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Application of Chitosan–Selenium Nanoparticles composite Biosynthesized by *Kytococcus schroeteri* as an adsorbent of pesticide pollutants

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The indiscriminate application of pesticides in agricultural practices has significantly increased pesticide concentrations in aquatic environments. This study focused on the feasibility of employing chitosan beads incorporating selenium nanoparticles to effectively remove pesticides (permethrin) from aqueous solutions and aquaculture. Selenium nanoparticles were produced using *Kytococcus schroeteri*, with diameters ranging from 10.1 to 22.4 nanometers. Se-NPs were synthesized utilizing a bacterial cell-free supernatant rich in enzymes and metabolic byproducts that catalyze nanoparticle formation. Therefore, the application of these nanoparticles for pesticide removal does not appear to pose a threat to living organisms. Selenium nanoparticles were immobilized in chitosan to produce a composite material (CS-Se-NPs) to remove pesticides. The CS-Se-NPs composite beads exhibited a remarkable 99% adsorption efficiency for permethrin. Under optimal conditions (room temperature, pH 7), 0.5 grams of beads removed 99% permethrin from a 25 ml pesticide solution (0.1 mg L-1) within 60 minutes. This method effectively eliminates any potential risks to human health. These findings suggest that the biosynthesized CS-Se-NPs composite is a promising, eco-friendly material for water purification.

Keywords: Nanoparticles; Selenium; Permethrin, Chitosan Bio-nanoparticles; *Kytococcus schroeteri*

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INTRODUCTION

While essential for agricultural productivity, pesticides have raised concerns due to their widespread and often uncontrolled use. Their indiscriminate application has resulted in significant environmental contamination and adverse human health effects. Exposure to pesticides has been linked to a variety of acute and chronic illnesses. While pesticides are crucial for reducing crop losses and controlling disease vectors, their potential harm to human health cannot be overlooked. The broad-spectrum nature of many pesticides often leads to the unintended harm of non-target organisms, further exacerbating the issue. A substantial amount of research supports pesticides' detrimental impact on human health and the environment (Heravizadeh *et al.*, 2019 & Richardson *et al*., 2019).

Agricultural lands, including fields and lawns, act as catchment areas for rainwater, which carries pesticides and fertilizers into surface and underground water bodies. Moreover, sewage and wastewater discharges, contaminated with agricultural chemicals and pesticides, significantly contribute to the indirect pollution of rivers and lakes. The cumulative impact of these pollutants severely threatens the health and survival of all aquatic flora and fauna (Singh *et al*., 2020) according to Wang *et al*. 2016 who mentioned that Permethrin, a synthetic insecticide belonging to the pyrethroid family, has been extensively employed worldwide due to its potent insect-killing properties and perceived low toxicity. Initially, it was considered relatively harmless to non-target organisms. However, with the escalating global use of permethrin, mounting evidence has unveiled its potential for causing a broad spectrum of adverse effects in both animals and humans. These detrimental impacts encompass a range of health issues, including damage to the nervous system (neurotoxicity), liver (hepatotoxicity), immune system (immunotoxicity), and cellular function (cytotoxicity).

Green synthesis of NPs is a technique that involves the use of biological sources like bacteria and fungi, etc. Many bacterial species can produce nanoparticles that differ in their dimensions, intracellular or extracellular (Mohesien et al., 2023; Abd El Hamid et al., 2024). The extracellular production of nanoparticles occurs when bacteria release enzymes to reduce the number of metals in the body. In some cases, producing nanoparticles extracellularly is more advantageous than accumulating them within the cell (Narayanan and Sakthivel, 2010 & Pandit *et al*., 2022). The innovative applications of nanoparticles have inspired researchers to develop simpler and more efficient biological methods for producing them. Hence, producing nanoparticles with precise size, structure, and shape is crucial for their effective use. (Koul *et al*., 2021).

Selenium nanoparticles (Se-NPs) can be produced through various methods, but biological synthesis is favored. This approach generates Se-NPs with enhanced beneficial properties and significantly

reduced toxicity compared to traditional selenium (Kumar and Prasad, 2021). Nano-encapsulation and nano-conjugation of bioactive particles within biopolymers such as chitosan can benefit NPs by increasing their physical stability, bioactivity, and delivery to their target (Argüelles-Monal *et al*. 2018). The immobilization of NPs using chitosan or alginate improves their applicability to direct use in environmental applications (Alghuthaymi, 2022).

Se-NPs have gained significant interest in recent decades due to their high biological activity and biosafety performance. Se-NPs can be prepared using a variety of physical, chemical, or biological procedures (Abdelhamid *et al* .2023). Because of their unique optical and biological features, SeNPs have great promise for different technological applications in various sectors. Despite their potential, we still need to find better ways to use selenium nanoparticles effectively in treating water on a large scale. So, in the present study, the distinctive bacterium was chosen to produce Se-NPs as a green biosynthesis, and the study objectives are to investigate Se-NPs that are immobilized by chitosan to remove permethrin from aqueous solutions. To achieve this goal, a suitable bacterial isolate was picked to produce Se-NPs efficiently, then immobilized by chitosan efficiently, and the investigations of Se-NPs in removing pesticides from aqueous solutions were applied.

MATERIALS AND METHODS

Bacterial isolation

Thirteen water samples were taken along the Kitchener drain in Egypt. It is also known as the main drainage of Gharbia Governorate. To preserve the integrity of the samples, sterile containers were used for collection. These containers were immediately transported to the laboratory under refrigerated conditions for subsequent analysis. For organizational purposes, the samples were systematically labeled with codes K1 to K13, corresponding to their specific collection sites as detailed Table 1.

Isolation and Screening of Selenium -resistant bacterial isolates

To isolate and screen the bacterial strains resistant to Selenium, 100 µL of each water sample collected from Kitchener drains were screened via pour plate methods on nutrient agar supplemented by Na_2SeO (Sigma-Aldrich) with 0.2% concentration. A sterilized selenium solution was passed through a filter and added after autoclaving. After that, the media was incubated aerobically at 30°C for 48 h. The colonies surrounded by a clear zone (indicating bacterial metal reduction) were separated onto fresh metal-containing nutrient agar plates. Then, one colony was selected from each plate and picked up. Then, the purification of the colony was implemented. 100 ml of nutrient broth was autoclaved and supplied with 0.5 g/l Na2SeO3 at pH 7 as a filter out-sterile solution, then inoculated with selected pure isolate; the plates of nutrient broth were incubated at 30 °C for 48 h. After that, the growth density was measured by UV-Spectrophotometer; this method is like El-Shanshoury *et al*. 2020.

