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SILVER NANOPARTICICLES FROM ENTOMOPATHOGENIC FUNGI AGAINST THE SPINY BOLLWORM, EARIAS INSULANA IN MAIZE CROP

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ABSTRACT

The spiny bollworm, (SBW) *Earias insulana* (Lepidoptera: Noctuidae) is considered one of the most important corn pests in the world. It causes severe damage, resulting in a great loss in both quality and quantity of Maize yield. The study aimed to evaluate the virulence of fungal spores and silver nanoparticles (AgNPs) from entomopathogenic fungi (EPF) on *E. insulana* under laboratory conditions. Concentrations of the fungal spores and the silver nanoparticles were prepared from *Metarhizium anisopliae*, *Beauveria bassiana*, and *Verticillium lecanii*. *Metarhizium anisopliae* isolated from larvae and adults of the beet moth and *Beauveria bassiana* isolated from the beet beetle. The compound was used Bio Catch (*V. lecanii*). The concentration of EPF used was (1x10^6 spores/ ml). Hundred larvae and pupae were used for each treatment, divided into 5 groups, and kept at 24±2 ºC and 65±5 % R.H. Silver nanoparticles were synthesized. The nano-particle solution was sprinkled over the filter paper and incubated at 24±2ºC for 3 days. The mortality rate was recorded 2 days after the treatment and mortality percentage was calculated. One-way ANOVA was used to compare the effects of the experimental and control treatments. The results showed that the three EPF achieved (47–70%) mortality rates within 6 days in larvae stage. *B. bassiana* and *M. anisopliae* were the most effective ones than *V. lecanii*. Spores of *B. bassiana* increased larvae mortality. The larvae mortality rate was (70 %) higher than the control. Pupae mortality rate was 65 % achieved in 6 days when treated with *B. bassiana*, (60 %) with *M. anisopliae* and (50%) with *V. lecanii*. *B. bassiana* was more effective on *E. insulana* than *M. anisopliae* and *V. lecanii*. The Ag NPs synthesized through applications of spore suspension and biosynthesized silver nanoparticles showed efficacy against *E. insulana*, different stages.

INTRODUCTION

The spiny bollworm, *Earias insulana* (Lepidoptera: Noctuidae) is considered one of the most important corn pests in the world. It causes severe damage, resulting in a significant loss in both quality and quantity of corn yield. Various conventional chemical insecticides are available which offer some protection against these pests, but they have created many problems (resistance, secondary pest outbreaks, environmental pollution, etc….) (Dhakal and Singh, 2019). The death or harm to microorganisms caused by pesticides affects the fertility rate of the soil.

Nanotechnology has become one of the most promising novel approaches for pest control in recent years, it also employs nanoparticles (NPs) having one or more dimensions in the order of 100 nm or less (Auffan *et al*., 2009).

The trials for evaluating nanotechnology in controlling insects are based on their size-dependent qualities high surface-to-volume ratio and increasing chemical reactivity and penetration in the living cells (Medina *et al*., 2007), A wide variety of nanoparticles materials are used against some insects in the laboratory. Goswami *et al*., (2010), Abdel-Raheem *et al*., (2019a, 2020a), Saad and Abdel-Raheem, 2020), studied that the applications of different nanoparticles such as silver nanoparticles (AgNPs), aluminum oxide nanoparticles, (Al2O3) zinc oxide, (ZnO) and titanium dioxide nanoparticles (TiO2), in pest control of rice weevil, Sitophilus oryzae, and the Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae). Also, Stadler *et al*., (2010) Successfully applied alumina against stored grain pest *Callosobruchus maculatus*. Debnath and Seth (2011) demonstrated the application of SNP could significantly increase the mortality effect of NPs by increasing the time after application.

Vinutha *et al*., (2013), Abdel-Raheem (2019b), Abdel-Raheem *et al*., (2009, 2016, 2020b) recorded the potential of nanoparticles against *Helicoverpa armigera*. Entomopathogenic fungi (EPF) have been studied as biological control agents. Two isolates of various microorganisms were isolated from *Cassida vittata, Metarhizium anisopliae* and *Beauveria bassiana* (Saleh *et al*., 2016 and Abdel-Raheem and Al-Keridis 2017). 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, (Zaki and Abdel-Raheem 2010, Sabry *et al*., 2011, Abdel-Raheem, *et al*., 2020 a, b, c, & d). Fungi, bacteria, algae, and plant extracts are known to synthesize silver nanoparticles (Ag NPs) (Sabbour and Abdel-Raheem 2016, Nisha *et al*. 2017, Dimetry *et al*. 2019, and Sabbour *et al*. 2020). Fungi such as Verticillium species are known to produce Ag NPs (Zonorodiam, *et al*., 2016). This study aimed to evaluate the bioefficacy of Nanoparticles of EPF, B. *bassiana*, M. *anisopliae* and V. *lecanii* as fungal spores and silver Nanoparticles on different life stages of E. *insulana* (larvae and Pupae) under laboratory conditions.

