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Research Article

Biological Activity of Ag and Cu Monometallic Nanoparticles and Ag-Cu Bimetallic Nanocomposites against Plant Pathogens and Seeds

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The antibacterial activities of silver (Ag) and copper (Cu) monometallic and bimetallic nanoparticles (MNPs and BNPs) against the most economically important and common plant bacterial diseases in Kyrgyzstan were tested, as well as their phytotoxic effects on wheat and corn seeds. To achieve this, a range of Ag and Cu MNPs and BNPs were synthesized using different methods and were tested at different concentrations to compare their biological effects. The antibacterial activities of Ag and Cu MNPs and Ag-Cu BNPs against the plant pathogenic bacteria *Erwinia amylovora*, *Pseudomonas syringae*, and *Pectobacterium carotovorum* were evaluated. Of the three pathogens, *Ps. syringae* was found to be the most sensitive to NPs. The antimicrobial activities of the tested NPs showed significant positive correlations with the concentration; hence, there was a dose-dependent effect. Due to their antibacterial properties, these Ag-Cu nanocomposites have high potential in plant protection applications.

1. Introduction

New materials (i.e., nanopowders, nanocomposites, and nanofibres) less than 100 nm in at least one dimension have been developed in the last decades. Due to their quantum size and a significant increase in the proportion of surface atoms compared to bulk materials, nanocomposites have unusual biological properties [1–3]. In particular, their antimicrobial properties allow them to be used in various human and industrial applications. The antimicrobial mechanisms of nanoparticles (NPs) are based on their penetrability, small size, chemical toxicity, and ability to damage the bacterial cell membrane and produce reactive oxygen species (considered the primary mechanism of action) [4, 5]. Silver-based NPs (Ag NPs) are mainly used as bactericidal and bacteriostatic agents in medicine [6–12]. It has been shown that the antibacterial activity is due to Ag + ions, not metallic Ag [13].

Nanocomposites, consisting of Ag and chitosan, in the form of nanofilms for covering wounds have been used to treat infections caused by *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [14, 15].

Copper NPs (Cu NPs) are also garnering considerable interest for practical applications in various economic areas [16]. Their favourable characteristics include high electrical and melting points, low electrochemical migration, increased bioavailability, and relatively low production cost.

In contrast to ionic biogenic metals, NPs have prolonged action, are less toxic than metal salts, and can activate physiological and biochemical processes in plants [17]. Among the studied polymer nanocomposites, those containing Ag and Cu have the greatest potential for use in humans and for improving human health, for example, in the food industry, biomedicine, and water purification applications [4]. Studies have shown that Cu NPs exhibit high cytotoxic

activity against Gram-negative (*E. coli*) and Gram-positive (*Bacillus subtilis*) bacteria compared with gold NPs (Au NPs) [18].

The antibacterial activity of nanocomposites containing Cu NPs in cellulose was studied against pathogenic human bacteria, such as *S. aureus* and *Klebsiella pneumonia*. It was found that the chemical nature and morphology of the nanomillers had a significant influence on the antibacterial activity; the higher the Cu content in the composition, the greater the antibacterial activity [19]. In another study, Cu NPs at 70 ppm were used to control fire blight disease in pear seedlings under greenhouse conditions. Treatment with Cu NPs led to increased morphological and physiological characteristics of the trees and improved fruit yield [20].

Nanocomposites of stabilized binary metal systems, combining the unique properties of different metal particles, have several qualities that are absent from materials that consist of one metal particle. Bimetallic NPs (BNPs) have drawn greater interest than monometallic NPs (MNPs) from scientific and technological perspectives [21–23].

The application of NPs is constantly expanding, and some attempts have been made to use them in agriculture, especially as potent antimicrobial agents for seed dressing [24, 25]. The effect of Ag nanocomposites based on humic substances and their precursors on the potato plant pathogen *Clavibacter michiganensis* subsp. *sepedonicum* strain Ac–1405 was studied [26]. With the dearth of published research on NPs in agriculture, it is evident that the potential use of NPs in agriculture as antimicrobial agents to combat plant phytopathogens remains largely unexplored. Moreover, the interactions of metal NPs with plant cells are poorly understood; the effects of NPs on plant cells when NPs are used as plant protection agents are unclear. There are scant data on the negative effect of metal NPs on seed germination, root elongation, and vegetable crops' biomass [2, 27, 28]. Such knowledge and data gaps lead to a misunderstanding of how NPs are transmitted and accumulated at various levels of the food chain [29].

This research is aimed at studying the biological activity of MNPs (Ag and Cu) and BNPs (Ag-Cu) against the most economically important and common plant bacterial diseases in Kyrgyzstan and the phytotoxic effect on wheat and corn seeds. To achieve this goal, a range of Ag and Cu MNPs and BNPs were synthesized using different methods and were tested at different concentrations to compare their biological effects.

2. Materials and Methods

2.1. Synthesis of the Ag and Cu MNPs. The Ag and Cu MNPs used in this study were synthesized at the Faculty of Biology and Chemistry, Arabaev Kyrgyz State University. Tables 1–3 show the chemical formulations and synthesis methodologies of the Ag and Cu MNPs [30, 31].

2.2. Synthesis and Composition of the Ag and Cu BNPs in Various Media. Promising materials include nanostructured composites stabilized by polymeric substances in aqueous solutions, which hinder NP aggregation. To obtain a nano-composite of Ag and Cu, chemical reduction in the presence of a stabilizer was performed. This method is simple and allows control of particle dispersion and morphology and, consequently, the properties of the final products. The solutions were prepared from chemically pure-grade silver nitrate ($AgNO_3$) and crystalline hydrate of copper sulphate ($CuSO_4 \cdot 5H_2O$). Gelatin was used to stabilize the BNPs as an efficient and environmentally safe material. A gelatin solution was added to a salt solution of Ag, and a solution of hydrazine hydrate $N_2H_4.H_2O$ served as a reducing agent.

The total reaction volume for the synthesis was 100 ml, which contained a mixture of aqueous Ag and Cu solution ($C_{Ag+} = 0.05$ H and $C_{Cu}{}^{2+} = 0.05$ H). A stabilizer and ammonia solution were added until pH = 11. The solution was heated, and then hydrazine hydrate was added in an amount exceeding the quantity of Ag and Cu ions.

The redox potential of hydrazine depends on the pH of the solution and has a more negative value in an alkaline medium (-1.15 V at pH = 14). For this reason, the synthesis was carried out in the presence of ammonia [11, 12].

The synthesis of the Ag and Cu BNPs was carried out in neutral (pH = 7), acidic (pH = 6), ammonia (pH = 11), and alkaline (NaOH, pH = 11) media in the presence or absence of a stabilizer (Table 4).

The reduction products were separated from the liquid phase and washed using centrifugation until neutral pH was reached. The resulting product was dried in a drying oven at 55–60°C.

The resulting products were characterized on a RINT-2500 HV diffractometer using filtered Cu radiation and a JOEL JSM-7600F emission scanning electron microscope (Kumamoto University, Japan). The BNPs were then tested at different pH values and concentrations, as indicated in Table 5.

2.3. Plant Pathogens. The following plant pathogens were used to test the antibacterial activity of the MNPs and BNPs: *Erwinia amylovora* (the causative agent of bacterial blight in apple and pear trees); *Pseudomonas syringae* (the causative agent of bacterial canker in fruit trees); and *Pectobacterium carotovorum* (the causative agent of soft rot in vegetable crops). The collection cultures of these pathogens isolated from diseased plants were preserved in meat peptone broth (MPB) at −18°C.

2.4. In Vitro Screening of Biological Activity of the MNPs and BNPs. The well diffusion method was used to estimate the antimicrobial activity of the MNPs and BNPs. The test cultures were grown on nutrient agar for 20–24 h; then, a suspension was prepared in a 0.9% sodium chloride solution according to the turbidity standard for 10 units (1×10^9 CFU/ml). Then, the bacterial culture was thoroughly mixed in molten nutrient agar medium and incubated at 27°C. After colony growth, holes (5 mm diameter) were made using a sterile metal drill under aseptic conditions. One ml of MNP or BNP solution (at different pH) was added to each well, and the Petri dishes with the cultures were incubated at 28°C. In the control, nystatin was used in the form of a 1.0% solution.

TABLE 1: The chemical formulations and synthesis methods of the Ag MNPs used in this study.

Sample no.	Chemical formulation	Synthesis method
Sample I°	Ag-H$_2$O-ERD (electrical erosion in distilled water)	The Ag nanopowder was derived using the electrical erosion method, in the presence of distilled water.
Sample II°	Ag-H$_2$O-gelatin, ERD	The Ag nanopowder was synthesized using the electrical erosion method with a mixture of 0.2% gelatin.
Sample III°		The Ag nanopowder was derived using the chemical recovery method with hydrazine at a neutral pH. The Ag nanopowder was extracted before the first reaction.
Sample IV°	Ag-NH$_4$OH-	The Ag nanopowder was derived using the chemical recovery method with hydrazine in the presence of ammonia. Then, the Ag powders were washed to reach neutral pH.
Sample V°	Ag-H$_2$O-gelatin	At neutral pH, the chemical recovery method was used to derive the Ag nanopowder in a 0.2% gelatin.
Sample VI°	Ag-NH$_4$OH-gelatin	The Ag nanopowder was derived using the chemical recovery method with 0, 25% gelatin in ammonium reaction.

TABLE 2: The concentration of Ag MNPs derived in different synthesis ways.

I°-Ag-H$_2$O-ERD	II°-Ag-H$_2$O gelatin- ERD	III°-Ag-H$_2$O	IV°-Ag-NH$_4$OH	V°-Ag-H$_2$O-gelatin	VI°-Ag-NH$_4$OH gelatin
I°-1-5 mg/ml	II°-1-5 mg/ml	III°-1-5 mg/ml	IV°-1-5 mg/ml	V°-1-5 mg/ml	VI°-1-5 mg/ml
I°-2-1 mg/ml	II°-2-1 mg/ml	III°-2-1 mg/ml	IV°-2-1 mg/ml	V°-2-1 mg/ml	VI°-2-1 mg/ml
I°-3-0.5 mg/ml	II°-3-0.5 mg/ml	III°-3-0.5 mg/ml	IV°-3-0.5 mg/ml	V°-3-0.5 mg/ml	VI°-3-0.5 mg/ml
I°-4-0.1 mg/ml	II°-4-0.1 mg/ml	III°-4-0.1 mg/ml	IV°-4-0.1 mg/ml	V°-4-0.1 mg/ml	VI°-4-0.1 mg/ml

TABLE 3: Different synthesized methods of Cu MNP samples at different concentrations.

I°-Cu-H$_2$O-ERD	II°-Cu-H$_2$O gelatin-ERD	III°-Cu-H$_2$O	IV°-Cu-NH$_4$OH	V°-Cu-H$_2$O-gelatin	VI°-Cu-NH$_4$OH gelatin
I°-1-5 mg/ml	II°-1-5 mg/ml	III°-1-5 mg/ml	IV°-1-5 mg/ml	V°-1-5 mg/ml	VI°-1-5 mg/ml
I°-2-1 mg/ml	II°-2-1 mg/ml	III°-2-1 mg/ml	IV°-2-1 mg/ml	V°-2-1 mg/ml	VI°-2-1 mg/ml
I°-3-0.5 mg/ml	II°-3-0.5 mg/ml	III°-3-0.5 mg/ml	IV°-3-0.5 mg/ml	V°-3-0.5 mg/ml	VI°-3-0.5 mg/ml
I°-4-0.1 mg/ml	II°-4-0.1 mg/ml	III°-4-0.1 mg/ml	IV°-4-0.1 mg/ml	V°-4-0.1 mg/ml	VI°-4-0.1 mg/ml

TABLE 4: The synthesis ways of the Ag-Cu BNPs.

