

Chemical Induced Grafting of Indole onto Chitin & Chitosan – and their Antimicrobial Activity

Samia M. Mokhtar, Tahia B. Mostafa and Maha A. Hewedy

Chemistry Department Faculty of Women, Ain-Shams University, Cairo, Egypt
Botany Department, Faculty of Women, Ain-Shams university, Cairo, Egypt

Abstract: The graft copolymer of indole with chitin and chitosan was prepared by two techniques, chemically using potassium persulphate as an initiator. Evidence of grafting was obtained by FTIR. The effects of initiator concentration, monomer concentration and temperature on the grafting percentage were studied. The grafted samples show a higher chelation capacity towards cation ions of Cu^{2+} , Ni^{2+} and Co^{2+} . The thermogravimetric analysis of grafted samples showed an improvement in the thermal stability. The antimicrobial activity of the grafted samples was investigated using the agar-disc diffusion method and the spectroscopic method. These two methods showed different effects against a diverse range of organisms comprising some Gram-positive, Gram-negative bacteria and fungi. The mechanism of antimicrobial activity of the grafted samples could make some of the tested micro-organisms flocculate. Also a weak antimicrobial activity was shown against the other organisms.

Key words: Chitin, chitosan, indole, grafting, Thermal stability antimicrobial activity fungi bacteria and streptomycetes.

INTRODUCTION

Chitin and chitosan are recommended as suitable functional materials because these natural polymers have excellent properties (Majeti, & Ravi Kumar, 2000) such as biocompatibility, biodegradability, non-toxic and adsorption.

The modification of natural polymers such as chitin or chitosan by grafting is considered the most important method for preparing new materials.

Considerable interest has been focused on chemical modification by grafting synthetic polymers onto chitin and chitosan (Longdi, & Seiichi, 1994; Singh, & Alok, 1994; Caner *et al.* 1998; Byung *et al.* 1999; Abduel Najjar *et al.* 2000; Mehrdad *et al.* 2000; El-Tahlawy, & Hudson, 2007; Yinghai *et al.* 2002). Graft copolymerization with several monomers (Singh, & Ray, 1994; Tao *et al.* 2003; Casimiro *et al.* 2005; Mostafa *et al.* 2005) such as, methylmethacrylate, acrylamide, 2-hydroxyethylmethacrylate acrylonitrile, methacrylic acid, styrene and itaconic acid can be carried out with different initiator systems and by different mechanisms. In a comprehensive review, Jenkins and Hudson (David, & Samuel, 2001) have discussed the advances toward controlling radical-based reactions of grafting chitin and chitosan with many acrylic and vinyl monomers under different experimental conditions. Another review dealing with the metal complexation of chitosan and its derivatives (Varma *et al.* 2004; El-Sherbiny, 2009) threw more light on the importance of chitosan and its value.

Polyindole have received a significant share of attention in the past several years and may be good candidates for application in various domains, like electronics, electro-catalyst anode materials in battery, anti-corrosion coatings and pharmacology. Polyindole have advantages of fairly good thermal stability and high redox-activity (Jingkun *et al.* 2005; Andrew *et al.* 1998)

Grafting of chitin or chitosan with N-containing heteroaromatic organic molecules such as polyindole, yields grafted copolymer that has good air and thermal stability, as well as, high potential in adsorbing metal ions (Juang, & Shao, 2002; Benguella, & Benaissa, 2002; Eric, 2004; Trimukhe A& Varma, 2009).

Several studies have demonstrated the antimicrobial and antifungal action of this polysaccharide (Moller *et al.* 2004). Several chitosan derivatives are known by or from their antimicrobial activity. They are used in agriculture, medicine, environment and food (Devlieghere *et al.* 2004). Much of the interest in the antimicrobial properties of chitosan has focused on its possible role in plant protection. It has been reported that both soil

Corresponding Author: Samia M. Mokhtar, Chemistry Department Faculty of Women, Ain-Shams University, Cairo, Egypt

and foliar plant pathogens fungal, bacterial and viral, may be controlled by chitosan application (Maher *et al.* 2008).

Moreover, the combined effect of the principal reaction variables for the grafting process was systematically studied. A series of grafted and ungrafted chitin and chitosan with different concentrations were prepared and used *in vitro* to control the growth of pathogenic fungus *Fusarium oxysporum* which is the causal agent of one of the most important diseases "root rot". These diseases affect the cultivation of many plants with limited loss of the beneficial effect of plant growth promoting bacteria (PGPR) used in this work which were: two isolates of *Azospirillum* spp., one isolate of *Peanbacillus polymyxa* and two isolates of streptomycetes (*S. ochraceiscleroticus* and *S. chibaensis*).

In this work, the graft copolymerization of indole onto chitin or chitosan with potassium persulfate as an initiator focused on the controlled grafted copolymerization conditions such as the effect of monomer concentrations and initiator concentrations.

MATERIAL AND METHODS

Experimental:

Chitosan and chitin were kindly supplied by prof. Dr. Furuhashi of Tokyo Institute of Technology (T.I.T), Indole, EDTA and eriochrome black T Initiators (potassium persulfate) were analytical grade reagents from Merck chemicals and were used as received. All solvents from Aldrich were purified according to the conventional methods.

