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Original paper

Virulence of Nano – Particle preparation of Entomopathogenic fungi and Entomopathogenic Bacteria against red palm weevil *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae)

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Abstract

Background: *Rhynchophorus ferrugineus* is one of the most severe pests on palm species, including date palms In Asia. **Purpose:** The purpose of the current study is to evaluate the virulence of bio efficacy of nano-particle of entomopathogenic fungi and entomopathogenic bacteria on red palm weevil, *Rhynchophorus ferrugineus.* **Methods:** Prepare concentrations of Fungal Spores and Silver Nano Particles were prepared from *Metarhizium anisopliae*, *Beauveria bassiana*, *Verticillium lecanii* and *B. thuringiensis*, *B. subtilis.* **Results:** *M. anisopliae* was the highest % mortality (90%), (95%) and (77%) against eggs, larvae and adults of *R. ferrugineus*, respectively when treated with bio synthesized or with fungal spores after seven days from treatment and the lowest % mortality was recorded when treated with *V. lecanii*.

Three concentrations, (10^3, 10^4, and 10^5 CFU/mL), formulated as bacterial suspensions and bio-synthesized silver nanoparticles, (Ag NPs) were evaluated for their ability to inhibit egg hatch. The 10^5 CFU/mL concentration, both as a bacterial suspension and formulated as Ag NPs, was tested for efficacy against 10 day-old larvae and adults. Egg hatch was significantly inhibited by all concentrations and formulations of *B. subtilis* and *B. thuringiensis*, and exhibited lethal concentrations for 50% mortality (LC_{50}) of 3.45 X 10^3 and 6.73 X 10^4 CFU/mL, respectively. The percent mortality of larvae six days after treatment was 85% and 77% for *B. thuringiensis* and *B. subtilis* Ag NPs, respectively. The percent mortality of adults six days after treatment was 75% and 67% for *B. thuringiensis* and *B. subtilis* Ag NPs, respectively.

Conclusion: The bio control efficiency of Ag NPs synthesized by five isolates of *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch on *R. ferrugineus* was proven to be effective through bio assay by spore suspension and bio synthesized silver nanoparticles. *M. anisopliae* had the highest efficiency on *R. ferrugineus* and was more effective than *B. bassiana*, Bio Magic, Bio Power and Bio Catch.

B. thuringiensis inhibited the hatching of *R. ferrugineus*, it is the highly effect than *B. subtilis* against all stages (eggs, Larvae and Adults) of *R. ferrugineus*. *B. thuringiensis* is the highly effect as a bio synthesized Ag NPs against larvae and adults of *R. ferrugineus* than bacterial suspensions. *B. thuringiensis* is the highest effect than *B. subtilis* as well bacterial suspensions or as a bio synthesized Ag NPs.

Keywords Virulence, Nano-particle, entomopathogenic fungi, entomopathogenic bacteria, *Rhynchophorus ferrugineus*.

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Introduction

Rhynchophorus ferrugineus is one of the most severe pests of palm species attacking date palms (Giblin-Davis, 2001). It develops within the trunk of date palm and subsequently destroys vascular system causing collapse then death of the tree. *R. ferrugineus* spreads in Europe Oceania, Africa and Asia. In Southeast Asia, *R. ferrugineus* has caused serious damage to coconuts, Giblin-Davis, (2001). In 1980s, it appeared in the Middle East. The first infestation in Jordan was 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). Eggs are laid on the surface of the palm tree. The life stages are found in the same palm tree, Ferry and Gomez (2002), and Soroker, et al (2005). Natural parasites and pathogens of *R. ferrugineus* have been studied as biological control agents, Salama, et al (2004), Mohamed Abdel-Raheem (2019) and Shamseldean (2004). About 95 isolates of various microorganisms isolated from *Rhynchophorus* spp found in dead *R. ferrugineus*, only three isolates were entomopathogenic fungi, Salama, et al (2004) and Zaki and Abdel-Raheem (2010). *M. anisopliae* and *B. bassiana* were isolated from *R. bilineatus* in New Guinea and in Iran, Ghazavi and Avand-Faghih, (2002). *Beauveria* sp. was found associated with cocoons of *R. ferrugineus*, Shaiju-Simon, et al (2003). The entomopathogenic fungi are infecting the host by contact, penetrating through the insect cuticle. The host can be infected by direct treatment, by transmission of inoculum from treated insects, cadavers to untreated insects, or by the new generation of spores, Quesada-Moraga, et al (2004), Batta (2004), Ihara, et al (2003). The populations have been observed for various entomopathogenic fungi, *e.g. B. bassiana, M. anisopliae* and *P. fumosoroseus*), Quesada-Moraga, et al (2004), and Furlong and Pell (2001). Larvae and adults were contaminated with *B. bassiana* and *M. anisopliae* which % mortality reached to 50-100, Glare, et al (2002).

