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Fredinandcohnia onubensis-Synthesized Silver Nanoparticles on Chitosan for Removal of the Pesticide Permethrin from Waste Waters for Aquaculture

Usama M. Tahoun*, Walaa T. Hamza, Sahar H. Hassan
National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt

***Corresponding Author: usamatahoun@yahoo.com**

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ABSTRACT

Pesticide residue levels in water bodies have increased due to the extensive use of pesticides in agriculture. To address this challenge, this study considered the possibility of using biogenic silver nanoparticles (Ag-NPs) embedded in chitosan beads to remove the pesticide. A simple, efficient method was used: Ag-NPs (diameter range: 8.02 to 27.2nm) were biosynthesized by *Fredinandcohnia onubensis* and then immobilized on chitosan to remove the permethrin from aqueous solutions. At 90%, the resultant chitosan silver nanoparticles composite (CS-Ag-NPs composite) showed a remarkable adsorption capacity. To be more precise, 1g of CS-Ag-NPs composite efficiently eliminated 90% of permethrin from a 25mL pesticide solution (0.1mg/ L) under controlled circumstances (35°C, pH 7-, and 60-minutes shaking). These results demonstrate the potential of *Fredinandcohnia onubensis*-biosynthesized CS-Ag-NPs composites as a novel, biocompatible, and eco-friendly method for the removal of pesticide in water treatment applications.

INTRODUCTION

Pesticides are used to eliminate pests from the environment; however, they can harm other living organisms including humans. Pesticides protect crops and fight illnesses, while being toxic to unintended targets since they target the same basic processes in those species. It has been widely investigated that pesticides are detrimental to both people and the environment **(Richardson *et al.*, 2019, Kelany *et al.*, 2024)**. Runoff from agriculture and aquaculture operations as well as gardens transports pesticides and other contaminants into the surface and ground water hurting all aquatic flora and fauna **(Singh *et al.*, 2020)**. Additionally, the permethrin (synthetic pyrethroid) is used widely, and there are growing evidence suggesting that it can harm both humans and animals in various ways, including nervous system damage, liver problems, weakened immune systems, and cell death **(Wang *et al.*, 2016)**.

Saleh *et al.* (2020) mentioned that the growing problem of water pollution caused by pesticides has led to the development of various treatment methods. These methods can be broadly categorized as chemical, physical, and biological, and the adsorption proving very effective at removing some nasty pollutants that harm the environment and public health. The

success of this method depends on the material used (adsorbent). The best ones have plenty of surface area, porosity, and numerous spots for the pollutants to latch onto.

Based on the previous investigations, **Ali *et al*. (2015)** explored using iron-based nanoparticles to remove atrazine (pesticide) from water. Their method achieved a remarkable 95% removal rate under specific conditions: 30 minutes; pH 7, temperature of 20°C, low starting concentration of atrazine (30µg/ l), and a specific amount of the nanoparticle material (2.5g/l). Further analysis suggested the atrazine removal happened in two stages: first it attached to a thin layer of liquid around the nanoparticles, then it was absorbed onto their surface. This approach proved to be cost-effective, fast, and reliable for removing atrazine from water. **Shalaby *et al*. (2018)** investigated a different type of nanoparticle composite made of chitosan and zinc oxide. They tested its ability to remove a pesticide called lambda-cyhalothrin from water. This method achieved a very high removal rate (nearly 98%) within 60 minutes.

Current techniques of producing some nanoparticles involve harmful chemicals, consume a lot of energy, and yield a small number of nanoparticles. Thus, there is an urgent need to find more environmentally responsible techniques to produce nanoparticles. In this respect, biogenics is a promising technology that uses bacteria or plants to produce nanoparticles **(Ahsan, 2020)**.

Nanoparticles can be made by microbes, either inside or outside their cells. These microbes are naturally tough against toxic metals because they can detoxify them in several ways. One way is by pumping the metals out of the cell using energy. Another way is by changing the metals' form to make them less harmful. This detoxification can happen outside the cell (through minerals, absorption, or precipitation) or inside the cell (accumulation). Making metal nanoparticles outside the cell is more useful for practical applications, but controlling their size and shape is a challenge. When the nanoparticles are made inside the cell, they tend to be more uniform in size **(Narayanan & Sakthivel, 2010)**.

Dehaghi *et al*. (2014) reported that, the scientists are developing nanostructures and nanoarrays with features that are not found in larger counterparts. Metal oxides are highly fascinating at the nanoscale due to their small size and the abundance of corners and edges, which give them unique physical and chemical properties. This has sparked considerable interest in employing chitosan in conjunction with metal nanoparticles as a new method of water purification.

In a previous study, **Saifuddin *et al*. (2011)** successfully developed a new material for removing pesticides from water. This material combined chitosan, a natural substance, with tiny silver oxide nanoparticles embedded within it using microwave technology. They tested this composite material and found it effective at adsorbing a common pesticide called permethrin. Silver nanoparticles are a popular choice for a whole bunch of things, from medical tests to filters. This is because they have unique properties that make them good for lots of different tasks, and they are not too expensive to produce **(Elamawi *et al*., 2018)**.

Silver nanoparticles (Ag-NPs) are fascinating because of their unique properties. This makes them very promising for various technologies across many fields. However, there is a need for better ways to use Ag-NPs in water treatment applications. In this context, this research aimed to develop an eco-friendlier method for using Ag-NPs in water treatment. The plan was set to use a specially chosen bacteria to produce the Ag-NPs through a green synthesis process. Then, these biogenic nanoparticles were immobilized (attached) with

chitosan, a natural material. Finally, the researchers tested the effectiveness of this chitosan-Ag-NP composite in removing permethrin, a pesticide, from water.

MATERIALS AND METHODS

Bacterial isolation

Authors took water samples from the Kitchener drain, the main agricultural drain in Gharbia, Egypt (Fig. 1). They collected the samples from various locations (listed in Table 1)) and were applied as the source of bacterial isolation, using sterilized bottles that were kept chilled in an icebox to be transported to the lab for microbial testing **Error! Reference source not found.**.

Fig. 1**. Location of Kitchener drain**

Fig. 1. Location of Kitchener drain

Table 1. Sampling sites from Kitchener drain

No.	Coordinates	Sample sites	Code
1	31°08'02.9"N 31°03'17.2"E	Nimrat Al-Basal village, El-Mahalla al-Kubra (Gharbia Province)	K1
2	31°04'55.8"N 31°04'40.3"E	Al-Banwan, Al-Mahalla al-Kubra (Gharbia Province)	K2
3	31°08'02.9"N 31°03'17.2"E	Kafr Dakhmis, Al-Mahalla al-Kubra (Gharbia Province)	K3
4	31°16'05.6"N 31°07'17.3"E	Ezbet Al-Manawafa, Al-Hamoul (Kafr El-Sheikh Province)	K4
5	31°19'01.8"N 31°08'45.2"E	Al-Hamoul City (Kafr El-Sheikh Province)	K5
6	31°21'42.8"N 31°10'38.1"E	Tambari, Al-Hamoul (Kafr El-Sheikh Province)	K6
7	31°24'42.5"N 31°10'48.5"E	Village No.7, Al-Hamoul (Kafr El-Sheikh Province)	K7
8	31°26'05.9"N 31°10'25.3"E	Village No.9, Al-Hamoul (Kafr El-Sheikh Province)	K8
9	31°27'08.0"N 31°10'10.7"E	Village No.11, Al Majaz Al Sharqiya , Al-Hamoul (Kafr El-Sheikh Province)	K9
10	31°28'10.3"N 31°09'39.5"E	Central Village 13, Al-Hamoul (Kafr El-Sheikh Province)	K10
11	31°29'22.4"N 31°09'07.9"E	Ezbet 51-Al-Zahraa, Al-Hamoul (Kafr El-Sheikh Province)	K11
12	31°31'15.1"N 31°09'23.2"E	Kafr Al-Sawahel, Ezbet Bahri, Al-Zawiya Sector, Burullus (Kafr El-Sheikh Province)	K12

