J. Pest Cont. & Environ. Sci. 15 (1): 69 -85(2007).

Effect of depolymerization degree of the natural biopolymer chitosan on some plant pathogenic bacteria and fungi

Mohamed E. I. Badawy
Department of Pesticide Chemistry, Faculty of Agriculture, 21545-El-Shatby, Alexandria University, Alexandria, Egypt.
E-mail: m_eltaher@yahoo.com

ABSTRACT

Three different molecular weights of chitosans were obtained from a commercial sample of chitosan by a method based on nitrous acid depolymerization. Molecular weights of chitosan samples were determined by measurements of intrinsic viscosity that were 0.5×10^4, 3.7×10^4 and 5.7×10^4 Dalton for the prepared compounds and 2.9×10^5 Dalton for the native chitosan sample. The effect of antimicrobial activities of these compounds against some economic plant pathogenic bacteria and fungi was investigated. The antibacterial assessment of the chitosan compounds was performed with Nutrient Agar dilution method against the bacteria cultures of crown gall disease (*Agrobacterium tumefaciens*) and soft mould disease (*Erwinia carotovora*) and the results are expressed as Minimum Inhibitory Concentration (MIC). In general, *E. carotovora* is more sensitive to these compounds than *A. tumefaciens*. The result also indicated that chitosan with a molecular weight of 0.5×10^4 Dalton exhibited a good antibacterial potency against *A. tumefaciens* with MIC = 2600 $mg.L^{-1}$ while chitosan with a molecular weight of 3.7×10^4 Dalton is the most active one against *E. carotovora* with MIC = 950 $mg.L^{-1}$. The antifungal assessment against the leaf spot and blight (*Alternaria alternata*), the grey mould (*Botrytis fabae*), the root rot disease (*Fusarium oxysporum*) and the black mould (*Rhizopus stolonifer*) was achieved using the mycelial radial growth inhibition technique and the results are expressed as Minimum Effective Concentration of 50% of mycelia growth (EC_{50}). Generally, the result demonstrated that the antifungal activity was increased with the decreasing of the molecular weight and a chitosan low molecular weight (0.5×10^4 Dalton) was exhibited a high antifungal potency against *B. fabae*, *F. oxysporum*, and *R. stolonifer* with EC_{50} of 1392, 1290 and

2232 mg.L^{-1}, respectively while the fungus of *A. alternata* was more sensitive to a chitosan of 3.7×10^{4} Dalton with EC_{50} = 1765 mg.L^{-1}.

Keywords: Chitosan; Depolymerization; Intrinsic viscosity; Average molecular weight; Antibacterial activity; Antifungal activity.

INTRODUCTION

Chitosan is a well studied linear polysaccharide, polymerized from the monomers 2-acetoamido-2-deoxy-β-D-glycopyranose (GlcNac) and 2-amino-2-deoxy-β-D-glycopyranose (GlcN), which contains a higher part of GlcN causing the solubility of chitosan in aqueous media and it is produced from crustaceous shell waste (No and Meyers, 1997).

Chitosan and its derivatives have been used in a wide variety of applications (Rabea *et al.*, 2003), but the properties and effectiveness of these materials are dependent upon their molecular size; thus a need is evident for a controlled method of manipulating this characteristic. Chitosan is, like other polysaccharides, susceptible to a variety of degradation mechanisms, including oxidative-reductive, free radical depolymerization, acid-, and alkaline- and enzymatic-catalyzed hydrolysis. Degradation occurs via cleavage of the glycosidic bonds. To control the depolymerization process of chitosan, it is useful to control properties like viscosity, solubility and biological activity (Boryniec *et al.*, 1997). Studies recommended nitrous acid, hydrochloric acid, phosphoric acid as well as hydrogen peroxide for chitosan depolymerization (Allan and Peyron, 1995a and b; Chang, *et al.*, 2001 and Varum *et al.*, 2001).

Chitosan depolymerization by reaction of nitrous acid (HONO) is particularly advantageous because the reaction between chitosan and HONO, Fig. 1, has been investigated, and the reaction products are established (Liu *et al.*, 1997 and Mao *et al.*, 2004). Liu *et al.*, (1997) found that $NaNO_2$ showed the best performance and this result was recently confirmed, but no detailed experimental information was provided (Janes and Alonso, 2003). Nitrosating species originating from HONO selectively attack the amine groups and subsequently cleave the glycosidic linkages of the polymer chain. One mole of HONO is consumed per mole of amine group reacted, and

a 2,5-anhydro-D-mannose unit is formed at the reducing end of the cleaved polymer (Sashiwa *et al.*, 1993 and Tømmeraas *et al.*, 2001).

