Biodegradation of chlorpyrifos pesticide using silver bio-nanoparticles *Bacillus thuringiensis israelensis* extracts

Labeeb A. Al-Zubaidi¹, Marwah Th. Alnuaimi¹, Zahraa Zahraw Aljanabi² and Manal M. Adel³

¹Ministry of Science & Technology/ Directorate of Environment and Water, Baghdad-Iraq.
²Environment Research Centre, University of Technology, Baghdad, Iraq.
³National Research Center -Pests & Plant Protection Dep. Agriculture Division, EGYPT

E-mail: Drmarwa520@gmail.com, zahraa.zfarhan@uotechnology.edu.iq

Abstract. Pesticides are vastly used for pests monitoring in agriculture and public health fields, causing severe depletion in quality of drinking water. Moreover, most insecticides resist biodegradation and carcinogen even at very low levels up to Parts per billion (ppb). This study was carried during April/2019 and extended to January/2020 in Directorate of Environment and Water, Baghdad-Iraq to synthesis and characterization silver bionanoparticles by using *Bacillus thuringiensis israelensis* extract (biological methods) and investigate this activity on the chlorpyrifos pesticide under laboratory conditions. The silver bionanoparticles were characterized using many techniques, X-ray Diffractometer (XRD) Fourier Transform Infrared (FTIR), atomic force microscopy (AFM) and zeta potential analyzer spectroscopy. The results of XRD technique confirmed the crystalline nature of the nanoparticles. AFM analysis revealed that particles were spherical, single or in aggregates. Determination the shifting of active groups sites was performed using FTIR. The zeta potential values were -36.33 mV for AgNPs. The biodegradation of chlorpyrifos using silver bionanoparticles was determined quantitatively using high performance liquid chromatography (HPLC) techniques. From the result, it can be suggested that silver bionanoparticles from *Bacillus thuringiensis israelensis* extracts lead to biodegradation of chlorpyrifos completely without forming harmful products confirmed by GC-MS analysis. We endorse that this process has scientific potential in the biodegradation of chlorpyrifos pesticide contaminated water using Green biosynthesis of nanoparticles.

Keywords: *Bacillus thuringiensis israelensis* extracts; biodegradation; chlorpyrifos pesticide; silver bionanoparticles.

1. Introduction

Pesticides occupy unparalleled place among contaminants found in the ecosystem, where they are intentionally used to control, insects in agribusiness [1]. Moreover, pesticides are poisonous to a lot of non-target species including, beneficial insects and humans too. Being commonly utilized, they leak into ground and surface water causing serious threat to quality of drinking water. Pesticides persist in the soil and have a significant health menace and the possible effects of pesticides exposure, can induce different types of cancer such as pancreatic and bladder cancer [2]. Chlorpyrifos, O, O- Diethyl- O- (3, 5, 6- trichloro-2-pyridyl) phosphorothioate (C₉H₁₁Cl₃ NO₃ PS), an organophosphate insecticide, is one of the most-widely used active ingredients for insects’ control in the world [3]. Like some pesticides, its action is due to the inhibition of the enzyme called acetylcholinesterase, resulting in the aggregation of the neurotransmitter, acetylcholine, at nerves endings [4]. This leads to extreme transmission of nerve impulses, which causes death [5]. Thus, it becomes necessary to develop novel methods, which are able to degrade pesticides even, at ppm or ppb levels.
The modern nanotechnology is increasingly clear in whole of sciences, including field of ecological studies. Nanoparticles referred to groups of atoms, with size ranging from 1 to 100 nm [6]. Actually, Nanoparticles show new and improved characteristics compared to larger molecules of material and, these new characteristics are derived from specific properties such as size, ionic state, distribution and morphology, of the particles [7]. Biotechnology uses nanoparticles that are manufactured on biological platforms such as algae, fungi, yeast, bacteria, radial fungi and plants. These allow their use in catalysis, antibacterial, anti-cancer, drug delivery as well as mechanical and optical applications [8]. The use of silver nanoparticles for environmental remediation is a new area of study in which only restricted research has happened so far [9]. The production of silver bio nanoparticles by microorganisms or biological materials may be synthesized using bacteria or fungi, or using plants extract. Nano techniques provide an advantage over other methods of synthesizing silver nanoparticles in an environment friendly manner with lesser requirement of input energy and can take place around room temperature [10]. Some types of fungi and bacteria have been successfully used in the green synthesis of silver nanoparticles, and it is can take place both extracellular and intracellular [11]. Based on this, a study was carried out to assess the biodegradation of the chlorpyrifos via silver bionanoparticles using Bacillus thuringiensis israelensis extracts.