Microbial Strains and Culture Conditions:

Potato dextrose agar medium (PDA) was used in the cultivation of pathogenic fungus *Fusarium Oxysporum* (ATTC, 1992), *Peanbacillus Polymyxa* was cultivated on nutrient agar medium (Dawson, 1957), both were obtained from the department of Microbiology, soil water and Environmental Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. Other bacteria were obtained from the collection of Botany department, Women's college, Ain Shams University: the Gram negative bacteria *Azospirillum* sp.1 and 2 were maintained on nitrogen – free agar medium (NFB) containing malate (5g L⁻¹) (Krieg, & Dobreiner, 1984), Gram positive filamentous bacteria *Streptomyces ochraceiscleroticus* and *Streptomyces chibeansis* were locally isolated from Tushka's Egyptian soil and characterized by (Hewedy, 2003), all bacteria are local isolates and used as plant growth promoting bacteria (PGPR).

Graft Copolymerization Procedure:

Chemically Induced Graft Copolymerization:

0.1 g of chitosan or chitin was mixed in 50 ml stopper round-bottomed flask with water –dioxane mixture (1:1) followed by the addition of indole (monomer) and initiator in the required order with constant stirring. After the desired time interval, the product was filtered and washed thoroughly with hot water to remove the unreacted monomer (There is no homopolymer formation), and then dried in an air oven at 60 °C till constant weight was achieved.

The percentage of grafting was calculated based on the following equation:

$$\%Gr = (W_1 - W_0) \times 100/W_0$$

Where W_0 is the weight of chitosan or chitin before grafting, and W_1 is the weight of chitosan or chitin after grafting.

Metals Uptake:

0.1 g of grafted chitosan or chitin was mixed with 50 ml of metal solution (0.1 M) in a (100 ml) flask agitated vigorously by magnetic stirrer. The above mixture was subjected to stirring overnight. In all cases, the working pH was that of the solution and was not controlled. At appropriate time intervals, stirring was briefly interrupted. The chitosan / metal or chitin / metal complexes were then filtered, washed with distilled water and dried in an oven at 60 °C. The absorbed amount of metal ion was calculated by analyzing the residual metal concentration in the aqueous solution. This was done by complexmetric titration using EDTA and eriochrome black T as an initiator at pH= 10.

FTIR Spectroscopy:

FTIR spectra were taken using FTIR spectrometer Bruker Vector 22 Germany in the range between 400 and 4000 cm^{-1} .

Thermal Analysis:

Thermogravimetric TGA analysis was carried out using shimadzu TGA -50H at a heating rate of 10 $^{\circ}\text{C}$ / min. under nitrogen atmosphere.

Evaluation of Antimicrobial Activity:

Antifungal Activity:

The effect of the four treatments (C_1 , C_1 -g- Ind, C_2 and C_2 -g- Ind) on the growth of fungus was studied using two methods: The first, 1.0 ml of different concentrations (0.5% and 1.5%) of the four treatments was placed onto the center of a PDA plate seeded with 1 ml of fungal spore suspension. The plates were prepared in triplicates and incubated at 32 $^{\circ}\text{C}$ for 72 hrs until they reached their maximum diameter. The inhibition zone was measured in mm. by using radial growth measurement (Roller, & Covill, 1999). In the second method, the substrate mycelium of fungus was weighed in the same plates in grams.

Antibacterial Activity:

One loop full of each isolate of bacteria was inoculated into 10 ml of its specific media and incubated at 32 $^{\circ}\text{C}$ for 24hrs for *Peanbacillus Polymyxa* and 48hrs for *Azospirillum* and *Streptomyces*. Each bacterial solution was diluted to 10^{-5} cells/ml and the surviving bacteria were determined by pour plate method by measuring the clear zone which surrounds the hole containing 1.0 ml of each treatment (Park *et al.* 1998). Moreover, the susceptibility of each organism to each compound was estimated by measuring it spectroscopically at 610 nm at spectronic 21D also, some morphological characters were examined.

RESULTS AND DISCUSSION

Graft copolymer based on chitin (C_1) or chitosan (C_2) was synthesized by grafting indole (Ind) onto a polysaccharide molecule using free radical initiation in H_2O . This technique enables the production of new polymer materials with desired properties. The effect of monomer concentration, initiator concentration, and temperature on the grafting percentage was studied.

Effect of Initiator Concentration:

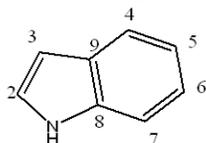
When chemical initiation is used, the effect of initiator concentration was changed from 0.05 – 0.6 keeping other reaction conditions constant at 60 $^{\circ}\text{C}$.

The results in Fig.1 indicated that, the percentage of grafting increases with increasing the initiator concentration for both the grafted indole onto chitin and chitosan. The sulphat radical SO_4^{+2} is known to be a strong redox initiator that can oxidize the polysaccharide with the production of a free radical on the ring. This higher concentration of SO_4^{+2} is expected to activate more sites at the systems in study.

Effect of Monomer Concentration:

Fig. (2) showed the effect of concentration of indole on graft copolymerization. The %gr increased with increasing the concentration of indole reaching a maximum value when the concentration of indole was 1.5 mol /L when grafted with chitin and 2.0 mol/L when grafted with chitosan, and then decreased. This behavior could be explained by the fact that an increase in monomer concentration led to the accumulation of monomer molecules in close proximity to the polymer backbone. The possibility of a large number of couplings may result in cyclic structures (Andrew, & Alastair, 1998). These are undesired side products of polymerization and they decrease the graft polymerization efficiency.

The grafting of indole in the back-bone of the polymer does not occur through the nitrogen species in indole. Thus and the polymerization takes place at the C_2 - C_3 positions (Talbi *et al.* 1998; Koleli *et al.* 2002).



