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Production of biopolymer based on nanocomposites as edible fruits and vegetables coating against Aspergillus sp. and its toxins

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Abstract: *The present study was carried out as the synthesising of the chitin bionano composites and the synthesis of silver nano composites. The synthesised silver nano composites were identified by the colour change of the solution and was characterized using the various methods -UV Vis spectrophotometer readings at the range of 300-600nm, FT-IR Fourier Transform- Infrared Spectroscopy studies for identification of unknown materials in the chitin silver nanocomposites, SEM (Scanning Electron Microscope) studies for morphological identification of the synthesized nanocomposites. This characterization helps in the studies of morphological, and functional group properties. The main aim is to checking the antimicrobial activity, mainly antibacterial activity using Minimum Inhibitory Concentration (MIC) method organism using Escherichia coli and Antifungal activity using well diffusion method of silver nano composites. To evaluating efficiency of the coated solution against various fruits (Sapota- Manilkara zapota) and vegetables (Cucumber-Cucumis sativus, Broad beans- Vicia faba) as edible coating. Shelf life analysis was done by carbohydrate test of fruits and vegetables, nutritive value analysis done by sodium, potassium, calcium test and noted.*

Keywords: *Silver nanoparticles, Characterization, Chitin, MIC, Nanocomposites.*

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Introduction

The nanoparticles which describe the nanosized atoms with dimensions (length, width or thickness) with their size range of 1 to 100 nm. Faraday (1857) he first found the existence of metallic nanoparticles where in solutions and Mie (1908) give a quantitative explanation of their colour. (C.A. Charitidis *et al* January 2014)

Chitosan is a linear homopolymer that is constituted of β-(1,4)-linked N-acetyl-glucosamine unit. It was incompletely deacetylated polymer which acquired from basic deacetylation of chitin, which is a glucose-based unbranched polysaccharide that is found extensively in the major components of crustaceans and insects exoskeletons, like wise some bacterial and fungal cell wall. The quality of the chitosan material depends upon the source of chitin material and its separation and the degree of deacetylation of chitin. Chitosan material has excellent biological properties which includes nontoxic, mucoadhesive, homo-compatible, biodegradable and possessing antitumor, antioxidant, and antimicrobial properties. The properties which make the chitosan was a very attractive biomaterial for different applications in the biomedical field.

Antitumor Activity

The antitumor activity of the chitosan derivatives is caused by an increase in the secretion of the interleukin (IL)-1 and interleukin 2, which results in the maturation and infiltration of the cytolytic T- lymphocytes.

Antioxidant Activity

It was well-known that the antioxidants have the high beneficial effects on human health. They prevent the damage of compounds such as membrane lipids, proteins, and DNA by the body's reactive oxygen radical molecules. Many of the studies that shows the chitosan and its derivatives have the ability to scavenge and the active oxygen free radicals in vitro. Low molecular weight chitosan molecules have the several advantages over of high-weight chitosan molecules in the elimination of free radicals.

Biodegradability

Chitosan in the biological organisms can be catalyzed using bioenzymes to depolymerize that molecule. The degraded products which are N-acetyl glucose and the glucosamine, which are nontoxic to the human cells. Degradation which intermediates they do not accumulate in the human body and they don't have immunogenicity. (Dongying Zhao *et al* 2018).

Materials and Methods

0.5% and 1% of the chitin solutions was prepared by two methods and were used to synthesis silver nanoparticles

UV Visible Spectrophotometer

The nanoparticles were primarily conformed by using UV-VIS spectrophotometer from nanometer of 300-600, with 1nm resolution in 2 ml quartz cuvette with 1 cm path length. UV-Visible study was done to identify the compounds and the diluted supernatent was analysed in the wavelength range from 200-400 nano meters.

