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Effect of Zinc Oxide nanoparticles on gene expression of penicillin production in *Penicillium chrysogenum*

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Abstract : The aim of this research ,evaluated the effect of ZnO NP on genes expression of the genes that produce of penicillin antibiotic in *Penicillium chrysogenum* and testing of activity of penicillin that produced in present of ZnONP. Results, show that ZnONP increased the gene expression of genes (pcbAB ,pcbC,pcbDE)that encoding to penicillin produce Also, indicated the activity of penicillin in present of ZnONP(12%)against of *Staphyllococcus epidermidis.*
Key words : ZnONP , Gene expression, *P.chrysogenum*, (pcbAB ,pcbC ,pcbDE).

Introduction :

Nanoparticles as those particles, which range in dimensions (100-1) nanometers and their small size, they are characterized by the qualities differ from molecules when they are large in size, where possesses qualities of magnetic , electronic and optical make them behave in a different [(1)]. The nanoparticles of zinc oxide one of nanoparticles which were extensively used in many fields[(2)] , including in the field of medicine, especially in the treatment of tumors where it was used against cancer cells where its works to penetrate the cell and link with DNA[(3,4)] many studies have also pointed to the role of nanoparticles to zinc oxide at the genetic level[(5,6)] .Also Lee[(7)] reported that exposure of nanoparticles to zinc oxide cause up regulated genes and increase the gene expression. Therefore, the present study was shed light on the effect of nanoparticles of ZnONP on the level of gene expression of the genes responsible for the production of penicillin antibiotic in *P. chrysogenum*-known produce it of this antibiotic .

Materials and Methods:

Samples collection:

193 clinical samples were collected from various ages and both sexes of patients with respiratory infections and who reviewed the Popular Clinic of Chest Diseases in Maysan province , patients were asked to do the mouth washed with distilled water three times and collected three replicates per patient using the collection of sputum samples sterile container and then transferred to the laboratory for testing them.

Isolation and identification :

Cultured samples on Sabourauds Dextrose Agar, streaking smear cotton on the surface of media, each sample worked three replicates , the dishes were incubated at 37 C ° for 7 days. After incubation period thespecies was classification by using the charactristic of the colony(color, texture and the pigments produced)and the microscopic characteristics that mentioned in[(8,9,10,11)]

Prepare concentration of zinc oxide nanoparticles :

The preparation of 12% of zinc oxide nanoparticles (≤ 50) nm. were purchased from (Hong. mater, China).by dissolving , 12 mg of zinc oxide nanoparticles in 100 ml of the Sabourauds Dextrose broth[12]

Effect of zinc oxide NP on the genes that production of penicillin (pcbAB, pcbC, penDE):

To test the effect of zinc oxide NP on the genes responsible for the production of penicillin (pcbAB, pcbC, penDE) in isolates *of Penicillium spp.(P.chrysogenum (1) , P chrysogenum (2) and P.citrinum)* using flasks 250 mL containing SDB with three replications for negative control and three replications for treatment of Oxide Zinc NP concentration of 12% for each of the three fungal species. These inoculated with disc (5 mm) from culture of *Penicillium* spp. in age 7 days and incubated for seven days then polymerization chain reaction in real time and conduct quantitative experience.

Quantitative Reverse Transcription Real-Time PCR(RT-qPCR):

Was an examination of polymerization chain reaction in real-time quantitative (reverse reproduction) to measure the levels of quantitative DNA reporter (mRNA) to denote the amount of gene expression of genes (pcbAB, pcbC, penDE) as well as the use of gene of (Act1) genestander record to calculate gene expression [15].as following:

Total RNA extraction:

The extraction of Total RNA using of Trizol kit equipped by the Korean company Pioneer and have been working with this kit according to the manufacturer's instructions

Table(1)Primer of DNA

source	**Target sizebp**	**Sequence(3'-5')(/**		**primer**
design in this experiment	127bp	**TTGTCCACCGCAAGTGTTTC**	**F**	Act1
		AATCCACCCACCGCAATTTC	**R**	
	143bp	**ATTCTCGCGATTTGGAAGGC**	**F**	pcbAB
		TGGCGTTCACATTTCGAAGC	**R**	
	147bp	**TGGCACACATCACCAACAAC**	**F**	pcbC
		TCTTGCCGTCTTCCTTGCTAG	**R**	
	85bp	**ATTCGCGGTATTGCAAAGGG**	**F**	penDE
		TGAGCCCGTATGCAAATTCC	**R**	

Quantitative Real-Time PCR (qPCR):

It was an examination of the qPCR by using Accupower 2x Green Star qPCR kit supplied by Korean Pioneer company, that containing the dye green Alsaybr which interact with bloated genes in the device of the Real-Time PCR.

