Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.7, No.4, 2017

Biological Effect of ZnO Nanoparticles on the Vitality of Genus Penicillium spp.

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Abstract:
The objective of this study was to clarify the biological effect of ZnO nanoparticles on the vitality of one of opportunistic fungi ,*Penicillium spp.* ZnONPs with size ≤50nm and concentrations of (0,3,6 and 12)% were used .Radial growth and dry weight ,were used to estimate the biological effects .Antibacterial effect of the fungus grow in present of ZnONPs were also investigated .Result ,showed nano-ZnO having stimulate activity on the *Penicillium spp.* and increasing in activity against of bacteria. Thus , this study indicates nanoZnO having stimulated activity for *Penicillium spp.*.
Keywords : ZnO, nanoparticles ,*Penicillium spp*., *Staphyllococcus sp.*,Antibacterial.

Introduction:

The genus *Penicillium* ,one of geneses that spread broadly, comprises about 200 species play different roles in the environment, agriculture and biotechnology. Some of it , such as *Penicillium chrysogenum* produces penicillin antibiotic and *P. griseofulvum* produces griseofulvin . And some species produces anti-cancer substance such as *P. albocoremium* and others will be important in the cheese industry and the production of certain enzymes, such as enzymes Cellulases, Xylanases .(Visagie *et.al.,*2014). In recent years, nanoparticles have received increasing attention due to their unique physical and chemical properties make them behave in a different .(Nel,*et.,al*.,2006) The nanoparticles of zinc oxide one of nanoparticles which were extensively used in many fields Such as chemistry, biology, engineering, physics and other.(Nowak,2010).Although many studies on the biological activity of ZnO NPs have been carried out of antibacterial effects (Nawaz *et.al*.,2011) only few studies have been performed for the mention effects on fungi . To the best of our knowledge, there is no study carried out for activity of this particles on fungi. this study, we investigate the effects of ZnO NPs compared with fungal antibiotics as ketoconazole . Also this study determined the antibacterial activity of the fungus which including in study against *Staphyllococcus*

Materials and Methods:

Fungal strain: Three strains (*P.chrysogenum (1) , P.chrysogenum (2),and P.citrinum*) isolated from patients with pulmonary infections who reviewed the Popular Clinic of Chest Diseases in Maysan province**.**

Prepare concentration of zinc oxide nanoparticles: -

The preparation of (0,3,6,and 12)% of zinc oxide nanoparticles (≤ 50) nm. were purchased from (Hong. mater, China).by dissolving ,3,6, 12 mg of zinc oxide nanoparticles in 100 ml of the Sabourauds Dextrose agar (Lili,*et.al.,*2011).

Prepare of antifungal concentration:

Melting 20 mg of anti-fungal in 100 ml of the media before it cools and then shake well to be prepared (20%) of the anti-fungal as a positive control used in radial growth and weight tests Dry.

Radial Growth estimation of fungus: This test was carried out by inoculating center of the dishes (which containing a different concentrations that mentioned in the preceding paragraph) with fungal disc (5mm) from terminal growth of fungus colony (7 days age).then this dishes incubated at 37c .After incubated n period measuring the radial growth.

Dry Weight estimation of fungus: Used conical flasks (sized 250ml) for this test, each one containing 50ml from liquid medium that contain different concentrations .Inoculated these by fungal disk from colony of fungus (7days in age) and incubated at 37c for seven days .After incubated period, the fungal growth was filtrated and dried at 60c for 24hr. and then was measured the dry weight (Pinto *et.al*., 2001)

Antibacterial activity of *P.chrysogenum (2);*

To test the antifungal activity of *penicillium* spp. in present of Oxide zinc nanoparticles on growth of bacteria , was conducted the test after growth the *P. chrysogenum*(2) on SDB added a zinc oxide nanoparticles with 12% and collected the filtered of the fungal growth then worked disks from the filter paper (Machery-Nagel) and submerged this disks in the filtered of fungus ,for 24 hours , put disks on the surface of dishes container the nutrient agar and growing the bacteria *Staphylococcus epidermidis* which obtained from laboratory of General Hospital in the province of Maysan . Dishes were incubated at $37C^0$ then measure the diameter of the inhibition zone .The same steps in the case of the control treatment except not to add zinc oxide nanoparticles.(Pal *et.al*.,2007).