13	31°32'53.7"N 31°06'23.7"E	Tahrir, Al-Rub' (Souk Al-Talat), Burullus (Kafr El-Sheikh Province)	K13

Screening of silver-resistant bacteria

Screening of silver-resistant bacteria was conducted by taking 100µl from each water sample and spreading it on plates containing nutrients and a specific concentration of silver nitrate ($AgNO_3$). The plates were incubated for 48 hours at 30°C, after that, investigation of colonies was performed, while the colonies surrounded by a clear zone (indicating potential silver resistance) were transferred to fresh plates with silver. A single colony from each plate was chosen and grown in liquid nutrient broth containing an even higher concentration of silver nitrate. After incubation, cell growth was monitored using a UV-spectrophotometer scanning a wavelength range of 200 to 800nm with a resolution of 1nm. This confirmed which bacteria could truly thrive in the presence of silver. This technique is similar to the one used by **Das *et al*. (2014)** and **Solís-Sandí *et al*. (2023)**.

Ag-NPs biosynthesis

After growing the selected isolated bacteria on nutrient broth media containing $AgNO_3$ at 30°C for 48 hours under shaking conditions (120rpm), the collection of the culture's supernatant by centrifugation (8000rpm for 10 minutes) and filtration (0.2 µm) was occurred. The supernatant was mixed with an equal amount of fresh $AgNO_3$ solution and incubated for 3 days at 30°C with a gentle shaking (150rpm). Ag-NP production was monitored through direct observation of color changes in the media and by using a UV/VIS spectrophotometer, following methods employed in previous studies **(Musarrat *et al.*, 2010; El-Shanshoury *et al.*, 2020)**.

Bacterial identification

To identify the most effective bacteria for producing silver nanoparticles (Ag-NPs), we classified the isolates using Bergey's Manual of Systematic Bacteriology based on biochemical analysis. This identification was then confirmed through molecular and phylogenetic approaches. We extracted genomic DNA from the most productive bacteria and amplified the 16S rRNA gene using PCR with a thermal cycler (Thermo Fisher Scientific, USA). The Egyptian Microbial Identification Center (EMIC) at El-Sadat University conducted the molecular identification following a published method **(Kim *et al.*, 2011)**, using universal primers: 27F-Forward primer (5′ AGA GTT TGA TCC TGG CTC AG 3′) and 1492R-Reverse primer (5′ CTA CGG CTA CCT TGT TAC GA 3′).

Finally, we analyzed the amplified gene sequence using the BLAST database and deposited it in GenBank, a genetic sequence library, with the unique accession number PP266981. A phylogenetic tree was then constructed to visualize the evolutionary relationships between the microorganisms.

Optimization of Ag-NPs production

The investigations of the optimal conditions for Ag-NP production using the most effective isolated bacteria *Fredinandcohnia onubensis* were implemented. After growing the *F. onubensis* and the supernatant was collected, the supernatants were divided into samples. These samples were incubated under various conditions: different temperatures (20, 25, 30, 35, and 40°C), incubation times (6, 12, 24, 48, 72 and 96 hours), and concentrations of a

silver compound (0.1, 0.2, 0.5,0.75, and 1g/ l). This method was applied twice. To find the optimal conditions, an equal volume of the supernatant was mixed with the silver solution, and the solution's absorbance was measured at 430nm using a UV/VIS spectrophotometer. This approach is similar to the method used by **El-Shanshoury *et al*. (2020)**.

Fabrication and purification of Ag-NPs by *Fredinandcohnia onubensis*

After identifying the optimal conditions for producing silver nanoparticles (Ag-NPs) using *F. onubensis*, two sets of flasks were prepared for further experimentation. One set contained only nutrient broth, serving as the control group, while the other set had silver supplementation. A 1ml aliquot of fresh *F. onubensis* suspension was used as inoculum. The flasks were incubated at 30°C for 24 hours, and then the suspensions were centrifuged. The supernatant was subsequently mixed with an equal volume of silver solution and incubated under optimal conditions, resulting in the production of Ag-NPs. The method used for the purification of Ag-NPs employed were described by **El-Shanshoury *et al*. (2020)**.

Ag-NPs characterization by TEM

The size and morphology of the biosynthesized silver nanoparticles (Ag-NPs) by *F. onubensis* were characterized using the transmission electron microscopy (TEM) available at the Research Laboratories Complex of the Faculty of Agriculture, Cairo University. A JEOL JEM (Japan, 80 kV) instrument was employed for this analysis. TEM sample preparation involved drop coating the Ag-NPs onto carbon-coated TEM grids. Once deposited, the film was allowed to dry, and excess solution was removed with blotting paper. TEM utilizes high-energy electrons to provide information regarding the morphology, composition, and crystallographic structure of the sample.

CS-bionanocomposite preparation

The chitosan bio-nanoparticles composite of silver nanoparticles (Ag-NPs) was prepared following the method reported by **El-Sheshtawy *et al*. (2021)**. A 1% chitosan solution was prepared by dissolving 0.5g of chitosan in 50mL of a 2% acetic acid solution. The mixture was then stirred for 24 hours at 1000rpm and 60°C. Subsequently, 0.5g of dried Ag-NPs were added, and pH of the solution was adjusted to 7. The solution was centrifuged at 5000rpm for 20 minutes to separate the solid product. This solid product was then repeatedly washed with distilled water and finally dried in an oven at 40°C for 24 hours.

Permethrin removal from aqueous solution by CS-bionanocomposite application as an adsorbent

Permethrin adsorption experiments were conducted in batch mode following the method of **Dehaghi *et al*. (2014)**. A shaker incubator was used to agitate a mixture of varying adsorbent dosages (0.01-1.5g) with 25mL of 0.1ppm permethrin solution at 150rpm and 25°C for 45 minutes. The distribution coefficient and the percentage removal of permethrin was determined using a UV-Vis spectrophotometer as described by **Dehaghi *et al*. (2014)**. All experiments were performed in triplicate at pH 7.