Real-Time PCR data analysis:

The analyze resulting from the polymerase chain reaction in real-time quantitative through the use of way livak method, which was developed by[10], which rely on the extraction of relative quantitative data and the absolute quantity through a correction and equalization process gene goal with control samples so that the results are meaningful biological each sample from the target samples corrected with a control sample to produce a specified level of relative expression.

Effect of the penicillin produced by *P.chrysogenum*(2) in present of 12% of ZnONP on Staphyllococcus *epidermidis*:

To test the effect of penicillin in present of Oxide zinc nanoparticles on growth of bacteria known sensitivity to penicillin[14] 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 ,after 24 hours , put disks on the surface of dishes container the nutrient agar and and growthing 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.

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.[16]

Results:

Isolation and identification:

The results of isolation show that 14 samples in percentage 7.25% containing *Penicillium spp.* distribution in three species of it , *P.chrysogenum* (1) with yellow pigment 5(6.67) ;*P.chrysogenum* (2) with blue pigment 7(9.33) and *P.citrinum* 2(2.67) .table (2)

Table(2) number and percentage of species of *Penicillium*

Percentage (%)	No.of isolates	species
6.67	5	*P.chrysogenum(1)*
9.33	7	*P.chrysogenum(2)*
2.67	2	*P.citrinum*

Effect of zinc oxide NP on the genes that production of penicillin (pcbAB, pcbC, penDE):

Figure (1, 2, 3, 4) explain the results of the effect of nanoparticles on the genes that production of penicillin in *P.chrysogenum*(1).Its show refers to the disparity between the treatment of 12% and control treatment .And fig.(5,6,7,8) explain that present of significant between control treatment and 12% of ZnONP in average of gene expression in genes that production of penicillin antibiotic in *P.chrysogenum (2)* .While the figures(9,10,11,12) refer increased insignificantly and is felt in gene expression of the genes in *P.citrinum*when compared with the control treatment.

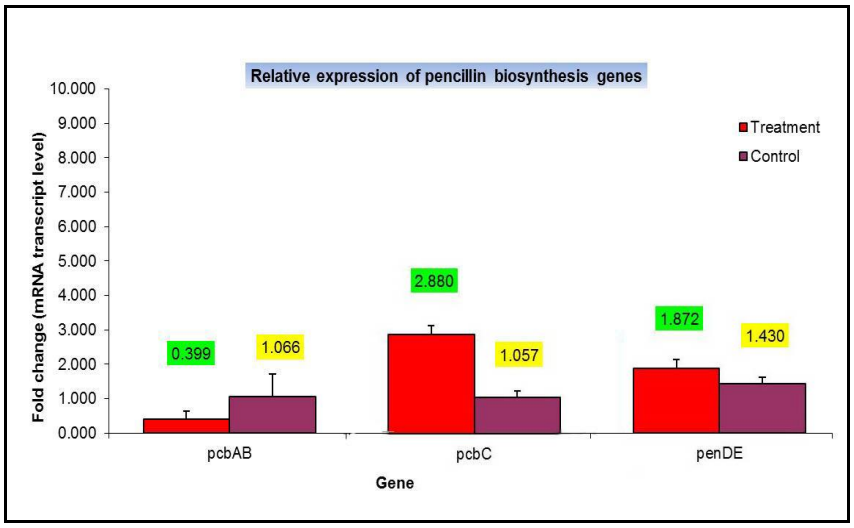

Fig.(1) Effect of ZnONP on genes expression in *P.chrysogenum*(1)

