Research Article

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Biosynthesis and characterization of selenium and silver nanoparticles using *Trichoderma viride* filtrate and their impact on *Culex pipiens*

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Abstract: Some of the significant globally prevalent vector-borne illnesses are caused by *Culex pipiens*. Synthetic pesticides have been widely utilized to eradicate *C. pipiens*, which has led to a number of health risks for people, insect resistance, and environmental contamination. Alternative strategies are therefore vitally needed. In the current investigation, the *Trichoderma viride* fungal culture filtrate was used to create selenium and silver nanoparticles (SeNPs and AgNPs, respectively) and test them on *C. pipiens* larvae in their fourth instar stage. The death rate increased significantly when SeNP and AgNP concentrations increased, according to the results. SeNPs and AgNPs significantly affected the developmental and detoxification enzymes in fourth instar larvae of *C. pipiens* at 24 h after being treated with the sublethal concentration of the tested NPs. As a result of their insecticidal effect on *C. pipiens* larvae, SeNPs and AgNPs are considered effective and promising larvicidal agents.

Keywords: green synthesis, nanoparticles, *Trichoderma viride*, *Culex pipiens*, larvicidal agents

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1 Introduction

Egypt has a large population of *C. pipiens*, which is thought to be a vector for a variety of illnesses such as filariasis, West Nile fever, and Rift Valley fever [1,2]. The current global plan to combat illnesses spread by mosquitoes includes controlling this vector [3]. The traditional class of insecticides has a number of significant drawbacks, including high dose per unit crop, drift risks, operational risks, and residues in the environment, plants, and marketable product, as well as an adverse impact on non-target vegetation and non-target species. In order to address the aforementioned gaps, they must be replaced with a different pest management method [4,5].

One effective approach to this is nanotechnology [6–8]. The use of environmentally friendly pesticides has drawn attention worldwide as a viable substitute. When the substance is prepared as nanoparticles, water solubility, dissolution rate, and diffusion uniformity are significantly increased upon administration without causing any chemical changes to the pesticide molecule [9]. The material's saturation solubility is increased as the particle size is reduced to the nanoscale. The surface area dramatically increases once a large reduction in particle radii is accomplished by nanosizing, leading to considerably quicker dissolution [10].

The nanosilver (nano-Ag) particle is the most widely utilized nanoparticle (NP) [11–13]. Silver, zinc, copper, and titanium are the metals that are most frequently used as NPs [14–20]. Researchers interested in nanotechnology like AgNPs because they have antibacterial and antiviral properties [21–23]. Their predilection is heightened by their low toxicity, intrinsic charge, greater surface area, and crystalline structure [24]. In a certain quantity, selenium (Se) is an essential element for individuals, plants, and animals. This element plays a crucial part in how plants normally function, protecting them from a variety of stressors [25,26]. Due to their effectiveness in reducing a number of biotic and abiotic stresses, such as metals, salt, dryness, and warmth, as well

as their capacity to inhibit phytopathogenic microorganisms, Se particles in the nanometer scale (SeNPs) have recently attracted increasing interest, especially for plants [27]. *Clausena dentata* leaf extracts were used in the effective green production of SeNPs. The NPs were shown to have potent mosquito larvicidal action in a dosedependent manner when applied at extremely low concentrations [28]. They can be utilized for making the pesticide formulation. In order to minimize the loss of water from the organism's tissues and avoid death from dryness, insect body walls include a variety of lipids in their cuticle. Insects die as a result of a NP being absorbed in the lipids of the coat by abrasion [29]. *Trichoderma viride* is a highly effective biocontrol agent due to its multienzyme production capacity [30]. This fungus, *T. viride*, grows quickly, is not harmful to humans, and is safe for the environment. For large-scale manufacturing, the synthesis of NPs using the *T. viride* extract is a suitable and straightforward procedure [31]. Herein, this study aimed to biosynthesize both AgNPs and SeNPs using *T. viride* filtrate through a green and ecofriendly method for the first time. Characterizations of biosynthesized AgNPs and SeNPs were done using different techniques. Finally, the toxic effect of SeNPs and AgNPs on the tissues of the fourth instar larvae of *C. pipiens* was examined and clarified with regard to the protein content and the activity of developmental and detoxification enzymes and proposed as promising agents for killing larvae.