FT-IR – Fourier Transform–Infrared Spectroscopy

FT-IR stand for Fourier Transform Infrared (FT-IR) is a technique based on the vibrations of the atoms within a molecule. An infrared (IR) spectrum is obtained by passing IR radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in the absorption spectrum appears corresponds to the frequency of vibration of a part of a sample. FT-IR analysis method uses infrared light to scan test samples and observe chemical properties. It is used to identify and characterize unknown materials.

SEM

SEM was used to identify the morphology of synthesized chitin silver nanoparticles. Sample was prepared by centrifugation and pellet was collected. The pellet was dried and used for the Scanning Electron Microscopy analysis.

MIC

The MIC of chitin silver nanoparticles against *Escherichia coli* was determined by using the different concentration of the solution. Nutrient broth was prepared by 13.0g in 1000ml of distilled water and sterilized under autoclave 121℃ for 15 minutes. The sterilized media was cooled and transferred to test tubes each tubes contain 1 ml of broth. After transferring 50,100,150 µl of nanoparticles was added with 10µl of *Escherichia coli* bacteria. After mixing the tube was incubated at 37℃ for 24 hours. Control was maintained with out adding sample.

Preparation of coating solutions

Based on the MIC and Antifungal activity we are preparing the coating solution. The 1% chitin solutions are prepared and the solution keep in the incubator shaker for sometimes for proper mixing of the solution. The chitin solution is filtered and separate residue from solution. This residue is dried in the room temperature. The dried residue is grind to make fine powder by using the Motor and pestle. The powder is transformed into a new beaker and addition of the 5ml of the 1% trisodium citrate in the residue separated from the solution. Then the 1mM of the AgNO3 solution for the synthesis of the chitin silver nanoparticles. keep the solution in water bath 60℃ for 30 minutes and primarily confirmed by using UV VIS spectrophotometer.

Coatings to the fruits and Vegetables

The available seasonal vegetables and fruits are used to coating the chitin silver nanoparticles. The prepared coating solution is transferred into the spray bottle. Then wipe the fruit and vegetable using sterile cotton to remove outer damage. Then the prepared solution is sprayed over the fruit and vegetables completely. The fruits and vegetables which are not sprayed is use as control to check the change between the sample and the control.

Shelf life analysis of fruits and vegetables

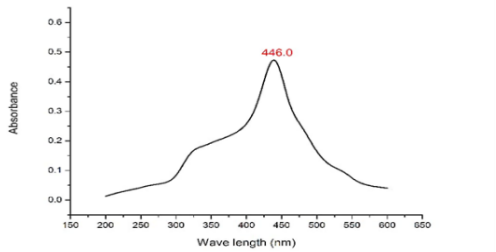
The test was performed continuously in five days for analysing the sample (the chitin silver nanoparticles coated) and control. The difference is analysed by using the shelf life analysis test. The test is performed in the nutrient

broth media. Preparation of nutrient broth, after that take the swab of the controls and samples and they inoculated into nutrient broth medium.

Results

UV -Vis spectrophotometer

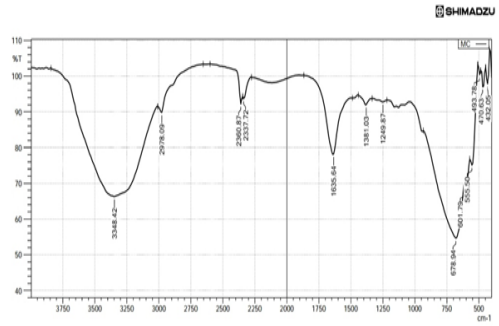
The primary conformation of chitin silver nano particles was confirmed by the UV VIS Spectrophotometer. The biosynthesis of Ag NPs was characterized by UV–visible spectroscopy. The colour of the solution that was changed from colourless to light yellow and finally to yellowish brown was noted by visual observation and this confirmed the synthesis of Ag NPs. The increase of the intensity of yellowish brown colour is due to the excitation of surface plasmon resonance (SPR) of AgNO3.



