Assessment Of Antibacterial Activity For Synthesized Zinc Oxide Nanorods Against Plant Pathogenic Strains

Elsayed E. Hafez, H. Shokry Hassan, M.F. Elkady, Eslam Salama

Abstract: Nano-ZnO has been successfully synthesized via hydrothermal technique to evaluate as plant pathogenic antibacterial agent. The crystalline and morphological structures of ZnO were examined using X-ray diffraction and scanning electron microscopy respectively. The morphological structure of synthesized ZnO was nano-rod with an average aspect ratio about 8. The antibacterial effect of ZnO nanorods on eight different hetero soft root plant pathogenic bacteria was investigated for inhibition and reduction the cell growth of examining strains using disc diffusion method. The minimum inhibitory concentrations of nanorods ZnO towards plant pathogens microbes were explored. The recorded inhibition zones using ZnO were ranged between 14 to 32 mm compared with 0 to 24 mm for antibiotics.

Keywords: ZnO nanorods, hydrothermal method, antibacterial activity and plant pathogenic bacteria.

1. Introduction

In the beginning of the twenty-first century, both of Nanoscience and nanotechnology became very important for revolutionary contributions in many fields [1]. Nanomaterials have specific structures which have novelties in physical, chemical, and biological properties, these properties acquired the nanomaterials a lot of functions due to their nano scaled size [2]. Both of metals and metal oxides (inorganic materials) have attracted lots of attention for many scientists working in this field due to their ability to withstand the great process [3]. It was demonstrated that various types of nanomaterials could be used as antimicrobial agents. Among these nanomaterials shows; silver [4, 5], copper [6], titanium dioxide [7] and zinc oxide [8-10]. Recently and especially in crop sciences, nanotechnology can be used as a substation for the chemical pesticides, fertilizers, and other Agrochemicals as well [11].

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Moreover, nanotechnology could be a suitable technique for control a wide range of plant diseases and this could be achieved by delivering the nanoparticles as functional molecules and/or diagnostic tool for specific plant disease, meanwhile, an assessment of nano-control for the plant disease branch will be developed [12]. A lot of management methods have been used for the control of plant pathogenic organisms, but each method has more one limitation. Also, due to dangerous effects resulted from the extreme use of pesticides; scientists are obligated to search for alternative safe pesticides. For that reasons nanoparticles could be used as antimicrobial agents against a wide range of plant pathogens [13]. ZnO nanostructure exhibits high catalytic efficiency, strong adsorption ability and are used more and more frequently in the manufacture of sunscreens, ceramics, rubber processing, wastewater treatment, and as a fungicide [14, 15]. Synthesis of nanomaterials is most commonly done based on three strategies: liquid phase synthesis, gas phase synthesis and vapor phase synthesis. Under liquid phase synthesis the techniques used for synthesis are: hydrothermal/solvo thermal, sol gel processing, micro emulsions, microwave and sono chemical [16-20]. Hydrothermal processing can be defined as any heterogeneous reaction in the presence of aqueous solvents or mineralizes under high pressure and temperature conditions to dissolve and recrystallize (recover) materials that are relatively insoluble under ordinary conditions [18]. There are many reports [21] on the considerable antibacterial activity of metal oxides, which is attributed to the generation of reactive oxygen species on the surface of these oxides. The advantage of using these inorganic oxides as antimicrobial agents is that they exhibit strong activity even when administered in small amount. Among them, ZnO and nano-ZnO were known to have strong inhibitory and antibacterial effects as well as a broad spectrum of antimicrobial activities. The availability of a wide range of nanostructures makes ZnO an ideal material for nanoscale optoelectronics [22] and piezoelectric nanogenerators [23] as well as an efficient material for biotechnology [24]. Furthermore, ZnO appears to be strongly resistant to microorganisms, and nano-ZnO is now widely used as antibacterial [25]. In this regard, as an innovative study the synthesized ZnO nanorods via hydrothermal technique will be evaluated as plant

pathogenic antibacterial agent. This investigation represents the first research study that utilizes nano-zinc oxide for plant disease control. In order to cover all other research areas of this study, the synthesized ZnO will be characterized using different techniques. Finally, the minimum inhibitory concentration (MIC) from the suspended ZnO was demonstrated for each plant isolate in a separate manner.