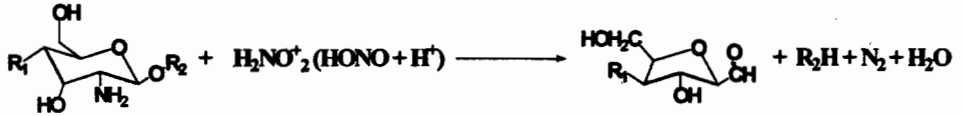

Figure 1. Depolymerization reaction of chitosan with nitrous acid

The antibacterial effect of chitosan and chitosan oligomers is reported to be dependent on its molecular weight (Jeon *et al.*, 2001); however, most studies involved only one or a few different molecular weights of chitosans or chitosan oligomers (Tsai and Su, 1999 and No *et al.*, 2002). Thus, special information is lacking on the antibacterial activity of chitosans and chitosan oligomers with widely different molecular weights. The main factors affecting the antibacterial activity of chitosan are molecular weight and concentration. There are some reports that chitosan is more effective in inhibiting growth of bacteria than chitosan oligomers (No *et al.*, 2002) and the molecular weight of chitooligosaccharides is critical for microorganism inhibition and required higher than 10,000 Da (Jeon and Kim, 2000). In addition, numerous studies on antifungal activity of chitosan against plant pathogens have been carried out (Allan and Hadwiger, 1979; El Ghaouth *et al.*, 1992 and Xu *et al.*, 2007) and reviewed (Bautista-Banos *et al.*, 2006 and Rabea *et al.*, 2003). Chitosan's inhibition of fungi was observed on different developmental stages such as mycelial growth, sporulation, spore viability and germination, and the production of fungal virulence factors (Xu *et al.*, 2007). It has been commonly recognized that antifungal activity of chitosan depends on its molecular weight, deacetylation degree, pH of chitosan solution and the target organism. Mechanisms proposed for the antifungal activity of chitosan focused mainly on its effect on fungal cell wall (Allan and Hadwiger, 1979; Bautista-Banos *et al.*, 2006; El-Ghaouth *et al.*, 1992 and Rabea *et al.*, 2003) and cell membrane (Leuba and Stossel, 1986).

In the present study, a series of chitosan compounds with different molecular weights were prepared by depolymerization reaction using nitrous acid from

a commercial chitosan sample. The biological activity (*in-vitro*) of the chitosan compounds was evaluated against the most economic plant pathogenic fungi of leaf spots and blights (*Alternaria alternata*), the grey mould (*Botrytis fabae*), root rot disease (*Fusarium oxysporum*) and black mould (*Rhizopus stolonifer*) and the bacteria of crown gall disease (*Agrobacterium tumefaciens*) and soft mould disease (*Erwinia carotovora*).

MATERIALS AND METHODS

Materials: Chitosan of low viscosity was purchased from Vanson Co. (Redmond Washington, USA). Acetic acid, hydrochloric acid, sodium acetate, sodium nitrite, sodium hydroxide were used without further purification. For viscosity determination, Ubbelohde viscometer (capillary section size 0.7 mm) was used to determine the intrinsic viscosity. For bacteria and fungi bioassay techniques, Nutrient Agar (NA), Nutrient Broth (NB) and Potato Dextrose Agar (PDA) media were purchased from Sigma-Aldrich Co. (Egypt). Plant pathogenic fungi of leaf spots and blights (*Alternaria alternata*), the grey mould (*Botrytis fabae*), root rot disease (*Fusarium oxysporum*) and black mould (*Rhizopus stolonifer*) and the bacteria of crown gall disease (*Agrobacterium tumefaciens*) and soft mould disease (*Erwinia carotovora*) were provided by Microbiology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

Depolymerization of chitosan: The low molecular weight chitosan compounds were prepared by oxidative degradation reaction with $NaNO_2$ at 70-80°C according to the method of Allan and Peyron (1995a and b). Briefly, 2.5% (w/v) chitosan was prepared in 1% aqueous acetic acid solution under magnetic stirring. When chitosan was completely dissolved, the appropriate amount of $NaNO_2$ (0.01, 0.02 and 0.05 mol/1 mol of chitosan) was dissolved in distilled water and added dropwise and the reaction was maintained at 70-80°C for 20 minutes. After addition of $NaNO_2$, the viscosity of the chitosan solutions rapidly decreased, and evolution of nitrogen gas was evident. The reaction mixture was cooled to room temperature and was poured into 1N NaOH solution to stop the reaction and precipitate the chitosan product. The precipitated chitosan was immediately filtered through a glass fiber filter and

washed several times with distilled water until neutralization and then oven-dried overnight at 60 °C.