2. MATERIALS AND METHODS
Chemicals: Chlorpyrifos: Technical grade chlorpyrifos was obtained from Ministry of Agriculture/ Plant Protection Department, Iraq. AgNO3: was bought from Sigma Aldrich. Nutrient agar, Nutrient broth from HI media, India. Microorganism: The bacterial isolate Bacillus thuringiensis israelensis were obtained from the Department of Plant Protection/ College of Agriculture/ University of Baghdad, Iraq. Their purity was verified using some primary (Appearance tests) and biochemical tests and save until being use.

2.1. Synthesis of AgNPs; Preparation of silver, nitrate (AgNO3) solution
The bacterial production extracts were dropped onto AgNPs solution (1 mmol/ L) and incubate for 48 h. 16.98 g of silver, nitrate (AgNO3) was dissolved in 100 ml of de-ionized distilled water (DW), 1ml of prepared AgNO3 which was added to 1000 ml of de-ionized distilled water to make 1 mM solution. Storage of AgNO3 solution. The B. Thuringiensis israelensis isolate was grown in suitable fermentation media at laboratory conditions (incubate at 36°C) for 3 days. Washing of biomass B. thuringiensis israelensis culture by using centrifugation at 4500 rpm for 20 minutes. 1 ml of Supernatant was used for synthesis of silver bionanoparticles while discarded the biomass. One ml of previously supernatant was added in to 99 ml of AgNO3 solution (1 mM). The pH was regulated to 8 by addition of sufficient quantity of NaOH. Then, all the flasks, was placed, in a shaking, water bath, (150 rpm/ 35°C) for, three days in darkness conditions at 35°C. The appearance of light yellowish-brown color was a signal for the production of Ag bioNPs [2].

The solution of Ag bioNPs solution was dried by using spray dryer. Finally, the Dried molecules were collected and then preserved, for more characterization procedure [12][13].

2.2. Characterization and description of AgNPs
The present research investigates the green synthesis of silver bio nanoparticles - AgNPs by utilizing isolated bacteria B. thuringiensis israelensis extracts (biological method). Silver nanoparticles were successfully synthesized, detected and carried out using X Ray Diffraction (XRD), Atomic Force Microscope (AFM), Fourier-transform infrared spectroscopy (FTIR) and zeta potential.

2.2.1 XRD-measurement (XRD). A thin film of uniformly water suspended of each type of nanoparticles was dried on a one glass, slide. X-ray, diffraction pattern was recorded by using x-ray diffractometer at (2θ/θ) scanning, mode and operational, voltage (40) kV and current (30) mA, Cu K (α) radiation, λ = 1.540 [14].
Data were recorded for the 2θ range of 10 to 80 degrees with a step of 0.0200 degree. The result gained from the XRD was explained with standard reference of Joint Committee on Powder Diffraction Standards for the characterization of bio AgNPs [15]. By using Debye–Scherrer equation the particles size of the samples was determined as follows:

Particles size = 0.9 / cos θ.

The crystal size is θ the diffraction angle (Braggs angle) in radians wavelength of x-ray, and is the full width at half maximum (FWHM) of the peak in radians [16].

2.2.2. Atomic Force Microscopy. From each, type of nanoparticles A thin, film of the, sample, were placed on a slide through dropping 100 μl of the sample (AgNPs) on the glass slide, and then, dried for 5 min [5]. The final step was scanning of glass, slides with, the AFM [16].

2.2.3. FTIR analysis. The Fourier transformed infrared (FTIR) spectra were recorded using Perkin Elmer-RS1 spectrometer in the diffuse reflection mode at resolution of 4/cm and all measurements were carried out in the range of 400–4,000/cm-1 [12].

2.2.4. Zeta potential. The stability and constancy of the synthesized silver nanoparticles was estimate in terms of zeta potential using the zeta potential analyzer allowing to run from (-160) mV to (+160) mV, and plotting the data in graph [18][19].