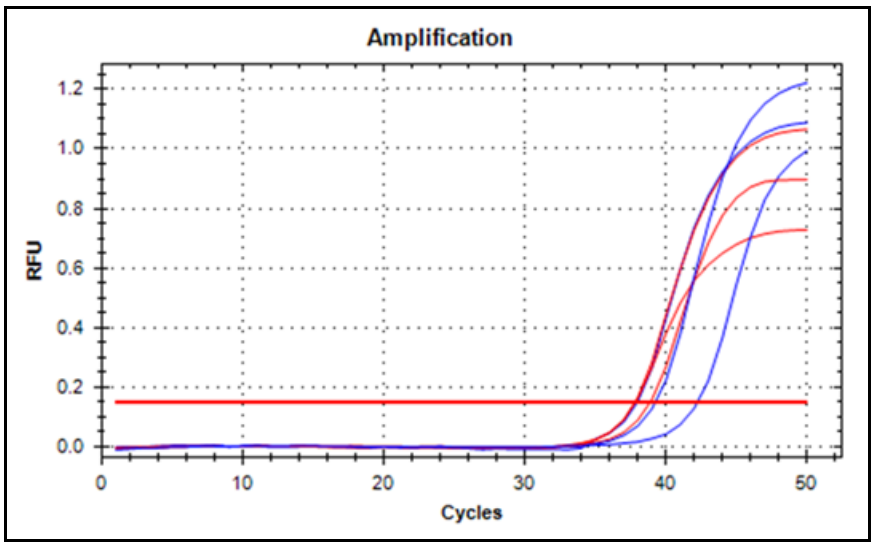

Fig.(2)Amplification curve to test the interaction of Real-Time PCR of pcbAB genein *P.chrysogenum* (1). Where blue curves represent treatment qroups and curves in red color represents control.

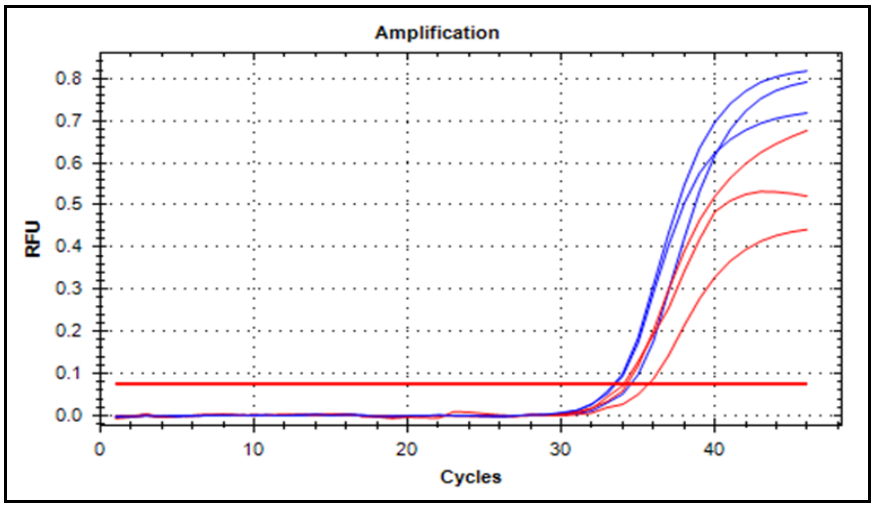

Fig.(3)Amplification curve to test the interaction of Real-Time PCR of pcbC genein *P.chrysogenum* (1). Where blue curves represent treatment qroups and curves in red color represents control.

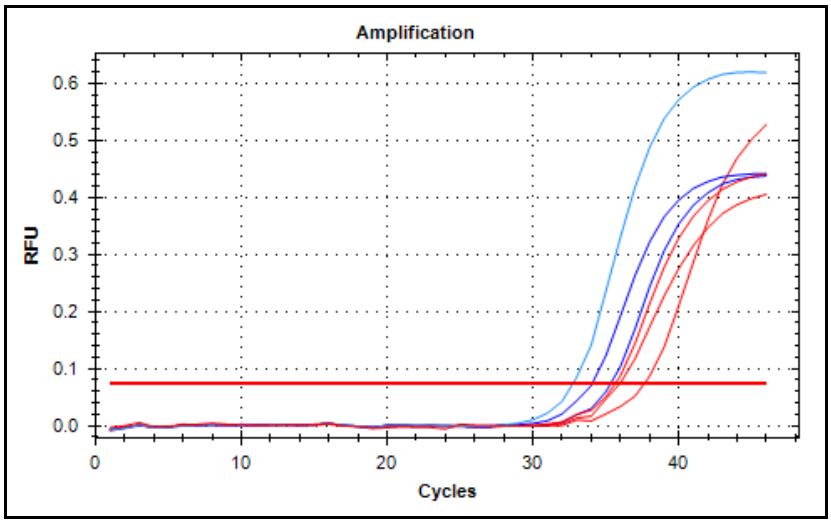

Fig.(4)Amplification curve to test the interaction of Real-Time PCR of pcbDE genein *P.chrysogenum* (1). Where blue curves represent treatment qroups and curves in red color represents control.