Extracellular biosynthesis of Se-NPs

To investigate the efficiency of isolates for Se-NPs synthesis, the isolate was inoculated in 100 ml of nutrient broth supplemented with (0.05 g/l Na_2SeO), incubated at 30 °C for 48 h. at 120 rpm shaking conditions. The culture supernatants were collected via centrifugation (8000 rpm for 10 min). The extracted supernatant was transferred and mixed with an equal volume of metal solution (0.5 g/l Na_2SeO) and, after that, incubated at 30 °C and 150 rpm for 72 hours. The examination by visible color changes and UV/VIS spectrophotometer at 420 nm was done. This method resembles other methods (Musarrat *et al*, 2010 and El-Shanshoury *et al,* 2020).

Characterization and Identification of the most efficient bacterial isolate

The most powerful bacterial isolate produced Se-NPs based on the clear zone diameter length. The bacteria were categorized using Bergey's Manual of Systematic Bacteriology, and their identification was based on their morphology and biochemical characteristics. Then, genomic DNA analysis was performed.

Molecular identification and phylogenetic analysis

The molecular identification was performed at the Egyptian Microbial Identification Center (EMIC) at El-Sadat University. According to (Kim *et al.* 2011), it was performed with universal primers: 27F-Forward primer 5’AGA GTT TGA TCC TGG CTC AG 3’ (20 mer) and 1492R-Reverse primer 5’CTA CGG CTA CCT TGT TAC GA 3’ (20 mer). PCR was performed using a thermal cycler (Thermo Fisher Scientific, USA). The nucleotide sequences of the 16S rRNA genes were compared to existing databases using BLAST software. These sequences were registered in the Genbank database under the accession number

Table 1 Sampling collection sites

Sample Code	Location	Coordinates
K1	Nimrat Al-Basal village – El-Mahalla al-Kubra Center, Gharbia Governorate	31° 3'30.86"N 31°4'51.39"E
K2	Al-Banwan- Al-Mahalla al-Kubra Center, Gharbia Governorate	31°04'55.8"N 31°04'40.3"E
K3	Kafr Dakhmis- Al-Mahalla al-Kubra Center, Gharbia Governorate	31°08'02.9"N 31°03'17.2"E
K4	Ezbet Al-Manawafa, Al-Hamoul Center, Kafr El-Sheikh Governorate	31°16'05.6"N 31°07'17.3"E
K5	Al-Hamoul city, Kafr El-Sheikh Governorate	31°19'01.8"N 31°08'45.2"E
K6	Tambari- Al-Hamoul Center, Kafr El-Sheikh Governorate	31°21'42.8"N 31°10'38.1"E
K7	Village No.7- Al-Hamoul Center, Kafr El-Sheikh Governorate	31°24'42.5"N 31°10'48.5"E
K8	Village No.9- Al-Hamoul Center, Kafr El-Sheikh Governorate	31°26'05.9"N 31°10'25.3"E
K9	Village No.11, Al Majaz Al Sharqiya – Al-Hamoul Center, Kafr El-Sheikh Governorate	31°27'08.0"N 31°10'10.7"E
K10	Central Village 13- Al-Hamoul Center, Kafr El-Sheikh Governorate	31°28'10.3"N 31°09'39.5"E
K11	Ezbet 51-Al-Zahraa- Al-Hamoul Center, Kafr El-Sheikh Governorate	31°29'22.4"N 31°09'07.9"E
K12	Kafr Al-Sawahel – Ezbet Bahri, Al-Zawiya Sector, Burullus Center, Kafr El-Sheikh Governorate	31°31'15.1"N 31°09'23.2"E
K13	Tahrir, Al-Rub' (Souk Al-Talat), Burullus Center, Kafr El-Sheikh Governorate 6975702	31°32'53.7"N 31°06'23.7"E

PP267320. Finally, a phylogenetic tree illustrating the evolutionary relationships between these sequences was constructed using MEGA 11 software.

Optimization of Se-NPs production

The biosynthesis of Se-NPs, by the most efficient isolated bacterial strain *K. schroeteri*, was examined under various conditions. The bacterial culture was grown in a medium containing a metal solution. After a period of growth, the liquid portion (supernatant) was separated and exposed to three different sets of conditions:

- A range of temperatures (20, 25, 30, 35, 40 °C).
- Various incubation times (6, 12, 24, 48, 72, and 96 hours).
- Different concentrations of selenium solutions (0.25, 0.5, 0.75, 1, and 1.5 g/l).

The bio-reduction process was started by adding an equal volume from the supernatant to the selenium solution, then measured by UV/VIS spectrophotometer at 420 nm to determine the optimal conditions for the selenium nanoparticle production process (El-Shanshoury *et al*, 2020).

Production and purification of Se-NPs

Optimal growth conditions were used to cultivate *K. schroeteri* in a nutrient broth for selenium nanoparticle synthesis. When *K. schroeteri* was cultivated in the nutrient broth containing sodium selenite (Na2SeO3) for 24 hours, the medium changed from yellow to red, signifying the production of selenium nanoparticles. Additionally, when the supernatant was mixed with sodium selenite solution, it also turned red. This color change confirmed that the bacteria reduced the selenium compound into elemental selenium nanoparticles. The distinct red hue resulted from the interaction of light with the nanoparticles and served as a clear indicator of their presence.

The inoculum of *K. schroeteri* was 1 ml of fresh suspension. Flasks were incubated at 30°C for 24 h. Suspensions were centrifuged (15 min at 6000 rpm), the supernatant was mixed with an equal volume of selenium solution and incubated under the optimum conditions, and Se-NPs were produced. The study adopted the same experimental methods as El-Shanshoury *et al*. (2020).