2.3. Estimate the quantitative loss of pesticide as a result of use the microbial silver nanoparticle, by using specialized chemical methods

2.3.1. Extraction of Pesticide Residues Samples for HPLC Analysis. Chlorpyrifos (25, 50 and 100) ppm with bio silver nanoparticles (25, 50 and 100 ml) separately incubated at 31°C in shaker at 150 rpm. Chlorpyrifos residues were reveal at 7 days from inoculation. Each sample was performed in 3 replicates. A medium without bio silver nanoparticles with an identical amount of chlorpyrifos was run simultaneously under same conditions.

Five milliliters from the previously mentioned samples were centrifuged at 4000 rpm for 15 min to get rid of precipitate and extracted 3 times with 50 ml of dichloromethane by shaking. For dehydration Dichloromethane extract was passed across anhydrous Na2SO3, and then dried using rotary evaporator at 30 C°. Dissolved the dry residue was in 1 ml acetonitrile and subjected to HPLC [20].

2.3.2. Gas chromatography-mass spectrometry analysis for biodegradation of Chlorpyrifos pesticide. GCMS analyses for the biodegradation chlorpyrifos pesticide were carry out in electron ionization (EI, 70 eV) mode in a Shimadzu GC 2010 plus coupled to a mass, selective detector (Shimadzu MS 2010 plus). The (GC-MS) (armed, with J&W, Scientific DB5 column that’s have 30 m × 0.25 mm × 0.25 μm), the temperature of oven started at 50 C° and kept for 1min and then increased 250 C’ and held for 11 min; both detector and injector temperature was maintained at 200 C°; and used helium as the carrier gas at 60 kPa pressure with a run time of 31 min. The retention times for CP and TCP were 12 and 17 min, respectively [21].

Liquid-liquid extraction procedure was used for extraction of residues chlorpyrifos from the culture. Chlorpyrifos culture was transferred to separating glass funnel and added 20 ml n hexane to medium. For 5 minutes the mixture, was shaken, and kept tranquil until, the separation of two liquids took, place eventually. All samples were extracted 3 times and n hexane layer was collected in 500 ml conical flasks. Rotary evaporator at 50°C used to evaporated extract to almost dry under pressure using vacuum pump and the residue of chlorpyrifos was re-dissolved in 5 ml n hexane in sterile glass vials for GC- MS analyses [22].
3. Results and Discussion

3.1. Synthesis of Ag bioNPs

The Ag bioNPs formation was change the mixture color from bright white to light yellow (after 72 h). As the color intensity increased the aggregation of bio AgNPs increased after 3 days incubation (dark brown) Fig. 1. The reduction property of silver ions and production of silver nanoparticles AgNPs were in fact still not clear, but can be related to enzyme and protein molecules includes the enzymes of nitrate reductase, that’s act as perfect agent in silver nanoparticles synthesis [23]. As elucidated by [24] green synthesis of AgNPs show strong absorption level of electromagnetic waves in the visible range due to their properties of optical, resonant, known as Surface Plasmon Resonance (SPR). The Surface Plasmon Resonance is highly affected by size and shape of the silver nanoparticles. as well, the bacteria, can produce, inorganic metal ions because it has the metal-microbe, interaction, and have numerous applications, in fields of sciences, includes bioremediation, bio-mineralization, and microbial, corrosion [25]. While [26] explain how the, NPs extracellular biosynthesis of NPs is mediated by many enzymes located on the cell membrane or released to the growth medium. Thus, NPs produced may be present in the medium or be adsorbed on the cell membrane.

![Figure 1](image1.png)

**Figure 1.** Visual observation of Synthesis of bio Silver nanoparticles. (A) AgNO3 (10mM) Solution. (B) After 48 h of preparation.

3.2. Characterization of AgNPs

The present research investigates the synthesis and production of silver nanoparticles (Ag NPs) by green synthesis method using *B. thuringiensis israelensis* extracts. The detailed characterization and description of the Ag NPs was done by using X Ray Diffraction (XRD), Atomic Force Microscope (AFM), Fourier-transform infrared spectroscopy (FTIR), and zeta potential.

3.2.1. X-Ray Diffraction (XRD)

The green synthesis of AgNPs was further supported by X-ray diffraction (XRD). In Figure 2 recorded four obvious diffraction peaks at values 38.20, 44.31, 64.39 and 97.90 for AgNPs which were corresponded to 111, 200, 220 and 400 planes of silver.

