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# Effect of nano copper and well water on the growth and epidemiology of *Pectobacterum carotovorum*

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**Abstract**
Soft rot disease of potatoes is an important disease that causes a major problem in potato fruit storage. Studies have focused on trying to prevent or reduce its occurrence through agricultural and preventive methods away from pesticides as much as possible in terms of food security or food safety because the disease is related to tubers that are consumed directly. The results showed that bacteria can grow at a wide range of temperatures (5-35°C). Among the most influential experiments on the growth and development of bacterial cells was the micronutrient experiment, where the growth inhibition rate was (5.5) compared to the control treatment, which was (0). Also, the effect of well water with an EC of (8.7) was clear on the number of growing colonies, where the rate of increasing colonies was (294) colonies when compared to the control treatment, which had some colonies (754). There are some micronutrients such as copper, which harm soft rot bacteria *P. carotovorum*, such as copper. Where he demonstrated its effectiveness in nano-use by conducting some important tests on it such as TEM, EDS, and XRD tests. In studying some properties of nano-composites.

**Keywords:** Nano copper, *Pectobacterium carotovorum*, soft rot disease, potato storage

## 1. Introduction
Potato Solanum tuberosum, which belongs to the Solanaceae family, is the fourth strategic crop after wheat, maize, and rice (AL-Razaq *et al*., 2018, Shayaa and Hussein, 2019) [1, 2], and the third most important food crop in terms of global consumption. Potato tubers are directly consumed in human and animal nutrition and are indirectly used in manufacturing industries after freezing or drying. The Food and Agriculture Organization of the United Nations has strongly recommended potatoes as an important crop for food security due to the challenges faced by the world as a result of the large increase in population numbers and problems related to food supplies (André Devaux *et al*., 2014) [22]. In addition, the potato crop is considered one of the most important vegetable crops globally because it is a rich food source of energy, and it is also an important source of many nutritional components as it contains a high percentage of starches, sugars, proteins, amino acids, organic acids, minerals, and fibers. It is a good source of many vitamins, the most important of which are vitamins C and B (Alaee, 2018) [3]. Many compounds in potatoes contribute to antioxidant activity (Akrimi *et al*. 2020; Hellmann *et al*. 2021) [4, 5]. Potatoes are grown in large areas around the world and occupy an important position in Iraq in terms of cultivated area and productivity. The average cultivated area in Iraq amounted to about 1,717,800 dunums, producing about 392,348.0 tons annually during the period from 2013-2018 (FAOSTAT, 2019) [7]. Potatoes are affected by many viral, fungal, nematode, and bacterial diseases. Bacterial soft rot, caused by the pathogenic bacterium *Pectobacterium carotovorum carotovorum*, is one of the most important diseases, as the infection begins in the field and develops rapidly during the transportation and storage of tubers (Youdkes *et al*., 2020) [8], resulting in significant crop losses (Guttman *et al*., 2021) [9], in addition to infecting several other economic crops and ornamental plants around the world (Yishay *et al*., 2008) [10].
Controlling plant diseases is one of the main goals that must be taken into consideration to maintain the quality and abundance of food, feed, and fiber produced by farmers who often rely on chemical pesticides to combat these diseases (Aboutorabi, 2018) [12]. However, the excessive and incorrect use of these pesticides has led to the emergence of many problems that were not expected due to the disruption of the ecological balance, which led to severe toxicity to a large number of parasites and predators (biological enemies) and weakening their role in the natural control process, in addition to poisoning non-target organisms such as pets, birds, bees and humans.

Therefore, the following experiments aimed to observe the effect of nutrients, temperature and acidity indicators, and well water on the activity of soft rot bacteria (Massi *et al*., 2021) [11].

## 2. Materials and Methods

### 2.1 solation and identification of *Pectobacterium carotovorum*

Potato tubers infected with soft rot were obtained from the stores of Al-Hashimiyah district, Babylon City, then transferred to the bacteriology laboratory at the College of Agriculture - University of Kufa, following the method of (Doololbeldieva *et al*. 2016) [15], where the tubers were washed with tap water to get rid of dirt, then sterilized with 70% ethyl alcohol, then small pieces were taken from the area separating the damaged tissue from the healthy tissue and crushed with a ceramic mortar after removing the outer peel, then a part of the stuck was taken with a loop (a wire ending with a loop) and inoculated with the Nutrient ager medium using the planning method, and the dishes were placed in the incubator at 28 +_ 2 °C, and after 24 hours the isolate was purified and then planted on the selective Crystal violet pectate medium (CVP). It was diagnosed morphologically according to what was mentioned by (Darrasse *et al*., 1994) [31].

### 2.2 Growth test of *P. carotovorum* bacteria in different temperature ranges

Prepare (NA) medium and sterilize it in an incubator at a temperature of 121 °C and a pressure of 15 pounds per inch for 20 minutes. After the temperature of the medium decreased to 40 °C, the medium was poured into petri dishes. The medium was then inoculated with 48-hour-old bacterial colonies using the planning method and incubated at a temperature of (5), 10, 15, 20, 25, 30, and 35 C) for 24 hours to determine whether growth is observed (Scchad, 1980) [14].