RESULTS

Ag-NPs manufacturing bacteria screening

This study investigated the ability of aquatic bacteria isolated from the Kitchener drain in 2023 to produce silver nanoparticles (Ag-NPs). Thirteen morphologically distinct isolates

(**Error! Reference source not found.**) were selected from water samples based on their formation of clear zones (halo zones) around them on agar plates containing silver nitrate. These purified isolates were then screened for their potential to produce Ag-NPs extracellularly. The supernatants of all 13 isolates (K1, K2, K3, K4, K5, K6, K7, K8, K9, K10, K11, K12, and K13) exhibited the ability to form Ag-NPs after being mixed with silver solution (

). Visual inspection (Fig. 2) and spectrophotometric analysis were used to monitor Ag-NP formation, with a color change in the solution (**Error! Reference source not found.**, **Error! Reference source not found.**) indicating their presence. Following a 72-hour incubation, the maximum absorbance of each isolate's solution was measured (Fig. 5). Finally, based on the UV-Vis spectrophotometer analysis, one isolate (K9) with the most promising Ag-NP production capability was chosen for further study.

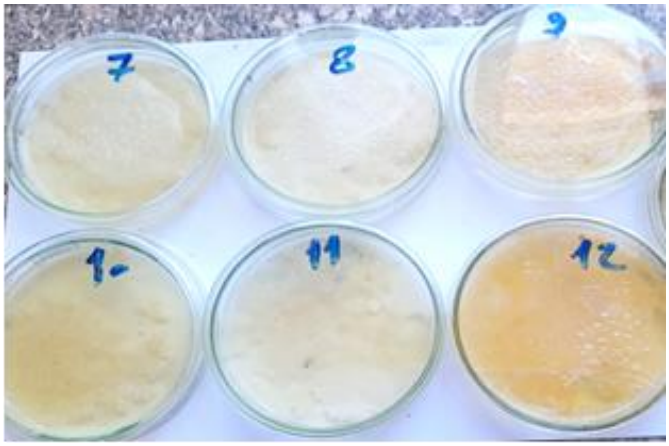

Fig. 2. Strains on the media supplemented with $AgNo_3$

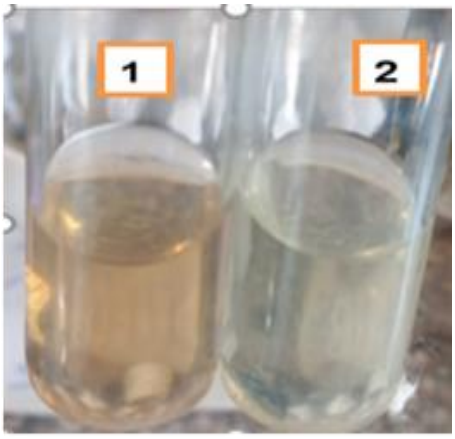

Fig. 3. 1) Media inoculated with bacterial strain; 2) Media without bacterial inoculation

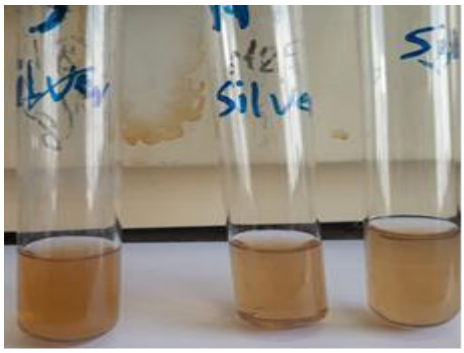

Fig. 4. Supernatants of isolates after incubation

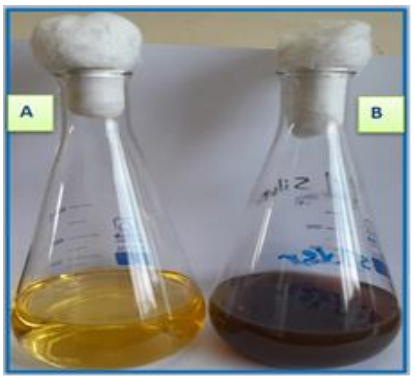

Fig. 5. Growth of isolates, A) in the absence of Ag, and B) in the presence of Ag

Table 2. Absorbance by bacterial strains measured by UV-spectrophotometer; identification of the most efficient isolates

Sample	Absorbance
K1	0.988
K2	1.400
K3	1.308
K4	1.012
K5	1.103
K6	1.214
K7	1.453
K8	1.752
K9	**1.802**
K10	1.300
K11	1.450
K12	1.003
K13	0.930

Bacterial isolate K9 was purified (Fig. 6) and identified as a rod-shaped, aerobic, motile, spore-forming bacterium, exhibiting yellowish, shiny colonies. Fig. (7) shows the agarose gel electrophoresis results for isolate K9. DNA sequencing followed by a BLAST search against the NCBI database revealed that the isolate had 99.6% similarity to *Ferdinandcohnia onubens* strain PP266981, indicating a close genetic match. A phylogenetic tree constructed using sequences from *F. onubensis* further confirmed this relationship (Fig. 8).

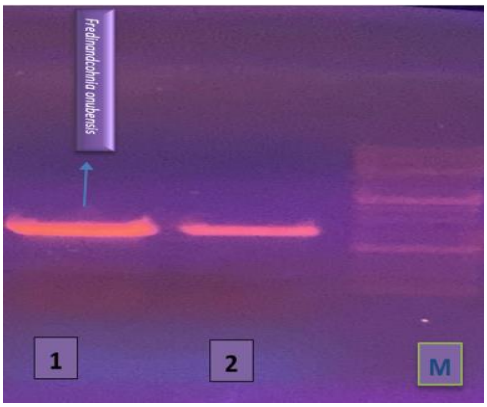

Fig. 6. Bacterial isolate purification

Fig. 7. Pattern of agarose gel electrophoresis of *F. onubensis*, Lanes: M, DNA molecular size marker; 1, amplicon of *F. onubensis*; 2, Amplicon of another microorganism

