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Comparison of Antibacterial Activity of Fungal Chitosan and Some Preservatives Against Some Foodborne Pathogenic Bacteria

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ANTIBACTERIAL activity of chitosan, sodium nitrite and sodium benzoate were studied by disc diffusion assay. Inhibition percentage, minimal inhibitory concentration and effect of MIC of chitosan on the survival of pathogenic bacterial strains were compared. Results showed that chitosan, sodium benzoate and sodium nitrite exhibited antibacterial activity against all the tested pathogens, namely *E. coli*, *S. typhimurium*, *B. cereus* and *Staph. aureus*. Inhibition of all strains increased with increasing concentrations of fungal chitosan and preservative.

Keywords: Chitosan, Antibacterial activity, Preservatives, Minimal inhibitory concentration, *E. coli*, *S. typhimurium*, *B. cereus* and *Staph. aureus*.

Chitosan is a copolymer of D-glucoseamine and N-acetyl-D- glucoseamine units, drived from the deacetylation of chitin in the presence of hot alkali. Chitosan and its derivatives can be variously used as a permeability control agent, an adhesive, a paper sizing agent, a fining agent, flocculating and chelating agents, an antimicrobial compound and a chromatographic support (Shahidi *et al*, 1999). Chitosan has antifungal activity against many plant pathogens (El-Mougy *et al.*, 2002). Chitosan is a non-toxic compound which was reported to induce resistance against soil born fungi (Benhamou & Theriault, 1992 and Abd-Elkareem, 2002). The aim of this work was to study the effect of fungal chitosan on some Gram-positive and Gram-negative food-borne pathogenic bacteria compared to selected chemical preservatives.

Materials and Methods

Preparation of chitosan solution

Chitosan was obtained from sphinx for international Trade Company, Cairo, Egypt. Stock chitosan suspension 2% (w/v) was prepared in 1% (v/v) acetic acid. The applied concentrations of chitosan were 750, 1000, 1500, 2000 ppm. The chitosan suspension was stirred overnight at room temperature, and filtered to remove potential impurities according to Wu *et al*. (2005).

Preparation of preservatives

Sodium benzoate and sodium nitrite were prepared by dissolving in liquid medium. The final concentrations of sodium benzoate were 5000, 7500, 10000 and 12500 ppm while, sodium nitrite concentrations were 500, 1000, 1500 and 2000 ppm according to the method described by Stanojevic *et al.* (2010). Preservative solutions were heat-treated at 80°C for 15 min before testing.

Determination of antibacterial activity of fungal chitosan and some preservatives by disc diffusion method

Two concentrations of chitosan (750 & 1000 ppm), sodium benzoate (5000 & 10000 ppm) and sodium nitrate (1000 & 2000 ppm) were applied for disc diffusion. Petri dishes containing trypton soy agar medium were individually inoculated with the pathogenic bacteria (*E. coli, Salmonella typhimurium, B. cereus and Staph. aureus* then the impregnated discs were added and the plates were incubated at 37 ˚C for 24 h according to Logesh *et al.* (2012). The zone of inhibition was measured and the results were recorded.

Determination of antibacterial activity of the produced chitosan as compared with some preservatives by optical density

The minimal inhibitory concentration was defined as the lowest concentration of an antimicrobial agent at which there was no visible growth of the microbe.

Chitosan was added to tryptic soy broth (TSB) to give a final chitosan concentration of 2000 ppm (0.2% w/v). The pH of the broth was adjusted to 4.5 with 1NHCl before autoclaving at 121 °C for 15 min according to Balicka *et al*. (2005) and dispensed at 10 ml per test tube. Different quantities of preservatives were added to test tubes to reach the final concentration. One tenth ml of the pathogenic bacterial culture approximately (10^9CFU/mL) was added to 10 mL broth medium supplemented with preservatives and incubated at 37 °C for 48 h. MIC was calculated as the concentration, which inhibits 95% of bacterial growth (Barakat, 2010). At the end of the incubation period, turbidity was determined by measuring the absorbance at 600 nm using a spectrophotometer according to the method described by Theis *et al.* (2003). The inhibition percentage was calculated using the following equation.

$$\text{Inhibition percentage} = \frac{\text{OD* control positive - OD test}}{\text{OD control positive}} \text{ X100}$$

*OD: Optical density

Inhibitory effect of chitosan and some preservatives on survival of pathogenic bacteria