2. Materials and Methods

2.1. Synthesis of ZnO Nanorods

ZnO nanorods were prepared via hydrothermal technique. 14 mM aqueous solution of zinc acetate dihydrate Zn (CH3COO).2H2O (purity 99%) (Rankem, India) were prepared by adding 6 g of zinc acetate in 50 ml distilled water. Polyvinylpyrolidone (PVP) (purity 95%) (0.25 µM) was added into the reaction flask at room temperature under magnetic stirring and then 50 mM of sodium hydroxide (NaOH) (purity 99%, Sigma- Aldrich, Germany) were added drop wisely into the reaction flask under continuous stirring. Then the resulting solution was aged into an autoclave at 70 oC and 50 Kpsi for three hours. The obtained white powders were washed several times with distilled water and absolute ethanol to remove any residual salts, and centrifuged at 4000 rpm. Finally nanopowders were dried at 60 oC under air atmosphere overnight.

2.2. Characterization of ZnO Nanorods

X-ray diffraction patterns of ZnO nanorods were obtained using (schimadzu-7000, USA) diffractometer, operating with Cu Kα radiation (λ=0.15406 nm) generated at 30 KV and 30 mA. The obtained ZnO nanopowders sample was packed into a flat aluminum sample holder. Scans were done at 2o min-1 for 2θ and from 10 to 80 degrees. The morphological structures and sizes of ZnO nanorods were obtained from scanning electron microscopy (SEM) (JEOL JSM 6360LA, Japan). ZnO nanopowders were stocked over a holder. Then it was gold-sputtered before the examination. The samples were scanned to identify the structure of prepared samples and estimate the average length and diameter at different magnifications.

2.3. Bacterial Isolates

The purified bacterial isolates were kindly provided from Faculty of Agriculture, Alexandria University, Egypt. Their identification and isolation sources were tabulated in Table 1. All the bacteria isolates were grown and maintain on nutrient broth.

Table 1. The bacterial strains examined in this study.

strain number	Bacterial strain	Geographical origin	accession number
Pcs 34	*Pectobacterium carotovorum* subsp	Egypt	SAPCCREC 38
Pcsw 39	*Pectobacterium carotovorum* subsp. *wasabiae*.	Iran	HQ 424871
Pa 45	*Pectobacterium atrosepticum*	Netherlands	-
Dc 54	*Dickeya chrysanthemi*	Iran	JF 972567
Pcs 59	*Pectobacterium carotovorum* subsp. *wasabiae*.	Iran	HQ 424869
Pc 63	*Pectobacterium carotovorum*	Iran	HQ 424862
Ds 65	*Dickeya solani*	Netherlands	JN 663794
Dd 69	*Dickeya dianthicola*	Netherlands	-

2.4. Bactericidal Activity of ZnO Nanorods

The bactericidal activity of the produced ZnO nanorods was tested against the previously mentioned bacterial strains. The purified ZnO nanorods were suspended in sterile distilled water with different concentrations (30, 15, 7.500, 3.750, 1.870 and 0.938 mg/ml). The disc diffusion technique was achieved to assay the activity of ZnO nanorods on the bacterial strains [26]. About, 20 ml of sterile molten and cooled media LB that composed from (peptone (10 g/L), sodium chloride (10 g/L), yeast extract (5

g/L), Agar (16 g/L)) was poured into a different sterilized Petri dish. After medium solidification about 200 µl of each strain was spread on the plate. After a bacterial inoculation, wells were performed with a sterilized stainless steel cork borer. At each well a 100 µl of freshly prepared ZnO suspension (with specific concentration) were added to each well. Then plates were incubated at 30 oC for 24 h. The measured inhibition zones were considered as bactericidal activity of the examined ZnO concentration. The minimum inhibitory concentration (MIC) was demonstrated for each strain, isolate in a separate manner.

3. Results and Discussion

3.1. Physical Mechanism of Nanorods Growth

It is known that supersaturation is the key driving force for crystal growth. In this case, the small metastable region between the two kinds of lines was suitable to produce ZnO nanorods without precipitation of other zinc species. More precisely, the amount of NaOH can only vary over a small range for a fixed Zn2+ concentration. For ZnO nanorods growth, the following equation (1), where n = 2 or 4, was suspected to happen in solution [27]:

$$Zn(CH_3COO).2H_2O + [Zn(OH)_n]^{2-n} \longrightarrow [Zn_2O(OH)_{2n-2}]^{4-2n} + H_2O \quad (1)$$

The crystal structure of ZnO was first constructed by this dehydration between OH− on the surface of the growing crystals and the OH− legends of the hydroxyl complexes [28]. The possible mechanism for the hydrothermal growth of ZnO nanostructures have been suggested by Shi and co-workers [29]. During hydrothermal process following chemical reactions may have been involved.

$$Zn^2+2OH- \longrightarrow Zn(OH)2 \quad (2)$$

$$Zn(OH)_2 \longrightarrow ZnO+H2O \quad (3)$$

It can be easily understood and obviously observed that at the beginning of this process, Zn (OH) 2 precipitate is obtained (reaction (2)).