Fungi, Bacteria, algae and plant extract are known to synthesize silver nanoparticles (Ag NPs), Borase (2014), Nisha, et al (2017), and Nadaf and Kanase (2016). Fungi such as *Verticillium* species are known to produce Ag NPs, Borase (2014), Praneehdevi, et al (2013) and Zonorodiam, (2016). The *Bacillus* genus contains key anatagonistic agents to many phytophagous insects (Salama et al 2004), many species of *Bacillus* have been used in biological control programs. Species of *Bacillus* synthesize proteins with insecticidal activity (Nicola et al 2015). Naji Mordi et al (2016) isolated and characterized *Bacillus* species from dead red palm weevil adults. NPs using microbes have advantages like being clean, non-toxic, eco-friendly, and it is also possible at ambient temperature and pressure. Recently, research efforts point out the potential of the green synthesis of metal NPs, chiefly Ag NPs, for use against a wide spectrum of noxious pest species either in the laboratory or in the field. For example, Jayaseelan et al (2011) reported that Ag NPs synthesized by leaf aqueous extract of *Tinospora cordifolia* (Thunb.) caused complete mortality of the head louse, *P. humanus capitis* De Geer adults after 1 h of exposure at 25 mg/l. Ag NPs was negatively influenced the growth (i.e., larval weight and period of development, pupal weight, and adult weight) of both species as a result of the physiological changes in the body of the insects due to the presence of NPs (Yasur and Usha Rani 2015). Ag NPs synthesized by extracellular filtrate of the entomopathogenic fungus *Trichoderma harzianum* Rifai (Hypocreales: Hypocreaceae) resulted in 92, 96, and 100% mortality of 1^{st}, 2^{nd}, and 3^{rd}–4^{th} instar larvae or pupae of *A. aegypti*, respectively, at 0.25% concentration after 24 h of exposure (Sundaravadivelan and Padmanabhan 2014).

The aim of this study was to evaluate the virulence of bio efficacy of Nano-particle of entomopathogenic fungi as Fungal Spores and Silver Nano Particles and Nano-particle preparation of entomopathogenic bacteria on *R. ferrugineus* (eggs, larvae and adults).

Materials and Methods

Insect host

Palm trees, *Phoenix dactylifera* were examined. Many stages of *R. ferrugineus* such as larvae, cocoons and adults were collected from damaged trees from Al-Ahsa, Saudi Arabia. Samples were collected and transferred to the laboratory of Biological Science Department, Faculty of Science, University, Jeddah, Saudi Arabia and examined carefully.

Insect rearing

R. ferrugineus colony was reared in the laboratory of Biological Science Department, Faculty of Science, University, Jeddah, Saudi Arabia on sugarcane as both food and oviposition substrate, Adults was put to mate and oviposit in groups of five pairs put on a substrate of moist sugar cane sawdust. From the 1^{st} larval stage to adult emergence, *R. ferrugineus* were reared individually at 27±2°C. For egg harvesting, the adults of both sexes were kept on sugarcane sawdust. The eggs were collected every 2 days. Newly hatched adults, males and females, were placed in rearing boxes with sugarcane to eat. The first eggs are laid after two to three days, and the high number of eggs per female has been counted after sixteen days. After three weeks, the ovipositing females were placed in new boxes daily with fresh sugarcane and one day old eggs were collected for treatments. We collected the larvae after ten days old and reared individually and fed on amounts of sugar cane.

Entomopathogenic Fungi

Egyptian Isolates

M. anisopliae isolated from larvae and adults of *S. ocellatella* and *Beauveria bassiana* (Balsamo) Vuillemin, isolated from *Cassida vittata* (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 minutes, and poured into Petri- dishes (10 cm diameter x 1.5 cm). Then the incubated the 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

The concentration of entomopathogenic fungi, Bio Magic (*M. anisopliae*) Bio Power (*B. bassiana*) and Bio Catch (*V. lecanii*) was $1x10^9$ spores / ml.

Concentrations Preparing

Spores harvested by rising with sterilized water and added 0.5% Tween 80 from culture Peptone media 14 day old. 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 mm^2). The concentrations used 1×10^9 spores/ml from all entomopathogenic fungi. The grown fungal cultures were centrifuged at 12000 rpm fungal for 30 min at 25°C and the supernatant was used for the synthesis of Ag NPs.

Silver nanoparticles bio synthesis

Silver nanoparticles were synthesized by using 50 ml aqueous solution of 1 mM Ag No_3 treated with 50 ml of fungi culture 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 dark condition. The control was maintained without adding the culture supernatant to the solution of Ag No_3.

Bioassay procedure

M. anisopliae, *B. bassiana*, Bio Magic, Bio Power, and Bio Catch were tested at concentrations (1×10^9 spores/ml to contaminate the eggs, larval and adults of *R. ferrugineus* 100 Eggs, larvae and adults were used for each treatment, divided into four groups each of twenty five 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 of *R. ferrugineus* was observed after seven days.

Bio assay studies

R. ferrugineus was placed in sterile Petri dishes having food and sterile filter paper. The nanoparticle solution was sprinkled over the filter paper. The filter paper was allowed to air dry aseptically and incubated at 27 ± 2°C at for three days. The experiment was replicated thrice. Mortality was taken five days after the treatment and % mortality was calculated.

