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Entomopathogenic Fungi against the Red palm weevil, *Rhynchophorus ferrugineus* Olivier

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ABSTRACT: The red palm weevil, known as *Rhynchophorus ferrugineus* Olivier (RPW) ranks as the primary pest affecting multiple species of palm trees. In light of environmental and regulatory issues, the quest for ecologically sound alternatives to synthetic chemical insecticides has become imperative in the quest to control this elusive insect species. Entomopathogenic fungi offer promising potential in combatting this pest. Enhancements in the management and efficacy of controlling the red palm weevil can be achieved through the direct application or horizontal spread of entomopathogenic fungal strains. The study conducted in the laboratory focused on examining the impact of the entomopathogenic fungi, *Metarhizium anisopliae* Var. acridum (Metchinkoff) Soroken and *Beauveria bassiana* (Bals.) Vuill, on *Rhynchophorus ferrugineus*. Two types of entomopathogenic fungi were tested across three different concentrations: 2 x 10 ^{4}, 2 x 10 ^{5}, and 2 x 10 ^{6} spores / ml (C_1, C_2 & C_3). Mortality rates were monitored on a daily basis. It was found that *M. anisopliae* exhibited greater virulence towards the larvae, pupae, and adult stages of *R. ferrugineus* compared to *B. bassiana*. These results suggest that both *M. anisopliae* and *B. bassiana* show promise as effective agents in pest management, thereby potentially reducing environmental pollution, especially when pest populations are below the economic threshold.

Keywords: *Rhynchophorus ferrugineus*, Evaluate, *Metarhizium anisopliae*, *Beauveria bassiana*.

INTRODUCTION

The *Phoenix dactylifera* L., commonly known as date palm, is a diploid perennial plant belonging to the Arecaceae family. It is revered as one of the most ancient and widely cultivated crops in the regions of Southwest Asia and North Africa. Through the course of history, its cultivation has expanded to encompass regions such as Australia, South America, southern Africa, Mexico, Pakistan, and the United States. *Rhynchophorus ferrugineus* (Olivier), also known as the red palm weevil (RPW), is identified as a destructive insect pest that targets various palm species. Originating in India, this invasive species has proliferated globally and is now widespread across Africa, America, Asia, Europe, and Oceania (Waleed *et al.,* 2024, Wakil *et al.,* 2015, Tagliavia *et al.,* 2014, Fiaboe *et al.,* 2012 and EPPO, 2024). The Red Palm Weevil (RPW) is responsible for extensive harm to coconut trees in the region of Southeast Asia. *Rhynchophorus ferrugineus* Olivier is known to be the most destructive among pests. Research has been conducted on the pathogens of *Rhynchophorus ferrugineus* in order to assess their viability as agents for biological control. *Metarhizium anisopliae*, sourced from *R. bilineatus*, was discovered subsequent to the treatment of young palm trees against the scarabaeid Scapanes australis using a formulation incorporating *M. anisopliae* spores (Salama *et al.,*

2004, Shamseldean, 2004 and Prior and Arura 1985). In Iran, *M. anisopliae* was obtained from adult insects, while *Beauveria bassiana* (Bals.) Vuill. was isolated from pupae. It was observed that Beauveria sp. was present in certain cocoons of *R. ferrugineus*. The effectiveness of using entomopathogenic fungi as biological control agents against various weevil species has been examined. Both adult insects and larvae were successfully infected with *B. bassiana* and *M. anisopliae*, leading to mortality rates ranging from 50% to 100% (Ihara *et al.,* 2003, Kehat, 1999 and Abdel-Raheem, 2005). In numerous nations, various authors have utilized Entomopathogenic fungi for biological pest control targeting a range of insect species (Abdel-Raheem, 2013, Sabbour and Abdel-Raheem 2014, Abdel-Rahman and Abdel-Raheem 2018). Specifically, some researchers have employed a variety of isolates, including *B. bassiana*, *M. anisopliae*, *Isaria fumosorosea*, and *Verticillum lecanii*, against the *Rhynchophorus ferrugineus* pest. Additionally, other studies have investigated the efficacy of fungal spores and Silver Nano-particles in combatting *Rhynchophorus ferrugineus*, with findings indicating that Silver Nano-particles demonstrated superior effectiveness across all life stages compared to fungal spores (Abdel-Raheem *et al.,* 2009, 2019, 2020 and Reyad, *et al.,* 2020). Several researchers have leveraged different entomopathogenic fungi in efforts to manage various pests, as documented by (Mohamed Abdel-Raheem, 2015, 2017, 2020, Sabry et. *al.,* 2011, Zaki and Abdel-Raheem 2010, and Sabbour *et al.,* 2012).