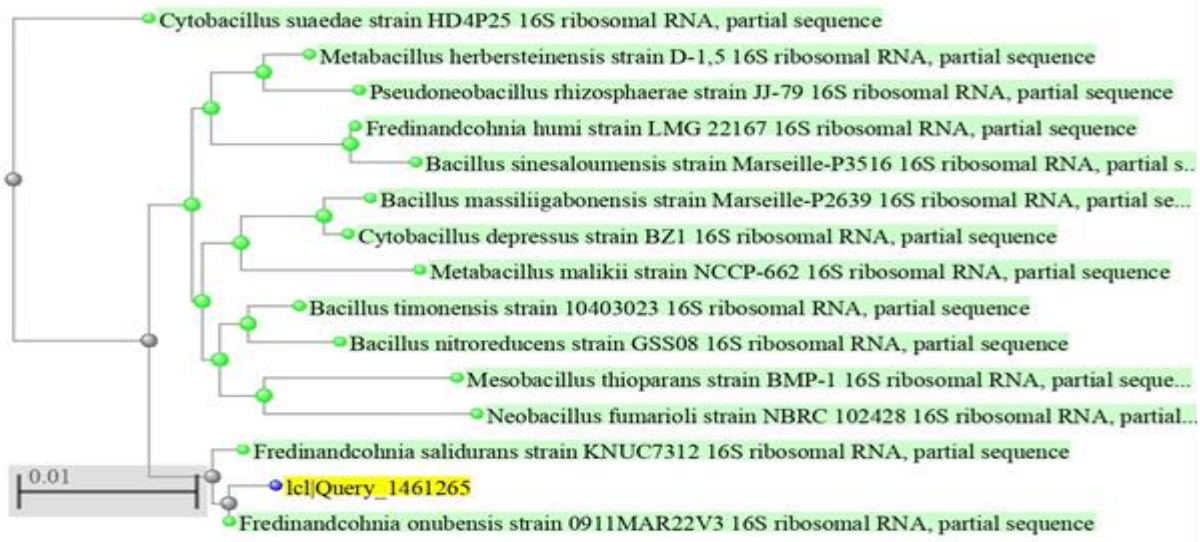

Fig. 8. Phylogenetic tree of *Fredinandcohnia onubensis*

Optimization of Ag-NPs production

The influence of various parameters on Ag-NP production by *F. onubensis* was investigated. Various incubation period (6, 12, 24, 48, 72 and 96 hours) were applied for Ag-NPs production by *F. onubensis*; the incubation time was found to be directly correlated with Ag-NP formation, with the highest productivity achieved after 48 hours, followed by a decrease (Fig. 9). Similarly, for Ag-NP production, optimal temperature was observed at 35°C, with a decline in productivity at higher temperatures (Fig. 10). Finally, the effect of silver concentration on Ag-NP production was assessed. The results indicated that 1g/ L was the most effective concentration for Ag-NP formation by *F. onubensis* (Fig. 11).

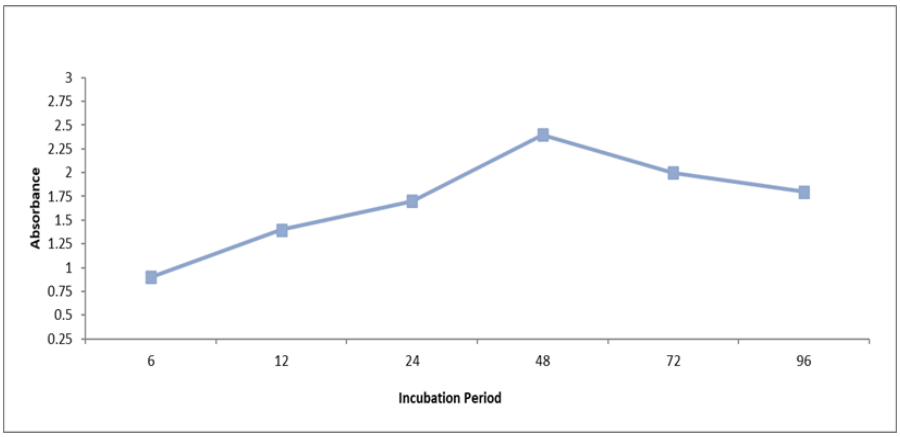

Fig. 9. Ag-NPs produced by *F. onubensis* at different incubation period

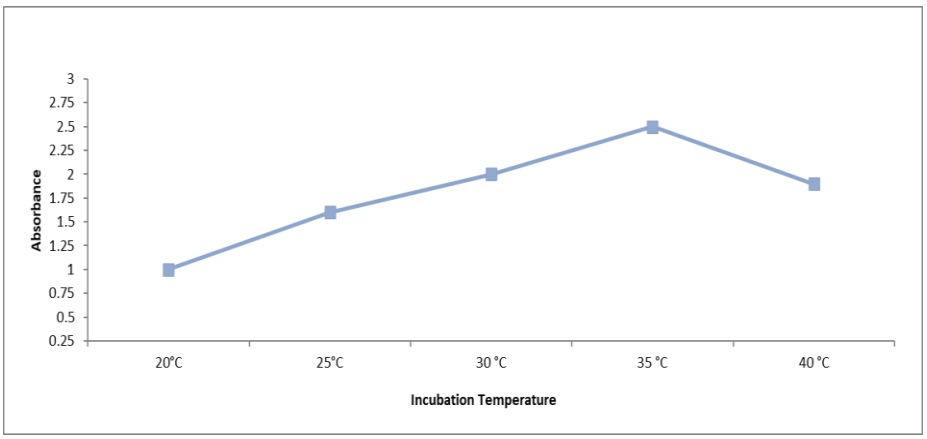

Fig. 10. Ag-NPs produced by *F. onubensis* at different temperature

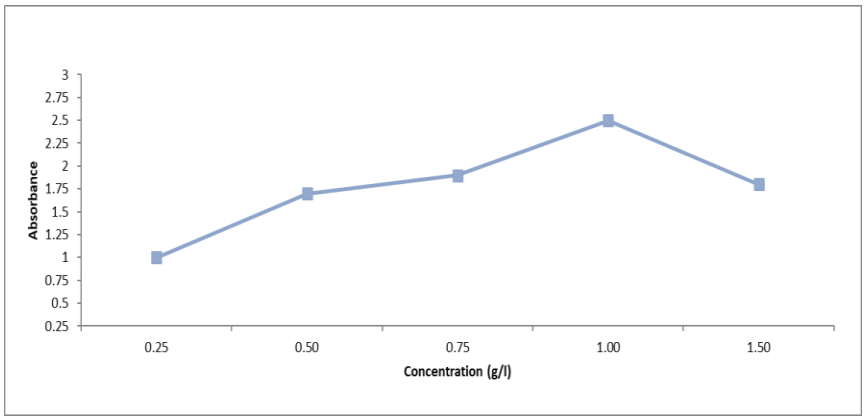

Fig. 11. Ag-NPs produced by *F. onubensis* at metals different concentration

Production and purification of Ag-NPs

F. onubensis was cultivated in nutrient broth media supplemented with silver metal for 24 hours. A color change in the medium was observed, indicating the production of nanoparticles. Additionally, when the cell-free supernatant was challenged with a metal solution, a further color change occurred. This color change suggests that a reduction reaction took place, leading to the formation of Ag-NPs within the solution, then Ag-NPs was purified to more further investigations (**Error! Reference source not found.**).

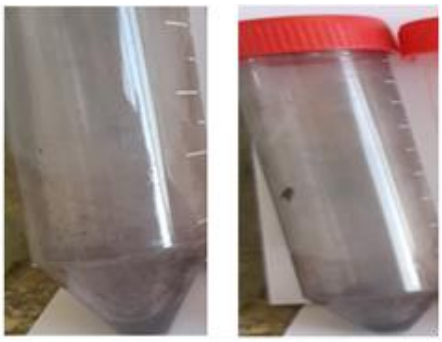

Fig. 12. Ag-NPs production

Ag-NPs characterization by TEM

In Fig. 13), transmission electron microscopy (TEM) images revealed the characteristics of the bio-synthesized silver nanoparticles (Ag-NPs) produced by *F. onubensis*. The Ag-NPs exhibited a polydisperse distribution, meaning they varied in size. However, they primarily displayed a spherical shape. Particle size measurements ranged from 8.02 to 27.2nm, with an average size of approximately 12.99 ± 5.1nm as determined from the diameter distribution analysis.