2 Materials and methods

2.1 *C. pipiens* laboratory rearing

The Entomology Dept, Faculty of Science, Benha University provided the *C. pipiens* for this study. In the insectary, they were kept at 27°C, 75% RH, and a photoperiod of 14 h light/10 h dark. The ratio of ground bread to fish food (TetraMin) for larvae was 3:1. The developed pupae were then transferred from the porcelain pans to a cup of water (dechlorinated) and put in examined cages (35 cm × 35 cm × 40 cm) where the adults eventually emerged. Female mosquitoes were occasionally fed, while the adult colony was given a 10% sugar solution. The same laboratory settings and continuous access to the fourth instar larvae were maintained during the study [32].

2.2 Biosynthesis of AgNPs and SeNPs using *Trichoderma viride* filtrate

Trichoderma viride filtrate was used in the production of AgNPs and SeNPs because it is a safe biological method, inexpensive, non-toxic, and environmentally friendly. The 2 mM $AgNO_3$ and Na_2SeO_3 were separately added to the *T. viride* filtrate and incubated, and their pH was subsequently adjusted to 9 and 6 to obtain AgNPs and SeNPs, respectively. Following incubation, AgNPs and SeNPs became brown and red, respectively. The final product was separated and dried at 90°C for 24 h.

2.3 Characterization of Se and AgNPs

Powder X-ray diffraction (XRD) patterns of AgNPs and SeNPs were obtained by an X-ray diffractometer with an X-ray source (Cu Kα; λ = 1.54178 Å). At the Faculty of Science, Benha University, FT-IR spectra of the as-prepared AgNPs and SeNPs were recorded using KBr pellets with a Thermo Scientific NicoletiS10 FT-IR spectrometer in the 4,000–400 cm^{-1} range. The as-prepared AgNPs and SeNPs were photographed using a transmission electron microscope (JEOL-JEM 2100).

2.4 Larvicidal bioassay activity

Under controlled laboratory conditions, two batches of AgNPs and SeNPs were evaluated on *C. pipiens* larvae in their fourth instar stage. In order to obtain different concentrations, 1 mL of Se or AgNPs was evenly scattered over 1,000 mL of DW using an ultrasonicator. At doses of 30, 50, and 70 ppm, fourth instar larvae were used to investigate the toxicity of Se and AgNPs. Transferring 20 larvae per concentration into a glass beaker with 250 mL of DW was the standard procedure for all tests. The experiment was run three times in comparison to a group that did not receive any nanomaterial treatment, and after the instar larvae were exposed to the treatments for 24 h, the mortality % was noted [33]. Biochemical analyses were carried out at non-kill concentrations of SeNPs or AgNPs.

2.5 Preparation of AgNPs and SeNPs for biochemical analysis

About 0.5–1 g of fourth-instar larvae equivalent to the weight of 100 larvae was taken from the handled larvae at a sublethal dose of the tested AgNPs and SeNPs and stored at −25°C for no longer than a week in order to undergo the biochemical test for assessing the detoxifying enzymes and protein in the body of larvae. Similar settings

were used for untreated larvae. For biochemical examination, all samples were transported in ice boxes (−20°C) to the Central Lab of the College of Veterinary Medicine.

2.5.1 Total proteins

Bradford's technique [34] was used to calculate the total amount of proteins. The protein reagent was made by combining 50 mL of 95% ethanol with 100 mg of Coomassie brilliant blue G-250. About 100 mL of 85% (w/v) phosphoric acid was added to this solution. A final volume of 1 L was achieved by diluting the obtained solution. Phosphate buffer (0.1 M, pH 6.6) was used to make up the test tube's volume to 1 mL. The test tube was filled with 5 mL of protein reagent, and the mixture was stirred by vortexing. After 2 min and before 1 h, the absorption value at 595 nm was calculated in comparison to a blank made from 1 mL of phosphate buffer and 5 mL of protein solution.

