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# Biosynthesis of selenium nanoparticles by *Penicillium griseofulvum* and their impact on *Spodoptra littoralis*

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***ABSTRACT*** **: Nanotechnology has emerged as the forefront of innovation, permeating numerous domains, notably agriculture, employing it as an eco-safe alternative for pest control. Selenium nanoparticles (SeNPs) was biosynthesized using fungal filtrates of *Penicillium griseofulvum, Penicillium expansum, Aspergillus candidus, Aspergillus tereus* and *Aspergillus flavus*. SeNPs were detected by *P. griseofulvum* using UV-visual spectrophotometer with red-yellowish color forming at strong peak 265 nm. The average size diameter of SeNPs was 91.25 nm. SeNPs resulted in the highest mortality rates for both larvae and pupae. *P. griseofulvum* selected for further study so verification of its identity was accomplished using the 18S rRNA gene of DNA and the resulting fungal sequence was deposited into NCBI under accession number OR672743. Cotton leafworm, *Spodoptra littoralis* causes great damages in cotton crop. SeNPs produced from *P. griseofulvum* was highly effective against *S. littoralis* than its spore suspension at all experimental conditions. The $LC_{50}$ and $LC_{90}$ were 4.79 and 8.89 PPM, respectively after 5 days post-treatment. While the slope value of toxicity linear attained 4.7742±0.9066. These findings suggest that SeNPs produced by *P. griseofulvum* was encourage the use in pest control systems as well as the development of environmentally friendly materials.**

***KEYWORDS:*** **Selenium nanoparticles, *Penicillium griseofulvum, Spodoptra littoralis.***

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## I. INTRODUCTION

*Spodoptra littoralis* poses a considerable threat to numerous crops, including potatoes. This pest is polyphagous nature and its larvae are particularly voracious, feeding extensively on the leaves of potato plants resulted in defoliation, ultimately leading to decreased yields **(Ghoneim *et al.*, 2020)**. Over time, *S. littoralis* has developed resistance to numerous chemical insecticides, posing challenges for pest management as traditional treatments may prove ineffective. Thus, controlling infestations in potato crops necessitates an integrated approach, combining biological control methods (**El-Sayed *et al.*, 2023 and Ofuya *et al.*, 2023)**.

Fungi play a crucial role in biocontrol against insects, serving as effective biological control agents in agriculture. This approach involves harnessing naturally occurring or cultivated fungi to manage pest populations **(Qin *et al.*, 2023)**. These fungi are formulated into mycoinsecticides, applied to target insects, and serve as alternatives to chemical insecticides, especially where pest resistance is a concern **(Gul *et al.*, 2023).** Their use is environmentally friendly, with minimal impact on non-target organisms and ecosystems, promoting sustainable agricultural practices **(Liang and Meng, 2024)**. Despite their benefits, challenges such as ensuring effectiveness under varying environmental conditions and consistent application exist. Success in fungal biocontrol depends on factors like the target pest, crop type, and local climate **(Dutta and Phani, 2023)**.

Selenium nanoparticles (SeNPs) have the advantages of high absorption rate, high biological activity and low toxicity **(Xu *et al.*, 2023)**. The selenium nanoparticles inserted in crop growth, also has antibacterial, antioxidant, anticancer and anti-tumor effects. In addition, selenium nanoparticles applied in selenium nutrition enhancement in animals, plants and humans **(Zhang *et al*., 2023)**.

## MATERIALS AND METHODS

### 2.1. Materials:

**Rearing technique of *S. littoralis*:**

The laboratory strain of *S. littoralis* used in this study was obtained from a laboratory of leaf worm Research Department, Plant Protection Research Institute, Sharkia branch, this strain was reared for several generations away from any contamination with insecticides on castor bean leaves, *Ricinus communis* L. (as a source of food which was provided daily until pupation) under controlled conditions in an incubator at 26 ± 1°C and 70 ± 5% RH. as described by( **El-Defrawi *et al.* 1964)**. Pupae were transferred individually to other clean tubes and incubated at the same previous conditions until moth emergence. The adults were sexed and placed in glass gars (500 mL volume) supplied with leaves of Tafla, *Nerium oleander* L. for eggs laying. Adults were fed on 10% sugar solution and changed by new one daily. The eggs were obtained and kept in glass gars (500 mL volume), incubated at the same previous conditions until hatching. The newly hatched larvae were used directly in experiments.