Sample no.	Samples	Stabilizer	Medium reaction
1	Ag-Cu	Gelatin	Neutral
2	Ag-Cu	Gelatin	NaOH
3	Ag-Cu	Gelatin	NH$_4$OH
4	Ag-Cu	—	NH$_4$OH
5	Ag-Cu	—	NaOH
6	Ag-Cu	—	Neutral
7	Ag-Cu	—	Acidic

After the specified time, the diameters of the growth inhibition zones were measured. Highly active NPs were considered to be those samples that exhibited more extensive zones of inhibition. Chemical colloidal Ag Xerox, copper sulphate preparations, and a natural preparation based on *Streptomyces* bacteria were used to compare the biological activities of the tested BNPs.

2.5. Tests Used to Determine the Effects of the MNPs and BNPs on Wheat and Corn Seeds

2.5.1. Seeds. The wheat (*Tríticum*) variety "Intensive" seeds and corn (*Zea*) "Pioneer" grains from the National Seed Gene Bank Kyrgyzstan were used.

2.5.2. Seed Germination and Exposure. To prevent seed-borne microorganisms, the seeds were sterilized with 5% sodium hypochlorite solution for 5 min and washed three times with sterilized distilled water. Then, seeds were soaked in NP solutions and a suspension of a biological product for 2 h for comparison.

Each NP sample was tested on 30 seeds. The experiment included three replicates. Germination was recorded daily for 12 days, at which time the germination percentage was calculated. After 8–12 days (because of different types of seed germinate at different times), depending on the type of grain, treatment was halted and seedling root and shoot length were measured. Distilled water was used as a control. To compare the phytoregulatory effect of the NP samples, *Streptomyces violaceoruber* and *Streptomyces diastochromogenes* bioproducts were used as biostimulators (Table 6).

2.5.3. Seed Germination Measurement. According to the International Rules for Testing Seeds, the final percentage

TABLE 5: The Ag-Cu BNPs tested for antibacterial activity.

Sample no	Ag-Cu BNP	Ag-Cu BNP concentration	
		AgNO$_3$ concentration	CuSO$_4$ concentration
1	Ag-Cu (neutral media)	0.05 mol, 20 ml, and pH = 7.0	0.05 mol, 20 ml, and pH = 7.0
2	Ag-Cu (ammonia media)	0.05 mol, 20 ml, and pH = 11.0	0.05 mol, 20 ml, and pH = 11.0
3	Ag-Cu (alkaline media)	0.05 mol, 20 ml, and pH = 11.0	0.05 mol, 20 ml, and pH = 11.0
4	Ag-Cu with gelatin (neutral media)	0.05 mol, 20 ml, and pH = 7.0	0.05 mol, 20 ml, and pH = 7.0
5	Ag-Cu with gelatin (ammonia media)	0.05 mol, 20 ml, and pH = 11.0	0.05 mol, 20 ml, and pH = 11.0
6	Ag-Cu with gelatin (alkaline media)	0.05 mol, 20 ml, and pH = 11.0	0.05 mol, 20 ml, and pH = 11.0

TABLE 6: The NP and control concentrations and the seed exposure times.

Test variant	Composition	NP or bioproduct concentration	Seed exposure time	Seed types
MNP	Ag	1 mg/l	2 h	Wheat and corn
MNP	Cu	1 mg/l	2 h	Wheat and corn
BNP	Ag-Cu in alkaline medium	1 mg/l	2 h	Wheat and corn
BNP	Ag-Cu in neutral medium	1 mg/l	2 h	Wheat and corn
BNP	Ag-Cu in ammonium medium	1 mg/l	2 h	Wheat and corn
Control	Distilled water		2 h	Wheat and corn
Biostimulator	*Streptomyces violaceoruber*	10^3 spores/ml	2 h	Wheat and corn
Biostimulator	*Streptomyces diastochromogenes*	10^3 spores/ml	2 h	Wheat and corn

TABLE 7: The inhibition of bacterial pathogen's growth under effects of Ag and Cu NPs in 1.0 mg/l doze ($n = 3$).

No.	Samples	Stabilizer	The reaction medium	Causative agents of plant diseases		
				Pseudomonas syringae	*Erwinia amylovora* Lysis zone, mm ($n = 3$)	*Pectobacterium carotovorum*
1	Ag-Cu	Gelatin	Neutral	6.1 ± 0.25	6.0 ± 0.10	7.1 ± 0.89
2	Ag-Cu	Gelatin	NaOH	8.0 ± 0.02	7.0 ± 0.14	7.1 ± 0.92
3	Ag-Cu	Gelatin	NH$_4$OH	7.0 ± 0.23	7.0 ± 0.13	7.2 ± 0.87
4	Ag	Gelatin	NaOH	2.0 ± 0.25	3.0 ± 0.51	5.0 ± 0.43
5	Cu	Gelatin	NaOH	4.0 ± 0.10	4.0 ± 0.97	4.7 ± 0.51

of germination and measurements of grown organs were carried out [32]. The following equations were used to calculate seed germination. Germination percentage (GP%) = (Gf/n) × 100 where Gf is the total number of germinated seeds at the end of experiment and n is the total number of seed used in the test. Mean germination time (MGT) = $\Sigma NiDi/n$ where Ni is the number of germinated seeds till ith day and Di is the number of days from start of experiment till ith counting and n is total germinated seeds. Germination rate (GR) = $\Sigma Ni/\Sigma TiNi$ where Ni is the number of newly germinated seeds at the time of $Ti = (a/1) + (b - a/2) + (c - b/3) + \cdots + (n - n - 1/N)$.

2.5.4. *Statistical Analysis.* The experiment was replicated thrice. Data were stated as the means of three replications. Statistical analyses were performed using SAS JMP Statistical Package version 13.0. The means of metallic nanoparticles, doses, and interactions were considered significant if $P < 0.05$.

3. Results and Discussion

3.1. *The Morphology and Dimensions of Ag and Cu NPs Synthesized in Various Media.* Diffraction patterns of the products of the combined reduction of silver and copper ions in the presence of gelatin are shown in Figure 1. Analysis of the diffraction patterns shows that the outcomes of the three samples consist of two phases: metallic silver and copper.

The average lattice parameter of silver and copper obtained in all three samples is 0.40 nm and 0.35 nm, respectively. Calculations show that the value of the crystal lattice parameter of metals corresponds to that for massive metals (JCPDS no. 04-0783 and no. 04-0836). This value indicates that the reduction of ions in stabilizers leads to the formation of silver and copper nanocomposites [33–35].

Diffraction patterns of the products of the combined reduction of silver and copper ions in the absence of gelatin are shown in Figure 2. Analysis of diffraction patterns of the
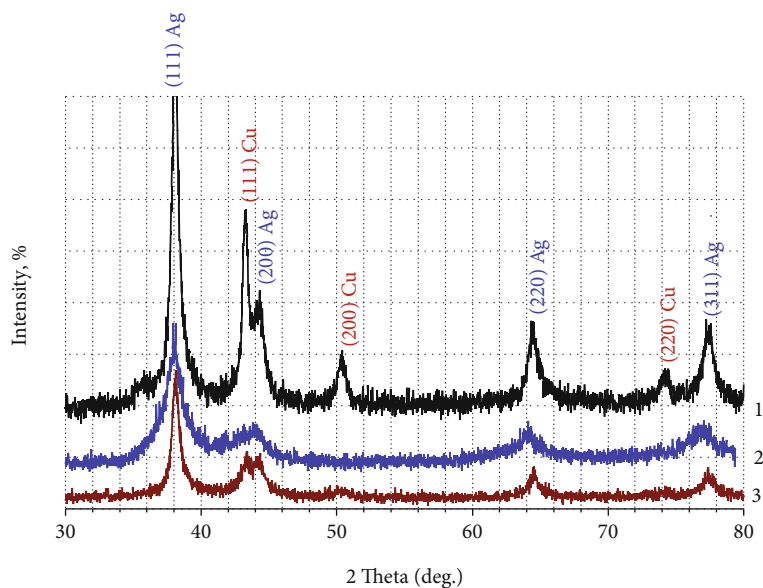
FIGURE 1: Diffraction patterns of the products of the combined reduction of silver and copper ions in the presence of gelatin in neutral (1), alkaline (2), and ammonia (3) media.
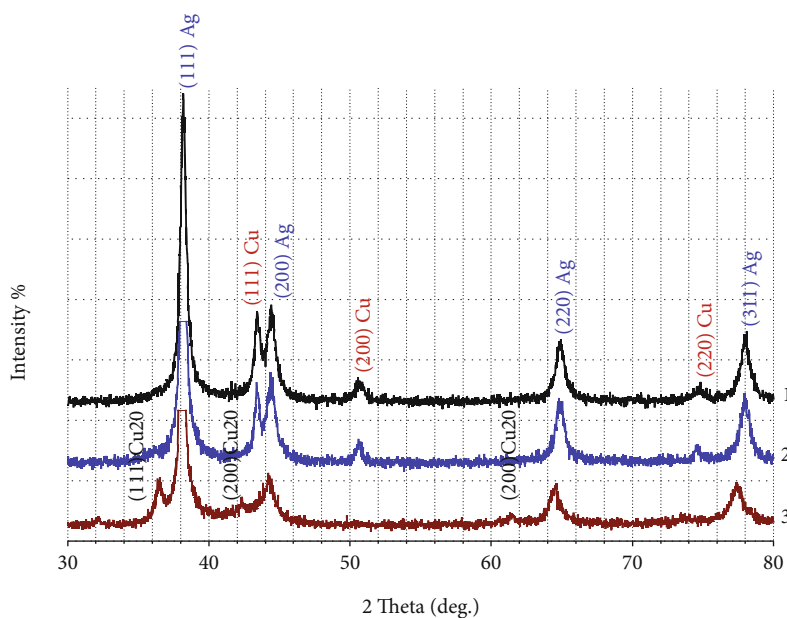

FIGURE 2: Diffraction patterns of the products of the combined reduction of silver and copper ions in ammonia (1), alkaline (2), and neutral (3) media.

joint reduction of silver and copper ions without a stabilizer indicates that the products obtained in alkaline and ammonia media consist of two phases: metallic silver and copper. The average lattice parameter is 0.4095 nm for silver and 0.362 nm for copper. The product synthesized in a neutral medium except the metallic phases also contains a third phase—copper oxide (I) [34, 36].

The morphology and dimensions of silver and copper nanocomposites were determined by using scanning electron microscopy. To determine the size of silver and copper nanoparticles, the histograms of the corresponding nanopowders were compiled from the analysis of their micrographs.

