A NOVEL METHOD OF LASER EFFECTING GROWTH OF *PENICILLIUM CHRYSOGENUM* AND *PENICILLIUM EXPANSUM* TREATED WITH BORIC ACID AND COLORED WITH GENTIAN VIOLET AND METHYLENE BLUE[+]

Asma'a A. Basher[*]

Abstract:

Culture of *P. chrysogenum* and *P. expansum* were isolated from wood surfaces and treated with different concentrations of boric acid (1% and 2%), colored with gention violet and methylene blue and exposed to He-Ne laser light with absorbed doses of 1.06 and 2.12 joules/cm^{2}respectively and survivors enumerated. The maximum inhibition (for 6min.and 2%boric acid concentration)of *P. chrysogenum* and *P.expansum* were 92.2 % and 90 % for coloring by Gentian Violet respectively. While the inhibition of these fungi for coloring by Methylene Blue were 85.5% and 87.7 % respectively.

Key Words:
Laser, Boric acid, *P. chrysogenum* and *P. expansum*, Gentian Violet, Methylene Blue

المستخلص:

تم عزل فطر البنسليوم كرايسوجينيوم والاكسبانسيوم من سطوح خشبية من مناطق مختلفة،أجريت طريقة جديدة لتأثير التلوين بصابغات (الجنتشن البنفسجية والميثيلين الزرقاء)على زيادة القتل بالتشعيع بالليزر حيث تمت معاملة البيئات المحضرة بتركيزات مختلفة من حامض البوريك(١% و٢%) وتشعيعها لفترات ٣ و٦ دقائق(بعد تلوينها بهذه الصابغات) . ولقد بينت النتائج أن ثمَّة اختلافات في استجابة الفطر لسمية هذا الحامض بالتشعيع ،حيث كانت أعلى نسبة تثبيط هي بالتلوين بصبغة الجنتشن البنفسجية (٩٢,٢% و٩٠%)على التوالي من البنسليوم كرايسوجينيوم و البنسليوم الاكس بانسيوم ،في حين كانت(٨٥,٥و٨٧,٧%)على التوالي بالتلوين بصبغة الميثيلين الزرقاء .

Introduction:

The perfect stage of genus *Penicillium* was full into the order Eurotiales. In this order, organisms were produced asci within cleistothecia. *Penicillium* was often referred to as Deuteromycetes, or fungi imperfecti. *Penicillium chrysogenum*, was classified as a psychrotrophic microorganism and this species was one of the best lipase producers among other fungi. *Penicillium chrysogenum* has high enzymatic activity and has the ability to produce alpha-amylase[1].

Concentrations were typically higher indoors than they were outdoors.It was not uncommon to find *Penicillium* growing on paper and wood surfaces in high-humidity basements and living spaces. It was grown well on wetted ceiling tiles. *Penicillium*

[+] **Received on 2006/7/4 , Accepted on 2007/1/17**
[*] **Lecturer - Dept. of Pharmacy, Institute of Technical Mosul**

species were reported to produce mycotoxins. There was no evidence that inhalation exposures pose any unique health risks. Several *Penicillium* species were pathogenic, that was, they pose significant health risks to immune-compromised individuals, such as those with AIDS or on chemotherapy[2].

P. expansium was worked by producing ethylene to accelerate ripening. It was covered the fruit with green conidia, causing the fruit to shrivel and dry out. *P. expansum* produces one called patulin. Most of these species were resembled each other in color characteristics, style of decay, and infection symptoms; they were full under a general category called blue mold. *P. expansum* was one of the most aggressive species. These fungi were lived a long time and were quite durable, even under adverse conditions. *Penicillium expansum* was known to attack apples. The most common treatment was to use fungicide on harvested produce *Penicillium*[3]. Boric acid was an extremely effective fungicide[4], and the effecting of boric acid on the growth of some fungi was studied by many investigators[5-6].

Lethal photosensitization of the yeast at exposure to laser radiations was studied with different dyes, toluidine blue and methylene blue with exposed to light from a helium/neon or gallium aluminium arsenide laser[7].

In this work and as apart of continuous program directed toward the studying of the isolation and identification of some indoor dust fungi and their effect on the respiratory system[8],we were comparative studied here the effect of boric acid on growth of the some fungus[5] in different concentrations (1% and 2%),and the lethal photosensitization toward helium/neon laser after colored with gentian violet.

