

Full Length Research Paper

## Antifungal activity of chitosan-silver nanoparticle composite against *Colletotrichum gloeosporioides* associated with mango anthracnose

P. Chowdappa\*, Shivakumar Gowda, C. S. Chethana and S. Madhura

Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bangalore-560 089, India.

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Chitosan loaded with various metal ions such as  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$  has been reported to exert strong antimicrobial activity. In this study, the silver-nanoparticles (AgNPs) were synthesized at  $95^\circ\text{C}$  using chitosan as the reducing agent and stabilizer. The UV-Vis spectrum displayed peak in a range between 415-420 nm, the characteristic surface plasmon resonance band of silver nanoparticles. The size, shape and aggregation properties of the resultant nanoparticles were examined using field emission scanning electron microscopy coupled with energy dispersive X-ray spectroscopy. The measurement results indicated that the chitosan-silver nanoparticle (chitosan-AgNP) composite having the mean hydrodynamic diameter range of 495-616 nm were apparently smooth and the silver nanoparticles with the size distribution was from 10 to 15 nm. Chitosan-AgNP composites had a zeta potential of +50.08 mV to +87.75 mV. *In vitro* conidial germination assay indicated that chitosan-AgNP composite exhibited significantly higher antifungal activity against *Colletotrichum gloeosporioides* than its components at their respective concentrations. *In vivo* assay using detached mango fruit cv. Alphonso showed that anthracnose was significantly inhibited by chitosan-AgNP composite. Therefore, this study suggests that postharvest decay in mango can be minimized by chitosan-AgNP composites and its application on a commercial scale needs to be exploited.

**Key words:** Chitosan, silver nanoparticle, antifungal activity, mango fruits, *Colletotrichum gloeosporioides*.

### INTRODUCTION

Chitosan, a deacetylated derivative of chitin, is the second most abundant natural hydrophilic linear polysaccharide found in the nature after cellulose. It is made up of N-acetyl-2 amino-2-deoxy-D-glucose (glucosamine) and 2-amino-2-deoxy-D-glucose (N-acetyl-

glucosamine) residues (Aranaz et al., 2010). Due to its unique biological properties, such as non-toxicity, biodegradability, biofunctionality and biocompatibility, many applications have been reported either alone or blended with other natural polymers (starch, gelatine and

\*Corresponding author. E-mail: [pallam22@gmail.com](mailto:pallam22@gmail.com). Tel: +91-80-28466420. Fax: +91-80-28466291.

alginate) in the food, pharmaceutical, textile, agriculture, water treatment and cosmetics industries (Harish Prashanth and Tharanathan, 2007). This biodegradable material can also improve the quality of agricultural products and extend shelf life by minimizing microbial growth in the product because of its positively charged (poly cationic) nature (Zhong et al., 2009; El Hadrami et al., 2010). Antimicrobial properties of chitosan have been shown against many bacteria, filamentous fungi and yeasts (Kong et al., 2010). It has got significant importance in plant protection because of its dual function; fungistatic or fungicidal effects and induction of defence responses (Kendra and Hadwiger, 1984; Sudarshan et al., 1992; Tsai and Su, 1999; Bautista-Banos et al., 2006). The antimicrobial activity of chitosan depends on its molecular weight, deacetylation degree, pH of chitosan solution and the target organism (Helander et al., 2001; Jeon et al., 2001; Zhong et al., 2009). The antifungal activity of chitosan against *Colletotrichum gloeosporioides* has been reported (Ali et al., 2010). Antifungal activity of chitosan has also been reported on *Alternaria alternata*, *Botrytis cinerea*, *Rhizopus stolonifer* and *Phytophthora capsici* (El Ghaouth et al., 1992; Xu et al., 2007). Chitosan possesses high chelating capacity with various metal ions such as  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Fe}^{2+}$  in acidic conditions and these chitosan metal complexes exert strong antimicrobial activity (Kong et al., 2010).

The antimicrobial property of elemental silver ( $\text{Ag}^+$ ) has been studied extensively and has found many applications in medicine than any other inorganic metal ion, while at the same time having shown no harm to human cells (Russell et al., 1994). Silver can be used for management of plant pathogens in a relatively safer way as compared to synthetic fungicides (Park et al., 2006) as it displays multiple modes of inhibitory action (Clement and Jarrett, 1994). Previous studies provided evidence of the applicability of silver for controlling plant pathogenic fungi such as *Bipolaris sorokiniana*, *Magnaporthe grisea* (Jo et al., 2009), *Golovinomyces cichoracearum* or *Sphaerotheca fusca* (Lamsal et al., 2011) and *Raffaelea* sp. (Kim et al., 2009). In recent years, considerable research has been done for incorporation of silver-nanoparticles into ultrathin fibers for many important applications, such as using wound dressing materials in the medical field (Zhuang et al., 2010). Chitosan has been used as both a reducing agent and stabilizer to form AgNPs (Murugadoss and Chattopadhyay, 2008). The binding interaction between chitosan and the silver-nanoparticles results in stabilization of the chitosan-AgNP composites and hence, the nanoparticles attached to the polymer chains will disperse in the solution when the composite dissolved.