B. thuringiensis and *B. subtilis* Concentrations Preparing

To prepare *B. subtilis* and *B. thuringiensis* for the bioassays, colonies of each species were grown overnight in 150 mL of nutrient broth at 32ºC with constant agitation (120 rpm). The harvested cells were rinsed twice with Ringer's solution and centrifuged for 5 minutes at 1,400 rpm between rinses, Bernhard Winkler et al 2016. The cell suspensions were diluted to obtain 10^5, 10^4, and 10^3 CFU/mL. The concentration of each *Bacillus* species used in the biosynthesis of Ag NPs was 10^5 CFU/ml. Mature cultures of each *Bacillus* species was centrifuged at 12,000 rpm for 30 minutes at 25°C. The supernatant was used in the synthesis of the Ag NPs.

The Ag NPs were synthesized by combining 50 mL aqueous solution of 1mM Ag NO_3 with 50 mL of *Bacillus* culture supernatant. The mixture was incubated at 40°C with agitation (200 rpm) for 7 days under dark conditions. Control particles were created without adding the culture supernatant to the Ag NO_3 solution.

The bioassay description

To assay eggs, 10 eggs were placed on a filter paper in a petri dish. The eggs were then sprayed with 2 mL of the appropriate cell suspension. The petri dishes were sealed with Parafilm® (Kaakeh 2005), and placed at 25°C and 75% R.H. The number of eggs hatching was counted daily for six days. As well as the control, in which eggs were placed in the presence of distilled water only.

To assay the larvae, 1mL of 10^5 CFU/mL suspension of each *Bacillus* species or 1 mL of Ringer's solution (control) was sprayed on a piece of apple for larvae in plastic cups individually and adult with shredded sugarcane pieces (3 × 3 cm). Larvae were placed in the container with the apple piece and held at 25°C and 75% R.H. The larvae were checked daily for 10 days, and the number that had died recorded. The nanoparticle solution was sprinkled over the filter paper, and the filter paper was allowed to air dry under aseptic conditions. The treated filter papers were placed in with sugarcane sawdust (10 gm / dish) for food with adds and change food when it need. The petri dishes containing the weevil (Eggs, Larvae and Adults) were placed at 27±2°C for 6 days. Morality was assessed 6 days after treatment and percent mortality calculated. For control treatment the individuals were immersed in distilled water.

Results and Discussion

The efficacy of nanoparticle preparations of *Metarhizium anisopliae*, *Beauveria bassiana*, and *Verticillium lecanii* against eggs, larvae, and adults of the red palm weevil.

Table 1 shows that the results of the eggs of *R. ferrugineus* which were treated with *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch as fungal Spores and Silver nano particles. After six days of treatment, the petri dishes plates were observed and the result was 90% mortality of *R. ferrugineus* eggs. Additionally, white, green and metallic muscardine were found on the dead eggs. The % mortality after six days, from treated subjects, recorded 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 after six days from treated subjects recorded 90, 84, 73, 70, and 58% by infection with bio synthesized Ag NPs from *M. anisopliae, B. bassiana*, Bio Magic, Bio Power and Bio Catch, respectively. *M. anisopliae* was the highest % mortality (90%) on the eggs of *R. ferrugineus* when treated with bio synthesized or with fungal spores after six days from treatment and the lowest % mortality was (58%) when treated with Bio Catch. The results according with Abdel-Rahman and

Abdel-Raheem, (2018), Abdel-Raheem, (2019), Mohamed Abdel-Raheem, et al (2016, 2018 & 2019), Abdel-Raheem, (2011), Abdel-Raheem, et al (2009, 2011, 2013, 2016 [a, b]), Abdel-Raheem and Lamya Ahmed Al-Keridis (2017), Mohamed Abdel-Raheem (2019), and Salem, et al (2016), mentioned when eggs were exposed to *M. anisopliae* spores, the total mortality of eggs and emerged larvae were reduced in comparison with the control group.

Table 1. Bio efficacy of entomopathogenic fungi on the eggs of *R. ferrugineus* using spore suspension and bio synthesized silver nanoparticles

Entomopathogenic fungi	Treated with	
	Fungal spores (Mean ± S.E)	Bio synthesized Ag NPs (Mean ± S.E)
M. anisopliae	80.0 ± 1.20	90.0 ± 2.10
B. 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

Table 2 shows that the 4th larvae of *R. ferrugineus* were treated with *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch as fungal Spores and Silver nano particles. After six days of treatment, the petri dishes plates showed up to 90% mortality of *R. ferrugineus* larvae. Also, white, green and metallic muscardine were found on the dead larvae. The % mortality after six days recorded 84, 75, 71, 65, and 55% by infection with fungal spores from *M. anisopliae*, *B. bassiana*, Bio Magic, *Bio* Power and Bio Catch, respectively. Furthermore, the % mortality after six days recorded 95, 87, 77, 73, and 60% by infection with bio synthesized Ag NPs from *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch, respectively. *M. anisopliae* was the highest % mortality (95%) against the larvae of *R. ferrugineus* when treated with bio synthesized or with fungal spores after six days and the lowest % mortality was (60%) when treated with Bio Catch, *V. lecanii*. The results according with Ekesi, (2001), Tefera, and Pringle, (2003) and Thomas, et al (1997), mentioned the bio efficacy of *M. anisopliae* in all stages of *R. ferrugineus* caused up to 48 to 95% mortality of adult and larvae.

