Nano-Metals Forming Bacteria in Egypt. II. Efficacy towards Some Bacterial Soft Rot/Blackleg Genera ex vivo and Bio-Control in vivo as Eco-Friendly Therapeutic against Dickeya Solani

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Nano-Metals Forming Bacteria in Egypt. II. Efficacy towards Some Bacterial Soft Rot/Blackleg Genera *ex vivo* and Bio-Control *in vivo* as Eco-Friendly Therapeutic against *Dickeya solani*

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Abstract

The antibacterial activity of Cu, Fe, Co and ZnNPs which were formed by *Enterococcus thailandicus, Pseudomonas putida, Marinobacter hydrocarbonoclasticus*, and *P. geniculate* sequentially was tested against some soft rot/blackleg genera. The effects of NPs were recorded on bacterial DNA, proteins and carbohydrates concentration of *Pectobacterium carotovorum, Enterobacter cloacae* (soft rot) and *Dickeya solani* (soft rot/blackleg). Treated cells showed degradation in isolated DNA, and decrease in proteins and carbohydrates concentration compared with untreated cells. The treated cells using SEM, showed collapsed and small pits in cell wall. Internal changes using TEM showed penetration of NPs inside the tested bacterial cells, appearance of periplasmic space, formation vacuoles and condensation of cytoplasm.

Disease severity *ex vivo* of tuber infected with tested genera demonstrated that NPs treatment didn't show any rotted tissue compared with untreated. FeNPs was tested to control of soft rot/blackleg disease caused by *D. solani* in comparison with copper pesticide. Present data recorded increase in shoot and root length, in addition increase in dry and fresh weight, compare with either infected or healthy plants. In studying the ability of treated potato (*Solanum tuberosum*) seedlings with NPs to uptake and accumulate FeNPs from soil, ICP-OES recorded a small increase in Fe content of treated plants compared with untreated. , FeNPs can be used to control of soft rot/blackleg disease caused by *D. solani* instead of copper pesticide, and can be considered as a new and alternative approach to traditional disease management methods, and also increase the nutritional value of the plant.

Introduction

Plant pathogenic bacteria cause different diseases and symptoms on different plant organs, e.g. galls, overgrowths, wilts, leaf spots, specks, blights, soft rots, scabs, and cankers. Some produce toxins, inject special types of proteins that lead to host-cell death or enzymes that break down key structural components of plant cells and their walls[1]. Ten most virulent and important bacterial plant pathogens based on their pathogenicity level, economic impact, and molecular aspects were listed[2]. In Egypt most of these devastating bacterial plant pathogens species were isolated which belong to genera *Pseudomonas syringae* pathovars[3], *Ralstonia solanacearum*, *Agrobacterium tumefaciens*[4], *Xanthomonas*[5], *Erwinia amylovora*[6] and *Xylella fastidiosa*[7].

Bacterial soft rot is common to all vegetables in the field and is readily found in the market when produce ceases to be fresh. Harvesting, handling and freezing injuries encourage development of soft rot bacteria in plant tissue[8]. Potatoes' crops exposed to the causative agents of bacterial soft rot as consider is an important disease in Egypt. These opportunistic bacterial plant pathogens species belong to genera *P. carotovorum, E. cloacae*[9,10].

Bacterial soft rot/blackleg in potato crops causes *D. solani* is a complex disease in Egypt[5]. Its symptoms are often indistinguishable from those caused by *Pectobacterium* but are more virulent, causing disease from lower levels of inoculum and spreading through the plant more effectively[11]. In warm climates, *Dickeya* has also been reported to cause both blackleg and soft rot of potato[12].

There are many ways to control of bacterial plant diseases such as antibiotics; it was not successfully used in diseases control due to the natural development of bacterial resistance making the antibiotic ineffective in disease management[13]. Using Chemical Pesticides cause hazardous effect on the environment, animals and human health[14]. At the end of 20th and the beginning of 21st century, nanotechnology has been increasingly applied to the development of novel antibacterial agent for the management of phytopathogenic bacteria affecting agricultural crops, humans and animals. Reduction of macro materials into nano-scale particles (1-100 nm) gives birth to new characteristics and the material behaves differently. Nanomaterials can be potentially used in the crop protection, especially in the plant diseases management[15].

The using of nanomaterials in plant disease management is a novel and fancy approach and alternative to traditional methods for disease management that may prove very effective in the future with the progress of application aspect of agro-nanotechnology[16]. The biological method of NPs synthesis is a relatively simple, cheap, and environmentally friendly than the conventional chemical and physical methods[17]. Metallic NPs in particular have demonstrated broad antibacterial-spectrum against both gram positive (G^{+ve}) and gram negative (G^{-ve}) bacteria due to ultra-small size, high reactivity and large surface area and different process to effects on bacterial bioavailability[18].

The objectives of this study were applied of metallic NPs from eco-friendly bacterial isolates collected from Egyptian ecosystem, and studying their effect on some soft rot/blackleg bacteria *ex vivo* and disease severity *ex vivo* and *in vivo*. The study concern the effect of metals NPs on DNA, carbohydrates and proteins of bacteria, ultrastructure studying for the interaction of metals NPs with bacterial cells and studying the presence of metals FeNPs inside the plant tissues.

Results

Collection of Nano-metals forming bacteria and soft rot/blackleg genera

Nano-metals forming bacteria *E. thailandicus, P. putida, M. hydrocarbonoclasticus*, and *P. geniculata* for Copper (Cu), Iron (Fe), Cobalt (Co) and Zinc (Zn) Nanoparticles (NPs) production sequentially, were tested for advanced studies on soft rot (*P. carotovorum* and *E. cloacae*) and blackleg (*D. solani*) genera, as followed**:**

Effect of metals NPs on biomolecules of soft rot/blackleg genera.

Effect on bacterial DNA

The effect of metals NPs on bacterial DNA was tested by agarose gel electrophoresis. The amount of total DNA isolated from untreated and treated bacterial cells with Fe, Cu, Co and ZnNPs are presented in Fig. 1. Results revealed that there are different effects of metals NPs on bacterial DNA (*P. carotovorum, D. solani,* and *E. cloacae*). Single DNA band was showed from untreated bacterial cells (C) and different effect in obtained DNA from treated cells was observed. FeNPs treatment showed total DNA degradation in case of *P. carotovorum* and *E. cloacae*, CoNPs showed total DNA degradation in case of *P. carotovorum* and *D. solani.* As well there was fragmentation effect of Cu-NPs and ZnNPs for all tested isolates.