The antimicrobial activity of chitosan solution against some pathogenic bacteria was determined by plate counts. Ten ml of tryptic soy broth supplemented with MIC of chitosan, sodium nitrate or sodium benzoate were

individually inoculated with 0.1 ml of the each of the pathogenic bacterial cultures. Non- supplemented medium was used as control. The inoculated tubes were incubated at 37˚C for 48h with cell counts taken after, zero, 6, 12, 18, 24, 30, 36, 42 and 48 hr .The inhibition ratios were calculated with the following formula according to the method described by Khalaf (2004):

$$\text{Inhibition percentage} = \frac{\text{Control count} - \text{treatment count}}{\text{Control count}} \text{ X100}$$

Results and Discussion

Antibacterial activity of chitosan and some preservatives

Data in Fig. 1a&b show that increasing the concentrations of chitosan and preservatives increased the antibacterial activity for *E. coli*, *S. typhimurium*, *B. cereus* and *Staph. aureus*. This result is in harmony with Guirguis *et al.* (2013) who found that increasing the concentration of chitosan led to increasing in the antimicrobial activity for *S. aureus* and *B. subtilis*.

Concentration of 1000 and 2000 ppm for sodium benzoate and sodium nitrite, respectively, gave higher inhibition zones for all tested bacteria compared to fungal chitosan at 1000 ppm. In general, sodium benzoate at 10,000 ppm gave the highest inhibition zone against *E. coli*, *S. typhimurium*, *B. cereus* and *Staph. aureus* being 19, 16, 15 and 13 mm, respectively, while, the fungal chitosan at 1000 ppm gave antibacterial activity being 8, 6, 13 and 11 mm, respectively.

This result is in agreement with Islam *et al.* (2011) who studied different concentrations of chitosan namely, 400, 600, 800 and 1000 ppm. They found that zone of inhibition (mm) was 10, 12, 13 and 13, respectively for *Staph. aureus* while the zone of inhibition (mm) was 8, 8, 10, 10, respectively for *E. coli*. Data recorded that fungal chitosan at concentration 1000 ppm exhibited antibacterial activity against Gram + pathogens (*B. cereus* and *Staph. aureus*) greater than Gram - pathogens (*E. coli* and *S. typhimurium*).