Characterization of Se-NPs by Transmission electron microscopy

TEM (Joel jem-Japan-80volt) was used to implement the size and morphology of biosynthesized Se-NPs. This analysis was conducted at the Research laboratories complex at the Faculty of Agriculture - Cairo University. TEM analysis was prepared by drop-coating Se-NPs onto carbon-coated TEM grids. The film on the TEM grids was allowed to dry, and the extra solution was removed using blotting paper. TEM analysis provided detailed information about sample morphology, composition, and crystalline arrangement.

Preparation of Chitosan Bio-nanoparticles (CS-Bionanocomposite)

According to El-Sheshtawy *et al*, (2021), A 1% (w/v) chitosan solution was prepared by dissolving 0.5 g of chitosan in 50 mL of 2% acetic acid. The resulting solution was subjected to mechanical agitation at 60 °C and 1000 rpm for 24 hours after adding 0.5 g of dried selenium nanoparticles. The pH of the solution was adjusted to 7 by 0.5 M NaOH solution, and pH was measured using a pH meter. The solution was centrifuged (5000 rpm for 20 min.), and the solid products were separated and repeatedly cleaned

with distilled water before being dried in the oven for 24 hours at 40 °C.

Application of CS-Bionanocomposite as adsorbent to remove permethrin from aqueous solution.

As described by Dehaghi *et al*. (2014), Batch experiments were conducted in a shaker incubator operating at 150 rpm and 25°C for 45 min. With a specified number of adsorbents (the dosage varied from 0.01 to 1.5 g) in contact with 25 ml of permethrin solution (0.1 ppm). A control sample was subjected to identical conditions as the pesticide-treated sample to assess potential pesticide loss due to evaporation. Consistent results between the control and treated samples indicated negligible evaporative losses of the pesticide. Permethrin (5 %, EPIC) was used to prepare the pesticide stock solution. The percentage of pesticide removed from the water solution after 45 min. at various adsorbent dosages were calculated by UV-Vis Spectrophotometer at 290 nm. Then, the following equations are used.

$$\text{Removal of pesticide } (\%) = \frac{(\text{Absi} - \text{Abst})}{\text{Absi}} \times 100\% \quad (1)$$

$$\text{Rd } (\text{ml g}^{-1}) = \frac{(\text{Amount of Permethrin onto sorbent})}{(\text{Residual amount of Permethrin in solution at equilibrium})} \times \frac{Volume\ of\ solution\ V\ (ml))}{\text{Amount of sorbent W } (\text{g}^{-1})} \quad (2)$$

$$\text{Qt} = \frac{(\text{C}_0 - \text{Ct})}{m} \times \text{V} \quad (3)$$

Whereas
Qt: adsorbed analyte amount
t: time
C0, Ct: initial and liquid-phase concentrations of permethrin, respectively.
V: volume of permethrin solution (ml).
m: adsorbent mass (g).

Experiments have been carried out in triplicate (at pH 7 and 25°C).

RESULTS

Isolation and screening of Nano-producing bacteria

In the present study, the aquatic bacterial strain was isolated and investigated in December 2023 to produce Se-NPs. A total of 13 morphologically distinct isolates were picked up, one from each plate, from water samples collected from the Kitchener drain (Table 2 & Figure 1). The isolates were selected based on the formation of halo zones on agar plates supplemented with sodium selenite. The purified isolates have been screened for their capacity for the extracellular formation of Se-NPs. The supernatant of 13 isolates could produce Se-NPs, as evidenced by

Table 2. Absorbance development by bacterial isolates measured by UV-spectrophotometer

Sample Code	Absorbance
K1	0.232
K2	0.965
K3	0.874
K4	0.895
K5	1.231
K6	0.365
K7	0.951
K8	**1.921**
K9	1.562
K10	1.233
K11	1.184
K12	0.632
K13	0.542

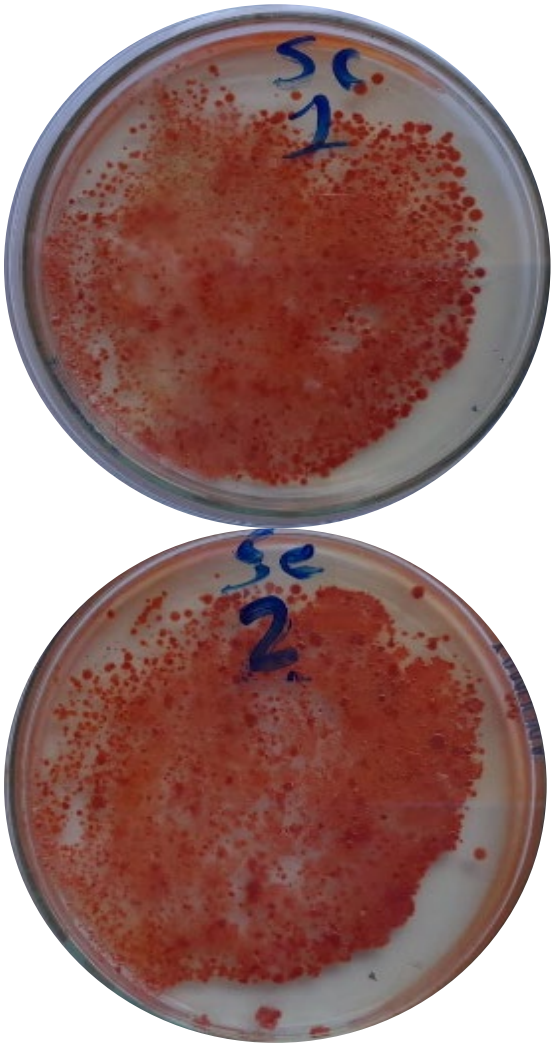


Figure 1. Bacterial isolates cultured in media containing sodium selenite (Na_2SeO_3).

the development of a red coloration, indicating the presence of Se-NPs in the solution. The codes of the positive isolates were K1, K2, K3, K4, K5, K6, K7, K8, K9, K10, K11, K12 and K13. Then, the supernatants of bacterial isolates were mixed with selenium solutions to investigate their capacity for Se-NPs formation. The appearance of the red color (Figures 2 & 3) indicated the prevalence of Se-NPs formation in the solution by spectrophotometric scanning after visual inspection to track each isolate's color change in the reaction solution. After incubation for 72 h., the color's maximum absorbance was measured. Isolates were tested after being purified, as shown in Figure 4 to produce Se-NPs, then one bacterial isolate was picked up depend on UV-Vis spectrophotometer analysis for producing Se-NPs (Isolate K8).