**Screening of fungal isolates for their mortality effect against *S. littoralis***

100 mL of PDA medium was inoculated with spore suspensions of five isolates that isolated from an agricultural soil sample (*Penicillium griseofulvum, Penicillium expansum, Aspergillus candidus, Aspergillus tereus* and *Aspergillus flavus* and) obtained by washing the 7-day-old slant of tested fungal strains and then incubated at 28±2°C for 7 days. All strains were tested for their pathogenicity toward the *S. littoralis* and its influence on insects' mortality **(Abd-ElAzeem *et al*., 2019)**.

**Screening of the most potent fungal spore suspension concentrations against larval mortality**

Spores of fungal isolate were harvested by rinsing with sterilized water containing 0.005% Tween80 from 7 days-old culture PDA medium grown at 25±1°C for *P. griseofulvum.* The suspensions were filtered through cheese cloth to reduce mycelium clumping. The mortality percentages were detected (calculated by Abbott's formula) after 1, 2, 3, 4 and 5days post-treatments after inoculation with different *P. griseofulvum* spore concentrations ($9.8x10^{12}$, $9.8x10^{11}$, $9.8x10^{10}$ and $9.8x10^{9}$ ppm) also, determination its $LC_{50}$ and $LC_{90}$. The spores were counted in the suspensions using a hemocytometer. The concentrations were adjusted to $10^{9}$, $10^{10}$, $10^{11}$ and $10^{12}$. The treated leaves were dispersed with two mL of each spore suspension concentration. The treatments and control were incubated for 7 days under laboratory conditions 25 ± 1°C, 65± 5 RH% and 12 hrs photoperiod **(Khattab *et al.* 2018).**

**Biosynthesis of Selenium nanoparticles:**

Five mL of spore suspensions for each fungal isolate were inoculated aerobically in 250 mL Erlenmeyer flasks containing 100 mL of PDA broth medium that composed of; 200g potato, 20g dextrose and up to 1000 mL distilled water, incubated at 30°C for 7 days under shaking condition at 150 rpm. The fungal biomass was harvested and washed three times with sterilized bi-distilled water using Whatman filter paper No 1 to remove any traces of medium. 10 g of wet biomass were suspended in 50 mL sterilized bi-distilled water then incubated at 30°C for 7days under shaking condition at 150 rpm The fungal suspension was separated by Whatman filter paper No 1 and fungal cell free filtrate (CFF) were used for biosynthesis of SeNPs. An equal volume of each fungal CFF was mixed with (3 mM) $SeO_2$.

**Visual observation:**

The prepared nanoparticles were firstly characterized by a visual observation of color change for the nanoparticle solution with the formation of red-yellowish color due to the reduction of selenium ions. The bio-reduction of selenium ions was estimated by UV–visible spectrophotometer. Absorption measurements were carried out at a resolution of 1 nm and the absorption spectrum of colloidal solution of nanoparticles was scanned in the range of 200–800 nm**.**

**Dynamic light scattering (DLS)**

Dynamic light scattering system was used for evaluation of hydrodynamic diameter and distribution of particles in the solution. The solid particles distribution of selenium nanoparticles in the liquid solution was determined by Nicomp Particle Sizing Systems (CW388 NICOMP 380, Inc., Santa Barbara, Calif., USA). The system uses the proprietary technology of Nicomp analysis algorithm to analyze the distribution of complex multi-modal with the strongest resolution. The results were expressed as mean ± standard deviation (SD) of three measurements. Zeta potential of nanoparticles was determined by Zeta sizer Nano ZS ZEN3600, Malvern Instruments Ltd (Worcestershire, UK). Measurement of Zeta potential indicates a concept about the charges and stability of SeNPs, and its determination based on laser doppler electrophoresis technique in the same instrument.