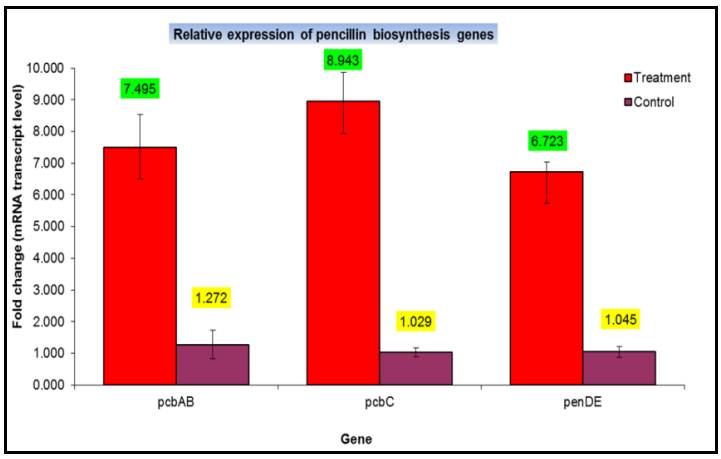

Fig.(5) Effect of ZnONP on genes expression in *P.chrysogenum(*2)

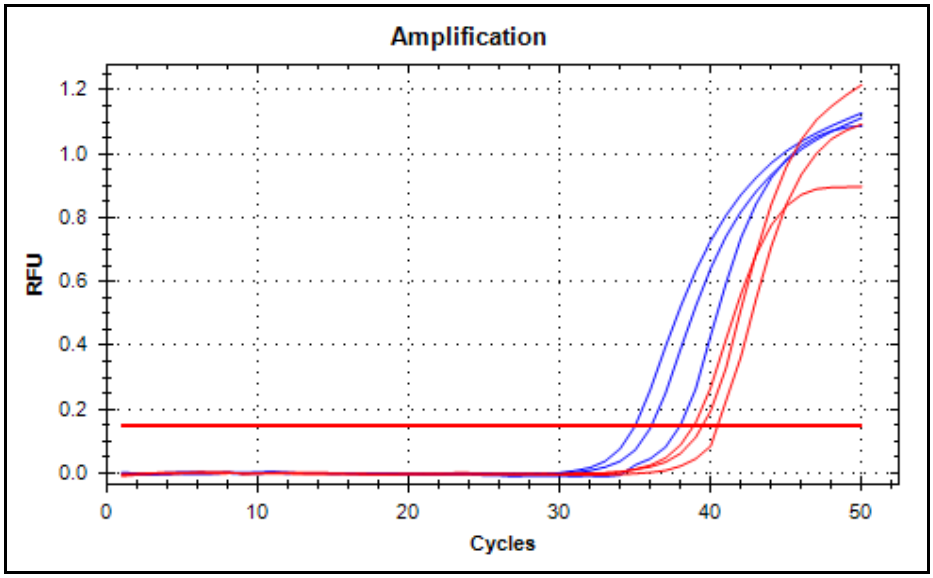

Fig.(6)Amplification curve to test the interaction of Real-Time PCR of pcb AB genein *P.chrysogenum* (2). Where blue curves represent treatment qroups and curves in red color represents control.

