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MUTUAL EFFECT OF CHITOSAN DERIVATIVES AND SOME MICROBES ON THE MICROBIAL ACTIVITY

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ABSTRACT

Blends of chitosan with different organic compounds such as carbohydrates and water-soluble chitosan derivative with glycidyltrimethylammonium chloride have been prepared. The obtained products have been cross linked with glutaraldehyde. Chitosan has been also treated with petroleum ether. The antimicrobial activity of the above mentioned materials were tested at different concentrations of 10 to 50 mg ml $^{-1}$ against some opportunistic microorganisms. Microorganisms were selected on the base of the diseases that may cause to humans. They are the yeast Candida albicans, the fungus Aspergillus niger and the bacterium Burkholderia cepaci. The effect of chitosan derivatives was variable and depends on the microbial species used which reached in some cases to a maximum extent of inhibition or lethality. In terms of the biological standpoint, the impact depends on the microbial cell wall components and the enzyme content. It can also be attributed to the degree of deacetylation and molecular weight of chitosan derivatives as well as pH of the nutritional media where chitosan derivative was used by some microbes as a carbon source. The mutual effect of microbes and chitosan derivative on biological activity of microbes and on nature of chitosan derivative has been studied by FT-IR analysis. Findings showed clearly

that the interaction of chitosan and microorganism was influenced by several factors such as the molecular size, molecular weight and functional groups of chitosan derivatives.

Keywords: Chitosan; Microbial activity; Biopolymers

1. INTRODUCTION

Chitosan is the most important derivatives of chitin, Chitin is a polysaccharide of animal origin found abundantly in nature and characterized by a fibrous structure. It formed the basis of the main constituent of the outer skeleton of insects and crustaceans like shrimp, crabs and lobster [1, 2, 3] as well as in the cell wall of some fungi such as yeast *Candida neoformans* [4]. Chitosan can be obtained from chitin by deacetylation of chitin [4, 5].

Chitosan is primarily characterized by its molecular weight (MW) and the degree of acetylation (DA). Commercially chitosan is available with > 85% deacetylated units (DA < 15%), and molecular weights (MW) between 100 and 1000 k Da. There is no a specific standard to define MW, but it is accepted that Low MW < 50 k Da, Medium MW 50 - 150 k Da, and High MW > 150 k Da.

Chitosan is polyglucosamine, polycationic, non-toxic and biodegradable .The presence of free amino groups helps in biological activities. One of these is the antimicrobial activity **[6, 7]**.

Omura measured the antimicrobial activity (Minimum Inhibitory Concentration, MIC) of chitosan and chitooligosaccharides with different molecular weights but without acetylated groups .They observed that chitosan with high molecular weight showed strong antimicrobial activity against Gram positive bacteria ,whereas chitosan of 11k Da and 20-30 k Da molecular weight were most effective against Gram negative bacteria **[8]**. It has been recently reported that the antibacterial activity of chitosan against *Escherichia coli* and *Staphylococcus aureus* increased with ionic strength and at a pH less than 6.0, while it decreased with the addition of metal ion. Additionally, they observed that the antibacterial activity of chitosan increased against *E. coli* in the presence of EDTA, but there was no significant change against *Staphylococcus aureus*. The antibacterial activity of chitosan was also found to be dependent on its charges and solubility **[9]**.

In this work, the mutual effect between some chitosan derivatives and microbial activity of some microorganisms was evaluated.

2. MATERIALS AND METHOD

2.1. Materials: Low molecular weight Chitosan and other chemicals were purchased from Aldrich, Milwaukee, USA unless otherwise mentioned and used without further purification. Blends with Chitosan have been prepared according to the method mentioned earlier **[1]**.

2.2. Microorganisms: Microbial isolates were obtained and used in the Laboratory of Microbiology, Faculty of Applied Sciences, Umm Al-Qura University in Makkah Al-Mukarramah. These were the yeast *(Candida albicans)*, the fungus *(Aspergillus niger*) and bacteria *(Burkholderia cepaci)*. They have been processed in a suitable environment for growth.