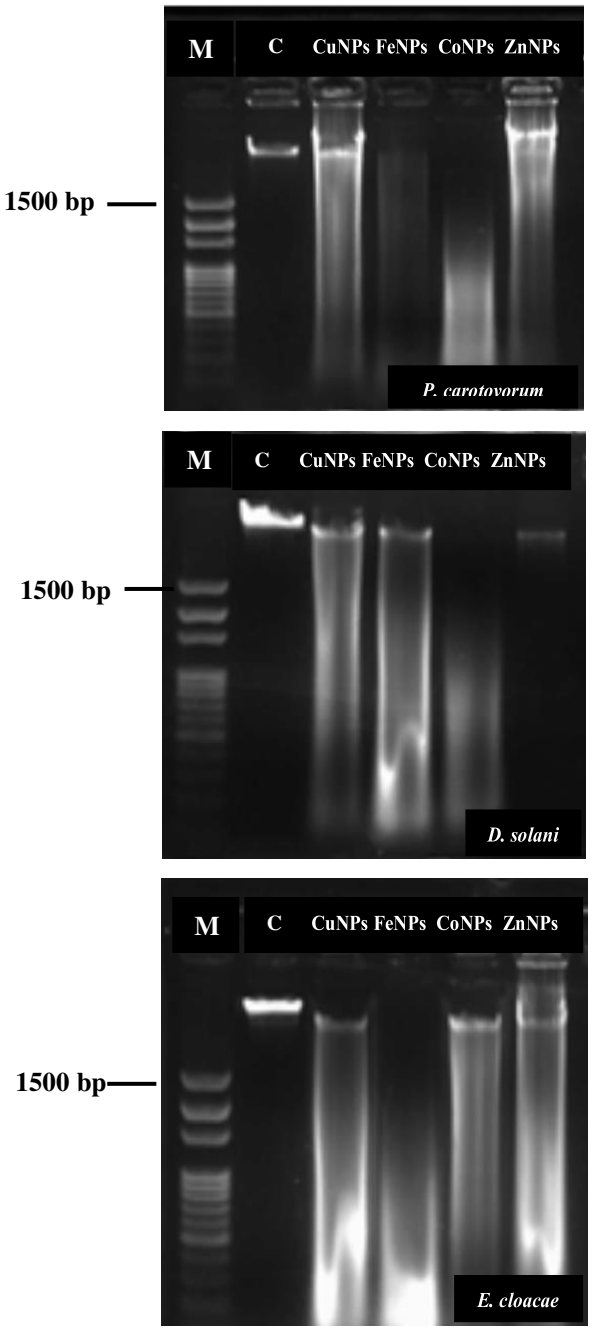

Figure 1. Effect of CuNPs, FeNPs, CoNPs, and ZnNPs on DNA of *Pectobacterium carotovorum, Dickeya solani*, and *Enterobacter cloacae*, C: DNA from untreated bacterial cells, M: 100 bp DNA ladder.

Effect on total carbohydrate and proteins

To validate the effects of metals NPs on metabolic activity of bacterial cells, the levels of intracellular macromolecules such as proteins and sugars, was analyzed. The effect and interaction of metals NPs on total cellular proteins and carbohydrates of phytopathogenic bacteria was shown in Fig. **2**., which illustrated the effect of FeNPs, CuNPs, CoNPs and ZnNPs on *P. carotovorum* Fig. (2A), Fig. (2B) *D. solani* and Fig. (2C) *E. cloacae* compared with untreated bacterial cells and it is demonstrated that all metals NPs showed effect on portion and carbohydrate degradations. ZnNPs showed high level of protein degradation followed by CuNPs and FeNPs in case of carbohydrate degradation ZnNPs and CoNPs were more effective.

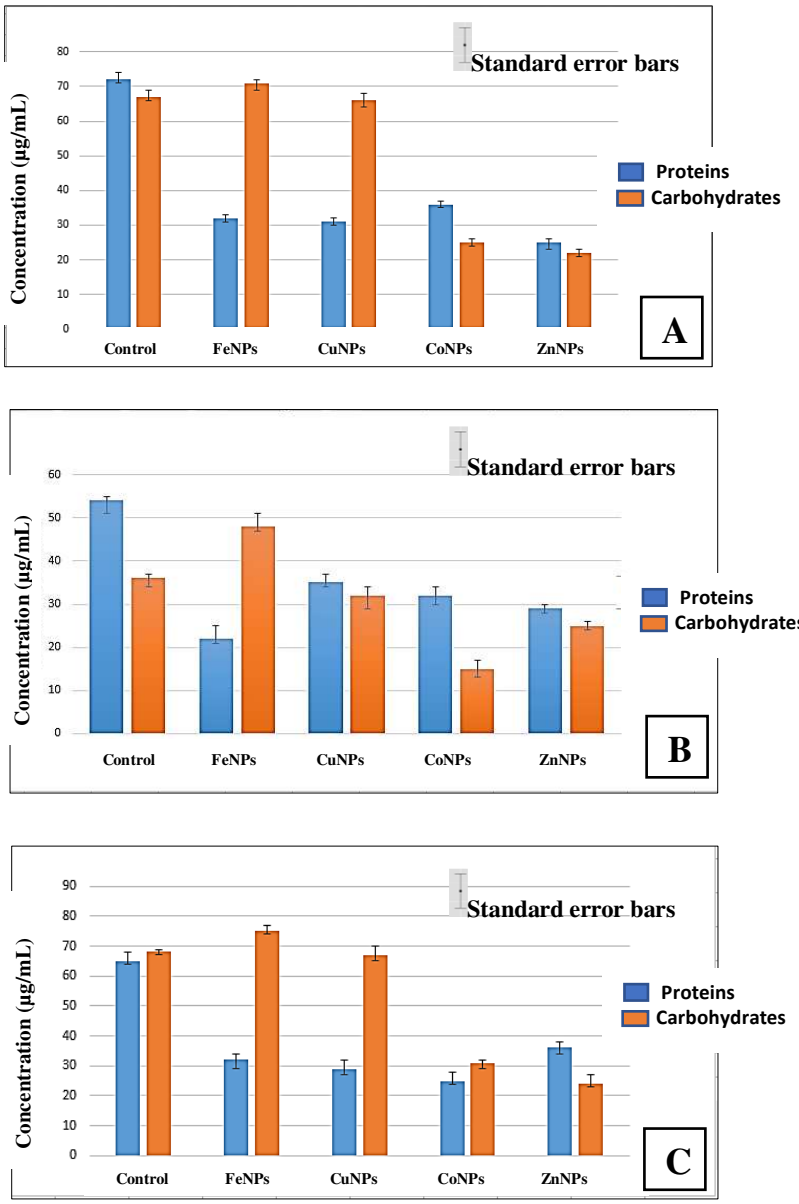

Figure 2. Effect of FeNPs, CuNPs, CoNPs, and ZnNPs on proteins and carbohydrate content of *Pectobacterium carotovorum* (A), *Dickeya solani* (B) and *Enterobacter cloacae* (C).