Viscosity measurement and molecular weight determination: The viscosity of polymer solutions in CH_3COOH/CH_3COONa buffer (0.5:0.2 mol/L, respectively) was measured by using the Ubbelohde capillary viscometer (capillary section size 0.7 mm) immersed in a constant temperature bath at 25.0 ± 0.1 °C. The stock solutions were prepared and diluted to yield lower concentrations made by adding the appropriate amount of the buffer to the stock solutions. The capillary was filled with 25 ml of sample and equilibrated in a water bath to maintain respective temperature. The sample was passed through the capillary once before the running time was measured. Each sample was measured 3 times. The running times of the solution and solvent were used to calculate the relative viscosity, specific viscosity, and reduced viscosity as follows:

$$\text{Relative viscosity } (\eta_{rel}) = t_{ch} / t_{sol}$$
$$\text{Specific viscosity } (\eta_{sp}) = (\eta_{rel}) - 1$$
$$\text{Reduced viscosity } (\eta_{red}) = \eta_{sp}/c$$

where t_{ch} is the running time of the chitosan solution, t_{sol} is the running time of the solvent, and c is the chitosan concentration in g/dl.

Intrinsic viscosity, defined as $[\eta] = C(\eta_{red})_{c=0}$, was obtained by extrapolating the reduced viscosity versus concentration data to zero concentration, the intercept on the ordinate is the intrinsic viscosity (Chen and Tsaih, 1998 and Rinaudo and Domard, 1989).

The molecular weight was calculated based on the Mark-Houwink-Sakurada (MHS) equation (Flory, 1953 and Tanford, 1961) as follows:

$$[\eta] = KM^a$$

Where $[\eta]$ is the intrinsic viscosity, K and a are viscometric parameters depending on the solvent. For chitosan in 0.5 M CH_3COOH/0.2 M CH_3COONa the K and a constant are found to be 3.5×10^{-4} and 0.76, respectively (Wang *et al.*, 1991).

Bactericidal assay: Antibacterial activity of chitosan compounds was assayed with Nutrient Agar dilution method as recommended by European Society of Clinical Microbiology and Infectious Diseases (ESCMID, 2000), for determination of minimum inhibitory concentration (MIC). Bacteria were grown in Nutrient Broth medium (peptone 0.5%, beef extract 0.3%, NaCl 0.8%, pH 6.7) and were incubated in the dark at 37°C for 24h using ISCO Incubator. Chitosan solutions were prepared in 0.5% (v/v) acetic acid/water before being applied to broth and each solution was added to Nutrient Agar medium to give a final chitosan concentrations ranged from 500 to 4000 $mg.L^{-1}$ and poured to Petri dishes. After solidifications, 5µl of bacterium (approximately 10^8 CFU/ml), which grown in a Nutrient Broth medium at 37 °C for 24 h, was inoculated as spotted (five spots per each plate) on the surface of medium. The inoculum spots were allowed to dry before inverting the plates followed by inoculum at 37 °C for 24 h. Each sample was tested in triplicate. The control was Nutrient Agar medium with a maximum volume of the solvent which added to the treatments. The MIC was defined as the lowest concentration of chitosan required to completely inhibit bacterial growth after incubation at 37 °C for 24 h.

Fungicidal assay: The antifungal assessment of chitosan compounds was conducted using a mycelia radial growth inhibition technique (El Ghaouth *et al.*, 1992) against four plant pathogenic fungi *A. alternata*, *B. fabae*, *F. oxysporum*, and *R. stolonifer* grown on PDA medium. Chitosan compounds were dissolved in aqueous 0.5 % (v/v) acetic acid and the pH was adjusted to 5.5-6.0 with 1M NaOH (Badawy *et al.*, 2004). For the mycelia radial growth determination, the solutions were added into PDA medium at the concentrations ranged from 250 to 5000 $mg.L^{-1}$ in sterile culture plates (9-cm diameter) and infected with 6-mm-diameter mycelia plugs taken from the fresh culture of the tested fungi. For each compound, three replicates were used for each fungus per concentration tested. The plates were incubated in the dark at 26°C using ISCO incubator. The mycelia radial growth measurements were determined when the mycelia in the control had grown up to the edge of the plate and EC_{50}'s with corresponding 95 % confidence limits was estimated by Probit analysis (Finney, 1971).

Statistical analysis: Statistical analysis was undertaking using a statistical software program, SPSS 12.0 (Statistical Package for Social Sciences, USA) according to Probit analysis (Finney, 1971). The log dose-response curves allowed determination of the concentration causing a 50% reduction in percentage of mycelia growth (EC_{50}) in fungi bioassay. The 95% confidence limits (CL) and standard error for the range of EC_{50} values were determined by least-square regression analysis of the relative growth rate (% control) against the logarithm of the compound concentration.