Table 2. Bio efficacy of entomopathogenic fungi on the 4th larvae of *R. ferrugineus* using spore suspension and bio synthesized silver nanoparticles

Entomopathogenic fungi	Treated with	
	Fungal spores (Mean ± S.E)	Bio synthesized Ag NPs (Mean ± S.E)
M. anisopliae	84.0 ± 2.10	95.0 ± 2.30
B. 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 shows that the adults of *R. ferrugineus* were treated with *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch as fungal Spores and Silver nano particles.

After six days of treatment, the petri dishes plates were observed and the parentage of mortality of *R. ferrugineus* adults was 77%. Also, white, green and metallic muscardine were found on the dead adults. The % mortality after six days 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. Moreover, the % mortality after six days of treatment recorded 77, 70, 63, 55, and 48% by infection with bio synthesized Ag NPs from *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch, respectively. *M. anisopliae* was the highest % mortality (77%) against the adults of *R. ferrugineus* when treated with bio synthesized or with fungal spores after six days and the lowest % mortality was (48%) when it treated with Bio Catch. Gothandapani et al (2015) and Ownley, et al (2008), stated that the entomopathogenic fungi are eco-friendly and have the bio control quality against insect pests. Biology synthesis of silver nanoparticles (Ag NPs) has given a new scope for a non-toxic environment (Hu, et al (2006), Moonjung, et al (2010), Navrotsky, (2000), Prakash et al (2013), Subha, et al (2017), Deeba Kamil, (2017), and Gindin, et al (2006). Monir M. El Husseini, (2019), treated the adults and larvae of *R. ferrugineus* with conidiospores of entomopathogenic fungus *Beauveria bassiana* the mortality reached to 100% mortality.

Table 3. Bio efficacy of entomopathogenic fungi on the adults of *R. ferrugineus* using spore suspension and bio synthesized silver nanoparticles

Entomopathogenic fungi	Treated with	
	Fungal spores (Mean ± S.E)	Bio synthesized Ag NPs (Mean ± S.E)
M. anisopliae	65.0 ± 2.00	77.0 ± 1.30
B. 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

The efficacy of nanoparticle preparations of *Bacillus subtilis, B. thuringiensis* against eggs, larvae, and adults of the red palm weevil

The mean number of unhatched eggs occurred at the highest concentration of each *Bacillus* species (Table 4). The highest concentration of *B. thuringiensis* resulted in a mean of 9.5 (± 0.4) unhatched eggs, and the highest concentration of *B. subtilis*, a mean of 7.5 (± 0.2) unhatched eggs. Probit analysis revealed that the LC_{50} or the Lethal Concentration to cause 50% mortality was greatest for *B. thuringiensis* while the LC_{90} was greatest for *B. subtilis*. Using of pathogens on *R. ferrugineus* showed more capacity in decreasing *R. ferrugineus* populations, Dembilio and Jacas, 2012 and Francardi et al, 2012. Bacteria collected from larvae and used for anti-hatching activity, Salama et al, 2004 and Butera et al, 2012. *B. subtilis* was the lowest LC_{50} and mortality at the highest concentration. *B. thuringiensis* was the highest inhibit of hatching eggs, Salama et al, 2004.

Table 4. Average number and Probit analysis of un hatching eggs of *R. ferrugineus*

Isolates	Inhibition on hatching (mean ± SE) concentration (CFU/mL)							
	Control	10^3	10^4	10^5	Slope ± S.E.	χ2 (P value)	LC_{50} (CFU/mL) (95 % FL)	LC_{90} (CFU/mL) (95 % FL)
B. subtilis	2.0±0.2	4.4± 0.3	7.0 ±0.0	7.5 ±0.2	0.3670±0.1 227*	1.923 (0.496)	$6.73x10^4$ ($5.87x10^3$ - $1.58x10^6$)	$2.68x10^8$ ($4.98x10^6$ - $6.77x10^{10}$)
B. thuringiensis	1.2±0.2	5.40 ±0.7	8.0 ±0.0	9.5 ±0.4	0.2538±0.1 220*	0.124 (0.954)	$3.45x10^3$ ($1.69x10^2$ - $7.25x10^4$)	$6.59x10^8$ ($4.28x10^6$ - $7.89x10^9$)

A significant difference between treated and control larvae were by using *B. thuringiensis* in (Table 5). The highest concentration of *B. thuringiensis* resulted in a mean of 2.37 (±0.27) larvae and the highest concentration of *B. subtilis*, a mean of 2.02 (±1.00) larvae. The total died of larvae treated with the highest concentration of *B. thuringiensis* resulted in 8 while the total died of larvae treated with the highest concentration of *B. subtilis* was 7.

Table 5. Treated the neonate larvae of *R. ferrugineus* with concentration 10^5 CFU/ mL from *B. subtilis* and *B. thuringiensis*

Isolates	Mean ± SE		Total died larvae		P value*
	Control	Treated	Control	Treated	
B. subtilis	0.00	2.02±1.00	0	7	0.0127
B. thuringiensis	0.00	2.37±0.27	0	8	0.1892

A significant difference between treated and control adults were by using *B. thuringiensis* in (Table 6). The highest concentration of *B. thuringiensis* resulted in a mean of 2.25 (±0.22) and the highest concentration of *B. subtilis*, a mean of 1.01 (±2.00). The total died of a treated with the highest concentration of *B. thuringiensis* resulted in 7 while the total died of adults treated with the highest concentration of *B. subtilis* was 5.

