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Mycosynthesis of Silver-Nanoparticles and their bio efficacy against *Spilosoma obliqua* (Walker)

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Abstract

Background: It is well established that insect pests and most notably, the *Spilosoma obliqua* is a major hindrance in the agricultural productivity of India, and that the very wide use of synthetic pesticides in agriculture contributes to the development of resistance and the destruction of the environment. Greener chemistry variant, e.g. the production of silver nanoparticles (AgNPs) using entomopathogenic fungi will be a more environmental-friendlier solution.
Purposes: The purpose of the experiment was (i) to prepare and fully characterize the AgNPs through producing them with the help of the fungus, *Trichoderma reesei* and (ii) to determine the larvicidal efficiency of the nanoparticles to third, fourth and fifth instar of *S. obliqua.*
Methods: The growth of *T. reesei* in a liquid broth medium liquid and subsequent production of extracellular AgNPs through the interaction of culture filtrate with silver nitrate (AgNO 3). The successful fabrication of the nanoparticles was assessed using ultraviolet- visible spectroscopy which showed the presence of a localized surface plasmon resonance peak at 390nm and Fourier- transform infrared spectroscopy which showed the presence of functional groups engaged in the reduction and capping processes. The third, fourth and fifth instar larvae were subjected to oral administration by feeding castor leaves sprayed with AgNPs at the concentration levels of 10 it 100mg/ml -1 per each time of 6, 12, or 24 hours. Two-way analysis of variance was performed on mortality data to be carried out with post-hoc test of Dunnett. There was also a complementary topical assay that tested the effectiveness of direct dermal contact. Findings: Spectroscopic analysis helped validate the existence of the desired 390nm plasmon band, whereas FT-IR spectra helped support the presence of biomolecule-containing hexafluorides under tying the nanoparticles. The larval activity was dose- and time-dependent, with a 100mg/ml concentration yielding an approximate of 99 percent of mortality in the third instar, 98 per cent in the fourth instar, and 87 per cent in the fifth instar after 24 hours. The mortality rate of the topical application was approximately 60 0 -150 0 after 24 hours, which made the organ exposure significantly more efficient.

Conclusions: The use of *T. reesei* makes the economical and environmentally friendly production of AgNPs economical yet effective in terms of larvicidal activities against the oblique insect, *S. obliqua*. These findings support fungus-mediated AgNPs as potential sustainable biopesticides; non-the less, additional research on them including a larger host spectrum and field-testing is necessary to be used in India in the future.

***Keywords: Trichoderma ressei*, Entomopathogens, Silver-Nanoparticles, *Spilosoma obliqua*, Biological Pest control, Larvicidal activity.**

INTRODUCTION

India's economy is largely dependent on agricultural productivity, with nearly two-thirds of the population related to and dependent on agriculture (https://www.pib.gov.in, 2022). Therefore, increasing and decreasing productivity is the main concern. Many sources that lead to crop loss but, insect pests are the agents responsible for major crop injuries and thus greatly affect agricultural productivity. Different research has revealed that the environmental hazards of these synthetic pesticides, (Subashini *et al.*, 2004). This has prompted people to look for some safer options and this follow-up has led to the search for some eco-friendly and bio-rational alternatives.

Nanoparticles are reported as significant task in biotechnology, pharmaceuticals, food products, medical services, electronics, personal hygiene, etc. Silver-nanoparticles (AgNPs) shows very effective for their antibacterial, antifungal, larvicidal and ant plasmodial properties (Elumalai *et al.*, 2010, Saxena *et al.*, 2010). However, rigorous methods are used to fusion of nanoparticles using physical and chemical methods including very high temperature pressures, substantial energy inputs, and various highly toxic chemicals. On the other hand, biosynthesis of nanoparticles using entomopathogenic agents has been found to be cheaper and eco-friendly (Casida *et al.*, 2005).

As the size decreases, the nanoparticles result in a larger surface area respected to volume ratio. Surface-area is not suitable for its catalytic activity and other related biological active characters such as antifungal (Ales Pana *et al.*, 2009), anti-HIV (Lara *et al.*, 2010), antimicrobial (Fayaz *et al.*, 2010; Wen-Ru *et al.*, 2010), antiviral (James *et al.*, 2008), mosquito larvicidal (Sap-Iam *et al.*, 2010) properties of AgNPs. Silver NPS is a nano-shaped particle of silver (Ag), with sizes ranging from 1 nm to 100 nm. Although they are frequently regarded as AgNPs, they are sometimes, formed by a very high percentage of silver oxide due to the proportion of large amounts of Ag atoms from the surface (Lu *et al.*, 2011).

Instead of using physical and chemical methods, nanoparticle N clichés are made from biological methods using various plant extracts, microorganisms, enzymes, etc., which is an eco-friendlier method. (Mohanpuria *et al.*, 2008). Nanoparticle synthesis by plants is often beneficial compared to various biological approve norms, as a result it excludes the flower process of maintenance of cell culture and may even be suitably scaled-up for important NP combinations. (Shankar *et al.*, 2004).

In central and north part of India, various pests affect the cotton crop, rice, wheat etc. but the major pest affecting these crops are *Spilosoma obliqua* also called as the Bihar hairy caterpillar or jute hairy caterpillar. The variety of chemical pesticides and insecticides are utilised for the effective management *S. obliqua*; consequently, harmful effects of these pesticides and insecticides, there is need for more eco-friendly alternative, this may be utilised to safeguard these crops from pests and insects. The *S. obliqua,* cotton bollworm, corn earworm is Lepidopteran moth, the larvae of which feed on a various plant, which include many important cultivated crops. It is a significant blighter in cotton and the foremost polyphagous and cosmopolitan blighter species, accounting for financial losses of about five crores in India (Manjunath *et al.*, 1985)

The objective of the present study was to synthesize and characterize silver nanoparticles (AgNPs) using *T. reesei* and to evaluate their larvicidal efficacy against different instars of *S. obliqua*.

MATERIALS AND METHODS

Preparation of *Trichoderma ressei* culture: The fungus *T. ressei* was obtained from MTCC. *T. ressei* plate culture in potato dextrose agar (PDA) media at 28 C with 6 different concentrations in Petri plates for 120 hrs.