RESULTS AND DISCUSSION

Depolymerization and molecular weight determination of chitosan compounds: The molecular size of chitosan can be precisely modified by depolymerization with nitrous acid. This reaction provides that the average molecular weight of chitosan before and after depolymerization is related to the mass ratio of nitrous acid added to chitosan originally present. The determination of viscosity-average molecular weight is relatively quick method and the intrinsic viscosity of a polymer solution is related to the polymer molecular weight according to the Mark-Houwink-Sakurada (MHS) equation (Flory, 1953 and Tanford, 1961) as follows:

$$[\eta] = KM^a$$

where $[\eta]$ is the intrinsic viscosity, **M** is the viscosity-average molecular weight; and K and a, are the constants for a given solute-solvent system.

Table 1 shows the $NaNO_2$/chitosan molar ratio, intrinsic viscosity and the molecular weight of chitosan products in this study. From these results, the concentration of sodium nitrite could be expected to play a significant role in the depolymerization process of chitosan and the data is clarified that the increasing of $NaNO_2$ added lead to decrease in the molecular weight of chitosan product compared to the original one. In order to facilitate the control of the molecular weight of chitosan fragment, the chitosan concentration was keeping constant at 2.5% (w/v) in 1% (v/v) aqueous acetic acid solution and the depolymerization process was carried out with addition of different $NaNO_2$/chitosan mol ratio (0.01, 0.02 and 0.05 mol of $NaNO_2$ to one mol of chitosan) using one type of chitosan as original material (Table 1).

The depolymerization process of chitosan produced three different molecular weights, 0.5×10^4, 3.7×10^4 and 5.7×10^4 Dalton for the prepared compounds and 2.9×10^5 Dalton for the native chitosan using of 0.05, 0.02, 0.01 and 0.0 mol of $NaNO_2$ to one mol of chitosan, respectively. The curves related to reduced viscosities and chitosan concentrations for the purified polymers are shown in Figure 2 and are indicated that all experimental points are very well aligned along straight lines ($r > 0.95$).

Table 1. $NaNO_2$/chitosan molar ratio and characterization of chitosan compounds

Reaction mol ratio (x mol $NaNO_2$/1 mol chitosan)	Intrinsic viscosity $[\eta]$	Molecular weight of chitosan product (Dalton)
0	4.9897	2.9×10^5
0.01	1.4384	5.7×10^4
0.02	1.0403	3.7×10^4
0.05	0.2360	0.5×10^4

The viscosity measurement performed with the purified chitosan samples allowed the determinations of their intrinsic viscosities and viscosity average molecular weights. The intrinsic viscosity $[\eta]$ is commonly used to evaluate the average molecular weight of macromolecules, as polymers and polysaccharides and use of purified polymer samples in the determinations of $[\eta]$ and molecular weight (MW) is called for obtaining a reliable relationship (Allan and Peyron, 1995a and b; Chen and Tsaih, 1998; Rinaudo and Domard, 1989 and Wang *et al.*, 1991).

Antibacterial activity of chitosan compounds: The antibacterial assessment of the chitosan compounds was performed as *in vitro* with Nutrient Agar dilution method against two plant pathogenic bacteria *A. tumefaciens* and *E. carotovora*. The results are expressed in terms of the Minimum Inhibitory Concentration (MIC) as shown in Table 2. As a result, all of the chitosan compounds exhibited higher activity towards *E. carotovora* than *A. tumefaciens* under the same experimental conditions.