Indole monomer

Effect of Reaction Temperature:

The effect of temperature was studied by changing the reaction temperature (50 -70 °C) and keeping other reaction conditions constant, as shown in Fig.(3). The % Gr reached its maximum at 60 °C. At low temperature, the amount of the radical generated was small and the % Gr was low. With the increase in temperature, the collision chance of chitin or chitosan and indole increased and resulted in the increase of chitin or chitosan macroradicals which enhanced the % Gr. The percentage grafting showed a decrease above this optimum temperature, which should be related to the following fact: At a higher reaction temperature, the thermal decomposition rate of persulfate increased the mobility of the macroradical, which led to increasing the termination rate.

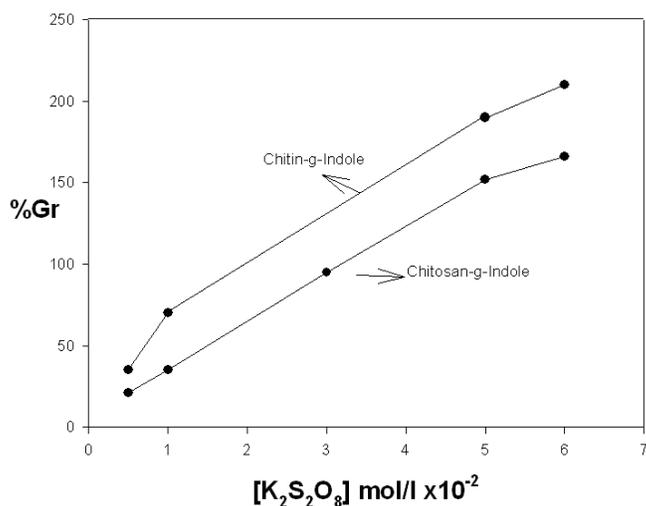


Fig. 1: Effect of initiator concentration (K₂S₂O₈) on the % graft at 60°C.

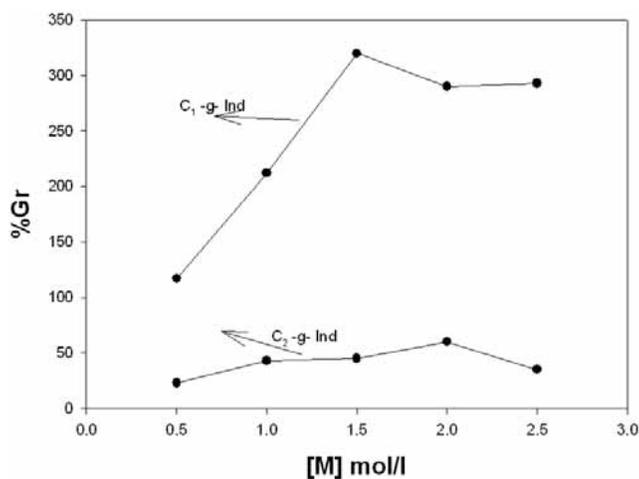


Fig. 2: Effect of monomer concentration on the % graft at 60°C and [K₂S₂O₈] =0.002 mol/l

Characterization of the Grafted Copolymers:

FTIR Analysis

The FTIR analysis was based on the identification of adsorption bands concerned with the vibrations of functional groups present in the molecules. Moreover, the spectrums confirm and assure the grafting processes. The infra - red spectra has proved the formation of a new absorption band at 1378.3 cm⁻¹ and 1377.5 cm⁻¹, corresponding to C-N stretching vibration in indole on grafting with chitin and chitosan respectively.

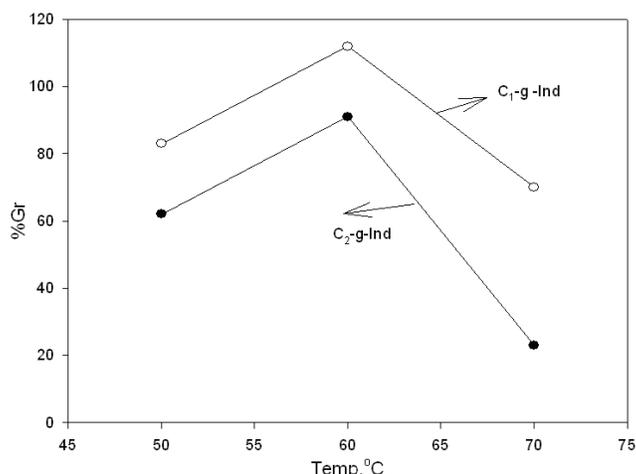


Fig. 3: Effect of temperature on the % graft, the monomer concentration = mole and $[K_2S_2O_8] = 0.002$ mol/l.

The appearance of these bands and the band at $767 - 748\text{ cm}^{-1}$ in grafted chitin and 747 cm^{-1} of grafted chitosan indicate that the benzene ring is not affected during the polymerization. The band located at 1455 cm^{-1} was assigned to the stretching of the benzene ring. The strong and broad peak at 3409 cm^{-1} was a characteristic adsorption of the N-H. This band together with the band at 1560 cm^{-1} can be ascribed to the stretching and deformation vibration of N-H bond. This proves that the nitrogen could not have been the polymerization site and the polymerization should have happened at the $C_2 - C_3$ positions.

In addition, the bands at 1075 cm^{-1} or at 1078 cm^{-1} and 1026.5 cm^{-1} in grafted chitin or chitosan are characteristics for the saccharide structure.

Thermogravimetric Analysis:

To evaluate the thermal properties of the grafted copolymer, the samples were characterized by TGA. Fig. (4) shows the weight loss curves of grafted chitin and grafted chitosan with indole.