Preparation of cultural extract: For nanoparticle synthesis, the fungus spore revives in 250 *mL* conical flask, in 100 *mL* of Potato Dextrose Broth (PDB) medium, The mixture was stirred continuously using rotary shaker

(IKA KS 260) at 25–28 *C* with 150 *rpm* for 72 hours. The reason to employ the PDB was to achive higher yield of *Trichoderma reesei.*

The mycelial part Separated from its culture by filtering it through a sterile filter paper with pore size about 11µm. The mycelial mass thus harvested was subsequently utilised for the synthesis process.

Synthesis of silver nanoparticles: The synthesis of AgNPs was accomplished by mixing silver nitrate solution in the filtrate. Silver nanoparticles were formed, as physical evidenced by a colour change and further endorsed by spectrophotometrically. The effect of concentration on silver-nanoparticle synthesis was observed by adding varying concentrations of $AgNO_3$ to the filtrate and oxidising at 40°C in the dark. The resulting silver nanoparticles were separated by centrifugation and used for further studies.

Characterization of AgNPs: The UV–visible bands were noted on double-beam spectrophotometer (SL- 210 double beam) from 300 to 800 nm. The double-distilled water served as a blank, and the sample for analysis was meticulously prepared on carbon-coated copper grids.

Culture and maintenance of Lepidopteran pest: Lepidopteran larvae (*Spilosoma obliqua*) samples were collected from diverse locations, encompassing urban, rural, and semi-urban areas of Bajali district Assam (26.49748° N, 91.17625° E), India and reared in purified buckets and fed with Castor leaves. From these field-collected larvae, the culture was established in laboratory at optimum temperature (25±3 ºC) and humidity (65±5%). The larvae of *Spilosoma obliqua* were maintained as per the regular method. For the present research work, *Spilosoma obliqua* was considered because it the major crop damaging polyphagous pests in India.

Implementation of synthesized silver nanoparticles on Lepidopteran larvae

The synthesized AgNPs was treated Castor leaves and feed these larvae. The different instars were feed separately in their favourable condition. Different concentrations were applied in different instars for different period.

RESULTS

Result after enrichment of *Trichoderma ressei* culture: *T. ressei* inoculates are enriched in PDA media for 7 days in 28° C after 7 days, good amount of fungus was grown in that plates. After observing those fungus under compound electronic microscope, these was confirmed that those fungus were *T.ressei* (filaments).

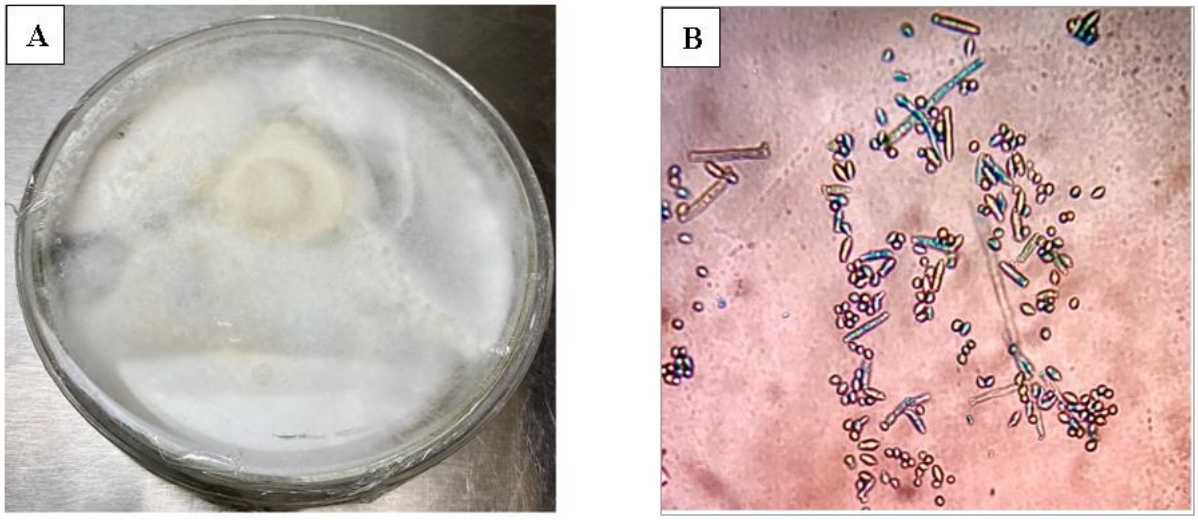


Figure 1: A. Culture plate of *Trichoderma ressi*. B. 40x View & C.10x view of *T. ressei* after enrichment.

Result after reviving of *T. ressei* in culture plates:

T.ressei strands are cultured for the colony formation in cultured plates for 14 days in 28°C. After 14 days some grayish colonies of filamentous fungi were observed. After observation of these fungi under microscope it was confirmed that cultured fungus was *Tricoderma ressei.*

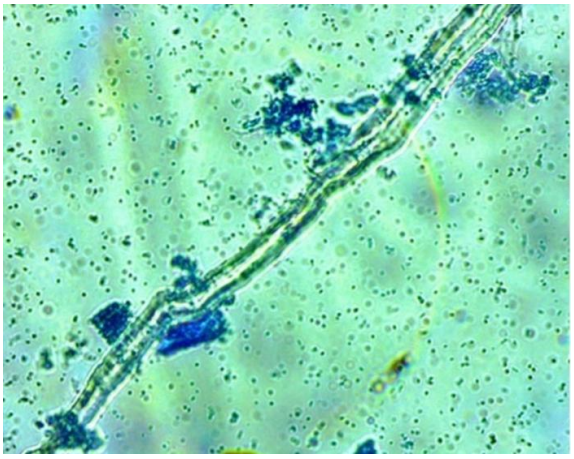


Figure 2: 100x view of *Trichoderma ressei* after reviving in PDA media in petri plate.

Figure 3: 40x view of *Trichoderma ressei* after reviving in PDA media in petri plate.

Primary conformation of Silver-Nanoparticle:

The primary conformation of AgNPs was done by its colour change. When the MFCF wet biomass of fungus integrated with Silver Nitrate solution and after incubation the colour of the solution changed from lime to deep brown. It confirms that bio reduction of $AgNO_3$ had taken place inside this bottle.