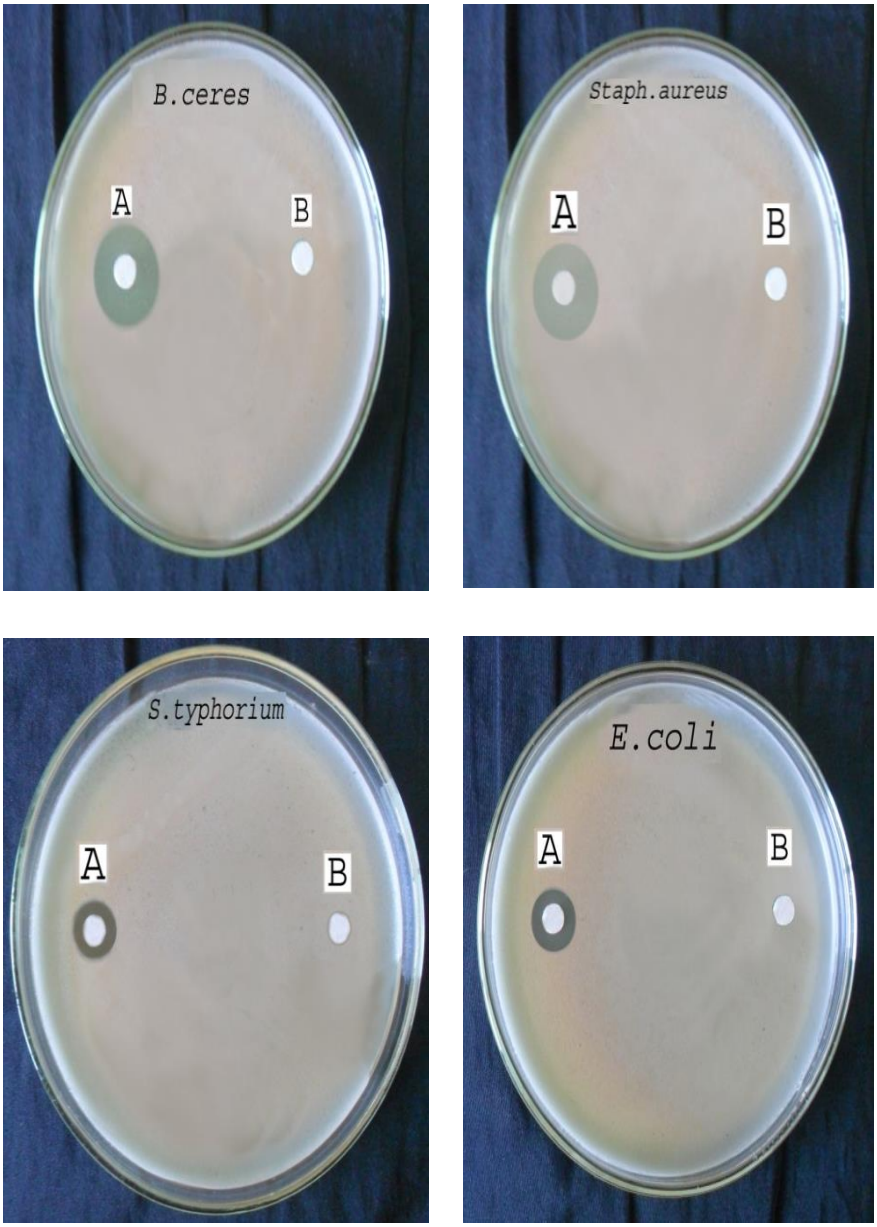

Fig.1.a.Antibacterial activity of fungal chitosan by disc diffusion method

A: Fungal chitosan (1000 ppm) B: Control

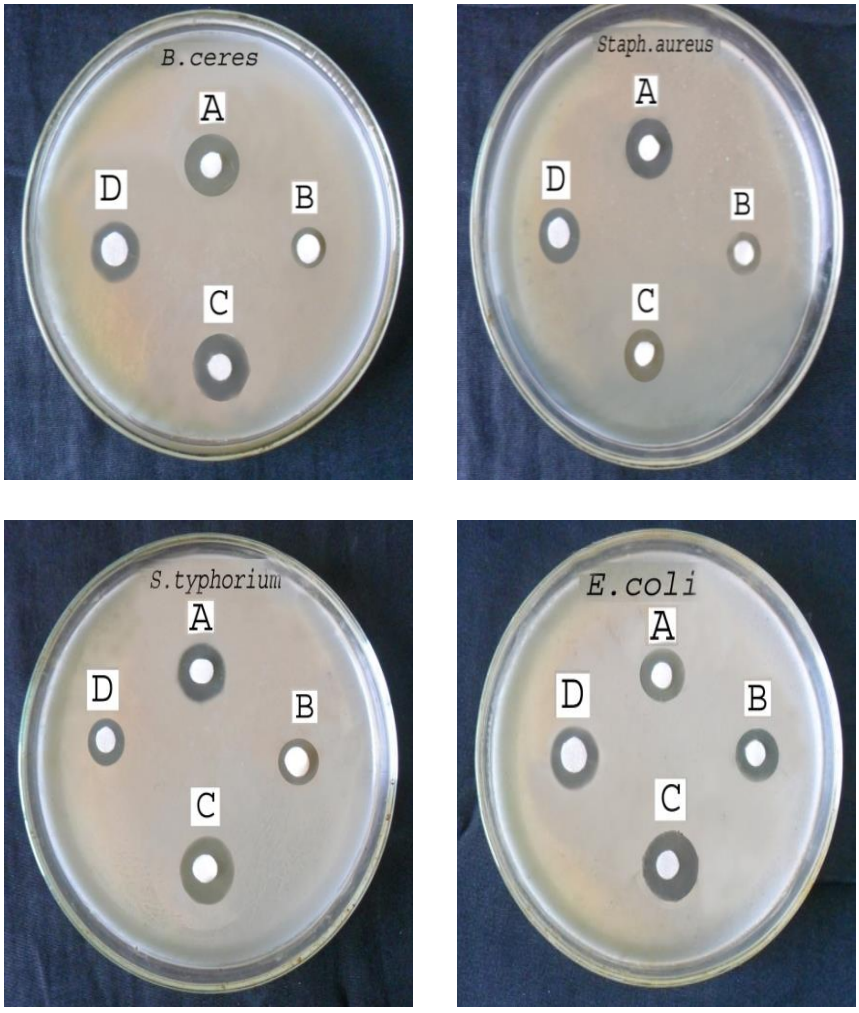

Fig. 1.b. Antibacterial activity of fungal chitosan by disc diffusion method. Fungal chitosan (1000ppm) of fungal chitosan compared to sodium benzoate and sodium nitrite