Statistical analysis: Results were analyzed statistically using Graph Pad Prism software (SAS Institute,

Inc.USA) 4th edition and Least significant differences (LSD)in 0.005 propability.(Motulsky ,2003).

Results:

Radial Growth estimation of fungus:

Rresults shown in the table (1) that the three concentrations of zinc oxide nanoparticles used , were no significant differences at probability of 0.05 between all treatment compare with negative control .In other hand there was significant differences positive control (ketoconazole). Table (2) show ,stimulate effective of ZnONP on growth of *P. chrysogenium(2)*. were no significant differences at probability of 0.05 between all treatment compare with negative control . On the contrary in ketoconazole treatment, where the radial growth of three concentrations (3,6,12)%, 2±0.3 , 2.46±0.08, 2.23±0.32 consequentially ,while in case of ketoconazole its 0.8±0.11.While table (3) explain influence of ZnONP on the radial growth of *P.citrinum* ,we noted that no significant effect between three concentration and control ,but there was significant with ketoconazole treatment.All of this results leads to the conclusion that nanoparticles stimulated the growth of fungus .

Table (1) effect of ZnONP on radial growth of *P. chrysogenium (1)*

Treatment	Radial growth (cm)*
Control	2.13±0.03[A]
Ketoconazole	0.93±0.06[B]
ZnO NP 3%	2.36±0.03[AC]
%6 ZnO NP	2.03±0.16[A]
ZnO NP 12%	2.63±0.23[C]

* Values represents the average values of three replicates ± standard error, the characters represent a statistical probability of reading at a level of 5%

Similar characters between any two groups means there is significant difference (P> 0.05) different characters between any two groups means that there were significant differences (p <0.05)

Table (2) effect of ZnONP on radial growth of *P. chrysogenium (2)*

Treatment	Radial growth (cm)*
Control	1.9±0.25[A]
Ketoconazole	0.8±0.11[B]
ZnO NP 3%	2±0.3[A]
%6 ZnO NP	2.46±0.08[A]
ZnO NP 12%	2.23±0.32[A]

* Values represents the average values of three replicates ± standard error, the characters represent a statistical probability of reading at a level of 5%

Similar characters between any two groups means there is significant difference (P> 0.05) different characters between any two groups means that there were significant differences (p <0.05)

Table (3) effect of ZnONP on radial growth of *P. c*itrinum

Treatment	Radial growth (cm)*
Control	1.23±0.08[A]
Ketoconazole	0.73±0.06[B]
ZnO NP 3%	1.13±0.03[A]
%6 ZnO NP	1.36±0.14[A]
ZnO NP 12%	1.4±0.20[A]

* Values represents the average values of three replicates ± standard error, the characters represent a statistical probability of reading at a level of 5%

Similar characters between any two groups means there is significant difference (P> 0.05) different characters between any two groups means that there were significant differences (p <0.05)

Dry weight estimation of fungus:

Tables(4,5,6) show the results of effect of ZnONP on dry weight of species of *Penicillium* ,these results confirm that has been getting it from stimulate affecting of the radial growth.There was no significant at 0.005 between all concentration and control treatment as well as there was significant with ketoconazole treatment.

