

Effects of Gentian Violet and Boric Acid on Growth of The Fungi: *Aspergillus flavus*, *Penicillium chrysogenum* and *P. expansum*

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ABSTRACT

The maximum Percentage inhibition of growth of *Aspergillus flavus* (which can cause a wide spectrum of diseases ranging from allergic to a life-threatening invasive disease Aspergillosis). *P. chrysogenum* and *P. expansum* whose mycotoxins can cause significant health risks ranging from the infections of fruits, vegetables and milk products to immune compromised individuals), by an organic dye (Gentian Violet) were 78.8, 77.7 and 76.6% respectively. While that of inorganic boric acid of (2%) maximum concentrations inhibition were (44.4, 52.2 and 61.1 %) respectively. This results was attributed to the amphiphilic properties of this dye i.e. its high solubility in both aqueous (hydrophilic) and organic (lipophilic) phases and became effective at site in the body (the cell membrane) and it must also be able to reach that sites.

Penicillium chrysogenum *Aspergillus flavus*
Penicillium expansum

) <i>Aspergillus flavus</i>	% 2	1		
<i>Penicillium Chrysogenum</i> (
		<i>Penicillium expansum</i>		
% 76.6	77.7	78.8		
	% 61.1	52.2	44.4	%2

INTRODUCTION

Aspergillus flavus are aflatoxigenic species that produce potent carcinogen aflatoxins Fig. (1) (Payne et al., 2006). These species grow on dead leaves, stored grain, compost piles, wood surfaces or in other decaying vegetation. (Kurtzmzn et al., 1987).

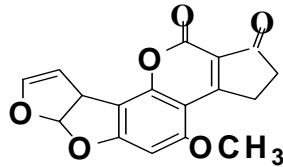


Fig. 1 :Chemical structure of Aflatoxin B1 (Payne et al., 2006)

Comparable to *Aspergillus*, *Penicillium* are also reported to produce mycotoxins, several *Penicillium* species are pathogenic and they pose significant health risks to immune-compromised individuals, such as those with AIDS or on chemotherapy (Bancerz et al., 2005).

Penicillium chrysogenum, was classified as a psychrotrophic microorganism. of the best lipase producers among other fungi they studied in the arctic tundra. *Penicillium chrysogenum* has high enzymatic activity and has the ability to produce alpha-amylase. *Pencillium* fungi were versatile and opportunistic (Barron, 2006).

In another hand *Penicillium expansum* was produced mycotoxins called patulin. Most of these species resemble each other in color characteristics, style of decay and infection symptoms; they fall under a general category called blue mold. *P. expansum* was one of the most aggressive species, these fungi live a long time and were quite durable, even under adverse conditions (Williams et al., 2006).

The toxicity effect of organic and inorganic compounds on the growth of some fungi was studied by many investigators (Al-Obaid et al., 1996) (Al-Sarrani, 2005).

However, comparative study between these compounds in different concentrations still needs further investigations. Therefore, the present studies were carried out to throw some light upon the effect of different concentrations of certain organic and inorganic compounds on the growth of the fungi.

MATERIALS AND METHODS TEST ORGANISM

Fungal isolates:

Aspergillus flavus, *P. chrysogenum* and *P. expansum*, were obtained from Dr. Faten Noori A refai, Department of Biology, University of Mosul (AL-Rrefai, 2006).

Inoculums preparation:

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 subculturing of the test organism as well as for preparation of fungal inoculum. 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, (Pitt and Hocking, 1997) and (AL-Rrefai, 2006).

Experimental media:

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

Expressions of results:

Fungal growth was determined by measuring the diameter of colony radial growth in mm. Data were recorded in the triplicates after 8 days of incubation at 28 °C .results were expressed as percentage inhibition control.

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 8 days of incubation at 28 °C.

Total dish diameter = 90 mm.

The colony radial growth diameter = 90 mm.

Growth % = $90 / 90 * 100 = 100 \%$ (control)

To calculate the %Inhibition of *A. flavus* by Boric acid (1% Conc) of 60mm colony diameter:

Inhibition diameter = Total dish diameter - colony diameter = $90 - 60 = 30\text{mm}$.

%Inhibition = Inhibition diameter / Total dish diameter * 100 = $30 / 90 * 100 = 33.30\%$ (AL-Rrefai, 2006).

RESULTS AND DISCUSSIONS

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 (Hitchcock, 1991) and as we were expected this inorganic compound was less effect on growth of these fungi (*Aspergillus flavus*, *Penicillium chrysogenum* and *Penicillium expansum*). Tables (1, 2 and 3) show the maximum inhibition, for 2% boric acid concentration (44.4, 52.2 and 61.1 %) respectively, while that of Gentian Violet (78.8, 77.7 and 76.6%) respectively as were appeared in Table (4).

Table 1 : Colony Diameter (mm) and % Inhibition of *Apergillus flavus* on (SAM) medium treated with Boric acid.

Boric acid Conc. %	Colony Diameter (mm)	% Inhibition
0.0	90	0
1	60	33.3
2	50	44.4

Table 2 : Colony Diameter (mm) and % Inhibition of *P. chrysogenum* on (SAM) medium treated with Boric acid.

Boric acid Conc. %	Colony Diameter (mm)	% Inhibition
0.0	90	0
1	52	42.2
2	43	52.2

Table 3 : Colony Diameter (mm) and % Inhibition of *P. expansum* on (SAM) medium treated with Boric acid

Boric acid Conc. %	Colony Diameter (mm)	% Inhibition
0.0	90	0
1	40	55.5
2	35	61.1

Table 4 : Colony Diameter (mm) and % Inhibition of Fungi on (SAM) medium treated with gentian violet

<i>Aspergillus flavus</i>		<i>P. chrysogenum</i>		<i>P. expansum</i>	
% Inhibition	Colony Diameter (mm)	% Inhibition	Colony Diameter (mm)	% Inhibition	Colony Diameter (mm)
78.8	19	77.7	20	76.6	21

We attributed all these results to the high solubility of the dye (compared to inorganic boric acid) in non polar organic lipids due to increasing of its organic structure.

This structure was amphiphilic, i.e. has property of solubility in both aqueous (hydrophilic) and organic (lipophilic) phases Fig. (2) (Block et al., 2004).

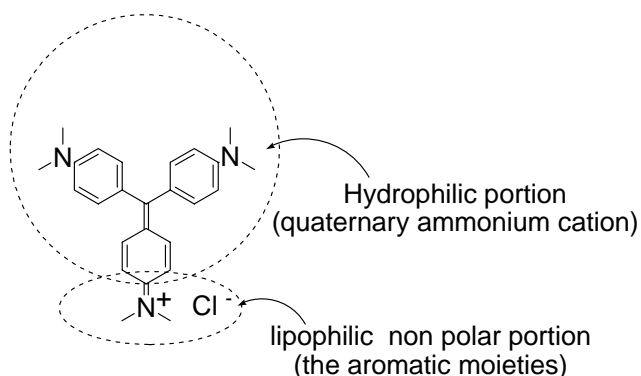


Fig. 2 : Gentian Violet (Block et al., 2004)

Thus, and in order for this compound to be effective at site in the body (the cell membrane) and it must also be able to reach those sites (Weininger and Stermitz, 1984). For this reason a lipophilic non polar portion (the aromatic moieties) carry this compound through the nonpolar cell membrane. While the polar portion (quaternary ammonium cation), hydrophilic portion was allowed for water solubility and reactive at that site. On the other hand the inorganic boric acid has only the hydrophilic property and different to reach the site, thus it was less active (Weininger and Stermitz, 1984).

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