## Fungal Identification:

### A. Morphological characterization:

From five fungal strains, *P. griseofulvum* was identified morphologically according to microscopic examination on PDA medium for 5 days of incubated at 30$^{o}$C and it was identified to genus and species using the taxonomic key proposed by **Pitt and Hocking (1997)**.

### B. Molecular identification

The morphological identification of *P. griseofulvum* was confirmed by the molecular identification of rRNA which was based on ITS rDNA sequence (18S–28S rRNA), flanking ITS1 (5.8S rRNA) and ITS 2 using ITS1/ITS4 primers. The universal sequences of the ITS1 and ITS4 primers were 5\'-TCCGTAGGTGAACCTGCGG-3\' and 5\'TCCTCCGCTTATTGATATGC-3\', respectively (**White *et al.*, 1990)**.

The obtained sequence of *P. griseofulvum* was registered to NCBI GenBank under the accession number OR672743. The obtained sequence was analyzed by BLAST program to detect its ratio of similarity with the other related sequences deposited at the GenBank. Molecular evolutionary genetic analysis (MEGA version X) software was used for phylogenetic analysis. Phylogenetic tree was constructed using the maximum composite likelihood method with 1000 bootstrap replicates based on ITS gene sequences**.** The closet homologous of the sequences were selected and multiple sequence alignments were carried out using the Clustal W program in the MEGAX software. The evolutionary distances were computed using the maximum composite likelihood method (**Tamura *et al*., 2004**).

**Statistical analysis:**

Data obtained were statistically analyzed according to completely randomized design. The appropriate methods were used for the analysis of data according to **Little and hills (1975)** and the proper "F" value was calculated as described by **(Fisher, 1944** and **Snedecor, 1970).**

## 3.RESULTS

### Biosynthesis and Characterization of SeNPs

The color of fungal filtrate, which were treated with Selenium ions, was changed to yellowish-red after its treatment with selenium salt. SeNPs was detected by UV-visual spectrophotometer at range of 200-800 nm. It was observed that the maximum absorbance peaks of UV-visual spectrophotometer, the surface plasmon resonance band, of SeNPs occurred at 265 nm Fig (1). Size distribution analysis of the biosynthesized SeNPs was performed using dynamic light scattering (DLS) in aqueous solution to reveal the distribution of different sizes of SeNPs in the solutions, Fig (2). The average size diameter of SeNPs which were found to be 91.25 nm.

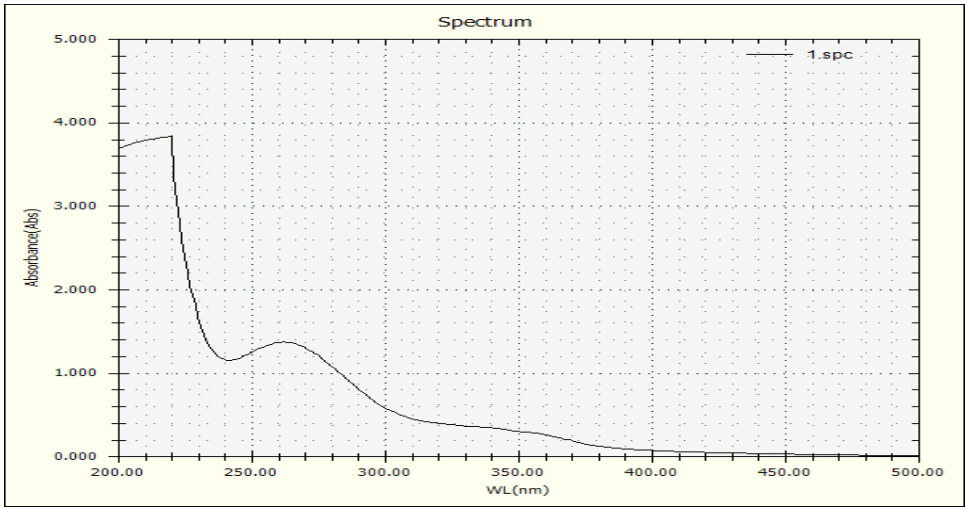


**Fig (1) UV spectroscopy of SeNPs**


**Fig. (2)** Zeta sizer analysis of S e N P s .

**The accumulative effect of fungal spore suspension against 2nd instar larvae of *S. littoralis.***

The data in Table (1) showed highly significant difference between the five fungal strains and control, recorded that *P. griseofulvum* was the most potent fungus for its pathogenicity causing highest mortality effect against the *S. littoralis* (72.33%) followed by *P. expansum* (53.00%) , control zero.