MATERIALS AND METHODS

Isolation and cultivation of fungi

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 which included (10g Peptone, 40g Dextrose, 2g yeast extract, 15g Agar and 500 ml. Chloramphenicol). The media were sterilized in autoclaved at 120 ºC for 20 min. and poured into Petri- dishes (10 cm diameter x 1.5 cm). Then, incubated at specific temperature at 24 ±2 ˚C and 65± 5% RH. The fungal cultures were periodically re-cultured every 14–30 days and stored at 4˚C.

Commercial Compound

A compound product called Bio Catch containing *V. lecanii*, was used in the experiment. The concentration of EPF used was (1x106 spores/ ml). Spores were harvested from 14-day-old cultures by rinsing with sterilized water and adding 0.5 % Tween 80. The suspensions were filtered to remove mycelium clumps, and spore concentrations were determined using a Hemocytometer (0.1mm x 0.0025mm2). The concentrations were (1 x 10 6 spores /ml) from each EPF. 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.

Insect rearing

The laboratory strain of *E. insulana* was obtained from laboratory of Pests & Plant Protection Department, National Research Centre, Cairo, Egypt. Where reared for several generations away from any contamination with insecticides on an artificial diet.

Bioassay

Larvae and pupae of *E. insulana* were treated by the *B. bassiana*, *M. anisopliae* and Bio Catch (*V. lecanii*). Each treatment group consisted of 100 larvae and pupae divided into 5 groups, with 20 individuals in each group. The control group was treated with sterilized water. The mortality rates of E. insulana were recorded daily, and kept at 24 ±2 ºC and 65 ±5 % R.H.

Biosynthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by using 50 ml aqueous solution of 1 mM Ag No3 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 ºC at 200 rpm for 6 days under dark conditions. The control was maintained without adding the culture supernatant to the solution of Ag No3.

Bioassay studies

E. insulana was placed in sterile Petri dishes having food and sterile filter paper. The silver nanoparticle solution was sprinkled over the filter paper. Which was allowed to air dry aseptically. The Petri dishes were then incubated at 24±2ºC for 3 days, and the mortality rate was recorded two days after the treatment and % mortality. The experiment was replicated three times.

Data Analysis

Mortality data were recorded and mortality percentages were calculated for nymphs and adults. The corrected percent mortality by use of Abbott's formula, 1925. 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 & DISCUSSION

Data of the treated nymphs of *E. insulana* with *B. bassiana*, *M. anisopliae* and Bio Catch (*V. lecanii)* as fungal spores and their Silver NPs particles were presented in Table (1). It appears that both treatments, fungal spores and bio-synthesized Ag NPs, have a positive impact on the EPF (Entomopathogenic Fungi) being studied, as compared to the control group.Six days post-treatment, up-to (70%) mortality rate of E. insulana was recorded in the treated larvae. The percent mortality rates attained 60, 50, and 47% by infection with fungal spores from *B. bassiana*, *M. anisopliae,* and *V. lecanii*, respectively. Furthermore, the percentage mortality rates were 70, 60, and 58% by infection with biosynthesized Ag NPs from *B. bassiana*, *M. anisopliae* and *V. lecanii* respectively, at the same time. *B. bassiana* recorded the highest mortality (70%) in the larvae of *E. insulana,* when treated with nanoparticles or with fungal spores after 6 days will the lowest was (47%) when treated with *V. lecanii* fungal spores. The control group, which did not undergo any treatment, maintained a constant value of 5.0. This suggests that there was no significant impact on the EPF's activity in the absence of treatment.

The standard error values (S.E) provided in the last row of the table indicate the variability or margin of error associated with the mean values. The S.E for fungal spores treatment is 1.3, while for bio-synthesized Ag NPs treatment, it is 1.40. These values represent the range within which the true mean values are expected to fall.

Table (1): Percent mortality of EPF on the larvae of E. insulana, using spore suspension and biosynthesized silver nanoparticles.

EPF	Treated with	
	Fungal spores (Mean ± S.E)	Bio synthesized Ag NPs (Mean ± S.E)
B. bassiana	60.0 ± 2.3	70.0 ± 2.2
M. anisopliae	50.0 ± 1.20	60.0 ± 1.2
V. lecanii	47.0 ± 1.1	58.0 ± 1.1
Control	5.0	5.0
S.E (m)	1.3	1.40

The pupae percent of mortality of *E. insulana*, treated with *B. bassiana*, *M. anisopliae* and *V. lecanii* as fungal Spores and Silver nanoparticles was presented in Table (2). Sex days post-treatment, mortality percent of *E. insulana* pupae was recorded: 57, 45, and 40 % by infection with fungal spores from *B. bassiana*, *M. anisopliae* and *V. lecanii*, respectively.

Will, at the infection with biosynthesized Ag NPs from *B. bassiana*, *M. anisopliae* and *V. lecanii* the mortality rates recorded 65, 60, and 50%, respectively. *B. bassiana* was the highest % mortality (65%) in the pupae of *E. insulana*, when treated with the nanoparticles or with the fungal spores (57%) after 6 days, while the lowest (50%) was when treated with *V. lecanii* biosynthesized.

