GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

# Efficacy of chitosan synthesized from shrimp (*Penaeus notialis*) shell against *Aspergillus flavus* of groundnut and wheat

Aremu Mariam Bukola [1,*], Ihum Temitope Adepeju [1], Rahman Mujeeb Olalekan [1], Kurrah Inikpi Amazinggrace [1], Olatunji Tope Joyce [3], Yusuf Kayode Abel [2], Ige Mercy Itohan [2], Oledibe Chioma Francisca [3] and Kayode Elizabeth Abiola [3]

[1] *Durable Crop Research Department, Nigerian Stored Products Research Institute, KM 3, Asa Dam Road, Ilorin, Kwara State, Nigeria.*

[2] *Perishable Crop Research Department, Nigerian Stored Products Research Institute, KM 3, Asa Dam Road, Ilorin, Kwara State, Nigeria.*

[3] *Research Operation Department, Nigerian Stored Products Research Institute, KM 3, Asa Dam Road, Ilorin, Kwara State, Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2023, 22(03), 235–242

Publication history: Received on 16 February 2023; revised on 24 March 2023; accepted on 27 March 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.22.3.0131

## Abstract

Chitosan is a natural safe biopolymer that has received great attention in agriculture, food, biomedical, pharmaceutical and environmental industries because of their biocompatible, biodegradable and non-toxic. Chitosan used in this study was synthesized using shrimp shell through chemical processes viz. demineralization, deproteinization, decolourization and deacetylation. Antifungal activity of Chitosan Synthesized from Shrimp Shells (CSSS) was evaluated at different concentrations of 0.25mgml$^{-1}$, 0.50 mgml$^{-1}$, 0.75 mgml$^{-1}$ and 1.0 mgml$^{-1}$ against *Aspergillus flavus*. Shrimp shell synthesized chitosan gave off-white to light brownish semi-crystallized product. The protocol for the synthesis yield chitosan with 98.9% degree of deacetylation and Ultra Violet-visible spectra (UV-vis) at a wavelength of 232 nm. There were variations in mean mycelia growth of *A. flavus* at different concentrations of CSSS and incubation days. With increasing incubation days there was accompanying increase in mean mycelial growth of *A. flavus*. At day one mean mycelia growth were 0.75±.05, 1.40±.10, 1.55±.15 and 1.60±.10cm whilst at the fifth day of incubation, mycelia radial growth were 7.55±.55 3.10±2.4 , 6.45±.15 and 6.95±.95cm respectively at concentrations of 0.25, 0.50, 0.75 and 1.0 (mgml$^{-1}$) of CSSS. Control had highest mycelia radial growth (8.05) as compared to the different concentrations of treated samples. Highest Percentage Inhibition of *A. flavus* (61.7%) by CSSS was recorded in 0.5mgml-1, however 6.80%, 20.4%, 14.2% inhibition were recorded in 0.25, 0.75 and 1.0mgml-1 concentrations respectively which were significantly different. It is evident from the study that shrimp shell synthesized chitosan are likely biocontrol agent capable of preventing *A. flavus* growth and toxin production.

**Keywords:** Chitosan; Shrimp; Shell; Antifungal; Inhibition; *A. flavus*

## 1. Introduction

Fungal invasion and mycotoxin contamination are considered the most global threat to food and feed (Mousavi *et al*., 2021). Fungal invasion affects nutritional quality, color and texture leading to food spoilage (Inbaraj *et al*., 2020). The presence of mycotoxins such as aflatoxins, ochrtaoxins etc in food and feed poses great hazards to humans and animal health. Aflatoxins (AFs) are fungal subsidiary products majorly produced by *Aspergillus flavus* and *Aspergillus parasiticus* strains on cereals, nuts, dried fruits, dairy, and animal feed under warm and humid conditions (Ehrlich *et al*., 2015). The significant source of AFs is *A. flavus* especially aflatoxin B1, which has received lots of attention in the food