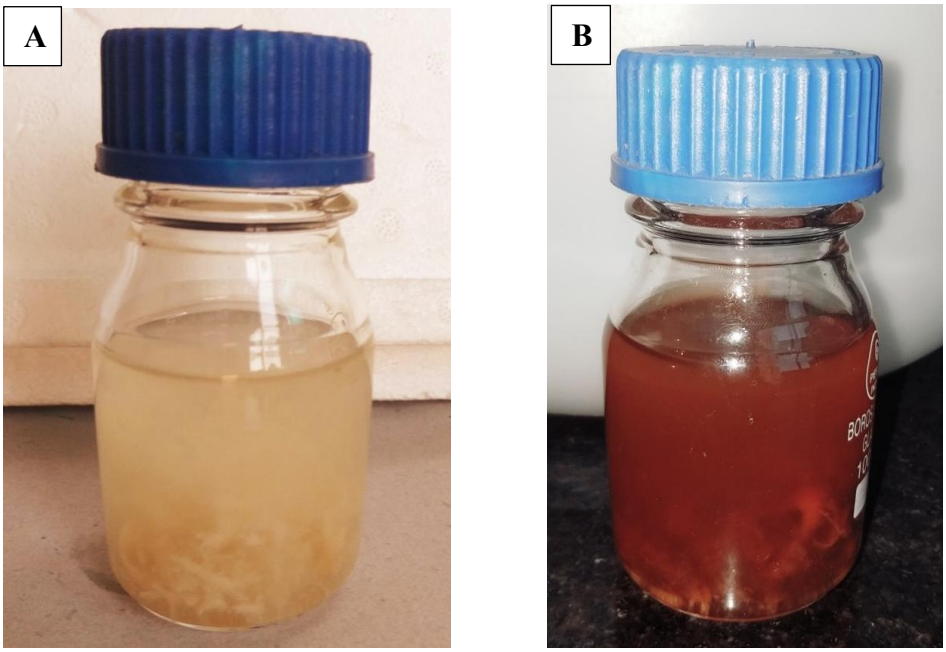


Figure 4: *Trichoderma ressei* biomass before **(A)** and after **(B)** mixed to Ag+ ions for 120 hours.

Secondary conformation of Silver-Nanoparticles with the help of optical Spectroscopy measurements:
Ultra-violet visible spectroscopy: The secondary conformation of silver nanoparticle was done firstly by UV Vis spectroscopy. This bio-reaction was monitored by SL- 210 double beam UV visible spectrophotometer at a range of 300nm to 800 nm.
Silver nanoparticle synthesized from the *T. ressei* and $AgNo_3$ showed a maximum absorption at λ_{max} 390nm. So, from this peak value we elucidated that the synthesized solution contains silver nanoparticles.

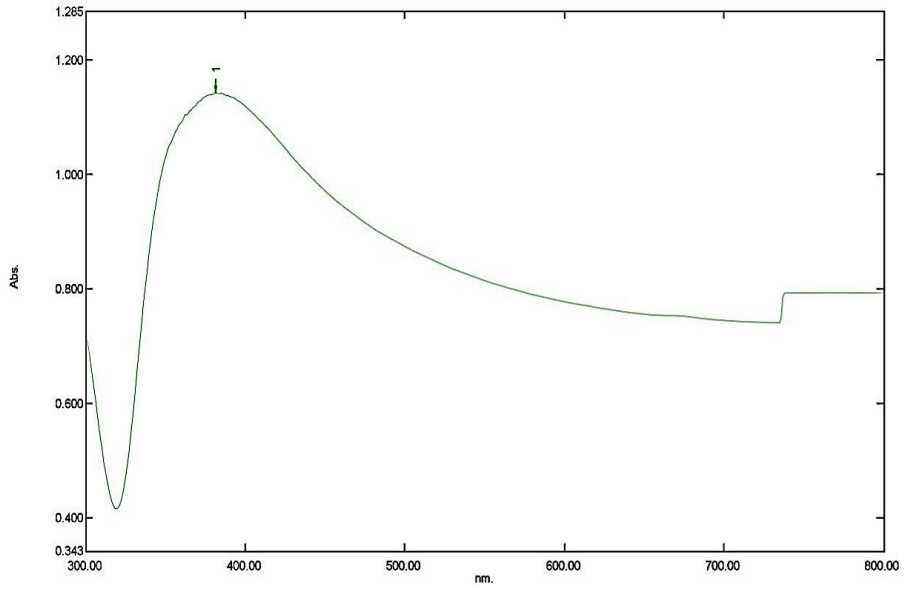


Figure 5: UV vis spectroscopy of AgNPs synthesized from *Trichoderma ressei*

Fourier-transform infrared spectroscopy (FTIR):

