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Virulence of fungal spores and silver nano-particles from entomopathogenic fungi on the red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae)

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Abstract

The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is one of the most severe pests of date palms. The study aimed to evaluate the virulence of fungal spores and silver Nano-particles from entomopathogenic fungi (EPF) on *R. ferrugineus* under laboratory conditions. Concentrations of the fungal spores and the silver Nano-particles were prepared from *Metarhizium anisopliae*, *Beauveria bassiana*, and *Verticillium lecanii*. The results showed that the 3 EPF achieved 70–90% mortality rates within 7 days in egg stage. *M. anisopliae* and *B. bassiana* were the most effective ones. The isolate *V. lecanii* was tested on eggs, larvae, and adults. Spores of *M. anisopliae* increased eggs' mortality and reduced their hatchability. The egg mortality rate was 80% higher than the control. Seventy-percent adult mortality rate was achieved in 7 days, when treated with *M. anisopliae*, 60% with *B. bassiana*, and 53% with *V. lecanii*. *M. anisopliae* was more effective on *R. ferrugineus* than *B. bassiana*.

Keywords: *Rhynchophorus ferrugineus*, Entomopathogenic fungi, Virulence, Fungal spores, Silver nano-particles

Background

The red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae), is one of the severe pests of date palms (Giblin-Davis, 2001). It develops within the stipe of the date palm and subsequently destroys the vascular system causing collapse tree death of the plant. *R. ferrugineus* spreads in Europe Oceania, Africa, and Asia. In Southeast Asia, it has caused serious damage to coconuts, Giblin-Davis, (2001). In 1980s, it appeared in the Middle East, (Murphy and Briscoe, 1999). The first infestation in Jordan was reported in 1999 (Khan and Gangapersad, 2001). The adults of *R. ferrugineus* are attracted to the damaged and dying parts of palm trees, (Ferry and Gomez, 2002). Entomopathogenic fungi (EPF) have been studied as biological control agents (Shamseldean, 2004, Salama et al., 2004, Abdel-Raheem, et al., 2009 & 2016 and Abdel-Raheem, 2018). About 95 isolates of various microorganisms were isolated from *Rhynchophorus* spp.; only 3 isolates were EPF (Salama et al. 2004). *Metarhizium anisopliae* and *Beauveria bassiana* were isolated from *R. bilineatus* in Iran (Ghazavi and Avand-Faghih 2002). *Beauveria* sp. was found associated with cocoons of *R. ferrugineus*, (Shaiju-Simon et al. 2003). The EPF are infecting the host by contact and penetrating through the insect cuticle. The host can be infected by direct treatment, transmission of inoculum from treated insects, cadavers to untreated insects, or by a new generation of spores. Larvae and adults were contaminated by *B. bassiana* and *M. anisopliae*, reaching 50–100% mortality.

Fungi, bacteria, algae, and plant extracts are known to synthesize silver nano-particles (Ag NPs) (Nisha et al. 2017). Fungi such as *Verticillium* species are known to produce Ag NPs (Zonorodiam et al., 2016).

The aim of this study was to evaluate bio efficacy of nano-particles of EPF, *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power, and Bio Catch as fungal spores and

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silver Nano-particles on different life stages of *R. ferrugineus* (eggs, larvae, and adults) under laboratory conditions.

Materials and methods

Entomopathogenic fungi (Egyptian isolates)

Metarhizium anisopliae isolated from larvae and adults of the beet moth, *Scrobipalpa ocellatella* (Boyd) and *Beauveria bassiana* (Balsamo) Vuillemin, and isolated from the beet beetle, *Cassida vittata* (Vill) (Abdel-Raheem 2005), were grown on peptone media (10 g peptone, 40 g dextrose, 2 g yeast extract, 15 g Agar, and 500 ml chloramphenicol). The media was autoclaved at 120 °C for 20 min and poured into Petri-dishes (10-cm diameter × 1.5 cm). Then, the incubated fungi were kept at 24 ± 2 °C and 65 ± 5% RH. The fungal isolates were re-cultured every 14–30 days and kept at 4 °C.