3.2. Characterization of ZnO Nanorods

3.2.1. Scanning Electron Microscopy (SEM)

The surface morphology of the as-synthesized product was observed using a scanning electron microscope (SEM) as shown in Fig. 1. The sample is composed of a large quantity of straight rods and a small amount of irregular ones. The average diameter of ZnO nanorods is between 40-80 nm. The suggesting mechanism for ZnO may be considered as the ZnO nanorods structure are formed from the decomposition of Zn (OH) 2 within 60 minutes heating, after that, the following time growth of the particles must result from a dissolution and re-precipitation of the existing ZnO particles. The results suggest that this is a slow process, as significant changes in the particle dimensions are only seen after 6 hours ageing [30].

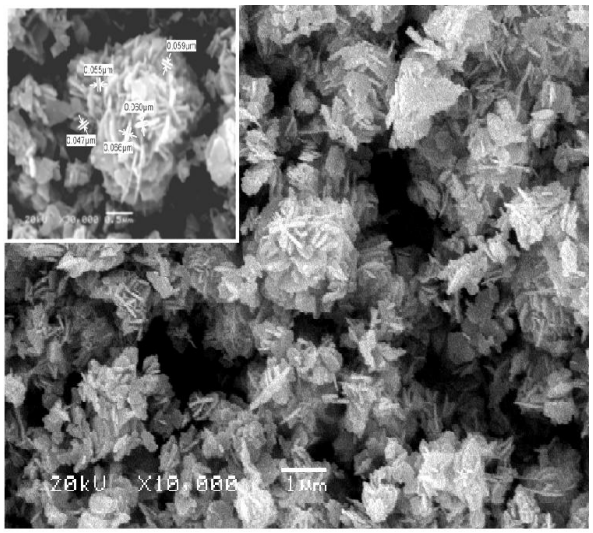


Fig. 1. SEM images of the ZnO nanorods.

3.2.2. X-ray diffraction (XRD)

XRD patterns of the obtained ZnO nanorods synthesized via hydrothermal technique are shown in Fig. 2. All the diffraction peaks can be indexed as the hexagonal wurtzite structure with high crystallinity. No characteristic peaks were observed for other impurities [31].

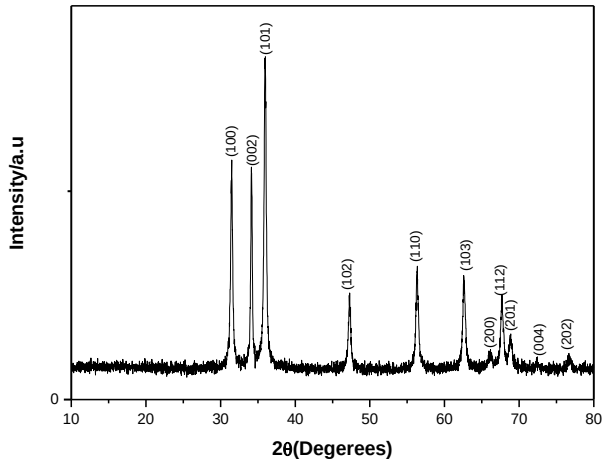


Fig. 2. XRD Patterns for ZnO nanorods.

3.3. Antibacterial Activity

The well diffusion technique was utilized to compare the plant pathogenic bactericidal activity in vitro against eight different strains using the synthesized ZnO nanorods. Table 2 has been investigated that the ZnO has a high bactericidal activity against all studied plant pathogenic strains. Moreover, (Fig. 3) Indicated that at the highest studied ZnO suspension concentration of 30 mg/ml, strain Pcsw 39 recorded the largest inhibition zone that's equivalent to 32 mm compared with 19 mm inhibition zone using Pcs 34 strains.