### 2.2.2 Test the effect of major elements on bacterial growth.

The effect of NPK fertilizer on bacterial growth was tested by dissolving 5 g of fertilizer per liter of sterile distilled water. After preparing and pouring the medium into Petri dishes and culturing the bacteria on them using the streak method, covered the entire medium, then holes were made in them using a cork drill to put 1 (ml) of the fertilizer in them and observe its effect on the levels of bacterial growth. As for the comparison treatment, 1 (ml) of sterile distilled water was put in for comparison, then incubated at 28°C. After 24 hours, the inhibition zone was calculated according to the method of (Korsten *et al*., 1995) [13].

### 2.3 Test the effect of nano copper element on bacterial growth.

Copper was used as one of the micro-elements needed by the plant, and the powder was diluted with 1.5 - (g) per liter of sterile distilled water and applied in the same way as mentioned in the test of the effect of major elements. According to the method of (Benjun Zhou and Zhen Wang, 2022) [16].

### 2.4 Test the effect of water well on bacterial growth.

Two water wells were used to observe their effect on the growth and development of *P. carotovorum* bacteria. The first well was designated as (B 1) and the second well as (B2). The well water was used instead of distilled water. After mixing the culture medium with it, the rest of the medium preparation steps were carried out, and then it was planted with 24-year-old bacterial colonies using the planning method according to the number of cells, in addition to using distilled water for comparison. The results were estimated according to (Korsten *et al*., 1995, Wanchaitanawong *et al*, 2005) [13]. By calculating the number of colonies in each of the treatments and the comparison, the percentage of inhibition was extracted according to the following equation:

$$\text{Growth inhibition index} = \frac{\text{Average colony diameter in control - Average colony diameter in treatment}}{\text{Average colony diameter in control}} * 100$$

### 2.5 Test the effect of organic fertilizer extract on bacterial growth.

The organic fertilizer extract was taken and prepared in the same way as the culture media. After poured the medium, it was inoculated with bacteria using the streak method.

### 2.6 Test the effect of acidity and alkalinity on the growth of soft rot bacteria on potatoes

Hydrochloric acid HCl was used in drops to make the culture medium acidic 4.5, and calcium carbonate CaCO3 was used to make the medium basic 7.5, and the effect of this on the growth and development of soft rot bacteria was observed.

### 2.7 Synthesis of copper oxide nanoparticles.

Aqueous solution of copper oxide was prepared by adding 1mM of CuO to 5 ml of filtrate at room temperature. The aqueous solution was mixed with 10 ml of filtrate of the copper element. The bio-reduced aqueous component was used for the UV-Vis spectroscopy characterization (figure 1).

**Fig 1:** Visual observation of the biosynthesis of CuO.

## 3. Results

3.1 *P.carotovorum* bacteria were isolated from potato tubers infected with soft rot, then purified and preserved in tubes with lactoferrin for further studies. Figure (2).

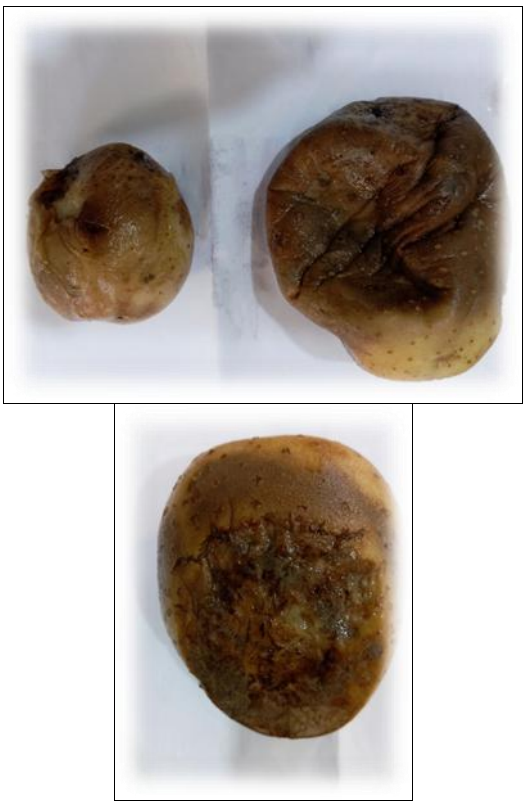

**Fig 2:** Tubers infected with soft rot disease.

### 3.2 Count the number of germinated colonies.

The number of germinated colonies was estimated using a colony counter to evaluate the effect of some treatments on the growth and development of bacteria, as shown in the figure below, where the concentration was (10).

### 3.3 Effect of temperature on the growth of *P. carotovorum*.

Different temperature ranges were taken to observe the effect of temperature on the growth and development of soft rot bacteria. There was a difference and variation in the impact between temperatures—fig (3 and 4).