2.5.2 Glutathione stransferase (GST)

The technique of Habig et al. [35] was used to detect the conjugate, S-(2,4dinitrophenyl)-L-glutathione. The mixture contained 200 µL of larval homogenate, 100 µL of GSH, and 1 mL of potassium (K) salt of a phosphate buffer (pH 6.5). About 25 µL of CDNB substrate solution was added to the reaction to begin the process. CDNB and GSH concentrations were 1 and 5 mM, respectively. For 5 min, enzymes and chemicals were left to incubate at 30°C. The nanomolar substrate-conjugated/min/larva was recognized as a molar extinction coefficient of $9.6 \cdot mM^{-1} \cdot cm^{-1}$, and the increase in absorbance at 340 nm was measured against a blank including all the components without the enzyme.

2.5.3 Quantitative determination of peroxidase

According to the method described by Hammerschmidt et al. [36], the peroxidase activity was assessed. About 1.5 mL of pyrogallol (0.05 M) and 100 µL of enzyme extract were added to a spectrometer sample cuvette. At 420 nm, the measurements were reset to zero. About 100 µL of hydrogen peroxide (1%) was poured into the specimen's cuvette to start the reaction. The shift in absorbance$\cdot min^{-1} \cdot g^{-1}$ sample was used to express the activity of the enzyme.

2.5.4 Determination of phosphatases

The Powell and Smith technique [37] was used to measure acidic and alkali phosphatases. In this process, 4aminoantipyrine reacts with phenol generated by disodium phenyl phosphate's enzymatic hydrolysis and gives a distinctive brown color when added with potassium ferricyanide. The amount of pnitrophenyl phosphate that an enzyme can hydrolyze in 1 min at 37°C at a pH of 10.4 for alkali phosphatase and 4.8 for acidic phosphatase is measured in units (U).

2.5.5 Nonspecific esterases

Using naphthyl acetate as the substrate, beta esterases (βesterases) and alpha esterases (αesterases) were identified in accordance with Van Asperen [38]. The chemical mixture contained 20 mL of larval homogenate and 5 mL of substrate solution (3×10^{-4} M or naphthyl acetate, 1% acetone, and 0.1 M phosphate buffer, pH 7). About 1 mL of diazo blue color reagent (made by combining two parts of 1% diazo blue B and five parts of 5% sodium lauryl sulfate) was added after the mixture had been incubated at 27°C for exactly 15 min. For hydrolysis of the substrate to provide either α- or β-naphthol, the developed color was read at 600 or 555 nm, respectively. The typical curves for α- and β- naphthol were created by combining 20 mg of – or -naphthol with 100 mL of phosphate buffer (stock solution) to achieve pH 7. The buffer was used to dilute 10 mL of stock solution to 100 mL. Aliquots of diluted solution in amounts of 0.1, 0.2, 0.4, 0.8, and 1.6 mL were transferred into testing tubes and made up to 5 mL with phosphate buffer. Following the addition of 1 mL of diazo blue reagent, the produced color was assessed as before.

2.6 Statistical analysis

The results of the susceptibility test were visually shown using a probit-log line for regression. The data were statistically analyzed using the probit analysis application (LdP Line). The biochemical analysis was performed using R version 4.2.1. $P < 0.05$ was used as the criteria of significance for each experiment.

3 Results

3.1 Characterization of Se and AgNPs

Figure 1 displays the XRD patterns of AgNPs and SeNPs. Characteristic distinct peaks can be seen in each spectrum. In AgNPs, four peaks appear at 2θ values of 32.5° (111), 46.1°

(200), 66.8° (220), and 76.3° (311) (Figure 1a), while in the case of SeNPs the peaks are observed at 2θ values of 22.8° (100), 30.5° (101), 41.1° (111), 51.1° (201), and 65.5° (210) (Figure 1b).