The XRD patterns indicated that the structure of bio AgNPs produced was spherical in shape. XRD pattern clearly showed that the AgNPs formed by the reduction of Ag+ ions using *B. thuringiensis israelensis* extracts are crystalline in nature. This agreed with [12] when they AgNPs, were successfully, green synthesizing, by using *B. thuringiensis*. 
3.2.2. Atomic Force Microscopy. The results of AFM analysis showed both the two dimensional and three-dimensional view of AgNPs they were spherical in shape, single or in aggregates, AFM analysis also displayed that the average size of AgNPs particles were 62.6 nm Figure 3 a, b.

![AFM images](image)

**Figure 2.** XRD patterns of Ag bio nanoparticles by using bacterial production extracts.

**Figure 3:** AFM images (a) AFM image showed 2 dimensional of AgNPs (2-D profile of AgNPs agglomeration 5x5um). (b) Showed column AFM diagram of size range of AgNPs

This finding is in agree with [13] where showed that the biosynthesized AgNPs were almost spherical, single or in aggregates. Nanomaterials are used for many biological treatments. When the nanoparticles are prepared, the surface area per unit mass of the material increases, thus, gaining more contact with the surrounding materials and this affects the speed and efficiency of the reaction. Nanoparticles show a quantum effect. Therefore, less activation energy is required for chemical reactions to occur. Surface plasmon resonance is another phenomenon shown by nanoparticles that can be used to detect toxic substances. As far as shape and size are concerned, the nanoparticles can diffuse or penetrate into a
contamination zone where the fine particles cannot reach and have higher oxidation-reduction interaction -
fractable contaminants [14].

3.2.3. Fourier Transform Infra-Red Spectroscopy. FTIR analysis measurement of the AgNPs synthesized
by *B. thuringiensis israelensis* extracts showed stabilization and reduction of metal nanoparticles and a band
at 3514.30, 3491.16 and 3452.58/ cm indicated bending N-H stretch. The band at 1747.51/ cm can assigned
to be C=O stretching vibration of esters while bands 1627.92/ cm-1, indicated N-H bend while peak at
1512.19/cm showed C=C which indicated the formation of aromatic ring and alkene. While the peak
at1384.89/cm revealed the formation of C-H stretching.

The analysis of FTIR provided evidence of protein coat on the stabilized and steady AgNPs. This implicit
that proteins of the *B. thuringiensis israelensis* extract has stronger affinity to bind with Ag⁺ ions and thus
could act as stabilizing and capping agents thereby decreasing the assemblage of NPs [27] Fig. 4.

![FTIR spectra results](image_url)

**Figure 4:** FTIR spectra results. (a) Fourier Transform Infra-Red Spectroscopy from *B. thuringiensis*
*israelensis* extracts. (b) Fourier Transform Infra-Red Spectroscopy from solid powder silver bio-
nanoparticles

FTIR analysis explain that capping material to be protein in nature. Biological particles performed
functions of stabilization and formation of silver nanoparticles in the aqueous medium [28].

The synthesis of AgNPs by biological material resulted from the presence of large number of organic
chemicals like fat, proteins, carbohydrate, enzymes, alkaloids, phenols, flavonoid gum, terpenoids etc. that’s
are capable of donating electron for the reduction process of Ag+ ions to Ag0. Depending upon extract microorganisms used the active component responsible for reduction of Ag+ ions is differed [29].

3.2.4. Zeta potential. The results of zeta potential values of the synthesized nanoparticles were -36.33 mV for AgNPs Fig. 5. Zeta potential is a key pointer of the steadiness and stability of colloidal nanomaterial. The size of the zeta potential refers to the degree of electrostatic repulsion between similarly charged particles. For particles and molecules that are small enough a high zeta potential will give stability and steadiness, i.e., the solution will resist aggregation. When the potential is small the attractive forces may exceed Therefore, colloids materials with elevated zeta potential (positive or negative) are electrically stabilized while colloids materials with low zeta potential Trend to flocculate or coagulate [30, 31]. Generally, the zeta potential of the silver nanoparticles should be either higher than +30 mV or lower than - 30 mV [32, 33]. So, the results showed stability while the rest nanoparticles were very near from normal stability range. This finding is agreed with [34], where found the zeta potential of green synthesized AgNPs -31.10 ± 0.42 mV with Bacillus Subtilis. The Zeta potential distributed with range of -18.9mV indicated the stable in nature of AgNPs synthesized using Bacillus thuringiensis extract [35].

