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Synthesis of Chitosan-Zirconium(IV) Complexes as an Antifungal Spraying Agent Against Tomato Infected *Aspergillus Niger*

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Abstract

Chitosan has been extensively reported as a potent antimicrobial agent either alone or composited with other materials. Its activity against a wide range of food borne microbes and its chelating properties makes it a potential material for food crop preservation via complex formation. In this study, different ratios of chitosan-zirconium(IV) complexes (1:1, 2:1, 3:1) were prepared via co-precipitation method. The complexes were evaluated for the inhibition of the growth of *Aspergillus niger* in post harvested tomato fruits. Spectroscopic (FT-IR and UV-Vis) studies of the chitosan-zirconium(IV) complexes confirmed interactions between chitosan and zirconium metal ion. Chitosan coordinated to Zr^{4+} through $–NH_2$ and –OH functional groups on its structure. Metal complex ratio 3:1 showed a higher antifungal activity compared to free chitosan on post harvested tomato fruits.

Key words: Anti-fungi Actiity, Chitosan-Zirconium(IV) Complex, Tomato Fruit

1.0 Introduction

Antimicrobial agents may be synthetic, either of plant or animal origin, or may be chemically modified natural compounds [1] that produces a significant impact on the outcome of an infected material [2].These antimicrobial agents inhibit the growth of a wide range of microbes such as bacteria, fungi, and viruses which are the major colonizers of the farm produce leading to crop spoilage and altering its nutritional properties.

Chitosan, a natural polysaccharide-based biopolymeric ligand has attracted considerable attention in various applications [3] such as catalysis [4-5], chemosensing [6-7], drug delivery [8] and optics [9] etc. Furthermore, unique biological properties of chitosan such as non-toxicity, bio-degradability, bio-functionality and bio-compatibility have been reported for the biopolymer with application in food, pharmaceuticals, agriculture and textile industries [10-11]. The bio-degradability of chitosan has also been shown to improve the quality of agricultural produce and prolong shelf life by reducing microbial growth due to the presence of a positively charge polycation in the polymer backbone [12-13]. In addition, the anti-microbial activity of chitosan depends on its molecular weight, degree of deacetylation, pH of the chitosan solution and the target organism [13].

Aspergillus niger is a common contaminant of food that causes black mould disease on certain fruits and vegetables. The characterization and antimicrobial activity of metal complexes have been studied and reported in literatures for different strains of bacteria and fungus [14-16]. Noteworthy, however, is our previous study where chitosan–cobalt(II) and nickel(II) chelates serve as effective antibacterial agents [8]. Based on our previous results, the objective of this study is to synthesize and characterize a sprayable form of chitosan coordinated 4*d* metal ion as an antifungal agent against *A. niger* in post harvested tomato fruit.

2.0 Experimental

2.1 Preparation of Chitosan-Zirconium(IV) Complexes

Low molecular weight (50-190kDa) chitosan was purchased from Sigma Aldrich (U.S.A.) while other reagents and solvents were of analytical grade and used without further purification. Different solutions of chitosan were prepared by dissolving 0.5 g, 1.0 g and 1.5 g of chitosan in 100 mL of 3 %(v/v) glacial acetic acid respectively; these were stirred with magnetic stirrer until homogenous gel like solutions were obtained. Then, 4.00 mmol of $Zr(SO_4)_2$ was dissolved in 25 mL of ethanol and added drop wise to the chitosan solution with stirring at 60 ^oC for 2 h. This was repeated for all the chitosan solutions to metal salt solution in ratios 1:1, 2:1, 3:1 respectively [6]. The chitosan-zirconium(IV) complexes was precipitated from solution using 0.5 M NaOH solution. The product was air dried at room temperature and stored for further analysis.

2.2 Anti-Fungi Studies

The *in vitro* antifungal activity of chitosan-zirconium(IV) complexes and chitosan alone were examined against *A. niger*, cultured and analyzed by the well diffusion method [8,17]. Five different tomatoes fruit samples with known weight were incubated with the *fungus* for 72 h at room temperature [8]. Furthermore, 1 mL of the different ratios of chitosan-zirconium(IV) complexes was sprayed onto already incubated tomatoes at three different spots. Chitosan alone was sprayed on another incubated tomato which served as control. After seven days, changes were observed and recorded.