Table 6. Treated adults of *R. ferrugineus* with concentration 10^5 CFU/ mL from *B. subtilis* and *B. thuringiensis*

Isolates	Mean ± SE		Total died adults		P value*
	Control	Treated	Control	Treated	
B. subtilis	0.00	1.01±2.00	0	5	0.0116
B. thuringiensis	0.00	2.25±0.22	0	7	0.1990

Differences in mortality for larvae treated with bacterial suspensions or with biosynthesized Ag NPs were observed for both species of *Bacillus* (Table 5). The biosynthesized Ag NPs treatments had higher mortalities than that found with the bacterial suspensions. *B. thuringiensis* caused the highest larval mortality, regardless of formulation (Table 4). Both *Bacillus* species caused greater larval mortality than the controls (Table 7).

After six days of treatment, the petri dishes plates were observed was 74% mortality of *R. ferrugineus* adults. The % mortality after six days from treated recorded 74% and 67% by infection with bacterial suspensions from *B. thuringiensis* and *B. subtilis*, respectively. Also, the % mortality after six days from treated recorded 85 and 77% by infection with bio synthesized Ag NPs from *B. thuringiensis* and *B. subtilis*, respectively. *B. thuringiensis* was the highest % mortality (85%) against the larvae of *R. ferrugineus* when treated with bio synthesized or with bacterial suspensions after six days and the lowest % mortality (77%) when it treated with *B. subtilis*.

Table 7. Efficacy of *B. thuringiensis* and *B. subtilis* against the larvae of *R. ferrugineus* using bacterial suspensions and bio synthesized silver nanoparticles

Entomopathogenic bacteria	Treated with	
	bacterial suspensions (Mean percent mortality ± S.E)	Bio synthesized Ag NPs (Mean percent mortality ± S.E)
B. thuringiensis	74.0 ± 1.41	85.0 ± 2.20
B. subtilis	67.0 ± 1.22	77.0 ± 2.33
Control	6.7	7.1
S.E(m)	2.2	2.90

The efficacy of the Ag NPs against adult red palm weevils was greater than that from the bacterial suspensions (Table 8). Again, *B. thuringiensis* imparted more mortality than *B. subtilis*, regardless of formulation. The mortality imparted by either *Bacillus* species was greater than the controls.

After six days of treatment, the petri dishes plates were observed was 61% mortality of *R. ferrugineus* adults. The % mortality after six days from treated recorded 61% and 55% by infection with bacterial suspensions from *B. thuringiensis* and *B. subtilis*, respectively. Also, the % mortality after six days from treated recorded 75 and 67% by infection with bio synthesized Ag NPs from *B. thuringiensis* and *B. subtilis*, respectively. *B. thuringiensis* was the highest % mortality (75%) against the adults of *R. ferrugineus* when treated with bio synthesized or with bacterial suspensions after six days and the lowest % mortality (67%) when it treated with *B. subtilis*

Table 8. Efficacy of *B. thuringiensis* and *B. subtilis* against the adults of *R. ferrugineus* using bacterial suspension and bio synthesized silver nanoparticles

Entomopathogenic bacterial	Treated with	
	bacterial suspensions (Mean percent mortality ± S.E)	Bio synthesized Ag NPs (Mean percent mortality ± S.E)
B. thuringiensis	61.0 ± 2.10	75.0 ± 1.40
B. subtilis	55.0 ± 1.10	67.0 ± 2.30
Control	7.2	8.2
S.E(m)	1.95	2.97

Conclusion

The bio control efficiency of Ag NPs synthesized by five isolates of *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch on *R. ferrugineus* was proven to be effective through bio assay by spore suspension and bio synthesized silver nanoparticles. *M. anisopliae* had the highest efficiency on *R. ferrugineus* and was more effective than *B. bassiana*, Bio Magic, Bio Power and Bio Catch.

B. thuringiensis inhibited the hatching of *R. ferrugineus*, it is the highly effect than *B. subtilis* against all stages (eggs, Larvae and Adults) of *R. ferrugineus*. *B. thuringiensis* is the highly effect as a bio synthesized Ag NPs against larvae and adults of *R. ferrugineus* than bacterial suspensions.

B. thuringiensis is the highest effect than *B. subtilis* as well bacterial suspensions or as a bio synthesized Ag NPs.

The author's stated that for control *R. ferrugineus* by entomopathogenic fungi or entomopathogenic bacteria you should use *M. anisopliae*, *B. bassiana* as a bio synthesized Ag NPs or *B. thuringiensis* as a bio synthesized Ag NPs because proven the highly virulence against *R. ferrugineus* in all stages.

Declarations

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Authors Contribution

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.

Competing interests

The authors declare that they have no competing interest.