A: Fungal chitosan (1000 ppm)
B: Fungal chitosan (750 ppm)
C: Sodium benzoate
D: Sodium nitrite

Inhibition percentage and minimal inhibitory concentration

Data in Fig.2 show that inhibition in liquid culture of all strains increased with increasing concentrations of fungal chitosan and preservatives. Also, the data gave MIC values of 750 ppm when fungal chitosan used against *E. coli*, *B. cereus* and *Staphylococcus aureus* while, the MIC was 1000 ppm when chitosan was used against *S. typhimurium*.

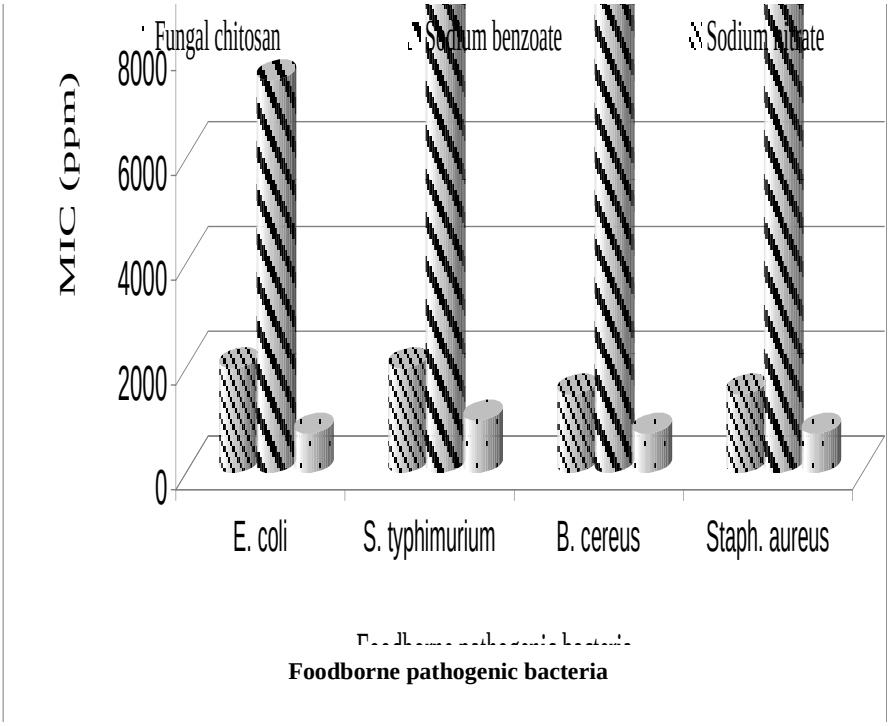

Fig. 2. Minimal inhibitory concentration (ppm) values of fungal chitosan compared to some preservatives against some foodborne pathogenic bacteria

These results are in harmony with Islam *et al.* (2011) who studied the effect of chitosan on the susceptibility of *Staph. aureus* and *E. coli*. They used different concentrations of chitosan namely 600, 800, 1000, 1200, 1300 and 1400 ppm. They found that the minimum inhibitory concentration (MIC) of the prepared chitosan was 1200 and 1300 ppm for *Staph. aureus* and *E. coli*, respectively.

It is worthily to mention that MIC of sodium benzoate was 7500 ppm for *E. coli* and 10000 ppm for *S. typhimurium, B. cereus* and *Staph. aureus*. While, MIC of sodium nitrite was 1500 ppm for *Bacillus cereus* and *Staph. aureus* and 2000 ppm for *S. typhimurium* and *E. coli*. These results are in agreement with Stanojevic *et al.* (2010) who investigated the antimicrobial effect of sodium benzoate and sodium nitrite on food-borne pathogenic bacteria such as *Bacillus subtilis, Bacillus mycoides, Staphylococcus aureus, Escherichia coli, Pseudomonas fluorescens, Proteus* sp. and *Pseudomonas aeruginosa*. They found the MIC for sodium benzoate was 5000 ppm while, MIC for sodium nitrite, it was 500 and 2000 ppm.