As shown in the micrograph (Figures 3–5), regardless of the presence of a stabilizer in various media, the particles of all six samples had a spherical shape. NPs obtained in the presence of gelatin in a neutral medium (Figure 3(a)) ranged from 10 to 170 nm, and particles with dimensions of 60–100 nm and 150–170 nm were predominant. In the presence of gelatin in an alkaline medium, the formed Ag and Cu NPs ranged from 20 to 120 nm and most were 20–40 nm in size (Figure 3(b)). Particles of a nanocomposite of Ag and Cu (Figure 4(a)) obtained in an ammonia medium in the presence of a stabilizer were 10–170 nm; aggregates 40–60 nm were predominant. The products obtained in ammonia in
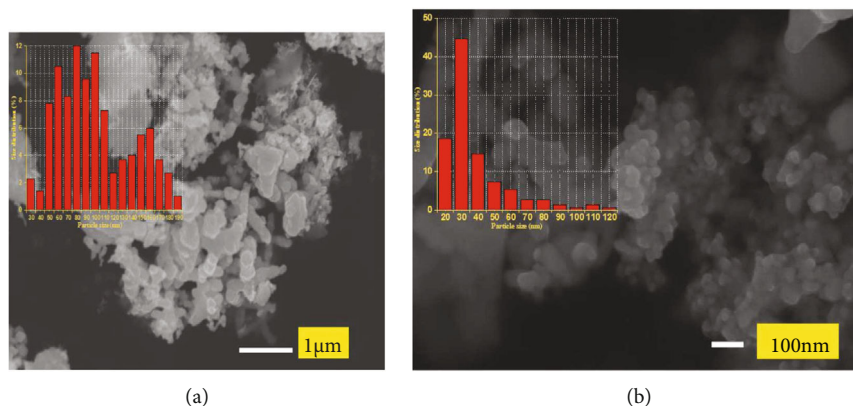
(a)

(b)

FIGURE 3: Micrograph and histogram of silver and copper nanocomposite particles synthesized in the presence of gelatin in a neutral medium (a) and the presence of gelatin in an alkaline medium (b).
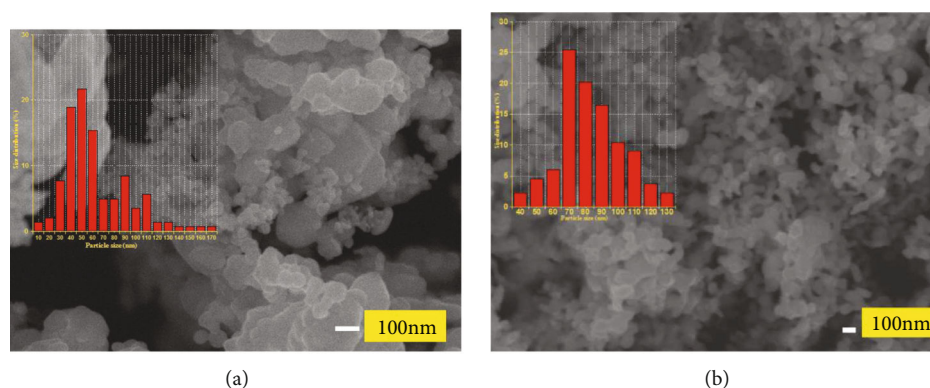

(a)

(b)

FIGURE 4: Micrograph and histogram of silver and copper nanocomposite particles synthesized in the presence of gelatin in ammonia (a) and the absence of gelatin in ammonia medium (b).

the absence of a stabilizer were spherical. Analysis of the particle histogram (Figure 4(b)) by size showed the predominance of particles that were 70–90 nm. In addition, the sample contained particles with dimensions of 40, 50, and 60 and from 100 to 130 nm.

The particle size in the nanopowder synthesized in an alkaline medium (Figure 5(a)) was, on average, 40–70 nm. The NPs obtained in a neutral medium were 40–60 nm in length (Figure 5(b)).

These findings show that the inclusion of gelatin makes it possible to obtain stable Ag-Cu nanocomposites with smaller sizes and a narrower size distribution than the absence of a stabilizer. The minor aggregates of NPs formed in an alkaline medium.

3.2. Effects of NPs on Plant Pathogens

3.2.1. Brief Characteristics of the Pathogenic Bacteria. E. amylovora is a Gram-negative bacteria consisting of rods that are 1.0–3.0 microns in length and 0.5–1.0 microns in diameter. They are mobile, peritrichous flagellated, heterotrophic, facultative anaerobes. They are associated with plants as epiphytes, saprotrophs, and phytopathogens. E. amylovora is a devastating bacterial plant pathogen of the Rosaceae family, causing the disease fire blight, spreading rapidly across many countries and causing substantial economic damage. The

bacterial cultures used in this work were isolated from diseased organs of apple and pear trees grown in orchards and forestry farms in northern and southern Kyrgyzstan. They were genetically identified, and their degree of pathogenicity was determined [37, 38].

The phylogenetic tree produced from the I6S gene sequences of the Erwinia amylovora KTMU 5-6 strain used in this study is presented in Figure 6.

Ps. syringae pv. syringae is a rod-shaped, Gram-negative bacterium with polar flagella. It can cause disease in more than 180 plant species, including annual and perennial plants, fruit trees, ornamentals, and vegetables [39]. In Kyrgyzstan, essential fruit crops, such as apricots, plums, cherries, and other stone fruits, suffer from bacterial canker caused by this bacterium [40] (Figure 7).

The phylogenetic tree produced from the I6S gene sequences of Pseudomonas syringae KTMU strain used in this study is presented in Figure 8.

P. carotovorum was named after the phytopathologist Erwin Frink Smith (1854–1927), the first to study this pathogen. This bacterium lives in colonies and as solitary rods, 1–3 microns in size, and forms short chains. A heterotrophic facultative aerobe is the causative agent of mild rot in potatoes (potato blight) and other vegetables (Figure 9). The optimum temperature range for this pathogen is 18–25°C, and it thrives in moist soil. The pathogen damages the
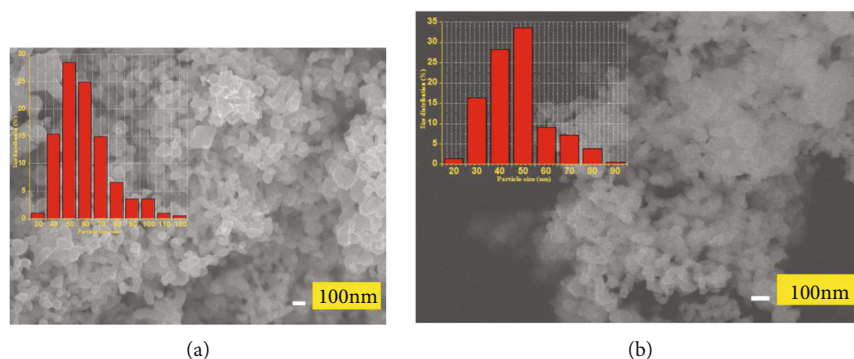
(a)

(b)

FIGURE 5: Micrograph and histogram of silver and copper nanocomposite particles synthesized in an alkaline medium (a) and in the neutral medium (b).
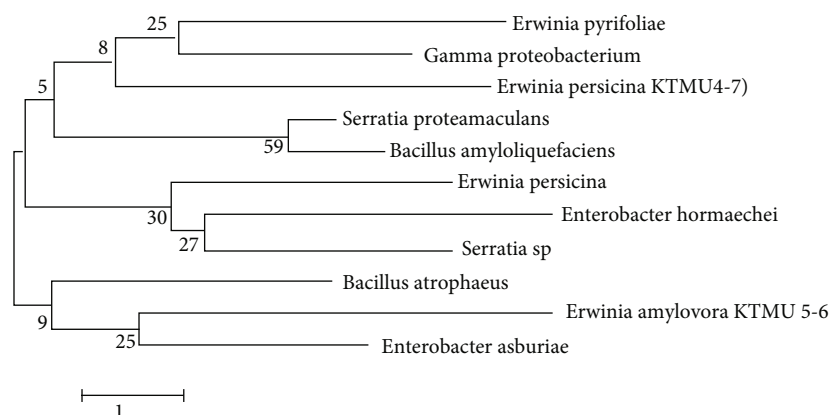

FIGURE 6: The phylogenetic tree produced from the I6S gene sequences showing the phylogenetic relationships of *Erwinia amylovora KTMU 5-6 strain* with Gram-negative and Gram-positive bacteria using the neighbor-joining method. Relevant bootstrap values (expressed as a percentage of 1000 replicates) are shown at branch points, and the bar (1.0) changes per sequence position.

aboveground plant parts and the underground organs and joints. It reduces yield by 50–75%. The phylogenetic tree produced from the I6S gene sequences of *Pectobacterium carotovorum* strain used in this study is presented in Figure 10.

3.2.2. In Vitro Biological Activity of Ag MNPs against Bacterial Pathogens. Of all the tested concentrations, only high-concentration Ag MNP solutions showed antibacterial activity. The 5 mg/l Ag-H$_2$O-ERD and 5 mg/l Ag-H$_2$O gelatin-ERD MNPs showed higher activity against the bacterial canker pathogen (*P. syringae* pv. *syringae*) than the other pathogens. The lysis zone of bacterial colony growth reached 7.0 ± 1.01 mm ($P > 0.05$). However, these solutions in any concentration had no antibacterial effect on *P. carotovorum* and *E. amylovora*. While the high concentration (5 mg/l) of Ag-H$_2$O and Ag-NH$_4$OH solutions also positively affected the bacterial canker pathogen (*Ps. syringae* pv. *syringae*) and the vegetable bacteriosis pathogen (*P. carotovorum*) (Figures 11–13).

3.2.3. In Vitro Biological Activity of Cu MNPs against Bacterial Pathogens. Different concentrations of Cu MNPs synthesized by various methods were tested for their antibacterial activity against plant pathogens. The antibacterial activities of the Cu NPs were also compared to those of the Ag NPs. Cu-H$_2$O, Cu-H$_2$O-gelatin, and Cu-NH$_4$OH-gelatin NPs at 1–5 mg/l showed insignificant activity against *P. carotovorum*. At all three tested concentrations, the lysis zone was 0.3 ± 0.01 mm. Lower concentrations did not show any effect at all.

Cu-H$_2$O-ERD, Cu-H$_2$O-gelatin, and Cu-NH$_4$OH-gelatin at 1–5 mg/l showed high activity against *Ps. syringae*. Significantly, at 5 mg/l, all the samples had high activity. The lysis zone reached 10.0 ± 0.95 mm.

At 5.0, 1.0, and 0.5 mg/l, Cu-NH$_4$OH, Cu-NH$_4$OH-gelatin, and Cu-H$_2$O-gelatin each exhibited good activity against *E. amylovora*. Cu-H$_2$O-gelatin-ERD at 5 mg/l resulted in a lysis zone of 12.0 ± 0.95 mm (Figures 14–17).

The use of the chemicals (Xerox colloidal Ag preparation and copper sulphate) resulted in no lysis zone. Thick masses of bacterial colonies were observed, despite the high concentrations (5 mg/l) used (Figure 18).

Furthermore, Cu NPs were tested under field conditions. Apricot trees were treated with this Cu NPs. The preliminary results were positive, with reductions in pathogen populations observed. However, these studies should be continued and repeated (see Figure S3 in the supplementary material).