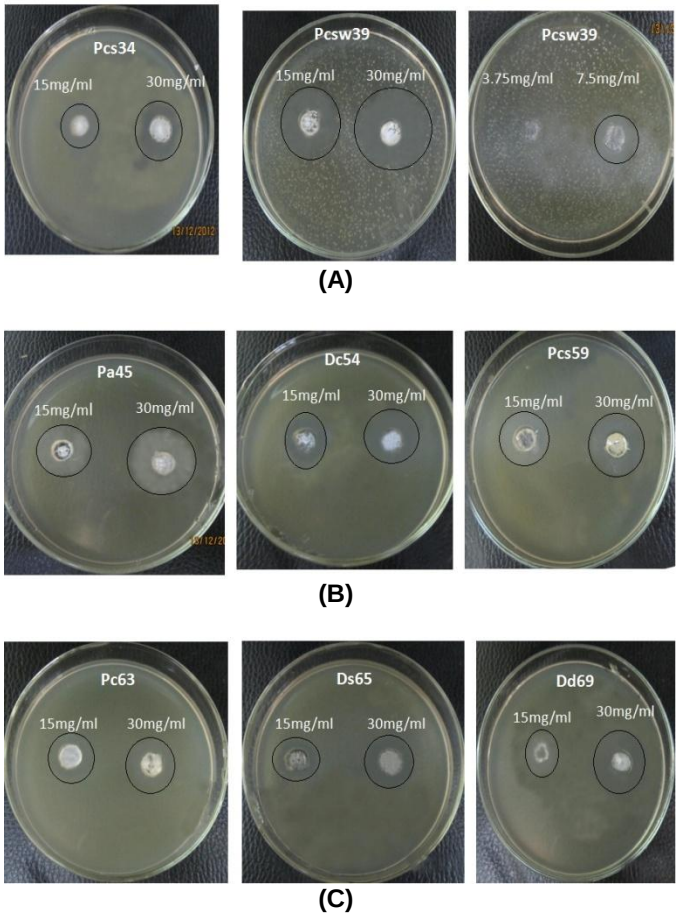


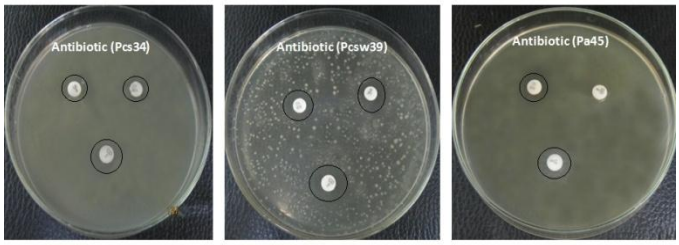
Fig. 3. (A, B, C) the bactericidal activity of the produced nanorods ZnO with different concentrations. A: Antibacterial activity of ZnO against strains Pcs 34 and Pcsw 39 where B: Antibacterial activity of ZnO against strains Pa 45, Dcs 54 and Pcs 59. C: Antibacterial activity of ZnO against strains Pc 63, Ds 65 and Dd 69.

Regarding to the minimum inhibitory concentration (MIC) from the synthesized ZnO against the different studied bacterial strains, Table 2 showed that for strains Pa 45 and Dd 69 the ZnO MIC value was 1.87 mg/ml compared with 15 mg/ml for strain Ds 65. Accordingly, it was established from these results that the plant pathogenic strain identified as Ds 65 represents the stronger ZnO anti-resistant plant pathogenic strain.

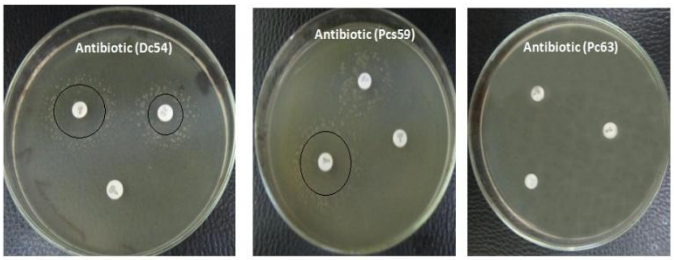
Table 2. The plant pathogenic activity as measurable inhibition zones against different bacterial strains using various concentrations of ZnO nanorods suspensions.