The control group, as before, shows a constant value of 5.0, indicating no significant change or treatment effect. The standard error values (S.E) provided in the last row of the table indicate the variability or margin of error associated with the mean values. The S.E for the fungal spores treatment is 1.31, while for the biosynthesized Ag NPs treatment, it is 1.51. These values represent the range within which the true mean values are expected to fall.

Table (2): Percent mortality of EPF on the pupae of *E. insulana* using spore suspension and biosynthesized silver nanoparticles.

EPF	Treated with	
	Fungal spores (Mean ± S.E)	Bio synthesized Ag NPs (Mean ± S.E)
B. bassiana	57.0 ± 1.2	65.0 ± 1.11
M. anisopliae	45.0 ± 1.2	60.0 ± 1.11
V. lecanii	40.0 ± 1.2	50.0 ± 1.10
Control	5.0	5.0
S.E (m)	1.31	1.51

The implementation of nanotechnology in agriculture has resulted in the development of efficient strategies for pest insect control. Abdel-Raheem *et al*. (2009, 2019, 2020a & b) demonstrated that the mortality rate of larvae significantly increased when exposed to M. anisopliae spores compared to the control group. Similarly, Saleh *et al*. (2016) and Abdel-Raheem (2019b) suggested that variations in pathogenicity rates among different fungal species may be attributed to the presence of certain polar compounds with antimicrobial activity, ranging between 1000 and 1500 Dalton, extracted from fungi. Additionally, Moustafa *et al*. (2019) discovered that newly hatched larvae of Earias insulana exhibited high susceptibility to the toxic effects of M. anisopliae. Entomopathogenic fungi have been shown to effectively induce mortality in P. gossypiella at different stages, as reported by Niu *et al*. (2019), Rizwan *et al*. (2019), and Omar *et al*. (2021).

CONCLUSIONS

The results proved that the use of the Ag NPs synthesized through the application of spore suspension and biosynthesized silver nanoparticles showed efficacy against *E. insulana* larvae and pupae stages. *B. bassiana* had the highest potential as well as it was more effective than *M. anisopliae* and *V. lecanii.*

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CONFLICT OF INTEREST

The authors state that there are no conflicts of interest with the publication of this work.

استخدام جزيئات الفضة النانوية في مكافحة دودة اللوز الشوكية على محصول الذرة الشامية

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الخلاصة

تعتبر دودة اللوز الشوكية (Lepidoptera: Noctuidae) *Earias insulana* (SBW) واحدة من أهم آفات الذرة في العالم. تتسبب في أضرار جسيمة، مما يؤدي إلى خسارة كبيرة في كل من جودة وكمية محصول الذرة. هدفت الدراسة إلى تقييم فاعلية جسيمات الفضة النانوية (AgNPs) والجراثيم الفطرية من الفطريات الممرضة للحشرات (EPF) على *E. insulana* تحت الظروف المعملية. تم تحضير تركيزات الجراثيم الفطرية والجسيمات النانوية الفضية من *Metarhizium anisopliae* و *Beauveria bassiana* و *Verticillium lecanii*. تم عزل *Metarhizium anisopliae* من يرقات وحشرات فراشة البنجر و *Beauveria bassiana* المعزولة من خنفساء البنجر. تم استخدام المركب (Bio Catch, *V. lecanii*). كان تركيز EPF المستخدم (61 10 X جراثيم / مل). تم استخدام مئات اليرقات والعذارى لكل معاملة، وتم تقسيمها إلى 5 مجموعات، وحفظها عند 24 ± 2 درجة مئوية و65 ± 5٪ رطوبة نسبية تم تصنيع جسيمات الفضة النانوية. رش محلول الجسيمات النانوية على ورق الترشيح ووضع بالحضان عند 24 ± 2 درجة مئوية لمدة 3 أيام. تم تسجيل معدل الموت بعد يومين من العدوى وتم حساب نسبة الموت. باستخدام ANOVA أحادي الاتجاه لمقارنة تأثيرات العلاجات التجريبية والمراقبة. أوضحت النتائج أن ثلاث انواع من الفطريات الممرضة للحشرات حققت (47–70٪) معدلات موت خلال 6 أيام في طور اليرقات. كانت *B. bassiana* و *M. anisopliae* الأكثر فاعلية من *V. lecanii*. أدت جراثيم الفطر *B. bassiana* إلى زيادة معدل موت اليرقات. كان معدل موت اليرقات أعلى بنسبة (70٪) من الغير معاملة بالفطر. بلغ معدل موت العذارى 65٪ في 6 أيام عند معاملتها بفطر *B. bassiana*، و (60٪) بجرثومة *M. anisopliae* و (50٪) بفطر *V. lecanii*. كان *B. bassiana* أكثر فاعلية على *E. insulana* من *M. anisopliae* و *V. lecanii*. أظهرت Ag NPs التي تم تصنيعها من خلال تطبيقات تعليق الجراثيم والجسيمات النانوية الفضية المصنعة حيويا فعالية ضد *E. insulana*، في مراحل مختلفة.

الكلمات المفتاحية: الفطريات الممرضة للحشرات، جزيئات الفضة النانوية، *Earias insulana*، الذرة.

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