* Corresponding author: Aremu Mariam Bukola; Email: mariambukola036@yahoo.com

and feed industry (Inbaraj *et al*., 2020). High concentrations of aflatoxin can cause aflatoxicosis, an infection that could lead to cancer in severe cases (Mauro *et al*., 2018). Additionally, chronic absorption of aflatoxins causes various adverse effects, such as increased susceptibility to various pathogens. For this reason, control measures and successive management of *A. flavus* growth and toxins production are important (Bandyopadhyay *et al.,* 2016). Chitosan, α (1-4) 2-amino-2-deoxyβ-D glucan, obtained by chitin deacetylation, is found in the exoskeleton of crustacean and several other organisms including insects and fungi (Sathiyabama and Muthukumar. 2020). Chitosan has favorable properties which have attracted the interest of researchers. It is non-toxic, biocompatible, edible and also possess antimicrobial properties (Mousavi *et al*., 2021). Chitosan is also used as a carrier for drug delivery and biomedical applications (Xing *et al*., 2015). The biopolymer has attracted attention cause of its antitumor, anti-allergic, anticoagulant, anti-inflammatory, antibacterial, antifungal, antiviral and immune-stimulating effects (Kritchenko *et al*., 2020). It is also considered a useful pesticide in control of plant diseases (Arthasarathy *et al.,* 2020). Inbaraj *et al*. (2020) reported that chitosan can inhibit fungal growth in the developmental stages such as mycelial growth, spore formation, spore viability and germination. Chitosan can also exert fungistatic or fungicidal properties against pathogens of different types ((El-Alfy *et al.,* 2020). Use of chitosan as an antifungal agent to reduce incidence of aflatoxin production in foods and feeds is of utmost necessity.

Hence, this study seeks to determine the inhibitory efficacy of Chitosan Synthesized from Shrimp Shell (SSC) against *Aspergillus flavus* and Aflatoxin Production.

## 2. Material and methods

Materials used include: Shrimp shell, Potato Dextrose Agar, Sodium hypochlorite, Hydrochloric acid, Sodium hydroxide, Acetone, Glacial acetic acid and other consumables. Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Media was prepared according to the manufacturer's guidelines.

### 2.1. Culture collection

*Aspergillus flavus* previously isolated from groundnut and wheat samples was obtained from the Microbial culture bank of the Nigerian Stored Products Research Institute.

### 2.2. Confirmation and identification of *A. flavus*

Inoculum was picked from stock cultures of *A. flavus* obtained from NSPRIs culture collection and subcultured on 9 mm petri dishes containing solidified potato dextrose agar medium under sterile conditions. Isolates that were not pure were continuously sub cultured until pure isolates were obtained. Pure fungal cultures were then identified using the method of Yafetto *et al*. (2019). A small amount of fungal mycelium was removed from a fungal colony, immersed in a drop of 70% alcohol on a glass slide, after which it was gently teased out with an inoculation needle. A drop of lacto phenol cotton blue dye was then added to the mycelium and gently covered with a glass coverslip and observed under the microscope (x 40) objective lens. Microscopic identifications of fungi was determined based on morphological and growth features using Leica DM500 Digital Microscope (Leica Microsystems, Switzerland) in consultation with relevant identification manuals (Kidd *et al*., 2017a).

### 2.3. Chitosan Synthesis

Synthesis of chitosan was carried out according to the method of Yafetto *et al*. (2019) with slight modification. Shell waste of shrimp was washed and dried in hot air oven (St. Louis, MO, USA) at 60 $^{o}$C for an hour. Oven dried shell was pulverized using a blender (Master Chef 3456.UK model). Blend shell was then deproteinized by addition of 3.5% (w/w) of NaoH for 2hrs with constant stirring at a solid to solvent ratio of 1:10 (w/v). These was washed seven times with distilled water, followed by demineralization with 1N HCL in a solid to solvent ratio of 1:15 (w/v) for 30 min after which the solution was washed seven times with distilled water. Decolourization was carried out using acetone for 10 minutes. Resultant substance was dried under ambient room temperature for 2 hours before bleaching with 0.32% (v/v) solution of sodium hypochlorite (hypo) and washed three times with distilled water. The solution was then filtered until neutrality was reached. Deacetylation was carried out at 15 psi and temperature of 121 $^{o}$C using 50% NaoH solution for 15minutes. The resultant particle was referred to as chitosan.

### 2.4. Characterization of Synthesized chitosan using UV Spectrophotometer

Degree of deacetylation of synthesized chitosan was determined by infrared spectroscopy method using UV spectrophotometer (UV-visible19002PC) (Kritchenko *et al*., 2020). The surface plasmon resonance (SPR) band was measured by diluting a small aliquot of the sample in 1ml of distilled water in a cuvette. The spectra between 200-800 nm ranges were scanned to find the absorbance peak of synthesized chitosan.

### 2.5. Fourier Transform Infrared (FTIR) Spectroscopy

The extracted chitosan samples were analyzed by Fourier transform infrared (FTIR) spectroscopy to determine the presence of the characteristic IR bands. Dried powdered samples were mixed with KBr and then pressed to create a homogenous sample/KBr disk. Chitosan samples infrared spectra were measured over the frequency range of 400 to 4000 cm−1 at a resolution of 4 cm−1 using a Bruker Vertex 70 spectrometer.