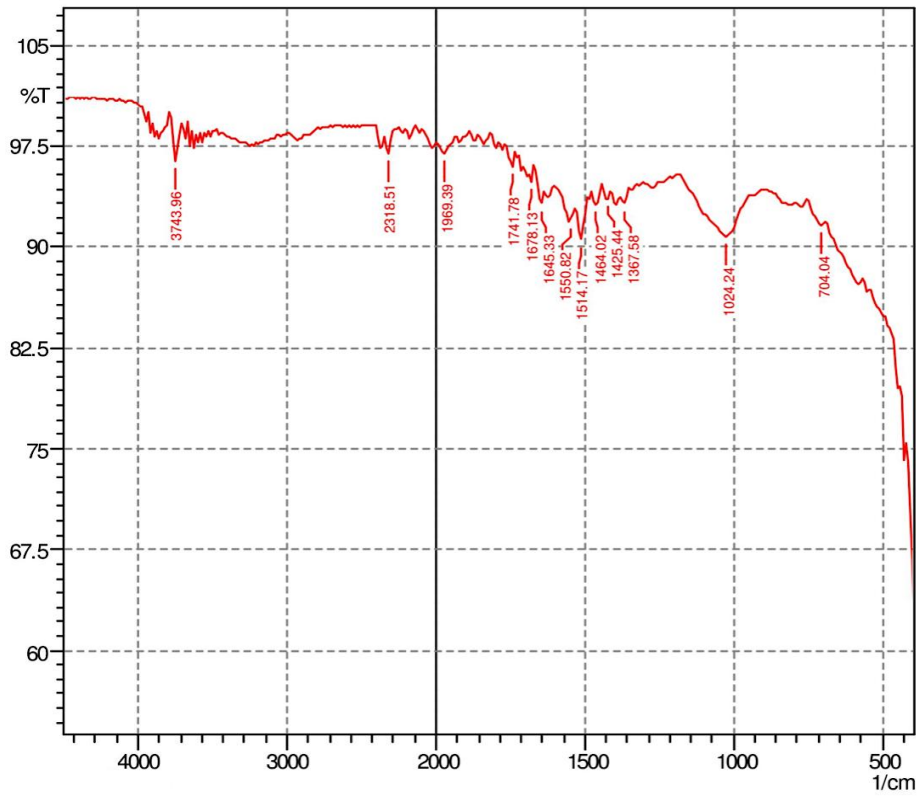


Figure 6. FTIR analysis for *T. ressei* synthesized AgNPs

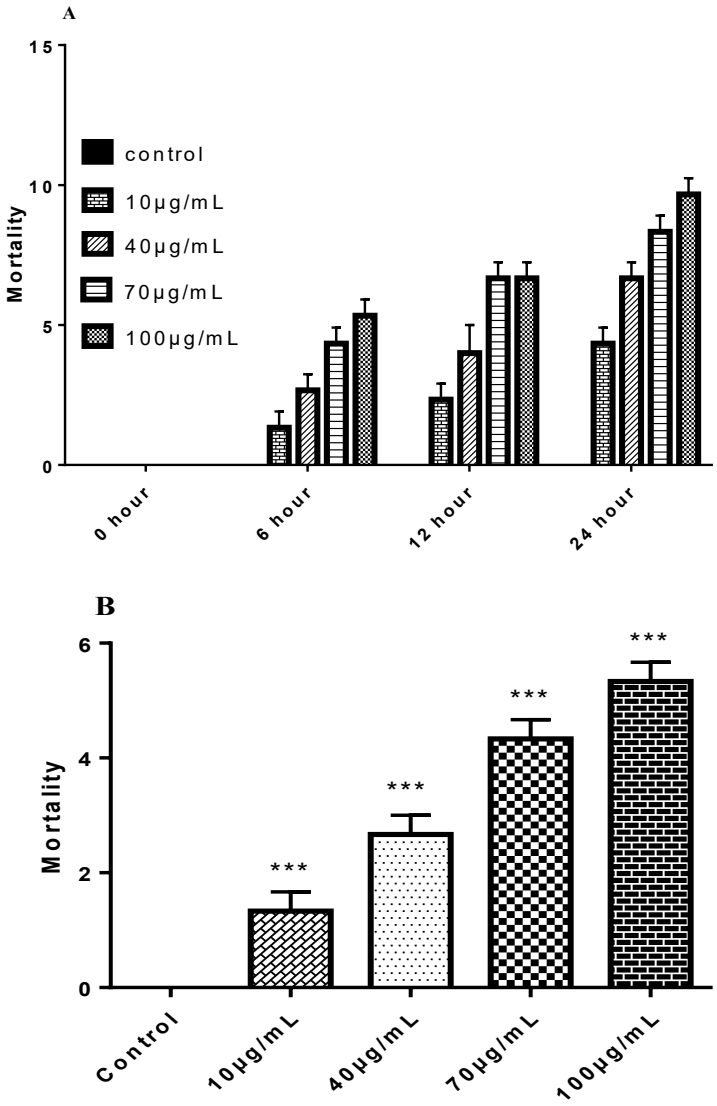
After performing the UV Vis Spectroscopy of synthesized AgNPs, the FTIR was done for the high-spectral-resolution data over a wide spectral range. FTIR analysis of the *T. ressei s*ynthesized nanoparticles showed the major peak in both fingerprint region as well as functional group region. This sample showed the major peak at 1024.24, 1514, 2318.51, 3743.96 cm^{-1}. The strong broad absorption peak at 1024.24 due to CO-O-CO stretching of anhydride, strong absorption peak at 1514 due to N-O stretching, the strong and sharp peak at 2318.51 due to O=C=O stretching of carbon di-oxide and medium sharp at 3743.96 cm^{-1} for the O-H stretching of alcohol group.

Result after implementation of silver nanoparticles on *Spilosoma obliqua* larvae:

After implementation of different concentrations of synthesized AgNPs on these Lepidopteran larvae (pest), we observed the mortality rate of these species in different instars after various exposure times.

Firstly, no mortality was observed in the larvae that were fed the normal diet, *i.e*, the castor leaves.

Secondly, by using 10µg/ml concentration in 3rd instar larvae, although the mortality was significant as compared to control, but only a few larvae were affected by AgNPs at 6hrs time duration. Therefore, it can be said that the efficacy is less in this concentration after 6 hrs. Further, we observed the application of higher concentrations like 40, 70 and 100 µg/ml on these larvae, the efficacy or mortality rate although increases but it is still not reasonable. Thereafter, we increased the exposure time and used the same concentrations for 12 hrs and then 24 hrs time duration (Figure 7.1 A). Increasing exposure time yielded better result in all the concentrations used. For third instar stage of this pest, observed that at 100 µg/ml concentration, the survival rate of these insects was very less after 12 hrs (Figure 7.1 C), and after 24 hrs (Figure 7.1 D), 99% mortality rate in this Lepidopteran species was recorded.