Table 1 shows the physical and chemical properties of some chitosan derivatives used for investigation of their biological activity.

Table 1: **Physical and chemical properties of some chitosan derivatives used for investigation of their biological activity**

Sample	Composition	pH	Active Groups
1	Chitosan blend with glucose	5	NH_2, C=O, OH
2	Chitosan blend with glucose and GT[1]	5	NH_2, C=O, OH
3	Chitosan treated with petroleum ether sand GT[1]	9	NH_2, C=O
4	Chitosan blend withGTMA[2]	7-9	NH_2, ,N^+R_3
5	Chitosan blend with GTMA[2] and GT[1]	7-9	NH_2, C=O,N^+R_3

[1]: Glutaraldehyde [2]: Glycidyltrimethyl ammonium chloride

3. RESULTS AND DISCUSSION:

From fig.1, most chitosan derivatives (10mg / ml) recorded an anti-bacterial activity and a total inhibition in the bacterial growth up to stop it completely in case of D2. The same concentration (**10** mg / ml) induced an increase in the growth of bacteria, as shown in the case of D**5** .A concentration of 10 mg / ml of the Chitosan derivatives accelerated the growth of fungus in all cases compared to the control as in fig.1.

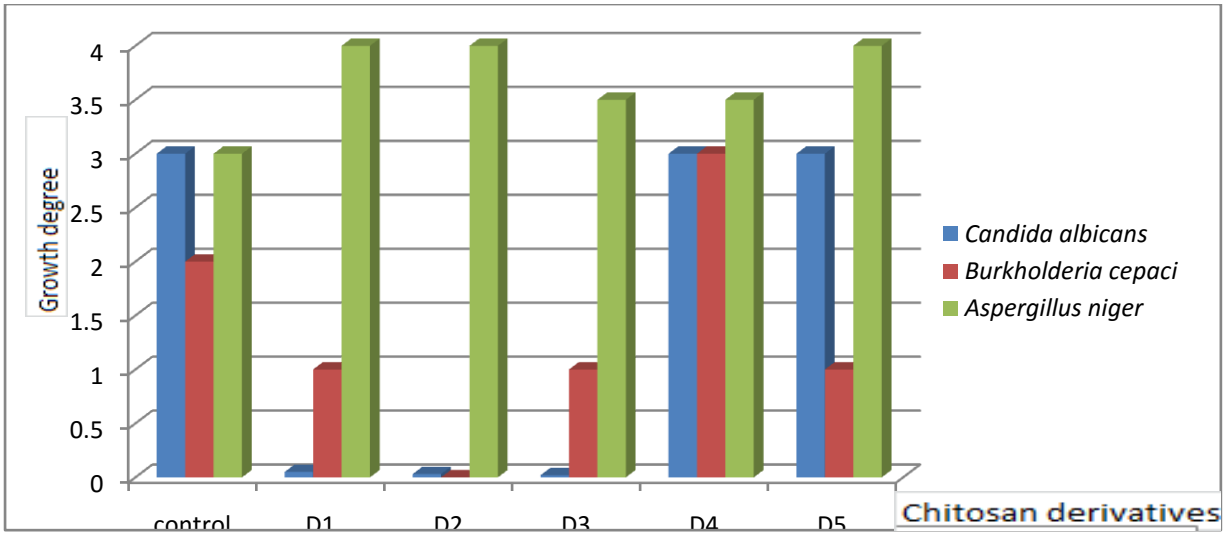

Fig1. Effect of Chitosan derivatives (10mg/ml) on the growth of the yeast *candida albicans* and the fungus *Aspergillus niger* in liquid Czapecks medium and the bacteria *Burkholderia cepaci* in mineral liquid medium

On contrary Chitosan derivatives accelerated the growth of *A. niger* especially D1, D2 and D5.The fungus may be contain enzyme like chitinase which degraded these compounds and exhausted as carbon and nitrogen sources.