Effect of MIC of chitosan on the survival of pathogenic bacterial strains compared to some preservatives

Data in Fig. 3, 4, 5 and 6 showed that the counts of all pathogenic bacteria in the control treatment (without antibacterial agents) gradually increased with the

increasing of incubation period to reach their maximum values after 42 h for *E. coli* and 48 h for *S. typhimurium*, *Staph. aureus* and *B. cereus*. Data graphically illustrated in Fig 3 emphasize that the inhibition percentage for *E. coli* was 95.26 % after 42 h when using MIC of chitosan while, 96.21% and 95 % after 24 h and 30 h when using MIC of sodium benzoate and sodium nitrite, respectively. Also, data graphically illustrated by Fig. 4 showed that inhibition percentage of *S. typhimurium* reached to 95.16 %, 95.15% and 95.01 % after 48, 24 and 30 h of incubation when using chitosan, sodium benzoate and sodium nitrite, respectively. On the other hand, data graphically illustrated by Fig. 5 emphasized that the inhibition percentage of *B. cereus* were 95.90, 95.79 and 95 % after 24, 30 and 18 h of incubation when using MIC of chitosan, sodium benzoate and sodium nitrite, respectively. In addition, data graphically illustrated by Fig. 6 showed that the inhibition percentage of *Staph. aureus* was 95.13 % after 30 h when chitosan applied, while the inhibition percentage was 95.93 % after 24 h when using MIC of sodium benzoate and 95.02 % after 30 h when using MIC of sodium nitrite.

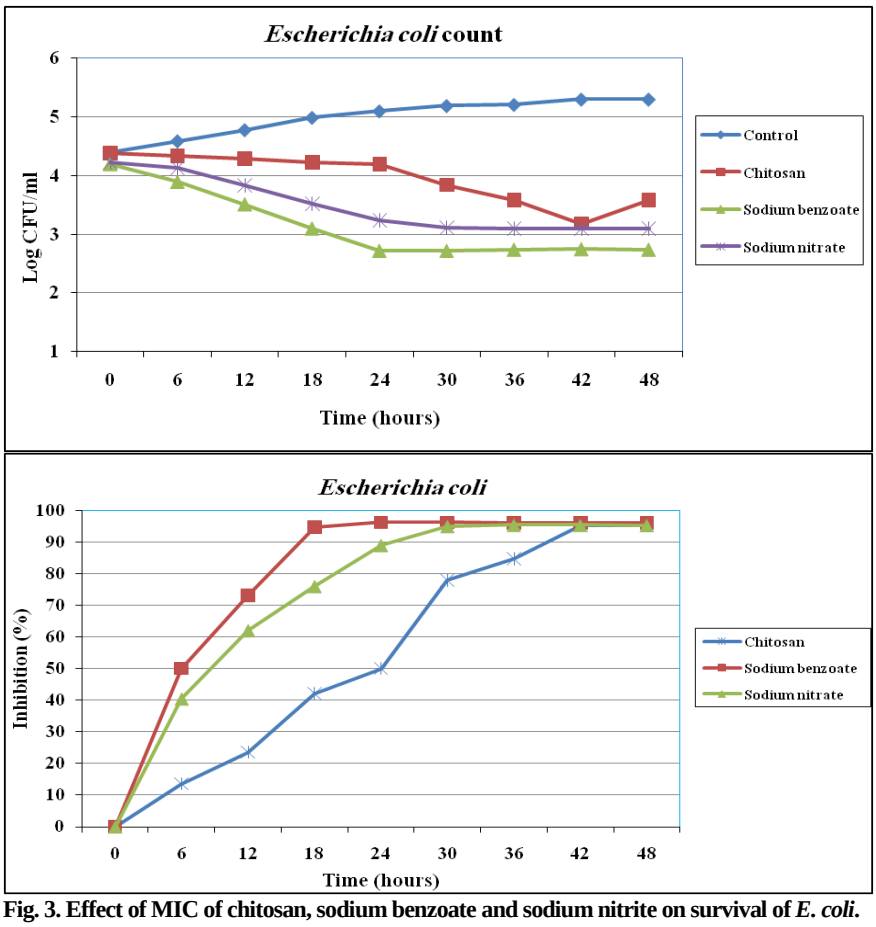

Fig. 3. Effect of MIC of chitosan, sodium benzoate and sodium nitrite on survival of *E. coli*.