### 2.6. Antimicrobial evaluation of the chitosan using food poisoning method

Antimicrobial evaluation of synthesized chitosan using food poisoning method was carried out using the modified method of Mousavi *et al.* (2021). Antimicrobial evaluation of shrimp synthesized chitosan was carried out by dissolving different concentrations of chitosan (0.25 g, 0.50 g, 0.75 g and 1.0 g) in 100 ml of distilled water containing 0.5 ml glacial acetic acid, 1ml solution of dissolved chitosan was pipetted into an empty Petri dish after which molten PDA was added to the solution contained in the petri dish. The plates were left to solidify, after which a 6mm diameter cork borer was used to create a well at the center of the solidified medium. Pure culture of *A.flavus* (same diameter) was then inoculated into the well. Media containing sterile water with glacial acetic acid served as control. Treatments were made in triplicates and incubated at room temp (28 ± $2^0$C). Daily radial mycelia growth was measured using a ruler until the control plate was full.

## 3. Results and Discussion

Physical appearance of chitosan synthesized from shrimp shell (Fig 1). The resultant particle was off-white to light brownish semi-crystallized product (Fig 1). This is in line with findings of Tamzi *et al.* (2020), who reported that yellow colouration means that the product is still chitin and when the deacetylation is complete, the resultant polymer will be off- white colour.

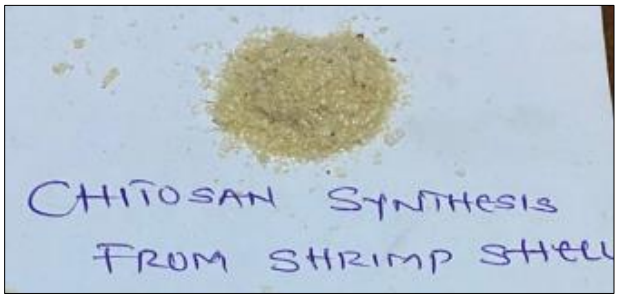



**Figure 1** Physical Appearance of Chitosan Synthesized from Shrimp Shell

### 3.1. UV-Visible Spectra scanning of Chitosan synthesis from shrimp shell (CSSS)

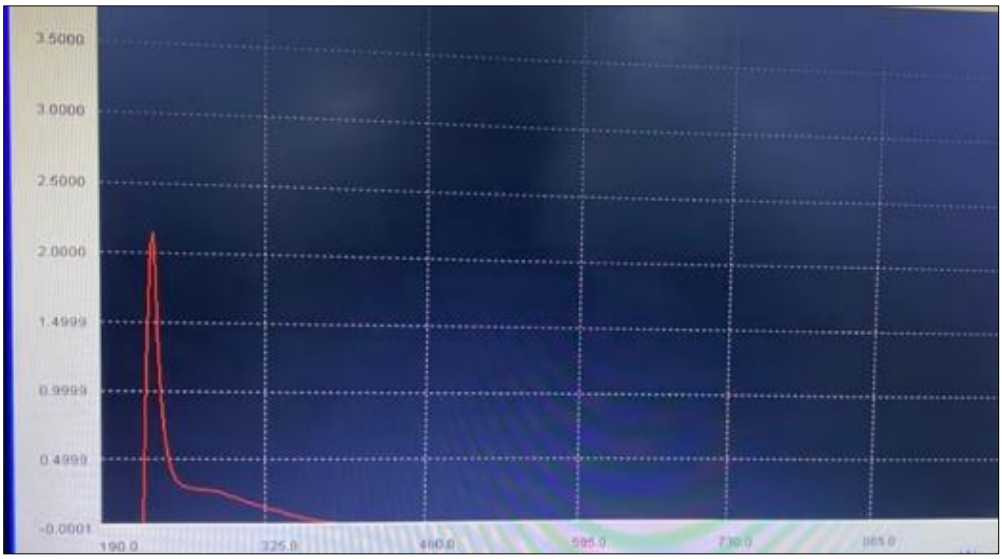



**Figure 2** Graph of UV Spectrophotometer of chitosan synthesis from shrimp shells

Graph of UV spectrophotometer and data chart display of chitosan synthesized from shrimp shells (Figure 2 and 3) showed Ultra Violet-visible spectra (UV-vis) of CSSS at a wavelength of 232nm and at an absorbance peak of 2.1457. The result is in line with Hao *et al*. (2021), who reported that a characteristic peak of 235nm could be due to n–π* transition. The UV-Vis spectra confirmed formation of a complex between sodium and chitosan. Kritchenko *et al*. (2020) reported that the range of 200-350 nm corresponds to electronic transition involving single pair of electrons on sodium, oxygen atom and hydroxyl group. Appearance of the broad band around 230-450 nm suggests formation of complex between oxygen, carbon and ammonium groups of chitosan. These results strongly suggest possible interaction between cationic amino groups of chitosan