Mango (*Mangifera indica* L.) is commercially important tropical fruit crop in India, accounting for > 54% of the total mango produced worldwide. India exports fresh

mangoes to more than 50 countries (Tharanathan et al., 2006). Anthracnose, caused by the *C. gloeosporioides* is the most important post-harvest disease of mango (Arauz, 2000) and cause decay during storage and transport. Chemical fungicides have been the most effective way to control postharvest decay of mango, but their use has caused the development of fungicide resistance, and increasing public conflict (Lin et al., 2011). Benzimidazole fungicides have been used for management of *Colletotrichum* diseases during the last 38 years and numerous cases of resistance have been reported due to its very specific mode of action (Hewitt, 1998; Peres et al., 2004). In view of wide-spread benzimidazole fungicide resistance across *Colletotrichum* species, alternative safe chemical control strategies need to be developed to manage pre and postharvest anthracnose diseases. Previous studies showed that chitosan significantly inhibited growth of *Colletotrichum* sp. even at lower concentrations (Bautista-Banos et al., 2003; Munoz et al., 2009). Sanpui et al. (2008) has demonstrated that chitosan-AgNP composites were highly effective in inhibiting certain bacteria than chitosan alone. As no work has been carried out on the antifungal activities of chitosan-AgNP composites, we hypothesized that chitosan can be useful in plant protection due to its dual function of defense response in plants and antifungal effect, and its activity can be enhanced in combination with metal ion in the form of nanoparticles. The objective of this study was to evaluate the chitosan-AgNP composite for its antifungal activity against *C. gloeosporioides in vitro* and its efficacy in reduction of anthracnose disease in mango fruit.

## MATERIALS AND METHODS

### Chemicals

Chitosan low molecular weight (85% deacetylated), and Whatman No. 1 filter paper were purchased from Sigma-Aldrich, USA. Silver nitrate ( $\text{AgNO}_3$ , 99.9%) was supplied by Merck, India. Acetic acid (glacial, 99–100%) was from SD fine chemicals, India. Tween-80 and sodium hydroxide were procured from HiMedia Laboratories, India. All the chemicals were used as received without further purification. We used double distilled and deionized water throughout the study (MilliQ/Millipore System, Billerica, MA, USA).

### Synthesis of the chitosan-AgNP composite

Nanoparticles were prepared by adopting the one-pot synthesis method of Sanpui et al. (2008) with slight modifications. Briefly, a 100 ml aqueous solution containing 0.2 g of chitosan was kept on a magnetic stirrer with hot plate at a temperature of  $95 \pm 1^\circ\text{C}$  with constant stirring. This was followed by addition of freshly prepared 2.0 ml solution of different concentrations (10, 15, 20, 25 and 30 mM) of silver nitrate and 300  $\mu\text{l}$  of 0.3 M NaOH, respectively. The pH of the solution was measured to be 10.0. Formation of AgNPs occurs spontaneously in about a minute, by turning the resultant solution to yellow indicating the formation of AgNPs in the medium.

The reaction was allowed to continue for additional 30 min and cooled to room temperature. Powdered yellow colored solid was then collected by filtration through Whatman No. 1 filter paper. The powder was washed with deionized water four times, then air dried and used for further studies.

### Analytical measurements and characterization

Ultraviolet-visible spectroscopy (UV-vis) spectra of all the synthesized nanoparticle samples were recorded at room temperature using a TECAN infinite 200 (Seestrasse, Mannedorf, Switzerland) in the range 250–550 nm. The dynamic light scattering (DLS) and Zeta potential measurements of the liquid samples were performed by using Zeta pals (Brookhaven Instrument Corporation, Holtsville, NY, USA) at 25°C. Size, shape and aggregation properties of the nanoparticles were examined by field emission scanning electron microscopy (FESEM). For FESEM studies, a drop of the aqueous suspension of nanoparticle was placed on aluminium stub with double sided carbon tape and kept for drying at 70°C for three hours. Then, samples were kept in desiccator containing silica gel for 72 h, and gold particles were sputtered over the coated material to prevent the charging effect and kept under vacuum pressure for 30 min before analysis. The samples were examined under FESEM using a Zeiss-Ultra 55 model microscope (Carl Zeiss Promenade, Jena, Germany) equipped with an energy dispersive X-ray spectroscopy (EDS) capability. The quantity of silver present in the nanocomposite was estimated using atomic absorption spectrophotometer. A stock solution of silver at a concentration of 1000  $\mu\text{g mL}^{-1}$  was prepared by dissolving 1.574 g of  $\text{AgNO}_3$  (equivalent to 1g of metallic silver) in nitric acid : water (1: 1) and diluted to 1000 ml with Milli Q water and stored in the dark. A Thermo M series atomic absorption spectro-photometer (Thermo Electron Corporation: Chromatography and Mass Spectrometry, River Oaks Parkway San Jose, CA, USA) was used with a silver hollow-cathode-lamp, at an operating current of 2 mA, and a wavelength and spectral band pass of 328.1 and 1 nm, respectively.

### Germination of treated-conidia of *C. gloeosporioides* with chitosan-AgNP composite

*C. gloeosporioides* isolate (Cm 50 NCBI accession number EF025937) recovered from mango was grown on potato dextrose agar (PDA) for 7 days under cool white fluorescent light (67.5  $\text{mmol m}^{-2} \text{s}^{-1}$ ) at  $25 \pm 1^\circ\text{C}$  to promote sporulation. Conidia were washed from the PDA plate with 5 ml of sterile distilled water and adjusted to  $1.5 \times 10^6$  conidia/ml using a haemocytometer (Than et al., 2008). A solution of 50  $\mu\text{l}$  containing different concentrations of chitosan-AgNPs composite (loaded with 30 mM  $\text{AgNO}_3$ ) and chitosan (0.1, 1, 10, 100 and 1000  $\mu\text{g/ml}$ ) dissolved in 0.1% (v/v) acetic acid was added to the well of the cavity slide containing 50  $\mu\text{l}$  spore suspension. An equal volume (50  $\mu\text{l}$ ) of sterile distilled water with 0.1% (v/v) acetic acid was added to the control well. All cavity slides were placed in a moisture chamber containing ~95% humidity and incubated for 12 h at  $25 \pm 1^\circ\text{C}$ , germination of conidia in 20 randomly chosen fields were determined at 400x magnification under Zeiss bright field microscope (Axio Scope.A1, Gottingen, Germany). Conidia were considered germinated when the length of the germ tube equalled or exceeded the length of the conidia. Percent inhibition of spore germination over control was calculated using the following formula.  $I = (C-T/C) \times 100$ , Where, I = percentage inhibition of conidial germination in test pathogen, C = number of germinated conidia in control and T = number of conidia germinated in treatment. Each treatment had three replicates, and each replicate

contained nine slides. The experiment was repeated three times.