Materials and methods test organism

P. chrysogenum and *P. expansum*, were previously isolated from wood surfaces of different places in Mosul during three months before this work(February- April). They were in abundance (80%) followed by other yeasts not interested. Their identification was dependent on different temperatures (5◦C, 25◦C and 37◦C) and three culture mediums[9]:

1-Czapex concentrate :

[30gm $NaNO_3$; 5 gm KCl; 5gm$MgSO_4.7H_2O$; 0.1 ml $FeSO_4.7H_2O$ and 100ml dist. Water]

2-Czapic Yeast Extract Agar(CYA):

[1gm K_2HPO_4, 10 ml Czapex concentrate, 5gm yeast extract powder ,30gm sucrose,15gm agar and 1000ml dist water]

3-Malt Extract agar(MEA):

[20gm (ME); 1gm peptone; 20gm glucose; 20gm agar; and 1000ml dist. Water]

4-25% Glycerol nitrate agar(G25%N)

[0.75gm K_2HPO_4; 7.5ml Czapek concentrate; 3.7gm yeast extract, 250ml glycerol(analytical grade); 12gm agar and 750 dist.water]

Inoculums preparation [9]

Sabauraud's Agar medium (SAM) was used, with the following composition (g/l): Glucose, 10; Agar, 10,Pepton10 and distilled water, 1000ml. The pH value of the medium was adjusted at 5.6 then autoclaving at 1.5 atm. for 15 minutes. (SAM) medium was also used for sub culturing of the test organism as well as for preparation

of fungal inoculum's. This was prepared in the form of fungal culture discs each of 7 mm diameter using 8 days old culture. (SAM) medium was also used as control medium for measuring the toxicity of boric acid on fungal growth.

Experimental media

This was prepared using (SAM) medium. It was supplied singly by different concentrations of boric acid of 1% and 2%. The dyes (gentian violet and methylene blue) was supplied as 0.0001%.

Statistical Expressions of results

%Inhibition of fungi:

Fungal growth was determined by measuring the diameter of colony radial growth in mm., data were recorded in triplicates after 8days of incubation at 28^{o}C,and 3-6minutes.

Total dish diameter = 90 mm

The colony radial growth diameter = 90 mm

Growth % = 90 / 90 * 100 = 100 %

To calculate the %Inhibition of *P. chrysogenum* by Boric acid(1% Conc) of 52mm colony diameter (as example, Table 1):

Inhibition diameter = Total dish diameter - colony diameter = 90 – 52 = 38mm

%Inhibition = Inhibition diameter / Total dish diameter * 100 = 38 / 90 * 100 = 42.2%

Irradiation absorbed dose:

Irradiation absorbed dose in joules/cm^2 = power(watt) × irradiation time (sec)/area(cm^2) = 0.001watt×5min.×60 / 0.3^2 × 3.14 = 1.06

=0.001watt ×10min ×60 /0.3^2 ×3.14 = 2.12

Laser used

Laser He-Ne of wave length 632.8 nm and power output of 0.001watt, irradiations techniques was provided in Laser Laboratory, Department of Physics, College of Education ,Mosul University.

Results and discussions:

In ancient literatures Stean and Stearn were indicated that the behavior of bacteria toward dyes could be explained and largely determined by its protein[10]. This behavior was increased when these dyes irradiate by laser, a photodynamic action of gentian violet, May apparently was mediated by oxidative laser irradiation. The used of dyes (gentian violet and methylene blue) was based on the ability of certain organic dyes to absorb the energy of a source and then re-radiates the energy as laser Radiation[11].

The dehydration power effects of boric acid on the fungal biochemistry was clearly associated with damage to the cell membrane with the loss of essential cellular components such as potassium ions and amino acids[12]. And this effects were increased by dye laser irradiations. The dye laser was based on the ability of certain organic dyes to absorb the energy of a source and then re-radiate the energy as laser radiation[13].

Results presented in Tables (1-4) were showed that the coloring by organic dyes (Gentian Violet and Methylene Blue) were increased the lethal photosensitization of these two fungi, and the maximum inhibition (for 6min.and 2%boric acid concentration)of *P. chrysogenum and P. expansum* growth were 92.2 % and 90 % for coloring by Gentian Violet respectively. While the inhibition of these fungi for coloring by Methylene Blue were 85.5% and 87.7 % respectively[5].

The different in the maximum inhibition of these experiments were attributed to the presence of high concentration of boric acid (2%), the dye Gentian Violet and the time of irradiation (6min.) that afford the required killing of these fungi. The same effect was observed for methylene blue.

Based on our data it was seemed that the coloring by organic dyes were the most important factors in the lethal photosensitization of these two fungi. There is however, need in future to investigate further the identity of the active compound(s) and other dyes so that a comparison can be made between them and our results.

.

Table (1) Colony Diameter (mm) & %Inhibition of *P. chrysogenum* on (SAM) medium Colored with Methylene Blue and supplied with different conditions (of Boric acid concentrations and radiation times).