It was observed that the $C_1\text{-g-Ind}$ exhibits two distinct stages. The first stage ranges between $41-117\text{ }^\circ\text{C}$ and is associated with the loss of adsorbed and bound water. This stage shows 10% weight loss. The end stage of the weight loss starts at $314\text{ }^\circ\text{C}$ and continues up till it reaches $450\text{ }^\circ\text{C}$ with maximum decomposition rate at $377\text{ }^\circ\text{C}$. These stages show that 56 % of weight loss is due to the bulk decomposition of the polymer residue.

Also, the TGA of the $C_2\text{-g-Ind}$ observed the same behavior with some changes in the 3rd stage. It started decomposing from $280\text{ }^\circ\text{C}$ until $468\text{ }^\circ\text{C}$ with two peaks at $292\text{ }^\circ\text{C}$ and $377\text{ }^\circ\text{C}$. The weight loss reached 55.6 % at $600\text{ }^\circ\text{C}$. This indicates that the $C_2\text{-g-Ind}$ is more stable than $C_1\text{-g-Ind}$.

Metal Uptake:

By considering the nature of the functional groups present in the chitin or chitosan grafted copolymers, a preliminary exploration on the adsorption of these grafted chitin or chitosan copolymers for Cu^{2+} , Ni^{2+} and Co^{2+} ions were investigated.

The metals uptake was calculated by the determination of the residual metal concentration in the aqueous solution. This was done by complex mature titration using EDTA and erachrom black T as indicator at pH 10 and the metal uptake g (mg ion metal /g chitin or chitosan) was determined as follows :

$$q = (C_2 - C_1) V / M$$

Where C_1 and C_2 are the initial and final metal ion concentration (mg /L) respectively, V is the volume of solution (ml) and M is the chitin or chitosan weight (g). The amount of metals uptake are listed in Table(1).

It is obvious from the obtained results that the chitin or chitosan modified copolymer has a great affinity for Cu^{2+} , Ni^{2+} and Co^{2+} metal ions compared with ungrafted chitin or chitosan.

Chitin and chitosan exhibit various interesting biological activities, which have made these polysaccharides and their derivatives increasingly important.

In our experiments, the influence of the four compounds C₁, C₁-g-Ind, C₂ and C₂-g-Ind was investigated separately. Their antimicrobial activity has been observed against *Fusarium oxysporum*, which caused disease and a major limiting factor in the productivity of legumes in all Nile Valley. They are also tested against some plant growth promoting rhizobacteria (PGPRs) (Oplinger *et al.* 1997; El-Sayed, & Saood, 2007).

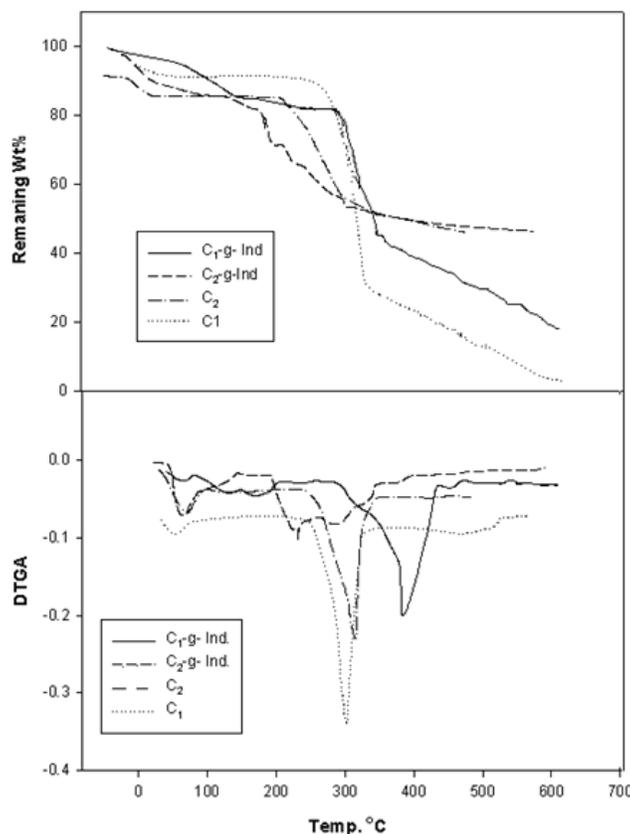


Fig. 4: Thermogravimetric analysis (TGA) and differential thermal analysis (DTGA) for ungrafted and grafted samples

Table 1: The amounts of metal up-take in mg/g of grafted and ungrafted samples

Samples	Cu ²⁺	Co ²⁺	Ni ²⁺
C1	4.0	8.0	3.0
C ₁ -g- Ind	81.5	62.0	73.0
C ₂	6.0	10.0	35.0
C ₂ - g - Ind	82.6	90.0	70.0

Antimicrobial Assessments:

Antifungal Activity:

F. oxysporum was determined by two methods: the first method recorded the inhibition zone by using radial growth measurement over a period of 72 hrs at 32°. The fungus showed no discernible sensitivity when exposed to PDA agar medium supplemented with chitin (C₁), while grafted chitin with indole (C₁-g-Ind) showed a very slightly inhibition (Fig 5 and 6). In case of chitosan (C₂) and the grafted chitosan (C₂ -g-Ind), a clear inhibition zone was observed of about 40 and 50 mm, respectively (Fig 5 and 6). In the second method, the substrate mycelium of fungus was weighed in each treatment at different concentrations (0.5% and 1.5%). The weight of *F. oxysporum* was a little lower than the average in case of C₁ and C₂. On the other hand, C₁ -g- Ind and C₂ -g-Ind were more active, and the weight of fungus was statically lighter compared with the untreated media (Table2).