### Coating preparation

The coating solutions were prepared by dissolving chitosan-AgNPs composite loaded with 30 mM  $\text{AgNO}_3$  and chitosan (0.5 and 1.0% w/v) respectively at 40°C in a 0.5% (v/v) acetic acid solution, since chitosan is only soluble in an acidic medium. Then, Tween 80 at 0.1% (v/v) was added for improving wettability. The resulting mixture was agitated vigorously with heating using a magnetic stirrer during 2 h until chitosan was dissolved (Garcia et al., 2010).

### Coating and antifungal activity of chitosan-AgNP composites on anthracnose development on mango fruits

The fruits of mango cv. 'Alphonso' collected from 15 year old tree grown in the experimental farm of Indian Institute of Horticultural Research, Bangalore, India were used in the experiments. The healthy fruits were selected at a semi-ripe stage with similar size and weight (250 g). The fruits were washed in running water and surface sterilized with 0.1% sodium hypochlorite solution for 2 min and then washed with sterile distilled water thrice and air dried. The fruits were dipped for 15 min in sterile water (control), 0.5% acetic acid, chitosan (0.5% and 1%) and chitosan-AgNPs composite loaded with 30 mM  $\text{AgNO}_3$  (0.5% and 1%) with and without addition of 0.1% Tween-80 (v/v). Chemical check of carbendazim at 0.001, 0.01%, 0.001 and 0.0001% were also done for coating mangoes and then coated fruits were air dried for 30 min at 25°C. Mango fruits were then wounded on the same side (distal and proximal) to a depth of 2 mm by puncturing them with a sterilized pin. Each wound site was then inoculated with 20  $\mu\text{l}$  of spore suspension ( $1.5 \times 10^6$  spore/ml) of *C. gloeosporioides*. Treated and control fruits were then placed onto wire mesh in plastic boxes (45 cm height x 40 cm length x 15 cm width) containing water, maintaining ~95% relative humidity and incubated at  $24 \pm 1^\circ\text{C}$  for 7 days. Lesion area ( $\text{Cm}^2$ ) was scored separately by measuring length and breadth of each infection site. The experiment was carried out with three replicates, each replicate contain 12 fruits, thereby, making a total of 36 fruits per treatment. The experiment was repeated three times.