Graph.1. UV VIS Spectrophotometer reading of chitin silver nanoparticles

FT-IR (Fourier Transformed- Infrared Spectroscopy)

FT-IR showed the structure, the respective bands of synthesized nanoparticles and the stretch of bonds.



Graph.2. FT-IR spectrum of chitin silver nanoparticls

SEM (Scanning Electron Microscope)

The Scanning Electron Microscopy Was carried out to find out the morphology. The size was in the range of 60nm-140nm.The shape was found to be cuboid, linear, and small round.

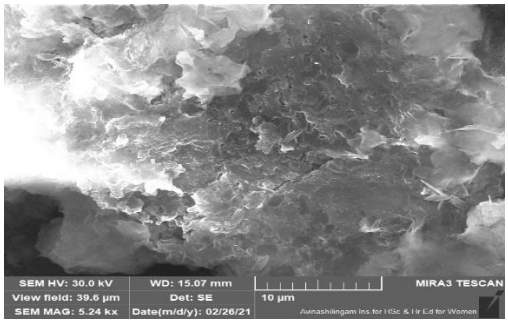


Fig .1. Image of the Scanning Electron Microscopy of chitin silver nanoparticles

Shelf life analysis of fruits and vegetables

The nutrient broth medium was inoculated with swabs of coated and non-coated samples like Sapota, Broad beans, Cucumber. Up to days 4 analysed the shelf life of fruits and vegetables. OD value is taken in 600 nm and recorded the readings.

Sample	Day 1	Day 2	Day 3	Day 4
Control cucumber	0.53	0.58	0.69	0.72
Control broad beans	0.72	0.66	0.82	0.76
Control sapota	0.45	0.48	0.71	0.85
Sample cucumber	0.30	0.08	0.11	0.29
Sample broad beans	0.21	0.19	0.25	0.25
Sample sapota	0.01	0.11	0.20	0.20

Table.1. Colorimetry reading for the shelf life analysis of chitin $AgNO_3$ coated and non coated samples.

Discussion

The initial growth of the bacterial population was high in the three groups of both the fruits, but after applying the chitin coating and the Chitin composites, bacterial populations were reduced significantly. Our results are on line with the findings of who found that chitosan based coating reduced microorganisms level in fish coated with chitosan.(V.Nisha*, *et al* 2016)

Ag nanocomposites coated in the outer layer of Fruits (Apple and Sapota) were done under the sterile condition at room temperature. It is concluded that when compared with non-coating, the coated fruits and vegetables showed the good shelf life period from 20 to25 days at room temperature. For apple shelf life period was about 25 days and for sapota it was 25 days .(Sharanya Devadiga B, *et al* 2016)

Summary and conclusion

Silver nanoparticles are importance due to their unique properties and its various applications in the field of food industry and medicine. Here the study was done to synthesis chitosan silver nanoparticles using 1mM $AgNO_3$.

After synthesizing the chitin nanoparticles was characterized using the UV-VIS spectrophotometer and the plasmon peak was observed at 446.0 nm. The functional group also confirmed that the silver nanoparticles formation using the FT-IR studies the bands of stretchs indicating the functional group of nanoparticles. The SEM was studied to find out the morphological features. The size was in the range of 60nm-140nm.The shape was found to be cuboid, linear, and small round .Followed by the sample was used for antibacterial activity against *Escherichia coli* and Antifungal activity against *Aspergillus flavus, Aspergillus niger, Aspergillus terrus* by well diffusion method. This was shown good antimicrobial activity against food pathogens. The shelf life analysis was studied using various fruits and vegetables (cucumber, broad beans, and sapota).and the chitin nanoparticles coated found to be better candidate.so chitin nanoparticles was used for preservation of cucumber, broad beans, sapota compared with controls (non coated). Nanoparticles coated shown higher shelf life.

In future the chemical coating will be replaced by chitin silver nanoparticles and used as an edible fruit coating. Future it will be benefited.

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