From our results, chitosan (C₂) and grafted chitosan (C₂-g-Ind) indicated an inhibitory effect on fungal growth; the inhibition zone diameter is an indication of the antifungal capacity of the polymer, the bigger the

zone the stronger the capacity, 1.5% concentration of C₂-g-Ind was enough to cause a significant retardation of the weighing growth of fungus followed by C₂. Generally, the grafting of indole to C₁ and C₂ enhances their antifungal activity. Numerous reports indicated that chitosan effectively delays the infection of fungi and slows down the infection process. In general, the reduction of rots increases with the increase in chitosan concentration (Bautista – Banos *et al.* 2006; Xiooying *et al.* 2006).

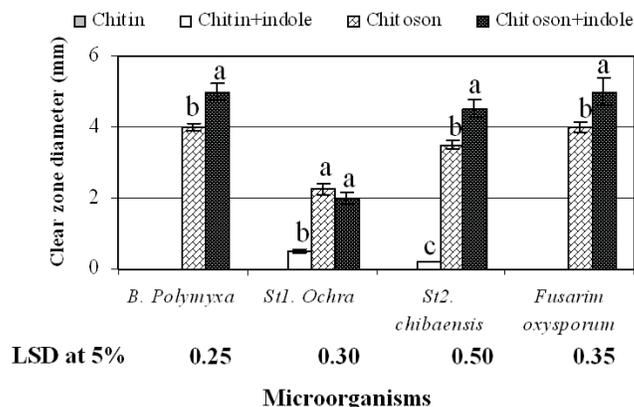


Fig. 5: Antimicrobial activity of C₁, C₁-g-Ind, C₂ and C₂-g-Ind on the growth of *P. polymyxa*, *S. ocraceischeroticus*, *S. Chibaensis* and *F. oxysporum* after 72 h of incubation at 28°C by a pour plate method (mean values of the diameter on the inhibition zones in mm).

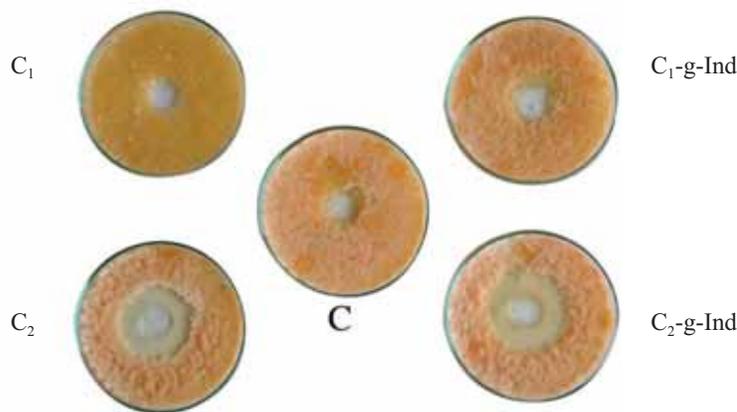


Fig. 6. Effect of C₁, C₁-g-Ind, C₂ and C₂-g-Ind on the mycelia growth of *F. oxysporum*

Table 2: Growth of Gram +ve and Gram –ve bacteria in traditional growth media with 0.5% and 1.5% concentrations of C C₁-g-Ind, C₂ and C₂-g-Ind at 32°C measured spectrophotometrically at 610 nm.

Microorganisms	Concentrations (mg/L)								LSD at5%	
	Cont.	C ₁		C ₁ -g-Ind		C ₂		C ₂ -g-Ind		
		0.5	1.5	0.5	1.5	0.5	1.5	0.5		1.5%
<i>P. polymyxa</i>	0.235a	0.23a	0.146c	0.162 b	0.038d	0.011e	0 e	0.14 c	0.028 d	0.015
<i>S. ocraceischeroticus</i>	0.022f	0.164d	0.176d	0.384a	0.331b	0.155d	0.176d	0.11e	0.272c	0.042
<i>S. Chibaensis</i>	0.27e	0.645d	0.764c	0.862b	0.996a	0.818b	0.844b	0.808b	0.972a	0.095
<i>Azospirillum Sp.1</i>	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	-
<i>Azospirillum Sp.2</i>	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	-

Means in the same raw with the same letter non-significant

Antibacterial Activity:

The absence of colonies was taken as a sign for total inhibition at different concentrations for *Azospirillum* spp. (Fig. 7A&B). The inhibition zone of gram- positive, *Pea bacillus polymyxa* was not detectable except for C₂ and C₂-g-Ind which has a clear zone of 25 mm and 20 mm, respectively



Fig. 7A: Effect of C_1 , C_1 -g-Ind, C_2 and C_2 -g-Ind on the growth of *Azospirillum* sp.1



Fig. 7B: Effect of C_1 , C_1 -g-Ind, C_2 and C_2 -g-Ind on the growth of *Azospirillum* sp.2

(Fig. 5 & 9 A&B). Spectrometrically, at 620 nm C_2 -g-Ind has the lowest statistic effect of inhibition than C_2 at 1.5% and 0.5% concentration. Both of the G-positive filamentous bacteria *S. ochraceiscleroticus* and *S. chibaensis* gave clear zones which elaborates that *S. ochraceiscleroticus* is more resistant (Fig.5, 8 &9). Spectroscopically, the results suggested that these four substances may have stimulated the growth of the two *streptomyces*, C_1 -g-Ind and C_2 -g-Ind at 1.5% concentration (Table 3).