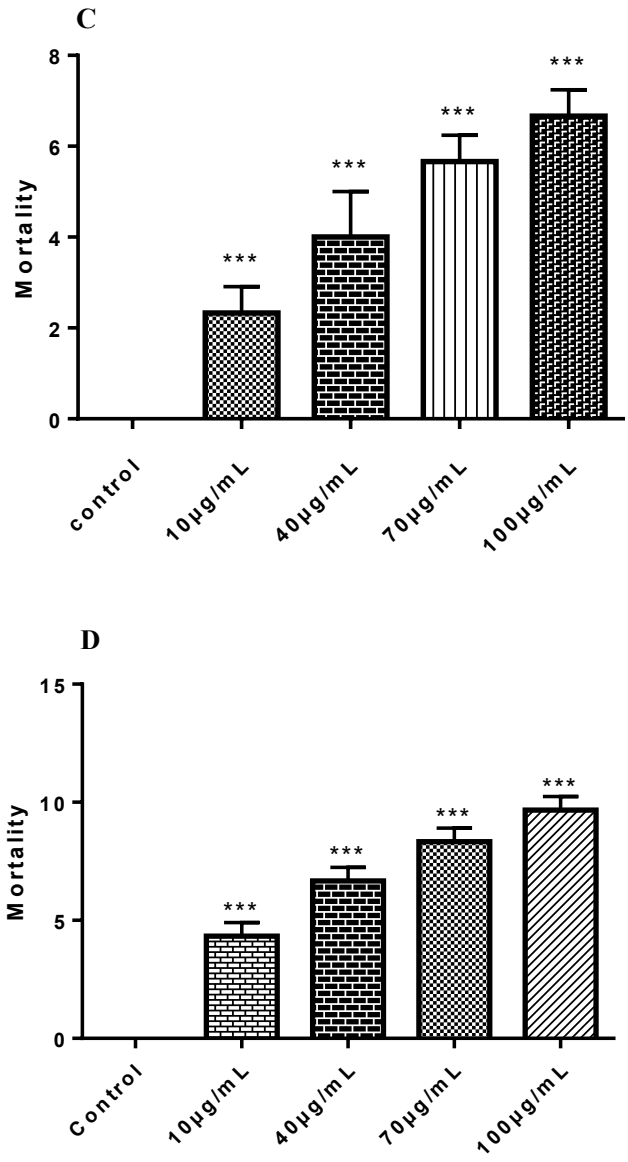
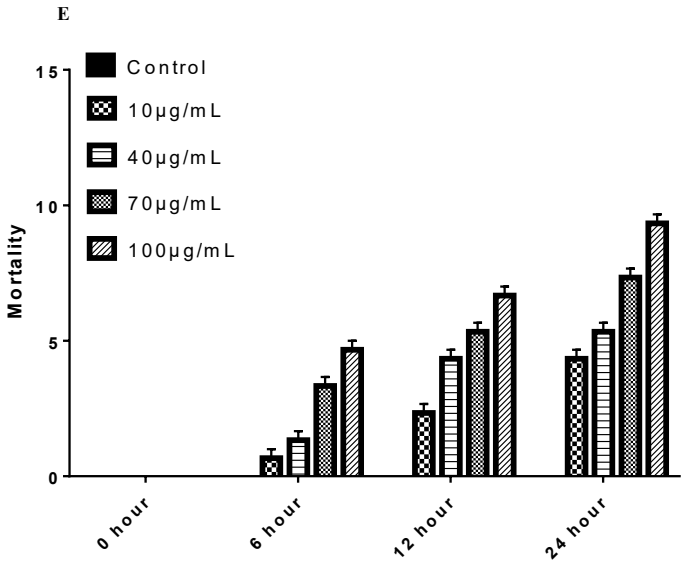


Figure 7.1 : The graph (A) represents the larvicidal activity of silver nanoparticle against fifth instar larva after 0hr, 6hr, 12hr, 24hr. The data represent the mean mortality (n= 30) induced by the treatment, the statistical analysis of which was done by two-way ANOVA test followed by Dunnett's comparison test , the graph (B) represents the effect after 6 hrs., the graph (C) represent the effect after 12 hrs., the graph (D) represent effect after24 hrs. *P≤0.5,**P≤0.01, ***P≤0.001 *vs* control.

Graph (7.1 A) indicates the comparison of mortality of 5th instar larvae; it was caused by different concentration of *T.ressei* synthesized AgNPs after different time intervals. No mortality found in controlled groups. In conc.10µg/ml, the mortality rate of the pest was increased with the time exposure. In 20µg/ml, high mortality rate was noted after 24 hrs as compared to 6 and 12 hrs. Same trend was followed by rest of all concentrations. Highest mortality was observed in 100µg/ml after 24 hrs. *i.e* 87%.
The graph (7.1 B) indicated the effect of AgNPs against lepidoptera larvae after 6 hrs. The mortality was observed in all concentration of AgNPs and it was also significant in all concentration. Similarly, the graph (7.1 C) indicates the larvicidal activity of AgNPs after 12 hrs of treatment. The mortality was significant in all concentration. The graph (7.1 D) represents the larvicidal activity of AgNPs after 24 hrs. The mortality was also significant in all concentration as compared to control.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6 hour
12 hour
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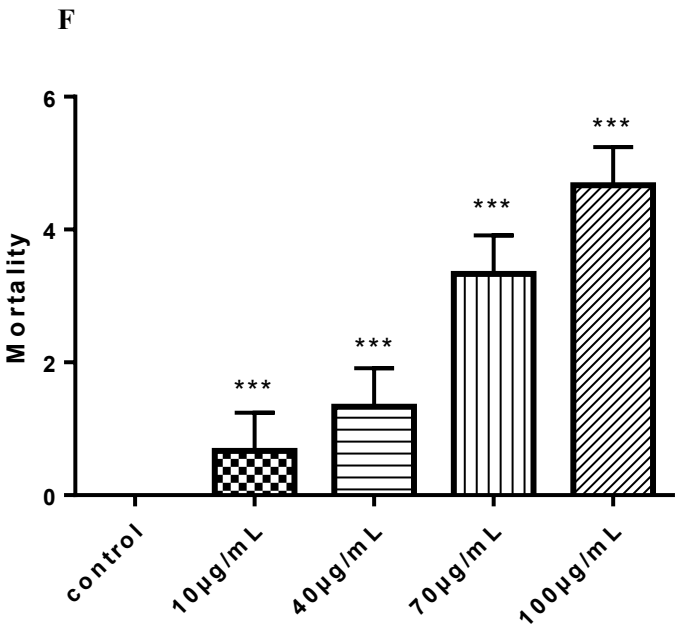
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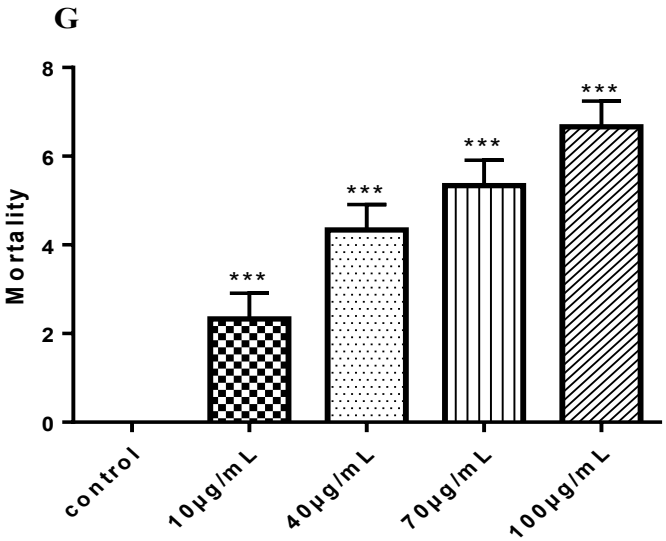
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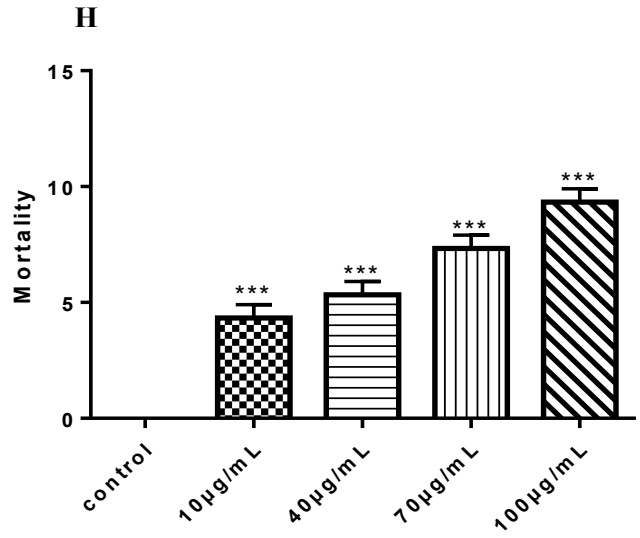
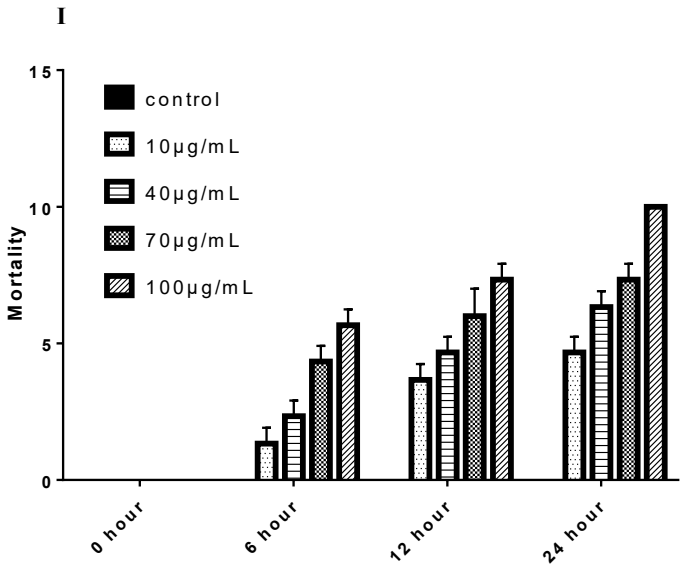