3.2.4. In Vitro Biological Activity of Ag-Cu BNPs against Bacterial Pathogens. The BNPs prepared in neutral,

(a)

(b)

(c)

(d)
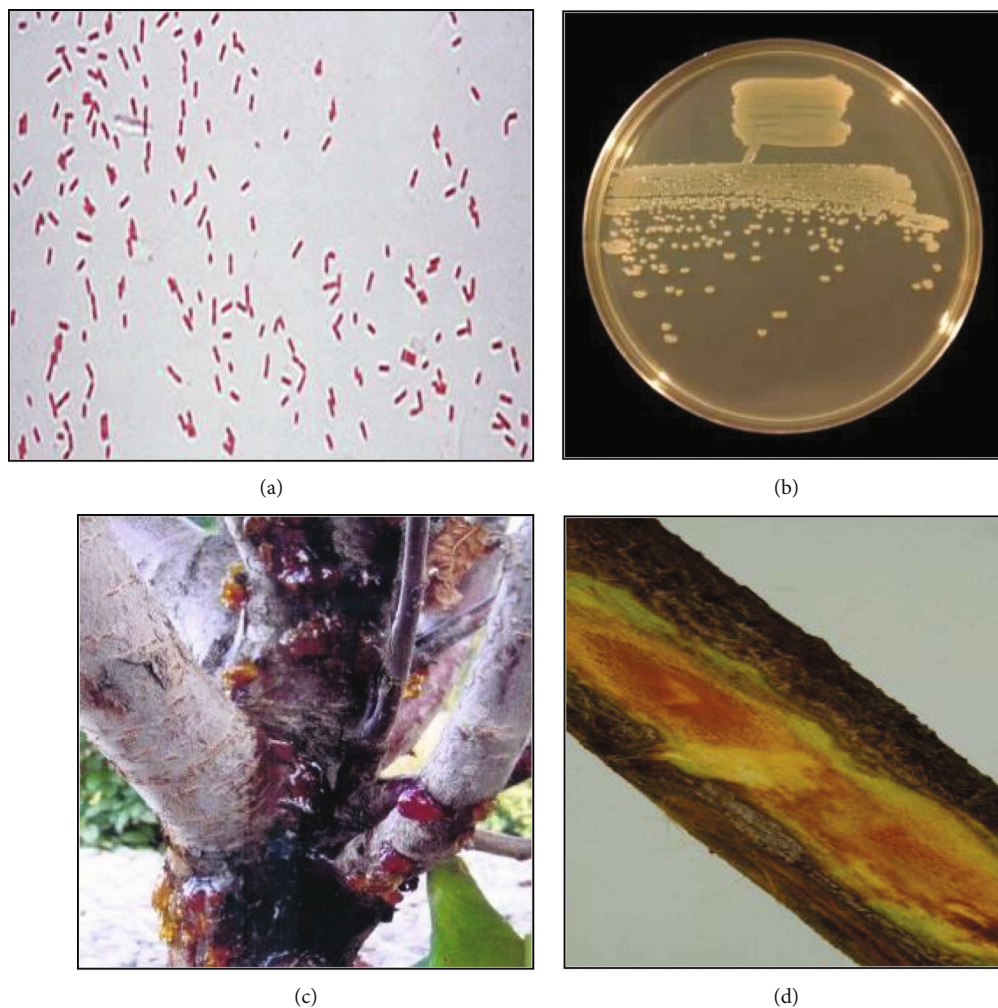
FIGURE 7: (a) Microscopic view of *Pseudomonas syringae* cells; (b) colonial growth on the agar medium; (c) adverse effects on the plants; (d) symptoms on an infected branch.

FIGURE 8: Phylogenetic tree produced from the I6S gene sequences showing the phylogenetic relationships of *Pseudomonas syringae KTMU strain* with Gram-negative and Gram-positive bacteria using the neighbor-joining method. Relevant bootstrap values (expressed as a percentage of 1000 replicates) are shown at branch points, and bar (0.02) changes per sequence position.

(a)

(b)

(c)

(d)

FIGURE 9: (a) Microscopic view of Pectobacterium carotovorum bacterium cells; (b) symptoms of cabbage bacteriosis; (c) symptoms of onion bacteriosis; (d) symptoms of wet potato rot.

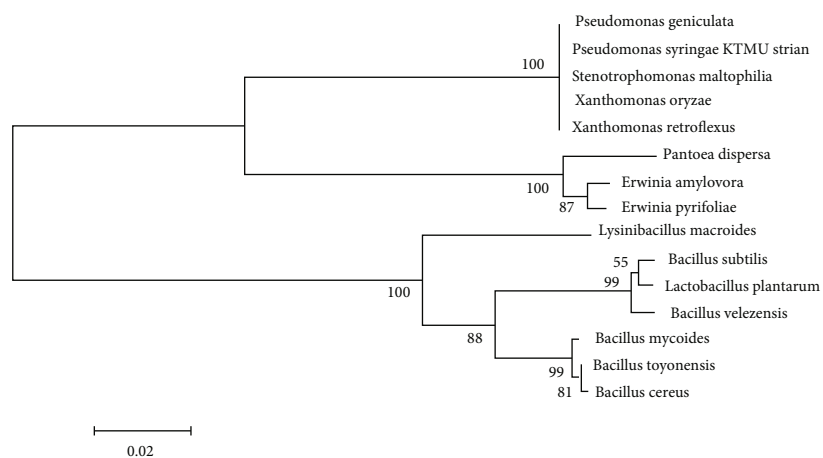FIGURE 10: Phylogenetic tree produced from the I6 S gene sequences showing the phylogenetic relationships of *Pectobacterium carotovorum strain* with Gram-negative and Gram-positive bacteria using the neighbor-joining method. Relevant bootstrap values (expressed as a percentage of 1000 replicates) are shown at branch points, and the bar (0.1) changes per sequence position.
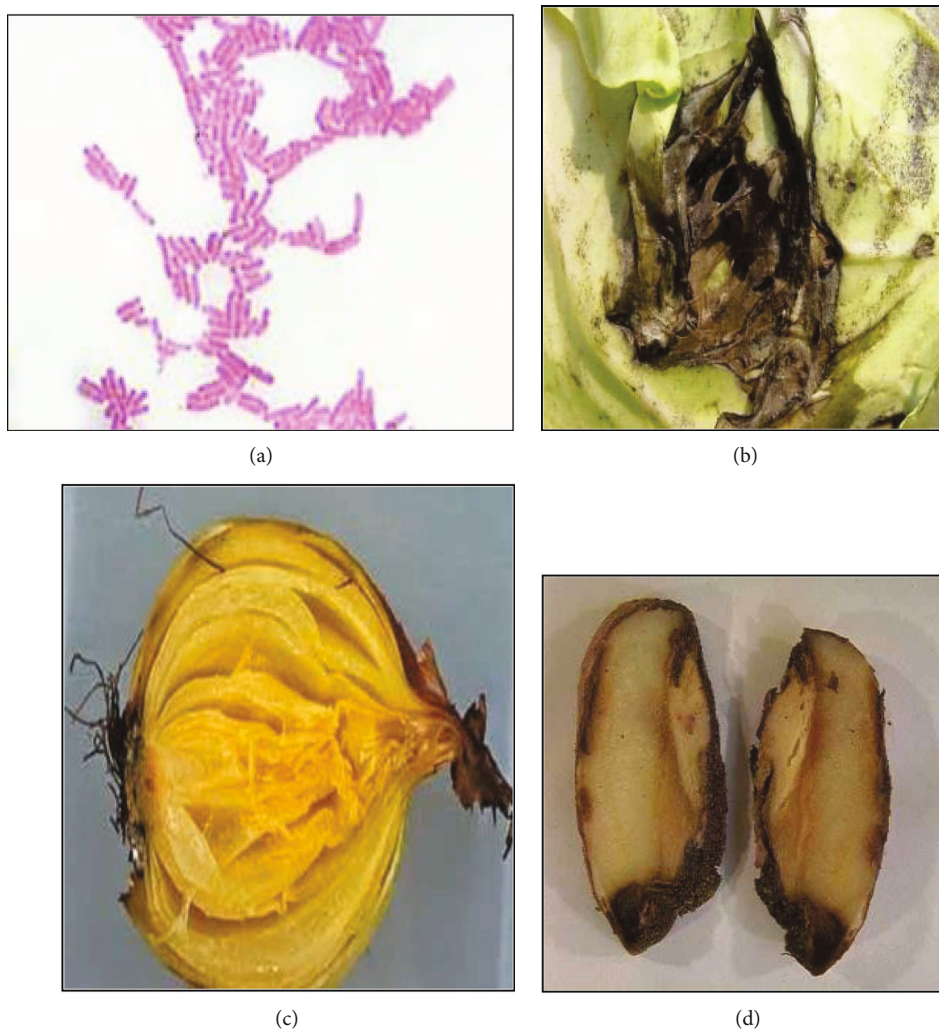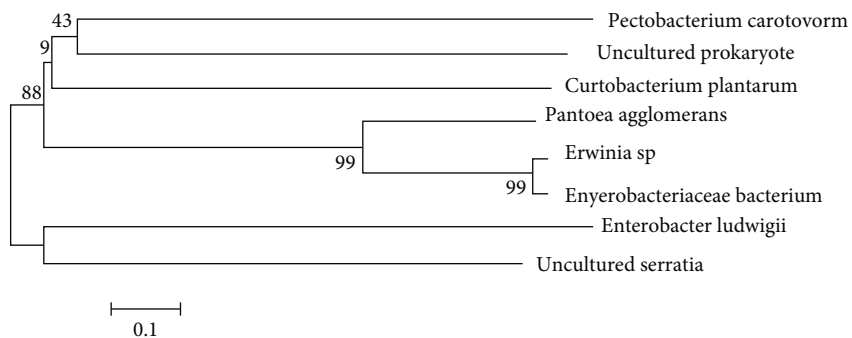
FIGURE 11: Effects of Ag NPs on bacterial diseases of various plants: 1–5 mg/l of AgH$_2$O EID; 2–5 mg/l of Ag-H$_2$O gelatin-ERD; 3–5 mg/l of Ag-H$_2$O; 4–5 mg/l of AgNH$_4$OH.

(a)   (b)
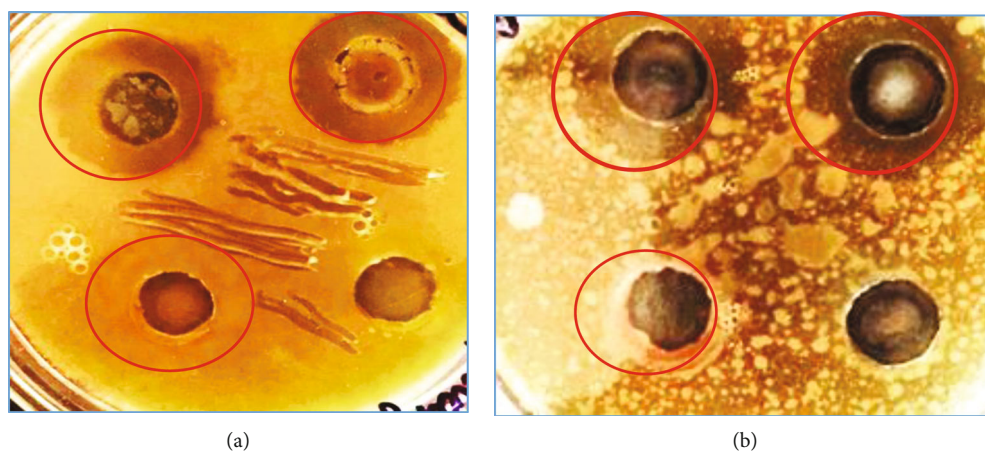
FIGURE 12: The pictures of lysis zones of bacterial growth around the well on agar medium with Ag NPs: (a) an activity of Ag-H2O-EID and Ag-H$_2$O gelatin-ERD samples against Pseudomonas syringae (bacterial canker of plums); (b) Ag-H2O-EID and Ag-H2O gelatin-ERD sample activity against Pectobacterium carotovorum (bacteriosis of vegetables).