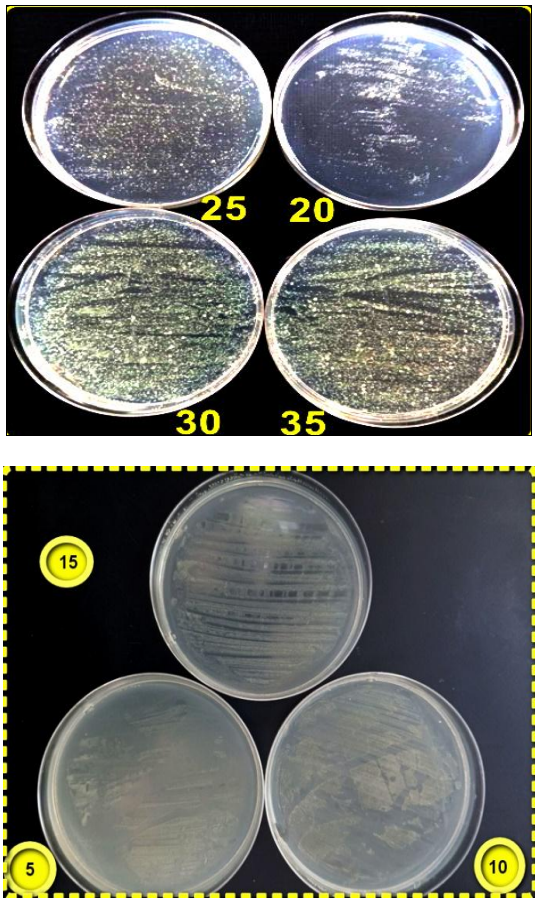


**Fig 3:** Effect of temperature on the growth of *P. carotovorum*

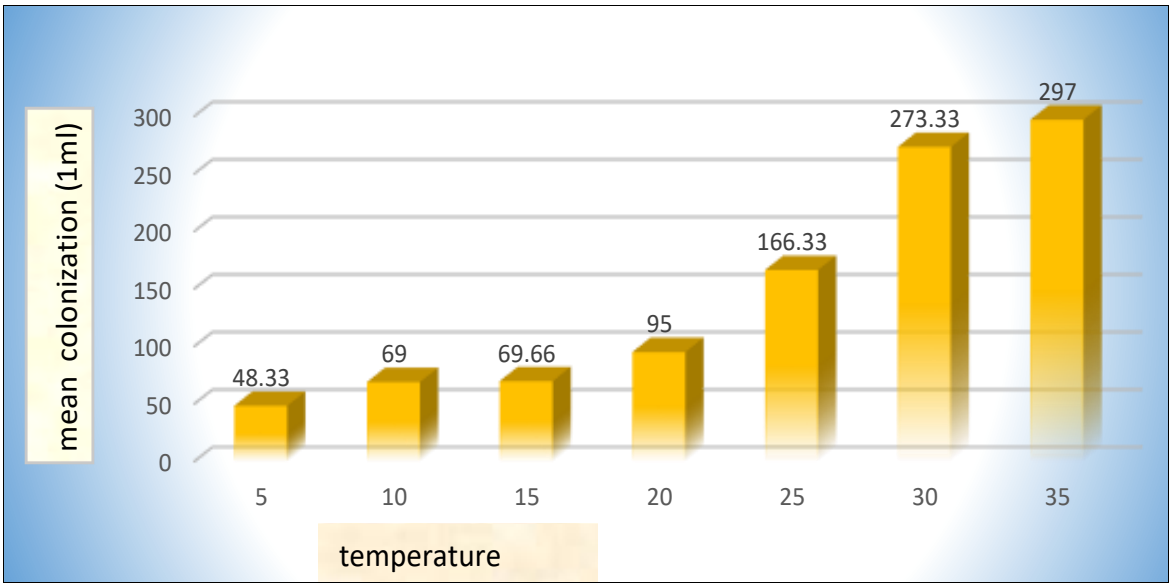


**Fig 4:** Effect of different temperature ranges on the formation of colony density of soft rot bacteria on potatoes 24 hours after the experiment.

**3.3.1 Effect of major elements on the growth of soft rot bacteria.**

The results showed that the treatment of major elements (NPK) did not record any effect on the growth and development of bacteria compared to the control.

**3.3.2 Effect of copper on growth indicators of soft mold bacteria:**

Copper treatment showed a significant effect on the growth of soft rot bacteria *P. carotovorum* compared to the control by measuring the inhibition zone. Fig (5 and 6).