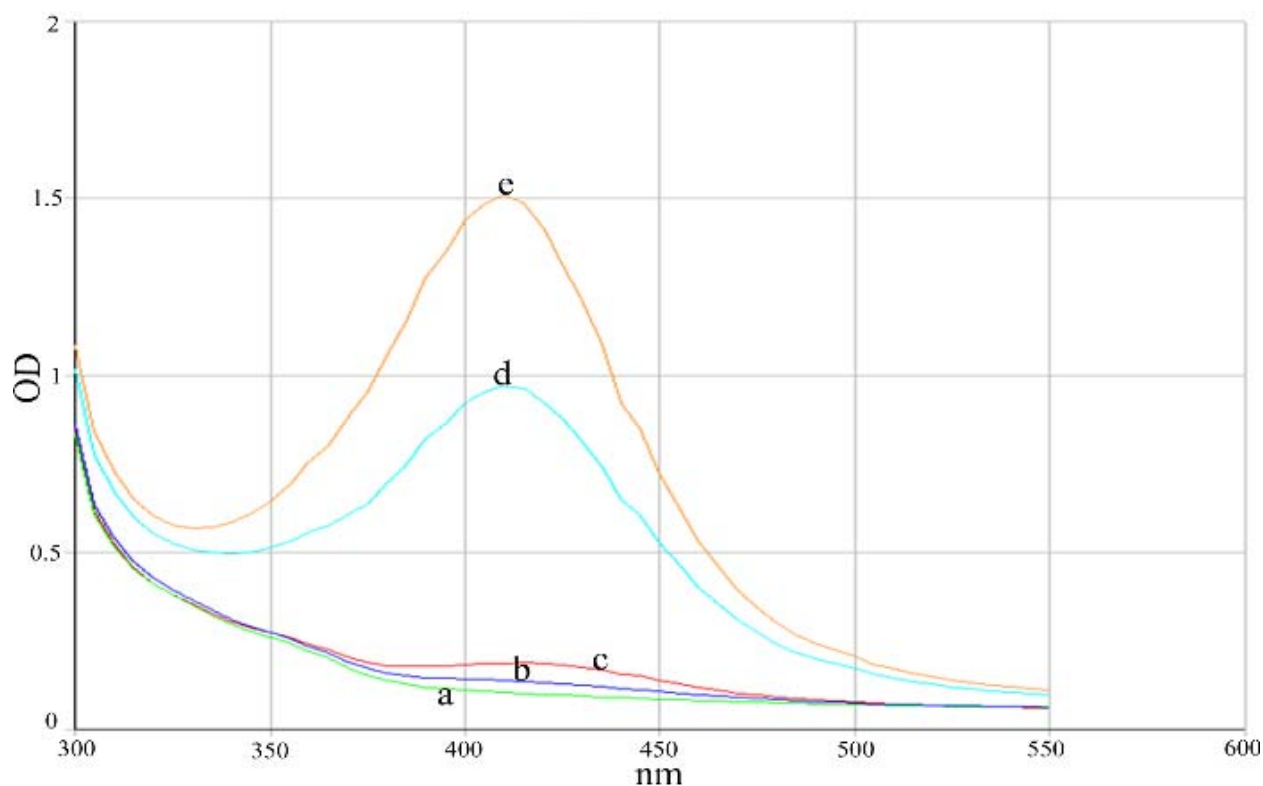
### Statistical analysis

All data were statistically analysed using one-way analysis of variance (ANOVA) to identify the origin of significance and followed by a Fishers test to separate means and treatments using Graph pad Prism V.500 for windows (Graph pad software, San Diego, California, USA). Means were compared between treatments by least significant difference (LSD) at the 1% level ( $p < 0.01$ ). Percentage data were arcsin-transformed before analysis according to  $y = \arcsin [\text{sqr} (\_/100)]$ .

## RESULTS

### Synthesis and characterization of chitosan-AgNP composites

As substantiated by the formation of yellow colored powder, reduction of  $\text{Ag}^+$  to Ag NPs was observed in the presence of NaOH and at an elevated temperature. This indicated chitosan produced Ag-NPs under alkaline



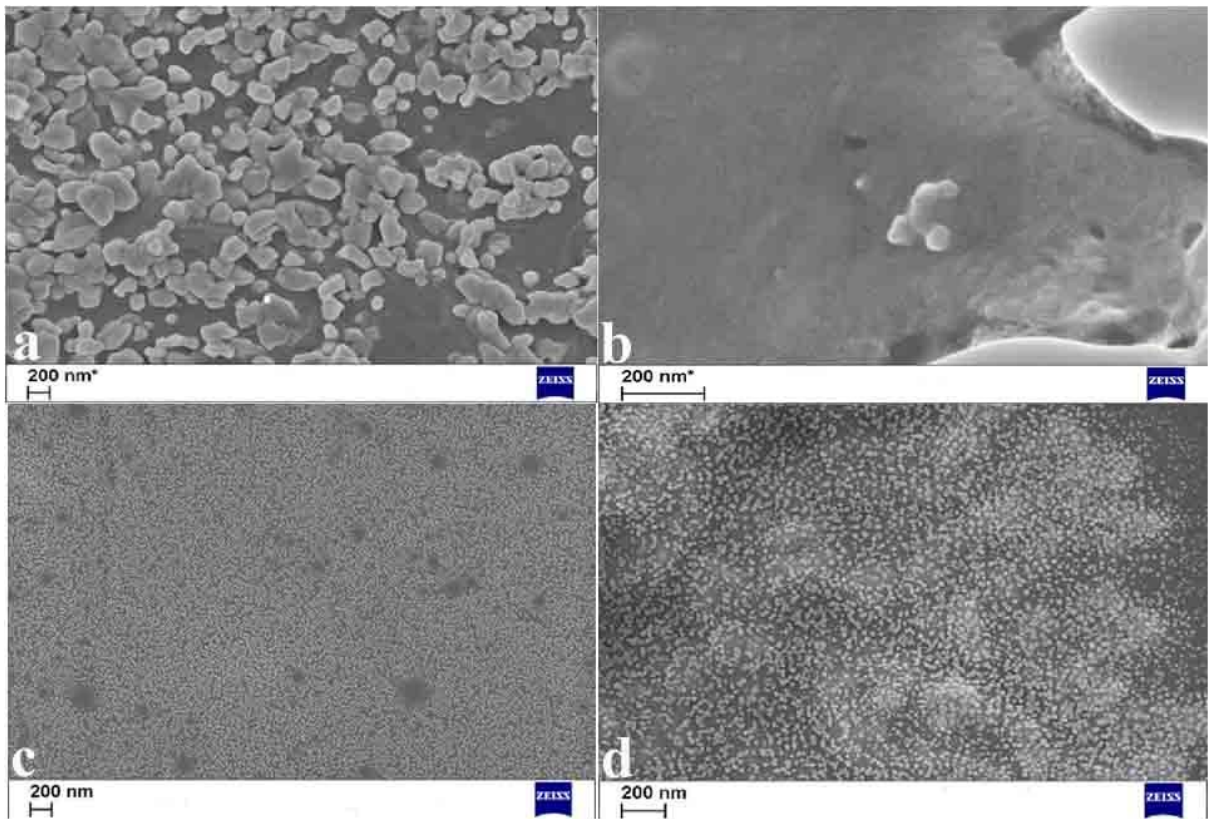
**Figure 1.** UV-Vis absorbance spectra of chitosan-AgNP composite dissolved in 0.1% (v/v) acetic acid in water. The concentration of silver ions in the original solution used for the preparation of NPs were a) 10 mM, b) 15 mM, c) 20 mM, d) 25 mM, and e) 30 mM  $\text{AgNO}_3$  respectively. The amount of chitosan used for synthesis was 0.2g in 100 ml.