(a)   (b)
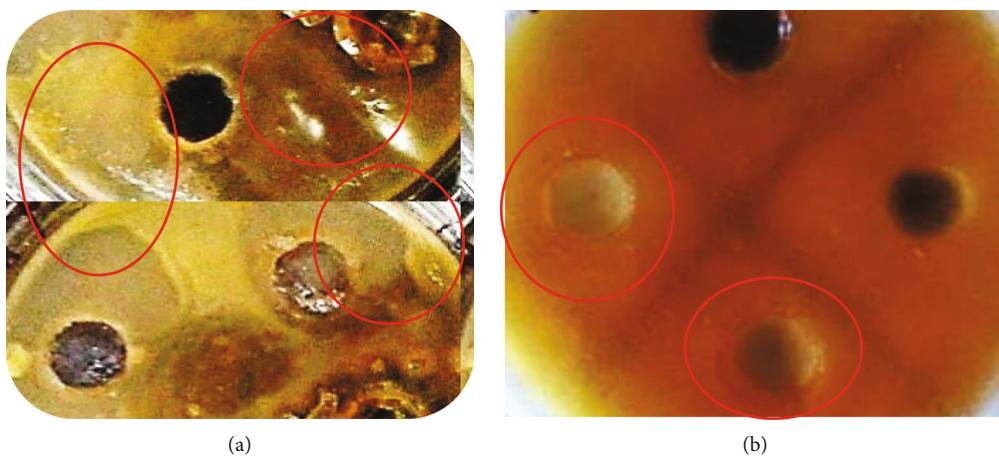
FIGURE 13: The pictures of lysis zones of bacterial growth around the well on agar medium with Ag NPs: (a) an activity of Ag-H$_2$O-EID, Ag-H$_2$O gelatin-ERD, and Ag-H$_2$O against *Ps. syringae* (bacterial canker of apples); (b) activity of Ag-H$_2$O and AgNH$_4$OH samples against *E. amylovora* (bacterial blight of pears).
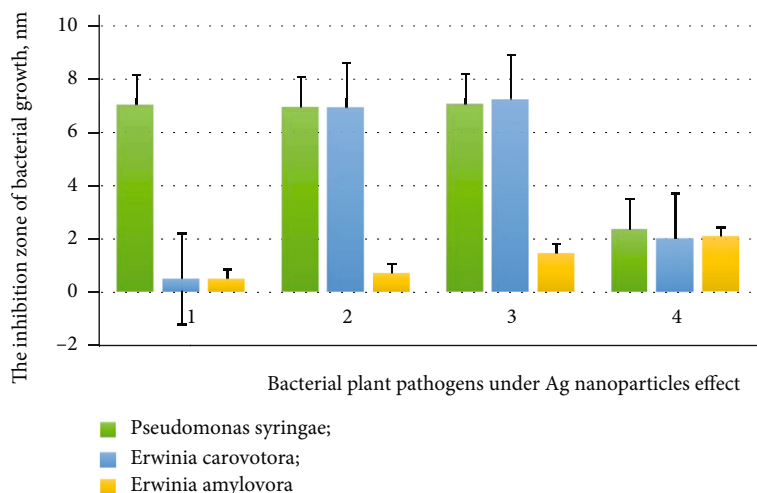
FIGURE 14: Effect of Cu NPs on bacterial diseases of various plants: 1–1–5 mg/l of Cu-H$_2$O–EID; 2–1–5 mg/l of Cu-H$_2$O gelatin-ERD; 3–1–5 mg/l of Cu-H$_2$O; 4–1–5 mg/l of Cu-H$_2$O-gelatin.

(a)

(b)

(c)
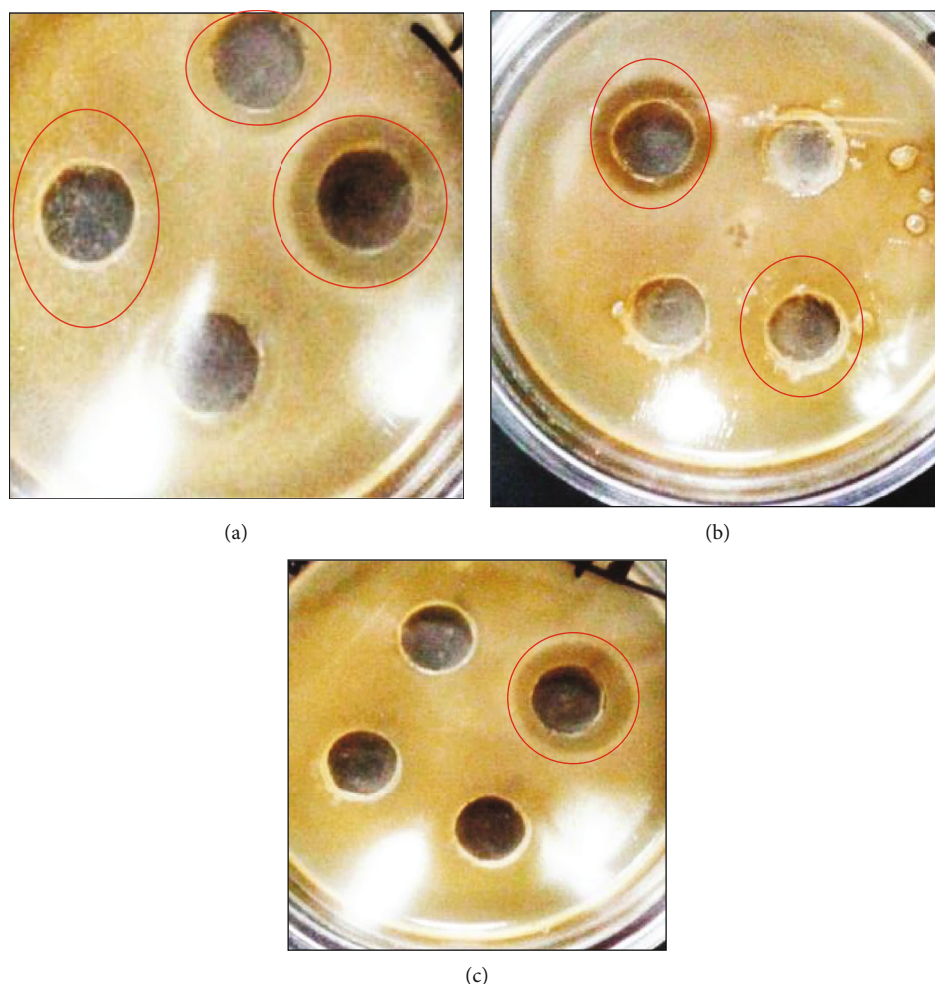
FIGURE 15: The pictures of lysis zones of *P. carotovorum* growth around the well on agar medium with Cu NPs: (a) Cu-H$_2$O- EID; (b) Cu-H$_2$O gelatin-ERD; (c) Cu-Cu-H$_2$O-gelatin.

(a)

(b)

(c)
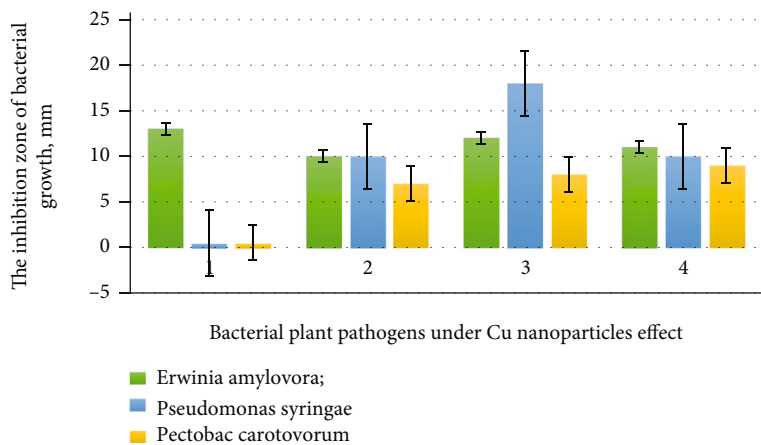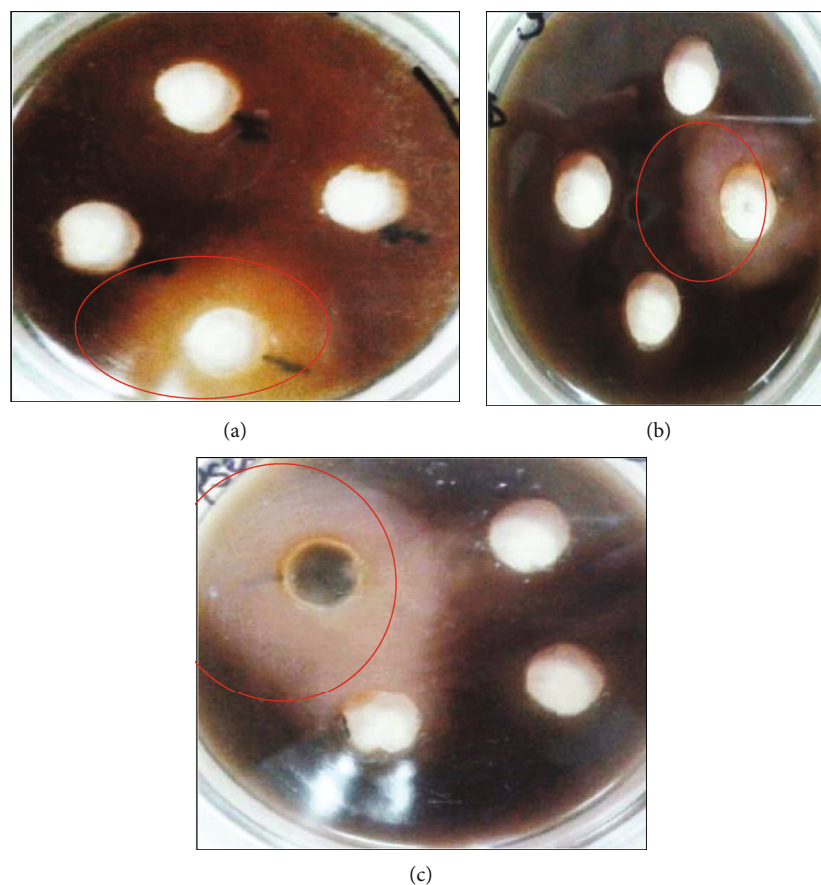
FIGURE 16: The pictures of lysis zones of *Ps. syringae* growth around the well on agar medium with Cu NPs: (a) Cu-H_2O-EID; (b) Cu-H_2O gelatin-ERD; (c) Cu-Cu-H_2O-gelatin.

(a)

(b)
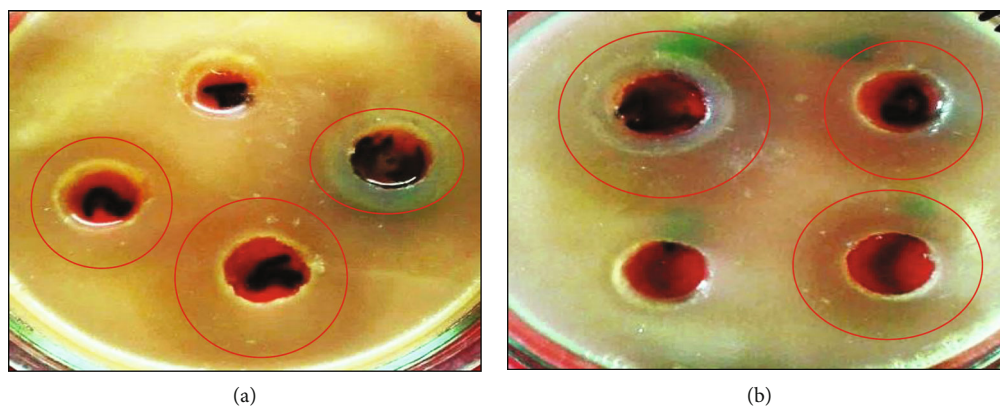
FIGURE 17: The pictures of lysis zones of *E. amylovora* growth around the well on agar medium with Cu NPs: (a) Cu-H_2O-EID; (b) Cu-H_2O-gelatin.

ammonia, and alkaline media, both with and without gelatin, showed pronounced activity against the fire blight pathogen bacteria, *E. amylovora*. The lysis zones ranged from 4.0 ± 0.10 mm to 7.0 ± 0.13 mm ($P \leq 0.05$). The absence of bacterial growth was noted at these distances, and vegetative cells formed with morphological deformities closer to the periphery. Microscopic examination revealed disrupted cell morphogenesis: bacterial cells were torn into small pieces and vegetative rods had transformed into cocci.