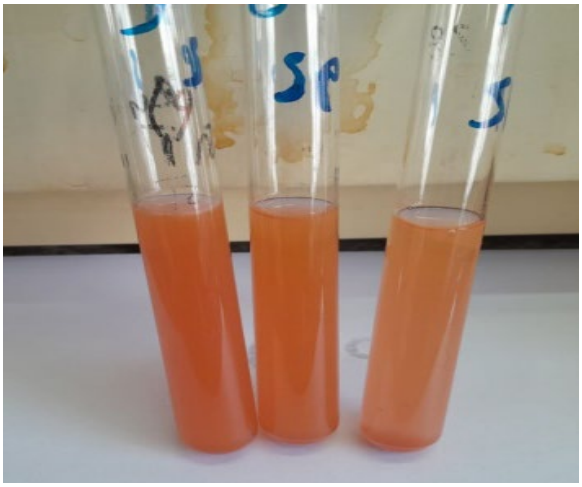


Figure 1. Supernatants of isolates after incubation.

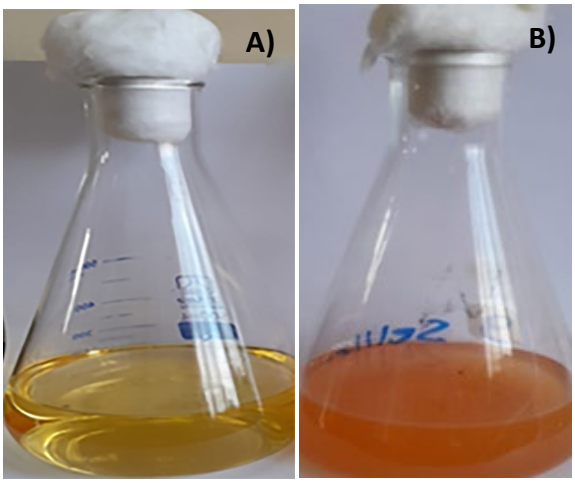


Figure 2. Growth of isolates, A) in the absence of selenium, and B) in the presence of selenium.

Identification of the most efficient isolates

After the purification processes of the K8 isolate, as shown in Figure 4. The isolate was characterized as a Gram-positive bacterium, coccoid, yellow-pigmented, aerobic, non-motile, non-spore-forming bacteria, predominantly existing singly, in pairs, in tetrads and sometimes in clusters. DNA of this isolation was isolated and purified, and the sequence data of amplified 16s rRNA were analyzed. Gel electrophoresis was applied to visualize and analyze the amplified DNA fragments (Figure 5), and the Nucleotides nucleotide sequences were identified. *Kytococcus schroeteri* strain had the highest percentage of similarity (99.6%), according to the NCBI database. Under accession no PP267320, the Gene Bank nucleotide database shows a 99.6% nucleotide identity for isolate K8. The phylogenetic tree of *Kytococcus schroeteri* is shown in Figure 6.

Optimization of Se-NPs production

Different incubation times (6, 12, 24, 48, 72 and 96 hours) have been applied for Se-NP production by *Kytococcus schroeteri*, as shown in Figure 7, and the results indicated that Se-NP production increased with incubation time, reaching a peak at 72 hours, followed by a decline. On the other hand, the results indicated that Se-NP production demonstrated a positive correlation with temperature, reaching a maximum at 35°C, followed by a subsequent decrease. (Figure 8). The supernatant was supplemented with varying selenium concentrations to investigate the influence of selenium concentration on Se-NP biosynthesis by *K. schroeteri*. The experimental results demonstrated that an optimal selenium concentration of 1 g/L yielded the highest Se-NP production by *K. schroeteri* (Figure 9).

Production and purification of Se-NPs under the optimized conditions

When *K. schroeteri* was cultured in nutrient broth supplemented with selenium for 24 hours, a color change in the medium was observed, indicative of nanometal formation. Furthermore, the exposure of the supernatant to a metal solution resulted in a color alteration, indicative of a reduction process and the subsequent formation of Se-NPs within the solution Figure 10.

Se-NPs Characterization by TEM

TEM images of Se-NPs Bio-synthesized by *K. schroeteri* are given in Figure 11. The Se-NPs were

