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Silver Nano-particles from entomopathogenic fungi against the cotton bollworms, *Earias insulana* in Okra

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Abstract
Earias insulana, a type of cotton bollworm belonging to the Noctuidae family of Lepidoptera, is widely regarded as a significant threat to corn crops worldwide. The damage it causes is extensive, leading to substantial losses in terms of both quality and quantity of Okra harvest. The aim of this investigation was to evaluate the effectiveness of spores from entomopathogenic fungi (EPF) and silver nanoparticles against E. insulana in a controlled laboratory setting. Various concentrations were created using Beauveria bassiana, Metarhizium anisopliae, and Verticillium lecanii. The mortality rate of E. insulana ranged from 55% to 80% in each concentration in larvae within a span of six days. Among them, *B. bassiana* and *M. anisopliae* demonstrated superior efficacy compared to *V. lecanii*. *B. bassiana* spores exhibited an enhanced ability to cause larvae mortality, resulting in an 80% mortality rate, which showed a considerable increase compared to the control group. Additionally, when exposed to *B. bassiana*, 75% of the pupae experienced mortality within six days, while the percentages for *M. anisopliae* and *V. lecanii* were 68% and 62%, respectively. Thus, it can be concluded that *B. bassiana* exhibited greater effectiveness against *E. insulana* as compared to *M. anisopliae* and *V. lecanii*.

Keywords: Entomopathogenic fungi, silver Nano-particles, *Earias insulana*, Okra

Introduction

Earias insulana, a type of cotton bollworm, is widely recognized as a major corn pest worldwide and has a detrimental impact on the quantity and quality of Okra yield. However, the use of chemical control methods in corn fields poses several disadvantages, including the presence of toxic residues, environmental pollution, and harm to non-target organisms. Now nanotechnology is emerged as a promising approach for insect pest control, utilizing nanoparticles (NPs) with dimensions of approximately 100 nm or smaller [1]. These nanoparticles display characteristics that are influenced by their size, such as a large surface area relative to their volume, increased reactivity with chemicals, and enhanced ability to enter living cells [2]. Various types of nanoparticles have been tested against insects in laboratory settings [3-15]. For instance, studies have investigated the effectiveness of silver nanoparticles in controlling pests such as the rice weevil, *Sitophilus oryzae* and the Red Palm Weevil, *Rhynchophorus ferrugineus* Olivier [16]. Additionally, alumina has proven successful in combating the stored grain pest *Callosobruchus maculatus*. Furthermore, application of SNP has been shown to significantly increase the mortality impact of nanoparticles over time [17]. Nano particles have also demonstrated potential in controlling the cotton bollworm, *Helicoverpa armigera* [18]. On the biological control front, entomopathogenic fungi (EPF) have been extensively studied as agents for pest control [9-11, 19, 20]. Various microorganisms, including *Metarhizium anisopliae* and *Beauveria bassiana*, have been isolated from *Cassida vittata*, and these EPF infect hosts by contacting and penetrating their cuticles [21- 30]. In experiments, *B. bassiana* and *M. anisopliae* have contaminated eggs, larvae, and adults, resulting in mortality rates of 50-100% [27-30]. It is worth noting that silver nanoparticles (Ag NPs) can be synthesized by fungi, bacteria, algae, and plant extracts, with Verticillium species being particularly known for Ag NP production [31-37]. The aim of study was to evaluate the effectiveness of EPF nanoparticle, particularly those derived from B. bassian, M. anisopliae, and V. lecanii, as well as silver nanoparticle, against different developmental phases (larvae and pupae) of E. insulana in a controlled laboratory environment.

Materials and Methods

Entomopathogenic fungi

M. anisopliae, which was derived from larvae and adults of *Scrobipalpa ocellatella*, and *B. bassiana*, isolated from *Cassida vittata* (Vill.) [21], were grown on Peptone media. The fungi were incubated at a temperature of 24 ±2 ˚C and a relative humidity of 65± 5%. The fungal isolates were regularly transferred to new cultures every 14 to 30 days and stored at a temperature of 4˚C.

Commercial Compound

Bio Catch (*V. lecanii*) was utilized as the agent. The concentration used was ($1x10^8$ spores/ ml). To retrieve spores, they were collected by flushing with sterilized water and supplemented with 0.5% Tween 80. To prevent mycelium clumping, the suspensions were filtered through cheese cloth. A Haemocytometer (0.1mm x 0.0025mm2) was employed to count the spores in the suspension. Each

EPF had a concentration of (1 x 10^8 spores /ml). The fungal strains were grown and subsequently centrifuged at a speed of 12,000 revolutions per minute for a duration of 30 minutes at a temperature of 25 degrees Celsius. The resulting liquid above the sediment was utilized to generate silver nanoparticles.

Insect rearing
The *E. insulana* strain utilized in this study was getting it from the laboratory (NRC). It was selectively bred for several generations in a controlled environment devoid of any contact with insecticides and was provided with an artificial diet.