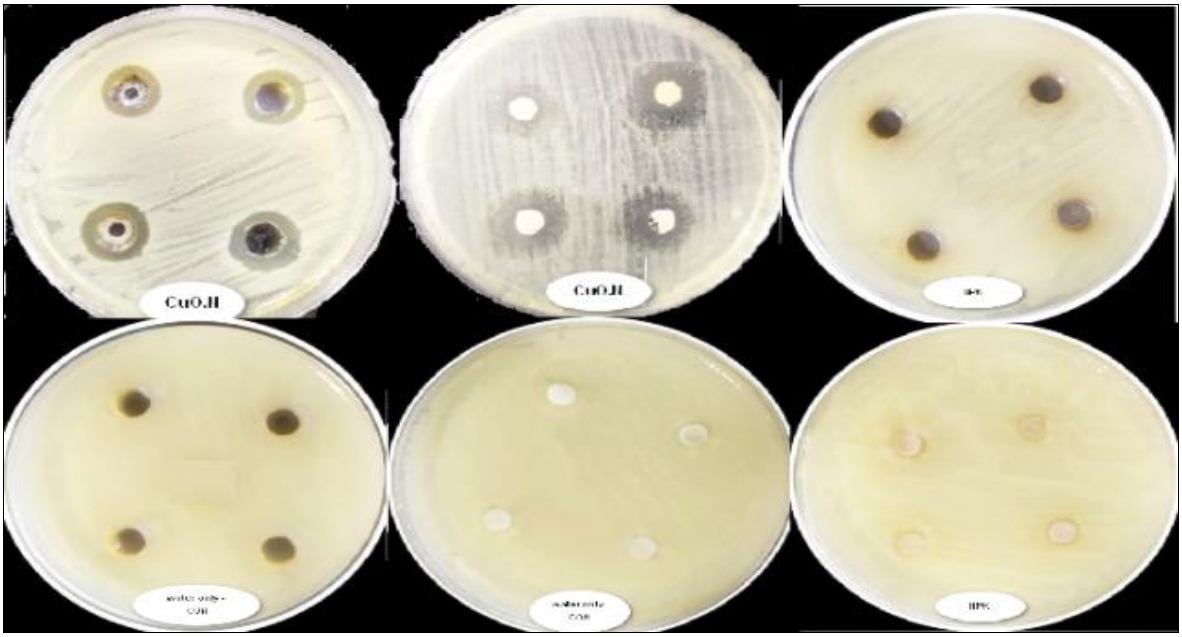


**Fig 5:** Effect of copper on growth indicators of soft mold bacteria

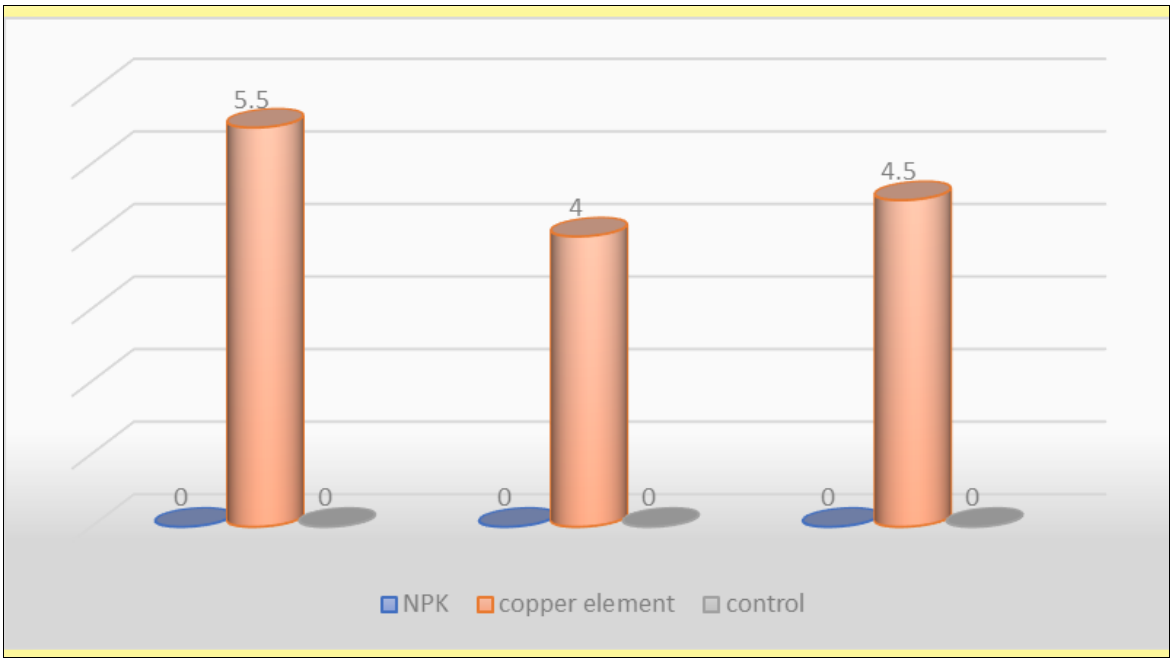


**Fig 6:** Measure the inhibition rate for the NPK fertilizer treatment and the copper element treatment 24 hours after conducting the experiment.

**3.3.3 Effect of water well on the growth of soft rot bacteria *P. carotovorum*:**

The results showed that the water well (B1) with a salinity ratio of (8.7) had an effect on the growth of soft rot bacteria, while the results of the water well (B2) with a salinity ratio of (4.6) led to an increase and encouragement of the growth of soft rot bacteria. Fig (7 and 8).


**Fig 7:** Effect of well water on the growth of soft rot bacteria, where (B1) represents the effect of the first well water on the growth of bacteria, (B2) represents the effect of the second well water on the growth of bacteria, while (C) represents the control treatment.