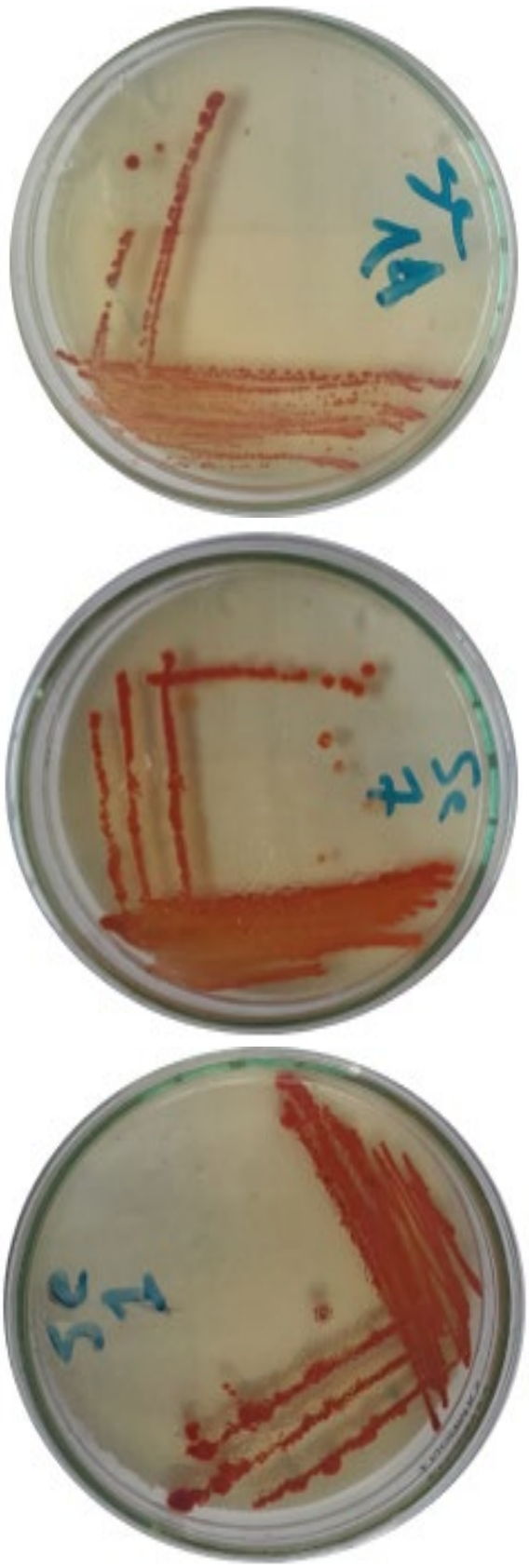


Figure 3. Bacterial isolates purification.

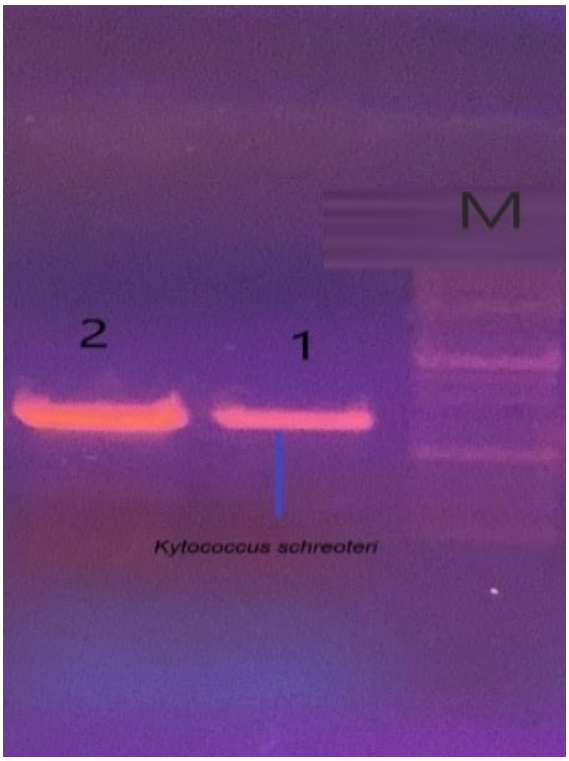


Figure 4. Pattern of agarose gel electrophoresis of *Kytococcus schreoteri,* Lanes: M, DNA molecular size marker; 1, amplicon of *Kytococcus schreoteri*; 2, Amplicon of another microorganism were used for other investigations.

polydisperse and spherical; measurement of the particles ranged from 10.1 to 22.4 nm, and the average particle size obtained from the corresponding diameter distribution was about 17.2 ± 3.2 nm.

Chitosan -Bionanocomposite and adsorption experiments

To increase the range of applications for Biosynthesized nanoparticles such as Se-NPs, they are immobilized on chitosan substance. Figure 12 shows Selenium nanoparticles (Se-NPs) synthesized by *Kytococcus schroeteri* and immobilized within chitosan to assess their efficacy in removing pesticide contaminants from an aqueous solution. The influence of sorbent amount on permethrin removal percentage was investigated while keeping constant values for other parameters (like permethrin concentration, temperature, and agitation time) by employing CS-Se-NPs composite at varying amounts (0.01–1.5 g). To evaluate the permethrin removal efficiency, adsorption experiments were conducted using 25 mL aqueous solutions containing 0.1 ppm of permethrin.

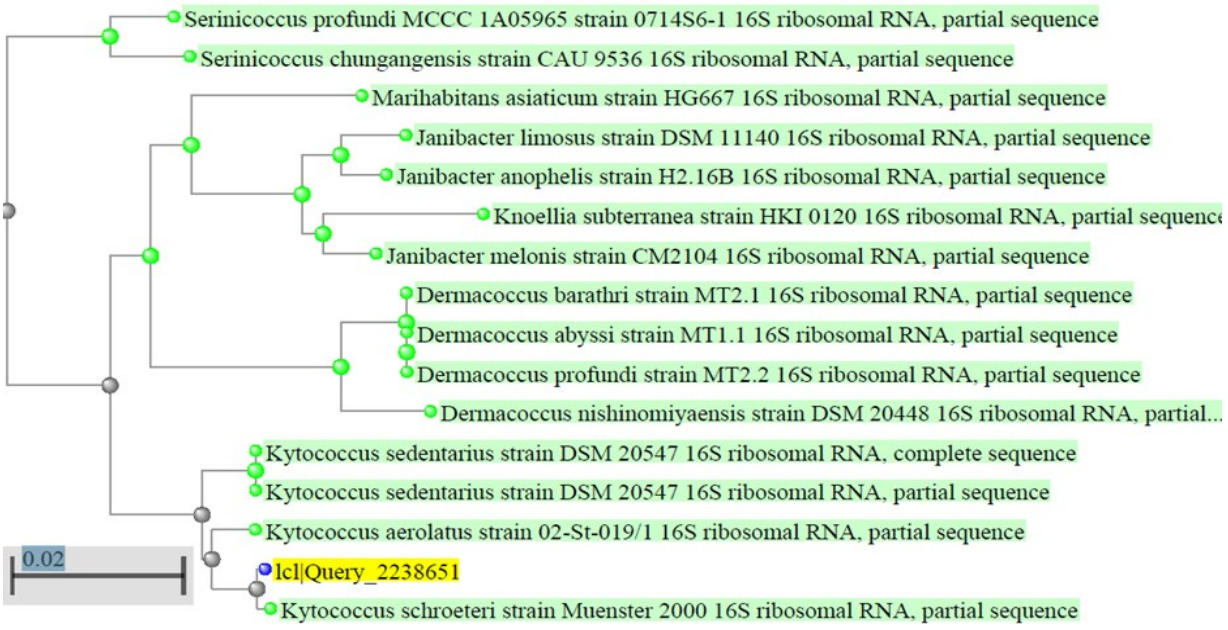


Figure 5. Phylogenetic tree of *Kytococcus schroeteri.*

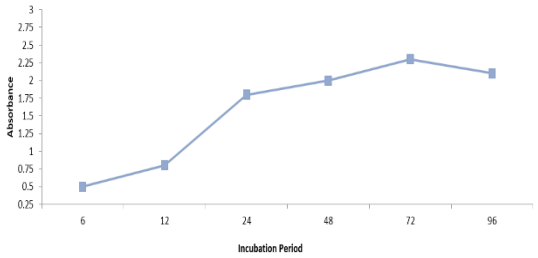


Figure 6. Extracellular production of Se-NPs produced by *K. schroeteri* at different incubation periods.