Figure 7.2: The graph (**E**) represents the larvicidal activity of silver nanoparticle against third instar larva after 0hr, 6hr, 12hr, 24hr. The data represent the mean mortality (n= 30) induced by the treatment, the statistical analysis of which was done by two-way ANOVA test followed by Dunnett's comparison test , the graph (**F**) represents the effect after 6 hrs, the graph (**G**) represent the effect after 12 hrs, the graph (**H**) represent effect after24 hrs. *P≤0.5,**P≤0.01, ***P≤0.001 *vs* control.

Graph (**7.2 E**) indicates the comparison of mortality of 4th instar larvae, caused by different concentration of *T. ressei* synthesized AgNPs after different time intervals. There is no mortality found in controlled groups. In conc.10µg/ml, mortality rate increases with increased time exposure. In 20µg/ml, high mortality was observed after 24 hrs as compared to 6 and 12 hrs. Same trend was followed by rest of the concentrations. Higher mortality was observed in 100µg/ml after 24 hrs. *i.e.*, 92%

The graph (**7.2 F**) indicated the effect of AgNPs against lepidoptera larvae after 6 hrs. The mortality was observed in all concentration of AgNPs, and it was also significant in all concentration. Similarly, the graph (**7.2 G**) indicates the larvicidal activity of AgNPs after 12 hrs of treatment. The mortality was significant in all concentration. The graph (**7.2 H**) represents the larvicidal activity of AgNPs after 24 hrs. The mortality was also significant in all concentration.