3.0 Results and Discussion

3.1 FT-IR Analysis

Functional group determination was recorded on a Schimazdu FT-IR spectrophotometer. The FT-IR spectrum of chitosan (Figure 1) shows a broad –OH and –NH stretching absorption band between 3435-3400 cm^{-1} and the aliphatic C-H stretching between 2382 and 2107 cm^{-1}. The absorption band at around 1017 cm^{-1} is attributed to the free primary amino group (NH_2) at C-2 position [18-19]. In addition, the peak at 1634 cm^{-1} represented the C=O stretch of the acetylated amino group of chitin, which indicated that the chitosan is not fully deacetylated [20].A chitosan-metal interaction was observed by a slight blue shift in –OH and –NH overlapping frequency at around 3428-3390 cm^{-1} as well as a corresponding slight red shift at around 1635 cm^{-1} (Figure 2). The observation suggests a possible chitosan-zirconium interaction with N and O as donor atoms (Figure 3) [21].

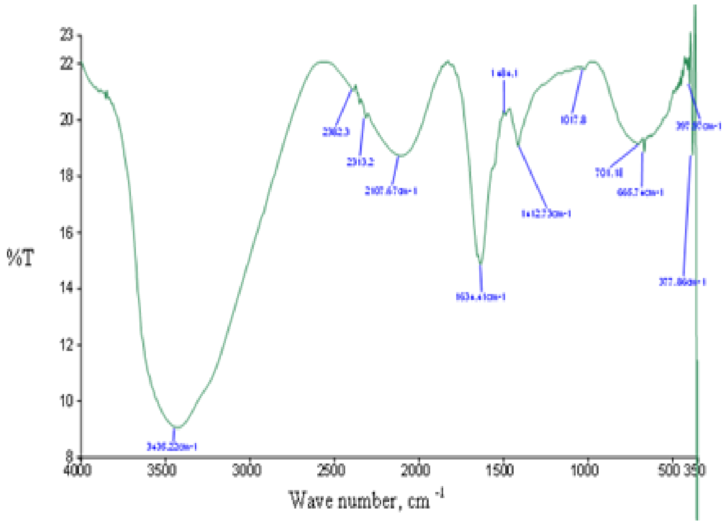

Figure 1: FT-IR Spectrum of Chitosan

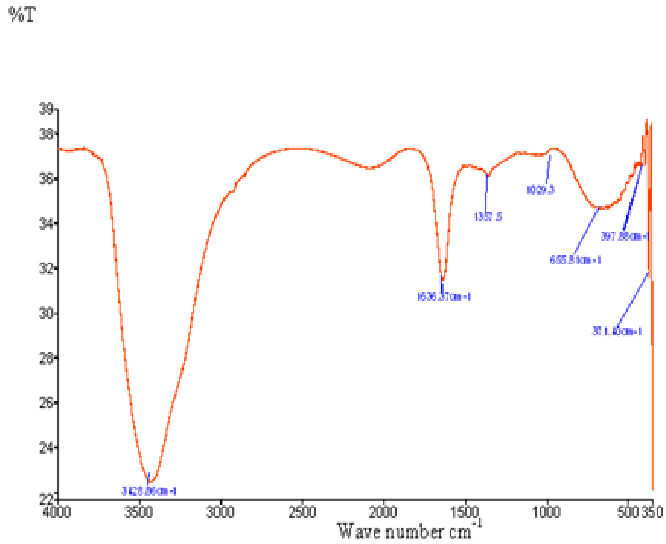

Figure 2: FT-IR of chitosan-Zirconium(IV) Complex

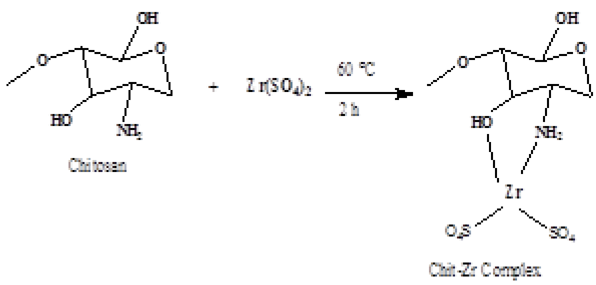

Figure 3: Suggested equation of reaction for the formation of Chitosan-Zirconium(IV) Complex

3.2 Uv-Visible Spectroscopy Analysis

According to previous reports from literatures, no peak is expected in the UV-Visible spectrum of chitosan at the range of 300-800nm [3].However, the band at around 280 nm can be attributed to the n→π* intra ligand electronic transition due to the C=O of the N-acetyl group of chitosan. Conversely, in the spectrum of Chitosan-Zr(IV) complex, a red shifted band was observed at 298 nm, with a hyperchromic shift from 0.91 to 1.20 (Figure 3). This could be tentatively attributed to the n→π* electronic transition due to the C=O of the N-acetyl group of chitosan, which suggest interaction between chitosan and zirconium metal. This result suggests the complexation of Zr^{4+} metal ion with chitosan to form the Chitosan-Zr(IV) complex [22].