The aim of this study is to evaluate the effect of the *M. anisopliae* and *B. bassiana* against the red palm weevil, *R. ferrugineus*.

MATERIALS AND METHODS

Entomopathogenic Fungi

Metarhizium anisopliae (Metchinkoff) Soroken isolated from larvae and adults of *Scrobipalpa ocellatella* and *Beauveria bassiana* (Balsamo) Vuillemin isolated from *Cassida vittata* (Abdel-Raheem, 2005) were grown on Peptone media (10g Peptone, 40g Dextrose, 2g yeast extract, 15g Agar and 500 ml. Chloramphenicol). The media was autoclaved at 120 ºC for 20 minutes, and poured in Petri- dishes (10 cm diameter x 1.5 cm). Then incubated the fungi and kept at 24 ±2 ˚C and 65± 5 % RH. The fungal isolates were re-cultured every 14 – 30 days and kept at 4 ˚C.

Preparing of concentrations

Spores of fungal isolates harvested by rising with sterilized water 0.5 % Tween 80 from 14 day old culture Peptone media. The suspensions were filtered through cheese cloth to reduce mycelium clumping. The spores were counted in the suspension using a Haemocytometer (0.1 mm × 0.0025 mm^2). The concentrations were used 2 × 10^4, 2×10^5 and 2 × 10^6 spore /ml. (C_1, C_2 & C_3).

Insect host

Field observations were recorded on the date palm trees, *Phoenix dactylifera* L. (Fam. Palmaceae) in Farm Agricultural global production at El-Behira (El-Nubaria) Governorate. A total of 100 palm trees were examined. Many different stages of RPW e.g., larvae, cocoons of coarsely constructed fibers and adults were collected from damaged trunks of infested trees. Samples were collected and transferred weekly to the laboratory and carefully examined.

Insect rearing

A RPW colony was established in the laboratory on sugarcane as both food and oviposition substrate, Adults were set to mate and oviposit in groups of at least five pairs placed on a substrate of moist sugar cane sawdust or on sugar cane logs. From the first larval stage to adult emergence, the RPWs were reared individually at 27- 29 °C. For egg harvesting, the adults of both sexes were kept on sugarcane sawdust. Eggs were collected every 2 days.

Bioassay procedure

The Entomopathogenic Fungi, *Metarhizium anisopliae* Var. acridum (Metchinkoff) Soroken and *Beauveria bassiana* (Bals.) Vuill. were tested at three concentrations (C_1, C_2 & C_3) to contaminate the larval instar, pupae and adults of the red palm weevil, *Rhynchophorus ferrugineus*. 100 larvae, pupae and adults were used for each treatment, divided into 4 groups each of 25 larvae, pupae and adults placed in Petri-dishes. One individual / dish, 25 /concentration. The fungi were applied in a suspension containing (C_1, C_2 & C_3) in the control treatment treated with sterilized water and kept at 25 ±2 ºC and 70 ±5 % RH. The mortality of all stages of *Rhynchophorus ferrugineus* was observed daily.

RESULTS

The larvae were infected with *M. anisopliae* and *B. bassiana* concentrations 2 × 10^4, 2 ×10^5 and 2 × 10^6 spore /ml., (C_1, C_2 & C_3). The mortality recorded 17.9 % by infection the 3rd concentration from *M. anisopliae* after 3rd day but was 12.9 % with the same concentration from *B. bassiana* and day. *M. anisopliae* was more virulence effect on the larvae of *R. ferrugineus* than *B. bassiana*. The mortality reached to 100 % after 9th day from the infection by *M. anisopliae* but reached to 100% after 10th days from the infection by *B. bassiana* Fig. 1.