FTIR analysis was performed on samples of biologically synthesized AgNPs and SeNPs. The peaks for vibrations of N–H or O–H bonds are observed at 3,350–3,210 cm^{-1} in the FTIR spectra of AgNPs and SeNPs (Figure 2a and b). The potential link between these bands is attributed to the stretching vibration of C–H. It is convenient to identify the amide bands (I and II) of proteins or polypeptides around 1,638 cm^{-1}. Peaks between 400 and 600 cm^{-1} are correlated with vibrations of the metal_oxygen bond. In the current study, the formation of AgNPs can be proved by the peaks observed around 420 and 470 cm^{-1} belonging to Ag–O. Moreover, a characteristic band is observed at 500 cm^{-1}, attributed to Se–O.

The TEM image shows that AgNPs are well-dispersed and nearly spherical (Figure 3a). For AgNPs, the particle size is found to be in the range of 14.28–30.58 nm (Figure 3a). In contrast, NPs of Se are noticed to be spherical in shape with a size of 25.4–80.6 nm (Figure 3b).

3.2 Larvicidal bioassay activity

It is clear from our results that SeNPs and AgNPs affect the percentage of observed larval mortality, increasing gradually with the increase of concentration. The estimated LC_{50} values, at 95% probability, were 39.2 and 52 ppm for larvae treated with SeNPs and AgNPs, respectively after 24 h (Table 1).

