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SILVER NANO-PARTICLES FROM ENTOMOPATHOGENIC FUNGI ON THE TORTOISE BEETLE, *CASSIDA VITTATA* (COLEOPTERA: CHRYSOMELIDAE)

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Abstract

The tortoise beetle, *Cassida vittata* (Coleoptera: Chrysomelidae) is one of the most severe pests of sugar beet plants. The study aimed to evaluate the virulence of fungal spores and silver Nano-particles from entomopathogenic fungi (EPF) on *Cassida vittata* 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 (47-95%) 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 (84%) higher than the control. 69% adult mortality rate was achieved in 7 days, when treated with *M. anisopliae*, (65%) with *B. bassiana* and (55%) with *V. lecanii*. *M. anisopliae* was more effective on *C. vittata* than *B. bassiana*.

Key words: *Cassida vittata*, Entomopathogenic Fungi, Silver Nano-particles.

Introduction

Sugar - beet, *Beta vulgaris* is one of the most important economic crops in Egypt. Therefore, farmers are encouraged to increase sugar beet production in order to increase the requirements for local sugar production. Sugar beet was used in the production of sugar as it contains 15-20% sugar. The sugar beet has currently been infested with many insect pests which cause high losses in the crop yield and decrease its sugar content (Bassyouny, 1993; Elnagar *et al*., 2000; Rabie *et al*., 2005; Abdel-Raheem, 2000 & 2005; Abdel-Raheem and lamyaa, 2017 and Abdel-Raheem and Samia, 2019). Among these insects, *Cassida vittata* (Vill) (Coleoptera: Chrysomelidae). Chemical insecticides were used to control these insect pests, but they were always causing a lot of pollution to the environment (Goodwin *et al*., 2007). Thereafter microbial control agents were advocated to be used against such pests. Entomopathogenic fungi (EPF) are the natural enemies of insect-pests (Lav Sharm *et al*., 2018; Salem *et al*., 2020 and Sabbour *et al*., 2020). Entomopathogenic fungi belonging to the genera *Beauveria*, *Lecanicillium*, *Metarhizium*, *Isaria* and *Hirsutella* are the best known and most frequently used in biological control of pests (Grent, 2011; Koubová, 2009). The advantage of entomopathogenic fungi is little likelihood of insect resistance development to them. Their disadvantage is that efficacy of fungi against pests is dependent on environmental conditions, particularly on temperature and humidity. *Beauveria bassiana* is soil fungus spread worldwide. *B. bassiana* can parasite on range of pest hosts and has also the ability of endophytic colonization of plants (Koubová, 2009; Domsch *et al*., 2007). Fungi of *Lecanicillium* genus are important pest pathogens. These genus was formerly classified in the genus *Verticillium* including species *V. lecanii*. These species has been dividend into a number of new taxonomic entities, including *L. lecanii*, *L. longisporum*, *L. attenuatum*, *L. nodulosum* and *L. muscarium*. Most isolates, formerly identified as *V. lecanii*, belong to *L. muscarium*, according

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to Zare and Gams, 2001 and Mohamed Abdel-Raheem, *et al*., 2020 a & b).

Entomopathogenic fungi (EPF) have been studied as biological control agents (Abdel-Raheem, *et al*., 2009, 2016, 2019, 2020 a&b and Abdel-Raheem, 2018). 2 isolates of various microorganisms were isolated from *Cassida vittata, Metarhizium anisopliae* and *Beauveria bassiana* (Abdel-Raheem, 2005; Saleh, *et al.*, 2016 and Mohamed Abdel-Raheem, 2019 a&b, 2020 a&b). 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. Eggs, 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 nanoparticles (Ag NPs) (Nisha *et al.*, 2017 and Abdel-Raheem *et al.*, 2016, 2019). 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 *a*nd Bio Catch as fungal spores and silver Nano-particles on different life stages of *Cassida vittata* (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, isolated from the beet beetle, *Cassida vittata* (Vill) (Abdel-Raheem, 2005) were grown on Peptone media (10g Peptone, 40g Dextrose, 2g yeast extract, 15g Agar and 500 ml. Chloramphenicol). The media was autoclaved at 120ºC for 20 min and poured into Petri- dishes (10 cm diameter × 1.5 cm). Then, the incubated the fungi were kept at 24 ±2ºC and 65± 5% RH. The fungal isolates were re-cultured every 14-30 days and kept at 4ºC.

Commercial Compounds

Three compounds, Bio Magic (*M. anisopliae*), Bio Power (*B. bassiana*) and Bio Catch (*V. lecanii*). 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 day old. The suspensions were filtered through cheese cloth to reduce mycelium clumping. The spores were counted in the suspension, using a Haemocytometer ($0.1mm \times 0.0025mm^2$). The concentrations were (1×10^9 spores /ml) from each EPF. The grown fungal cultures were centrifuged at 12000 rpm fungal for 30 min at 25ºC and the supernatant was used for the synthesis of Ag NPs.

Insect rearing

Cassida vittata was reared in Pests & Plant Protection Department, National Research Centre, Giza, Egypt on sugar beet leaves. Five pairs of adults were placed on a plant of sugar beet mate and oviposit. *Cassida vittata* was reared individually from the 1st larval instar to emergence of adults, at 24 ±2ºC. 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 *C. vittata*. One 100 eggs, larvae and adults were used for each treatment, divided into 5 groups, each of 20 eggs, larvae and adults were placed in Petri-dishes. The fungi were applied in a suspension in the control group, treated with sterilized water and kept at 24 ±2ºC and 70 ±5% R.H. The mortality rates of *C. vittata* were daily.