The large lysis zones observed show that the tested Ag-Cu nanocomposites have sufficiently high biological activity against *E. amylovora*, *Ps. syringae*, and *P. carotovorum* (Table 7). Among the studied nanopowders, the Ag-Cu nanocomposites synthesized in an alkaline medium showed the best results against *E. amylovora*, *Ps. syringae*, and *P. carotovorum*. The maximum zone of inhibition reached for each bacteria was 8.0 ± 0.02 mm for *Ps. syringae*, 7.1 ± 0.92 mm for *P. carotovorum*, and 7.0 ± 0.14 mm for *E. amylovora*

FIGURE 18: The pictures of no lysis zones of *Ps. syringae* growth around the well on agar medium with chemical preparations: (1) Xerox colloidal silver preparation; (2) copper sulfate.

($P \leq 0.05$). The antibacterial activity of the Ag-Cu nanocomposites was three to four times higher than the antibacterial activity of the Ag MNPs at 1 mg/ml.

Thus, the combined chemical reduction of Ag and Cu ions in the presence of gelatin leads to an increase in the antibacterial activity of Ag-Cu nanocomposites. The minor NP aggregates formed in an alkaline medium exhibited the highest antibacterial activity against all tested bacterial pathogens (Figures 19 and 20).

When smears were taken from bacterial colonies around the lysis zones and the bacteria examined and compared to those from colonies of control plates, significant differences were observed in the places of contact between NPs and bacteria. As shown in Figures 21 and 22, the bacterial morphology changed dramatically when the bacteria were exposed to NPs. The *E. amylovora* rod-shaped cells were crushed into cocci shapes or became shapeless. Moreover, the *Ps. syringae* cells lost their normal form and turned into an amorphous mass. In this case, the bacteria also lost their normal activity. It is reasonable to assume that the processes of division and reproduction were disrupted, given that metallic NPs are known to affect the bacterial DNA structure.

3.2.5. The Effects of Ag and Cu NPs on Wheat and Corn Seed Germination and Growth. Seed germination and seedling growth are widely used to test the phytotoxicity of chemical species, such as engineered nanomaterials that may be released into the environment. Measurement of seed germination and shoot and root elongation are relatively rapid methods for determining acute phytotoxicity, and they have several advantages: sensitivity, simplicity, low cost, and suitability for reactive chemicals and contaminated soil samples [27].

The greatest wheat germination and seedling growth resulted from exposure to the *S. diastochromogenes* suspension. Root length reached 17.1 ± 1.02 cm in 10 days, and shoot size 15.0 ± 1.09 cm. The seed germination rate was $99.9 \pm 0.09\%$ ($P \leq 0.05$). An interesting phenomenon was observed in this treatment compared with other specimens: the stems were very thick. The second biological agent—*S. violaceoruber*—also showed a significant growth-stimulating effect; however, it was less than that of the first natural agent (Table 8).

Of all the tested NPs, the Ag-Cu BNP (acidic) composition exhibited the greatest growth-stimulating effect on the

wheat roots and stems. In 10 days, the root length reached 15.0 ± 1.05 cm and the stem length reached 15.0 ± 1.25 cm ($P < 0.05$) ($n = 3$). The seed germination was also higher ($95.4 \pm 0.13\%$, $P < 0.05$) than that observed for other NP samples, but it was lower than that for the biological products. The Ag MNP solution was found to positively affect the germination of wheat seeds; the germination rate was $99.8 \pm 0.05\%$ ($P < 0.05$) (see Figure S1 in the Supplementary Material).

The effectiveness of the Ag-Cu (neutral) and Ag-Cu (alkaline) BNPs was much lower than that of the Ag-Cu (acidic) BNPs and the Ag MNPs. However, both of these compositions stimulated wheat stem growth but not root growth (see Figure S2 in the Supplementary Material). The thickness of the stems was very different from that observed in the controls, reaching some mm in diameter. The root length of the control (water) was 3.0 ± 0.15 cm, the shoot length was 15.0 ± 1.09 cm, and the germination was $90.0 \pm 0.08\%$ ($P < 0.05$) (Figures 23–25).

In general, there was a decrease in seed germination when NPs were used, except for Ag MNPs. In terms of phytotoxicity, the NP solutions (1 mg/l) were more phytotoxic than the biological products (1×10^3 spores/ml) for wheat seeds.

The results were significantly different when corn seeds were used. The Ag MNPs showed the highest growth-stimulating effect. The root and stem lengths reached 15.0 ± 0.78 cm and 15.0 ± 0.92 cm, respectively, in 10 days with three repetitions. However, the germination of corn seeds was less than that of wheat seeds, at $80.1 \pm 0.12\%$ ($P < 0.05$) (Table 9).

The Ag-Cu (neutral) BNP composition also had a significant effect on the growth of the plant vegetative organs. The root length was 12.0 ± 0.67 cm and the stem size was 15.0 ± 1.41 cm ($P < 0.05$). However, the germination was even lower than that resulting from exposure to the Ag MNPs. The Ag-Cu (alkaline) BNP composition was found to be considerably phytotoxic, with the lowest germination rate of 60.0 ± 0.16 cm ($P < 0.05$).

The bioproducts of *S. diastochromogenes* induced a corn seed germination rate of $99.7 \pm 0.09\%$, although the growth-stimulating effect was lower than that of the NPs. The germination of corn seeds in the control was $80.2 \pm 0.31\%$, the root length was 4.0 ± 0.17 cm and the shoot size was 15.0 ± 1.06 cm ($P < 0.05$). The Ag MNPs and Cu MNPs had the same effect on germination as the water control; that is, the toxic effect was not noticeable (Figures 26–28).

4. Discussion

The antibacterial effects of Ag and Cu have been known since ancient times. Cu is involved in the metabolism of all living organisms, and it is primarily involved in fermentation [19]. Cu salts are used in agriculture for seed treatment as chemical fungicides and in prophylactic therapies against phytopathogens of fruit crops and other agricultural plants. Chemical compounds containing Ag and Cu are also used in medicine, food processing, environmental protection, and other areas [12, 13, 19, 28, 41].

Bacterail plant pathogen growth under Ag-Cu bimetalic compostion

- → - Pseudomonas syringae;
- → - Erwinia amylovora;
- → - Erwinia carovotora
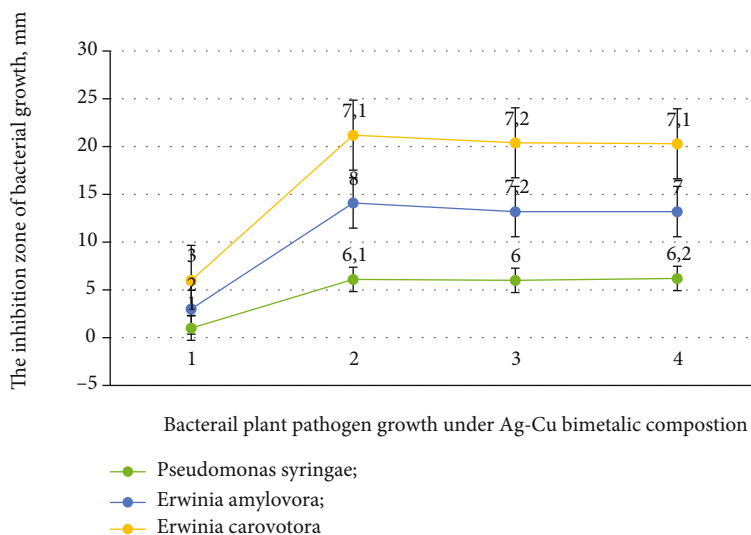
FIGURE 19: Effects of Ag and Cu NPs (1 mg/l) on bacterial diseases of various plants: 1: Ag and Cu MNPs; 2: Ag-Cu, gelatin in NaOH; 3: Ag-Cu, gelatin in NH$_4$OH; 4: Ag-Cu, gelatin in neutral reaction medium.
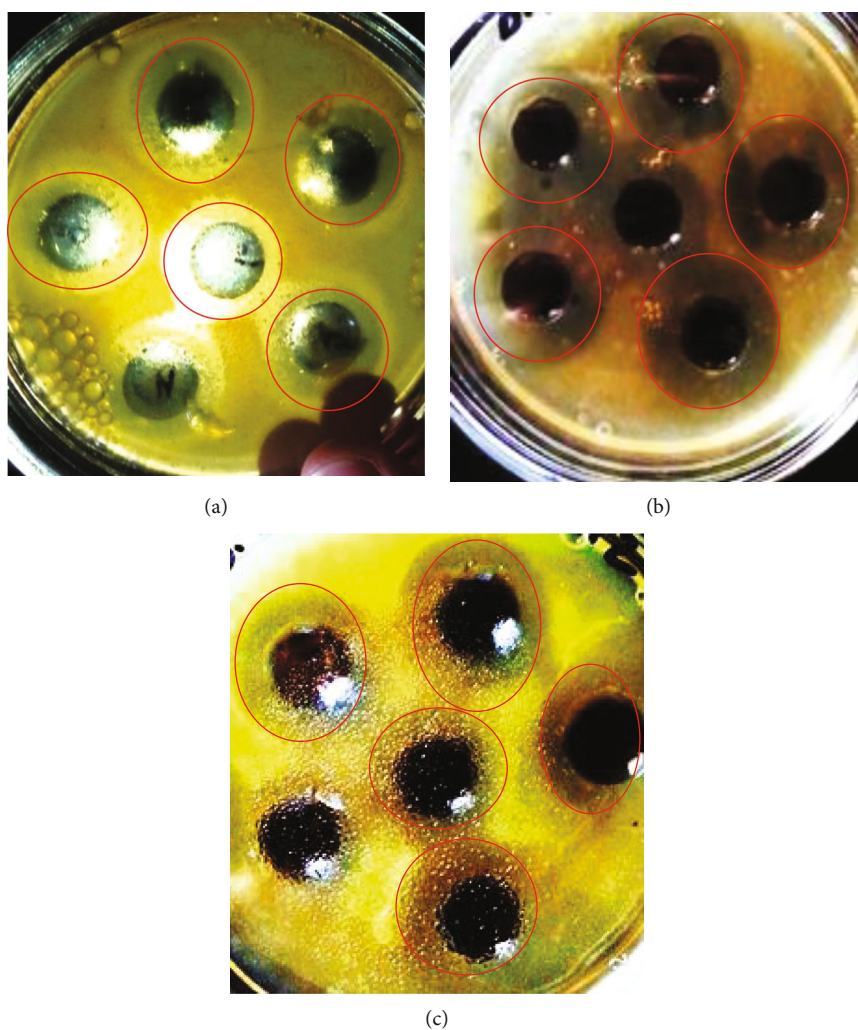