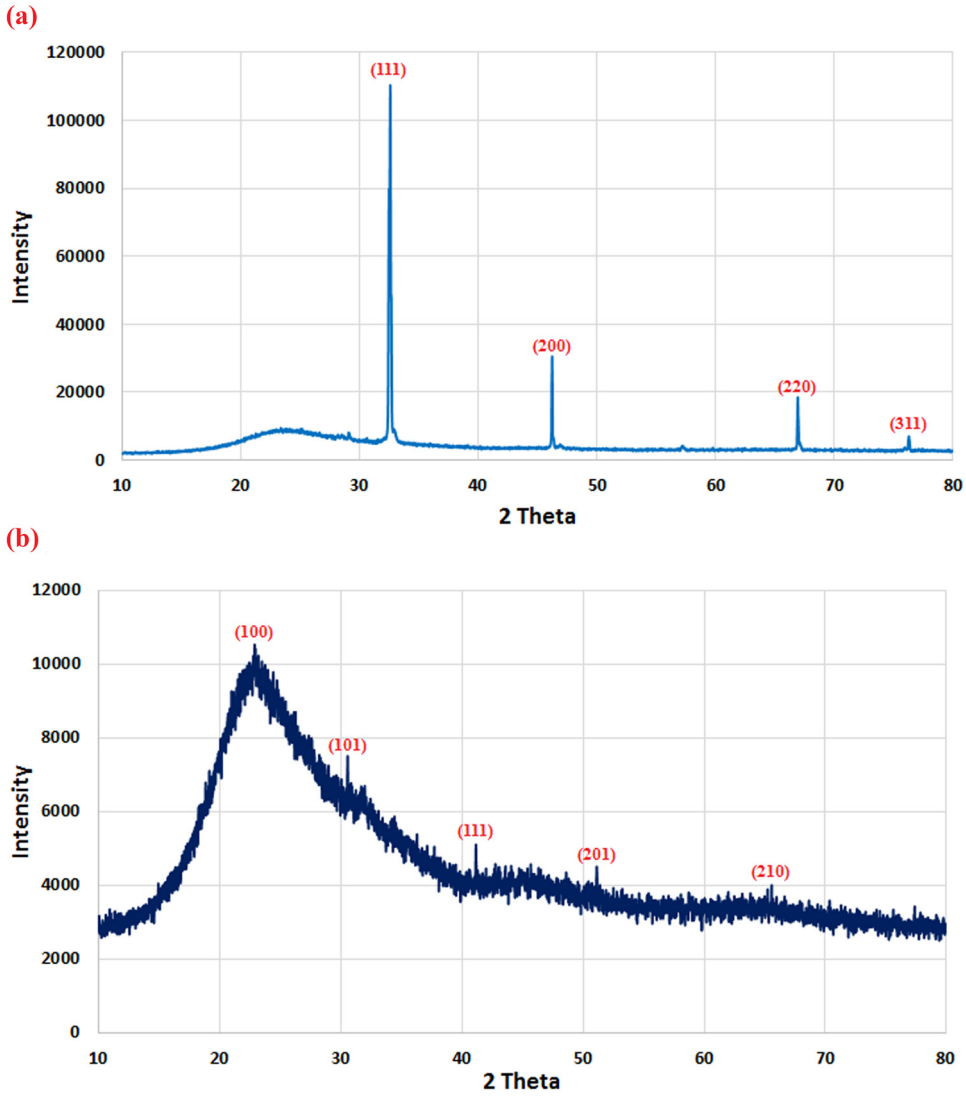

Figure 1: XRD patterns of AgNPs (a) and SeNPs (b) biosynthesized by *T. viride*.