Three hypotheses can take into account the mechanical work of chitosan and its derivatives as antimicrobial. First hypothesis is the most widely accepted, is the interaction positive charges for chitin or chitosan particle with negative charges of the microbial cell membrane through electrostatic force between molecule + NH_3 with negative charge on the surface of the membrane and thus these compounds operated on the inhibition of microbial growth. The second assumption is that the link between chitosan and nucleic acids of the microbe which leads to inhibition of ribosomal DNA and messenger RNA which leads to inhibition of protein synthesis **[10-12]**. The third hypothesis is a mechanical antagonism by grab the minerals and suppression of the elements that the microbial cell growth need **[13-14]**.This method depends on the pH, if the pH value is high, the microbial cell linked to positive ions of chitosan and if the pH is low, its negative ions united with chitosan**[15]**.

Chitosan as well as derivatives worked anti-microbial activity and not as a germicide .Since the mechanical interaction is still under study and interferes with several factors, interactions antibacterial, it is clear from the previous results that the work of chitosan and its derivatives depends mainly, in terms of chemical, on particle size and weight as well as the functional groups. It also depends on pH and the degree of acetylation.

The proportion NH groups for Chitosan derivatives have demonstrated a large scale of variation shown in fig.2 and 3. The results showed that the Chitosan derivatives have significant changes in the values of the proportion of NH and C= O groups when contacted with yeast or bacteria. The acetylation of these derivatives has to proceed with varying degrees completely different. Table 2 showed a summary of the values of the ratio NH / C= O samples under study.

The analysis of X-infrared (FT-IR) illustrated in fig.2a and 3a, it indicated the control before treatment with yeast or bacterium in medium recorded a peak of the absorption at 3366 cm-1 characterized the NH group, and other peak of absorption at 1725cm-1 characterized the carbonyl group.

The analysis X-infrared (FT-IR) of Chitosan derivatives 1 and 3 has indicated in fig. 2b and 3b after treated with *C. albicans* .The peak raised to the top of the absorption at 3339 cm-1 characterized the NH group, the other peak at 1667cm-1 indicated the carbonyl group.

IR analyses of Chitosan derivatives after exposure to the growth of bacterium *B. cepaci* in a nutritional mineral medium at concentration of 10mg/ml were shown in the fig 2c and 3c .IR spectrum has pointed to the emergence of an absorption peak at 3339cm-1that characterized a NH group. The peaks of absorption appeared at 1667 and 1219 cm-1 absorption, which marked the carbonyl group.

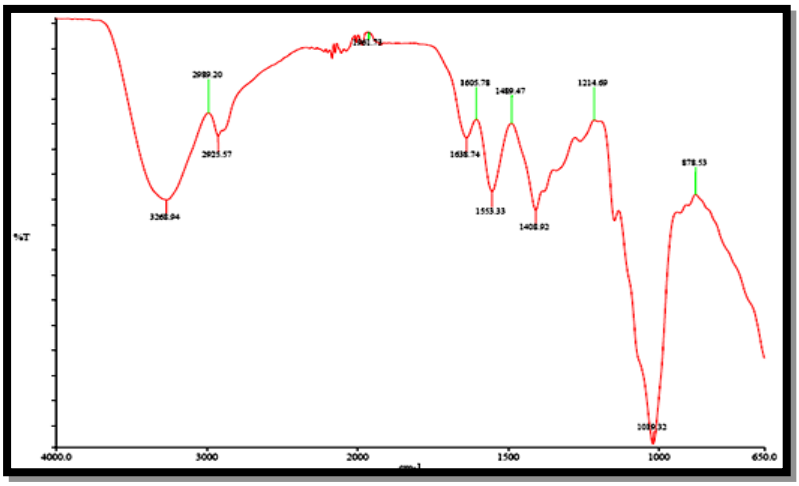

Fig.2, (a) Infrared Spectrum Analysis of Chitosan derivatives 1 without treatment