(a)

(b)

(c)

FIGURE 20: The pictures of the lysis zone of bacterial growth around the well agar medium with BNPs (Ag-Cu, gelatin in NaOH): (a) *Erwinia amylovora*; (b) *Pseudomonas syringae*; (c) *Pectobacterium carotovorum*.
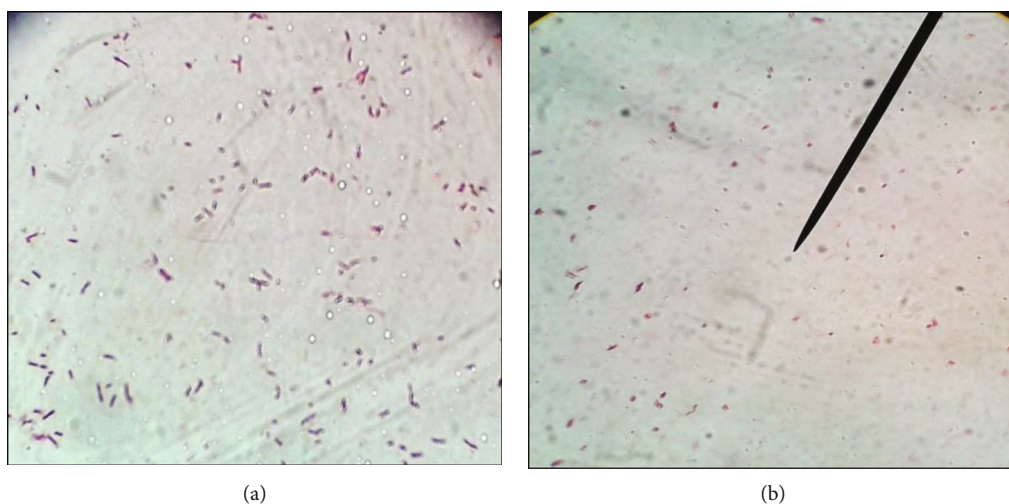
(a)

(b)

FIGURE 21: (a) Microscopic rods of the bacterium Erwinia amylovora in control; (b) bacterium Erwinia amylovora in contact with nanoparticles.

(a)

(b)
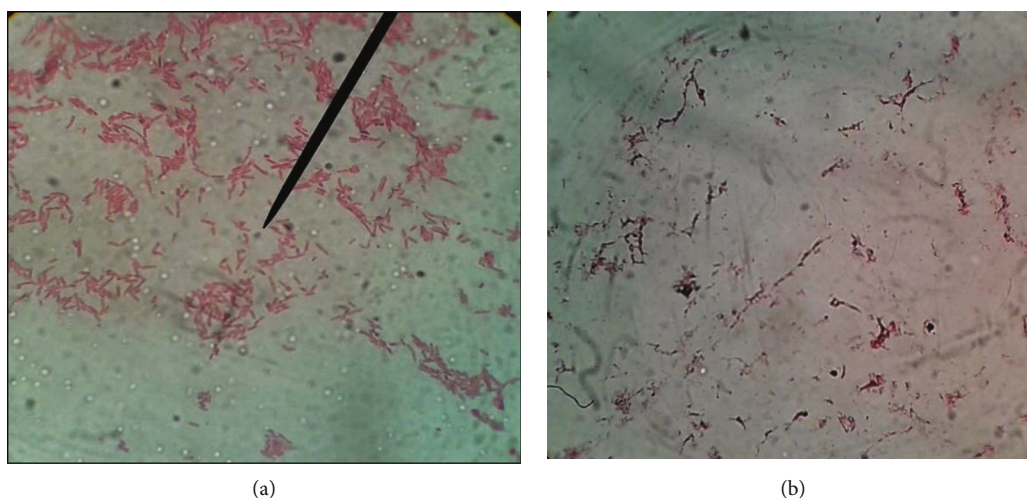
FIGURE 22: (a) Microscopic rods of the bacterium Pseudomonas syringae in control; (b) bacterium Pseudomonas syringae in contact with nanoparticles.

TABLE 8: Effects of Ag and Cu MNPs and Ag-Cu BNPs on the germination and growth of wheat seeds compared with bioproducts ($n = 3$).

Variant	Concentration	Root length (cm)	Stem length (cm)	Germination (%)
Control (water)		3.0 ± 0.15	15.0 ± 1.09	90.2 ± 0.08
Ag MNP	1 mg/l	10.1 ± 0.97	12.0 ± 1.03	99.8 ± 0.05
Cu MNP	1 mg/l	8.0 ± 0.76	14.0 ± 1.21	90.2 ± 0.07
Ag-Cu BNP (acidic)	1 mg/l	15.0 ± 1.05	15.0 ± 1.25	95.4 ± 0.13
Ag-Cu BNP (neutral)	1 mg/l	5.0 ± 0.89	15.0 ± 1.31	90.1 ± 0.16
Ag-Cu BNP (alkaline)	1 mg/l	8.0 ± 0.15	13.0 ± 1.07	95.0 ± 0.15
Streptomyces violaceoruber	1×10^3 spores/ml	10.2 ± 0.94	15.0 ± 0.92	99.8 ± 0.04
Streptomyces diastochromogenes	1×10^3 spores/ml	17.1 ± 1.02	15.0 ± 1.09	99.9 ± 0.09
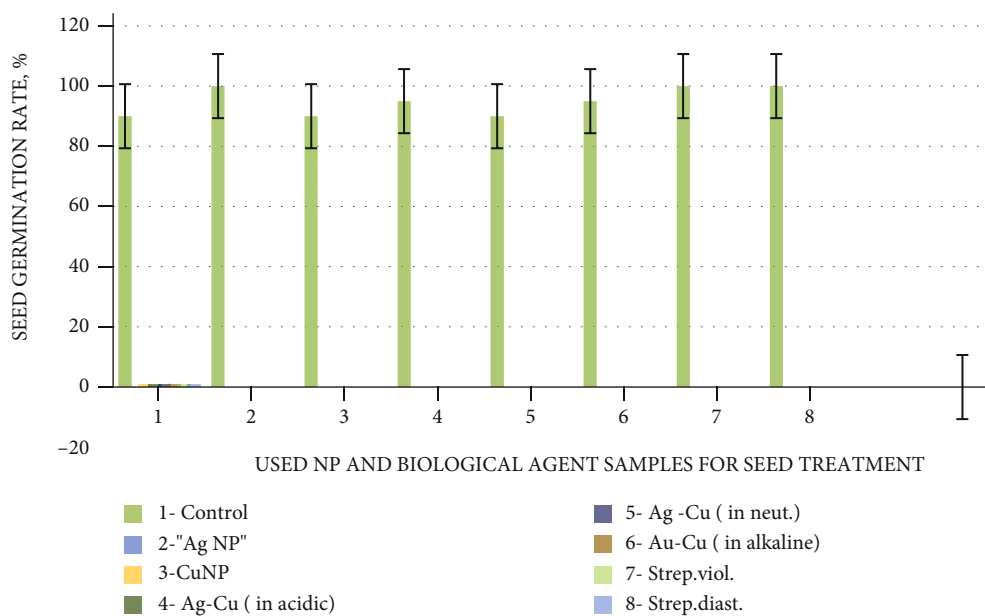
FIGURES 23: The germination rate of wheat seeds under treatments by MNPs, BNPs, and biological agents.
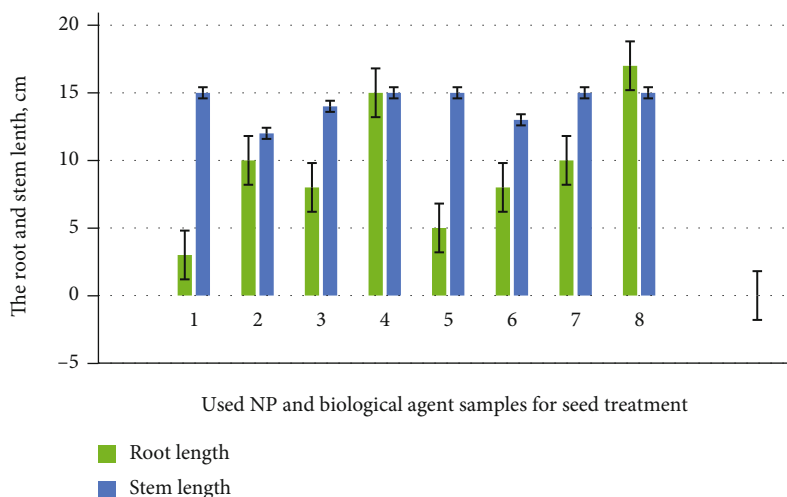
FIGURES 24: The growth-regulator effects of metallic nanoparticles and biological agents on wheat seedlings by treatments: 1: control; 2: Ag NP; 3: Cu NP; 4: Ag-Cu (in acidic); 5: Ag-Cu (in neutral); 6: Ag-Cu (in alkaline); 7: *Streptomyces violaceoruber*; 8: *Streptomyces diastochromogenes*.

The development of modern nanotechnology is aimed at producing NPs and nanoscale structures of metals.

In the present study, Ag and Cu MNPs and BNPs were synthesized using different methods and media and their antibacterial activities were studied. For the first time, Ag-Cu BNPs were investigated for their antibacterial activities against Gram-negative plant pathogenic bacteria: *Ps. syringae*, *P. carotovorum*, and *E. amylovora*.

When Ag and Cu MNPs were tested, it was found that only MNPs at a high concentration (5 mg/l) that were synthesized in a neutral reaction and stabilized in gelatin showed significant activity ($P < 0.05$) against *Ps. syringae*. The Ag MNPs had low activity against *P. carotovorum* and *E. amylovora*. Statistical analysis of variance with 0.05 significance showed that a 5 mg/l Ag MNP concentration had a common inhibition effect on *P. carotovorum* and *E. amylovora*. Ag MNPs synthesized in a neutral medium (Ag-H_2O) and in an ammonia medium (Ag-NH_4OH) at 5 mg/l manifested antibacterial properties against *Ps. syringae* and *P. carotovorum* ($P < 0.05$).

The antibacterial activity of the Ag MNPs was enhanced when gelatin was included as a stabilizer in the neutral pH reaction. These results confirm the importance of including stabilizers for the production of biocompatible NPs with physiological activity; they enhance the biological properties of NPs [42]. It is worth noting that in the current study, the enhancing role of gelatin for Ag MNPs was only observed against Gram-negative *Ps. syringae*. Others have reported a higher antibacterial effect on Gram-negative bacteria due to electrostatic interactions between positively charged NPs
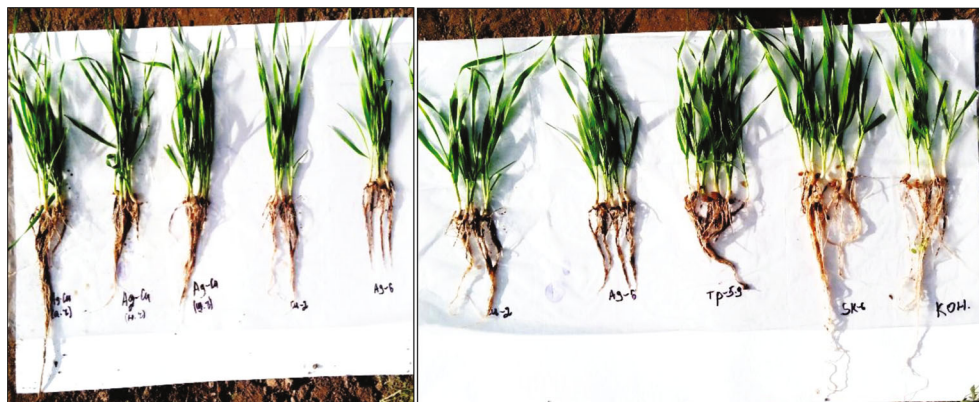
FIGURES 25: The growth of roots and aboveground parts of wheat under the treatment by Ag, Cu nanoparticle solutions, and bioproducts of *Streptomyces* bacteria.