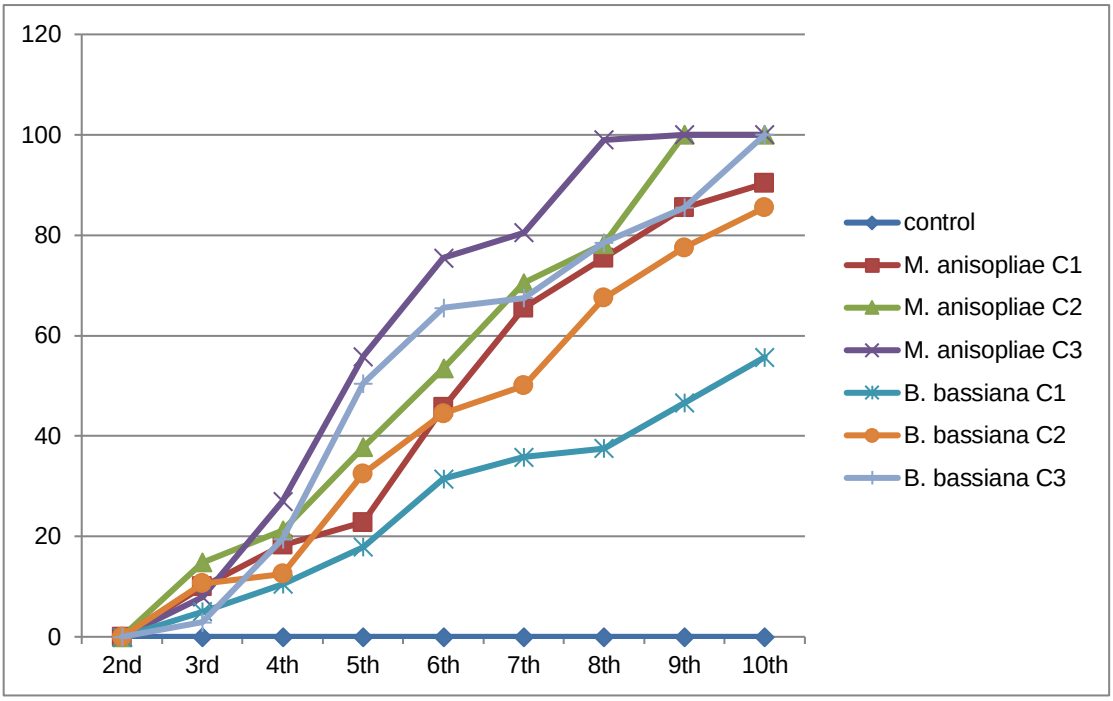

Fig. 1. Percent Mortality of the larvae of *Rhynchophorus ferrugineus* infected with *M. anisopliae* and *B. bassiana* at 25±2 ºC and 70 ±5 % RH.

The pupae were infected with *M. anisopliae* and *B. bassiana* concentrations 2 × 10^4, 2 ×10^5 and 2 × 10^6 spore /ml., (C_1, C_2 & C_3). The mortality recorded 10.0 % by infection the 3rd concentration from *M. anisopliae* after 4th day but was 8.0 % with the same concentration and day from *B. bassiana*. *M. anisopliae* was more effect on the pupae of *R. ferrugineus* than *B. bassiana*. The mortality reached to 100 % after 12th day from the infection by *M. anisopliae* but reached to 100% after 13th days from the infection by *B. bassiana* Fig. 2. The adults were infected with *M. anisopliae* and *B. bassiana* concentrations (C2 × 10^4, 2 ×10^5 and 2 × 10^6 spore /ml., (C_1, C_2 & C_3).

