, with *T. virens*. These decreases in antioxidant enzyme activities indicate that the fungi induce oxidative stress, further compromising the larvae's physiological functions and enhancing the effectiveness of the biocontrol treatment. Antioxidant enzymes play a crucial role in the defense mechanism against reactive oxygen species (Al-Khayri and Khedr, 2024; Al-Khayri et al., 2024; Khedr and Khedr, 2024).

The digestive physiology of *R. ferrugineus* must adapt to reduce the harmful effects of reactive oxygen species (ROS) produced during the ingestion. Superoxide dismutase (SOD) plays a crucial role in this process by preventing the buildup of superoxide anions and producing hydroxyl radicals (OH^-), which are highly reactive. These radicals are further converted into hydrogen peroxide (H_2O_2), the most toxic ROS (Kolawole et al., 2014; Oni et al., 2019). According to Hamed et al. (2019), the fat body serves as a site for ROS detoxification. This phenomenon is also observed in other insect species, such as *Spodoptera littoralis* (Krishnan and Kodrík, 2006). These findings suggest that the fat body is primarily responsible for scavenging superoxide anions, with each tissue having a specific role in this process.

The current study found low activities of GPO. These antioxidant enzymes work together to neutralize ROS. Glutathione peroxidase reacts with hydrogen peroxide (H_2O_2) or lipid/organic hydroperoxides, reducing them to water or corresponding alcohols, while simultaneously oxidizing two molecules of glutathione (GSH) to glutathione disulfide (Jovanovic-Galovic et al., 2004; Bamidele et al., 2017). Similar GPO activity levels were reported by Krishnan and Kodrík (2006) in different gut tissues of *S. littoralis*, particularly in larvae fed on plants. The GPO activities observed in this study were lower than those recorded for both diapausing and non-diapausing larvae of *Ostrinia nubilalis* (Jovanovic-Galovic et al., 2004), various instars of *Lymantria dispar* (Perić-Mataruga et al., 1997), and different beetle species from heavy metal-contaminated sites (Migula et al., 2004). Additionally, the GPO activity in *R. ferrugineus* was significantly lower than that found in the fat body, gut, and head of African palm weevil (*Rhynchophorus phoenicis*) larvae (Bamidele et al., 2017).

The field evaluations demonstrated that *T. virens* facilitated the recovery of treated palm trees within 3 wk. This substantial recovery rate highlights *T. virens* as an effective and practical solution for managing RPW infestations in real-world scenarios. The obtained results also clearly show the effectiveness of injecting fungal isolates into a date palm under field conditions in agreement with Sutanto et al. (2023).

Some entomopathogenic fungi have a narrow host range, while others, such as *Metarhizium anisopliae*, have a broad host range. Our findings demonstrated the pathogenicity of *M. anisopliae*. This aligns with the results of Tamai et al. (2002), who studied the effects of 45 isolates including *M. anisopliae*, and *Paecilomyces farinosus* on *Tetranychus urticae*. Similarly, Bugeme et al. (2014) investigated the effects of various concentrations of *M. anisopliae* and observed the highest mortality rate with *M. anisopliae* isolates.

CONCLUSIONS

In conclusion, *Trichoderma virens* and *Metarhizium anisopliae* have the potential as effective biocontrol agents against the red palm weevil (*Rhynchophorus ferrugineus*) (RPW), a significant pest in date palm cultivation. *Trichoderma virens* demonstrated superior efficacy in both laboratory and field conditions, achieving the highest mortality rates among larvae and adult weevils and disrupting their defense mechanisms through biochemical alterations. Field trials confirmed *T. virens* superior performance in promoting recovery in infested palm trees compared to *M. anisopliae* and untreated controls. The study also explored the efficacy of the local isolate of *M. anisopliae* and the entomopathogenic fungus *T. virens*, which showed potential in controlling RPW through contamination with conidiospores. Additionally, research into the adaptability of RPW larvae to oxidative stress and detoxification mechanisms provides insights for future pest management strategies. The findings support the inclusion of these fungi in integrated pest management programs, aiming to reduce synthetic insecticide use and enhance the overall efficacy of RPW control.

Author contributions

Conceptualization: E.H.K., H.S.E.-B., M.G. Methodology: E.H.K., M.A.-H., B.A.K., M.G. Software: E.H.K., A.M.I., B.A.K. Validation: W.E., M.A-H., A.A.R. Formal analysis: E.H.K., M.G., M.A.-H. Investigation: E.H.K., M.A-H. Resources: H.S.E.-B., E.H.K. Data curation: E.H.K., B.A.K., M.A.-H., M.G. Writing-original draft preparation: E.H.K., M.G., B.A.K. Writing-review and editing: E.H.K., M.A-H., M.G. Supervision: E.H.K., H.S.E.-B. All authors have read and agreed to the published version of the manuscript.

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