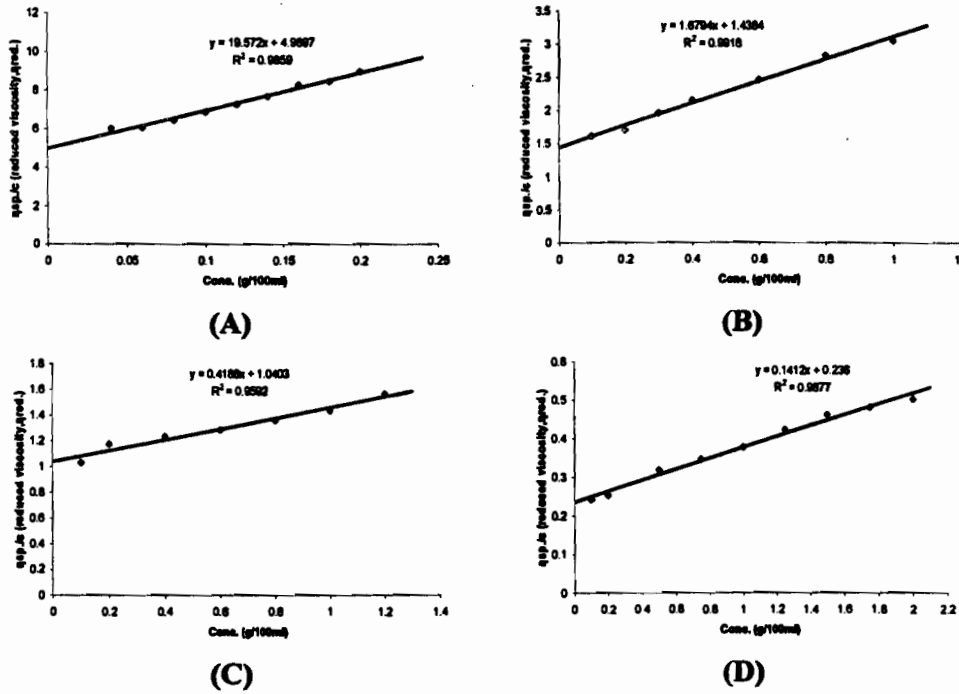

Fig. 2. Curves of reduced viscosity (η_{red}) against concentrations for a commercial chitosan, 2.9×10^5 Dalton (**A**) and depolymerized chitosan compounds, 5.7×10^4 Dalton (**B**), 3.7×10^4 Dalton (**C**) and 0.5×10^4 Dalton (**D**).

This result confirms that the bacterium of *E. carotovora* is more sensitive to these compounds than the bacterium of *A. tumefaciens*. Chitosan with a molecular weight of 0.5×10^4 Dalton exhibits a good antibacterial potency against *A. tumefaciens* with MIC = 2600 $mg.L^{-1}$ while chitosan with a molecular weight of 3.7×10^4 Dalton is the most active one against *E. carotovora* with MIC = 950 $mg.L^{-1}$. In general, the antibacterial activity is increased with the decreasing of molecular weight of chitosan compounds. This finding is in agreement with that reported by various investigators (Jeon *et al.*, 2001; Tsai and Su, 1999 and Zheng and Zhu, 2003); they found that with the decrease of molecular weight of chitosan, the antibacterial activities were

increased. Zheng and Zhu (2003) added that the low molecular weight chitosan appeared to better adsorb to and penetrate the cell walls of microorganisms.

From a comparison of our results and previously published data on bactericidal activity of different molecular weights chitosan compounds, we could conclude that in spite of lack of knowledge on the antibacterial action mechanism, the origin of low molecular weight chitosan as well as the method used for preparation of low molecular weight chitosan and its derivatives are the important issue especially in agriculture applications (Badawy *et al.*, 2006; Rabea *et al.*, 2003 and Zheng and Zhu, 2003).

Table 2. Minimum inhibitory concentrations (MICs, $mg.L^{-1}$) of different molecular weights (MW) chitosan compounds against bacteria of *Agrobacterium tumefaciens* and *Erwinia carotovora*

Chitosan MW (Dalton)	MIC ($mg.L^{-1}$) of bacteria	
	A. tumefaciens	*E. carotovora*
0.5×10^4	2600	1000
3.7×10^4	3100	950
5.7×10^4	2750	1050
2.9×10^5	3200	1350

Antifungal effect of chitosan compounds: The antifungal assessment of different molecular weights of chitosan compounds was assessed as *in vitro* using a mycelia radial growth inhibition technique against four plant pathogenic fungi *A. alternata*, *B. fabae*, *F. oxysporum*, and *R. stolonifer*. The result is expressed in the term of Minimum Effective Concentration of 50% of mycelia growth (EC_{50}) with the corresponding 95% confidence limits (CL) and is presented in Table 3. Generally the result demonstrates that the antifungal activity increased with the decreasing of molecular weight and chitosan with low molecular weight (0.5×10^4 Dalton) exhibited a good antifungal potency against *B. fabae*, *F. oxysporum*, and *R. stolonifer* with EC_{50} of 1392, 1290 and 2232 $mg.L^{-1}$, respectively while the fungus of *A. alternata* is more sensitive to a chitosan molecular weight of 3.7×10^4 dalton with EC_{50} = 1765 $mg.L^{-1}$.

Chitosan and oligochitosan compounds, have attracted much attention as an environmentally safe means of plant disease control (Hadwiger *et al.*, 1994 and

Lin *et al.*, 2005). However, little attention has been paid to its antifungal activity and related mode of action. In the present study, we have successfully obtained different molecular weight chitosan compounds by a nitrous acid depolymerization method and investigated their *in vitro* antifungal activity. Our result demonstrates that the decreasing in the molecular weight led to the increasing of the activity against the tested microorganisms and this fact is in agreement with recent studies which reported that oligochitosans, obtained by hydrolysis or degradation of chitosan, is not only water-soluble but also have shown to be more effective than chitosan to elicit multiple plant defense responses (Kim and Rajapakse, 2005). Moreover, antifungal activities of oligochitosans have also been observed against several fungi (Zhang *et al.*, 2003) and were higher in activity than the high molecular weight. Interestingly, oligochitosan (hexamer unit) that elicited maximal pisatin formation also exhibited higher antifungal activity against *F. solani* than the lower with degree of polymerization (Kendra and Hadwiger, 1984).