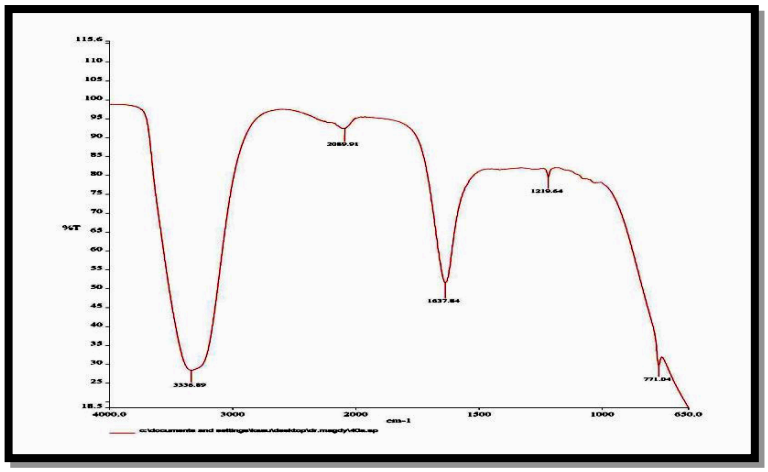

Fig.2, (b) Infrared Spectrum Analysis of Chitosan derivatives **1** exposed to the growth of *B. cepaci*. of *C. albicans*.

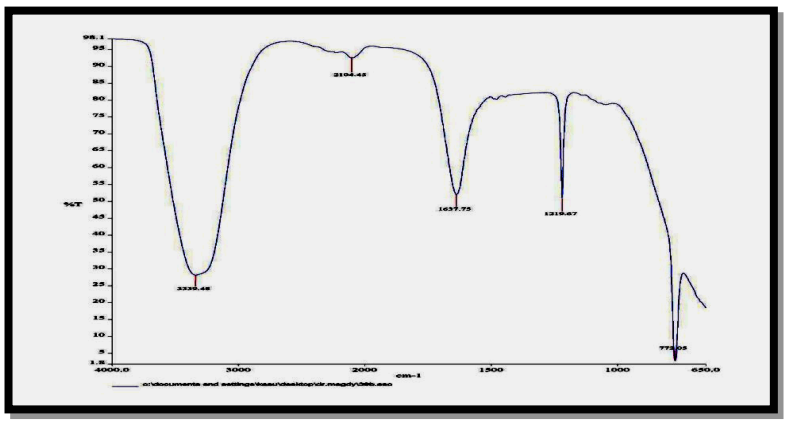

Fig.2, (c) Infrared Spectrum Analysis of Chitosan derivatives **1** exposed to the growth of *B. cepaci*.

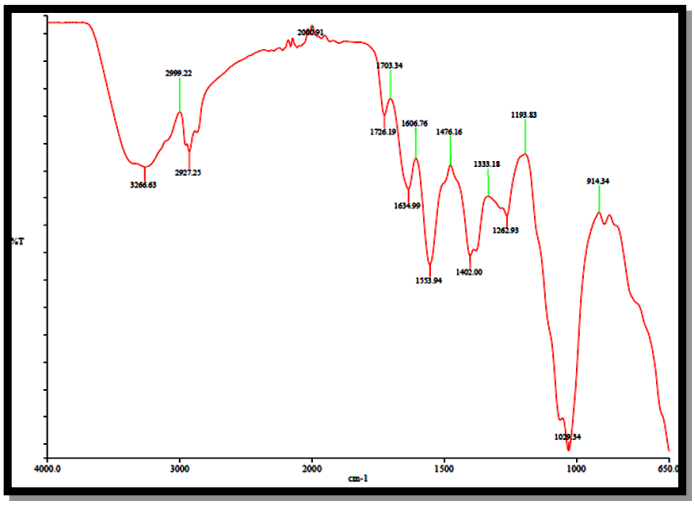

Fig.3, (a) Infrared Spectrum Analysis of Chitosan derivative **3** without treatment