Our results showed that the effects of antibacterial activity of the four compounds varied greatly. The diameter of a clear zone varied according to the active group in the copolymer and also the tested bacteria. The two Gram – negative *Azospirillum* sp. seemed to be very sensitive for all treatments. All concentrations could kill all of the two *azospirillum* isolates, though it had limited effect against Gram-positive bacteria *P. polymyxa* especially at 0.5% concentration of $C_1 > C_1$ -g-Ind $> C_2$ -g-Ind $> C_2$, respectively, this showed that the antibacterial activity had

a relationship with concentration. In accordance with us, (Devlieghere *et al.* 2004). said that Gram -ve bacteria seemed to be very sensitive against chitosan, while G +ve bacteria varied greatly. On the other hand (Xiooying *et al.* 2006) had an opposite opinion. By contrast, the optical absorption at 610 nm showed a stimulation growth of Gram–positive filamentous bacteria more than the control set. A clear zone was observed in case of solid media. This observation may be due to the chitinase enzyme produced by the 2 streptomyces isolates which reduced chitin and its derivatives with good efficiency and also the limited inhibition of *P. polymyxa* which can produce chitinase enzyme with moderate efficiency (Hewedy, 2003; El- Tahlawy, 2006). This enzyme can reduce the four compounds to more simple materials that can be easily used as soluble nutrients. In addition, the antibacterial activity of chitosan strongly suggested that chitin and chitosan induced a series of defense reactions correlated with enzymatic activities. This was in harmony with some authors who indicated that the concentrations of 50 and 100 ppm could promote the growth of *E. coli* (Maher *et al.* 2008; Saito *et al.* 2007; Nan Liu, 2006).

Some Morphological Characters of PGPBRS Bacteria:

Morphologically, the surface of *Peanbacillus polymyxa* transferred from cream color to a very faint pink one, while the color of the substrate mycelium and aerial spore mass of streptomyces were varied from dark grey color to light grey (Fig. 9A&B). All treatments inhibit the diffusible brown pigment produced by *S. chibaensis* (Fig. 10). and species dependency (Moller *et al.* 2004).

Table 3: Growth of *F. oxysporum* in traditional growth media with 0.5% and 1.5% concentrations of C₁-g-Ind, C₂ and C₂ C₂-g-Ind at 32°C (Growth weight (g)).

Microorganism	Growth weight (g)								LSD at 5%	
	Cont.	C ₁		C ₁ -g-Ind		C ₂		C ₂ -g-Ind		
		0.5	1.5	0.5	1.5	0.5	1.5	0.5		1.5%
<i>F. oxysporum</i>	14.88a	12.25b	9.29c	11.5b	8.35c	9.05c	6.31ef	7.95ce	5.29f	1.82

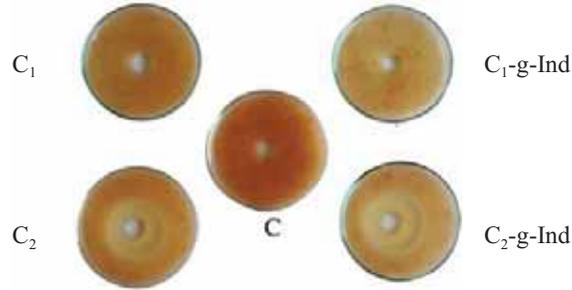


Fig. 8: Effect of C₁, C₁-g-Ind, C₂ and C₂-g-Ind on the growth of *P. Polymyxa*

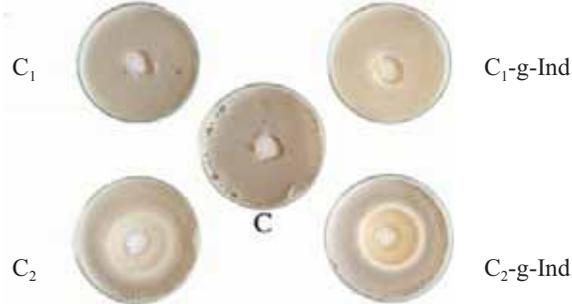


Fig. 9A: Effect of C₁, C₁-g-Ind, C₂ and C₂-g-Ind on the growth of *S. ochracaiscleroticus*

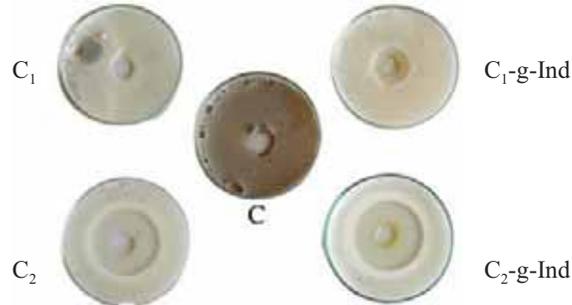


Fig. 9B: Effect of C₁, C₁-g-Ind, C₂ and C₂-g-Ind on the growth of *S. Chibaensis*



Fig. 10: Effect of C1(c), C1-g-Ind(d), C2(c) and C2-g-Ind(f) a, control, b, untreated media seeded with *St.chibaensis* on the melanin production of *S. chibaensis*

It can be concluded that comparing the antimicrobial activity of different chitin and chitosan studies is difficult because of possible differences in (1) characteristics (deacetylation and polymerization degree) of chitin and chitosan used in these studies, (2) type of solvents in which they were dissolved in, (3) experimental conditions (incubation time, temperature and pH value), (4) Strain and species dependency (Chung *et al.*, 2003 and Devlieghere *et al.* 2004).

