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, (103, 104, and 105 CFU/mL), formulated as bacterial suspensions and bio-synthesized silver nanoparticles, (Ag NPs) were evaluated for their ability to inhibit egg hatch. The 105 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 (LC50) of 3.45 X 103 and 6.73 X 103 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.
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 Shamseldin (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*, Shaju-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, *B. bassiana*, *M. anisopliae* and *P. fumosocephalus*, 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 antagonistic 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 1st, 2nd, and 3rd-4th 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 new boxes daily with fresh sugarcane and one day old eggs were collected for treatment. 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 1 x 10⁶ 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 mm²). The concentrations used 1 x 10⁵ 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 NO₃ treated with 50 ml of fungal 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 NO₃.

**Bioassay procedure**

M. anisopliae, B. bassiana, Bio Magic, Bio Power, and Bio Catch were tested at concentrations (1 x 10⁹ 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 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 5% 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 32°C 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 10⁴, 10³, and 10¹ CFU/mL. The concentration of each Bacillus species used in the biosynthesis of Ag NPs was 10³ 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 NO₃ 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 NO₃ 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 10⁶ CFU/mL suspension of each Bacillus species or 1 mL of Ringer’s solution (control) was sprayed on a piece of apple for larva in plastic cups individually and adult with shredded sugarcane pieces (3 x 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. Mortality 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,
Virulence of Nano – Particle preparation of Entomopathogenic fungi and Entomopathogenic Bacteria

(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 LC₉₀ or the Lethal Concentration to cause 50% mortality was greatest for B. thuringiensis while the LC₉₀ was greatest for B. subtilis. Using of pathogens on R. ferrugineus populations, Dembilo 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 LC₉₀ 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) | Slope ± S.E. | χ² (P value) | LC₉₀ (CFU/mL) (95 % FL) | LC₉₀ (CFU/mL) (95 % FL) |
|----------------------|------------------------------------|--------------|--------------|-------------------------|-------------------------|
|                      | concentration (CFU/mL)             |              |              |                         |                         |
| B. subtilis          | Control 10³                         | 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)         | 6.73×10⁶ (5.87×10⁷ - 1.58×10⁸) | 2.68×10⁸ (4.98×10⁹ - 6.77×10⁹) |
|                      | 10⁵                                | 5.40±0.7     | 8.0 ±0.0     | 9.5 ±0.4               | 0.2538±0.1 (220*)      | 0.124 (0.954)         | 3.45×10⁶ (1.69×10⁷ - 7.25×10⁸) | 6.59×10⁹ (4.28×10⁸ - 7.89×10⁹) |
| B. thuringiensis     |                                    |              |              |                         |                         |

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 10⁵ 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.
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.

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.

| 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 |

### 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                                                 |

### 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.
Virulence of Nano – Particle preparation of Entomopathogenic fungi and Entomopathogenic Bacteria

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. fusariumus 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. fusariumus 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.

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