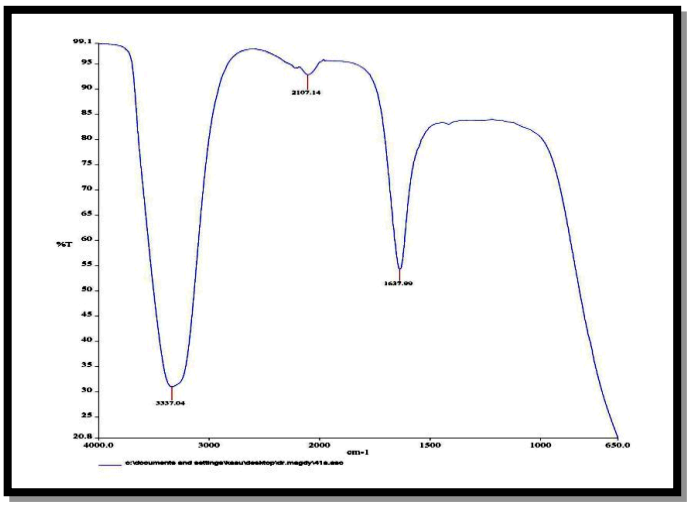

Fig.3, (b) Infrared Spectrum Analysis of Chitosan derivative **3** exposed to the growth of *C.albicans*

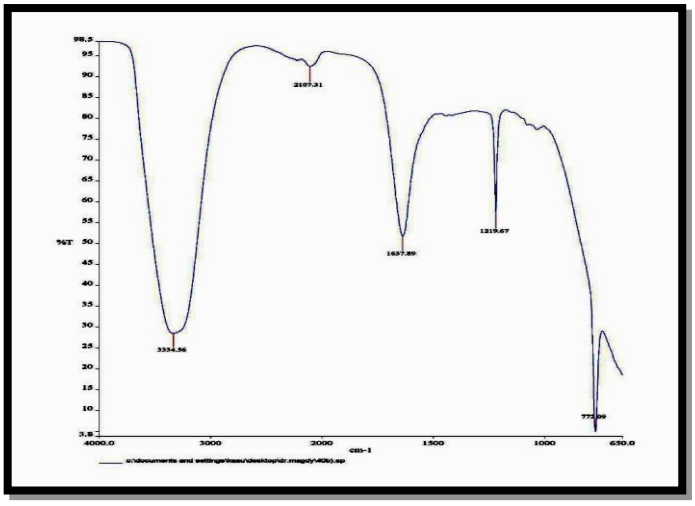

Fig.3, (c) Infrared Spectrum Analysis of Chitosan derivatives **3** exposed to the growth of *B. cepaci*

The ratio NH / C=O of Chitosan derivatives before and after treated with yeast and bacterium illustrated in table 2. Ratio NH / C=O of the control sample were calculated and found to be equal to 1.73, but with media of yeast equal 2.6, and with media of bacteria equal to 2.9. Ratio NH / C=O of the control sample were calculated and found to be equal to 1.73, but with media of yeast equal 2.6, and with media of bacteria equal to 2.9. The growth *C. albicans* and *B. cepaci* enhanced the ratio NH / C=O in media in all Chitosan derivatives used. It ranged from 1.81 to 1.90 before treatment but after growth, the ratio jumped up between 2.10 and 3.12. *C. albicans* recorded the least increase of ratio by14.75% in media contained Chitosan derivative1 and *B. cepaci* recorded the highest increase by 64.21% in media contained derivative 3.

Table 2: The ratio NH / C=O of Chitosan derivatives before and after treated with yeast and bacterium

Treatment	NH / C=O ratio of the treated chitosan derivatives					
	Control (media without Chitosan)	**1**	**2**	**3**	**4**	**5**
After *C. albicans*	2.68	2.10	2.70	2.90	3.54	3.02
After *B. cepaci*	2.00	2.80	2.90	3.12	3.02	2.93
Before treatment	1.70	1.83	1.84	1.90	1.81	1.90

CONCLUSION:

In biological terms, The activities of chitosan, whether disinfect or fatal depended on the type of organisms, composition of cell wall, enzymes contained, concentration or dosage used for the material under study .This area needed to a broad and comprehensive study of biological effect of chitosan on the growth of micro-organisms and how to use in the medical and industrial fields. Chitosan and most derivatives showed a clear influence on some microorganisms, where it had gradual influence, sometimes reached to the full extent of inhibition or lethality, depending on the type of microorganisms as well as the type of

chitosan derivatives. This area needs to comprehensive study to determine the impact of chitosan on biological growth of micro-organisms and how to use.

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