Plant pathogenic strain	Pcs 34	Pcsw 39	Pa 45	Dc 54	Pcs 59	Pc 63	Ds 65	Dd 69
ZnO concentration	Zinc oxide inhibition zones (mm)							
30 mg/ml	18	32	30	20	23	20	19	29
15 mg/ml	17	27	26	18	21	17	17	20
7.50 mg/ml	15	18	18	15	18	14	0	19
3.75 mg/ml	0	16	17	0	15	0	0	17
1.87 mg/ml	0	0	14	0	0	0	0	14
0.938 mg/ml	0	0	0	0	0	0	0	0

The main observation of these results was that the high nano-rods ZnO concentrations always combined with the high inhibition growth of the tested bacteria. On the other hand, the different studied plant pathogenic strain isolates were antigonsted using three different economics antibiotics. Fig. 4 indicated that the different examined antibiotics have diverse antibacterial behavior toward the various studied plant pathogenic bacterial strains. Where, the antibiotic Clindamycin inhibited the growth of strain Pcsw 39 only with inhibition diameter 15mm, while Levofloxacin antibiotic inhibit the growth of strains Pcs 34, Pcsw 39, Dcs 54, Pcsw 59 and Ds 65 in inhibition zone diameter ranged from 0 to 24 mm. However, the Nalidixic acid antibiotic showed antibacterial inhibition zones ranged from 0 to 19 mm.



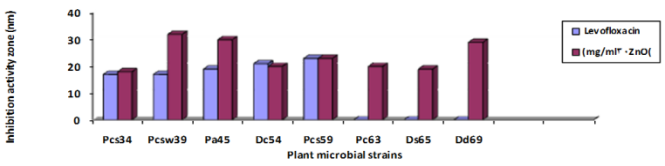
(A)



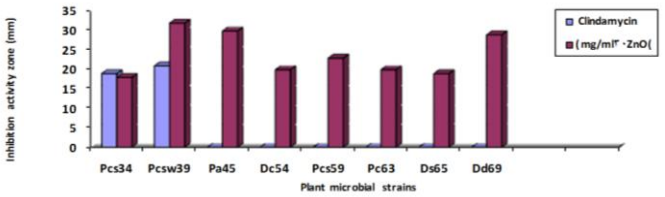
(B)

Fig. 4. (A, B) the bactericidal activity of the economic antibiotics Levofloxacin, Nalidixic acid and Clindamycin A: Antibacterial activity of antibiotics against strains Pcs 34 and Pcsw 39 and Pa 45. B: Antibacterial activity of antibiotics against strains, Dcs 54, Pcs 59 and Pc 63.

In order to establish the comparable plant pathogenic bactericidal of the synthesized ZnO nano-rods with the three different antibiotics onto the different studied bacterial strain isolates, Fig.5 showed that ZnO has bactericidal activity higher than that recorded using the comparable traditional antibiotics.



(A)



(B)

Fig.5. (A, B) the activity of various antibiotics compared with nano ZnO against some plant pathogenic bacterial strains (inhibition zone in mm).

Accordingly, it can be predicted that the synthesized ZnO nano-rods may be diffused to enter the bacterial cells through the cell walls and the pelli, and inhabited the mitochondrial DNA and the ribosomes as well. Till now there is no research literature deal with nano- ZnO utilization as bactericidal against the plant pathogenic bacteria. But there are many scientists used ZnO as antibacterial against human pathogenic bacteria and fungi [10, 32]. Where some researchers observed that nano zinc oxide has antimicrobial activity against the human pathogenic and they concluded that the growth inhibition was demonstrated against Bacillus subtilus (24 mm), Escherichia coli (24 mm) Staphylococcus aureus (22 mm) and Pseudomonas aeruginosa (22 mm) respectively[9]. Moreover, other researchers were succeeded to utilize the nano-MgO, nano-FeO and nano-ZnO as antifungal [13]. Their results agreed with the results we obtained. Regarding to the impact of nano-materials as plant pathogenic antibacterial agent, recent research has been studied nanosilver utilization of bio-control of plant pathogenic bacteria [33]. They investigated the effect of three different liquid nano-silver against four different plant pathogenic bacteria (Clavibacter, Erwinia, Pseudomonas, Ralstonia, and Xanthomonas genera) and they revealed that nano-silver could be used to control the plant pathogenic bacteria.

4. Conclusions

Zinc oxide nanorods were successfully synthesized using the hydrothermal method. It was examined using X-ray diffraction and scanning electron microscopy to be sure that is in nanoscale. The synthesized Zinc oxide nanorods have a strong antibacterial activity against some plant pathogenic strains compared with commercial economical antibiotics. Accordingly, the synthesized ZnO nanorods can be utilized as nano-control materials against the plant pathogenic bacteria. In the future work we will load the ZnO on suitable carrier and used it as bactericide in the soil. The mode of action of the nanorods ZnO on the bacterial organelles will be studied at the molecular level.

5. References

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