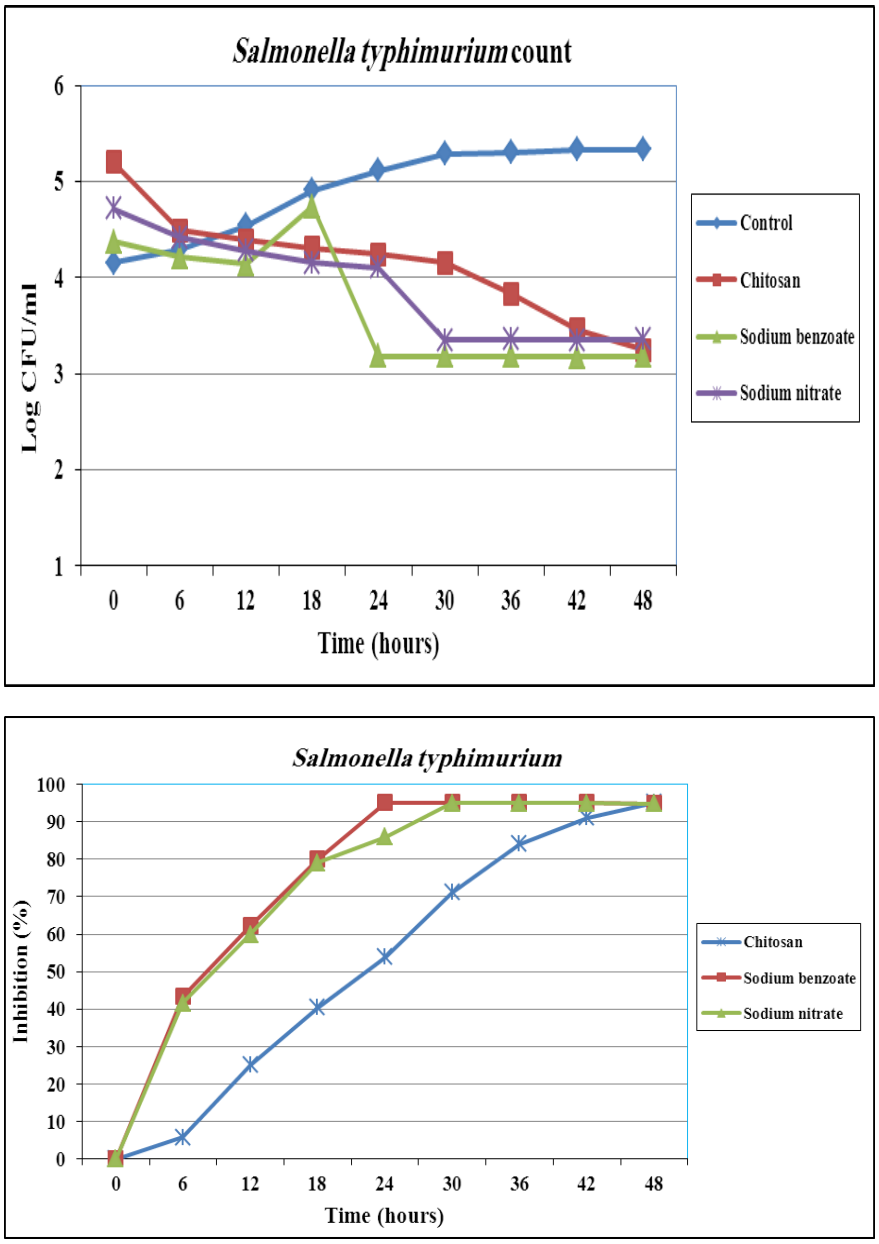

Fig. 4. Effect of MIC of chitosan, sodium benzoate and sodium nitrite on survival of *S. typhimurium*