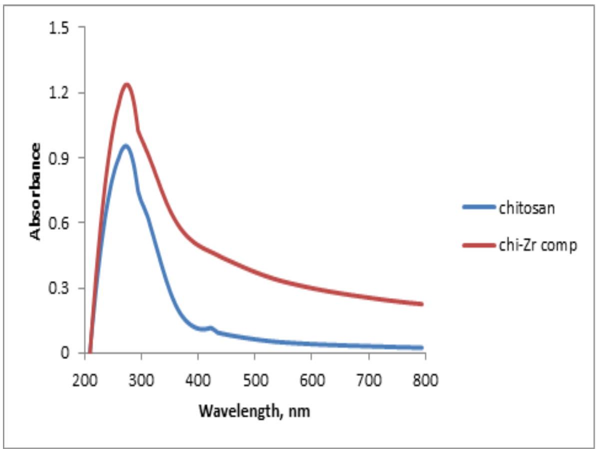

Figure 3: UV-Vis spectrum of chitosan and chitosan-zirconium complex

3.3 Antifungal Activity Test Result of Chitosan-Zr(IV) Complexes

The effect of chitosan-zirconium(IV) complex on the germination of *A. niger* on post harvested tomato fruit were determined and recorded in Table 1. It was observed that, when the complex was applied on the tomato fruits, every spot present on the tomatoes dried after 3 days, which accounted for the loss in weight (Table 2). The tomato fruit used as control became rotten after 7 days, however, the chitosan:Zr^{4+} ratio 3:1 was still fresh with about 0.52 % weight loss against chitosan:Zr^{4+} complex ratio 2:1 and 1:1 with about 0.94 and 0.99 % weight loss respectively. The increased weight loss in chitosan:Zr^{4+} complex ratio 2:1 and 1:1 could be due to the build-up of the *A. niger* colony in tomato fruit as against the inhibited activity in the chitosan:Zr^{4+} ratio 3:1. This implies the effective inhibition of the growth of the fungus with an increased amount of chitosan in the metal complex structure. The inhibitory activity of chitosan-zirconium(IV) complexes *A. niger* was due to the positively charged nitrogen atom (electron deficient nitrogen atom) which has the tendency to bind with the walls of the fungi, hence enhancing its antimicrobial activity [8].

Table 1: Antifungal Activity of Chitosan-Zr(IV) Complexes After 7 Days

Samples (Chit:Zr^{4+})	Day 3 (g)	Day 5 (g)	Day 7 (g)
(1:1)	Fresh	Wrinkle	Spoiled
(2:1)	Fresh	Wrinkle	Wrinkle
(3:1)	Fresh	Fresh	Fresh
CH	Fresh	Wrinkle	Wrinkle
Control	Fresh	Wrinkle	Rotten

Table 2: Weight Loss on Observed Tomato Fruits After 7 Days

Samples (Chit:Zr^{4+})	W1	D3	D5	D7	% L
(1:1)	2.01	1.99	1.99	1.99	0.99
(2:1)	3.20	3.18	3.17	3.17	0.94
(3:1)	1.92	1.92	1.90	1.91	0.52
CH	2.30	2.30	2.30	2.28	0.28
Control	3.20	3.20	3.20	3.20	0

Key: Chit = Chitosan, W1=Initial weight of tomatoes (g), D3=Weight loss (g) at day 3, D5=Weight loss (g) at day 5, D7=Weight loss (g) at day 7, %L=Percentage weight loss (g)

Conclusion

Chitosan-zirconium(IV) complexes were successfully prepared using co-precipitation method and then employed as an antimicrobial agent against *Aspergillus niger*. Spectroscopic studies confirmed an interaction between chitosan and zirconium metal ion, leading to a complex formation. The resultant complexes effectively inhibited the growth of the studied fungi on post harvested tomato fruits which could prevent enormous losses and spoilage in the food crop.

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