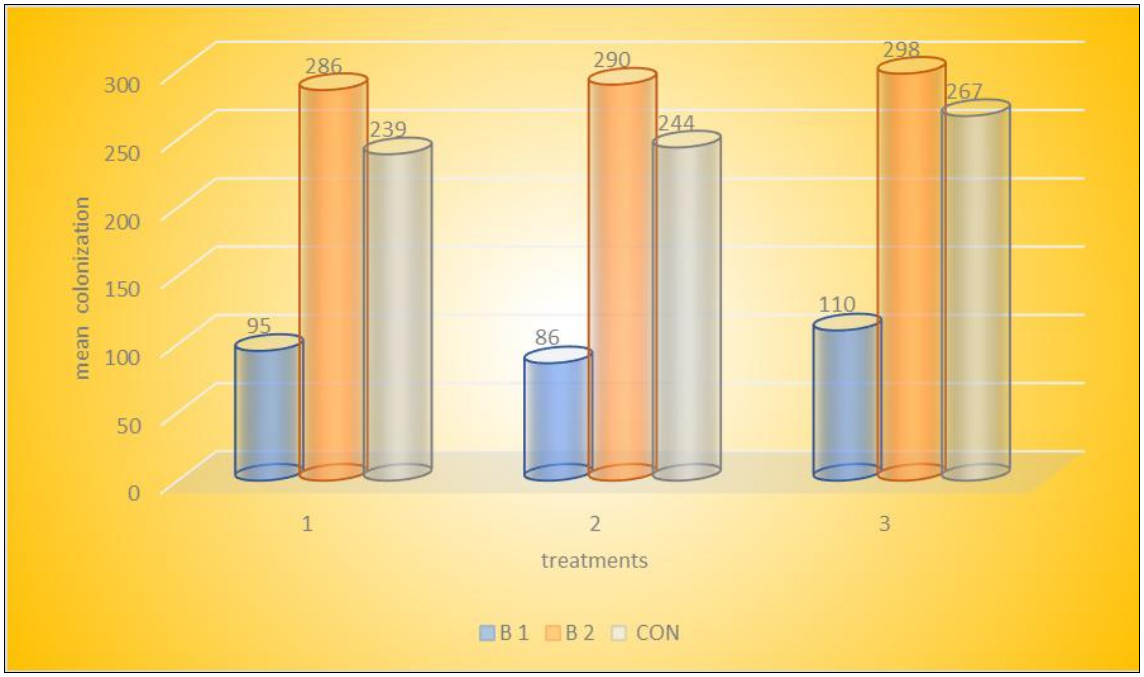


**Fig 8:** Calculating water-well treatment on the density of soft rot bacteria colonies on potatoes after 24 hours of the experiment.

**3.4: Effect of organic fertilizer extract on the development of soft rot bacteria growth on potatoes.**

The results of this experiment indicated that the organic fertilizer treatment encouraged an increase in the growth and density of bacterial cells compared to the control treatment. Fig (9 and 10).

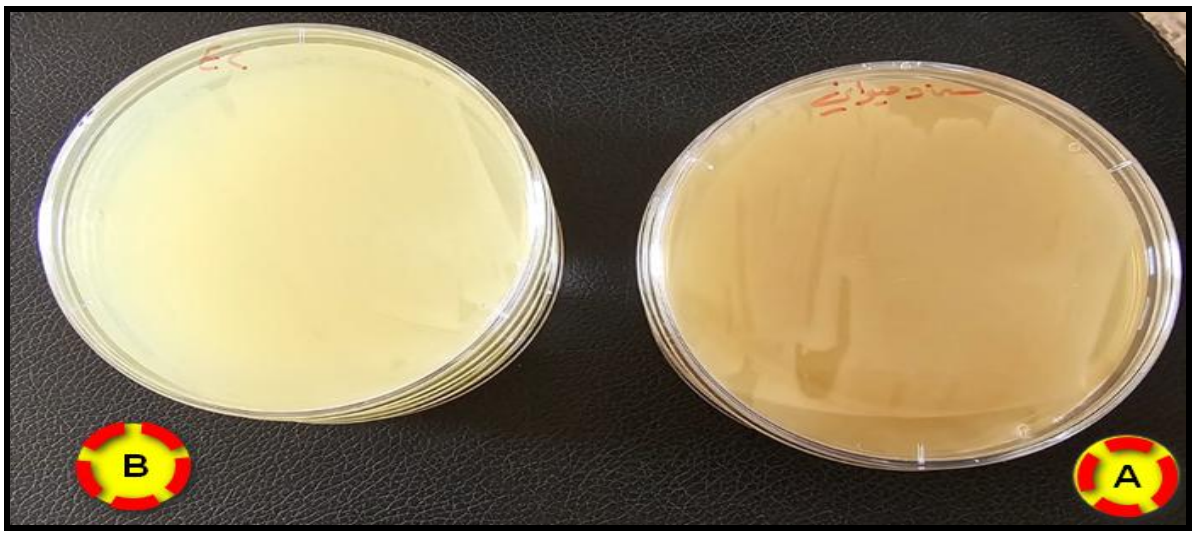


**Fig 9:** Organic fertilizer treatment on the growth and development of soft rot bacteria on potatoes, where (A) represents the organic fertilizer treatment, while the symbol for the control treatment is (B).

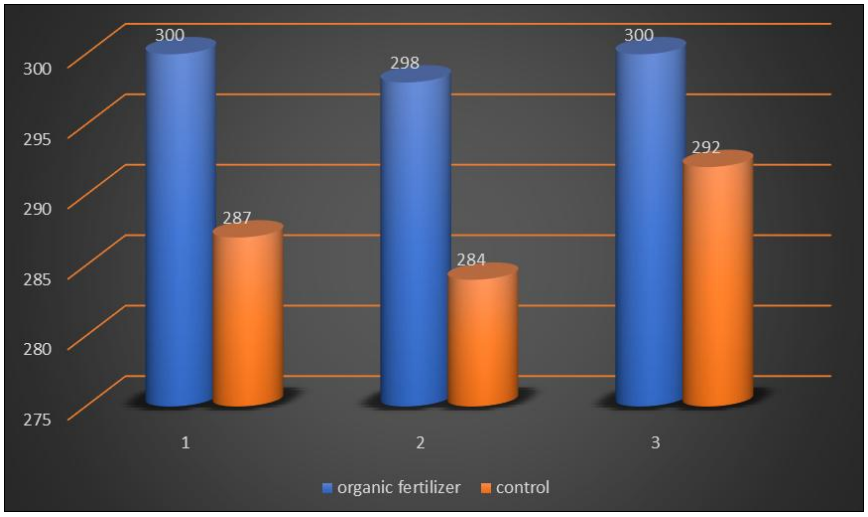


**Fig 10:** Organic fertilizer treatment on the density of the colonies formed by soft rot bacteria after 24 hours.

**3.5:** Effect of acidity and alkalinity of the culture medium on the growth and development of soft rot bacteria on potatoes.