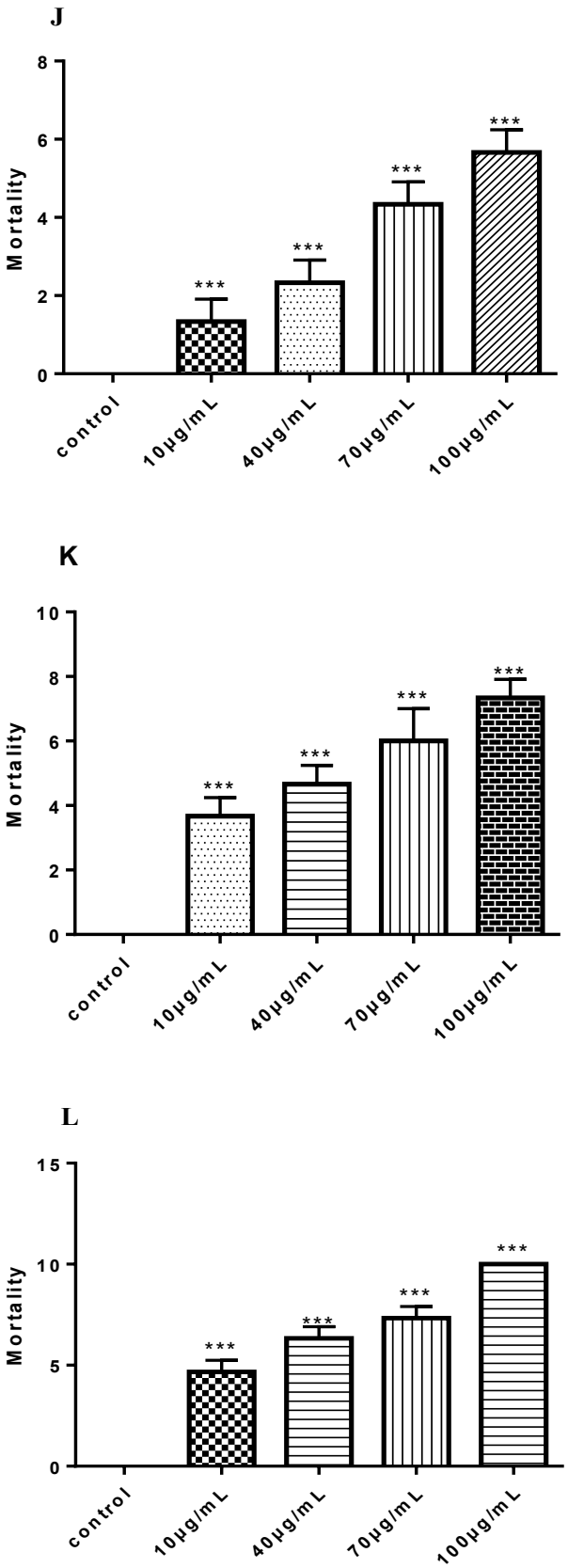


Figure 7.3: The graph **(I)** represents the larvicidal activity of silver nanoparticle against third instar larva after 0hr, 6hr, 12hr, 24hr. The data represent the mean mortality (n= 30) induced by the treatment, the statistical analysis of which was done by two-way ANOVA test followed by Dunnett's comparison test , the graph **(J)** represents the effect after 6 hrs., the graph **(K)** represent the effect after 12 hrs., the graph **(L)** represent effect after24 hrs. *P≤0.5, **P≤0.01, ***P≤0.001 *vs* control.

Graph **(7.3 I)** indicated the comparison of mortality of 4th instar larvae, caused by different concentration of *T.ressei* synthesized AgNPs after different time intervals. There is no mortality found in controlled groups. In conc.10µg/ml, mortality rate increases with increase the time exposure. In 20µg/ml, high mortality was observed after 24 hrs as compared to 6 and 12 hrs. Same trend followed by rest of all concentrations. Higher mortality was observed in 100µg/ml after 24 hrs. *i.e* 97 %. Both time dependent and dose dependent responses were observed.

The graph (**7.3 J**) indicated the effect of AgNPs against lepidoptera larvae after 6 hrs. The mortality was observed in all concentration of AgNPs and it was also significant in all concentration. Similarly, the graph (**7.3 K**) indicates the larvicidal activity of AgNPs after 12 hrs of treatment. The mortality was significant in all concentration. The graph (**7.3 L**) represents the larvicidal activity of AgNPs after 24 hrs. The mortality was highly significant in all concentration against control.

Figure 8. The larvicidal activity of synthesized AgNPs in 3rd instar stage at 100 concentrations for 24 hours (Stereo microscopic image)

Similar observations were made in the case of fourth instar larvae of this pest. By using 10 µg/ml AgNPs concentrations in 4th instar larvae, only a few larvae were affected at 6hrs time duration (Figure 7 E), and using 40, 70 and 100 µg/ml on those larvae, the efficacy or mortality rate increased with increasing concentration. For this instar stage it was observed that efficacy at 100 µg/ml concentration (Figure 7 H). The mortality was increased gradually with increasing the time and concentrations (Figure 7 F, G, H), and at 24 hrs time for100 µg/ml, it shows 98% mortality rate of 4th instar of this Lepidopteran pest (Figure 7 H).

We also performed the experiments on the 5th instar larvae, and it was observed that the mortality rate is a little bit less on this 5th instar as compared to 3rd and 4th instar (Figure 7 I). Before 12 hour and 70 µg/ml conc., the synthesized AgNPs was not much effective, but in 100 µg/ml conc. for 24 hrs. it was observed a high significant result on these larvae (Figure 7 L).

From the above-mentioned results, it can be suggested that the synthesized AgNPs have the potential to act as a larvicide, and they specifically target the larval life stages.

Lastly, the synthesized AgNPs were directly applied (topical application) on these larvae. After 24 hrs, after the treatment it was observed a significant result. There are about 60% mortality occurred due to this topical application on these larvae.

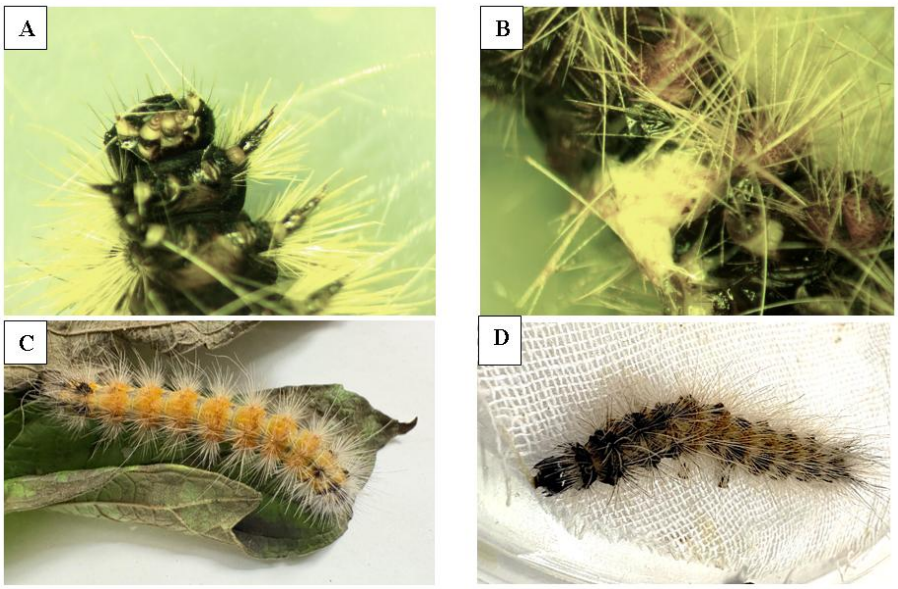


Figure 9. (A) & (C) Normal anterior portion of 4th instar before treatment and (B) & (D) Damage of anterior portion and external morphology of 4th instar after the direct treatment of AgNPs.