Commercial Indian compounds

Three compounds (Indian productions), Bio Magic (*M. anisopliae*), Bio Power (*B. bassiana*), and Bio Catch (*V. lecanii*), were obtained from the company of Seif Gaarah, Cairo, Egypt. The concentration of EPF used was (1×10^9 spores/ml). Spores were harvested by rising with sterilized water and by adding 0.5% Tween 80 from culture peptone media 14 days old. The suspensions were filtered through cheese cloth to reduce mycelium clumping. The spores were counted in the suspension, using a hemocytometer (0.1 mm × 0.0025 mm^2). The concentrations were (1×10^9 spores/ml) from each EPF. The grown fungal cultures were centrifuged at 12,000 rpm fungal for 30 min at 25 °C and the supernatant was used for the synthesis of Ag NPs.

Insect rearing

R. ferrugineus was reared in the Pests & Plant Protection Department, National Research Centre, Giza, Egypt, on sugarcane as food and site (Rahalkar et al. 1985). Five pairs of adults were placed on a substrate of sugarcane sawdust or on sugarcane logs to mate and oviposit. *R. ferrugineus* was reared individually from the first larval instar to emergence of adults, at 27 ± 2 °C. The eggs were collected every other day.

Bioassay

M. anisopliae, *B. bassiana*, Bio Magic, *M. anisopliae*, Bio Power, *B. bassiana*, and Bio Catch, *V. lecanii* were tested by infecting the eggs, larval, and adults of *R. ferrugineus*. 100 Eggs, larvae and adults were used for each treatment, divided into 4 groups each of 25 eggs, larvae and adults placed in Petri-dishes, one individual/dish. The fungi were applied in a suspension in the control group, treated with sterilized water, and kept at 27 ± 2 °C and 65 ± 5% R.H. The mortality rates of *R. ferrugineus* were observed after 7 days.

Biosynthesis of silver nano-particles

Silver Nano-particles were synthesized by using 50 ml. aqueous solution of 1 mM Ag No_3-treated with 50 ml of fungi culture (these particles prepared for all fungal isolates and commercial products) supernatant in a 250-ml conical flask and the PH was adjusted to 8.5. The whole mixture was incubated at 40 °C at 200 rpm for 7 days under a dark condition. The control was maintained without adding the culture supernatant to the solution of Ag No_3.

Bioassay studies

R. ferrugineus was placed in sterile Petri dishes having food and sterile filter paper. The nano-particle solution was sprinkled over the filter paper. The filter paper was allowed to air dry aseptically and incubated at 27 ± 2 °C for 3 days. The experiment was replicated thrice. Mortality rate was recorded after 2 days from the treatment and % mortality was calculated.

Data analysis

Mortality data were recorded and percents of mortality in eggs, larvae, and adults were calculated. Corrected percent mortality was by use of Abbot's formula. Student's *t* test or one-way ANOVA was used to compare the effects of the experimental and control treatments. Statistical analyses were performed by the Stat View for Power PC software, version 4.5 (Abacus Concepts, Inc., Berkeley, CA, USA).

Results and discussion

Data of the treated eggs of *R. ferrugineus* with *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power, and Bio Catch as fungal spores and their Silver NPs particles was presented in Table 1. Seven days post treatment, up to 90% mortality rate of *R. ferrugineus* was recorded in the treated eggs. The % mortality rates attained 80, 73, 65, 60, and 45% by infection with fungal spores from *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power, and Bio Catch, respectively. Furthermore, the % mortality rates were 90, 84, 73, 70, and 58% by infection with biosynthesized Ag NPs from *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power, and Bio Catch, respectively during the same time. *M. anisopliae* recorded the highest mortality (90%) in the eggs of *R. ferrugineus*, when treated with nano-particles or with fungal spores after 6 days and was the lowest (58%) when treated with Bio Catch. According to Abdel-Raheem (2019a, 2019b) and Abdel-Rahman and Abdel-Raheem et al. (2018) the total mortality of eggs and larvae were reduced than

Table 1 Mortality percent of entomopathogenic fungi on the eggs of *Rhynchophorus ferrugineus*, using spore suspension and biosynthesized silver nano-particles

Entomopathogenic fungus	Treated with	
	Fungal spores (mean ± S.E)	Biosynthesized Ag NPs (mean ± S.E)
Metarhizium anisopliae	80.0 ± 1.20	90.0 ± 2.10
Beauveria bassiana	73.0 ± 1.00	84.0 ± 1.30
Bio Magic	65.0 ± 0.20	73.0 ± 0.10
Bio Power	60.0 ± 3.10	70.0 ± 0.20
Bio Catch	45.0 ± 0.20	58.0 ± 1.20
Control	6.0	7.0
S.E (m)	1.14	1.98

the control group when eggs were exposed to *M. anisopliae* spores.