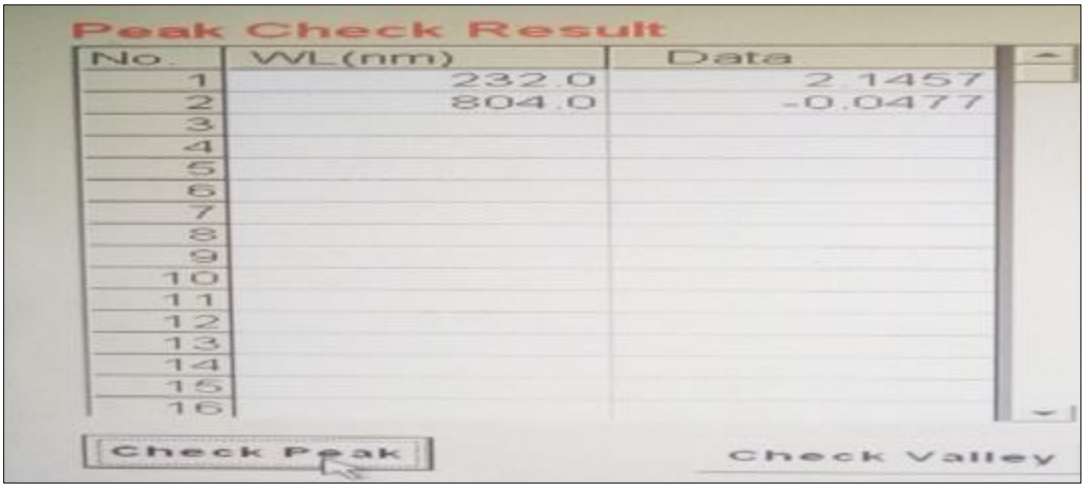



**Figure 3** Result of UV Spectra Wavelength Peak of Chitosan Synthesis from Shrimp Shells

### 3.2. Chemical Structure and Bonding Pattern of Chitosan Synthesize of Chitosan Synthesize from Shrimp Shell (CSSS)

The result of the FTIR analysis (Figure 4) show an absorption peak ranging from 3911.77, 3842.33, 3718.88, 3572.27, 3464.24, 3255.95, 3217.37, 3109.35, 2708.15, 2638.71, 2538.41, 2476.68, 2175.78, 1944.31, 1790.00, 1681.98, 1527.67, 1411.94, 1334.78, 1211.34, 1064.74, 949.01, 887.28 to 794.70. The FTIR spectral bands at 3109.35–2708.15cm-1 corresponded to OH– stretching and were assigned to the intra-molecular hydrogen bonds –OH-OC. The band at 3109.35cm$^{-1}$ characteristic to α-chitin corresponded to NH– stretching, which is involved in intermolecular and intramolecular hydrogen bonds. Furthermore, the amide-I band in the α-chitin spectrum split at 1790.00 and 1681.98cm, which were attributed to the intermolecular hydrogen bonds –CO-HO– and –CO-HN–, respectively. The absorption bands at approximately 1527.68 and 1411.94cm-1 were attributed to the chitosan characteristic peaks for amide II and amide III. The FTIR spectroscopy result is in line with the report of Hao *et al.* (2020) whose absorption band range from 3443 to 500.