References

1. ABDEL-RAHEEM, MA (2005). Possibility of Using the Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* for controlling the Sugar-Beet Insects *Cassida vittata* Vill. and *Scrobipalpa ocellatella* Boh. in Egypt, Ph.D. Faculty of Agriculture, Cairo University, Cairo., P 86.
2. ABDEL-RAHEEM, MA (2011). Impact of Entomopathogenic Fungi on Cabbage Aphids, *Brevicoryne brassica* L. in Egypt. Bull. NRC, 36(1): 53-62.
3. ABDEL-RAHEEM, MA (2013). Susceptibility of the red palm weevil, *Rhynchophorus ferrugineus* Olivier to some Entomopathogenic Fungi Bull. NRC, 2013, 38(1):69 -82.
4. ABDEL-RAHEEM, MA (2019). Pathogenicity Comparative of Some Egyptian Isolates and Commercial Indians Compounds of Entomopathogenic Fungi Against Some Insect Pests. Plant Archives, 19, supplement (1):1061-1068, 2019
5. ABDEL-RAHEEM, MA and LAMYA AHMED AL-KERIDIS A (2017). Virulence of three Entomopathogenic Fungi against White fly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) in Tomato Crop. Journal of Entomology, 14(4):155-159.
6. ABDEL-RAHEEM, MA, SABRY, KH, ZAKIA A RAGAB (2009). Effect of different fertilization rates on control of *Bemisia tabaci* (Genn.) by *Virticillium lecanii* and *Beauveria bassiana* in potato crop. J. Biol. Pest control, 19(2), 129-133.
7. ABDEL-RAHEEM, M A; I.A. ISMAIL; NA FARAG, RS ABDEL-RAHMAN and H H ELBEHERY (2016[a]). Isolates, Virulence of two Entomopathogenic Fungi as biological control agent on sugar beet fly, *Pegomyia mixta* in Egypt. Der Pharma Chemica, 8(18):132-138.
8. ABDEL-RAHEEM, MA; NAGLAA F REYAD; AL-SHURAYM, LAILA A and ABDEL-RAHMAN, IE (2016[b]). Nano Entomopathogenic Fungi As biological Control Agents on Cabbage Worm, *Pieris rapae* L. (Lepidoptera: Pieridae). Der Pharma Chemica, 8(16):93-97.
9. ABDEL-RAHMAN, IE, ABDEL-RAHEEM, MA (2018). Using entomopathogenic fungi as bio agents control on the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), Journal of Entomology and Zoology Studies; 6(6): 387-390. http://www.entomoljournal.com/archives/2018/vol6issue6/PartG/6-5-342-932.pdf
10. BATTA, YA (2004). Control of rice weevil (Sitophilus oryzae L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae.* Crop Prot., 23:103-108.
11. BERNHARD WINKLER, DAVID REINEKE, PAUL PHILIP HEINISCH, FLORIAN SCHÖNHOFF, CHRISTOPH HUBER, ALEXANDER KADNER, LARS ENGLBERGER and THIERRY CARREL (2016). Graft preservation solutions in cardiovascular surgery, *Interactive CardioVascular and Thoracic Surgery*, 23(2):300-309, https://doi.org/10.1093/icvts/ivw056
12. BORASE, HP, SAHMKE, B.K., SALUMKHE, RB, PATIL, CD, HALLSWORTH,J E , KIM, BS, PATIL, S.V. (2014). Plant extract: a promising biomatrix for ecofriendly, controlled synthesis for silver nanoparticles. Appl. Biochem Biotech, 173.1.
13. BUTERA G, FERRARO C, COLAZZA S, ALONZO G, QUATRINI P (2012). The culturable bacterial community of frass produced by larvae of *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) in the Canary island date palm. Lett Appl Microbiol 54(6):530-536.
14. DEEBA KAMIL, T PRAMEELADEVI, SANKAR GANESH, NPRABHAKARAN, R NRESHKUMAR, SHAN P THOMAS (2017). Green Synthesis of Silver nano particles by entomopathogenic fungus *Beauveria bassiana* and their bio efficacy against mustard aphid (*Lipaphis erysimi* kal.), Indian Journal of experimental Biology, 55: 555-561.
15. DEMBILIO O, JACAS JA (2012). Bio-ecology and integrated management of the red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), in the region of Valencia (Spain). Hell Plant Prot J 5: 1-12
16. EKESI, S (2001). Pathogenicity and antifeedant activity of entomopathogenic hyphomycetes to the cowpea leaf beetle, *Ootheca mutabilis* Shalberg. Insect Sci. Appl. 21:55-60.
17. FERRY, M, GOMEZ, S (2002). The red palm weevil in the Mediterranean area. *Palms* 2002, 46.