Ultrastructure effects of metals NPs on bacterial soft rot/blackleg genera

The morphological and internal changes in bacterial cells treated with metals NPs, were examined using Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM) and as shown in Fig. 3&4.

SEM observation

The morphological changes of metallic NPs on phytopathogenic bacteria in comparison with untreated cells were performed using SEM as shown in Fig. 3, the untreated cells showed smooth, healthy Fig. (3 A, C, E). The treated *P. carotovorum* with **FeNPs** Fig. (3B) and *D. solani treated* with CuNPs Fig. (3D) *showed* change in the size of the cells and appearance of big pits in cells, where *E. cloacae* treated with CoNPs showed totally lysis and deformation of cells, and also lost its rod shape as shown in Fig. (3F).

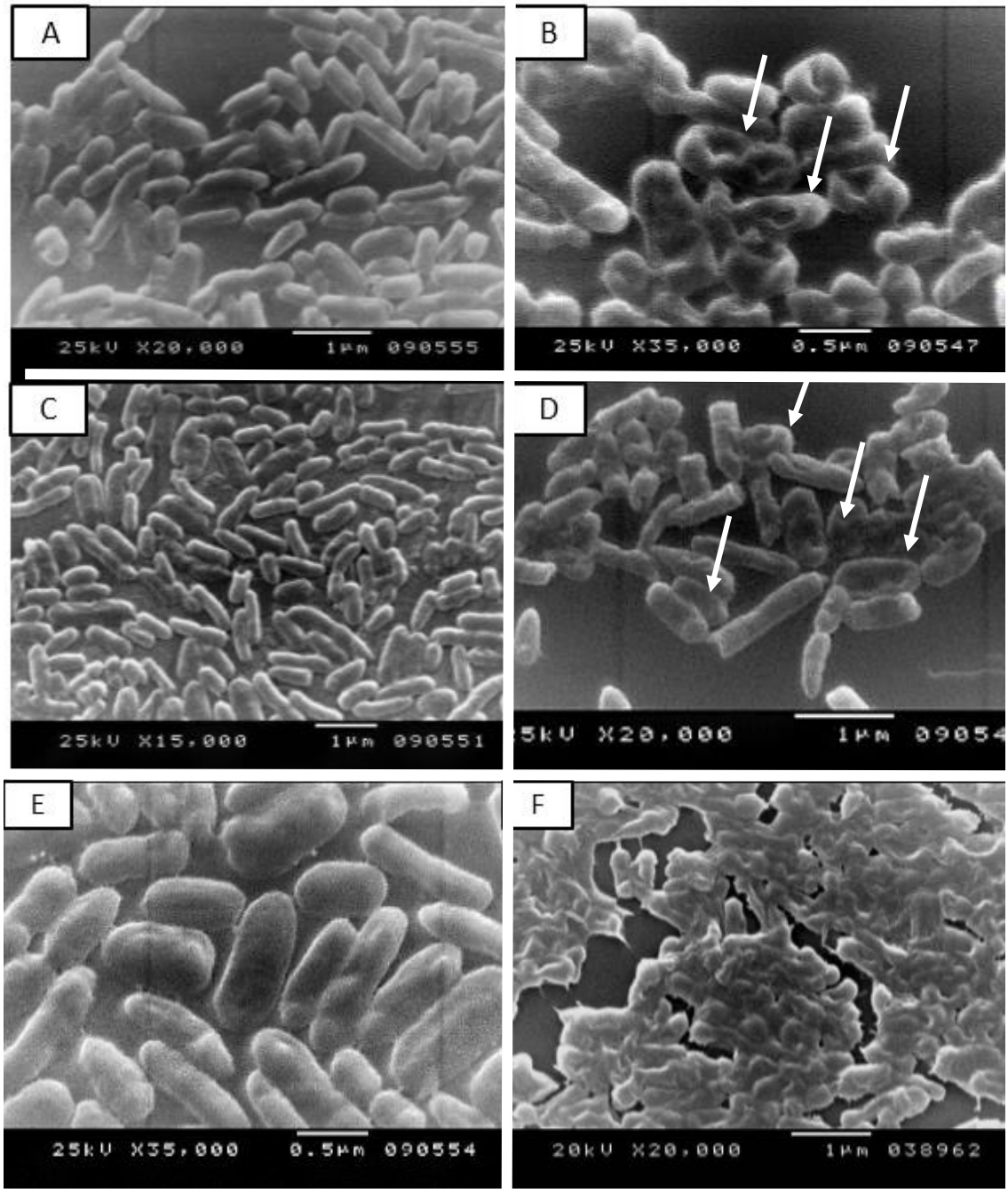

Figure 3. Scanning electron microscope analysis of phytopathogenic bacteria treated with metlas NPs. untreated *Pectobacterium carotovorum* (A), treated with FeNPs (B), untreated *Dickeya solani (C),* treated with CuNPs (*D*) and untreated *Enterobacter cloacae* (E), treated CoNPs (F)

TEM observation

The internal morphology of treated bacteria was shown in Fig. 4, the untreated cells Fig. (4A, C, E) showed healthy cells and normal in rod- shape with high cytoplasmic density, also normal contents, cell wall, and plasma membrane was no noticeable changes in morphological structure. Treated cells of *P. carotovorum* with FeNPs (Fig. 4B) were showed variation in cytoplasmic density compared with untreated cells, also showed big two vacuoles in the center. As well *D. solani* treated with CuNPs in Fig. 4D showed condensation of cytoplasm in the center of cell trapped with NPs in addition to formation spacious periplasmic space and plasma membranes were separated from cell wall and collapsed also *E. cloacae* treated with cobalt NPs Fig. 4F showed condensation of cytoplasm, formation cytoplasmic space and degradation in cell

wall and swelling in some cells which were most probably due to a change in cell permeability. All treated bacterial cells showed penetration and perception of NPs in cells.