Table 3. Antifungal activity of different molecular weight (MW) chitosan compounds against fungi of *Alternaria alternata*, *Botrytis fabae*, *Fusarium oxysporum*, and *Rhizopus stolonifer*

Chitosan MW (Dalton)	EC_{50} (mg.L^{-1})			
	A. alternata	*B. fabae*	*F. oxysporum*	*R. stolonifer*
0.5×10^4	1765 (1265-2417)* (2.50 ± 0.22)**	1392 (1147-1657) (1.52 ± 0.18)	1290 (1012-1585) (1.27 ± 0.18)	2232 (1409-3398) (3.69 ± 0.31)
3.7×10^4	1581 (1368-1817) (1.97 ± 0.19)	1520 (1258-1816) (1.49 ± 0.18)	1941 (1592-2413) (1.33 ± 0.18)	2857 (1957-6007) (2.24 ± 0.24)
5.7×10^4	2237 (1715-3028) (2.65 ± 0.24)	1998 (1665-2444) (1.45 ± 0.19)	2228 (1856-2760) (1.46 ± 0.19)	3527 (2706-6665) (3.45 ± 0.40)
2.9×10^5	2338 (1686-3519) (2.63 ± 0.24)	2407 (1953-3139) (1.27 ± 0.18)	2910 (2301-4068) (1.20 ± 0.18)	3843 (2952-7900) (3.33 ± 0.41)

*Ninety-five percent lower and upper fiducial limits. ** Slope ± SE.

Xu *et al.*, (2007) reported the preparation of oligochitosans by enzymatic depolymerization and investigated *in vitro* antifungal activity against nine phytopathogens *Fusarium graminearum*, *Phytophthora capsici*, *Verticillium dahliae*, *Alternaria solani*, *Botrytis cinerea*, *Colletotrichum orbiculare*, *Exserohilum turcicum*, *Fusarium oxysporum* and *Pyricularia oryzae*. They found that oligochitosans were more effective than the original chitosan in

inhibiting mycelial growth of *P. capsici* and its inhibition on different stages in life cycle of *P. capsici* was observed.

The present results show that chitosans with low molecular weight exhibited a higher antifungal activity than the original chitosan sample against four plant pathogenic fungi *A. alternata*, *B. fabae*, *F. oxysporum*, and *R. stolonifer*.

CONCLUSION

Different molecular weights chitosan compounds were obtained by a method of nitrous acid depolymerization. Average molecular weights of chitosan compounds were determined by measurements of intrinsic viscosity and were found to be 0.5×10^4, 3.7×10^4 and 5.7×10^4 Dalton for the prepared compounds and 2.9×10^5 Dalton for the native chitosan sample. All of the prepared chitosan compounds had good *in vitro* antimicrobial activities against the most economic plant pathogenic bacteria and fungi than the native compound and the antimicrobial activity was affected by the molecular weight of chitosan.

ACKNOWLEDGEMENTS

This material is based on work supported by the Bibliotheca Alexandrina - Center for Special Studies and Programs (CSSP), under Grant No. 215.

REFERENCES

Allan, C. R. and L. A. Hadwiger (1979). The fungicidal effect of chitosan on fungi of varying cell wall composition. Experimental Mycol., 3: 285-287.

Allan, G. G. and M. Peyron (1995a). Molecular weight manipulation of chitosan I: kinetics of depolymerization by nitrous acid. Carbohydr. Res., 277: 257-272.

Allan, G. G. and M. Peyron (1995b). Molecular weight manipulation of chitosan II: prediction and control of extent of depolymerization by nitrous acid. Carbohydr. Res., 277: 273-282.

Badawy, M. E. I., E. I. Rabea; T. M. Rogge; T. M. Stevens; G. Smagghe; W. Steurbaut and M. Höfte ((2004). Synthesis and fungicidal activity of new N,O-acyl chitosan derivatives. Biomacromol., 5: 589-595.

Badawy, M. E. I.; S. M. Ahmed and E. I. Rabea (2006). Bactericidal and fungicidal activities of different molecular weight chitosan samples. J. Pest Control Environ. Sci., 14: 19-34.

Bautista-Banos, S.; A.N. Hernandez-Lauzardo; M.G. Velazquez-del Valle; M. Hernandez-Lopez; E. Barka; E. Bosquez-Molina and C.L. Wilson (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Prot., 25: 108-118.