No significant differences were recorded between the pH and comparison treatments.

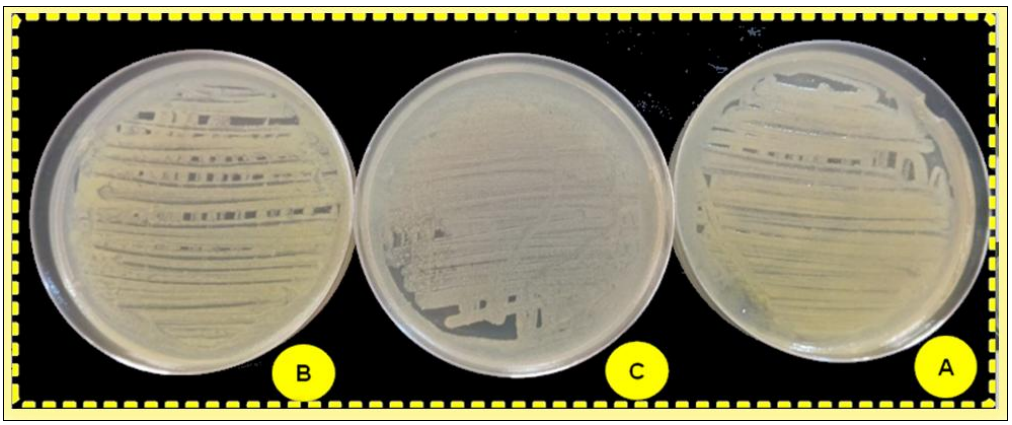


**Fig 11:** Acidity and alkalinity of the culture medium on the growth and development of soft rot bacteria on potatoes.

**3.6 Powder X-ray Diffraction (XRD) Study of CuO.**

The powder XRD patterns of the bio-synthesized copper oxide nanoparticles were documented using Bruker D8 Advance XRD using Cu radiation (λ = 1.5406 Å), 20 kV to 40 mA, 2θ/θ scanning mode. The information was collected for the 2θ range of 10 to 80° with a 0.020° stage. The diffraction data (Fig. 12).

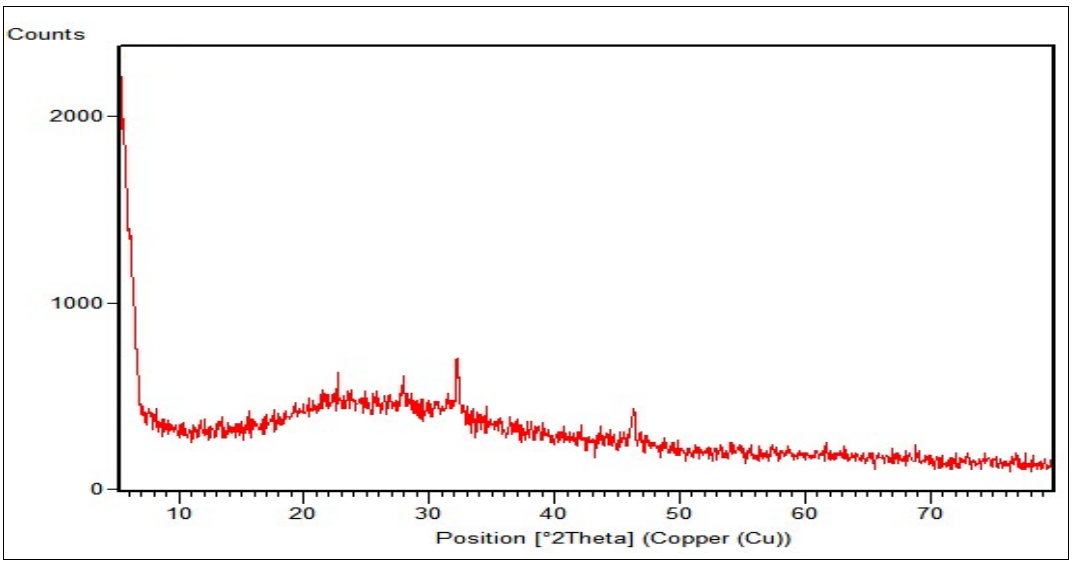


**Fig 12:** XRD analysis of copper oxide nanoparticles synthesized from copper element.

### 3.7 Atomic force microscopy (AFM).

The outer surface of the nano-fungal filtrate was studied using an atomic force microscope. The figure shows a two-dimensional image of copper oxide, in which minute aggregates with spherical shapes appear. Fig (13).