TABLE 9: Effects of Ag and Cu MNPs and Ag-Cu BNPs on the germination and growth of corn seeds compared with bioproducts ($n = 3$).

Variant	Concentration	Root length (cm)	Stem length (cm)	Germination (%)
Control (water)		4.0 ± 0.17	15.0 ± 1.06	80.2 ± 0.31
Ag MNP	1 mg/l	15.0 ± 0.78	15.0 ± 0.92	80.1 ± 0.12
Cu MNP	1 mg/l	10.0 ± 0.78	15.0 ± 1.1	80.0 ± 0.17
Ag-Cu BNP (acidic)	1 mg/l	8.0 ± 1.13	13.0 ± 1.14	70.2 ± 0.34
Ag-Cu BNP (neutral)	1 mg/l	12.0 ± 0.67	15.0 ± 1.41	70.3 ± 0.70
Ag-Cu BNP (alkaline)	1 mg/l	8.0 ± 0.14	15.0 ± 1.12	60.0 ± 0.16
Streptomyces diastochromogenes	1×10^3 spores/ml	10.2 ± 1.05	12.0 ± 1.14	99.7 ± 0.09
Streptomyces violaceoruber	1×10^3 spores/ml	10.2 ± 1.79	14.1 ± 1.09	80.2 ± 0.07

1- Control
2-"Ag NP"
3-CuNP
4- Ag-Cu (in acidic)
5- Ag -Cu (in neut.)
6- Au-Cu (in alkaline)
7- Strep.viol.
8- Strep.diast.

FIGURES 26: The germination rate of corn seeds under treatments by MNPs, BNPs, and biological agents.

FIGURES 27: The growth-regulator effects of metallic nanoparticles and biological agents on corn seedlings by treatments: 1: control; 2: Ag NP; 3: Cu NP; 4: Ag-Cu (in acidic); 5: Ag Cu (in neutral); 6: Ag-Cu (in alkaline); 7: *Streptomyces violaceoruber*; 8: *Streptomyces diastochromogenes*.
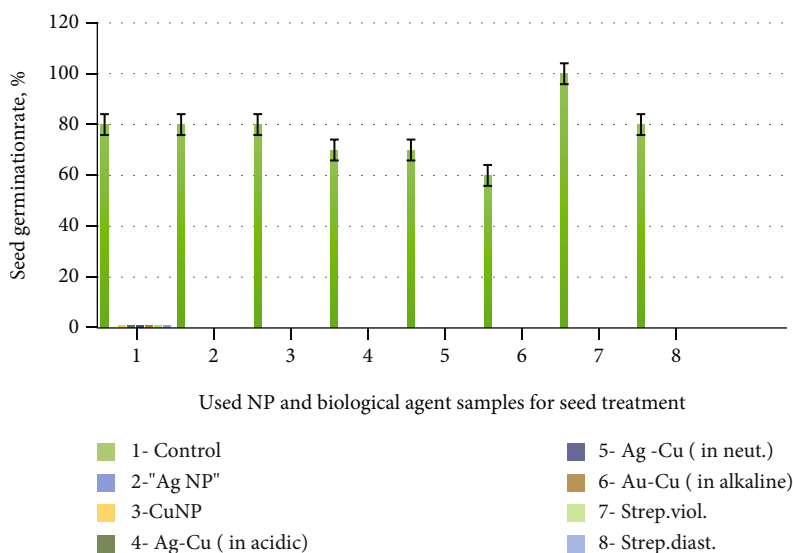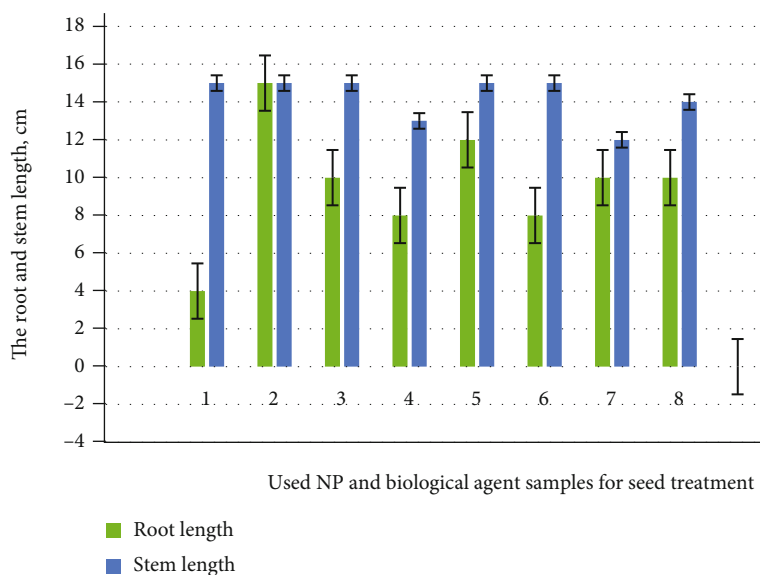

FIGURES 28: The growth of roots and aboveground parts of corn under the treatment by Ag, Cu nanoparticle solutions, and bioproducts of *Streptomyces* bacteria.

and the negatively charged bacterial membrane. Because of the exchange, the permeability of cell membranes is disrupted, destroying the cell wall and damaging DNA replication [36, 43–45]. However, in this study, even high concentrations of Ag MNPs did not have a bactericidal effect on the bacterial fire blight pathogen *E. amylovora*. Hence, the findings indicate that not all Gram-negative bacteria are sensitive to Ag MNPs. Of the three bacteria tested, *Ps. syringae* was the most susceptible. As noted by others, the response or sensitivity of each bacterial species to NPs depends on their metabolic characteristics [46–49].

When the Cu MNP results were analyzed, it was found that the biological properties and effective antibacterial concentrations of these MNPs differed sharply from those of the Ag MNPs. Unlike Ag MNPs, Cu MNPs at 0.5, 1.0 and 5.0 mg/l exhibited antibacterial activity. Where Ag MNPs were found to be active mainly against the causative agent of bacterial canker (*Ps. syringae)*, Cu MNPs showed high activity against the causative agent of bacterial fire blight

(*E. amylovora*). In addition, in contrast to the Ag MNPs, the Cu NPs synthesized in different media and stabilized and unstabilized showed activity against all three tested pathogens. In particular, the Cu-H_2O MNPs at 1–5 mg/l showed the highest activity of all the Cu MNPs.

The Ag-Cu BNPs exhibited significantly higher activity than the MNPs. Even at a low concentration (1 mg/l) the composites showed significant activity against all three tested pathogens. The Ag-Cu nanocomposites synthesized in an alkaline medium demonstrated the highest activity against all three pathogens. The minor aggregates produced in the alkaline medium had the highest antibacterial activity against *E. amylovora* and *Ps. syringae*. Thus, these results have confirmed other reports of a significant difference in the biological activity of MNPs and BNPs. This suggests that, compared to MNPs, BNPs contain unique and additional characteristics that complement each other in terms of physiological properties. For example, electron transfer from Ag NPs to second metal NPs can enhance the

antibacterial activity of nanocomposites [23]. Therefore, it is more desirable to use BNPs at low concentrations in agricultural practice than MNPs.

We also examined the phytotoxic and phytoregulatory effects of the NPs on wheat and corn seeds to clarify the possibility of using NPs for presowing seed treatment. At 1 mg/l, the NPs were not phytotoxic to wheat seeds; in fact, the opposite was found. At this concentration, the NPs showed a growth-stimulating effect on the germination of seeds and the growth of young roots and shoots, although the NPs were inferior to the *Streptomyces rhizosphere* biological agents. However, when tested on corn seeds, the bimetallic compositions showed greater phytotoxicity than the monometallic materials; this feature should be considered in practical applications.

Some studies have demonstrated the protective and stimulating effects of Ag nanocomposites on the growth and development of wheat and other crops [27, 49, 50]. Others have confirmed that, unlike ionic biogenic metals, NPs have prolonged action and lower toxicity than metal salts and can activate physiological and biochemical processes in plants [24]. The Ag NP concentrations that are toxic to plant seeds have also been determined in some cases; for example, 10 mg/l suppressed the germination and growth of seedlings of *Hordeum vulgare* and *Linum usitatissimum* [3].

According to existing data, the effects of NPs on plants depend on the plant species and age, NP concentration and duration, and method of exposure. There are different and often conflicting reports on the accumulation and toxicity of NPs in plant species [29]. Our corn seed germination and corn seedling growth results are in alignment with those of other studies [29] that showed that Ag NPs had a toxic effect on corn root elongation. In contrast, the same Ag NP concentration has been shown to have a positive effect on watermelon and zucchini seedling growth, leading to the conclusion that Ag NPs exhibit both positive and negative effects on plant growth and germination.

Our results have shown that 1 mg/l is the ideal concentration of Ag MNPs and Ag-Cu BNPs for the treatment of wheat. The application of NPs at this concentration improved the percentage of seed germination and the growth of seedlings. This concentration was found to be slightly toxic for corn when the seeds were soaked for 2 h. According to existing reports, NPs promote the absorption of water by grains, increase the content of nitrate reductase, and support the development of antioxidants [51, 52].

5. Conclusions

The current study has shown that Cu and Ag MNPs and BNPs are effective against several pathogens that pose a threat to fruit trees and vegetable crops in Kyrgyzstan: *E. amylovora* (bacterial fire blight), *Ps. syringae* (bacterial canker), and *P. carotovorum* (rotten vegetable bacterium).

At 5 mg/l, the Ag MNPs Ag-H_2O and Ag-NH_4OH showed high antibacterial activity against *Ps. syringae*, whereas Ag-H_2O-ERD at 1 mg/l and Cu-H_2O-ERD at 0.1 mg/l exhibited a high inhibition effect against *E. amylo-*

vora. Ag-Cu bimetallic compositions were found to be very active against all tested bacterial pathogens. In particular, the Ag-Cu gelatin (neutral medium), Ag-Cu gelatin (ammonia medium), and Ag-Cu gelatin (alkaline medium) samples showed high antibacterial activity against the most harmful pathogen—*E. amylovora*.

Our results suggest that, due to their antibacterial activity, Ag-Cu nanocomposites could be useful additions to numerous agricultural applications that are aimed at protecting crops from dangerous diseases, such as seed coating and the treatment of plant roots and aboveground organs. We have also shown that advances in nanotechnology now allow for the development of new, safe, ecologically friendly, multifunctional material formulations. Therefore, bimetallic nanocomposites should be considered as alternatives to the harmful chemicals used in agriculture to protect crops from pathogenic bacteria.

This study has shown that a nanocomposite based on Ag and Cu can be used as an environmentally friendly, protective, growth-stimulating, new-generation antimicrobial formulation for treating agricultural plants.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

Supplementary Materials

Biological activity of Ag and Cu monometallic nanoparticles and Ag-Cu bimetallic nanocomposites against plant pathogens and seed article. *(Supplementary Materials)*

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