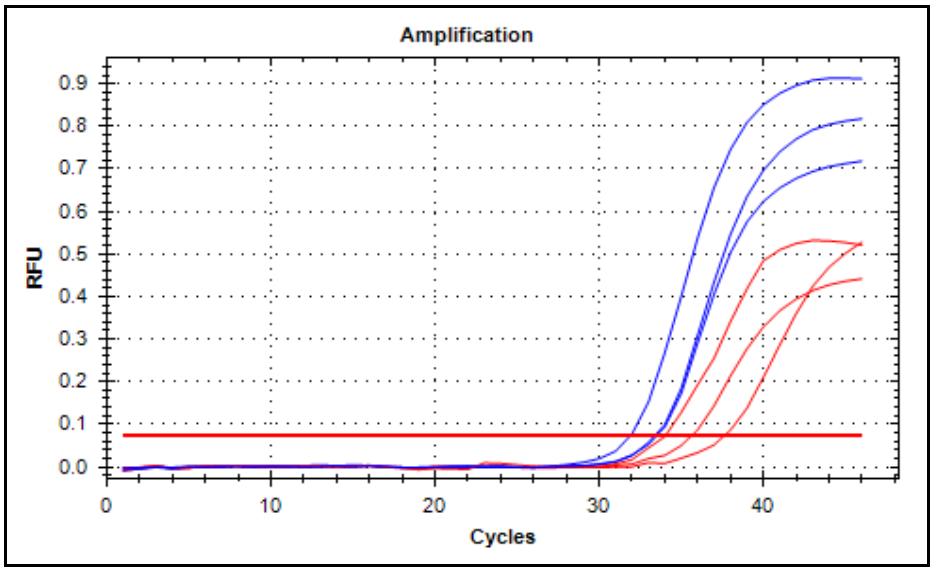

Fig.(7)Amplification curve to test the interaction of Real-Time PCR of pcbC genein *P.chrysogenum* (2). Where blue curves represent treatment qroups and curves in red color represents control.

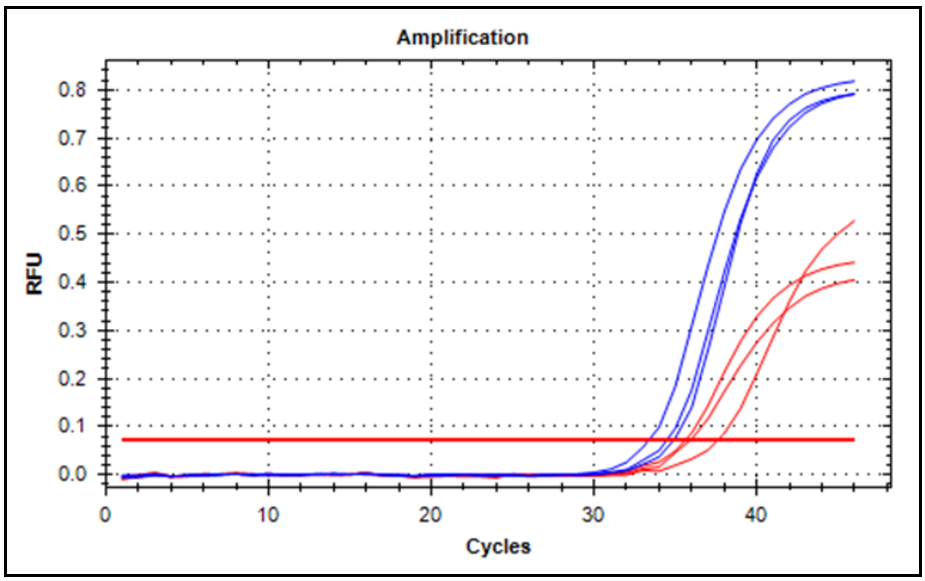

Fig.(8)Amplification curve to test the interaction of Real-Time PCR of pcbDE genein *P.chrysogenum* (2). Where blue curves represent treatment qroups and curves in red color represents control.