Bioassay
We assessed the efficacy of *B. bassiana*, *M. anisopliae*, and *V. lecanii* against *E. insulana* larvae and pupae by infecting them with these fungi. Each treatment involved a total of 100 larvae and pupae. The experiment involved the partitioning of larvae and pupae into 5 groups, where each group contained 20 individuals and was placed in individual Petri dishes. The control group did not receive any application of fungi, but instead, the larvae and pupae were treated with sterilized water. The Petri dishes in all groups were maintained at a temperature of 21 ±2 ºC and R.H. 70 ±5 %. The mortality rates of *E. insulana* were recorded on a daily basis.

Biosynthesis of Silver Nano-particles
To create silver nanoparticles, the scientists mixed a 50 ml Ag No3 solution with a 50 ml solution of supernatant from fungi culture in a 250 ml conical flask. This process was repeated for all fungal isolates and commercial products. The pH of the mixture was then adjusted to 8.5. The flask containing the mixture was placed in a dark environment and incubated at a temperature of 40 ºC, with constant agitation at 200 rpm, for a period of 6 days. To serve as a control, a sample without the addition of culture supernatant to the Ag No3 solution was also maintained.

Bioassay studies
E. insulana was introduced into clean Petri dishes that contained both nourishment and uncontaminated filter paper. The solution of nano-particles was dispersed onto the filter paper. Next, the filter paper was left to air dry in a sterile fashion and positioned in an incubator at a temperature of 24±2ºC for 3 days. This procedure was replicated three times. The mortality rate was observed 2 days after the treatment and the percentage of mortality was subsequently computed.

Data Analysis
The mortality data was recorded and the mortality rate of both nymphs and adults was calculated. The adjusted mortality rate was determined using the method described in [38]. To assess the effects of the experimental and control treatments, either the Student's t-test or one-way ANOVA was used. Statistical analyses were conducted using the Stat View for Power PC software, version 4.5 (Abacus Concepts, Inc., Berkeley, CA, USA).

Results and Discussion
Data on the mortality rates of *E. insulana* larvae infected with *B. bassiana*, *M. anisopliae*, and *V. lecanii* as fungal spores and their Silver NPs particles are presented in Table 1. Aftr 6 days of treatment, a mortality rate of up to 80% was observed in the treated larvae. The mortality rates achieved by infecting with fungal spores from *B. bassiana*, *M. anisopliae*, and *V. lecanii* were 67%, 61%, and 55% respectively. Similarly, the mortality rates were 80%, 75%, and 68% when infecting with bio-synthesized Ag NPs from the same fungi. Amongst the treatments, B. bassiana exhibited the highest mortality rate (80) in the larvae, whether treated with nanoparticles or fungal spores after 6 days. In contrast, *V. lecanii* resulted in the lowest mortality rate (68%). Previous studies [10, 11, 19, 21, 39, 40] have shown that exposure to *M. anisopliae* spores led to lower total larval mortality compared to the control group. The variation in pathogenicity rates between different fungi may be attributed to certain polar compounds with molecular weights ranging from 1000 to 1500 Dalton in the fungal extraction that contain antimicrobial activity [4, 22].

Table 1: The Percent mortality of the larvae of *E. insulana,* using of Entomopathogenic fungi as spore suspension and silver nanoparticles

Entomopathogenic fungi	Infected with	
	spores (Mean ± S.E)	**silver nanoparticles Ag NPs (Mean ± S.E)**
B. bassiana	67.0 ± 2.2	80.0 ± 2.5
M. anisopliae	61.0 ± 1.24	75.0 ± 1.7
V. lecanii	55.0 ± 1.3	68.0 ± 1.6
Control	6.0	6.0
S.E (m)	1.58	1.93

Table (2) depicts the percentage of mortality observed in *E. insulana* pupae after being infected with spores and silver nanoparticles of *B. bassiana, M. anisopliae,* and *V. lecanii*. After a duration of 6 days, the mortality rate in the *E. insulana* pupae was documented as 68%, 56%, and 50% when infected with fungal spores from *B. bassiana*, *M. anisopliae,* and *lecanii*, respectively. Conversely, when exposed to bio-synthesized Ag NPs from *B. bassiana*, *M. anisopliae,* and *V lecanii*, the mortality rates were observed to be 75%, 68%, and 62%, respectively. *B. bassiana* exhibited the highest mortality percentage (75%) in *E. insulana* pupae following treatment with either nanoparticles or fungal spores after 6 days, whereas *V. lecanii* displayed the lowest mortality rate (62).

Table 2: The Percent mortality of the pupae of *E. insulana,* using of Entomopathogenic fungi as spore suspension and silver nanoparticles

Entomopathogenic fungi	Infected with	
	Spores (Mean ± S.E)	**silver nanoparticles Ag NPs (Mean ± S.E)**
B. bassiana	68.0 ± 1.24	75.0 ± 2.33
M. anisopliae	56.0 ± 1.47	68.0 ± 2.44
V. lecanii	50.0 ± 1.33	62.0 ± 1.25
Control	6.0	6.0
S.E (m)	1.35	2.06

Conclusion
The results indicated that using Ag NPs produced during the application of spore suspension and bio synthesized silver nanoparticles effectively combated *E. insulana* at different stages. *B. bassiana*, out of the three options, displayed the highest efficacy and was more efficient when compared to *M. anisopliae* and *V. lecanii*.

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