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Using entomopathogenic fungi as bio agents control on the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae)

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Abstract

The red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) is the most pests of various palm species. Effects of the entomopathogenic fungi, *Metarhizium anisopliae* Var. *acidum* (Metchinkoff) Soroken and *Beauveria bassiana* (Bals.) Vuill on *Rhynchophorus ferrugineus* was studied in Laboratory. Two entomopathogenic fungi were tested at three concentrations 2.2×10^3 , 2.2×10^4 and 2.2×10^5 spore /ml. (C₁, C₂ & C₃). Mortalities were observed daily.

The susceptibility of RPW to two entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, strains of the former were found to be more virulent than those of the latter, achieving 100% larval mortality after 9 days by using the high concentration, 2.2×10^5 spores /ml. of *Metarhizium anisopliae* but after 10 days by using of the high concentration, 2.2×10^5 spores /ml. of *Beauveria bassiana*. The most virulent strains of *M. anisopliae* were then tested on RPW larvae then adults and Pupae.

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

Introduction

The red palm weevil (RPW), *Rhynchophorus ferrugineus* Olivier is the most pests of various palm species. The RPW causes severe damage to coconuts in Southeast Asia^[10].

Recently, pathogens of *Rhynchophorus ferrugineus* have been extensively studied to evaluate their potential as biological control agents^[25, 26, 31]. *Metarhizium anisopliae* was isolated from *R. bilineatus*, after treatment of young palms against the scarabaeid *Scapanes australis* with a formulation based on *M. anisopliae* spores^[12]. In Iran, *M. anisopliae* was isolated from adults and *Beauveria bassiana* (Bals.) Vuill. from pupae^[9]. *Beauveria* sp. was associated some cocoons of *R. ferrugineus*^[31]. Using entomopathogenic fungi as biocontrol agents against many weevil species has been evaluated^[9, 15, 17]. Adults and larvae were contaminated with *B. bassiana* and *M. anisopliae* which resulted in 50–100% mortality^[14]. Used some entomopathogenic fungi to control many pests such as *Rhynchophorus ferrugineus*, *Scrobipalpa ocellatella*, *Cassida vittata*, *Pegomyia mixta*, *Bemisia tabaci*, *Myzus persicae*, *Aphis gossypii*, *Brevicoryne brassica*, *Nezara viridula*, *Schistocerca gregaria*, *Tuta absoluta*, and *phthorimaea operculella*,^[1, 3, 4, 6, 8, 7, 23, 25, 21, 24, 16, 33]

The potential of entomopathogenic fungi as bio control agents against several weevil species has been evaluated^[7, 13, 15]. Passive mechanical transmission of fungi within insect populations has been observed for various entomopathogenic fungi, e.g. *B. bassiana*, *M. anisopliae* and *Paecilomyces fumosoroseus*^[10, 18, 20]. Adults were contaminated with *B. bassiana* or *M. anisopliae* spores transferred the fungal infection to larvae, which resulted in 50.100% larval mortality^[13, 22, 32].

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

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*^[1] were grown on Peptone media (10g Peptone, 40g Dextrose, 2g yeast extract, 15g Agar and 500 ml. Chloramphenicol). The media was autoclaved at 120 °C for 20 minutes, and poured in

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Petri- dishes (10 cm diameter x 1.5 cm). Then incubated the fungi and kept at 24 ± 2 °C and $65 \pm 5\%$ 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 x 0.0025 mm²). The concentrations were used 2.2×10^3 , 2.2×10^4 and 2.2×10^5 spore /ml. (C₁, C₂ & C₃).

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 two Entomopathogenic Fungi, *Metarhizium anisopliae* Var. acridum (Metchinkoff) Soroken and *Beauveria bassiana* (Bals.) Vuill. were tested at three concentrations (C₁, C₂ & C₃) 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, Twenty-five /concentration. The fungi were applied in a suspension containing (C₁, C₂ & C₃) in the control treatment treated with sterilized water and kept at 21 ± 2 °C and $65 \pm 5\%$ RH. The mortality of all stages of *Rhynchophorus ferrugineus* was observed daily.

Results

The larvae were infected with *M. anisopliae* and *B. bassiana* concentrations (C₁, C₂ & C₃). The mortality recorded 7.5% by infection the 3rd concentration from *M. anisopliae* after 3rd day but was 2.5% with the same conc. from *B. bassiana* and day. *M. anisopliae* was more 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* Table (1).

Table 1: The% Mortality of the larvae of *Rhynchophorus ferrugineus* infected with *M. anisopliae* and *B. bassiana* at 21 ± 2 °C and $65 \pm 5\%$ RH.

day after infection	control	<i>M. anisopliae</i>			<i>B. bassiana</i>		
		C ₁	C ₂	C ₃	C ₁	C ₂	C ₃
2 nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3 rd	0.0	3.5	4.5	7.5	0.0	0.0	2.5
4 th	0.0	4.5	10.5	22.0	3.5	6.5	13.5
5 th	0.0	7.5	15.5	45.5	5.5	14.5	46.5
6 th	0.0	10.5	22.5	69.5	8.5	21.5	62.5
7 th	0.0	12.5	55.5	78.5	9.99	47.0	63.5
8 th	0.0	25.5	70.3	95.5	17.5	67.5	78.5
9 th	0.0	55.5	75.8	100	26.6	77.5	85.5
10 th	0.0	60.3	90.9	100	35.7	75.5	100

2.2×10^3 , 2.2×10^4 and 2.2×10^5 spore /ml. (C₁, C₂ & C₃).