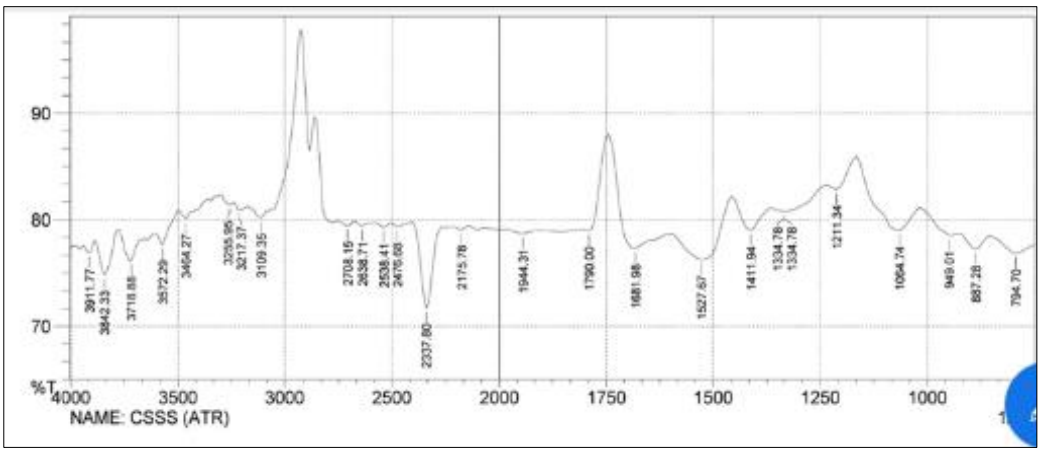



**Figure 4** Fourier Transforms Infrared (FTIR) spectra graph of Synthesize Chitosan for determination of DDA

### 3.3. The Degree of Deacetylation of Synthesized chitosan

The degree of Deacetylation was determined using a standard formular (Gaikwad *et al*., 2015). DDA = $100 - \frac{A_{1429}}{A_{3444}} \times 1.15$, where Area of peak of 1790.00=78.944T and Area of peak of 3109=80.022T

$A3109 = \frac{-\log T}{100} = \frac{-\log 80.022}{100} = 0.0967$; $A1790 = -\frac{logT}{100} = \frac{-log 78.944}{100} = 0.1026$

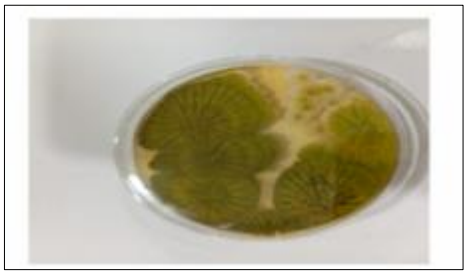
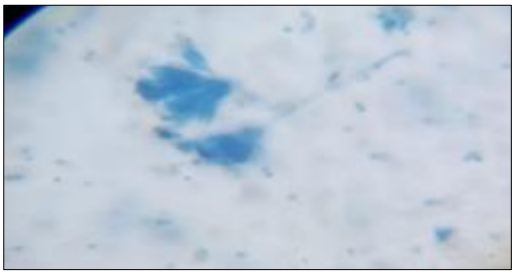
$DDA = 100 - \frac{0.0967}{0.1026} \times 1.15 = 98.9\%$ The degree of deactylation (DDA) of the chitosan synthesize from Shrimp Shells (CSSS) was 98.9%.

The protocol used for the extraction of chitosan that yield chitosan with 98.6% degree of deactylation was similar to the work of Gaikwad *et al*. (2015), who reported different protocol for the synthesis of chitosan. The result shows that the best method that produces the highest degree of deacetylation is deproteinization, demineralization, decolourization and deacetylation (DPMCA)

### 3.4. Fungal Identification

Photomicrograph of *Aspergillus flavus* isolate got from NSPRIs microbial culture collection. *Aspergillus flavus* has Velvety texture with light green to yellowish colony bound by white to cream edge on the Potato Dextrose Agar plate. The microscopic characteristic shows a nonsepate hyphae, unbranched conidiophores with scanty sterigmata. Conidia with varying sizes that are slightly roughened (Table 1).

**Table 1** Photomicrograph of *Aspergillus flavus* isolates

| Appearance on Potato Dextrose Agar Plate | Photomicrograph | Probable fungi |
|---|---|---|
|  |  | *Aspergillus flavus* |

### 3.5. Fungal Inhibition Test of chitosan synthesized from shrimp shell (CSSS)

Table 2 shows mean mycelia radial growth of *A. flavus* in CSSS treated plate at different concentrations and incubation days. At day 1 there was no significant difference in mean mycelia growth (0.75±.05$^{a}$ 1.40±.10$^{a}$, 1.55±.15$^{a}$, 1.60 ±.10$^{a}$) of *A. flavus* in CSSS. Highest mean mycelial growth of 4.25 ± 1.15$^{a}$ and 6.50 ± .50$^{c}$ was recorded respectively at day two and three at concentration of 0.25 (mgml$^{-1}$) of CSSS with least mycelia growth of 1.70 ± 0.10$^{a}$ recorded at a concentration of 0.50 (mgml$^{-1}$).

Lowest mean mycelial growth (3.10 ± 2.4$^{a}$) was recorded at the fourth day of incubation at CSSS concentration of 0.50 (mgml$^{-1}$) while highest mean mycelial growth of 6.70 ± .39c at concentration of 0.25 (mgml$^{-1}$) was recorded at day 4 closely followed by mean mycelial growth of 6.60 ±.70$^{c}$ and 5.95 ± .25$^{b}$ at 1.0 and 0.75 (mgml$^{-1}$) concentration of CSSS respectively (Table 2). There was no significant difference in mean mycelial growth at 0.25 (mgml$^{-1}$) and 1.0 (mgml$^{-1}$) concentration of CSSS. On the other hand, there were significant differences in radial mycelial growth at the different concentrations at day 5 with 0.50 (mgml$^{-1}$) and 0.25 (mgml$^{-1}$) CSSS concentration having mean mycelial growth of 3.10 ± 2.4$^{a}$ and 7.55 ± .55 respectively.