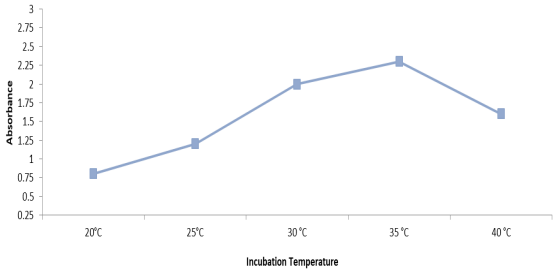
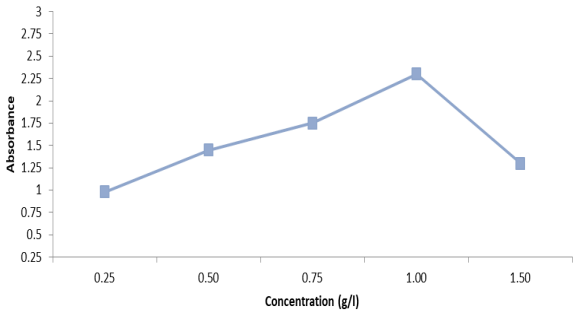


Figure 7. Se-NPs produced by *K. schroeteri* at different temperatures.

The impact of varying CS-Se-NPs composite dosages (0.1, 0.25, 0.5, 1, 1.24, and 1.5 g) on permethrin removal was assessed after 45-minute agitation. Permethrin removal percentages were determined for each composite dosage, as shown in Figure 13. The results revealed that permethrin removal effectiveness improved with a rise in the adsorbent amount of up to 0.5 g in a 25 mL solution.



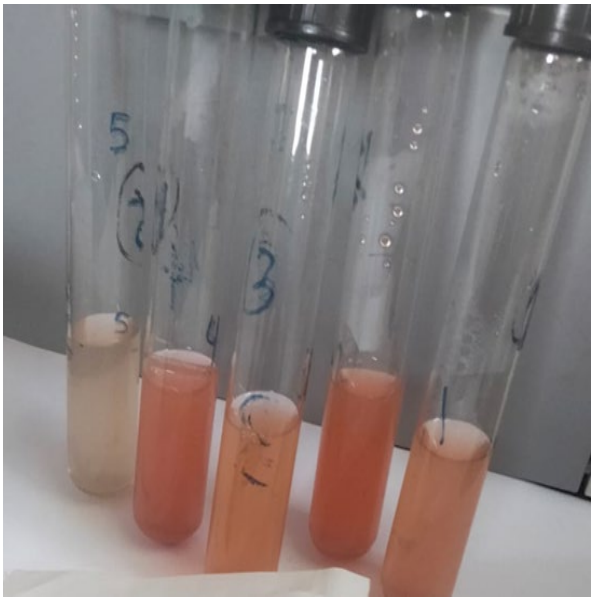


Figure 8. Se-NPs produced by *K. schroeteri* at different concentrations of selenium metal.

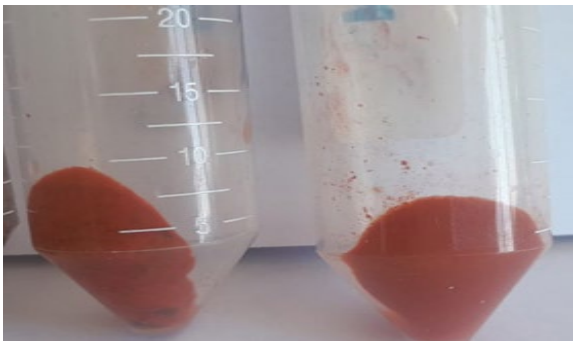


Figure 9. Se-NPs production

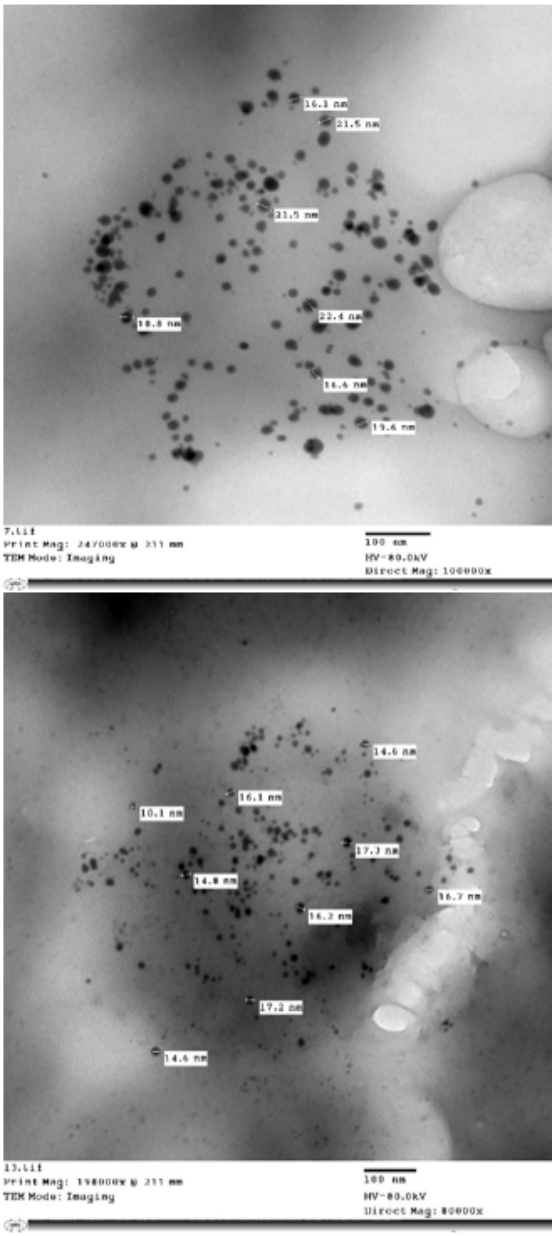


Figure 10. TEM micrograph of Se-NPs produced by *Kytococcus schroeteri.*

Figure 11. Se-NPs bead formation.