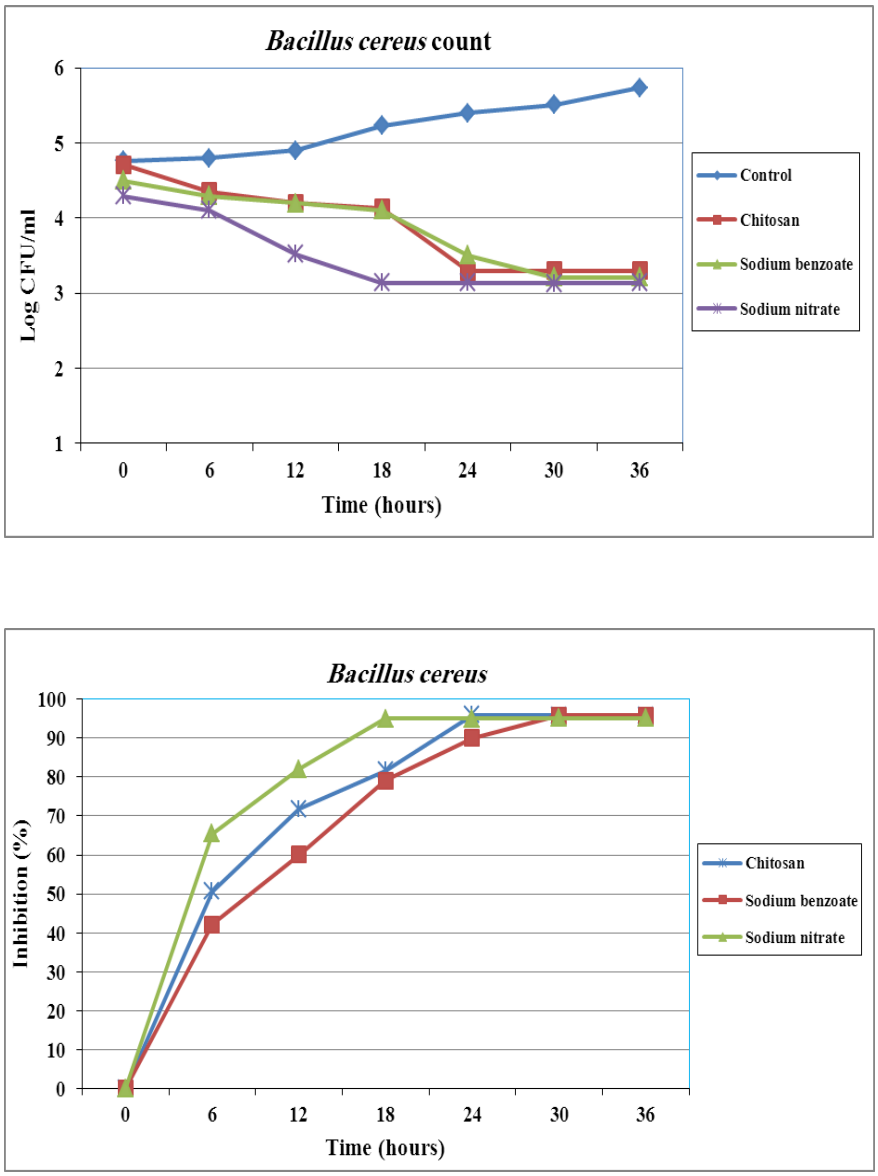

Fig. 5. Effect of MIC of chitosan, sodium benzoate and sodium nitrite on survival of *B. cereus*.