Generally, there were significant differences in mean mycelial growth of *A. flavus* in all the concentrations with the lowest growth in 0.5mgml$^{-1}$. Control sample containing *A. flavus* without chitosan shrimp shell treated had the highest mean mycelial growth (Table 2). Results revealed variation in sensitivity of *A. flavus* to CSSS. These variations in percentage inhibition of *A. flavus* at different concentration of CSSS may be due to difference in fatty acid composition. It is well known that genetic composition of fungal species plays an important role in chitosan sensitivity. Variation in

fungal resistance to chitosan was suggested by Abbas *et al*. (2016) who reported that fungi that have chitosan as one of the components of their cell wall are the most resistant to externally amended chitosan.

**Table 2:** Mean Mycelia Radial growth of *A. flavus* in Chitosan synthesized from shrimp shell treated plates

| **Days of Incubation** | **Mean mycelial growth of *A. flavus* in different concentration (mgml$^{-1}$) of chitosan synthesis from shrimp shell** | | | | |
|---|---|---|---|---|---|
| | **Control** | **0.25** | **0.50** | **0.75** | **1.0** |
| 1 | 1.7±.00$^{a}$ | 0.75±.05$^{a}$ | 1.40±.10$^{a}$ | 1.55±.15$^{a}$ | 1.60±.10$^{a}$ |
| 2 | 6.15±.05$^{c}$ | 4.25±1.15$^{b}$ | 1.70±.10$^{a}$ | 3.10±.40$^{b}$ | 3.35±.25$^{b}$ |
| 3 | 7.45±.05$^{c}$ | 6.50±.50$^{c}$ | 3.10±2.4$^{b}$ | 4.75±.75$^{b}$ | 5.75±.25$^{b}$ |
| 4 | 8.05±.05$^{d}$ | 6.70±.39$^{c}$ | 3.10±2.4$^{b}$ | 5.95±.25$^{b}$ | 6.60±.70$^{c}$ |
| 5 | 8.05±.05$^{d}$ | 7.55±.55$^{c}$ | 3.10±2.4$^{b}$ | 6.45±.15$^{c}$ | 6.95±.95$^{c}$ |

Figures having common superscript alphabet along rows are not significantly different at P≤0.05

### 3.6. Percentage Inhibition of *A. flavus* at Different Concentrations of CSSS

Table 3 shows percentage inhibition of *A. flavus* at different concentrations of chitosan synthesized from shrimp shell (CSSS) at the different days of incubation. Highest percentage inhibition (61.7%) of *A. flavus was* recorded in CSSS concentration of 0.5 mgfml$^{-1}$ at day 5 of incubation. Percentage inhibition 6.80%, 20.4% and 14.2% were recorded at CSSS concentration of 0.25, 0.75 and 1.0 mgfml$^{-1}$ respectively at the end of incubation period which is significantly different from percentage inhibition in 0.5mgml$^{-1}$. Results from this study showed that CSSS exhibited varying inhibitory levels against *A. flavus* with significant difference, however, at low concentration (0.5 mgml$^{-1}$) of CSSS, percentage inhibition was maxima at 61% for *A. flavus*. The result contradict the conclusion of Mauro *et al*. (2018), that the degree of fungicidal activity has a direct relationship with concentrations of antimicrobial formulation.

Effect of chitosan as defense enzyme inducer may be another mechanism of higher antifungal activity of chitosan (Xing *et al*., 2015). Varied tolerance of *A. flavus* isolate to different concentrations of chitosan may also be due to difference in unsaturated fatty acid composition which is key part of phospholipids in the cell membrane's lipid bilayer and represent an important factor that influence membrane stability and fluidity. Generally, antifungal activity of chitosan is attributed to larger surface area which enables chitosan adsorb more tightly to surface of fungal cells and disrupt membrane integrity (Ing *et al*., 2012). Chitosan might be able to diffuse into fungal cell and hence disrupt synthesis of DNA as well as RNA (Mousavi *et al*., 2021), consequently inducing a high antifungal activity. Ing *et al*. (2012) also reported that chitosan synthesized using silver nanoparticles showed antifungal activity against *Candida albicans, Fusarium solani* and *Aspergillus niger*. Fungi have tendency to produce different level of acids during growth resulting in acidic pH which induces protonation of amino groups of chitosan leading to damage biomolecules ((Ehrlich *et al*., 2010; Sathiyabama and Muthukumar, 2020).