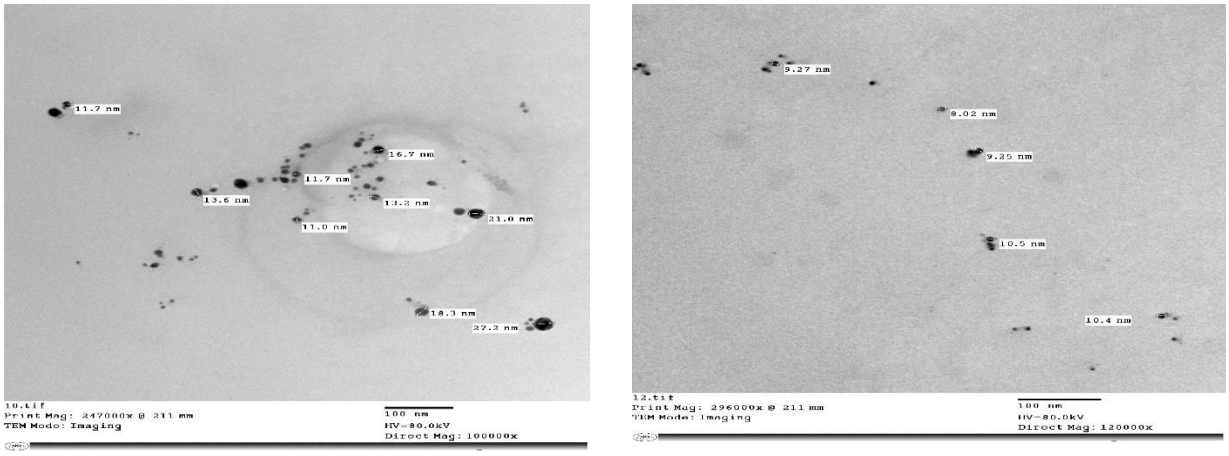

Fig. 13. TEM micrograph of Ag-NPs produced by *F. onubensis*

Adsorption experiments by CS-bionanocomposite

To broaden the applications of bio-synthesized nanoparticles of Ag-NPs, they were immobilized on chitosan. Fig. 14) shows Ag-NPs produced by *F. onubensis* immobilized with chitosan for further investigation into their ability to remove pesticide pollutants from aqueous solution.

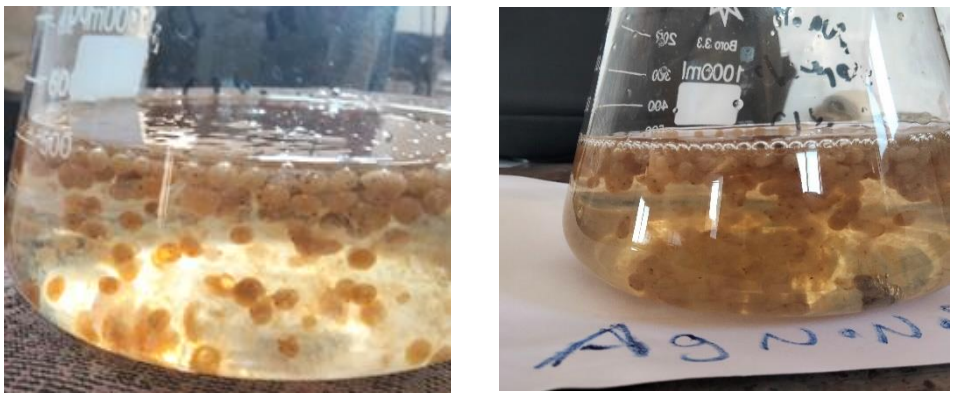

Fig. 14. Bead formation of Ag-NPs

In this study, the impact of sorbent quantity on the percentage removal of permethrin was explored. Constant parameters were maintained while varying the amount of CS-Ag-NPs composite employed (0.01-1.5g). Permethrin removal experiments were conducted using 25mL of solution with an initial permethrin concentration of 0.1ppm and an agitation time of 45 minutes. The influence of CS-Ag-NPs composite dosage (0.1, 0.25, 0.5, 1, 1.25, 1.5g) on permethrin removal percentage was then evaluated. The findings revealed an enhancement in permethrin removal efficiency with increasing the adsorbent quantity up to 1g in the 25mL solution as shown in Fig. 15). It was subsequently observed that exceeding 1g of adsorbent did not lead to a significant increase in the removal percentages. A constant removal percentage was achieved within the dosage range of 1-1.5g, resulting in approximately 88% pesticide elimination. As the most efficient removal was achieved with 1g of CS-Ag-NPs composite, this specific dosage was adopted for all subsequent investigations.

The impact of varying agitation times (20, 30, 40, 50, 60, 80 and 90 minutes) on permethrin adsorption by 1g of CS-Ag-NPs composite was investigated (Fig. 16). Experiments were conducted using 25mL of permethrin solution with an initial concentration of 0.1ppm and a constant temperature of 25°C and shaker speed of 150rpm. This illustrates the influence of agitation period on permethrin adsorption. As depicted in the Fig. (16),

permethrin adsorption increased progressively with time, eventually reaching a plateau, signifying saturation of available binding sites on the CS-Ag-NPs composite. This initial rapid adsorption can be attributed to the ample presence of vacant sites on the adsorbent surface. The sorption percentage rose from 50% at 20 minutes to 90% after 60 minutes, followed by a plateau despite extended contact time.

Furthermore, the effect of increasing permethrin concentration (0.05-0.3mg/ l) on the uptake by 1g of CS-Ag-NPs composite was explored (Fig. 17). The results indicated that the composite achieved a high removal efficiency, reaching up to 90%.