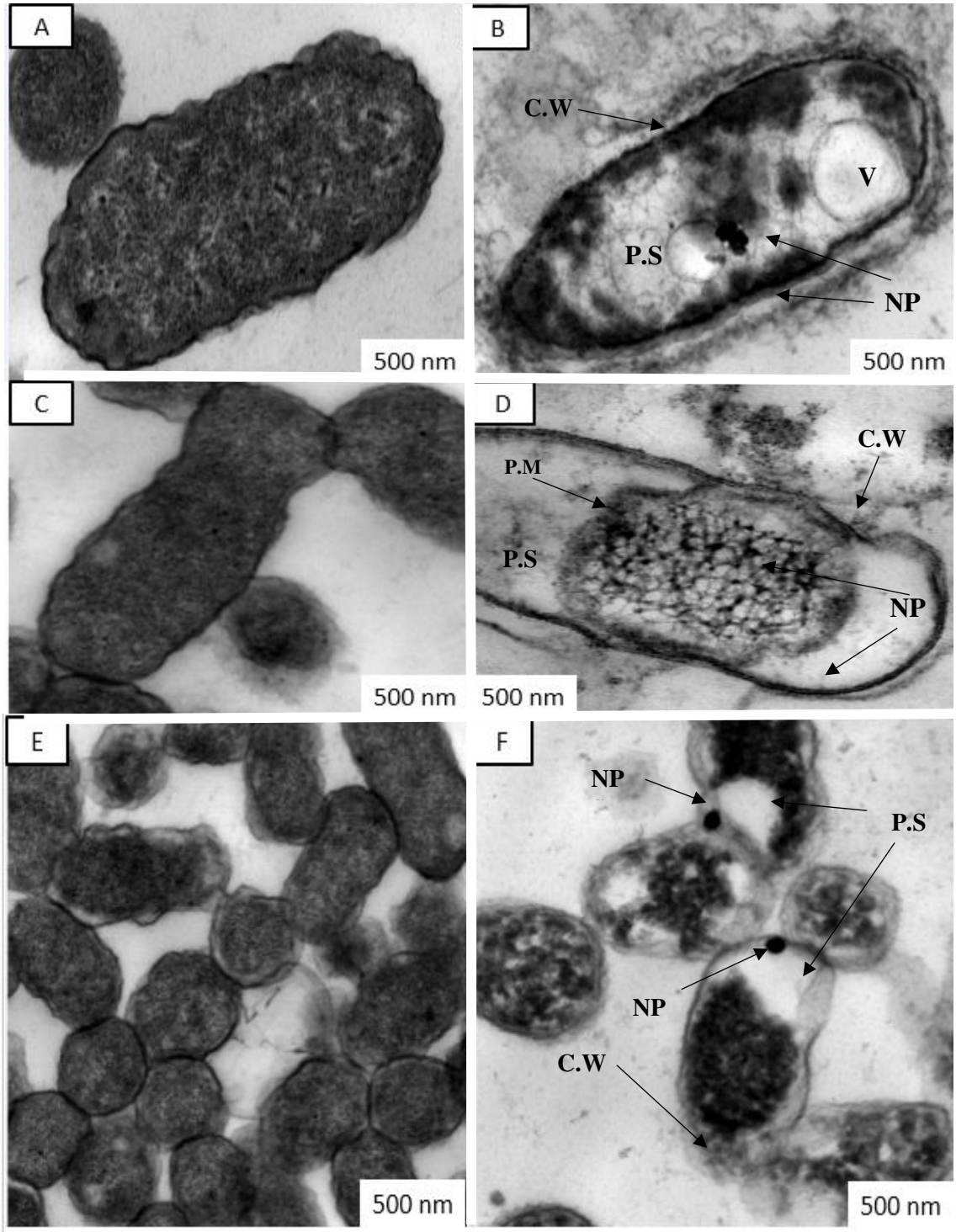

Figure 4. Transmission Electron Microscope analysis of phytopathogenic bacteria treated and untreated with metals NPs. untreated *Pectobacterium carotovorum* (A), treated with FeNPs (B), untreated *Dickeya solani* (C), treated with CuNPs (D) and untreated *Enterobacter cloacae* (E), treated with CoNPs (F). NP: NPs, C.W: cell wall, P.S: preplasmic space, P.M: plasma membrane, V: vacuole.

Effect of metallic NPs on disease severity *ex vivo*

Presented data (Table 1 & Fig. 5) showed the effect of Fe, Cu and CoNPs on disease severity of potato soft rot/blackleg bacteria. Table (1) showed the highest disease severity was in infected of potato tuber with *P. carotovorum* (47.12) followed by *D. solani,* on the other hand potato tuber treated with FeNPs, CuNPs and CoNPs showed decrease in disease with percentage 100 % as shown in Fig. 5.

NPS / Isolate	Negative control	Positive control	FeNPs	CuNPs	CoNPs
Pectobacterium ***carotovorum***	**0.0**	**47.12**	**0.0**	**0.0**	**0.0**
Dickeya ***solani***	**0.0**	**37.12**	**0.0**	**0.0**	**0.0**
Enterobacter ***cloacae***	**0.0**	**32.12**	**0.0**	**0.0**	**0.0**

Table 1. Effect of FeNPs, CuNPs, and CoNPs on ex vivo disease severity of *P. carotovorum, D. solani and E. cloacae.*

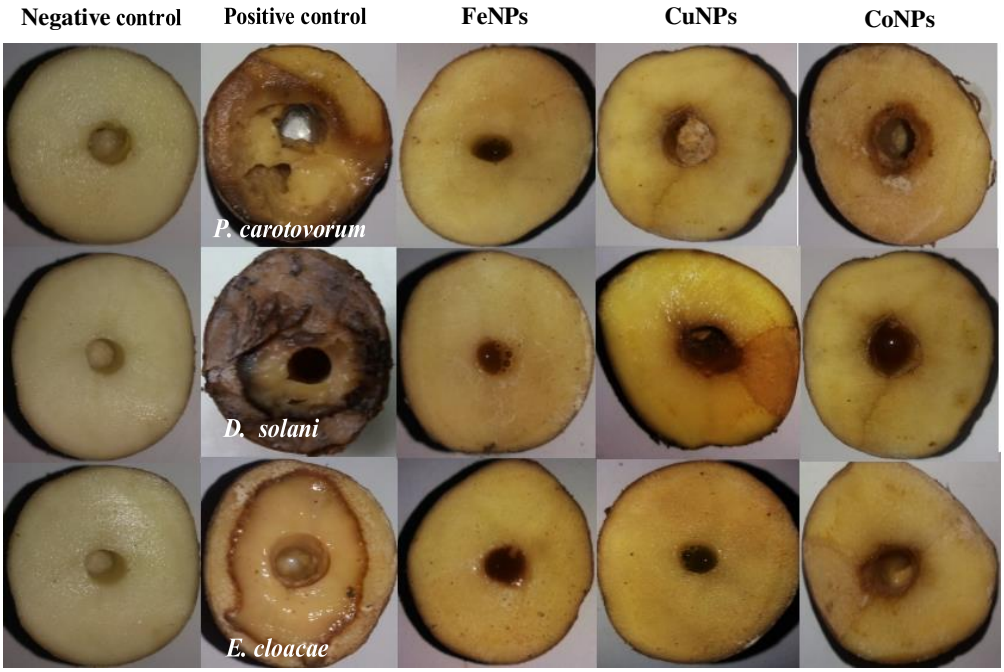

Figure 5. *Ex vivo* disease severity of inoculated potato tubers 'Lady Balfor cv.' with different sot rot/blackleg bacterial Isolates *Pectobacterium carotovorum, Dickeya solani, Enterobacter cloacae,* treatment with FeNPs, CuNPs and CoNPs.