**Table 3** Percentage Inhibition of *A. flavus* in different concentration of chitosan synthesis from Shrimp shell (CSSS)

| **Days of Incubation** | **Percentage Inhibition(%) in different Concentration (mgfml$^{-1}$) of Chitosan synthesis from shrimp shell** | | | |
|---|---|---|---|---|
| | **0.25** | **0.5** | **0.75** | **1.0** |
| 1 | 26.5$^{b}$ | 35.3$^{a}$ | 20.6$^{b}$ | 5.80$^{c}$ |
| 2 | 30.6$^{a}$ | 45.2$^{a}$ | 50.0$^{a}$ | 45.2$^{a}$ |
| 3 | 13.5$^{b}$ | 50.0$^{a}$ | 36.6$^{a}$ | 10.0$^{b}$ |
| 4 | 17.3$^{b}$ | 38.3$^{a}$ | 26.0a | 8.10$^{c}$ |
| 5 | 6.80$^{c}$ | 61.7$^{a}$ | 20.4$^{b}$ | 14.2$^{b}$ |

Figures having common superscript alphabet along rows are not significantly different at P≤0.05

Figure 5 shows growth of *A. flavus* in chitosan synthesized from shrimp shell (CSS) treated and control plates at two and four days of incubation

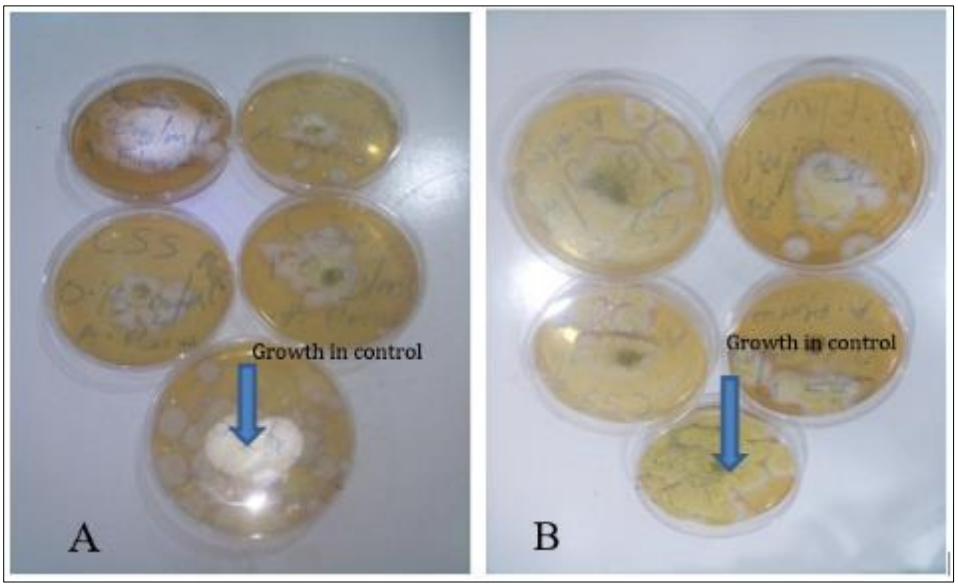



**Figure 5** Growth of *A flavus* in chitosan synthesized from shrimp shell (CSSS) treated plates and control at 2(A) and 4(B) days of incubation

## 4. Conclusion

Characterization of CSSS showed a UV-Visible Spectra of 232 nm which resulted in increase of positive charges due to free amino groups thus facilitating coupling process with cell membrane of fungi (*Aspergillus flavus*). The protocol for the synthesis yield 98.9% degree of deacetylated chitosan. Chitosan synthesized from shrimp shell showed varying inhibitory levels against *Aspergillus flavus* in the mycelia growth developmental stages. Consequently, CSSS could be a promising biocontrol against *A flavus* isolates and its toxins.

## Compliance with ethical standards

### *Acknowledgments*

We acknowledge the management of Nigeria Stored Products Research Institute for the opportunity to carry out the research in the institute Laboratory.

### *Disclosure of conflict of interest*

Authors have no conflicts of Interest.

## References

[1] Abbas, H. K., Accinelli, C., Vicari, A. Shier, W. T. Leaf application of a sprayable bioplastic-based formulation of biocontrol *Aspergillus flavus* strains for reduction of aflatoxins in corn. Pest Management Science, 2016; 72, 1521–1528.