Conclusion:

Polyindole could be grafted onto chitin or chitosan by using potassium persulfate as chemical initiator. The percent graft was controlled by varying the monomer concentration, initiator concentration and temperature. Thermal analyses have indicated that the grafted chitin or chitosan is more thermally stable than the ungrafted samples. Grafted chitin or chitosan enhancement of the metal complexes and the extent of metal ions uptake of Co^{2+} , Cu^{2+} and Ni^{2+} were determined.

C_1 -g-Ind and C_2 -g-Ind have reduced the antifungal activity and they have limited the antibacterial effect against PGPRs. This study helps to obtain a clean and economic system of cultivation with limited loss of the beneficial effect of plant growth promoting rhizobacteria (PGPRs).

REFERENCES

- Abduel Majid, K., Najjar, Wan Md Zin Wan Yunus, B. Mansor Ahmad, Ab. Mohamad Zaki Rahman, 2000. Preparation and characterization of poly(2-acrylamido-2-methylpropane-sulfonic acid) grafted chitosan using potassium persulfate as redox initiator. *Journal of Applied Polymer Science*, 77(10): 2314-2318.
- Andrew R. Mount and Alastair D. Thomson, 1998. Electrooxidation of N-methylindole. *Journal of the Chemical Society, Faraday Transactions*, 94: 553-558.
- ATTC, 1992. *Catalogue of Bacteria and Bacteriophages*, 18th ed, pp: 428.
- Bautista - Banos, S., A.N. Hernandez - Lauzardoa, M.G. Velazquez-del Valle, M. Hernandez - Lopez, E. Ait Barka and E. Bozquez - Molinac, 2006.
- Benguella, B., H. Benaissa, 2002. Cadmium removal from aqueous solutions by chitin: kinetic and equilibrium studies. *Water Research*, 36: 2463-2474.
- Byung-Ok Jung; Chun-Ho Kim; Kyu-Suk Choi; Young Moo Lee; Jae-Jin Kim; 1999. Preparation of amphiphilic chitosan and their antimicrobial activities. *Journal of Applied Polymer Science*, 72(13): 1713-1719.
- Caner, H., H. Hasipoglu, O. Yilmaz, E.Yilmaz, 1998. Graft copolymerization of 4-vinylpyridine on to chitosan-1. by ceric ion initiation. *European. Polymer. Journal*, 34: 493-497.
- Casimiro, M .H., M.L. Botelho, J.P. Leal, M.H. Gil, 2005. Study on chemical, UV and gamma radiation-induced grafting of 2-hydroxyethyl methacrylate onto chitosan. *Radiation Physics and Chemistry*, 72(6): 731-735.
- David W. Jenkins and Samuel M. Hudson, 2001. Review of Vinyl Graft Copolymerization featuring recent advances toward controlled radical-based reactions and illustrated with Chitin/Chitosan Trunk Polymers. *Chem. Rev.*, 101(11): 3245-3274.
- Dawson, W.J., 1957. "Plant disease due bacteria". Seconded, Cambridge, the university press, London, pp: 231.
- Dinesh Singh, K., R. Alok Ray, 1999. Controlled release of glucose through modified chitosan membranes. *Journal of Membrane Science*, 155: 107- 112.
- Devlieghere, F., A. Vermeulen, J. Defever, 2004. Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables *Journal .Food Microbiology*, 21:703-714.
- El-Sherbiny, I.M., 2009. Synthesis characterization and metal uptake capacity of a new carboxymethyl chitosan derivative *European. Polymer*, 45: 199.
- Eric Guibal, 2004. Interactions of metal ions with chitosan-based sorbents a review. *Separation. Separation and Purification Technology*, 38: 43- 74.
- El-Sayed Saood, Y.S., 2007. Utilization of some biological resources in biocontrol and promotion of some legume plants growth. M. Sc. Thesis, Women's College. Ain Shams University Egypt.
- El- Tahlawy, Y.A. Gh., 2007. Microbial impact productivity of some medicinal plants. M. Sc. Thesis, Fac. of agric Ain Shams University Egypt x.
- Hewedy Maha, A., 2003. Isolation and characterization of some streptomycetes Spp. From Touthka's Egyptian soil with preliminary indication of unusual N_2 fixation in a local strain. *N. Egypt. Journal of Microbiology*, 6: 15 - 32.