**Table 1.** Result of dynamic light scattering and zeta potential.

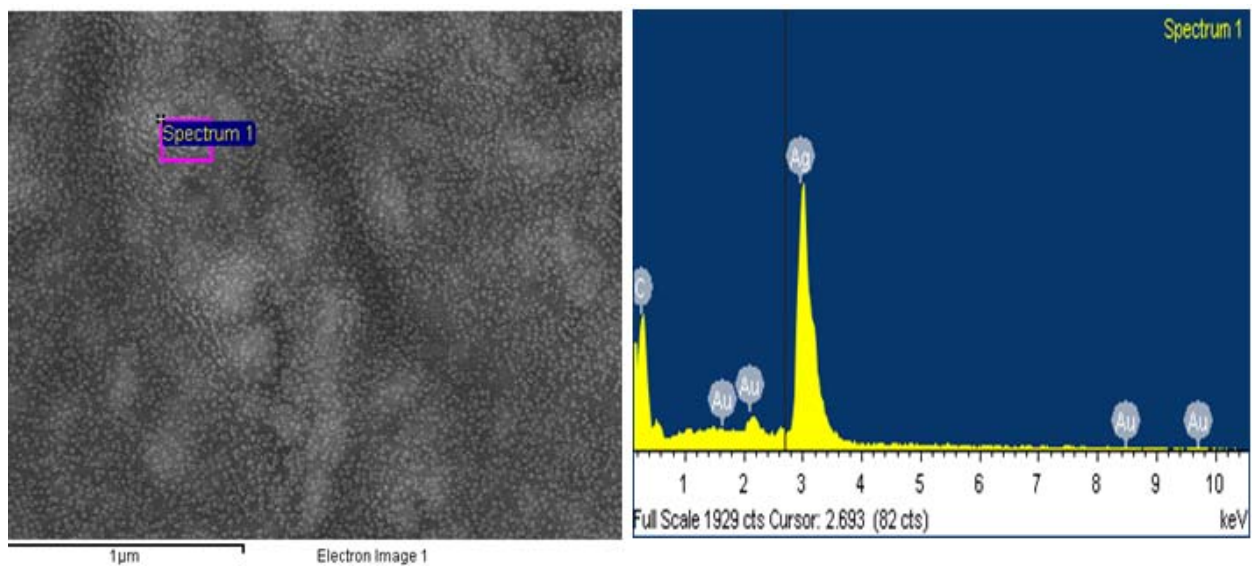
Concentration of $\text{AgNO}_3$	Particle size (nm)	Zeta potential (mV)
10 mM	494.5	50.08
15 mM	589.7	62.11
20 mM	616.1	76.52
25 mM	592.2	85.37
30 mM	594.8	87.75

conditions. The chitosan worked as both reducing agent and also stabilizer for the production of NPs. The yellow powder was found to be insoluble in water. The yellow powder of composite was dissolved in acetic acid and the UV-Vis spectrum was measured. The spectra showed that with the increase in concentration of  $\text{AgNO}_3$ , there was a gradual increase in the intensity of peak in a range between 415-420 nm (Figure 1), the characteristic surface plasmon resonance (SPR) band of silver nanoparticles, indicating the formation of silver nanoparticle. The result showed that with the increase in concentration of silver ion, the concentration of silver nanoparticle

formation has increased. Table 1 showed the size distribution profiles of the chitosan-AgNP composites loaded with different concentrations of  $\text{AgNO}_3$ . The mean hydrodynamic diameters of nanoparticles loaded with 10, 15, 20, 25 and 30 mM of  $\text{AgNO}_3$  was 495, 590, 616, 592 and 595 nm, respectively when analysed with DLS. The zeta potentials were enhanced significantly with the increase in concentration of  $\text{AgNO}_3$  (Table 1). Chitosan-AgNP composites had a zeta potential varying from +50.08 mV to +87.75 mV. When the same samples used for DLS studies were analysed through SEM, chitosan-AgNP particles showed spherical shape with solid dense structure having particle sizes in the range between 10-15 nm (Figure 2). When the elemental composition of chitosan-AgNP was determined by EDS, silver signal was detected, indicating the presence of significant amounts of silver in nano composite (Figure 3). In addition, carbon signal originated from the carbon tape used for coating the material and gold signal because of the gold sputtering on the surface of the film to prevent charging effect and to improve conductivity (Table 2). The nano-composite prepared from 30 mM  $\text{AgNO}_3$  had 30.281 mg of silver per gram of chitosan-Ag nanoparticle composite when analysed through atomic absorption spectrophotometer.



**Figure 2.** SEM micrograph; a) Chitosan aggregates, b) AgNO<sub>3</sub>, c) Chitosan-AgNP composite (30 mM AgNO<sub>3</sub>) at 50 K X, and d) 300 K X magnification.



**Figure 3.** SEM image and corresponding EDS spectra for chitosan-AgNP composite.

### Antifungal activity of chitosan-AgNP composite

In this study, the effect of chitosan and chitosan-AgNP

composite (Figure 4) loaded with 30 mM AgNO<sub>3</sub> (zeta potential of 87.75 mV) on conidial germination of *C. gloeosporioides* were determined and shown in Figure 6.



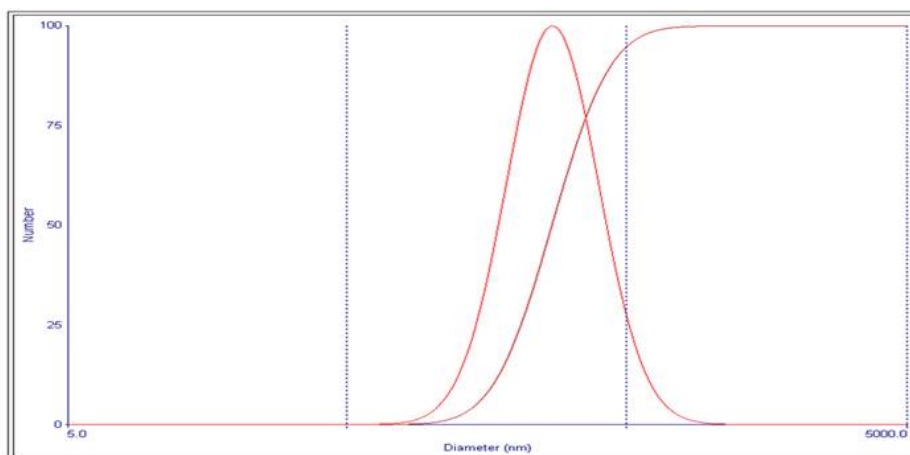
**Table 2.** Chemical composition of elements present in the chitosan-AgNP composite determined by EDS.