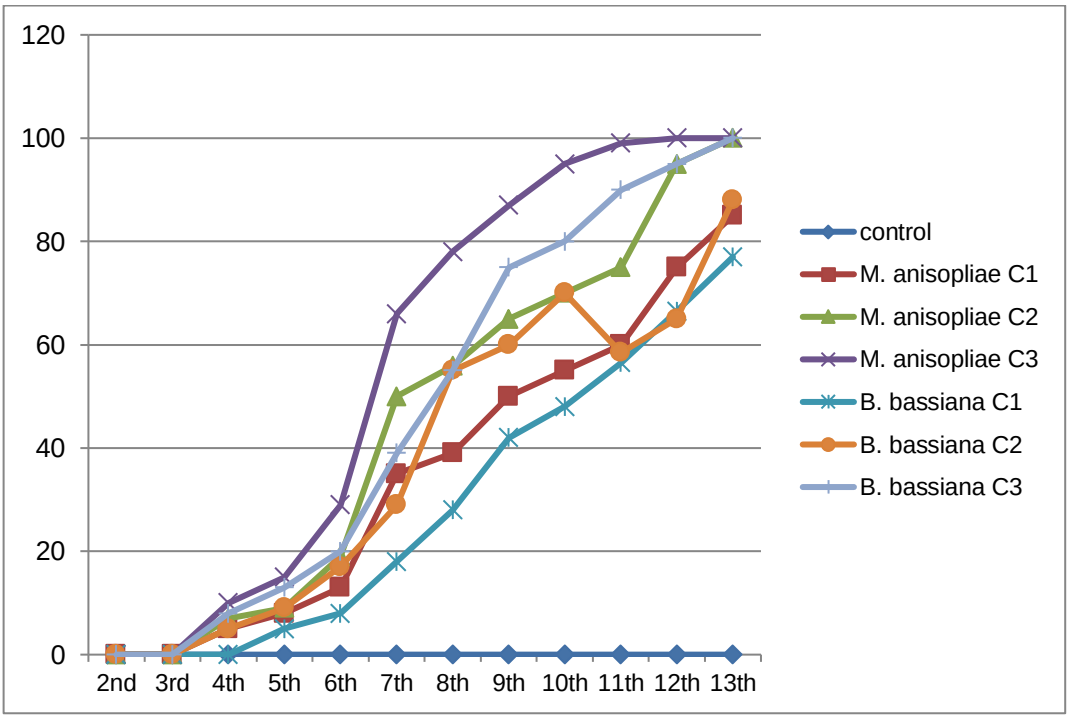

Fig. 2. Percent Mortality of pupae of *R. ferrugineus* infected with *M. anisopliae* and *B. bassiana* at 25 ±2 ºC and 70 ±5 % RH.

The mortality recorded 4.0 % by infection the 3rd concentration from *M. anisopliae* after 3rd day but was 0.0 % with the same concentration and day from *B. bassiana*. *M. anisopliae* was more effect on the adults of *R. ferrugineus* than *B. bassiana*. The mortality reached to 100 % after 10th day from the infection by *M. anisopliae* but reached to 100% after 13th days from the infection by *B. bassiana* Fig. 3.