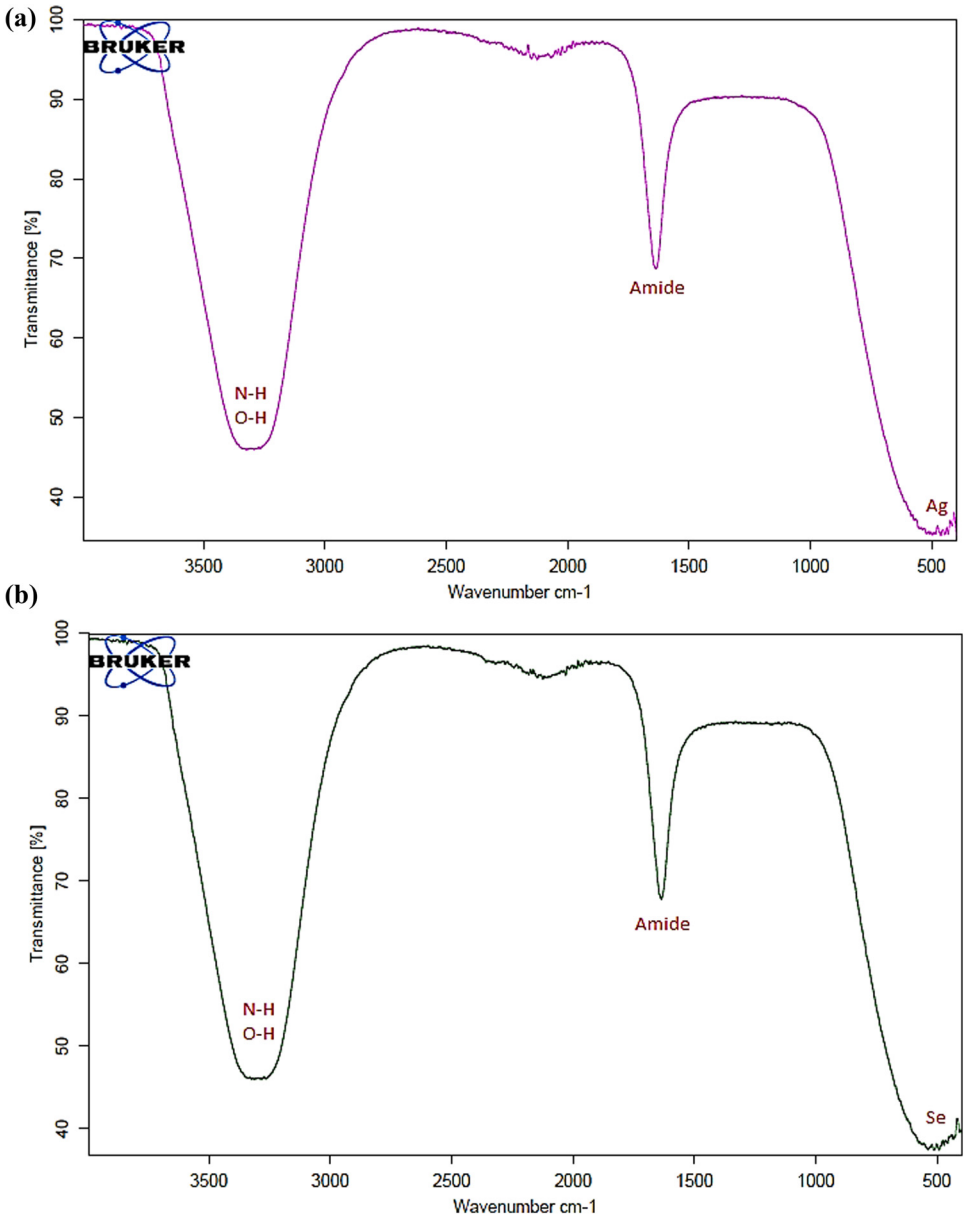

Figure 2: FTIR spectra of AgNPs (a) and SeNPs (b) produced by *T. viride*.

3.3 Biochemical studies

The information in Table 2 demonstrates the developmental and detoxifying enzyme activity in *C. pipiens* larvae in their fourth instar stage at 24 h after exposure to the non-killing concentration of the investigated NPs.

The SeNP data show that in treated larvae (developmental enzymes), there is a substantial reduction in the protein (total) content as well as alkaline and acid phosphatase enzyme activity. In addition, the activity of detoxification enzymes, such as GST, αesterase, and β-esterase, decreased significantly after treatment compared to the control. Insignificant effect was reported regarding peroxidase activity.

Regarding the effect of AgNPs on treated larvae, the amount of total protein and the activity of developmental enzymes, and GST decreased significantly. Meanwhile, α-esterase, β-esterase, and peroxidase showed insignificant change in activity. The outcomes of the larvicidal trials show that SeNP therapy is more successful than AgNPs in controlling mosquitoes.

4 Discussion

Despite the fact that a number of NPs have been successfully produced by biological agents such as bacteria and

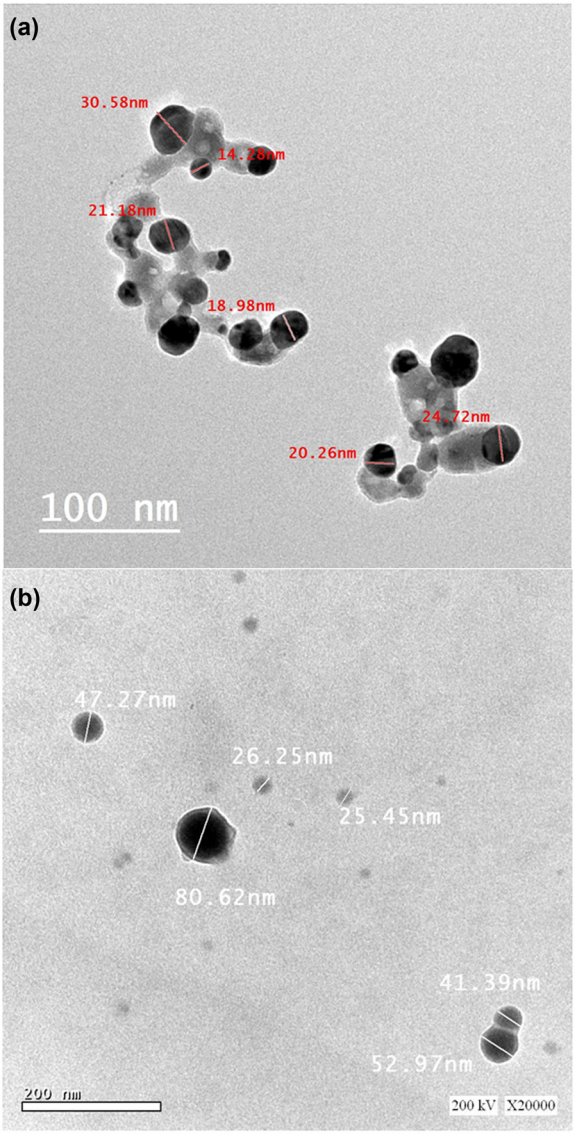

Figure 3: TEM images of biosynthesized AgNPs (a) and SeNPs (b).