**Table (1):** Accumulative effect by different fungal isolates against 2nd instar larvae of *S. littoralis*.

| **Fungal strains** | ***Penicillium griseofulvum*** | ***Penicillium expansum*** | ***Aspergillus candidus*** | ***Aspergillus tereus*** | ***Aspergillus flavus*** | **Control** | **P** | **Ftest** | **$LSD_{0.05}$** |
|---|---|---|---|---|---|---|---|---|---|
| **Mortality** | **72.33a** | **53b** | **41.6c** | **24d** | **15.53e** | **0f** | **0.000** | ******* | **1.624** |

Control is untreated with any fungal isolate.

The mean value followed by different letters (a, b, c, d &e) with in the same row are significantly different (One-way ANOVA., $P \leq 0.05$).

LSD least significant difference.

*** means highly significant difference.

**The accumulative effect of** SeNPs **produced from the fungal strains against 2nd instar larvae of *S. littoralis*.**

Effect of SeNPs biosynthesized by the five fungal strains on *S. littoralis* that shown in Table (2) illustrated that biosynthesis of SeNPs by each fungal strain causing higher mortality effect against the *S. littoralis* than extract fungal filtrate for the same fungal species i.e (biosynthesis of SeNPs by *P. griseofulvum* (80.00 %) higher mortality effect against the *S. littoralis* than extract fungal filtrate of *P. griseofulvum* (48.00%**)** control zero.

**Table (2**): Accumulative effect of SeNPs biosynthesized by different fungal strains against 2nd instar larvae of *S. littoralis*.

| Fungal strains | Mortality% | | | P | F test | $LSD_{0.05}$ |
|---|---|---|---|---|---|---|
| ***P. griseofulvum*** | **Control** | **Control 1** | ***P. griseofulvm*** | **0.001** | *** | **1.631** |
| | **0C** | **48b** | **80a** | | | |
| ***P. expansum*** | **Control** | **Control 2** | ***P. expansum*** | **0.001** | *** | **1.631** |
| | **0C** | **42b** | **61a** | | | |
| ***A. candidus*** | **Control** | **Control 3** | ***A. candidus*** | **0.001** | *** | **1.884** |
| | **0C** | **31b** | **55a** | | | |
| ***A. tereus*** | **Control** | **Control 4** | ***A. tereus*** | **0.001** | *** | **1.332** |
| | **0C** | **17b** | **30a** | | | |
| ***A. flavus*** | **Control** | **Control 5** | ***A. flavus*** | **0.001** | *** | **1.884** |
| | **0C** | **12b** | **23a** | | | |

Control is untreated with any fungal isolate.

Control followed by different number (1, 2, 3, 4 & 5) is extract fungal filtrate for each fungal isolates in the same row.

The mean value followed by different letters (a, b &c) with in the same row are significantly different (One-way ANOVA., $P \leq 0.05$).

LSD least significant difference. *** means highly significant difference

**The toxicity effect of spore suspension of *P. griseofulvum* on *S. littoralis***

Results in Table (3) investigated the $LC_{50}$ and $LC_{90}$ of *P. griseofulvum* spore suspension against *S. littoralis* 2nd larval instars. The present results showed the mean larval mortality percentages were increased with the increasing of spore's concentrations and mortality percentages after 5 days post-treatment was; 52.33, 61.66, 67.00 and 72.33%, respectively. A linear relationship between the tested spores' concentrations and the mean mortality percentages after 5 days post-treatment. The obtained results revealed that, the $LC_{50}$ and $LC_{90}$ were 4.793957 and 8.894841 PPM, respectively after 5 days post-treatment. While the slope value of toxicity linear attained 4.7742±0.9066.