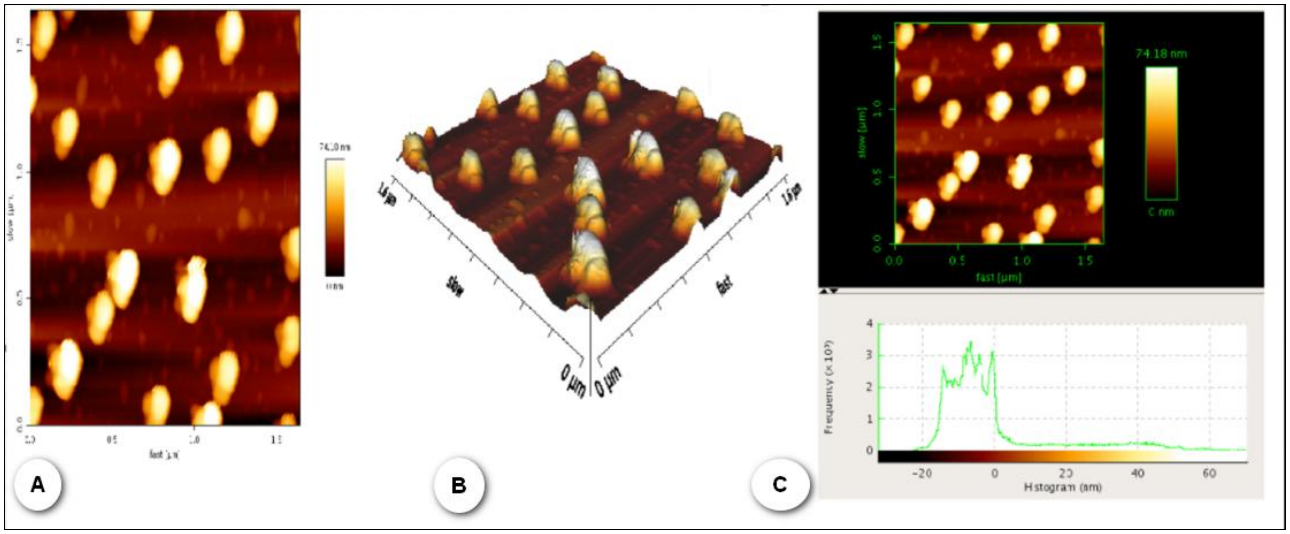


**Fig 13:** AFM images of synthesized CuO from (a) 2D image, (b) Size distribution, and (c) Curve showing distribution of CuO nanoparticles.

### 3.8 The energy dispersive spectroscopy (EDS)

The density of silver nanoparticleswas confirmed using a Company: IROST, energy dispersive spectroscopy (EDS) at 20 kV**.** The energy dispersive spectroscopy analysis of the copper oxide nanoparticles revealed that copper (40.11%) at 3 kV was the second major constituent element (Fig. 14).

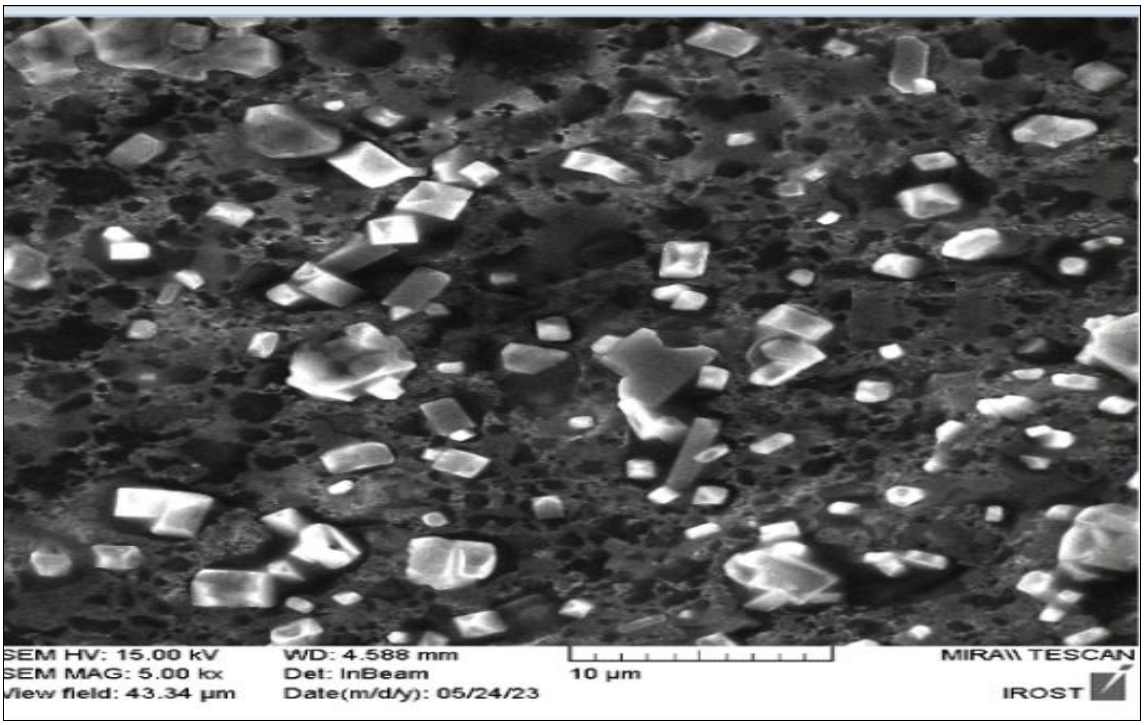


**Fig 14:** Characterization of the synthesized copper oxide after 14 days of incubation: Scanning electron microscopy. Scale bar = 200 nm.