Element	Weight (%)
Carbon*	19.64
Silver	75.60
Gold**	4.76

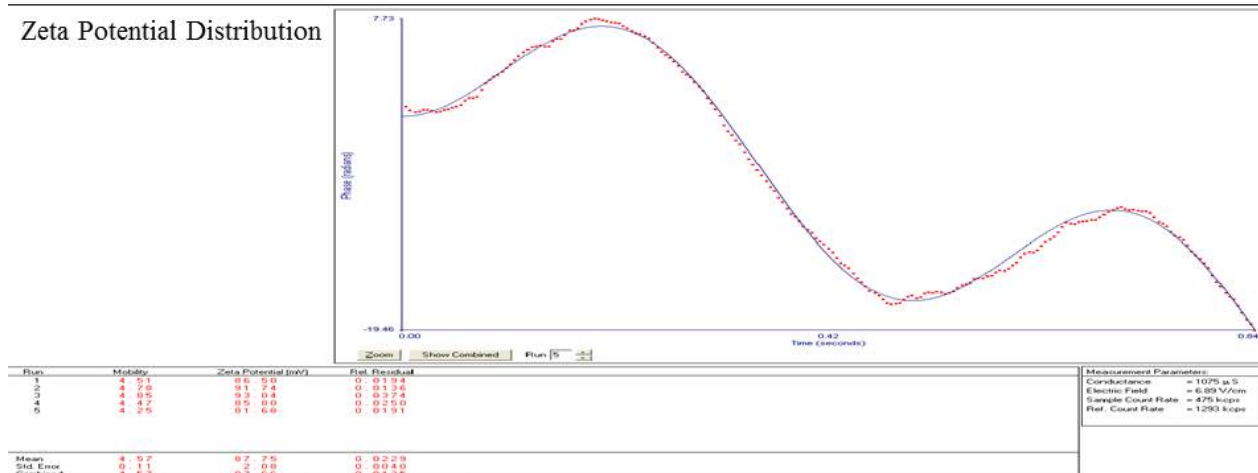
\*Carbon signal originated from carbon tape; \*\*Gold signal from gold sputtering on the surface of the film.

**Size Distribution**

**Effective Diameter:** 594.8 nm  
**Polydispersity:** 0.174  
**Avg. Count Rate:** 480.1 kcps  
**Baseline Index:** 0.0/ 98.32%  
**Elapsed Time:** 00:05:00



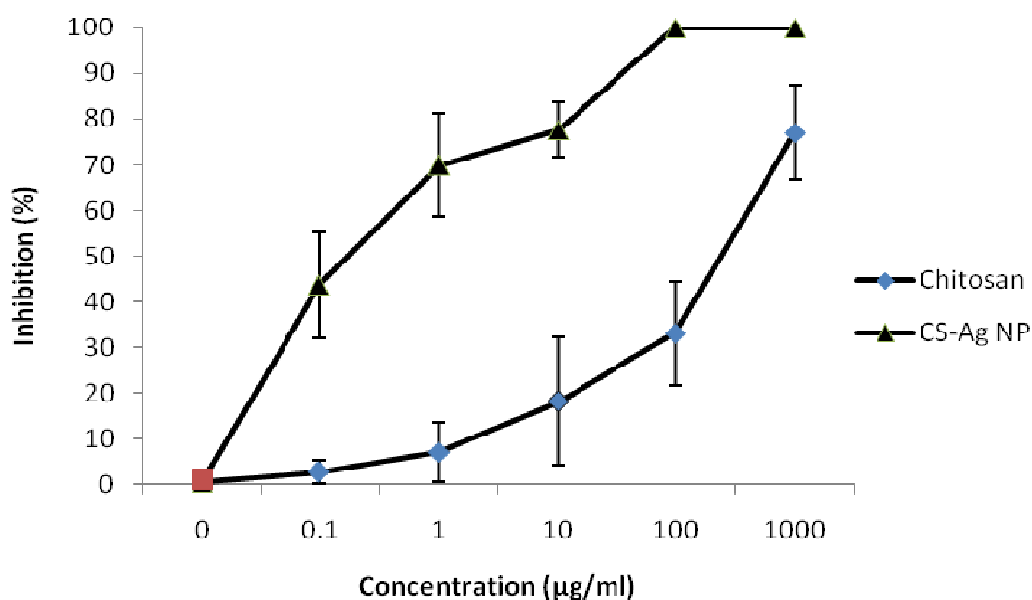
**Zeta Potential Distribution**



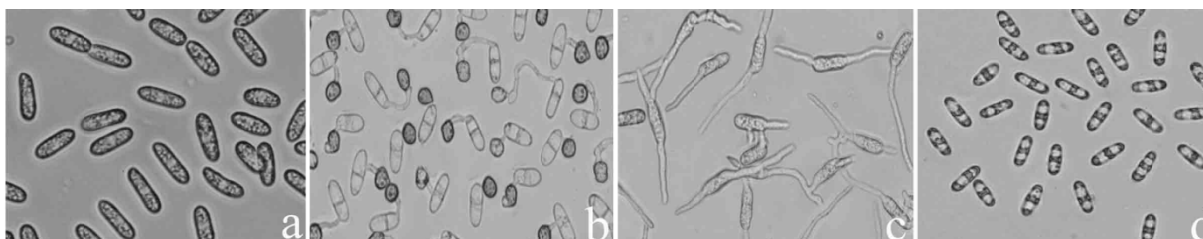
**Figure 4.** Size distribution and zeta potential distribution of 30 mM AgNO<sub>3</sub> treated chitosan-AgNPs composite.