fungi, substantial challenges still remain [39–41]. Because the procedure is safe and natural capping agents are inexpensive, the biogenic production of NPs using fungal extracts has received a lot of attention [42,43]. The green synthesis of SeNPs and AgNPs using the *T. viride* extract involves the reduction of Se and Ag ions to elemental Se and Ag, respectively, by the metabolites present in the fungal extract. XRD analysis confirmed that the synthesized SeNPs and AgNPs were very pure and crystalline, with no evidence of an impurity peak. These specific peaks correspond to the reflections of the (100), (101), (111), (200), (201), (210), (220), and (311) planes of the phase of Ag and Se NPs. SeNPs displayed a wide peak at about 2θ = 20–23° with no sharp Bragg reflections, revealing the nature of SeNPs. Therefore, the XRD examination clearly shows that the formed AgNPs and SeNPs were crystalline. Green synthesis of SeNPs and AgNPs [44,45] has been described before, and the XRD pattern shown here is consistent with those reported earlier. FTIR analysis was used to analyze the chemical groups on the surface of green-synthesized AgNPs and SeNPs. It is possible to identify the components in the *T. viride* extract that are accountable for stabilizing and reducing the SeNPs and AgNPs. FTIR analysis was used in a number of investigations to describe green-synthesized AgNPs and SeNPs [46–48]. TEM examination demonstrated a strong distinction between the fungal extract-derived AgNPs and manufactured SeNPs. TEM images show that the great majority of NPs are spherical and evenly dispersed, which is consistent with earlier findings [23,49]. In the current study, the particle sizes of Se and Ag ranged between 25.4 and 80.6 nm and between 14.28 and 30.58 nm, respectively, which were prepared from the *T. viride* extract.

The successful management of several insect pests has been done in recent years, thanks to the application of nanotechnology in all disciplines, including pesticide preparations. Due to their low toxicity, environmental friendliness, and affordability, green-synthesized AgNPs are employed more frequently than other metal NPs [50,51]. The effective

Table 1: Toxicity of SeNPs and AgNPs on fourth instar larvae of *C. pipiens* at different concentrations after 24 h

Product	Concentration (ppm)	Mean of mortality (%)
SeNPs	Control	0
	30	36.667 ± 0.94
	50	63. 333 ± 0.47
	70	80.000 ± 0.82
AgNPs	Control	0
	30	16.667 ± 0.94
	50	46.667 ± 1.88
	70	70.000 ± 0.00

Table 2: Developmental and detoxifying enzymes in *C. pipiens* larvae in their fourth instar

	Protein	Acid phosphatase	Alkaline phosphatase	Alpha esterase	Beta esterase	GST	Peroxidase
Control	55.12^{a} ± 1.20	62.0333^{a} ± 2.89	190.333^{a} ± 5.62	99.6667^{a} ± 3.54	36.2^{a} ± 8.32	$1{,}989.33^{a}$ ± 6.21	9.34000^{a} ± 7.58
SeNPs	46.52^{b} ± 2.30	53.4000^{b} ± 6.25	97.333^{b} ± 2.63	60.3333^{b} ± 2.65	25.7^{b} ± 4.36	$1{,}080.33^{b}$ ± 6.87	8.96667^{a} ± 5.69
AgNPs	47.60^{b} ± 1.06	49.2333^{b} ± 4.89	150^{b} ± 7.01	91.3333^{a} ± 1.85	37.5^{a} ± 5.27	$1{,}051.33^{b}$ ± 4.56	8.23000^{a} ± 1.56

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functionality group of a plant chemical embedded with an Ag ion-containing liquid during the reduction stage resulted in the formation of tiny size NPs, which is the mechanism of this green synthesis [52]. As a result of this tiny size, AgNPs are able to readily cross the cellular barrier of the insect, harm their inner cellular organelles, or interfere with their regular physiological processes, altering every organ system in turn, subsequently causing the death of the insect, according to some researchers [53]. The denaturation of DNA or sulfurcontaining proteins may be the cause of the SeNPs' larvicidal activity. This process also results in the denaturation of structures and enzymes, which decreases the membrane's permeability and inhibits ATP synthesis, both of which lead to the death of cells and the loss of cellular function [28].