Effect of FeNPs on growth parameter of infected potato plants *in vivo*

The effect of FeNPs on growth parameter of infected potato plants was presented in Tables 2 & 3 and Figs. 6, 7 & 8 compared with treated with copper pesticide and healthy plants without any treatment, the NPs treatment showed increase in root and shoot length of infected plants 106.51 (%) and 41.12 (%) as well fresh and dry weight showed significant increase 389.19 (%), and 139.29 (%) in compared with infected plants, on the other hand the copper pesticide treatment showed increase in root and shoot length of infected plants 19.67 (%) and 15.57 (%), as well fresh and dry weight showed significant increase 217.45 (%), and 78.58(%).

Treatment	Root length (cm)	Increase %	Shoot length (cm)	Increase %
Healthy plant (Negative control)	**19.22^{c*}**	**26.44**	**32.21^{c}**	**48.65**
Infected plant by *Dickeya solani* (Positive control1)	**15.20^{e}**	**-**	**21.19^{e}**	**-**
FeNPs treatment (Positive Control2)	**29.43^{b}**	**93.61**	**38.32^{b}**	**80.84**
***D. solani* + FeNPs treatment**	**31.39^{a}**	**106.51**	**41.12^{a}**	**94.05**
***D. solani* + Copper pesticide treatment**	**18.19^{d}**	**19.67**	**24.49^{d}**	**15.57**

Table 2. Effect of FeNPs treatment on growth parameter (root and shoot length) of potato seedlings 'Lady Balfor cv.' infected with *Dickeya solani.*

*Means with Common letters are not significant (i.e. Means with Different letters are significant), statistically significant at $p \leq 0.05$

Treatment	Fresh weight (g)	Increase %	Dry weight (g)	Increase %
Healthy plant (Negative control)	45.38^{c}*	387.51	12.07^{d}	47.73
Infected plant by *Dickeya solani* (Positive control 1)	11.36^{e}	-	8.17^{e}	-
FeNPs treatment (Positive control 2)	58.18^{a}	412.14	21.40^{a}	161.93
***D. solani* + FeNPs treatment**	55.56^{b}	389.08	19.55^{b}	139.29
***D. solani* + Copper pesticide treatment**	36.04^{d}	217.45	14.59^{c}	78.58

Table 3. Effect of FeNPs treatment on growth parameter (fresh and dry weight) of potato seedlings 'Lady Balfor cv.' infected with *Dickeya solani.*

*Means with Common letters are not significant (i.e. Means with Different letters are significant), statistically significant at $p \leq 0.05$

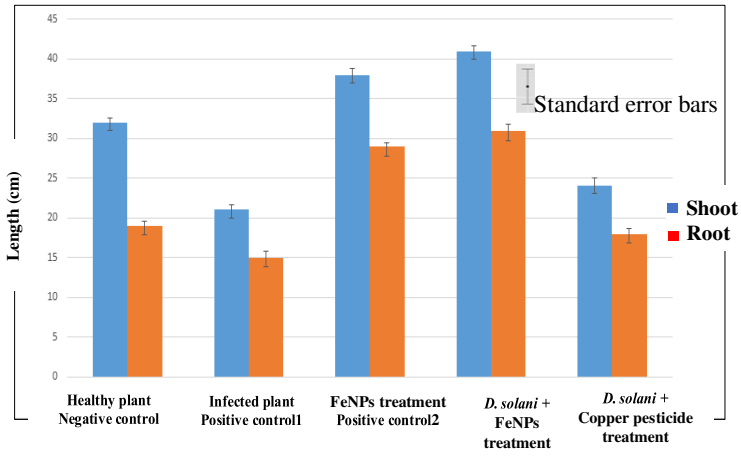

Figure 6. *In vivo* effects of FeNPs on growth parameter shoot and root length of potato seedlings 'Lady Balfor cv.' infected with *Dickeya solani.*

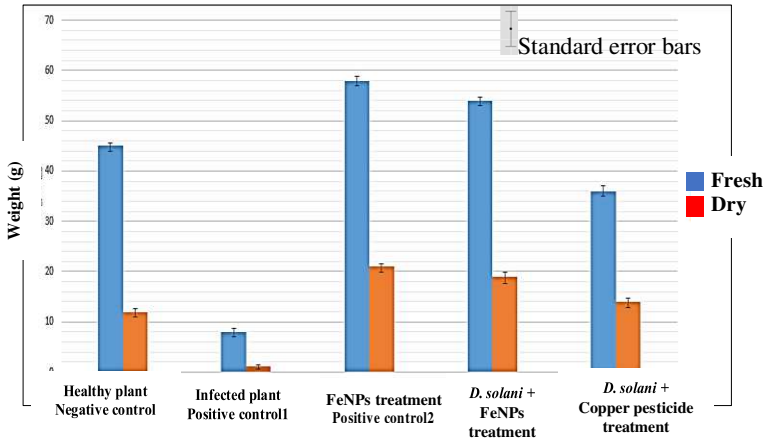

Figure 7. *In vivo* effect of FeNPs on growth parameter fresh and dry weight of potato seedlings 'Lady Balfor cv.' infected with *Dickeya solani*