## 4. Discussion

Soft rot disease caused by *P. carotovorum* is a well-known disease of potato tubers as well as tomatoes. A set of treatments were conducted to determine their results in reducing the damage of this disease as well as to know some environmental conditions in their positives and negatives towards stopping it or its development. The application of different temperature ranges showed that there is an effect of the temperature that is below the suitable temperature for bacterial growth, which ranges between 26-30°C, as a higher density of germinated colonies was recorded at temperatures of 20 °C and above compared to the rest of the temperature treatments (5, 10, 15) which gave the bacteria lower germination rates. The soft rot bacteria on potatoes *P. carotovorum* is characterized by a wide temperature range, as some studies indicated that it grows in a temperature range of (4-41°C) and causes soft rot at 20-35 °C, while other studies indicated that it causes soft rot at a temperature between 18-30°C depending on the species and strains. (Kushalappa and Zulfiqar 2001) [24]. Temperature directly affects enzyme expression, which is considered one of the most important virulence factors, as it leads to the

inactivation of enzymes and their destruction (De Boer, 2004) [25]. It has been shown that the different enzymes responsible for tissue degradation, pectate lyase, pectin lyase, pectin methyl esterase, polygalacturonase and endopolygalacturonase transamylase, are affected differently by temperature in different species of soft rot bacteria (Laurent *et al*. 2000; Smadja *et al*. 2004) [29, 26]. At the same time, the activation of these enzymes depends on the collective sensing, which is considered the most important virulence factor for bacteria, as well as the regulation dependent on the density of bacterial cells (Pérombelon, 2002; Toth *et al*. 2003) [28, 27]. A decrease in temperature below the optimum temperature leads to a decrease in the activity of enzymes responsible for stimulating and germination of bacterial cells, specifically the enzyme responsible for pectin degradation (pectine lyases) (Membre and Burlor, 1994, Laurent *et al*., 2001) [21, 17]. The results of the pH effect test showed no clear effect on the growth of soft rot bacteria *P. carotovorum*, as the bacteria have the ability to grow in wide ranges of pH, although optimal growth occurs whenever the pH approaches (pH7). (Laurent *et al*., 2001) [17]. The results of the nutrients also showed the effect of the treatment of micronutrients (copper element nanoparticle) on the rate of inhibition of bacterial growth and development, while no significant effect was recorded for the factor of macronutrients (NPK). This may be due to the presence of elements that have a strong effect on inhibiting bacterial growth, such as copper, which prevents bacterial reproduction and often causes the death of large numbers of bacteria as a result of the toxic effect of the divalent copper ion, as copper ions work in a non-specific manner at the level of the cell membrane, which leads to affecting structural and enzymatic proteins and changing the permeability of the membrane. Dissolved copper ions can enter the cell protoplasm. (Ordax *et al*., 2006; Aguirre and Pilon, 2016) [19, 18]. The degree of electrical conductivity or the degree of soil salinity (EC) affects microorganisms by increasing the osmotic potential on the cells, leading to their explosion and depriving them of the benefit of nutrients by affecting the activity of enzymes (Bergstrand, 2009; Sammar Khalil, 2011) [20, 21]. It was noted from the results that the animal manure extract encouraged the growth of soft rot bacteria of potatoes *P. carotovorum*. This is due to the availability of nutrients needed by the cause of soft rot and their ease of decomposition by the enzymes it secretes, in addition to the lack of uric acid in animal manure, the main nitrogenous component in organic fertilizers, which is found in poultry waste and which severely affects the growth of many bacteria, including soft rot bacteria on potatoes, which are considered unable to analyze and break it down. If it decomposes, it turns into ammonia detected. X-ray diffraction study confirmed that the particles were copper oxide nanoparticles with face-centered circular crystal structures. Organic bio-impurities may cause the peak in the diffraction pattern at 27°. AFM of NP5 copper oxide nanoparticles shows polydisperse nature, with few particles. Similar AFM reports of copper oxide nanoparticles very small, and the smaller the size of the nanoparticles, the easier it is for them to penetrate into the bacterial cell membrane. (Singh *et al*, 2017) [33]. The synthesized copper oxide mages from SEM exhibited identical crystalline structures aggregated closely with identical shapes and morphology. The crystals of copper oxide exhibit maximum absorption at 3 keV (Bindhu *et al*, 2015) [35].

## 5. Conclusion

The potato soft rot bacteria *P. carotovorum* has a wide temperature range for growth from 5 - 35 °C. The results gave a clear picture of the effect of micronutrient fertilizer in its nano formula on the growth and development of potato soft rot bacteria. The well water with EC (8.7) had an effect in determining the growth and development of bacterial cells, while (B2) increased their growth density. The bacteria have the ability to grow in acidic and basic ranges. The organic fertilizer extract encouraged the growth and development of the bacterial cells causing soft rot *P. carotovorum*. The fertilizer mixture (NPK) did not give any inhibitory results on the growth and development of the pathogen *P. carotovorum* cells.

**Conflict of Interest**
Not available

**Financial Support**
Not available

## 6. References

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