Biosynthesis of Silver Nano-particles

Silver Nano-particles were synthesized by using 50 ml. aqueous solution of 1 mM $AgNo_3$ 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 40ºC at 200 rpm for 7 days under dark condition. The control was maintained without adding the culture supernatant to the solution of $AgNo_3$.

Bioassay studies

C. vittata 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 24±2ºC 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 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

Table 1: % Mortality of EPF on the eggs of *C. vittata*, using spore suspension and bio synthesized silver nanoparticles.

EPF	Treated with	
	Fungal spores (Mean ± S.E)	Bio synthesized Ag NPs (Mean ± S.E)
M. anisopliae	84.0 ± 1.22	95.0 ± 2.11
B. bassiana	75.0 ± 1.10	87.0 ± 1.20
Bio Magic	68.0 ± 0.10	75.0 ± 0.11
Bio Power	65.0 ± 2.13	72.0 ± 1.11
Bio Catch	47.0 ± 1.12	61.0 ± 1.25
Control	5.0	5.0
S.E (m)	1.13	1.16

Stat View for Power PC software, version 4.5 (Abacus Concepts, Inc., Berkeley, CA, USA).

Results and Discussion

Data of the treated eggs of *C. vittata* 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 (95%) mortality rate of *C. vittata* was recorded in the treated eggs. The % mortality rates attained 84, 75, 68, 65 and 47% by infection with fungal spores from *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch, respectively. Furthermore, the % mortality rates were 95, 87, 75, 72 and 61% by infection with bio synthesized 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 (95%) in the eggs of *C. vittata*, when treated with Nano-particles or with fungal spores after 6 days and was the lowest (61%) when treated with Bio Catch. According to (Abdel-Raheem, 2005; Abdel-Raheem and Samia, 2019 and Abdel-Raheem *et al.*, 2009, 2019, 2020 a&b) the total mortality of eggs and larvae were reduced than the control group when eggs were exposed to *M. anisopliae* spores.

(Saleh *et al.*, 2016 and Abdel-Raheem, 2019) 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 Dalton in the extraction of fungi.

Data of larval mortality of *C. vittata*, 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, 97% mortality of *C. vittata* larvae was recorded as: 85, 78, 72, 67 and 58% by infection with fungal spores from *M. anisopliae*, *B. bassiana*, Bio Magic, *Bio* Power and

Table 2: % Mortality of EPF on the larvae of *C. vittata* using spore suspension and bio synthesized silver nanoparticles.

EPF	Treated with	
	Fungal spores (Mean ± S.E)	Bio synthesized Ag NPs (Mean ± S.E)
M. anisopliae	85.0 ± 2.17	97.0 ± 1.11
B. bassian	78.0 ± 2.42	87.0 ± 2.22
Bio Magic	72.0 ± 1.13	80.0 ± 1.11
Bio Power	67.0 ± 1.22	76.0 ± 1.11
Bio Catch	58.0 ± 1.22	64.0 ± 1.10
Control	6.0	6.0
S.E (m)	1.63	1.33

Bio Catch, respectively. By infection with bio synthesized Ag NPs from *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch, the mortality rates recorded were 87, 87, 80, 76 and 64%, respectively. *M. anisopliae* was the highest % mortality (97%) in the larvae of *C. vittata*, when treated with the Nano-particles or with the fungal spores after 6 days, while the lowest (64%) was when treated with Bio Catch, *V. lecanii*.

Data of adult mortality of *C. vittata*, 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 parentage of mortality of *C. vittata*, adults reached 80%. The % mortality rates recorded 69, 65, 62, 58 and 55% by infection with fungal spores from *M. anisopliae*, *B. bassiana*, Bio Magic, *Bio* Power and Bio Catch, respectively. While, by infection with bio synthesized Ag NPs from *M. anisopliae*, *B. bassiana*, Bio Magic, Bio Power and Bio Catch, the % mortality recorded were 80, 77, 69, 65 and 60 %, respectively. *M. anisopliae* was the highest % mortality (80%) in the adults of *C. vittata*, when treated with the Nano-particles or with fungal spores after 6 days, while the lowest was (60%), when they were treated by Bio Catch. Zaki and Abdel-Raheem, 2010; Gothandapani *et al.*, 2015 and Abdel-

Table 3: % Mortality of EPF on the adults of *C. vittata* using spore suspension and bio synthesized silver nanoparticles.

EPF	Treated with	
	Fungal spores (Mean ± S.E)	Bio synthesized Ag NPs (Mean ± S.E)
M. anisopliae	69.0 ± 2.11	80.0 ± 1.22
B. bassiana	65.0 ± 1.20	77.0 ± 1.00
Bio Magic	62.0 ± 2.11	69.0 ± 1.10
Bio Power	58.0 ± 1.12	65.0 ± 3.20
Bio Catch	55.0 ± 1.11	60.0 ± 1.20
Control	6.0	7.0
S.E(m)	1.53	1.54

Raheem and Lamya, 2017, 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 Kamil, 2017). Sabbour and Abdel-Raheem, 2016 and El-Husseini, 2019), treated the adults and larvae of *C. vittata* with conidiophores of EPF *B. bassiana* and reported that the mortality reached to 100%.

Conclusion

The results proved that use of the Ag NPs synthesized through application of spore suspension and bio synthesized silver Nano-particles showed an efficacy against *C. vittata*, different stages. *M. anisopliae* had the highest potential and was more effective than all the others.

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