18. FINNEY DJ (1971). Probit Analysis (3rd edition). Cambridge University Press, Cambridge, UK.
19. FRANCARDI V, BENVENUTI C, ROVERSI PF, RUMINE P, BARZANTI G (2012). Entomopathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin isolated from different sources in the control of *Rhynchophorus ferrugineus* (Olivier) (Coleoptera Curculionidae). REDIA 95:49-55.
20. FURLONG, MJ, PELL, JK (2001). Horizontal transmission of entomopathogenic fungi by the diamondback moth. *Biol. Control,* 22:288-299.
21. GHAZAVI, M, AVAND-FAGHIH, A (2002). Isolation of two entomopathogenic fungi on red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Col., Curculionidae) in Iran. Appl. Entomol. Phytopathol., 9:44-45.
22. GIBLIN-DAVIS, RM (2001). Borers of palms. in: Howard, F.W., Moore, D., Giblin-Davis, R.M. and Abad, R.G. [Eds.] Insects on Palms. CABI Publishing, Walling ford, UK.: 267-305.
23. GINDIN,G, LEVSKI, S, GLAZER, I, SOROKER, V (2006). Evaluation of the Entomopathogenic Fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the Red Palm Weevil *Rhynchophorus ferrugineus*, Phytoparasitica, 34(4):370-379.
24. GLARE TR, PLACET, C, NELSON, TL, REAY, SD (2002). Potential of *Beauveria* and *Metarhizium* as control agents of pinhole borers (*Platypus* spp.): 73-79.
25. GOTHANDAPANI S, BOOPALAKRISHANAN G, PRABHAKARAN N, CHETHANA BS, ARAVINDHAN M, SARAVANAKUMAR M, GIRIJA GANESHAN (2015). Evaluation of entomopathogenic fungus against *Alternaria porri* (Ellis) Causing purple blotch disease of onion Arch phytopathol pfl, 2015, 48, 135.
26. HU C, LAN YQ, QU JH, HU XX,WANG AM, (2006). Ag / Ag Br/ Ti O_2 visible light photo catalyst for destruction of azodyes and bacteria. J. physical chem, B 110, 4066.
27. IHARA, F, TOYAMA, M, SATO, T (2003). Pathogenicity of *Metarhizium anisopliae* to the chestnut weevil larvae under laboratory and field conditions. Appl. Entomol. Zool. 38:461-465.
28. JAYASEELAN C, RAHUMAN AA, RAJAKUMAR G, VISHNU KIRTHI A, SANTHOSHKUMAR T, MARIMUTHU S, BAGAVAN A, KAMARAJ C, ZAHIR AA, ELANGO G (2011). Synthesis of pediculocidal and larvicidal silver nanoparticles by leaf extract from heartleaf moonseed plant, Tinospora cordifolia Miers. Parasitol Res 109:185-194.
29. KAAKEH W (2005). Longevity, fecundity, and fertility of the red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) on natural and artificial diets. Emirates Journal Agriculture Science 17:23-33.
30. KHAN, A, GANGAPERSAD, G (2001). Comparison of the effectiveness of threeentomopathogenic fungi in the management of the banana borer weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). Int. Pest Control, 2001, 43:208-213.
31. MOHAMED ABDEL-RAHEEM (2019). Entomopathogenic Fungus, *Beauveria bassiana*, Lambert Academic Publishing P148. https://www.morebooks.shop/gb/search?utf8=%E2%9C%93&q=978-613-9-44933-0
32. MOHAMED ABDEL-RAHEEM, LAILA ALI M. AL-SHURAYM, LAMYA AHMED AL-KERIDIS (2016). Date Palm Pests and Their Control, Lambert Academic Publishing, Pp. 76. https://www.morebooks.de/store/de/book/date-palm-pests-and-their-control/isbn/978-3-659-97440-3
33. MOHAMED ABDEL-RAHEEM, NAGLAA REYAD, IBRAHIM ABD EL-RAHMAN (2018). Entomopathogenic Fungi, Effective, Safe and Cheap Applications, Lambert Academic Publishing, p. 80. https://www.morebooks.de/store/us/book/entomopathogenic-fungi,-effective,-safe-and-cheap-applications/isbn/978-613-9-85211-6
34. MOHAMED ABDEL-RAHEEM, SADEK SALEM and AHMED ABD EL- SALAM (2019). Red Palm Weevil, *Rhynchophorus ferrugineus* and Their Control, Lambert Academic Publishing, p. 188. https://www.morebooks.de/gb/search?utf8=%E2%9C%93&q=978-620-0-23387-5
35. MONIR M. EL HUSSEINI (2019). Efficacy of the fungus *Beauveria bassiana* (Balsamo) Vuillemin on the red palm weevil *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) larvae and adults under laboratory conditions, Egyptian Journal of Biological Pest Control, 29:58, https://doi.org/10.1186/s41938-019-0155-3
36. MOONJUNG, C, KYOUNG-HWAN S, JYONGSIK J. (2010). Plasmonic photocatalytic system using silver chloride/ silver nano structures under visible light. J. colloid Interface Sci, 2010, 341, 83.
37. NADAF, NY, KANASE, SS (2016). Bio synthesis of gold nanoparticles by Bacillus molisflavi and its potential in catalytic dye degradation, Arab J. Chem, Article in Press.
38. NAJI MORDI N. AL-DOSARY, SHOKI AL-DOBAI, JOSE ROMENO FALEIRO (2016). Review on the management of red palm weevil *Rhynchophorus ferrugineus* Olivier in date palm *Phoenix dactylifera* L, Emirates Journal of Food and Agriculture. 2016. 28(1): 34-44.
39. NAVROTSKY, A (2000). Technology and applications Nano materials in the environment, agriculture, and technology (NEAT). J Nano part Res, 2, 321.
40. NICOLA FRANCESCA, ANTONIO ALFONZO, GABRIELLA LO VERDE, LUCA SETTANNI, MILKO SINACORI, PAOLO LUCIDO AND GIANCARLO MOSCHETTI (2015). Biological activity of Bacillus spp. evaluated on eggs and larvae of red palm weevil Rhynchophorus ferrugineus, Ann Microbiol (2015) 65:477-485.
41. NISHA, C, BHAWONA, P, FULEKAR, MH (2017). Antimicrobial Potential of Green synthesized silver nanoparticles using sida acuta leaf extract, Vano Sci. Nano Technol, 11. 111.

