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Production of mycotoxin and antimicrobial agent by *Penicillium* sp.and there effect on the growth of some pathogenic bacteria

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ABSTRACT

This study aimed to determine the ability of five species from *Penicillium* include *P.chrysogenum* ,*P.griseofulvum*, *P.citrinum*, *P.camembertti* and *P.arenicola* to produce toxin and detect the antibacterial activity of *Penicillium* sp.on the growth of some pathogenic bacteria include *Staphylococcus aureus* ,*Enterococcus fecalis* ,*E.coli* ,*Enterobacter* ,*Klebsiella pneumonia* ,*Proteus mirabilis, Acinetobacter , and Pseudomonas aeruginosa*. The mycotoxin extracted by chloroform , while the second method extracted by ethyl acetate,in the first method used chloroform to extraction the toxin from the broth culture in equal volume and used chloroform and methanol 98:2 as mobile phase in isolation the toxin on TLC , in the second method, used ethyl acetate to extraction the toxin from the broth culture in equal volume and used toluene 90%, ethyl acetate and formic acid 1:2:3 as mobile phase in isolation the toxin on TLC .The antibiotic was extracted by used ethyl acetate and used ethyl acetate with acetone 75:25 as the mobile phase to isolation the antibiotic on TLC plate. This study showed *penicillium* species can produce many types of toxin such as citrinin , patulin, and moniliformin .also antibacterial agent extracted by ethyl acetate and the some active compound produced by *P. camemberti* and *P. griseofulvum* and the active compound produced by *P. griseofulvum* was higher activity in inhibition the growth of bacteria with a diameter of inhibition zone was 23.5 mm , while the active compound produced by *P. camemberti* gave the diameter of inhibition zone was 11.3 mm.

Keywords: Penicillium spp. ,fungi ,pathogenic bacteria ,toxin ,antibiotic

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INTRODUCTION

Antimicrobial resistance is when a microbe evolves to become more or fully resistant to antimicrobials which previously could treat it.(1) Resistant microbes are increasingly difficult to treat, requiring alternative medications or higher doses—which may be more costly or more toxic. Microbes resistant to multiple antimicrobials are called multidrug-resistant (MDR); or sometimes superbugs.(2) Antimicrobial resistance is on the rise with millions of deaths every year.(3) A few infections are now completely untreatable due to resistance.(4)For this reason we most begin to research on new source for antibiotic or research on source produce new antibiotic , the fungi consider one source of antibiotic and Penicillium first fungi produce antibiotic ***Penicillium*** is a genus of ascomycetous fungi of major importance in the natural environment and it is one of the most common fungi occurring in a diverse range of habitats ,from soil to vegetation to air ,indoor environments and various food products . It has a worldwide distribution and a large economic impact on human life(5).Some members of the genus produce penicillin , The thallus (mycelium) typically consists of a highly branched network of multinucleate, septate, usually colorless hyphae. Many-branched conidiophores sprout on the mycelia, bearing individually constructed conidiospores. The conidiospores are the main dispersal route of the fungi, and often are green in color.(6)*Penicillium* as another fungus produce secondary metabolism ,mycotoxins are secondary metabolites produce by filamentous fungi which may contaminate food ,the genera of mycotoxin are mainly produced by *Aspergillus*,*Penicillium,* and *Fusarium , Penicillium* produces many types of toxins include Ochratoxin A ,citrinin ,patulin, citreoviridin, griseofulvin and moniliformin (7).Antibiotics are chemical substances ,produced by microorganisms that can inhibit the growth or kill other microorganisms. Penicillin was the first antibiotic discovered by Alexander Fleming (1929) in *Penicillium notatum*. Amoxycillin and Ampicillin are a semisynthetic modification of penicillin (8).

MATERIALS AND METHODS

Collection and identification the samples

The fungi isolated from a different sources such as soil ,apple , citrus and contamination of laboratory refrigerator ,after the isolation the samples culture on PDA media and incubation at 25 c for 7 days , after the growth of fungi identification the fungi according to the macro and micro characteristics of fungi .
The bacteria was taken from the laboratory of pathogenic bacteria in faculty of since,the bacteria diagnosis by microscopic examination and biochemical tests.

Extraction the toxins

The five species of *Penicillium* cultured on PDB (potato dextrose broth) by take five-disc from culture with 7 days old for each species and inoculated PDB media with these disc and incubated at 22 ± 2 for 14 days, after 14 days used two methods for extraction toxin in the first method taken the filtrated of fungi without the mycelium of fungi and this filtrated mixed with equal volume of chloroform in separator funnel and shaking the mixture for 10 -15 min. and then toke the chloroform layer which containing on toxin , the extraction concentration on room temperature and the toxin isolated from extraction by taken small amount from extraction by using micropipette and put the extraction on TLC plate at 2cm from lower edge and let it to dry , after then used chloroform with methanol 98:2 as mobile phase to isolation the toxin (9). In the second method applied same steps in the first method but in this method used ethyl acetate to extraction toxin and used toluene 90% ,ethyl acetate and formic acid 1:2:3 as the mobile phase to isolation the toxin (10).