**Effect of SeNPs produced by *P. griseofulvum* against different parameters of immature stage of *S. littoralis***

Effect of biosynthesized SeNPs by *P. griseofulvum* against *S. littoralis* 2nd larval instars recorded great effect on larval duration, larval mortality, pupal period, pupation, pupal mortality and adult emergence than spore suspension of *P. griseofulvum* against *S. littoralis* 2nd larval instars (Table, 4). Highly significant differences were found between larval duration of *S. littoralis* the treated with biosynthesized SeNPs than spore suspension compared to the untreated samples. The highest effective nanoparticle was SeNPs, which the larval durations to 14 days while spore suspension 12 days compared to larval duration of control which was 8.67 days. It was also noticed that biosynthesized of SeNPs highly significantly increased the larval mortality of *S. littoralis*. SeNPs was the most potent compared with spore suspension at all experimental conditions. The percentage of larval mortality treated with SeNPs was 80.00% whereas percentage of larval mortality of the treatment with spore

suspension and water was 72.33% and 0.00%, respectively. The pupation percentage decreased to 15.00 % in the case of treatment with SeNPs than spore suspension and water significantly increased (25.00% and 95.33 %) respectively, where SeNPs gave the highest mortality percentage, 11.2 % than spore suspension and water significantly increased (9.33% and 4.00 %). The adult emergence of *S. littoralis* treated with SeNPs was reduced to 3.8% compared with spore suspension and water which was 15.67 and 91.33 %, respectively. Highly significant effect of SeNPs was observed on the percentage of adult emergence compared with spore suspension and control.

**Table (3):** Toxicity effect of different concentrations of *P. griseofulvum* spores against 2nd larval instar of cotton leaf worm

| **Concentrations (ppm)** | **Mean mortality percentages %** | **$LC_{50}$** | **$LC_{90}$** | **Slope ±SE** |
|---|---|---|---|---|
| **$9.8x10^{12}$** | **72.33** | **4.793957** | **8.894841** | **4.7742±0.9066** |
| **$9.8x10^{11}$** | **67.00** | | | |
| **$9.8x10^{10}$** | **61.66** | | | |
| **$9.8x10^{9}$** | **52.33** | | | |

SE Standard error

$LC_{50}$ lethal concentration kills 50% of treated larvae. $LC_{90}$ lethal concentration kills 90% of treated larva

**Table (4):** The effect of *P. griseofulvum* spore suspension against larval and pupal stages.

| **Treatments** | **Larval duration (days)** | **Larval mortality%** | **Pupal duration (days)** | **Pupation %** | **Pupal mortality %** | **Adult emergence%** |
|---|---|---|---|---|---|---|
| **SeNPs** | **$14^{a}$** | **$80^{a}$** | **7.2** | **$15^{c}$** | **$11.2^{a}$** | **$3.8^{c}$** |
| **$9.8x10^{12}$** | **$12^{b}$** | **$72.33^{b}$** | **6** | **$25^{c}$** | **$9.33^{a}$** | **$15.67^{d}$** |
| **Control** | **$8.67^{c}$** | **$0.00^{c}$** | **6.67** | **$95.33^{a}$** | **$4^{b}$** | **$91.33^{a}$** |
| **P** | **0.0018** | **0.0001** | **0.3961** | **0.0001** | **0.0003** | **0.0001** |
| **F. test** | ** | *** | **n.s** | *** | *** | *** |
| **$LSD_{0.05}$** | **1.998** | **1.6312** | | **1.998** | **1.998** | **1.998** |

Control is untreated with any fungal isolate.

The mean value followed by different letters (a, b, c, d & e) with in the same column are significantly different (One-way ANOVA., $P \leq 0.05$).

LSD least significant difference. *** means highly significant difference.

## Identification of fungal strains

*P. griseofulvum* was selected in this study as the most potential strain for highest mortality percentage against larvae of *S. littoralis*.