The pupae were infected with *M. anisopliae* and *B. bassiana* concentrations (C₁, C₂ & C₃). The mortality recorded 7.5% by infection the 3rd concentration from *M. anisopliae* after 4th day but was 4% with the same conc. 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* Table (2).

Table 2: The% Mortality of pupae of *R. ferrugineus* infected with *M. anisopliae* and *B. bassiana* at 21 ± 2 °C and $65 \pm 5\%$ RH.

Day after infection	control	<i>M. anisopliae</i>			<i>B. bassiana</i>		
		C ₁	C ₂	C ₃	C ₁	C ₂	C ₃
2 nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3 rd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4 th	0.0	2.5	4.5	7.5	0.0	3.5	4.0
5 th	0.0	3.0	5.9	15.5	2.0	8.0	10.5
6 th	0.0	10.0	14.0	20.5	5.5	11.5	13.9
7 th	0.0	30.5	45.0	50.5	11.5	25.5	31.5
8 th	0.0	32.7	55.9	73.8	22.0	47.5	55.0
9 th	0.0	45.5	65.5	87.6	22.0	50.0	65.5
10 th	0.0	48.5	65.5	93.5	28.5	50.0	80.5
11 th	0.0	50.0	70.6	93.5	33.5	58.5	90.5
12 th	0.0	50.0	85.7	100	36.5	65.9	95.0
13 th	0.0	55.6	90.0	100	47.0	68.0	100

2.2×10^3 , 2.2×10^4 and 2.2×10^5 spore /ml. (C₁, C₂ & C₃).

The adults were infected with *M. anisopliae* and *B. bassiana* concentrations (C₁, C₂ & C₃). The mortality recorded 10.5% by infection the 3rd concentration from *M. anisopliae* after 4th day but was 11% with the same conc. 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 11th day from the infection by *M. anisopliae* but reached to 100% after 13th days from the infection by *B. bassiana* Table (3).

Table 3: The% Mortality of adults of *R. ferrugineus* infected with *M. anisopliae* and *B. bassiana* at 21 ±2 °C and 65 ±5% RH.

day after infection	control	<i>Metarhizium anisopliae</i>			<i>Beauveria bassiana</i>		
		C ₁	C ₂	C ₃	C ₁	C ₂	C ₃
2 nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3 rd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4 th	0.0	5.5	8.5	10.5	4.5	7.5	11.0
5 th	0.0	7.0	12.5	15.5	5.5	8.0	14.0
6 th	0.0	10.5	15.0	25.5	11.0	14.5	20.5
7 th	0.0	20.9	35.5	53.5	11.5	30.5	45.5
8 th	0.0	45.5	56.0	73.0	22.0	47.5	65.5
9 th	0.0	65.0	65.5	90.5	50.0	66.0	85.5
10 th	0.0	70.0	75.0	95.5	60.5	66.0	90.0
11 th	0.0	75.0	80.0	100	65.0	70.0	97.0
12 th	0.0	80.0	100	100	75.0	82.5	99.0
13 th	0.0	89.0	100	100	78.0	90.0	100

2.2 x 10³, 2.2 x 10⁴ and 2.2 x 10⁵ spore /ml. (C₁, C₂ & C₃).

Discussion

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 according [13, 11, 27].

M. anisopliae was more promising than *B. bassiana* on (larvae, Pupae, and Adults) *R. ferrugineus*. The data revealing that according with, [19, 29, 30, 2, 5, 28].

The susceptibility of RPW to two entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, strains of the former were found to be more virulent than those of the latter, achieving 100% larval mortality after 9 days by using the high concentration, 2.2 x 10⁵ spores /ml. of *Metarhizium anisopliae* but after 10 days by using of the high concentration, 2.2 x 10⁵ spores /ml. of *Beauveria bassiana*. The most virulent strains of *M. anisopliae* were then tested on RPW larvae then adults and Pupae.

This data according with Gindin, 2006 mentioned that *Metarhizium anisopliae* and *Beauveria bassiana*, strains more virulent achieving 100% larval mortality within 6.7 days. *M. anisopliae* is the most virulent strains on RPW eggs and adults. The % mortality of eggs and hatched larvae was 80.82%, compared with 34% in the controls.

Conclusion

Using of Entomopathogenic fungi, *M. anisopliae* was more promising than *B. bassiana* on (larvae, Pupae, and Adults) *R. ferrugineus*.

Also, the authors advice the farmers should be use *M. anisopliae* in the high concentration, 2.2 x 10⁵ spores /ml. against *R. ferrugineus*.

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