Antibacterial agent production and activity of it

Used two methods in this experiment in the first method growth each specie from *Penicillium* on PDB at 22± c for 8 days , after 8 days of incubation the liquid culture of *Penicillium* species were filtered through two layers of Whatman No. 1 filter paper. and test the ability of it on inhibition growth of eight genus of bacteria by culture each genus of bacteria on Muller Hinton agar and made five well on each plate and added the filtered of each specie from *Penicillium* in these well after then incubator all plate at 37 C for 24 h. and measured the inhibition zone to determine the present of antibiotic(11) .

In the second method used modify PDA to growth fungi and bacteria together because on PDA alone the fungi growth well but bacteria cannot growth while on Nutrient agar the bacteria growth but the growth

of fungi was very weak ,so that used modify PDA which containing on PDA ,sugar 40% and peptone 4% ,this media allowed to bacteria and fungi growth well , taken disc with 6mm from each species of *Penicillium* and place in the center of the media and incubated at 22± C for 2-3 days after then spreading the suspension of each genus of bacteria around the growth of fungi disc and incubated at 37 C for 24h. . and measured the inhibition zone to determine the present of antibiotic, this step repeated with each species of *Penicillium* (12).

Extraction the antibiotic

To extraction the antibiotic growing each species of *Penicillium* on PDA at 22± 2C for 7days , after growth the test fungi the media in each plate cut into small pieces and put in blender and add 200ml from ethyl acetate for each five plates and mixture in blunder on middle speed for 15 min. after then filter the mixture and take the ethyl acetate layer which may be containing on antibiotic , the extraction concentration at room temperature and the antibiotic isolation from extraction by take small amount from extraction by micropipette and put on TLC plate at 2 cm from the lower edge,used ethyl acetate and acetone 75:25 as mobile phase to isolation the antibiotic ,after obtain on antibiotic test the ability of isolation antibiotic on inhibition the growth of bacteria(13) .

RESULT AND DISCUSSION

This study showed three species of *Penicillium* can produce toxin ,*P.griseofulvum* and *P.citrinium* produce fumonisin B1(rf 71) and citrinin (rf 75)(14) respectively when used chloroform as organic solvent in extraction the toxin and used chloroform with methanol as mobile phase ,*P.griseofulvum* produce patulin (rf 60)(15) when used ethyl acetate as organic solvent in extraction toxin and toluene 90% ,ethyl acetate and formic acid 1:2:3 as mobile phase to isolation the toxin (Table 1).

Table :1 ability of Penicillium species on produce the toxin by using tow organic solvent in extraction the toxin

solvent / Pnecillium spp.	Chloroform	Rf	Ethyl acetate	Rf
P. griseofulvum	Fumonisin B1	71	NI	57
P.chrysogenum	NI	77	NC	
P.citrinum	Citrinin	75	NI	27
P.camembrti	NI	86	NI patulin	27 60
P.arenicola	NI	86	NC	

NI: non-identification , NC : non-compound

Also this study showed when used the first method in test the ability of *Penicillium* species on producing antibiotic the active compounds produced by this method effect on gram-positive bacteria while the gram-negative bacteria were resistance to this compound ,and Staph.aureus was higher sensitivity to the active compounds from Enterococcus which was lees sensitivity (table 2) this may be back to the ability of this method to produce antibiotic effect on gram-positive bacteria only these mean produce extra secondary metabolism.

Table :2 effect active compound produced in the first method by *Penicillium* species in inhibition the growth of bacteria

Penicillium sp / Bacteria	Penicillium Camemberti	Penicillium chrysogenum	Penicillium arenicola	Penicillium citrinium	Penicillium Griseofolvum
Staph. aureus	22mm	18mm	14mm	20mm	24mml
Enterococcus fecalis	10mm	16mm	12mm	20mm	16mm
E. coli	0	0	0	0	0
Enterobacter	0	0	0	0	0

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Pseudomonas aeruginosa	0	0	0	0	0
Klebsiella pneumonia	0	0	0	0	0
Proteus mirabilis	0	0	0	0	0
Acintobacter	0	0	0	0	0