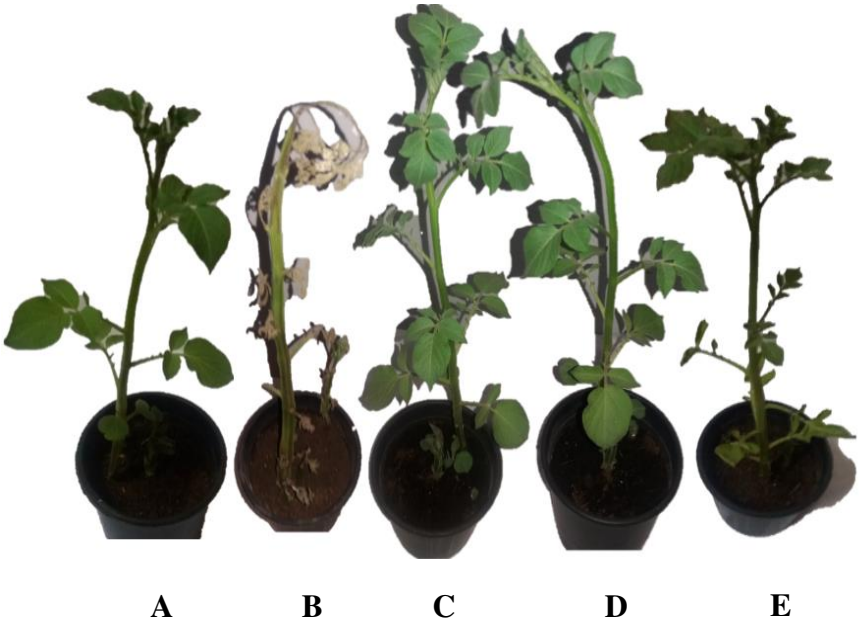

Figure 8. Effect of NPs treatment on shoot system of potato seedlings 'Lady Balfor cv.' inoculated with *Dickeya solani.* A: Healthy plant (Negative control), B: Infected plant by *D. solani* (Positive control1), C: FeNPs treatment (Positive Control2), D: *D. solani* + FeNPs treatment, and E: *D. solani* + Copper pesticide treatment

The ability of potato plant to uptake FeNPs

ICP-OES was used to measure the ability of uptake FeNPs by potato plant. This ability was tested by using treated roots and shoots of seedlings in comparison with untreated ones. The iron content (%) in root and shoot tissues was assayed in either infected plant with D. *solani* (treatment), treated plants with FeNPs (Positive control) in compare of healthy plant (Negative control). The results recorded, as shown in Fig. 9, a small increase significantly in Fe content of treated seedlings compared with untreated.

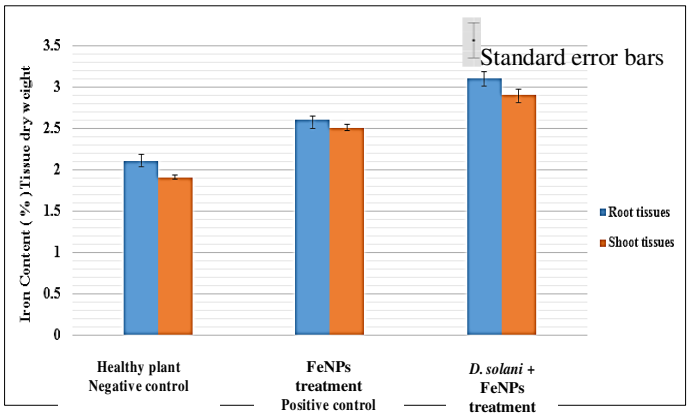

Figure 9. Iron uptake (%) in potato seedlings 'Lady Balfor cv.' as negative control, treated seedlings with FeNPs as positive control and infected seedlings with *Dickeya solani* + treated with FeNPs.

Discussion

Effect of metal NPs on DNA degradation was studied on *P. carotovorum, D. solani* and *E. cloacae* treated with Fe, Cu and CoNPs and present results appeared that metal NPs had negatively effect on genomic DNA, lead to degradation and fragmentation. **Jose *et al.,*** [19] proposed mechanism of DNA damage which was through generation of singlet oxygen as reported in the case of CuNPs. **Wang *et al.,***[18] reported that antibacterial activity include

Fe, Fe oxide, Cu, Zn, and CoNPs with different mechanisms as cellular leakage, Reactive oxygen species production (ROS) and the cell membrane, inhibit the formation of bacterial biofilms, inhibit the synthesis of bacterial proteins and DNA, binding also damage the cellular DNA and DNA repair, effect on sulfur-related proteins and effect of metabolic genes. **Nejdl *et al.,*[20]** reported that "platinum" PtNPs inhibit the DNA replication and interacted with bacterial DNA of *Salmonella enteritidis*. Also reported that DNA secondary structures as a result from DNA degradation could block transcription and replication with subsequent apoptosis. **Rafi *et al.,*[21]** reported that antibacterial activity of iron oxide NPs (IONPs) is via oxidative stress generated by ROS, resulting in the damage of the proteins and DNA in the bacteria.

Effect of metal NPs on biomolecules contents of *Pectobacterium carotovorum, Dickey solani and Enterobacter cloacae* demonstrated that all tested NPs had effect on proteins and carbohydrates degradations. As well as, tested ZnNPs in present study showed high level of protein degradation followed by Cu and FeNPs. In case of effect on carbohydrates, Zn and CoNPs were more effective on carbohydrate degradation. In addition, FeNPs treatment showed increase in carbohydrate content of *P. carotovorum and E. cloacae.* The initial hypothesis was that the increase in carbohydrate was due to an increase in capsular carbohydrates[22], this result was reported with *Escherichia coli* B23 cell treatment with streptomycin and kanamycin[23]. **Yuan *et al.,* [24]** recorded that the amounts of protein released in the suspension the AgNP treated G^{-ve} *P. aeruginosa* and *E. coli* showed significantly higher protein leakage compared to G^{+ve} *S. aureus* cells, suggesting that the G^{+ve} had lower antibacterial sensitivity than that of the G^{-ve}. In addition the antibacterial activity of Copper oxide-based NPs is reported to be attributed to generation of ROS, protein oxidation, lipid peroxidation, destruction of cell membrane and DNA degradation in bacteria cells[25]. **Li *et al.,* [26]** reported that *E. coli* cells treated with AgNPs showed leakage of reducing sugars of bacterial dry weight, which could be due to the higher concentration of AgNPs. Altogether, these findings clearly indicated that AgNPs exert antibacterial effects by influencing membrane permeability and inducing the leakage of reducing sugars, leading to the bacterial cell death[27]. Obtained results from **[24]** suggest that metals NPs decrease membrane permeability of cells and induce the release of intracellular materials, such as sugars and proteins and it one of the important mechanisms of metals NPs and bacteria interactions.