Interestingly, poisoning may result from NPs entering the body via the exoskeleton [54]. According to a study, surface charge-modified NPs killed insects by dehydrating them after absorbing into their cuticular lipids [55]. To summarize, there are many different theories about NP toxicity, some of which attribute the toxicity to the factors that lead to oxidative damage in arthropods. [56].

In accordance with the findings provided by Koodalingam et al., the current results showed that the total protein content was reduced following treatment with LC_{50} concentration [57]. The total protein presumably reduced during insecticidal stress as a result of RNA loss and protein degradation into free amino acids [58]. In addition, a possible drop in hemolymph quantity brought on by insecticidal treatment may result in a decrease in the amount of total protein [59]. Protein deficiency might be a result of a physiological process that helps tissues and cells to adapt to insecticidal stressors [60].

Alkaline/acid phosphatases were found to be significantly reduced after treatment with AgNPs and SeNPs in comparison with the control samples. Notably, the consumption of any xenobiotic or toxic chemical that might alter the functioning of the lysosome could be the cause of this decrease in acid phosphatase lysosomal enzyme [61]. Alkaline phosphatase's decrease might be ascribed to the binding of NPs to the gastrointestinal enzymes' active site or to the decreased enzyme production [62], similar to the results reported by Durairaj et al. [63].

A vast and varied set of hydrolases known as general esterases hydrolyze a wide range of molecules, including esters and nonester chemicals. A number of investigations have shown that esterases are crucial in causing or assisting in the detoxification of insecticides in many insect and arthropod species. Esterases are hydrating enzymes that break down ester molecules when water is added, producing alcohol and acids [64]. The alpha and beta esterase activity in *Culex pipiens* fourth instar larvae showed nonsignificant change with the LC_{50} concentration of AgNPs and significant reduction with that of SeNPs. The effect of both spinetoram and rynaxypyr on αesterase and β-esterase activity in the total homogenate of *Spodoptera littoralis* fifth instar larvae was also demonstrated by El-Kawas et al. [65]. They found a significant reduction of 31.71% in αesterase activity and 11.18% in β-esterase activity. The earlier research likewise produced similar findings. The present study observed a widespread decline in enzyme activity, which may suggest that general esterases do not participate in the detoxification of rynaxypyr and spinetoram. These results concur with those of Fahmy and Dahi [66], who discovered that GST and esterases may not have a significant role in inhibiting the *Spodoptera exigua* field species.

GST is a vital key enzyme for determining whether an organism developed resistance or susceptibility after exposure to specific bioinsecticides. It has also been established that because this enzyme is highly abundant in a variety of insect pests, particularly mosquitoes, it is crucial for the detoxification process [67]. The GST enzyme expression was much lower in this study, which suggest that AgNPs and SeNPs may be engaged in the redox response and may cause harm due to oxidative stress to the tissues of larvae when they were exposed to NPs [68].

Data indicated that therapy with SeNPs rather than AgNPs significantly decreased the expression of enzymes associated with antioxidants and peroxidase. Our findings are consistent with those reported by Hussein et al. [69], who discovered that the application of SeNPs to several groundnut cultivars significantly reduced the activity of some antioxidant agents, including peroxidase. The authors hypothesized that selenium's crucial function in detoxification, which resulted from oxidative stress, is responsible for the decline in antioxidant enzyme activity.

5 Conclusions

In conclusion, SeNPs and AgNPs were successfully synthesized from *T. viride*. The SeNPs and AgNPs were characterized by XRD, FTIR, and TEM. The NPs were shown to have substantial mosquito larvicidal action in a dose-dependent way when applied at extremely low concentrations. The outcomes of the larvicidal trials show that using SeNPs as a therapy is more efficient in controlling mosquitoes than AgNPs. The likelihood that next-generation NPs might be a more effective agent in controlling mosquitoes makes the current work important. Before marketing, more research is required to determine how the NPs affect nontarget living things and to evaluate their effectiveness in this field.

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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