Table (4) effect of ZnONP on Dry wieght of *P. chrysogenum(1)*

Treatment	Dry wieght (mm)*
Control	0.599±0.07^{A}
Ketoconazole	0.283±0.05^{B}
ZnO NP 3%	0.481±0.03^{A}
%6 ZnO NP	0.502±0.03^{A}
ZnO NP 12%	0.565±0.06^{A}

* Values represents the average values of three replicates ± standard error, the characters represent a statistical probability of reading at a level of 5%

Similar characters between any two groups means there is significant difference (P> 0.05) different characters between any two groups means that there were significant differences (p <0.05)

Table (5) effect of ZnONP on Dry wieght of *P. chrysogenum(2)*

Treatment	Dry wieght (mm)*
Control	0.476±0.05^{A}
Ketoconazole	0.208±0.02^{B}
ZnO NP 3%	0.455±0.02^{A}
%6 ZnO NP	0.459±0.02^{A}
ZnO NP 12%	0.463±0.02^{A}

* Values represents the average values of three replicates ± standard error, the characters represent a statistical probability of reading at a level of 5%

Similar characters between any two groups means there is significant difference (P> 0.05) different characters between any two groups means that there were significant differences (p <0.05).

Table (6) effect of ZnONP on Dry wieght of *P. citrinum*

Treatment	Dry wieght (mm)*
Control	0.617±0.12^{A}
Ketoconazole	0.194±0.02^{B}
ZnO NP 3%	0.394±0.04^{AB}
%6 ZnO NP	0.549±0.06^{A}
ZnO NP 12%	0.476±0.04^{A}

* Values represents the average values of three replicates ± standard error, the characters represent a statistical probability of reading at a level of 5%

Similar characters between any two groups means there is significant difference (P> 0.05) different characters between any two groups means that there were significant differences (p <0.05).

Antibacterial activity of *P.chrysogenum (2);*

It is noted from the Fig.(1) that there was significant effects between activity of fungus in present of nanoparticles against bacteria, compared with the control treatment (without nanoparticles)

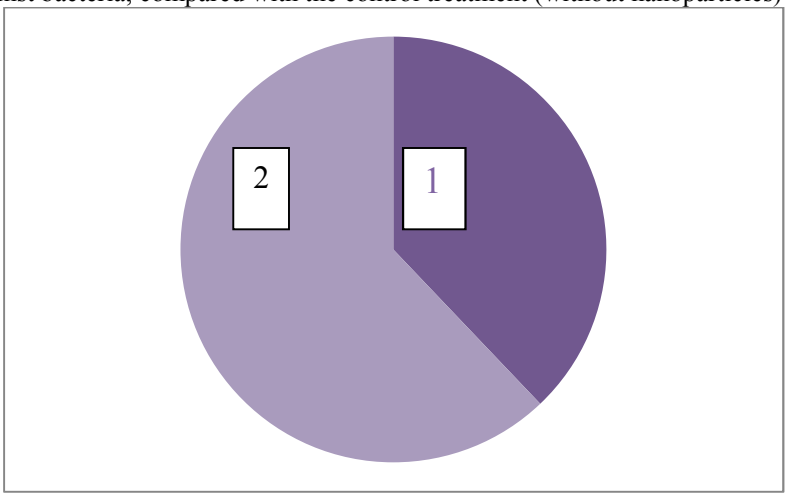

Fig.(1) Effect of P.chrysogenum on growth of *S. epidermidi* in present of ZnONP,1=control treatment,2=12%ZnONP

Discussion:

The current study sheds light on one of nanoparticles and their effect on the growth of one One of the industrially important fungus ,Penicillium spp.The results reveal the nano-ZnO having stimulate activity on the

Penicillium spp. and increasing in activity against of bacteria. Thus , this study indicates nanoZnO having stimulated activity Unlike of the many research confirmed the inhibitory effectiveness of these molecules against many bacterial species.(yamamoto,2001;Brayner,*et.al*.,2006).Also, this is not consistent with the findings of(Lili,*et al*.2011) of the nanoparticles zinc oxide inhibitor against the growth of fungus *Botrytis cinerea* and disagreed with the findings of the (Ramy &Osama, 2013) that nano zinc oxide inhibitor against the fungus *Fusarium oxysporum*..

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