DISCUSSION AND CONCLUSSION:

There are many farmers who have been using parasites and predators for biological pest control. Certain microorganisms also have the potential to kill insects and can be thus used in various pest management programmes. These entomopathogenic agents include fungi, bacteria, nematodes, and viruses. Entomopathogens act as natural regulation method of controlling various insect populations. (Hajek, *et al,.*2004). Most of the research in this direction is concerned with the finding of entomopathogenic agents and their use as biological pest control. Various entomopathogens equivalent to chemical pesticides are used to control various insect inhabitants. (Dentrt *et al*., 2000; Hajek *et a.,* 2004). Use of Entomopathogens is a traditional process, and it acts as an organic controlling agent of unfamiliar insect pests (Hajek, *et al.,* 2004).
In the present study, we tried to select a perfect entomopathogen for the synthesis of silver nanoparticle, one that is easy and cost-effective to synthesize. This led us to the synthesis of AgNPs from entomopathogenic fungus.
In green synthesis of AgNPs there are some parameters that should be maintained by a researcher. First, rate of production, secondly, it should be eco-friendly and lastly, it should be cheap, so that people can easily apply the product for pest control.
In the present research, we used entomopathogenic fungus, *Trichoderma ressei*. Different researchers have used different entomopathogenic fungus for the synthesis of different Nano metals. Some have used *Epicoccum nigrum,* isolated from *Phellodendron amurenso* to synthesize AgNPs, and it showed toxic activity against the pathogenic fungi. The synthesis of gold nanomaterial's (AuNPs) has also been achieved by the bio-reduction of Chloroauric acid (HAuCl4) using the fungal culture filtrate (FCF) of *Alternaria alternata*. Synthesis of AgNPs using, *Trichoderma asporellum*, an entomopathogenic fungus showed effective larvicidal and pupicidal activity against the dengue vector, *Aedes aegypti* (Mukherjee *et al.,* 2008).
Trichoderma has an advantage over other fungi because it assists to defend plants against molds and bacteria. *T. ressei* creates a barricade that makes it impossible for harmful bacteria and pathogens to pass through it. *Trichoderma* work well with other microbes, binding up with anything and, thus, increases the health of the rhizosphere. It will exactly dissolve any pathogenic fungi without any adverse effects.
Trichoderma has a lifespan of about 28 days. It reproduces itself repeatedly all on its own, but the quality of its enactment will fade over time. The basic significance is that *Trichoderma* is natural, and it doesn't hurt plants in the suggested dosages. (Sundaravadivelan and Padmanabhan, 2013)
After enrichment of *T. ressei*, we cultured it in PDA media for growing the colony. After growing the fungus successfully, the vegetative part was taken into Glucose casein hydrolase broth for further high amount of production. GC medium was employed because the growth of *Trichoderma reesei* is more in the GC hydrolase broth than any other culture media (Kabath *et al.,* 2011). 10g/L of *T. ressei* biomass was produced. In the current research, using this broth, approx. 8g/L of fungal biomass was obtained. This outcome may vary with the purity of GC broth and the purity of the mycelia used for the mass culture.

During this biological synthesis of silver nanoparticles by a fungus, many enzymes are formed which decrease the salt to its Ag^+ solid nanoparticles through the catalysis. As compared to the other filamentous fungi, the *T. ressei* is measured to be the most operative extracellular enzyme manufacturer, and it has a long antiquity in the production of commercial enzymes (T.Oksanen *et al., 2000*).

Based on present study, it can be concluded that the biological synthesis of AgNPs by *T. reesei* is favoured for its safety, being inexpensive and its extensive production potential.

As discussed above, we can synthesize AgNPs biologically on a large scale by using *T. reesei*, which have the great profit over any other fungus culture method. Some studies reveal that *T. reesei* is not injurious to humans. From *T. reesei*, the production of extracellular enzymes and nanoparticles is more effective than other fungi also it is noticeable that *T. ressei* is much easier to handle and culture and for its high growth rate it is useful to both industrial and laboratory condition as well as having low cost in large scale production. There is different process to produce AgNPs, out of them chemical vapour deposition, liquid solution reduction, irradiation usually produce large particles which are micrometres in size. These other methods have lower production and high-cost value to produce AgNPs when compared to the process that we have used to synthesis AgNPs (Baker *et al* 2000, Balaprashad *et al* 2005, Mukherjee *et.al*2008)

After the synthesis of AgNPs, it was observed that the solution turns from colourless to brown. This indicates the bio reduction of Ag ions into that solution. But it can't confirm that the formed solution contains Ag nanoparticle because the fungus contains different enzymes that may be extra cellular or intracellular. These enzymes may have reacted with the free Ag^+ ion present in $AgNO_3$ and thus resulted in the change in coloration. For further confirmation, we used UV Vis spectroscopy, which confirmed λ_{max} at 390 nm. According to some previous studies, AgNPs have their absorbance between 300 to 800 nm. So, from this point of view the synthesized nanoparticle were indeed AgNPs. The absorbance range may vary due to the clearance of test sample. So, before mixing $AgNO_3$ with *Trichoderma* fungal biomass, it should be made sure that no mycelia are present in the wet biomass.

After characterization of AgNPs, the synthesized AgNPs was applied on Lepidoptera larvae. For our experiments, *Spilosoma obliqua*, commonly known as The Bihar Hairy Caterpillar were employed because, now a days *S. obliqua* has taken the top position for crop damage in India. This lepidopteran pest species gregariously feeds on the juvenile leaves of pea, wheat, maize, rice plants etc. This causes a high economical loss to the farmers. There are different lepidopteran pests like *Helicoverpra armigera*, *Spodoptera litura,* etc. But due to the high resistibility to other pesticides, nowadays chemical control of pest has become an environmental hazard. In the present research, the notable observation was, NPs have high efficacy on Lepidopteran species. Further, these NPs cause time and dose dependent mortality.

Firstly, the efficacy was observed after the topical application of the synthesized NPs and then the insects were exposed to these NPs orally via their food. It is noteworthy to mention that the direct or the topical application on these NPs was not as efficient as when they were provided via food. This may be because these fungi directly act in the intestine of the larvae and damage their intestine (shown in Figure. 9)

Based on present study, the mycological extraction of AgNPs by *T. reesei* is favoured for its safety, being inexpensive and its extensive production potential.

Further, the results of this research make it clear that entomopathogen mediated synthesis of silver nanoparticle have ther ability to resist the Lepidopteran pests by showing their larvicidal activity. During this research we took 4 different concentrations of synthesized AgNPs. Out of them 100µg/ml conc. shows the most effective result. The mortality of lepidopteran pests was higher in 100µg/ml solution than other concentrations. In this study, we have reported the efficacy of AgNPs on a Lepidopteran pest species, but their efficacy also needs to be evaluated in other pests belonging to different order such as, Hymenoptera, Coleopteran, Hemiptera, etc. Therefore, a further scope of the study to see whether the green-synthesized entomopathogenic AgNPs are also efficient for the control of other pest species, or they act only on a specific group of insects only.

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Ethics statement: All experiments were conducted following institutional and national guidelines for the ethical use of insects in research

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Data Availability Statement
The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

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