- Jingkun Xu, Guangming Nie, Shusheng Zhang, Xuejun Han, Jian Hou, Shouzhi Pu, 2005. Electrosyntheses of freestanding polyindole films in boron trifluoride diethyl etherate *Polym.Sci. Part A Chem.*, 43: 1444-1453
- Juang, R.S., H.J. Shao, 2002. A simplified equilibrium model for sorption of heavy metal ions from aqueous solutions on chitosan. *Water Research*, 36(12): 2999.
- Johanna, R. Evans, G. William Davids, D. Jean MacRae, Aria Amirbahman, 2002. Kinetics of cadmium uptake by chitosan-based crab shells *Water Research*, 36 (13): 3219-3226.
- Khaled El-Tahlawy, M. Samuel Hudson, 2001. Graft copolymerization of hydroxyethyl methacrylate onto chitosan. *Journal of Applied Polymer Science*, 82(3): 683-702.
- Krieg, N.R. and J. Döbereiner, 1984. Genus *Azospirillum*. In *Begey's Manual of systematic Bacteriology*. Eds. NR Krieg and JG Holt., 1: 94-104. Williams and Wilkins MD. Baltimore. London.
- Koleli, F., Y. Arslan, M. Dudukcu, 2002. Preparation and SEESR-spectroscopic investigations of indole/pyrrole copolymers in aprotic medium *Synthetic Metals*, 129: 47.
- Longdi, Ren., Seiichi Tokura, 1994. Structural aspects of poly methyl methacrylate grafted β -chitin copolymers initiated by ceric salt. *Carbohydrate Polymers*, 23:125.
- Majeti, N.V. and Ravi Kumar, 2000. A review of chitin and chitosan applications. *Reactive and functional polymers*, 46, 1-27.
- Mehrdad Yazdani-Pedram, Jaime Retuert, Raul Quijada, 2000. Article information hydrogels based on modified chitosan, 1. Synthesis and swelling behavior of poly acrylic acid grafted chitosan. *Macromolecular Chemistry Physics*, 201: 923.
- Mostafa, T.B., H.F. Naguib, M.W. Sabaa, S.M. Mokhtar, 2005. Graft copolymerization of itaconic acid onto chitin and its properties. *Polymer International Journal*, 54(1): 221-225.
- Maher Z. Elsabee, Entsar S. Adou, Khaled S. A. Nagy and Mohamed Eweis, 2008. Surface modification of polypropylene films by chitosan and chitosan /pectin multilayer. *Carbohydrate polymers*, 71(1):187-195.
- Moller, H., S. Grelier, P. Pardon, V. Coma, 2004. Antimicrobial and physico-chemical properties of chitosan - HPMC - based films. *J. Agriculture Food Chemistry*, 54:3290-3296.
- Nan Liu Xi - Guang Chen, Hyun - Jin Park, Chen - Guang Liu, Cheng -Sheng Liu, Xiang-Hong Meng, Le -Jun Yu, 2006. Effect of MW and concentration of chitosan on antibacterial activity of *Escherichia coli* *Carbohydrate Polym*, 64:60.
- Oplinger, E.S., L.L. Hardman, E.A. Oelke, A.R. Kamins Ki, E.E. Schulte, J.D. DO all 1997. *Alternative field crops manual*. University of Wisconsin Madison, WI 53706.
- Park, J.S kim., J.H. Nho, Y.C and kwon O.H, 1998. Antibacterial activities of a cyclic acid - grafted polypropylene fabric and its metallic salt. *Journal of Applied polymer science*, 69:221.
- Roller, S., N.Covill, 1999. The antifungal properties of chitosan in laboratory media and apple juice. *Journal of International Food Microbiology*, 47: 67-77.
- Saito, A., T. Shinya, K. Miyamoto, T. Yokoyama, H. Shibuya, H. Tsujibo Y. Nagata, A. Ando, T. Fujii and K. Miy, 2007. The dasABC gene cluster, adjacent to dasR, encodes a novel ABC transporter N,N'-Diacetylchitobiose in *Streptomyces coelicolor* A 3:2.
- Singh, D.K., R. Alok Ray, 1994. Graft copolymerization of 2-hydroxyethylmethacrylate onto chitosan films and their blood compatibility. *Journal of Applied Polymer Science*, 53(8): 1115-1121.
- Singh, D.K., A.R. Ray, 1994. Graft copolymerization of 2- hydroxyethylmetha Crylate onto chitosan films and Their blood compatibility *Journal of Applied Polymer Science*, 53(8): 1115-1121.
- Sang,kook Kim, Nobukazu Miyagawa, Hiroko Seki, Tsutomu Ishikawa, Katsuyoshi Hoshino, 2008. Novel polyindole film prepared via the Meisenheimer complex of indole with trinitrofluorenone. *Journal of Electroanalytical Chemistry*, 615: 12-18.
- Syed, Abthagir P., K. Dhanalakshmi, R. Saraswathi, 1998. Thermal studies on polyindole and polycarbazole. *Synthetic Metals*, 93: 1-7.
- Tao, Sun., Peixin Xu, Qing Liu, Jian Xue, Wenming Xie, 2003. Graft copolymerization of methacrylic acid onto carboxymethyl chitosan. *European. Polymer Journal*, 3: 189- 192.
- Trimukhe, K.D., A.J. Varma, 2009. Metal complexes of crosslinked chitosans: Correlations between metal ion complexation values and thermal properties. *Carbohydrate Polymers*, 75(1): 63-70.
- Talbi, H., G. Monard, M. Loos, D. Billaud, 1998. Theoretical study of indole polymerization *J. Molecular. Structure. Theo. Chem*, 434: 129-134.
- Varma, A.J., S.V. Deshpande, J.F. Kennedy, 2004. Metal complexation by chitosan and its derivatives: a review. *Carbohydrate Polymers*, 55(1): 77-93.
- Xiooying, Wang., Yumin Du, Jianhong Yang, Xiaohui Wang, Xiaowen shi and Ying hu, 2006. Preparation, characterization and antimicrobial activity of chitosan/layered silicatenano.composites *Polymer*, 47(19): 6738-6744.

Xiaoying, Wang., Yumin Du, Jianhong Yang, Xiaohui Wang, Xiaowen Shi, Ying Hu, 2006. Preparation, characterization and antimicrobial activity of chitosan/layered silicate nanocomposites *Polymer*, 47(19): 6738-6744.

Yinghai, Liu., Zhenghao Liu, Yanzhe Zhang, Kuilin Deng, 2002. Graft copolymerization of methyl acrylate onto chitosan initiated by potassium diperiodatonickelate IV. *J. Macromolecular Science-Pure Applied Chemistry*, 39: 129-14.