Boryniec, S.; G. Strobin; H. Struszczyk; A. Niekraszewicz and M. Kucharska (1997). GPC studies of chitosan degradation. Int. J. Polym. Anal. Charact., 3: 359-368.

Chang, K. L.; M. C. Tai and F. H.Cheng (2001). Kinetics and products of the degradation of chitosan by hydrogen peroxide. J. Agric. Food Chem., 49: 4845-4851.

Chen, R. H. and M. L. Tsaih (1998). Effect of temperature on the intrinsic viscosity and conformation of chitosans in dilute HCl solution. Int. J. Biol. Macromol., 123: 135-141.

El Ghaouth, E. A.; J. Arul; J. Grenier and A. Asselin (1992). Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. Phytopathol., 82: 398-402.

European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). (2000). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Clinical Microbiol. Infection., 6: 509-515.

Finney, D. J. (1971). Probit analysis, 3rd Ed, Cambridge University Press, Cambridge, pp.318.

Flory, P. J. (1953). Principles of polymer chemistry, Cornell University Press, Ithaca, NY, pp. 266-316.

Hadwiger, L. A.; T. Ogawa and H. Kuyama (1994). Chitosan polymer sizes effective in inducing phytoalexin accumulation and fungal suppression are verified with synthesized oligomers. Mol. Plant Microb. Interact., 7: 531-533.

Janes, K. A. and M. J. Alonso (2003). Depolymerized chitosan nanoparticles for protein delivery: preparation and characterization. J. Appl. Polym. Sci., 88: 2769-2776.

Jeon Y. J.; P. J. Park and S. K. Kim (2001). Antimicrobial effect of chitooligosaccharides produced by bioreactor. Carbohydr. Polym., 44: 71-76.

Jeon, Y. J. and S. K. Kim (2000). Production of chitooligosaccharides using an ultrafiltration membrane reactor and their antibacterial activity. Carbohydr. Polym., 41: 133-144.

Kendra, D. F. and L. A. Hadwiger (1984). Characterization of the smallest chitosan oligomer that is maximally antifungal to Fusarium solani and elicits pisatin formation in Pisum sativum. Exp. Mycol., 8: 276-281.

Kim, S. K. and N. Rajapakse (2005). Enzymatic production and biological activities of chitosan oligosaccharides (COS): A review, Carbohydr. Polym., 62: 357-368.

Leuba, J. L. and P. Stossel (1986). Chitosan and other polyamines: antifungal activity and interaction with biological membranes. In: R. Muzarelli, C. Jeuniaux and G.W. Graham, Editors, Chitin in Nature and Technology (pp. 215-222). Plenum Press, New York, USA.

Lin, W.; X. Hu; W. Zhang; W. I. Rogers and W. Cai (2005). Hydrogen peroxide mediates defence responses induced by chitosans of different molecular weights in rice. J. Plant Physiol., 162: 937–944.

Liu, Y.; S. Chen and P. Yu (1997). Preparation of water-soluble low molecular weight chitosan and its complex with calcium ions. Fujian Shifan Daxue Xuebao., 13: 67-70.

Mao, S.; X. Shuai; F. Unger; M. Simon; D. Bi and T. Kissel (2004). The depolymerization of chitosan: effects on physicochemical and biological properties. Int. J. Pharm., 281: 45-54.

No, H. K. and S. P. Meyers (1997). Preparation of chitin and chitosan. In R.A.A Muzzarelli and Peter, M.G., eds., Chitin Handbook, European Chitin Society, Grottammare AP, Italy, pp. 475-489.

No, H. K.; N. Y. Park; S. H. Lee and S. P. Meyers (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int. J. Food Microbiol., 74: 65-72.

Rabea, E. I.; M. E. T. Badawy; C. V. Stevens; G. Smagghe and W. Steurbaut (2003). Chitosan as antimicrobial agent: Applications and mode of action. Biomacromol., 4: 1457-1465.

Rinaudo, M. and A. Domard (1989). In: Skjak-Bræk G., Anthonsen T., Sandford P. Ed., Chitin and chitosan: Sources, chemistry, biochemistry, physical properties and applications (pp. 71-86). London: Elsevier Applied Science.

Sashiwa, H.; H. Saimoto; Y. Shigemasa and S. Tokura (1993). N-Acetyl group distribution in partially deacetylated chitins prepared under homogeneous conditions. Carbohydr. Res., 242: 167-172.

Tanford, C. (1961). Physical chemistry of macromolecules (pp. 390-412), John Wiley Press, New York.

Tømmeraas, K.; M. Kjell; B. E. Varum and O. S. Christensen (2001). Preparation and characterisation of oligosaccharides produced by nitrous acid depolymerisation of chitosans. Carbohydr. Res., 333, 137-144.