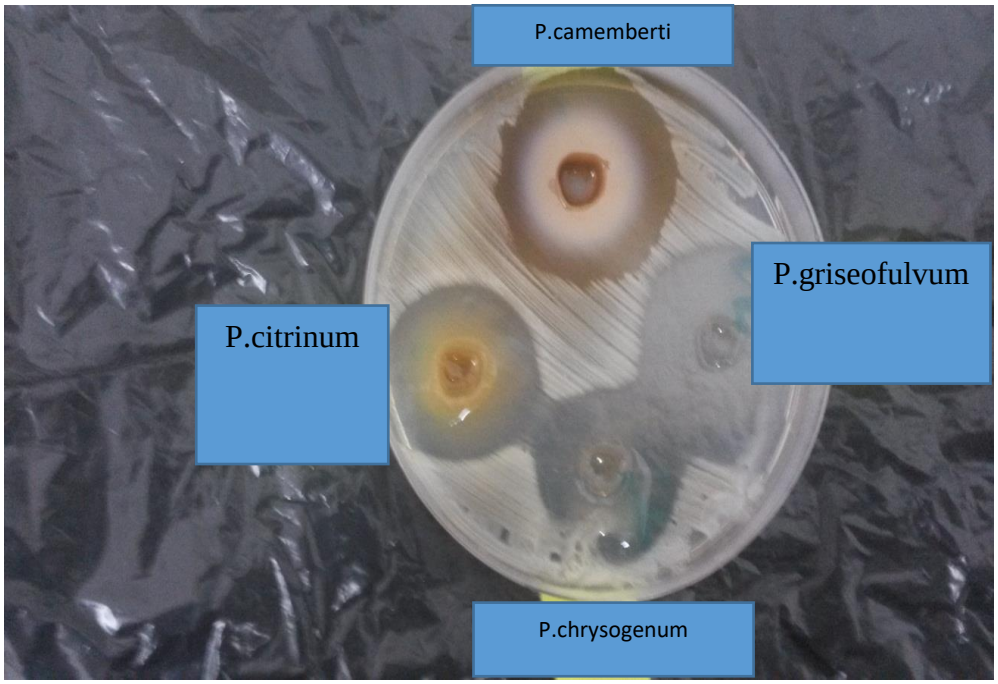

Fig. 1 :effect the active compound produced by *Penicillium* species in the first method on *Staph.aureus*.

Also this study showed the second method was higher activity from first method in produce active compounds which were high effect in inhibition growth of bacteria especial the compounds produce from *P.chrysogenum* which was most common in produce the antibiotic such as penicillin ,xanthocillin and sorbicillin (16) (table 3) this result may back in present the fungi with bacteria in same media and the compound can effect directly on bacteria.

Table :3 effect active compound produce in the second method by Penicillium species in inhibition the growth of bacteria

Penicillium sp. / Bacteria	Penicillium camembertti	Penicillium Chrysogenum	Penicillium arenicola	Penicillium citrinium	Penicillium Griseofulvum
Staph.aureus	34mm	44mm	8mm	32mm	30mm
Enterococcus fecalis	16mm	36mm	12mm	34mm	34mm
E.coli	15mm	50mm	10mm	0	20mm
Enterobacter	10mm	50mm	0	0	30mm
Pseudomonas Aeroginusa	24mm	45mm	0	0	40mm
Klebsiella Pneumonia	6mm	30mm	0	0	40mm
Proteus mirabilis	20mm	40mm	20mm	18mm	30mm
Acintobacter	20mm	40mm	6mm	16mm	40mm

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Fig.2 : effect the active compound produced by P.chrysogenum in second method on the growth of Klebsiella pneumonia.

This study showed when extraction toxin four species of *Penicillium* produced antibiotic ,*P.chrysogenum* ,*P.griseofulvum* ,*P.citrinum* and *P.camemberti* while *P.arenicola* don't produce antibiotic , and when test the activity of antibiotic production by *Penicillium* species on inhibition the growth of bacteria observed the antibiotic produced from *P.griseofulvum* and *P.camemberti* gave activity against bacteria and the antibiotic produced from *P.griseofolvum* was higher effect on bacteria while the antibiotic production from *P.camemberti* was lees activity, the activity of *P.griseofulvum* may be back to ability of it on produce penicillin and cyclopiazonic acid (17) and the activity of *P.camemberti* back to the antibacterial and antifungal properties of it (18) in addition to ability of it on producing cyclopiazonic acid (19),also this study showed the antibiotic production from *P.citrinum* and *P.chrysogenum* were inactive on the growth of bacteria (table 4) the inactivity of antibiotic produce from *P.chrysogenum* which was most common in ability of it in produce active antibiotic may be back to the method of extraction which was unsuitable to extraction and isolated the active antibiotic .

Table 4: effect the antibiotic extraction from Penicillium species in inhibition growth of bacteria

Penicillium sp / Bacteria	Penicillium camemberti	Penicillium chrysogenum	Penicillium citrinium	Penicillium Griseofolvum
Staph. aureus	11mm	0	0	28mm
Enterococcus fecalis	11mm	0	0	22mm
E. coli	8mm	0	0	22mm
Enterobacter	14mm	0	0	23mm
Pseudomonas aeruginosa	15mm	0	0	25mm
Klebsiella pneumonia	13mm	0	0	26mm
Proteus mirabilis	11mm	0	0	20mm
Acintobacter	12mm	0	0	21mm

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