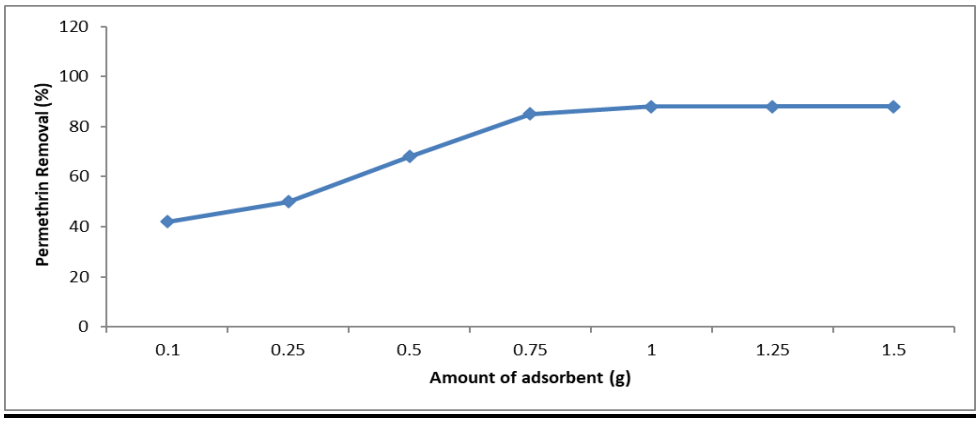

Fig. 15. Amount of sorbent effects on permethrin removal percentage

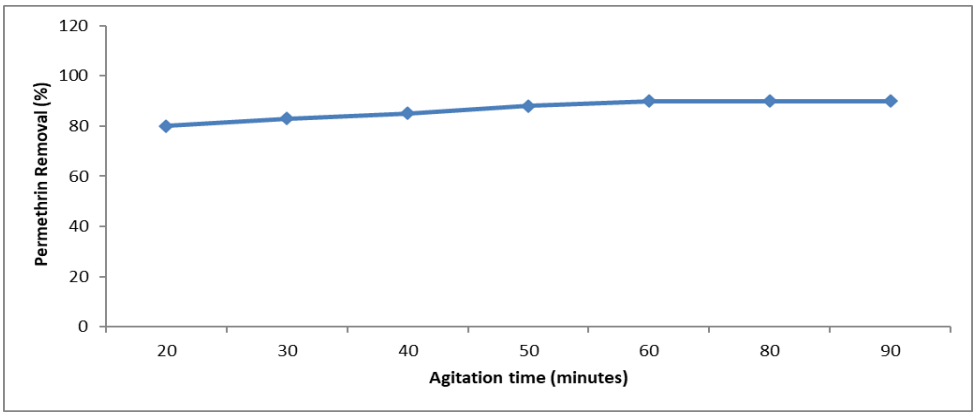

Fig. 16. Effect of agitation time on removal percentage

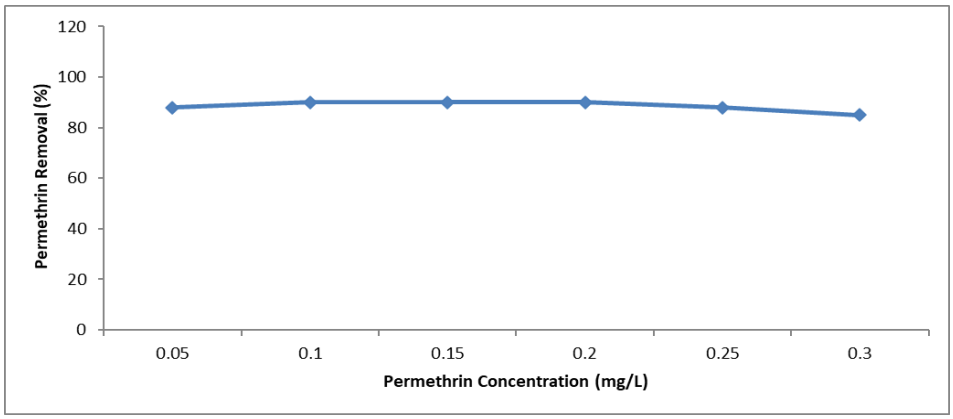

Fig. 17. Impacts of permethrin concentration on percentage of removal process

DISCUSSION

The rise in pesticide application within contemporary agricultural practices has led to a corresponding increase in their concentration within water sources. Consequently, the selection of an appropriate water treatment methodology for pesticide elimination is contingent upon both the specific type of pesticide contaminant and the demonstrated effectiveness of the treatment process itself **(Al-Ghouti & Da'ana, 2020)**. Adsorption has become a widely accepted water treatment process due to its economic viability; this approach is highly effective in the removal of a variety of environmentally significant pollutants. Several factors influence an adsorbent's efficiency, including the availability of active sites, porosity, total surface area, and potential interactions with the target pollutant **(Saleh *et al*., 2020)**.

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, Ag-NPs have attracted technological interest and supporting biotechnological and biomedical applications **(Piacenza *et al*., 2021)**.

Nanotechnology strives to engineer nanostructures with unique properties that are absent in their bulk or single-particle counterparts. Notably, nanoparticles exhibit distinct physical and chemical characteristics due to their limited size and high concentration of active sites at corners and edges **(Cestari *et al*., 2008; Dehaghi *et al*., 2014)**. In recent years, chitosan-based composites containing metal nanoparticles have gained an increasing attention as alternative adsorbents for water treatment. Studies, such as those by **Saifuddin *et al*. (2011)** explored embedding silver nanoparticles within chitosan using microwave irradiation to facilitate pesticide removal from water. This research aimed to develop an adsorbent comprised of a chitosan-silver nanoparticle composite. The investigations successfully demonstrated the potential application of Ag-NPs -chitosan beads for the adsorption of permethrin, a model pesticide.

Microbial strains, including various fungi and bacteria are recognized for their ability to produce various metal nanoparticles through extracellular biosynthesis; this process has been documented by numerous authors such as **El-Sheshtawy *et al*. (2021)**. The size variations observed in microbially produced nanoparticles are attributed to the different types of enzymes secreted by these organisms, as suggested by **Ovais *et al*. (2018)**.

In the present study, the isolated strain from Kitchener drain coded by K9 has a powerful ability to produce Ag-NPs; this isolate was identified as *Fredinandcohnia onubensis*. *Fredinandcohnia onubensis* is also known as *Bacillus onubensis* **(Dominguez-Monino, *et al*., 2018; Gupta *et al*., 2020)** referring to the place where it was found, Huelva, Spain (Onuba was the Roman name for Huelva).

It is a rod-shaped, aerobic, motile bacteria, and it can form oval-shaped spores; these single cells, pairs, or chains are between 0.6 and 0.9µm wide and 2.8 to 3.8µm long, they form yellowish, shiny colonies that are 1-3mm in diameter with uneven edges. These bacteria break down certain compounds and can survive in a wide range of temperatures (from 10 to 47°C), with a preference of 37°C. They can also tolerate a range of salt concentrations (up to 8%) and acidity levels (between pH 6 and 9.5). It hydrolyzes aesculin and Tween 20, but cannot hydrolyze gelatin, Tween 40 & 80, in addition to converting nitrate to nitrite but does

not produce indole from tryptophan. **Dominguez-Monino *et al*. (2018)** mentioned that these bacteria cannot ferment glucose for energy.

Furthermore, a prevalent method for bacterial identification involves sequencing the highly conserved 16S rRNA gene. This technique offers a reliable, uncomplicated, and expeditious approach to elucidating the evolutionary and phylogenetic relationships between bacterial strains **(Hu *et al.*, 2018)**. In line with a research by **Dominguez-Monino *et al.* (2018)**, 16S rRNA gene sequencing was extensively applied in the present study to enable the discrimination of *Bacillus onubensis* from other bacterial species and to produce the Phylogenetic tree.

According to **Saifuddin *et al*. (2009)** and **Ibrahim *et al*. (2021)**, a substantial body of research has documented the production of silver nanoparticles (Ag-NPs) by various microorganisms, including *Verticilium* sp., *Fusarium oxysporum*, *Bacillus cereus, E. coli, Staphylococcus epidermidis, Pseudomonas stutzeri, Salmonella enterica*, *Staphylococcus aureus*, *Porteus mirabilis* and *Thermomonospora* sp. These microbial sources exhibit the capacity to generate Ag-NPs with diverse morphologies and sizes, therefore there are many applications of Ag-NPs.