After establishing a constant removal percentage, it was discovered that increasing the adsorbent dose by more than 0.5 g had no meaningful effect on removal percentages. The 0.5-1.5 g dosage resulted in 95% pesticide elimination. Since the most efficient removal of permethrin happened with 0.5 g of CS-Se-NPs composite, all other investigations were carried out with the same amount of adsorbent.

On the other hand, the consequences of various agitation periods (20, 30, 40, 50, and 60 minutes) on 0.5 g CS-Se-NPs composite in 25 ml permethrin concentration at 0.1 ppm were tested. The effect of agitation time on permethrin adsorption on the produced CS-Se-NPs composite at 25°C and 150 rpm shaker speed is depicted in Figure 14. The graphic illustrates how Permethrin's adsorption rises over time and eventually reaches a constant value that prevents additional pesticide removal from the solution. The initial rapidity of adsorption processes is most likely caused by the abundance of accessible binding sites on the adsorbent's surface. The sorption process exhibited a rapid initial phase, with a 50% removal efficiency achieved within the first 20 minutes. Subsequently, the sorption rate increased, reaching a plateau at 99% removal after 60 minutes. No further significant enhancement in sorption was observed beyond this point, indicating the attainment of equilibrium. Conversely, Figure 15 illustrates the impact of varying permethrin concentrations (0.05-0.3 ppm) on the adsorption capacity of 0.5 g of CS-Se-NPs composite. The results demonstrate a high removal efficiency, approaching 99% at 0.1 ppm of permethrin concentration.

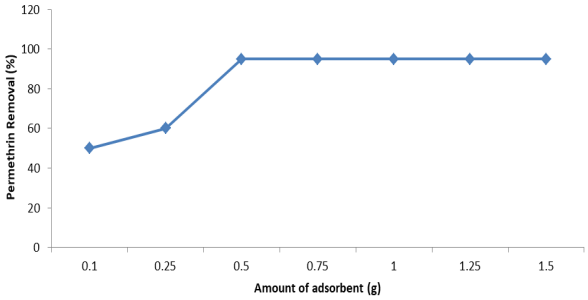


Figure 12. Amount of sorbent effects on permethrin removal percentage.

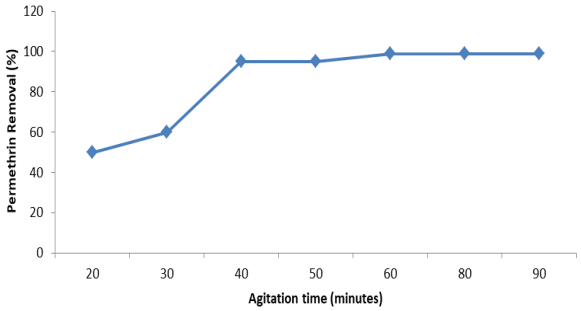


Figure 13. Effect of agitation time on removal percentage.

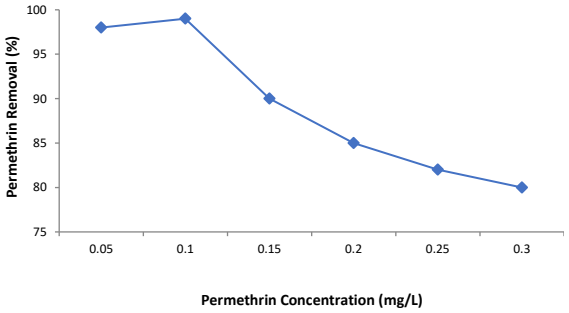


Figure 14. Impacts of permethrin concentration on the percentage of removal process.

DISCUSSION

Nanotechnology has grown at an exponential rate over the last 40 years, prompting the development of several synthetic techniques to manufacture Nanomaterials of varying sizes, forms, and compositions for a wide range of biotechnological uses, and due to their physical-chemical adaptability and effectiveness, Se-NPs have attracted technological interest and supported biotechnological and biomedical applications (Piacenza *et al*., 2021). Biosynthesis describes biological procedures or enzymatic reactions. These environmentally friendly technologies, known as green technology, can be applied to generate more effective nanoparticles employing microorganisms (El-Sheshtawy *et al,* 2021). Extracellular biosynthesis of metal nanoparticles is widely used by different microorganisms, either fungi (unicellular fungi and filamentous fungi such as *Candida albicans* and *Fusarium avenaceum*) or bacteria (Gram-negative and Gram-positive such as *Salmonella typhimurium*, *Staphylococcus aureus*) as described by many authors such as El-Sheshtawy *et al*. (2021). The extracellular biosynthesis by microbial strains results in NPs production of different sizes, which might be attributed to different types of enzymes excreted from these organisms (Ovais *et al*., 2018; Tahoun *et al*, 2024). In the present study, Se-NPs formation indicated the appearance of red color within the solution because the activation of the surface plasmon vibrations of the Se-NPs was responsible for the red color observed, which served as a spectroscopic marker of their biosynthesis, as mentioned by Oremland *et al* .2004. Many authors have reported that one of Se-NPs' distinguishing characteristics biosynthesized by the green approach is amorphous (El-Shanshoury *et al*, 2020).

In the present study, the isolated strain from the Kitchener Drain coded by K8 can produce Se-NPs. This isolate was identified as *Kytococcus schroeteri,* and it was characterized as a Gram-positive bacteria coccoid, yellow-pigmented, aerobic non-motile- no spore-forming, Gram stains of colonies appeared on spherical cells, predominantly existing singly, in pairs, in tetrads and sometimes in clusters, the results in accordance with Becker *et al*. (2002). Conversely, one of the most widely used methods for identifying bacteria is sequencing the highly conserved 16S rRNA gene. This technique is effective, straightforward, and quick for figuring out the evolutionary and phylogenetic relationships among bacteria (Hu et al., 2018). As mentioned by Church *et al*. (2020), the sequencing of the 16S rRNA gene has been employed to facilitate the differential identification of the genus Kytococcus.