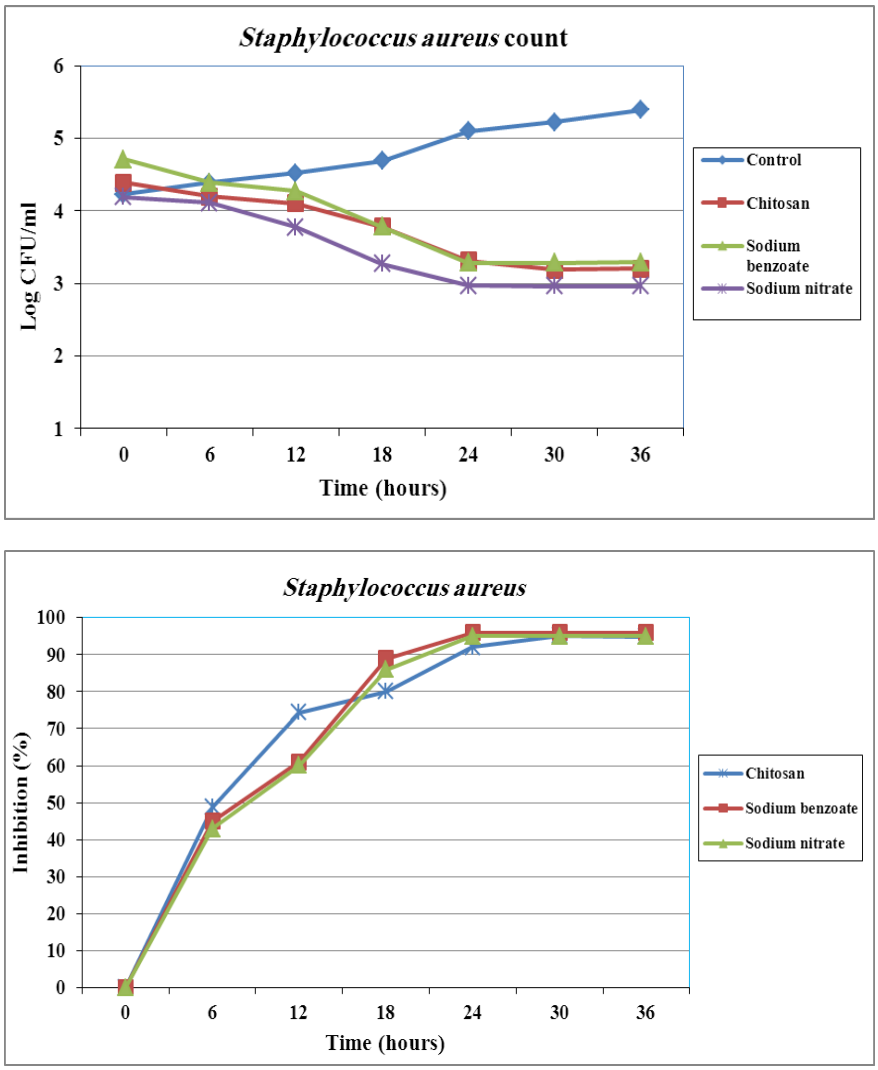

Fig. 6. Effect of MIC of chitosan, sodium benzoate and sodium nitrite on survival of *Staph. aureus*.

From the obtained data, it was worthwhile to mention that as has been previously reported (Tayel *et al*., 2011) chitosan is more effective on Gram positive bacteria than Gram negative bacteria.

Conclusion and Recomendation

In view of the obtained results, it was clearly that fungal chitosan showed good antibacterial activity against food borne pathogenic bacteria. Although this

chitosan was still less active than other chemical preservatives, it is edible and non-toxic for human health and likely a great deal more expensive.

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مقارنة النشاط التضادى للفطريات المنتجة للشيتوزان وبعض المواد الحافظة ضد بعض البكتريا المرضية المحمولة عن طريق الغذاء

راشد عبدالفتاح زغلول حامد السيد أبوعلى، طلعت محمد الحسينى ، غنيمى عبدالفتاح غنيمى و نهى محمد عشرى
كلية الزراعة بمشتهر- جامعة بنها و *معهد بحوث وتكنولوجيا الأغذية- مركز البحوث الزراعية – القاهرة – مصر .

تم إجراء هذاالبحث لدراسة النشاط التضادي للشيتوزان الفطري وبعض المواد الحافظة ضد البكتريا المرضية المحمولة عن طريق الغذاء بواسطة طريقة الانتشار القرصي، بالاضافة إلى دراسة نسبة التثبيط والحد الأدنى من التركيز المثبط وتأثير أقل تركيز مثبط من الشيتوزان على بقاء السلالات البكتيرية المسببة للأمراض بالمقارنة ببعض المواد الحافظة. أوضحت النتائج أن كل من الشيتوزان ، بنزوات الصوديوم ونتريت الصوديوم أظهرت نشاط تضادي ضد جميع المسببات المرضية .وأيضا أظهرت النتائج أنه بزيادة تركيز كل من الشيتوزان والمواد الحافظة يزداد النشاط التضادي ضد البكتريا المرضية وهي
E. coli, S.typhimurium, B.cereus and Staph.aureus

أيضا زادت نسبة التثبيط لكل السلالات بزيادة تركيزات الشيتوزان والمواد الحافظة. ومن ناحية أخرى أظهرت النتائج أن عدد البكتريا الممرضة في معاملة الكنترول يزيد مع زيادة فترة التحضين حتى يصل عدد الخلايا لأقصاها بعد 42 ساعة من التحضين لبكتريا بينما بعد 48 ساعة لكل من
E. coli., S.typhimurium, B.cereus and Staph. aureus