Boric acid Conc. (%)	8 Days without radiation				8 Days radiation after colored with Methylene Blue			
	Uncolored		colored with Methylene Blue		3 minutes radiation		6 minutes radiation	
	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition
0.0	90	0	40	55.5	30	66.6	28	68.8
1%	52	42.2	38	57.7	20	77.7	18	80
2%	43	52.2	35	61.1	16	82.2	13	85.5

Table (2) Colony Diameter (mm) & %Inhibition of P. chrysogenum on (SAM) medium Colored with gentian violet and supplied with different conditions (of Boric acid concentrations and radiation times).

Boric acid Conc. (%)	8 Days without radiation				8 Days radiation after colored with gentian violet			
	Uncolored		colored with gentian violet		3 minutes radiation		6 minutes radiation	
	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition
0.0	90	0	20	77.7	16	82.2	13	85.5
1%	52	42.2	19	78.8	11	87.7	9	90
2%	43	52.2	18	80	8	91.1	7	92.2

Table (3) Colony Diameter (mm) & %Inhibition of *P. expansum* on (SAM) medium Colored with gentian violet and supplied with different conditions (of Boric acid concentrations and radiation times).

Boric acid Conc. (%)	8 Days without radiation		8 Days radiation after colored with gentian violet	
	Uncolored	colored with gentian violet	3 minutes radiation	6 minutes radiation

	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition
0.0	90	0	21	76.6	18	80	16	82.2
1%	40	55.5	20	77.7	15	83.3	14	84.4
2%	35	61.1	19	78.8	11	87.7	9	90

Table (4) Colony Diameter (mm) & %Inhibition of *P. expansum* on (SAM) medium Colored with Methylene Blue and supplied with different conditions (of Boric acid concentrations and radiation times).

Boric acid Conc. (%)	8 Days without radiation				8 Days radiation after colored with Methylene Blue			
	Uncolored		colored with Methylene Blue		3minutes radiation		6 minutes radiation	
	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition	Colony Diameter(mm)	% Inhibition
0.0	90	0	23	74.4	18	80	17	81.1
1%	40	55.5	21	76.6	16	82.2	15	83.3
2%	35	61.1	20	77.7	13	85.5	11	87.7

Acknowledgment

I thank Dr. Abdulla, A.I.; Asst. professor of Laser Physics, department of physics, College of Education , Mosul University, for providing the laser irradiations techniques

References

1- Bancerz R, Ginalska G, Fiedurek J, Gromada A. "Cultivation conditions and properties of extracellular crude lipase from the psychrotrophic fungus *Penicillium chrysogenum* 9'" *J Ind Microbiol Biotechnol*. (2005).

2- Texas, J. *;(How dangerous is Penicillium mold?*); Indoor *Environment Notebook; Technical comments to the Webmaster (2004)*

3- *Penicillium*. (Classification, Description and Significance Cell Structure and Metabolism); htm # classif (last modified 2006)

4-RoyalWebHosting.com (Boric acid is an extremely effective fungicide) Nov 22, (2005)

5- Saied,S.M.,Basheer,A.A.and Al-Obaydi,Gh.F.;Organic Microbiological Studies (The Effects of Gentian Violet and Boric Acid on Growth of the Fungi *Aspergillus flavus, Penicillium chrysogenum and Penicillium expansum) ;*Dept. Of Pharmacy Institute of Technical/Mosul;(under publication)

6- Hashem,A.R.andAl-Obaid,A.M.;(Effect of Cadmium on The Growth of *Aspergillus flavus* and Ulocldium chlamydosporium) ;International Journal of Experimental Botany 59 (1/2): 171–175. (1996)

7-Lasers-in-Medical-Science CO: LMSCE(1994)

8- Basher ,A.A.*"Isolation and identification of some indoor dust fungi and their effect on the respiratory system"* MSc Thesis, College of Sciences; Univ. of Mosul;Iraq(2005)

9-Pitt, J.I. and Hocking,A.D.*"Fungi and Food Spoilage";*2nd Gaithersburg,Maryland:Chapman and Hallpp593(1997)

10-Stearn,E.W. and Stearn,A.E.'"The chemical mechanism of bacterial behavior" *.J.of Bact*., v.9(5),(1925)
11- Docampo-R; Moreno-SNJ,Department of Veterinary Pathobiology, University of Illinois, 2001 *S. Lincoln Ave., Urbana, IL 61801*, United States (1990)
12-Hitchcock, C.A.; Biochem.Soc.Trans, 199:782}. ((Boric Acid/ Borates/ Borax, Beyond Pesticides Rating: Least Toxic, Use and Mode of Action, BEYOND PESTICIDES, email info@beyondpesticides.org(2006)
13- Alexander,R. (The Dye Laser), the Laser Electro-Optic Program at Albuquerque Technical-Vocational Institute in 1992. Dye_Laser.htm