Chitosan-AgNP composite (treated with 30 mM AgNO<sub>3</sub>) markedly reduced conidial germination of *C. gloeosporioides* than chitosan. Chitosan-AgNP composite with 0.1 (0.00001%), 1.0 (0.0001%) and 10.0  $\mu$ g/ml (0.001%) concentration inhibited spore germination by 44, 70 and 78%, respectively. Spore germinations were completely inhibited with concentrations of 100.0  $\mu$ g/ml (0.01%) (Figure 5). Normal conidial germination of *C.*

*gloeosporioides* was found in sterilized distilled water with 0.1% (v/v) acetic acid after 12 h of incubation on the glass slide. Complete spore germination was observed at 0.1% acetic acid, so dispersion of composites with low concentration of acetic acid did not cause any adverse effect. The results showed that chitosan-AgNP composite treatment suppressed germination and was found to be more effective than its counterpart.



**Figure 5.** Effect of chitosan, and chitosan-AgNP composite on conidial germination of *C. gloeosporioides* after 12 h of incubation.



**Figure 6.** Effect of chitosan-AgNP composite on conidial germination of *C. gloeosporioides* a) Normal conidia, b) Water control with 0.1% (v/v) acetic acid (appressoria formation), c) Conidial germination in chitosan (100 µg/ml) with 0.1% (v/v) acetic acid, d) Complete inhibition of conidial germination by chitosan-AgNP composite.

### Effect of chitosan-AgNP composite on anthracnose of mango

The chitosan-AgNP composite, at 0.5 and 1% concentration, showed a reduction of anthracnose by 45.7 and 71.3% respectively. Chitosan at 0.5 and 1% concentration showed 35.5 and 41.8% reduction of disease (Table 3). On the other hand, combination of chitosan-AgNP composite with 0.1% Tween-80 reduced the disease by 75.8% at 0.5% and 84.6% at 1% concentration. Chitosan and Tween-80 combinations at 0.5 and 1% concentrations showed 51.9 and 65.7% reduction of disease incidence, respectively. While carbendazim at 0.0001 and 0.001% showed 49.3 and 63.0% inhibition when compared with untreated fruits (Table 3). The anthracnose incidence was significantly ( $P < 0.01$ ) lower in all chitosan-AgNP particle treatments as compared to other treatments (Table 3 and Figure 7).

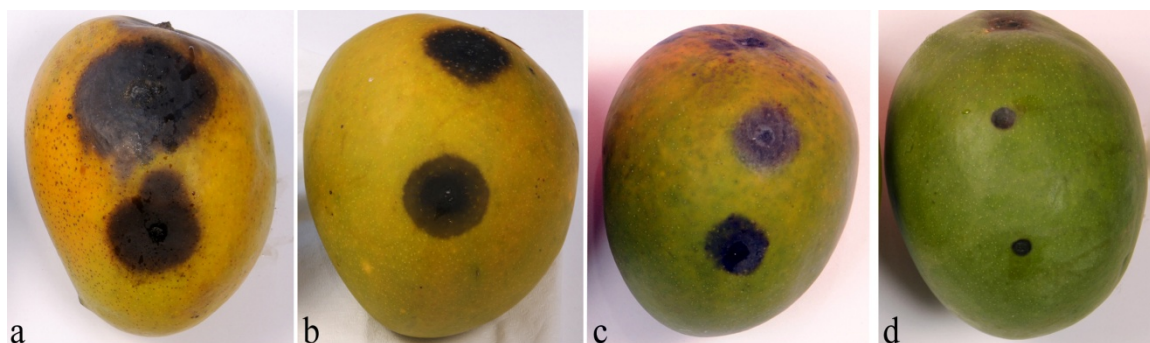
### DISCUSSION

Chitosan structure differs in molecular weight and degree of deacetylation. The primary amine and hydroxyl group in chitosan shows high affinity towards metal ions by chelation. Chitosan a biodegradable and nontoxic polymer in the presence of NaOH and at an elevated temperature reduces and stabilizes  $\text{AgNO}_3$  to silver nanoparticles. Different concentrations of silver nitrate were reduced to corresponding silver nanoparticles at particular temperature. The nanoparticle formation was characterised by UV-Vis spectrum. The UV-Vis absorption spectrum showed sharp peak in a range between 415-420 nm, the characteristic surface plasmon resonance (SPR) band of silver nanoparticles (Wei et al., 2009), which supports the formation of silver nanoparticles on chitosan matrix. For nano suspensions, size distribution and zeta potential are important characteristic parameters

**Table 3.** Efficacy of chitosan-AgNP composite and treatments on anthracnose of mango caused by *C. gloeosporioides*.

Treatment <sup>B</sup>	Lesion size <sup>A</sup> (Cm <sup>2</sup> )
Control	2.38± 0.08a
Acetic acid	2.23± 0.07 (14.84)b
Chitosan 0.5%	1.57± 0.08 (35.53)c
Chitosan 1%	1.32± 0.05 (41.78)d
Chitosan 0.5% + Tween 80	0.91± 0.04 (51.87)g
Chitosan 1% + Tween 80	0.40± 0.03 (65.74)i
Chitosan-AgNP 0.5%	1.12± 0.07 (45.67)e
Chitosan-AgNP 1%	0.25±0.01(71.28)j
Chitosan-AgNP 0.5%+Tween 80	0.14± 0.01 (75.79)k
Chitosan-AgNP 1%+Tween 80	0.03± 0.04 (84.55)l
Carbendazim 0.0001%	1.01± 0.04 (49.26)f
Carbendazim 0.001%	0.49± 0.03 (63.04)h
CD1%	0.26