This study investigated the biosynthesis of silver nanoparticles (Ag-NPs) using *Fredinandcohnia onubensis* cultivated on media supplemented with silver nitrate. *F. onubensis* was selected based on its potential to form Ag-NPs. The cultures were grown under various optimized conditions to maximize Ag-NP production. A combination of visual color change and UV-spectrophotometer analysis was employed to monitor Ag-NP formation. Following the approach described by **Saifuddin *et al*. (2009)**, the synthesized Ag-NPs were collected via high-speed centrifugation. This study identified the optimal conditions for silver nanoparticle (Ag-NP) production by *F. onubensis* as 48 hours incubation at 35°C with a metal concentration of 1g/ L. This aligns with previous work suggesting that bacterial populations can tolerate low metal concentrations, and even enhance nanometals production under such conditions **(Debieuxa *et al*., 2011)**.

Silver nanoparticles (Ag-NPs) produced by *Fredinandcohnia onubensis* were immobilized on chitosan in this study to evaluate their potential application in pesticide removal. Chitosan's inherent amenability to chemical modifications makes it a popular choice for researchers seeking to optimize its functionality and tailor specific characteristics. The presence of amine (NH2) and hydroxyl (OH) groups on the chitosan structure facilitates interaction with nanoparticles through coordination bonding. This approach has become increasingly prevalent, as evidenced by the work conducted by **Olajire and Bamigbade (2021)**.

Bioremoval approaches rely heavily on adsorbent amount. It determines the sorbent's capability for a specific starting sorbate concentration. This concept is consistent with prior findings **(Saifuddin *et al*., 2011; Dehaghi *et al*., 2014)**. As a result, the current study investigated pesticide removal using different quantities of Ag-NP adsorbent. The best dosage was found to be 1g of CS-Ag-NPs. This is due to two opposing effects: at greater dosages (> 1g), the number of active sites on the composite reduces. However, increasing the dose results in a larger overall active surface area. Furthermore, as proven in other studies **(Dehaghi *et al*., 2014; Rahmanifar & Dehaghi, 2014)**, sorbent-sorbent interactions become more prominent than sorbate-sorbent interactions when sorbent amounts increase.

These investigations determined that a 60-hour agitation duration was ideal for obtaining 90% permethrin elimination. Pesticide adsorption began quickly, but gradually

slowed as equilibrium neared. This behavior can be explained by the initial profusion of accessible surface adsorption sites. As these sites are occupied, the repulsive forces between adsorbed permethrin molecules and incoming molecules prevent additional adsorption, a concept investigated in earlier studies **(Sokkera *et al*., 2011; Dehaghi *et al*., 2014; Rahmanifar & Dehaghi, 2014)**.

This study identified 1mg/ L as the optimal concentration for permethrin removal. Interestingly, increasing the initial permethrin concentration resulted in a decrease in the removal percentage. While more permethrin is adsorbed onto the sorbent surface at higher initial concentrations, the removal efficiency declines. This phenomenon can be explained by the saturation of higher-affinity sites on the sorbent. As these sites become occupied, the remaining permethrin molecules encounter lower-affinity sites, leading to a reduction in removal efficacy. This aligns with observations of previous authors **(Saifuddin *et al*., 2011)**.

This study successfully demonstrated the ability of *Fredinandcohnia onubensis* to biosynthesize silver nanoparticles (Ag-NPs). These findings suggest a promising future and potential applications for Ag-NPs derived from *F. onubensis*.

CONCLUSION

This study investigated the potential of *Fredinandcohnia onubensis* isolated from the Kitchener drain (Village No. 11, Al Majaz Al Sharqiya, Al-Hamoul, Kafr El-Sheikh Province, Egypt) for biomanufacturing silver nanoparticles (Ag-NPs). The isolated *F. onubensis* strain demonstrated a remarkable ability to produce Ag-NPs with diameters ranging from 8.02 to 27.2nm. Furthermore, the research explored the application of these biogenic Ag-NPs for the removal of permethrin from an aqueous solution. Chitosan-Ag-NPs (CS-Ag-NPs) composite was employed as an adsorbent, exhibiting a high adsorption capacity of up to 90%. Notably, 1g of CS-Ag-NPs beads effectively removed 90% of permethrin from a 25 mL pesticide solution (0.1mg/ L) under controlled conditions (room temperature, pH 7-, and 60-minutes shaking time).

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LIST OF ABBREVIATIONS

16S rRNA	16S ribosomal ribonucleic acid
Ag-NPs	Silver Nanoparticles
BLAST	Basic Local Alignment Search Tool
CS-Ag-NPs	Chitosan-Sliver Nanoparticles Composite
mg L^{-1}	milligram/liter
NCBI	National Center for Biotechnology Information
nm	Nanometer
ppm	Part per million
TEM	Transmission Electron Microscopy

REFERENCES

Ahsan, T. (2020). Biofabrication of silver nanoparticles from Pseudomonas fluorescens to control tobacco mosaic virus. Egyptian journal of biological pest control, 30(1), 66. https://doi.org/10.1186/s41938-020-00268-3

Al-Ghouti, M.A. and Da'ana, D.A. (2020). Guidelines for the use and interpretation of adsorption isotherm models: A review. Journal of hazardous materials, 393, 122383. https://doi.org/10.1016/j.jhazmat.2020.122383 .

Ali, I.; AlOthman, Z and Al-Warthan, A. (2015). Sorption, kinetics and thermodynamics studies of atrazine herbicide removal from water using iron nano-composite material. Int. J. Environ. Sci. Technol. 13: 733-742. https://doi.org/10.1007/s13762-015-0919-6

Cestari, A. R.; Vieira, E. F.; Tavares, A. M. and Bruns, R. E. (2008). The removal of the indigo carmine dye from aqueous solutions using cross-linked chitosan-Evaluation of adsorption thermodynamics using a full factorial design. Journal of Hazardous Materials, 153(1-2), 566-574. https://doi.org/10.1016/j.jhazmat.2007.08.092.

Das, V.L.; Thomas, R.; Varghese, R.T.; Soniya, E.V.; Mathew, J. and Radhakrishnan, E.K. (2014). Extracellular synthesis of silver nanoparticles by the Bacillus strain CS 11 isolated from industrialized area. 3 Biotech, 4: 121-126. https://doi.org/10.1007/s13205-013-0130-8

Debieuxa, C.M.; Dridgea, E.J.; Muellera, C.M.; Splatt, P.; Paszkiewicz, K.; Knight, I.; Florance, H.; Love, J.; Titball, R.W.; Lewis, R.J.; Richardson, D.J. and Butler, C.S. (2011). A bacterial process for selenium nanosphere assembly. *PNAS* **2011,** 108*:* 1–6, https://doi.org/10.1073/pnas.1105959108

Dehaghi, S.M.; Rahmanifar, B.; Moradi, A.M. and Azar, P.A. (2014). Removal of permethrin pesticide from water by chitosan–zinc oxide nanoparticles composite as an adsorbent. Journal of Saudi Chemical Society, 18(4): 348-355. https://doi.org/10.1016/j.jscs.2014.01.004

Dominguez-Moñino, I. ; Jurado, V.; Gonzalez-Pimentel, J.L.; Miller, A.Z. and Hermosin, B. *et al*. (2018). *Bacillus onubensis* sp. nov., isolated from the air of two Andalusian caves. Syst Appl Microbiol, 41:167–172.