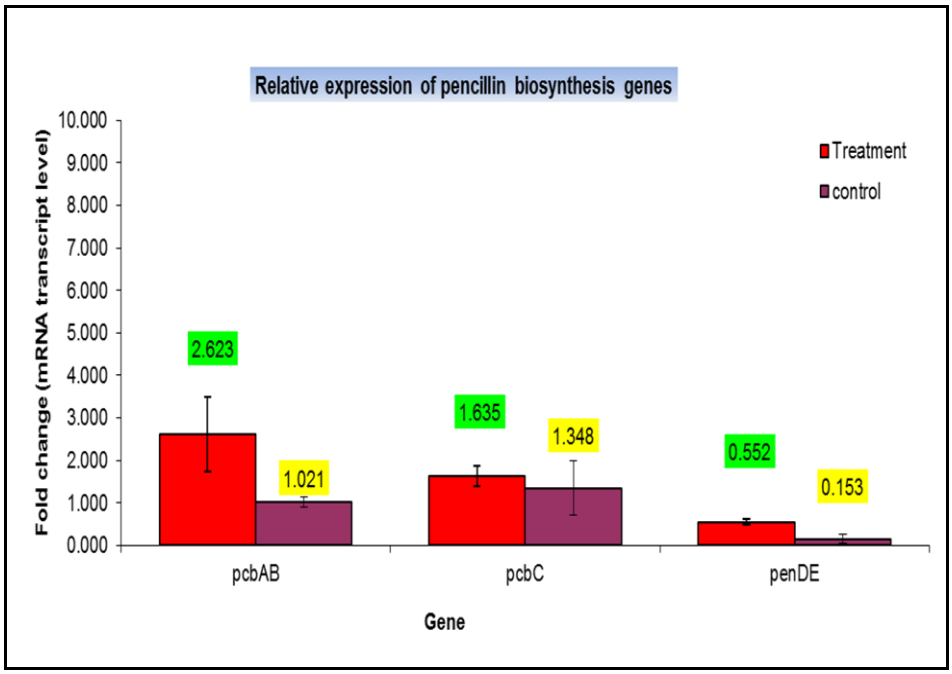

Fig.(9) Effect of ZnONP on genes expression in *P.citrinum*

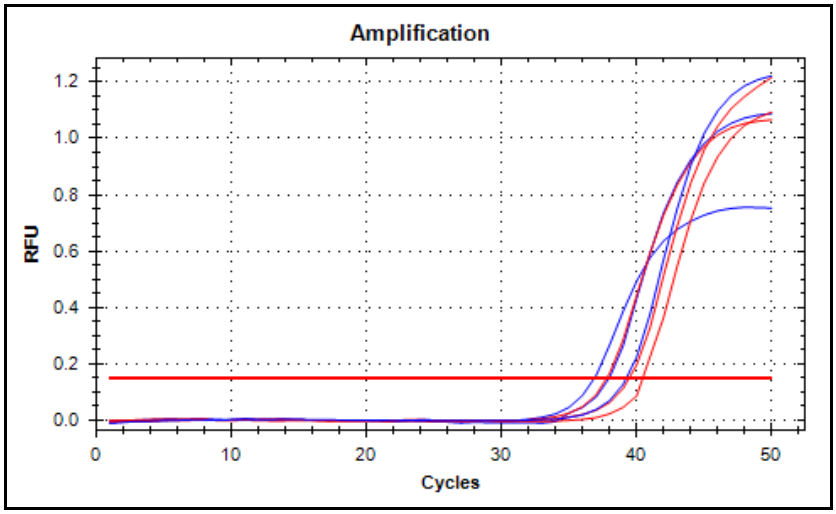

Fig.(10)Amplification curve to test the interaction of Real-Time PCR of pcbAB genein *P.citrinum.* Where blue curves represent treatment qroups and curves in red color represents control.

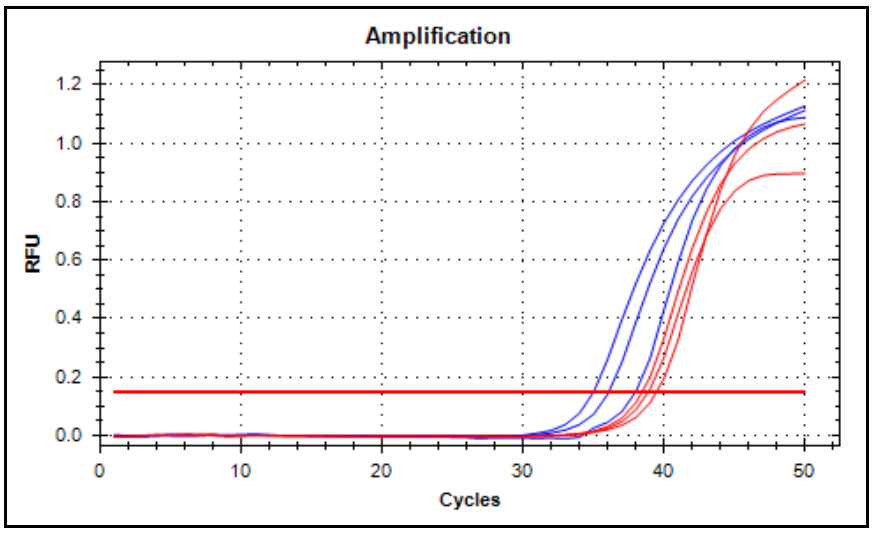

Fig.(11)Amplification curve to test the interaction of Real-Time PCR of pcbC genein *P.citrinum.* Where blue curves represent treatment qroups and curves in red color represents control.