Molecular identification:

The morphological identification of the most powerful two SeNPs biosynthesizing strains was confirmed by molecular methods, based on the 18S–28S rRNA sequence and nomenclature as *P.*

*griseofulvum*. This fungal strain represents 100% similarity with closely related fungi submitted in the Gen Bank. *P. griseofulvum* was deposited in the GenBank under the accession number OR672743.

## Discussion

For decades, artificial insecticides have been employed to manage insect pests, these chemical pesticides lead to various health issues for all living organisms **(Sabry *et al.*, 2023)**. In recent times, there has been significant progress in utilizing nanoparticles for enhanced pest control in plant protection management **(Ammar and Abd-ElAzeem, 2021)**. The objective of this research is to diminish the reliance on chemical pesticides and explore safer alternatives, such as selenium nanoparticles, for pest management. Selenium is chosen for its safety profile, affordability, and widespread availability compared to other metals. Therefore, this study delves into a novel pest control approach utilizing SeNPs. A free form of SeNPs was biosynthesized by *P. griseofulvum* so, it selected for further study not only for its nano-particles production but also its efficacy as insecticidal agent causing the highest mortality.

*P. griseofulvum* spore suspension caused highest larval mortality (72.33%) at highest concentration ($9.8x10^{12}$ ppm) while when concentration decrease, the mortality percentage decrease. **Khattab *et al.* (2018)** recorded same result showed that different conidial concentrations of *Trichoderma asperolides* ($10^5$-$10^8$spore/ml) against *Aphis crassivora* and mortality percentages after 7 days of application were ranged between 30 to 88%. Concentration is an important factor on the pathogenicity of entomopathogenic fungi **(Demirci *et al.*, 2011).** Also, **Geremew *et al.* (2024)** recorded the highest mortality rate of *Galleria melonella* (100%) by *Metarhizium robertsii* K-61 and K-102 at a concentration of $1x10^8$ conidial ml $^{-1}$ at 7 days post-inoculation.

The initial indication of SeNPs biosynthesis through fungal filtrate was the emergence of a reddish-yellow color that arises from the activation of free electrons, leading to the formation of surface plasmon resonance absorption bands via the synchronization of electron vibrations with light waves. **(Roy *et al.*, 2013).** UV-visible spectroscopy was employed to validate the biosynthesis process, revealing maximum absorbance peaks at 265 nm. These peaks signify the excitation of electrons within the conductive band situated around the surface of the nanoparticles **(Busi *et al.*, 2014)**. **Arunthirumeni *et al.* (2022)** agreed with these results showed the presence of maximum peak at 259 nm indicates to the formation of SeNPs by *Trichoderma* sp.

Our study illustrated the effect of SeNPs on *S. littoralis* more effective than its spore suspension in all biological parameters. Larval mortality and pupal mortality using SeNPs were 80 and 11.2% while using ($9.8x10^{12}$ ppm) were 72.33 and 9.33% compared to control 0.00 and 4%. On the other hand, pupation and adult emergence of the *S. littoralis* treated with SeNPs were 15 and 3.8% while using spore suspension ($9.8x10^{12}$ ppm) increased to 25.00 and 15.57% compared to control 95.33 and 91.33%. Also, larval and pupal duration greatly affected by SeNPs than spore suspension. These results confirmed that the small size and large surface area of metal nanoparticles increase the chemical reactivity and penetration in the living cells. The anti-insect activity of SeNps gave higher inhibition for all tested parameters explained that when the particles are smaller in size, their penetration into the living cells will be faster **(Ammar and El-Desouky 2016).** Therefore, the nanoparticles with fine size can enter rapidly inside the cells and disrupt some physiological function of the insect causing inhibitory effect **(Yang *et al* 2012 & Grillo *et al.*, 2016)**. The same results revealed by **Ammar and Abd-ElAzeem (2021)** that the smallest sized copper nanoparticles produced by *Asperigillus wentti* and *Asperigillus motte* was the strongest effective against all tested variables of spiny bollworm *Earias insulana* than its spore suspension.

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