Elamawi, R. ; Al-Harbi, R. and Hendi, A. (2018). Biosynthesis and characterization of silver nanoparticles using Trichoderma longibrachiatum and their effect on phytopathogenic fungi. Egyptian journal of biological pest control, 28(1): 1-11. https://doi.org/10.1186/s41938-018-0028-1

El-Shanshoury, A.R.; Darwesh, O.M.; Sabae, S.Z.; Awadallah, O.A. and Hassan, S.H. (2020). Bio-manufacturing of selenium nanoparticles by Bacillus subtilis isolated from Qarun Lake and evaluation their activity for water remediation. Biointerface Res. Appl. Chem., 10: 5834-5842. https://doi.org/10.1007/s40995-021-01147-8.

El-Sheshtawy, H.; Hefni, H.; Aboutaleb, W., Elaasser, M.; Mady, M. and El-Shiekh, H. (2021). Green Synthesis of Chitosan Bio-Nanocomposites and Investigation of their Antimicrobial and Antitumor Effects. *Iranian Journal of Science and Technology, Transactions A: Science*, 45: 1247-1261. https://doi.org/10.1007/s40995-021-01147-8.

Gupta, R. S.; Patel, S.; Saini, N. and Chen, S. (2020). Robust demarcation of 17 distinct Bacillus species clades, proposed as novel Bacillaceae genera, by phylogenomics and comparative genomic analyses: description of Robertmurraya kyonggiensis sp. nov. and proposal for an emended genus Bacillus limiting it only to the members of the Subtilis and

Cereus clades of species. International journal of systematic and evolutionary microbiology, 70(11): 5753-5798. https://doi.org/10.1099/ijsem.0.004475

Hu, D.; Cha, G.; Gao, B. (2018). A phylogenomic and molecular markers based analysis of the class Acidimicrobiia. Frontiers in microbiology, 9: 987. https://doi.org/10.3389/fmicb.2018.00987

Ibrahim, S.; Ahmad, Z.; Manzoor, M.Z.; Mujahid, M.; Faheem, Z. and Adnan, A. (2021). Optimization for biogenic microbial synthesis of silver nanoparticles through response surface methodology, characterization, their antimicrobial, antioxidant, and catalytic potential. Scientific Reports, 11(1): 770. https://doi.org/10.1038/s41598-020-80805-0

Musarrat, J.; Dwivedi, S.; Singh, B.R.; Al-Khedhairy, A.A.; Azam, A.; Naqvi, A. (2010). Production of antimicrobial silver nanoparticles in water extracts of the fungus Amylomyces rouxii strain KSU-09. Bioresource Technology, 101, 8772–8776, https://doi.org/10.1016/j.biortech.2010.06.065.

Narayanan, K.B. and Sakthivel, N. (2010). Biological synthesis of metal nanoparticles by microbes. Advances in colloid and interface science, 156(1-2), 1-13. https://doi.org/10.1016/j.cis.2010.02.001

Olajire, A.; Bamigbade, L. (2021). Green synthesis of chitosan-based iron@ silver nanocomposite as adsorbent for wastewater treatment. Water Resources and Industry, 26: 100158. https://doi.org/10.1016/j.wri.2021.100158

Ovais, M.; Khalil, A.; Ayaz, M.; Ahmad, I.; Nethi, S. and Mukherjee, S. (2018). Review: Biosynthesis of metal nanoparticles via microbial enzymes: A mechanistic approach. Intern J Mole Sci 19(12): 4100–4120. https://doi.org/10.3390/ijms19124100

Piacenza, E.; Presentato, A.; Ferrante, F.; Cavallaro, G.; Alduina, R.; Chillura Martino, D.F. (2021). Biogenic selenium nanoparticles: a fine characterization to unveil their thermodynamic stability. Nanomaterials, 11(5): 1195. https://doi.org/10.3390/nano11051195

Kelany, M.S.; , Attia, H.A.; Sharawy, Z.Z.; Abbas, E.M.; Elsaied, H.E. and Mustafa, F.H.A. (2024). Utilizing Magnetic Chitosan Hydrogel Nanocomposite for the Processing, and Treatment of Aquaculture Effluent. Egyptian Journal of Aquatic Biology & Fisheries, 28(4): 595 – 615

Rahmanifar, B. and Moradi Dehaghi, S. (2014). Removal of organochlorine pesticides by chitosan loaded with silver oxide nanoparticles from water. Clean Technologies and Environmental Policy, 16: 1781-1786. https://doi.org/10.1007/s10098-013-0692-5

Richardson, J.R.; Fitsanakis, V.; Westerink, R.H. and Kanthasamy, A.G. (2019). Neurotoxicity of pesticides. Acta neuropathologica, 138: 343-362. https://doi.org/10.1007/s00401-019-02033-9

Saifuddin, N.; Nian, C.Y.; Zhan, L.W. and Ning, K.X. (2011). Chitosan-silver nanoparticles composite as point-of-use drinking water filtration system for household to remove pesticides in water. Asian Journal of Biochemistry, 6(2): 142-159. https://doi.org/10.3923/ajb.2011.142.159 .

Saifuddin, N.; Wong, C.W. and Yasumira, A.A. (2009). Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. Journal of Chemistry, 6: 61-70. https://doi.org/10.1155/2009/734264.

Saleh, I.A.; Zouari, N. and Al-Ghouti, M.A. (2020). Removal of pesticides from water and wastewater: Chemical, physical and biological treatment approaches. Environmental Technology & Innovation, 19: 101026. https://doi.org/10.1016/j.eti.2020.101026

Shalaby, T.; Abdalla, S. and El-Segini, H. (2018). Efficient adsorption of heavy metals and pesticides onto chitosan nanoparticles and chitosan-zinc oxide nanocomposite. Acad. J. Agric. Res. 6 (5): 133–149. https://doi.org/10.4038/tar.v26i2.8102

Singh, J.; Yadav, P.; Pal, A.K. and Mishra, V. (2020). Water pollutants: Origin and status. Sensors in water pollutants monitoring: Role of material, 5-20. https://doi.org/10.1007/978-981-15-0671-0_2.

Sokkera, H.H.; El-Sawy, N.M.; Hassan, M.A. and El-Anadouli, B.E. (2011). Adsorption of crude oil from aqueous solution by hydrogel of chitosan based polyacrylamide prepared by radiation induced graft polymerization, J. Hazard. Mater. 190: 359–365. https://doi.org/10.1016/j.jhazmat.2011.03.055 .

Solís-Sandí, I.; Cordero-Fuentes, S.; Pereira-Reyes, R.; Vega-Baudrit, J. R.; Batista-Menezes, D.; de Oca-Vásquez, G.M. (2023). Optimization of the biosynthesis of silver nanoparticles using bacterial extracts and their antimicrobial potential. Biotechnology Reports, 40, e00816. https://doi.org/10.1016/j.btre.2023.e00816 .

Wang, X.; Martínez, M. A.; Dai, M.; Chen, D.; Ares, I.; Romero, A. ; Yuan, Z. (2016). Permethrin-induced oxidative stress and toxicity and metabolism. A review. Environmental Research, 149: 86-104. https://doi.org/10.1016/j.envres.2016.05.003.