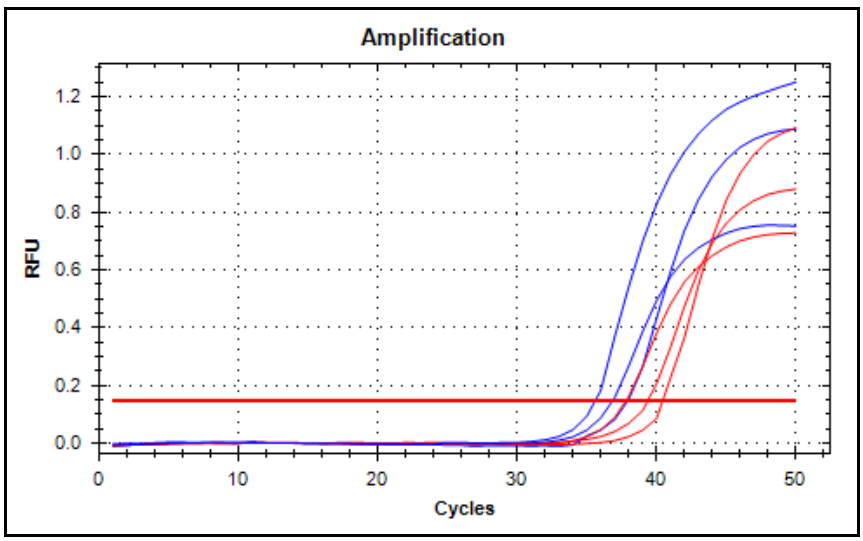

Fig.(12)Amplification curve to test the interaction of Real-Time PCR of pcbDE genein *P.citrinum.* Where blue curves represent treatment qroups and curves in red color represents control.

Effect of the penicillin producted by P.chrysogenum(2) in present of 12% of ZnONP on Staphyllococcus *epidermidis*

The results that present in table(3) and fig.(13) show the activity of filtrated of P.chrysogenum(2)(that contain of penicillin antibiotic) in present 12% of ZnONP against *S.epidermidis* compare with control treatment .

Table (3)Effect of penicillin production in present of 12% ZnONP on *S. epidermidi*s

Zone of inhibition (mm)	treatment
1.15^{A}±12	Control
19.66 -$^{+}$**1.76**^{B}	12%

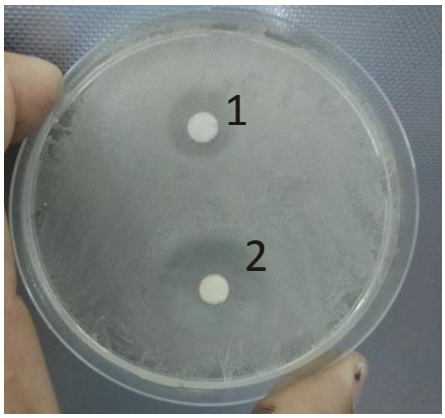

Fig.(13) Effect of penicillin production in present of 12% ZnONP on *S. epidermidis* (1):control treatment, (2): 12% of ZnONP treatment

Discussion:

The current study sheds loght on one of nanoparticles and their effect on one important antibiotics that produce by industrials fungi ,*P.chrysogenum*.The present results demonstrate that the ZnONP. Effect on the gene expression of genes(**pcbAB, pcbC, penDE)** which responseto production of penicillin in concentration 12% .This can be interpreted that nanoparticles have unique properties ,the most important ,their small size makes them enter thecell and reaches to the genetic material, then effect on it .As well as ,ZnONP was cytotoxicity and have ability to change of the genetic materials[5,6,17,18] .It is possible due to mutation in promoter regions of one gene or three genes ,as these genes are working as cluster for production one antibiotic or the mutation occurred in suppressor gene that causing increase in gene expression .Lee[7]and[19] reported that exposure to nanoparticles of ZnO causing up-regulate of the genes and increasing in gene expression .Also ,Nel[3], explain that the genetic influences that urges by nanoparticles as a result of its presence in the nucleus and linked to DNA capable inducing mutations.The test of activity of penicillin produced in present ZnONP is to prove that the mutation that occurred were not present inside the gene structure of any of the three genes responsible for antibiotic -production because it leads to fake an increase in the production , that there are high production, but the antibiotic is not effective to the presence of a mutation in it.It has been testing the effectiveness of the filter which containing antibiotic after the treated with nanoparticles against the bacterium *S.epidermidi s* known sensitivity to penicillin. Results of this test show significant effect of 12% ZnONP compared with the control treatment without adding ZnONP .,this according with Thati[20] which reported increasing of effect of penicillin G in present of ZnONP against of *S.aureus*.

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