[2] Arthasarathy, A., Vijayakumar, S., Malaikozhundan, M. B. Chitosan-coated silver nanoparticles promoted antibacterial, antibiofilm, wound-healing of murine macrophages and antiproliferation of human breast cancer MCF 7 cells. Journal of Polymer Test. 2020; 90,106- 675.

[3] Bandyopadhyay, R., Ortega-Beltran, A., Akande, A., Mutegi, C., Atehnkeng, J. Kaptoge, L. Biological control of aflatoxins in Africa: current status and potential challenges in the face of climate change. World Mycotoxin Journal. 2018; 9,771–789.

[4] Ehrlich, K. C., Yu, J. "Aflatoxin-like gene clusters and how they evolved." In Mycotoxins in Food, Feed and Bioweapons, M. Rai and A. Varma, Edition., pp. 65–75, Springer, Berlin, Germany, 2010

[5] Ehrlich, K. C., Moore, G. G., Mellon, J. E., Bhatnagar, D. Challenges facing the biological control strategy for eliminating aflatoxin contamination. World Mycotoxin Journal. 2015; 8,225–233.

[6] El-Alfy, E. A., El-Bisi, M. K. Taha, G. M. Preparation of biocompatible chitosan anoparticles loaded by tetracycline, gentamycin and ciprofloxacin as novel drug delivery system for improvement the antibacterial properties of cellulose based fabrics. International Journal of Biomacromolecule. 2020; 161, 1247–1260

[7] Gaikwad, B. V., Koli, J. M.,Desai, A. S. Isolation and characterization of chitosan from crab (Scylla serrata)) shell waste. International Journal of Sciences & Applied Research. 2-015; 2(8), 78-84

[8] Hao, G., Hu, Y., Shi, L., Chen, J., Cui, A., Weng, W.Osako, Kazufumi. (2021). Physicochemical characteristics of chitosan from swimming crab (Portunus trituberculatus) shells prepared by subcritical water pretreatment. Scientific Reports. 2021; 11. 10.1038/s41598-021-81318-0.

[9] Inbaraj, B.S., Chen, B.Y. Liao, C.W. Green synthesis, characterization and evaluation of catalytic and antibacterial activities of chitosan, glycol chitosan and poly (γ-glutamic acid) capped gold nanoparticles. International Journal of Biology Macromolecules, 2020; 161, 1484–1495.

[10] Ing, L.Y., Zin, N. M. Sarwar, A. (2012). Antifungal activity of chitosan nanoparticles and correlation with their physical properties. International Journal of Biomaterial, 2012; 56, 632-698

[11] Kidd, P. S., Álvarez-López, V., Becerra-Castro, C., Cabello-Conejo, M., Prieto-Fernández, Á. "Potential role of plant-associated bacteria in plant metal uptake and implicationsin phytotechnologies," Journal in Advances in Botanical Research, 2017a; 83, 87–126.

[12] Kritchenkov, A.S., Egorov, A.R. and Artemjev, A. A. Novel heterocyclic chitosan derivatives and their derived nanoparticles: catalytic and antibacterial properties. International Journal of BioMacromolecule, 2020; 149, 881–885

[13] Mauro, A., Garcia-Cela, E., Pietri, A., Cotty, P. J., Battilani, P. Biological control products for aflatoxin prevention in Italy: commercial field evaluation of atoxigenic *Aspergillus flavus* active ingredients. Toxins. 2018; 10, 30-39

[14] Mousavi, S.A., Ghotaslou, R., Kordi, S.G. Antibacterial and antifungal effects of chitosan nanoparticles on tissue conditioners of complete dentures..International Journal of BioMacromolecule. 2021; 118 (Pt A): 881–885

[15] Sathiyabama, M., Muthukumar, S. Chitosan guar nanoparticle preparation and its in vitro antimicrobial activity towards phytopathogens of rice. International Journal of Biomacromolecule. 2020; 153,297–304

[16] Tamzi, N. N., Faisal, M., Sultana T., Ghosh, S. K (2020). Extraction and Properties Evaluation of Chitin and Chitosan Prepared from Different Crustacean Waste. Bangladesh Journal of Veterinary and Animal Sciences. 2020; 8(2), 69-76.

[17] Xing, K., Zhu, X., Peng, X. Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review. Agronomic Sustain Development, 2015; 35(2), 569–588

[18] Yafetto, L., Adator, E. H., Ebuako, A. A., Ekloh, E. and Afeti, F. Y. (2019) Microbial quality of raw beef and chevon from selected markets in Cape Coast, Ghana. Journal of Biology and Life Science 10 (1): 78 – 97.