Kytococcus genus was differentiated initially from Micrococcus species in 1995. It is now considered a member of the Actinomycetales order and family, Dermacoccaceae. The genus is now known to include three species: *K. schreoteri*, *K. sedentarius* and *K. aerolatus.* However, the NCBI taxonomy browser lists more unclassified and uncultured variants *K. schroeteri* is a normal microflora existing on human skin, which may cause uncommon lethal infections in immunosuppressed persons. Just twenty publications were recorded about it in the last 17

years (Bagelman and Zvigule-Neidere, 2021). There are few reports about Kytococcus species' role in human diseases due to misidentifying these microorganisms as unspecified micrococci or misdiagnosis as environmental contaminants (Mnif *et al.,* 2006).

Many investigators recorded many species that can produce Se-NPs with different sizes and shapes, such as P*. alcaliphila*, *S. bikiniensis*, *C. fruendii*, *B. subtilis*, *B. mycoides*, *B. cereus*, *K. pneumonia*, *P. agglomerans*, *E. coli*, and Shewanella sp. Accordingly, there are numerous applications for these Se-NPs (Lee *et al,* 2007; Sardar and Alam, 2018). In the present study the biosynthesis of Se-NPs was done by growing *K. schroeteri* on a nutrient-containing precursor chemical selenite at different concentrations. They are grown under various optimized conditions to find the maximum production of Se-NPs. The color intensity of selenium changes from colorless to red color, measured at regular intervals and different temperatures. The purified Se-NPs were collected after centrifugation at high rpm, as mentioned by Menon *et al*. (2019). The present study demonstrated that the optimal conditions for Se-NPs production by *K. schroeteri* were (incubation time 72 h., 35 °C; and 1 g/l metal concentration). These results are comparable to El-Shanshoury *et al*. (2020). The findings indicated that several bacteria could continue to survive in low concentrations of metals, producing a larger amount of Se-NPs (Debieuxa *et al*. 2011).

In the present study, Se-NPs were immobilized on chitosan to estimate their application on pesticide removal, where the choice of chitosan was picked because chemical alterations to the material are commonly chosen to improve the polymer process's capabilities and change certain features. Furthermore, the functional groups in chitosan, such as NH2 and OH, can interact with nanoparticles by forming coordination interactions; this approach was used by many authors, such as Olajire and Bamigbade (2021).

The amount of adsorbent is a significant feature in bioremoval processes that determines the capacity of a sorbent for a particular initial concentration of sorbate, as mentioned by Saifuddin *et al*. (2011). Dehaghi *et al*. (2014), so that, in this study, investigations of pesticide removal by Se-NPs were implemented at different amounts of adsorbent. The study demonstrated that the best amount of adsorbent was 0.5 g of CS-Se-NPs composite, and the best amount of adsorbent was 0.5 g of CS-Se-NPs composite. The availability of the active sites on these composite decreases with increasing sorbent dosages (>0.5 g) while increasing the dosage leads to a rise in total active surface area. At the same time, when the amount of sorbent increases, sorbent-sorbent interactions dominate over sorbate-sorbent interactions, as established by many investigators, like Dehaghi *et al*. (2014); Rahmanifar & Dehaghi (2014).

In the present experiments, the optimal agitation periods recorded for the highest percentage of permethrin removal were 60 hours, reaching 99%. The pesticide adsorption slowed down close to equilibrium after starting quickly during the contact period. This occurrence can be attributed to the abundance of available surface sites for adsorption in the first phase, followed by the difficulty in absorbing more vacant sites because of the repulsive forces between the adsorbate molecules on the adsorbent as described by Sokkera, *et al*. (2011); Rahmanifar & Dehaghi (2014); and Dehaghi *et al*. (2014).

However, the present results recorded that the best concentration of permethrin removal is 0.1 mg/l, and an inverse relationship was observed between the initial permethrin concentration and removal efficiency. As the initial permethrin concentration increased, the percentage of permethrin removed by the adsorbent decreased. Furthermore, the percentage removal decreases as the concentration of a pesticide solution rises because lower energy sites will be exploited when higher energy sites become saturated (Saifuddin *et al*. 2011).

Finally, *K. schroeteri* has been recorded as being able to produce Se-NPs. At the same time, most articles on *K. schroeteri* reported its role in human diseases. The present study confirmed that the applications of Se-NPs biosynthesised by *K. schroeteri* have a novel and promising future.

CONCLUSIONS

The possibility of *K. schroeteri* manufacturing Se-NPS was demonstrated. Furthermore, the diameter of the biosynthesized Se-NPS ranged from 10.1 to 22.4 nm. Also, permethrin was removed from its aqueous solution by studying the behavior of the CS-Se-NPs composite as an adsorbent. It was discovered that this composite had an adsorption capacity of up to 99% for CS-Se-NPs beads. In conclusion, 0.5 g of CS-Se-NPs composite beads were able to remove 99 % of permethrin in a 25 ml pesticide solution (0.1 mg L^{-}

[1]) at room temperature, pH 7, and shaking time of 60 minutes.

LIST OF ABBREVIATIONS

16S rRNA	16S ribosomal Ribonucleic acid
BLAST	Basic Local Alignment Search Tool
CS-Se-NPs	Chitosan-Selenium Nanoparticles Composite
DNA	Deoxyribonucleic acid
mg L -1	milligram/liter
NCBI	National Center for Biotechnology Information
nm	Nanometer
NPs	Nanoparticles
PCR	Polymerase Chain Reaction
ppm	Part per million
Se-NPs	Selenium Nanoparticles
TEM	Transmission Electron Microscopy

COMPETING INTERESTS

No conflict of interest

AUTHORS' CONTRIBUTIONS

The authors confirm their contribution to the paper: Usama M. Tahoun collected all samples from the various sites and supervised the project's completion and manuscript writing. All authors (Usama M. Tahoun, Walaa T. Hamza, and Sahar H. Hassan) participated in all experiments, investigations, and analyses inside the laboratories and approved the final version of the manuscript.

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