Abdel-Raheem (2019a, 2019b) mentioned that the reason of different pathogenicity rates between one fungus and another may due to the fraction exhibiting antimicrobial activity of some polar compounds ranging between 1000 and 1500 Da in the extraction of fungi.

Data of larval mortality of *R. ferrugineus*, treated with *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power, and Bio Catch as fungal Spores and Silver Nano-particles was presented in Table 2. Seven days post treatment, 90% mortality of *R. ferrugineus* larvae was recorded as 84, 75, 71, 65, and 55% by infection with fungal spores from *M. anisopliae*, *B. bassiana*, Bio Magic, *Bio* Power, and Bio Catch, respectively. By infection with biosynthesized Ag NPs from *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch, the mortality rates recorded were 95, 87, 77, 73, and 60%, respectively. *M. anisopliae* was the highest % mortality (95%) in the larvae of *R. ferrugineus*, when treated with the nano-particles or with the fungal spores after 6 days, while the lowest (60%) was when treated with Bio Catch, *V. lecanii*. The results agree with Tefera and Pringle (2003) who reported that the bio efficacy of *M. anisopliae* in all stages of *R. ferrugineus* caused up to 48 to 95% mortality of adult and larvae.

Data of adult mortality of *R. ferrugineus*, treated with *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power, and Bio Catch as fungal spores and silver nano-particles was presented in Table 3. Seven days post treatment, the percentage of mortality of *R. ferrugineus* adults reached 77%. The % mortality rates recorded 65, 61, 56, 52, and 35% by infection with fungal spores from *M. anisopliae*, *B. bassiana*, Bio Magic, *Bio* Power, and Bio Catch, respectively, while by infection with biosynthesized Ag NPs from *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power, and Bio Catch, the % mortality recorded were 77, 70, 63, 55, and 48%, respectively. *M. anisopliae* was the highest % mortality (77%) in the adults of *R. ferrugineus*, when treated with the Nano-particles or with fungal spores after 6 days, while the lowest was (48%), when they were treated by Bio Catch. Gothandapani et al. (2015) stated that the EPF are eco-friendly and have the bio control potential against insect pests. Biology synthesis of silver nano-particles (Ag NPs) had given a new scope for a non-toxic environment (Subha et al. 2017 and Deeba et al. 2017). El Husseini (2019) treated the adults and larvae of *R. ferrugineus* with conidiospores of EPF *B. bassiana* and reported that the mortality reached to 100%.

Table 2 Mortality percent of entomopathogenic fungi on the larvae of *Rhynchophorus ferrugineus* using spore suspension and biosynthesized silver nano-particles

Entomopathogenic fungus	Treated with	
	Fungal spores (mean ± S.E)	Biosynthesized Ag NPs (mean ± S.E)
Metarhizium anisopliae	84.0 ± 2.10	95.0 ± 2.30
Beauveria bassiana	75.0 ± 2.12	87.0 ± 2.22
Bio Magic	71.0 ± 2.20	77.0 ± 1.10
Bio Power	65.0 ± 1.10	73.0 ± 1.20
Bio Catch	55.0 ± 0.10	60.0 ± 1.00
Control	6.2	7.3
S.E (m)	2.10	3.00

Table 3 Mortality percent of entomopathogenic fungi on the adults of *Rhynchophorus ferrugineus* using spore suspension and biosynthesized silver nano-particles

Entomopathogenic fungus	Treated with	
	Fungal spores (mean ± S.E)	Biosynthesized Ag NPs (mean ± S.E)
Metarhizium anisopliae	65.0 ± 2.00	77.0 ± 1.30
Beauveria bassiana	61.0 ± 1.20	70.0 ± 2.10
Bio Magic	56.0 ± 2.20	63.0 ± 0.10
Bio Power	52.0 ± 1.20	55.0 ± 3.20
Bio Catch	35.0 ± 0.00	48.0 ± 0.20
Control	7.0	8.0
S.E (m)	1.94	2.90

Conclusion

The results proved that use of the Ag NPs synthesized through application of spore suspension and biosynthesized silver nano-particles showed an efficacy against *R. ferrugineus* at different stages. *M. anisopliae* had the highest potential and was more effective than all the others.

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Authors' contributions
The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. All authors read and approved the final manuscript.

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The authors declare that they have no competing interest.

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