<sup>A</sup>Values in parentheses indicate percent inhibition over control. Percentage of inhibition was calculated based on data collected after seven days of inoculation. Percentage of inhibition was calculated using formula  $[C-T/C] (100)$ , where C is the lesion size on control fruit and T is the lesion size on treated fruit (cm<sup>2</sup>). Percentage data were arcsin-transformed before analysis according to  $y = \arcsin [\text{sqr. } (./100)]$ . Data are the means and standard deviation of three independent experiments. Each experiment contained three replicates. Each replicate contained 12 fruits and two inoculation points. Each row values followed by a different lower case letter are significantly different at  $p < 0.01$ , according to Fishers LSD test. <sup>B</sup>Healthy mangoes cv. Alphonso, treated with different concentrations of chitosan-AgNP composite in comparison with other test chemicals and control, placed in plastic boxes (45 cm height x 40 cm length x 15 cm width) containing water to maintain humidity and used for bioassay against *C. gloeosporioides* Cm 50.



**Figure 7.** Effect of nanocomposite on mango against anthracnose a) Control, b) carbendazim (0.001%), c) 1% chitosan, and d) 1% Chitosan-AgNP composite with Tween-80.

parameters (Muller et al., 2001). When the Chitosan-AgNP composites loaded with different concentrations of AgNO<sub>3</sub> were analysed with DLS, samples showed the size distribution in range between 495 - 616 nm. The zeta potential of ± 30 mV is required as a minimum for electrostatic repulsion of the physically stable nanosuspension, which indicates the degree of repulsion between adjacent, similarly charged particles and also stability of nanoparticles in a solution (Muller et al., 2001; Du et al., 2009). The zeta potentials were enhanced significantly

with the increase in loading of silver into nanocomposite ranging from +50.08 to +87.75 mV. These data indicated that nanocomposites prepared with different concentrations of AgNO<sub>3</sub> were highly stable. To conform the presence of silver nanoparticles in the composite, SEM measurements were carried out, chitosan-AgNP composites showed spherical particles with solid dense structure having particle sizes in the range between 10-15 nm. The significant decrease in size of nanocomposite can be justified by two major explanations, first is association of



large number of water molecules with nanocomposite when we investigate via DLS, while in the case of SEM imaging water was removed during sample preparation. The second significant difference is because of large chitosan particles present in bulk solution during DLS analysis, while in SEM no bigger particles has been considered for diameter measurement. The EDS taken during SEM imaging showed prominent silver signal, indicating the presence of silver in nanocomposite.

The major postharvest losses of mango are due to fungal infection, physiological disorders, and physical injuries, chitosan coating has the potential to prolong the storage life and control decay of mangoes (Kittur et al., 2001). Researchers have used some plant essential oils as post-harvest botanical fungicides in the management of anthracnose disease of mango fruits (Abd-AIIA and Haggag, 2013). Mohamed et al. (2013) showed antifungal activity of chitosan film on inhibition of *C. gloeosporioides* associated with mango. In the present study, we have evaluated the applicability of the chitosan-AgNP composite as a fruit coating material to inhibit the fungal growth of *C. gloeosporioides*. Nanocomposites showed a greater effect in reducing the percentage of rotting fruit tissue. Tween alone has no significant effect on inhibition of spores, but the addition of non-ionic surfactant Tween 80 to nanocomposite enhanced the wettability and adhesion property of coating solution, which did not allow the normal development of spores and exhibited higher disease reduction as compared to other treatments.

Previously, chitosan nanoparticles with the enhanced zeta potential have shown excellent inhibitory effects on microorganisms (Qi et al., 2004). In the present study, chitosan-AgNP composite markedly reduced conidial germination of *C. gloeosporioides* as compared to control and other counterparts. In mango, the post-harvest phase of anthracnose caused by *C. gloeosporioides* is the most devastating and economically significant phase, and this phase is directly linked to the field phase where the infection takes place on developing fruit and infections remain quiescent in the form of appresoria and subcuticular hyphae until the onset of ripening (Arauz, 2000). At present, the elimination of quiescent infection is achieved commercially by thermal and chemical treatments or a combination of both (McMillan, 1987). Temperature and time control is critical, because fruit can be easily damaged by over exposure to heat, and the process is time consuming and labour intensive.

In the present study, we demonstrated that chitosan-AgNP composites were more effective than chitosan in inhibiting spore germination of *C. gloeosporioides* and inhibition of anthracnose on mango. In their nanosized form, silver appear to be more toxic than their bulk sized counterparts. Thus, these nanocomposites can be utilized as coating material in preventing quiescent infections of *C. gloeosporioides* on mango to prevent post-

harvest losses.

## Conclusion

Silver nanoparticles with size of 10-15 nm were prepared using low molecular weight chitosan as a reducing and stabilizing agent at 95°C. The results of UV-Vis spectrum, EDS and FESEM confirmed the presence of silver nanoparticles and also structure of chitosan-AgNP composite. The resultant composite successfully inhibited conidial germination of *C. gloeosporioides* and also reduced the anthracnose incidence on mango. This could find applications in preventing quiescent infections of *Colletotrichum* on mango to prevent enormous crop losses and promote exports.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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