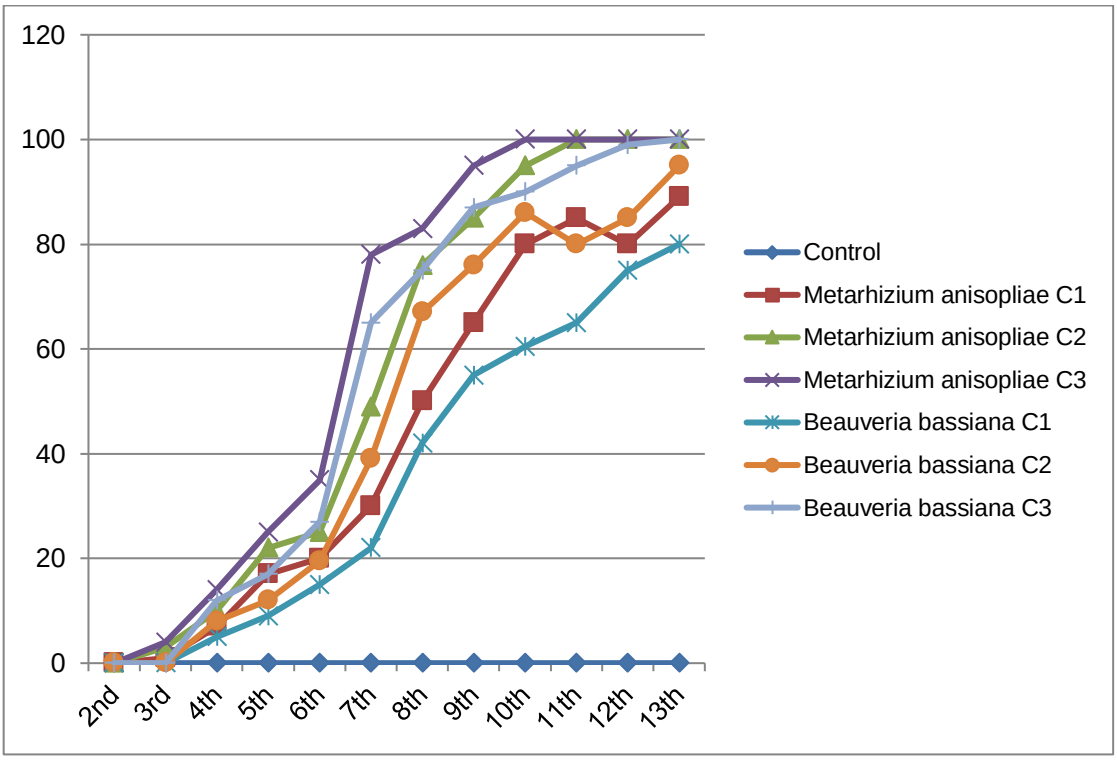

Fig. 3. Percent Mortality of adults of *R. ferrugineus* infected with *M. anisopliae* and *B. bassiana* at 25 ±2 ºC and 70±5 % RH.

Larva infected with *B. bassiana* Larva infected with *M. anisopliae*

Fig. 4. Larvae of *R. ferrugineus* infected with *B. bassiana* and *M. anisopliae.*

Adult infected with *B. bassiana* Adult infected with *M. anisopliae*

Fig. 5. Adults of *R. ferrugineus* infected with *B. bassiana* and *M. anisopliae.*

DISCUSSION

The data uncovered the potential of *M. anisopliae* and *B. bassiana* as effective agents in pest management to mitigate environmental pollution, particularly when pests were below their economic threshold. *M. anisopliae* showed greater promise than *B. bassiana* in controlling *R. ferrugineus* at various stages of its lifecycle, including larvae, pupae, and adults (Glare *et al.,* 2002; Sewify, 2007; Sewify and Fouad 2006). The study demonstrated the significant potential of various EPF isolates in combating specific life stages of *R. ferrugineus*. Field-based investigations are necessary to evaluate the effectiveness of fungal isolates in varying environmental conditions. It is imperative to examine the effects of temperature, humidity, and persistence post-application of these isolates to determine the optimal conditions for infecting the targeted insect pests. Utilizing these potent fungal isolates could aid in the development of a sustainable integrated management strategy for controlling the red palm weevil in date palm production systems. Exotic fungal isolates that have been previously used to combat various insect pests in different regions have often proven inadequate due to differences in hosts, isolates, and climatic factors. Therefore, in this study, native EPF isolates at different developmental stages of *R. ferrugineus* were employed, taking into account this limitation. It is crucial to identify highly virulent isolates through laboratory screening bioassays before implementing them in field conditions (Bidochka *et al.,* 1998; Qayyum *et al.,* 2021; Sun, 2016). The results of our screening test demonstrated that all examined isolates exhibited pathogenicity towards *R. ferrugineus*, corroborating the findings of previous researchers who investigated various isolates of *B. bassiana* and *M. anisopliae*. While *R. ferrugineus* proved to be susceptible to all isolates, variations in virulence were observed among them. This variability in virulence is a commonly observed occurrence, with genetic diversity among isolates from distinct geographical locations being cited as a probable cause. Two of the isolates utilized in the virulence analysis belonged to the species *B. bassiana* and *M. anisopliae*. Previous studies have suggested that pathogens obtained from *R. ferrugineus* display greater virulence towards this invasive pest in comparison to isolates not normally found in the host (Yang *et al*., 2023, Serna-Domínguez *et al.,* 2019; Qayyum *et al.,* 2021; Ullah *et al.,* 2022; Gindin *et al*., 2006; Güerri-Agulló *et al.,* 2011; Francardi *et al.,* 2013; Abdel-Raheem, 2011; Abdel-Raheem *et al.,* 2011).

CONCLUSIONS

These data revealed that *M. anisopliae* and *B. bassiana* can be used as a promising agent in pest control to reduce the Environmental pollution especially when the pests were under the economic threshold. *M. anisopliae* was more promising than *B. bassiana* on (larvae, Pupae, and Adults) *R. ferrugineus*.

FUTURE SCOPE OF STUDY

This study will allow us in the future to prepare bioactive compounds from entomopathogenic fungi to combat the various stages of the red palm weevil and eliminate it without using pesticides.

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