42. OWNLEY BH, GRIFFIN MR, KLINGEMAN WE, GWINN KD, MOULTON JK, PEREIRA RM (2008). *Beauveria bassiana*: Endophytic colonization and plant disease control. J Invertebr pathol, 98, 267.
43. PRAKASH D, MAHALE V, BANKAR A, NAWANI N, ZINJARDE S, KAPADNIS B (2013). Bio synthesis of colloidal gold nano particles by *Streptomyces* sp NK52 and its anti –lipid peroxidation activity. Indian J Exp Biol, 51, 969.
44. PRANEEHDEVI, T, KULANTHAIVEL, S, KAMIL, D, BORAH, J L, PRATHAKARAN, N, SRINIVASA, N (2013). Bio synthesis of silver nanoparticles from Trichoderma species, India J. Exp. Biol., 51, 543.
45. QUESADA-MORAGA, E, SANTOS-QUIROS, R, VALVERDE-GARCIA, P, SANTIAGO-ALVAREZ, C (2004). Virulence, horizontal transmission, and sub-lethal reproductive effects of *Metarhizium anisopliae* (anamorphic fungi) on the German cockroach (Blattodea: Blattellidae). J. Invertebr. Pathol. 87:51-58.
46. SALAMA HS, FODA MS, EL-BENDARY MA, ABDEL-RAZEK A (2004). Infection of red palm weevil, *Rhynchophorus ferrugineus*, by spore-forming bacilli indigenous to its natural habitat in Egypt. J Pest Sci 77(1):27-32.
47. Salem SA; Abd El-Salam, AME, El-Kholy MY (2016) Field Evaluation of Red Palm Weevil *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Curculionidae) Responses to its Fermenting Date Tree Volatiles. International Journal of ChemTech Research. 9(5):12-17.
48. SHAIJU-SIMON, KUMAR, RK, GOKULAPALAN, C (2003). Occurrence of *Beauveria* sp. on red palm weevil, *Rhynchophorus ferrugineus* (Oliv.) of coconut. *Insect Environ.* 9:66-67.
49. SHAMSELDEAN, MM (2004). Laboratory trials and field applications of Egyptian and foreign entomo-pathogenic nematodes used against the red palm weevil, *Rhynchophorus ferrugineus* Oliv. *Int. J. Nematol.* 2004, 14:44-55.
50. SOROKER, V, BLUMBERG, D, HABERMAN, A, HAMBURGER-RISHARD, M, RENEH, S, TALEBAEV, S (2005). Current status of red palm weevil infestation in date palm plantations in Israel. Phytoparasitica, 2005, 33:97-106.
51. SUBHA PK, ESTHER RM, GUNASEELI R, HUSSAIN MM (2017). Extracllular synthesis of silver nanoparticles by the fungus *Emericella nidulans* EV4 and its application. Indian J Exp Biol, 55, 262.
52. SUNDARAVADIVELAN C, PADMANABHAN MN (2014). Effect of mycosynthesized silver nanoparticles from filtrate of Trichoderma harzianum against larvae and pupa of dengue vector Aedes aegypti L. Environ Sci Pollut Res 21:4624-4633.
53. TEFERA, T, PRINGLE, K.L. (2003). Food consum-ption by *Chilo partellus* (Lepidoptera: Pyralidae) larvae infected with *Beauveria bassiana* and *Metarhizium anisopliae* and effects of feeding natural versus arti_cial diets on mortality and mycosis. *J. Invertebr. Pathol.*, 84:220-225.
54. THOMAS, MB, BLANFORD, S, LOMER, CJ (1997). Reduction of feeding by the variegated grasshopper, *Zonocerus variegatus*, following infection by the fungal pathogen *Metarhizium avoviride*. *Biocontrol Sci.*, *Technol.* 3:327-334.
55. YASUR J, USHA RANI P (2015). Lepidopteran insect susceptibility to silver nanoparticles and measurement of changes in their growth, development and physio-logy. Chemosphere 124:92-102
56. ZAKI, FN AND ABDEL-RAHEEM, MA (2010). Using of Entomopathogenic fungi and insecticide against some insect pests attacking peanuts and sugar beet. Archives of phytopathology and plant protection, 43(18):1819-1828.
57. ZONORODIAM, K, POURSHAHID, S, SADAT-SHARIFI, A (2016). Bio synthesis and characterization of silver nanoparticles by Aspergillus species, Bio Med Res Int, 1.