![Figure 5. The zeta potential value of bio AgNPs.](image)

3.3. Estimate the quantitative loss of pesticide as a result of using the microbial silver nanoparticle by using specialized chemical methods.

3.3.1 High performance liquid chromatography (HPLC). Mineralization of pesticide using silver bionanoparticles is an upcoming work field. Very few research works have been done and are discussed here. HPLC technique that also used to detect of Chlorpyrifos pesticide exhibited that samples which treated with AgNPs had low concentration of Chlorpyrifos pesticide compared with control (pesticide samples that not treated with nanoparticles). The extent of biodegradation was evident from the reduction of the peak and it is also observed from the decrease of area of the peak (Figure 6). The results showed that the AgNPs was able to degrade chlorpyrifos after 7 days of incubation compared with Bacillus sp had less efficiency for
chlorpyrifos with degradation rate 40% [36]. This study is the first report about the use of metal nanoparticles in laboratory control of Chlorpyrifos pesticide contamination.

Fig. 6. HPLC results. (a) HPLC standard of chlorpyrifos, (b) HPLC Image of sample 1 in 25ppm concentration after 7 days Treatment, (c) HPLC Image of sample 2 in 50 ppm concentration after 7 days Treatment, (d) HPLC Image of sample 2 in 100 ppm concentration after 7 days Treatment.
3.3.2. Gas Chromatographic-Mass Spectrometry. The GC-MS analysis results of bio silver nanoparticles samples activity show chlorpyrifos peak was disappear with time 11.45 mins (of chlorpyrifos peak), indicating biodegradation of chlorpyrifos amount in liquid mineral medium. The [37] reported that degradation of chlorpyrifos pesticides for strain Bacillus sp. reached 93.4%, by 14th day. One probable metabolite i.e. Di butyl phthalate was identified at RT 17.367 mins. The presence of 5-Octadecene, 7,10-Hexadecadienal, 2,13-Octadecadien-1-ol, was observed at R. Time. (20.496, 22.054, 22.129 and 23.799) mins respectively. These compounds are less toxic than chlorpyrifos pesticides. Fig. 7 & 8. Dibutyl phthalate is used in making flexible plastics that are found in a variety of consumer products [38]. While 5-Octadecene is bioactive Compounds in plants [39]. 3,13-Octadecadien-1-ol are pheromones [40].

**Figure 7.** gas chromatographic-mass spectrometry of chlorpyrifos after 7 days incubation with silver bio nanoparticles of *Bacillus thuringiensis israelensis* extracts.

**Figure 8:** proposed pathway of chlorpyrifos biodegradation by bio silver nanoparticles of *Bacillus thuringiensis israelensis* extracts

4. CONCLUSION
The main funding of this research was the manufacture of nanoparticles for degradation of pesticide by using bacterial extracts, were this method considered to be safe for ecosystem and also inexpensive. As mentioned above we used several techniques for characterization of bio-nanoparticles that has led to demonstrate the positive action of silver bio-nanoparticles in degradation of Chlorpyrifos pesticide. For sustainable
development, the use of biodegradation is fundamental and urgent for the control and management of pollution by pesticide and protect of the environment as well as public health by using ecofriendly techniques. The salient features of biological nanoparticles, their construction method, and examples are given. It mainly focusses was on the importance of nanoparticles in the decomposition of waste and toxic materials, including pesticides, which will also reduce the cost of treating waste and toxic materials. The nanoparticles not only directly stimulate the processing of waste and toxic substances, and are toxic to microorganisms, but they also help to enhance the efficiency of microorganisms in the decomposition of waste and toxic materials. It can be said that it has enormous applications in biological treatment as well. Due to its strong potential, its application is expected to increase greatly in the near future, and it will play an important role in sustainable development.

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Acknowledgment
The authors are gratefully acknowledged Dr. Abdul-Jabar Abass Ali Director of pollutant treatment center/ Directorate of Environment and Water /Ministry of Science& Technology, Baghdad-Iraq for the laboratory facilities.

Declaration of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this research.