Based on TEM and SEM studies on the ultrastructure effect of metal NPs which carried out on *P. carotovorum, D. solani* and *E. cloacae,* treated with Fe, Cu and CoNPs concluded that NPs could attack the bacterial cell making pits, deformation, lysis and cellular leakage in addition to making some vacuoles and periplasmic space these results confirmed that metal NPs had lethal biocide effect on G^{-ve} phytopathogenic bacteria. The same disruptive effect of NPs was observed by [28]**. Kamal *et al.,* [17]** demonstrated that AgNPs lead to the formation of "pits" in the cell walls of the bacteria and could enter into the periplasm through the pits and destroy the cell membrane, as well as, reported that NPs anchor into the cell membrane and enter the cells, leading to osmotic collapse and subsequent release of intracellular materials. On the other side AgNPs disrupt the bacterial membranes cause protein leakage by increasing membrane, consequently leading to intracellular leakage of macromolecules permeability in bacteria. Generally there were several mechanisms by which NPs exert their antimicrobial activity once deposited on microbial surface including: cell wall perforation, morphological changes as irregular-shaped pits[29]. For cell

membrane, NPs causes its detaching from the cell, destabilization, break or pits which rapidly increased cell permeability and intra component leakage[30]. Once NPs penetrate cell barriers into the entire cell, NPs interact with phosphorus containing compound as DNA, which causing losing its replication ability and inhibiting DNA unwinding[31]**.** The damaged cells were viewed using TEM imaging and it was revealed that the cell wall had physically separated from the internal cellular environment and that electron dense aggregation of compounds were surrounding the lysed cell[32]. Ag-NPs are able to create a barrier between the cell wall and the cytoplasm more effectively in G^{-ve} *E. coli* than in G^{+ve} *S. aureus*, indicating that perhaps the thick peptidoglycan layer present in G^{+ve} bacteria plays a role in protecting the cell from NP impregnation, but only at specific NP concentrations[33,34] .

The effect of treatment with NPs *Ex vivo* on disease severity of potato tuber infected with *P. carotovorum, D. solani* and *E. cloacae* concluded that Fe, Cu and CoNPs decreased disease severity 100% due to their antibacterial activity. **El-Batal [35]** reported that the impact of Ag and SeNPs of decrease disease severity of early blight disease in potato caused by *Alternaria solani* and improve plant parameters included physiological parameters and yield. Wide range of applications of nanotechnology were also emerged into the 'agri-food sector' which include the nanosensors, tracking devices, targeted delivery of required components, food safety, new product developments, precision processing, smart packaging and others[36].

Effect of FeNPs on growth parameters of infected potato plant treated with FeNPs had positive effect on controlling of soft rot/blackleg disease caused by *D. solani.* In addition to positive impact on growth parameter such as increase in root and shoot length therefor increased in fresh and dry weight. Different studies reported the importance of Fe to plant as an essential micronutrient for almost all living organisms because of it plays critical role in metabolic processes such as DNA synthesis, respiration, photosynthesis, a prosthetic group constituent of many enzymes, chlorophyll synthesis, and it is essential for the maintenance of chloroplast structure and function[37]. The Iron oxide NPs (Fe_3O_4 NP) at lower concentrations were observed to have beneficiary impact on plant and improves germination[38].

NPs uptake in plant tissue was examined using ICP-OES which demonstrated that increase in Fe content in infected plant with *D. solani* followed by healthy plant treated with FeNPs in comparison with negative control treated with pure distilled water. It might be refer to infected plant which uptake high amount of Fe NPs. **Nwugo, *et al.,* [39]** pointed that stressed plant tissues could accumulate more nutrients/unit mass than unstressed tissues. Extension potato plant infected with *Candidatus* Liberibacter solanacearum induced nutrient accumulation was observed for micronutrients especially iron in leaf and root tissues. Plant cell walls were a structure which was composed of cellulose which permits the entry of small particles and restricting the larger one, therefore smaller NPs can go through this layer in a comparatively easy way in respect to larger NPs. The size exclusion limit for the plant cell wall is between 5 and 20 nm[40]. **Etxeberria *et al.,* [41]** informed that NPs might move through endocytosis and further, through the symplastic transport, it might travel to different plant tissues[42]. **Wang *et al.,* [43]** indicated that size, magnitude, and zeta potentials are key in determining the transport of NPs inside the plant.

Conclusion and recommendations:

From data obtained we concluded that FeNPs is effective in control of soft rot/blackleg diseases in potato plants and additionally, promote growth of potato plants in comparison with copper pesticides that used in control of bacterial plant diseases.
Also, we have reached from our previous study that a biological synthesis of NPs using bacterial cells is eco-friendly, fast and inexpensive as well the highly toxicity of Fe, Cu, Co, and ZnNPs against phytopathogenic bacteria, in addition their bactericidal effect.
Generally, Nano-materials will increase the efficacy of pesticides and antibiotics, allowing decrease its doses to be used. So we recommend studying the applicability of metals NPs in bactericidal industry, also study the toxicity and safety concentrations of metal NPs to animals and human cells.

MATERIAL AND METHODS

Source of Nano-metals forming bacteria: Bacterial isolates were obtained from [44], which isolated from water samples collected from harsh condition locations in Egypt. Four selected isolates were identified as *E. thailandicus, P. putida, M. hydrocarbonoclasticus*, and *P. geniculate* for Copper (Cu), Iron (Fe), Cobalt (Co) and Zinc (Zn) Nanoparticles (NPs) production sequentially (**S1**)

Source of soft rot/blackleg genera: The antibacterial activity of selected metals NPs was tested against some bacteria causing the following diseases: soft rot (*Pectobacterium carotovorum, Enterobacter cloacae*) and blackleg (*Dickeya solani*)[44]. The metals NPs were evaluated for antibacterial activity against 3 molecular identified phytopathogenic bacteria (**S2**).

Ultrastructure effect of metals NPs on some bacterial soft rot/blackleg genera:

Phytopathogenic bacterial isolates were harvested after being treated with metals NPs for preparing and examination by transmission and scanning electron microscopy according to[45, 46]. The bacterial isolates after accumulation and reduction of metal ions were collected and fixed with Mix of 2% glutaraldehyde and 4% formaldehyde in phosphate buffer saline (PBS) at 4°C overnight. The fixed cells were washed three times (each for 10 min) with 0.1 M sodium cacodylate buffer (pH 7.4). The samples were post-fixed with 1% (v/v) osmium tetroxide at 4°C for 2 h. The post-fixed cells were washed again three times (each time for 10 min) with 0.1 M sodium cacodylate buffer (pH 7.4).

In case of prepare ultra-sections; the post-fixed samples were dehydrated in an ascending acetone concentration from 35 to 95% (each for 10 min). The samples were dehydrated in acetone 100% three times (each for 15 min). Sample embedded in Epon 812, then polymerized in oven at 60°C for 24-48 h. Epon was cutting by glass knives, staining using uranyle acetate for 10 min and lead citrate stain for 10 min. Ultra sections were then examined with TEM (JSM 1400 plus -JEOL).

While, using scanning electron microscope, the post-fixed samples were dehydrated in a series of graded ethanol series (from 30 to 90%) each for 10 min, and then dehydrated in absolute ethanol three times (15 min each). The dehydrated samples were critical point dried (Samdri PVT-3B Critical Point Dryer) for 30 min. The dried sample was coated with gold 90%/10% w/w using sputter coater (Jeol Fine Coat JFC-1100E). The Samples were observed using SEM (JEOL 5300 JSM)

Effect of metals NPs on biomolecules: Bacterial isolates with ca.1x 10^6 CFU/mL were treated with either of Fe, Cu, Co or ZnNPs and incubated overnight at 30°C. Culture free of metals NPs were incubated as a control sample in the same time. To prepare the cell lysate**,** the method by [47] was followed.