Tsai, G. J. and W. H. Su (1999). Antibacterial activity of shrimp chitosan against Escherichia coli. J. Food Prot., 62: 239-243.

Varum, K. M.; M. H. Ottoy and O. Smidsrod (2001). Acid hydrolysis of chitosans. Carbohydr Polym., 46: 89-98.

Wang, W.; S. Bo; S. Li and W. Qin (1991). Determination of the Mark-Houwink equation for chitosans with different degrees of deacetylation. Int. J. Biol. Macromol., 13: 281-285.

Xu, J.; X. Zhao; X. Han and Y. Du (2007). Antifungal activity of oligochitosan against Phytophthora capsici and other plant pathogenic fungi in vitro. Pesticide Biochem. Physiol., 87: 220-228.

Zhang, M. I.; T. Tan; H. Yuan and C. Rui (2003). Insecticidal and fungicidal activities of chitosan and oligo-chitosan. J. Bioactive Compatible Polym., 18: 391-394.

Zheng, L. Y. and J. F. Zhu (2003). Study on antimicrobial activity of chitosan with different molecular weight. Carbohydr. Polym., 54: 527-530.

J. Pest Cont. & Environ. Sci. 15 (1): 69 -85(2007).

تأثير درجة البلمرة للبوليمر الطبيعى كيتوزان على بعض البكتيريا والفطريات الممرضة للنبات

محمد الطاهر إبراهيم بدوى
قسم كيمياء المبيدات - كلية الزراعة (الشاطبى) - جامعة الاسكندرية - مصر

تم الحصول على ثلاث أوزان جزيئية مختلفه من الكيتوزان التجارى بطريقة إزالة البلمرة بواسطة حامض النيتروزو. تم تقدير متوسط الوزن الجزيئي لمركبات الكيتوزان المحضرة عن طريق قياس اللزوجة الجوهرية وكانت الاوزان هى0.5×10^4 و3.7×10^4 و5.7×10^4 دالتون للمركبات المحضرة بينما عينة الكيتوزان الأصلية كانت 2.9×10^5 دالتون . تم دراسة التأثير الميكروبى لمركبات الكيتوزان ضد البكتريا والفطريات الممرضة للنبات. وتم تقييم النشاط الإبادى البكتيرى بطريقة الأجار المخفف ضد البكتريا المسببة لمرض التدرن التاجى الأجروبكتيريوم تيموفيشنس و البكتريا المسببة لمرض العفن الطرى إروينيا كاروتوفورا وتم التعبير عن النتائج عن طريق التركيز التثبيطى الأدنى. و عموما أوضحت النتائج أن البكتريا إروينيا كاروتوفورا كانت أكثر حساسية لهذة المركبات من البكتريا الأجروبكتيريوم تيموفيشنس. وأوضحت النتائج أيضا أن الكيتوزان ذو الوزن الجزيئي 0.5×10^4 دالتون أظهر أفضل نشاط إبادى بكتيرى ضد بكتريا الأجروبكتيريوم تيموفيشنس حيث كانت قيمة التركيز التثبيطى الأدنى له 2600 ملليجرام/لتر بينما الكيتوزان ذو الوزن الجزيئي 3.7×10^4 دالتون كان له أقوى نشاط إبادى بكتيرى ضد بكتريا إروينيا كاروتوفورا حيث كانت قيمة التركيز التثبيطى الأدنى له 950 ملليجرام/لتر. وكذلك تم تقييم النشاط الإبادى الفطرى ضد فطر التبقع الورقى الترناريا ألترناتا و بوتريتس فابى و فيوزاريوم أوكسيسبوريم وريزوبس ستولونيفير بطريقة قياس قطر نمو الميسيليوم ، وتم التعبير عن النتائج عن طريق أقل تركيز تثبيطى ل50 % من النمو. وعموما أوضحت النتائج أن النشاط الإبادى الفطرى يزيد بإنخفاض الوزن الجزيئى وأن الكيتوزان ذو الوزن الجزيئي المنخفض (0.5×10^4 دالتون) أظهر أفضل نشاط إبادى فطرى ضد فطر بوتريتس فابى و فيوزاريوم اوكسيسبوريم وريزوبس ستولونيفير حيث كانت قيمة أقل تركيز تثبيطى ل50 % لهم هى 1392 و 1290 و2232 ملليجرام/لتر على التوالى بينما فطر ألترناريا ألترناتا كان أكثر حساسية للكيتوزان ذو الوزن الجزيئي 3.7×10^4 دالتون وكانت قيمة أقل تركيز تثبيطى ل50 % له هى 1765 ملليجرام/لتر.