Effect of metals NPs on total carbohydrate: Total soluble carbohydrates were determined using the anthrone technique. Three mL of clear supernatant was transferred to clean test tube, 6 mL of freshly prepared anthrone reagent (2g anthrone / L of 95% sulphoric acid) was added, the tubes were heated in a boiling water bath for 3 min and left to cool. The developed color was measured using spectrophotometer (T60 UV/VIS Spectrophotometer) at 620 nm. A blank mixture containing distilled water and reagent was measured under the same condition. A standard curve was constructed using Glucose as standard carbohydrate from which sugar concentrations were determined[48].

Effect of metals NPs on total proteins: Protein was determined according to the Lowry method[49] using bovine serum albumin (BSA) as a standard protein. Test was done with solutions A, B, C and D [A: 2% Na_2CO_3 in 1%M NaOH, B: 0.5% $CuSO_4$ in 1 % (w/v) sodium tartarate, C: Mix of 50ml of reagent A with 1ml of reagent B and D: Folin's reagent (BOH) diluted with water 1:3]. A protein sample (0.1) of the cell free extract was added to 5 ml of solution C, mixed well and allowed to stand for 10 min. Half mL of solution D was added with mixing and allowed to stand for another 30 min to allow the color to develop. Absorbance of the sample was measured at 750 nm in T60 UV/VIS spectrophotometer.

Effect of metals NPs on bacterial DNA: To study the effect of metallic NPs on bacterial DNA, cells were treated with Fe, Cu, Co, and Zn NPs separately, and incubated for overnight at 30 °C. DNA was extracted from treated and untreated cells as control with AMSHAGE DNA extraction kit[50].

***Ex vivo* Effect of metals NPs on disease severity:** Disease severity was estimated according to[51], as percentage of rotted tissue weight according to the change of tuber weight before and after treatment divided on weight of tuber before treatment as following formula:

$$^{*}PDS = (W1-W2)/W1 \times 100$$

*** Whereas: PDS = percentage of disease severity, W1= weight of whole tuber before treatment and W2= weight of tuber after removal of the rotten tissue.**

The soft rot bacterial isolates *(P. carotovorum,* and *E. cloacae*) and soft rot/blackleg (*D. solani*) were used for inoculating healthy potato (*Solanum tuberosum*) tuber 'Lady Balfor cv.', which were obtained from fresh market potatoes at Alexandria Governorate. This market is available to the public to buy from it, and they do not need permission to obtain the product, also **all study/experimental protocols involving plant materials was conducted in accordance with institutional, national, and international guidelines and legislation**. This was done by surfaced-sterilized potato tuber for 10 min with 1% (v/v) sodium hypochlorite solution, rinsed thoroughly, and allowed to air dry. For each isolate, 3 tubers were cutting in half and a hole was made in half tuber center approximately 1 cm deep with a sterilized Cork borer (1 cm in diameter), and 250 µL of culture ca.1x 10^{6} CFU/mL bacterial suspensions prepared from 24 h and then placed into the wound[52].

In the case of NPs treatment, potato tubers were submerged with Fe, Cu and Co NPs separately, before tubers were treated with a bacterial suspension. Sterile distilled water was used as negative controls. Potato tubers were placed randomized in plastic trays supplemented with sterilized moist cotton to maintain high humidity, and incubated for 48 h at 28+2˚ C after inoculation. Rotting tissue was removed from the potato tubers with a sterile spatula[53].

***In vivo* effect of FeNPs on growth parameter of infected potato seedling:** FeNPs and soft rot/blackleg bacteria *D. solani* was tested as model of *in vivo* experiment for control of bacterial plant diseases in potato plant. Surfaces of the aforementioned cultivar potato tubers were

sterilized with 1% sodium hypochlorite for 5 minutes washed with sterile water and planted (one tuber per pot) 15 cm diameter filled with sterile peat moss and clay (1:1)[54]. When potato plants reached 15-20 cm in length, 0.5 mL of 1x10^6 CFU/mL)/pot of *D. solani* was injected in soil. Inoculated seedlings were placed directly in a greenhouse at 25±2ºC. Four replicates were used and plants irrigated with sterile distilled water served as control. In case of iron NPs treatment potato tubers were immersed in iron NPs solution for 2 h and let in open air for dry, as well of iron NPs were add to soli after 2 days from bacterial inoculation in compare with copper pesticide: index (77 % copper hydroxide)[55]. Different growth parameter, root and shoot length, in addition fresh and dry weight were recorded after 14 days from inoculation.

Accumulation of NPs: The ability of potato plant to accumulate NPs was tested by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) according to[56]. For samples preparation**,** all the plant parts leaves, roots, stems and tubers were washed with double distilled water and placed in beakers, covered with watch glasses and dried for a period of 12 h in an oven at 110°C, later these samples were triturated to be homogenized well. For sample digestion**,** approximately 0.50±0.01 g of each dry sample was added into 50-mL cleaned and air-dried folin tube; finally 5 mL of concentrated nitric acid were added. The samples were kept at room temperature for 2-3 h, then folin tube were kept in heating block at 120-130ºC for 14-16 h. Samples let cool for several minutes, 30% hydrogen peroxide was added to samples at a ratio of 1 mL per sample. Samples were placed back onto the heating block for 20-30 min. Water was added to the 50 mL mark and let sit for 30 min. For **ICP-OES** analysis**,** samples were diluted and analyses were performed on Agilent ICP-OES 5110 VDV. The ICP-OES system was calibrated by serial dilutions of Fe, with limits of detection (10–1000 ppm). The emission lines used for the analyses were 238.20 nm, under Argon plasma with the concentric nebulizer.

Statistical analysis: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**.** (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using mean and standard deviation significance of the obtained results which was judged at the 5% level. The used test was F-test (ANOVA), for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (LSD) for pairwise comparisons.

Data availability

The datasets used as well as the materials are available in this study.

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Data availability

The datasets used as well as the materials are available in this study.

Author contributions

The authors A. A. S, N. A. A., A. K. & S. A. Z. proposed the research concept, design, and the statistical analysis, analyzed and interpreted the data. As well as performed all the experiments of antibacterial activity of nanoparticles against phytopathogenic bacteria *in vivo*, and they have carried out experiments for preparing nanoparticles. All authors contributed to the writing, revising, and approved the final manuscript to be published.

Competing interests

The authors declare no competing interests.

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