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Biosynthesis and characterization of chitosan nanoparticles, determination of its antifungal and antiaflatoxigenic against *Aspergillus flavus* isolates

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ABSTRACT

Aflatoxin is a mycotoxin generated mostly by the fungus *Aspergillus flavus*, which may be found in food and feed. It is a carcinogenic poison for both humans and animals. The goal of this investigation was to see if toxicogenic *A. flavus* strains might be found on nuts in the Kurdistan Region. A total of Fifty nut samples. The colony color of *A. flavus* and texture were used to identify macromorphological qualities, whereas the spore color, size, structure, conidiophore structure, and vesicle shape were used to assess micromorphological properties. Direct imaging of the UV fluorescence of *A. flavus* colonies on coconut agar media (CAM) was used to detect aflatoxin formation. Natural compounds, such as chitosan, have been suggested as a way to prevent fungal infection because of their extensive and well-known antibacterial action against a variety of microorganisms. The goal of this entry was to morphologically characterize and determine aflatoxigenic *A. flavus* isolates, characterize nanoparticles using UV–visible, X-ray diffraction (XRD), Energy-dispersive X-ray (EDX), and scanning electron microscope (SEM) analyses, and determine the formulations' in vitro antifungal activity on the treated fungus. Individual concentrations were created by altering the percentages of ingredients such chitosan solution, chitosan nanoparticles, glycerol, and canola oil. The final concentrations of chitosan solution and chitosan nanoparticles were 20% and 2% respectively. Chitosan

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nanoparticles completely inhibit the growth of *A. flavus* at 2%.

1. Introduction

The mycotoxins in foods and feeds have highly toxic effects on both humans and animals and can lead to some adverse health impacts (e.g., central nervous system disorders, hepatotoxicity, cardiotoxicity, nephrotoxicity, gastrointestinal tract damage, carcinoma, and even death), which have been an enormous threat to the public health worldwide (Horky, Skalickova, Baholet, & Skladanka, 2018; Khaneghah, Fakhri, Gahruie, Niakousari, & Sant'Ana, 2019). Mycotoxin producers mainly include *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, and *Claviceps* (Cunha, Sa, & Fernandes, 2018). By now, there are approximately 10,000 fungi identified and more than 500 species of mycotoxins have been reported. In addition, It is estimated that there are another 1,000 species undiscovered (Haque et al., 2020).

Aspergillus flavus is a common mycotoxigenic fungus that can cause infections in the field, during postharvest, and during storage (Nagur, Sukarno, & Listiyowati, 2014). Different toxigenic fungus, particularly *A. flavus*, can easily infect injured seeds. In addition to causing harm to seedlings, the pathogen lowers grain prices (Nagur et al., 2014).

Aflatoxins (AFTs) are secondary metabolites mainly produced from *Aspergillus* spp. (e.g., *parasiticus* and *flavus*), have been considered as the most predominant and highly toxic mycotoxins (Xue et al., 2019). AFG_1, AFG_2, AFB_1, and AFB_2 are inevitable in the foods and feeds. Notably, AFM_1 and AFM2 are the hydroxylated metabolites generated from AFB_1 and AFB_2, respectively. AFM_1 and AFM_2 usually occur in the meat of animals that eat the feed containing AFTs and some animal products such as cheese, eggs, and milk (Kumar, Mahato, Kamle, Mohanta, & Kang, 2017).

According to (Marín, Hodžić, Ramos, & Sanchis, 2008; Varga, Kocsubé, Péteri, Vágvölgyi, & Tóth, 2010), the most popular antifungal alternative for controlling different species of *Aspergillus*, including *A. flavus*, is chemically created pesticides by a variety of fungicides, including benzimidazoles and aromatic hydrocarbons, among

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others. However, frequent use of these products increases the danger of toxic remains in agricultural products and the environment.

Furthermore, because aflatoxins and chemicals are generally thought to have negative health consequences, it is critical to create nonchemical alternatives to reduce their contamination. As a result, natural compound active formulations have grown in importance as a way of safely delivering agricultural goods to consumers. There are a number of biodegradable plant and animal derivatives with antibacterial activity that have been thoroughly studied (Ramos-García *et al.*, 2010).

Nanoparticles are frequently employed in the healthcare and industrial sectors for a variety of purposes, including antimicrobials (Wang & Wang, 2014).

Chitosan is a positively charged linear polysaccharide with a random arrangement of β-(1–4)-linked d-glucosamine and N-acetyl-d-glucosamine. Chitosan itself may be found in the mycelia of certain fungi in association with other polysaccharides, but is mostly obtained by deacetylation of chitin (Nurunnabi, Revuri, Huh, & Lee, 2017). Because of its antibacterial and antifungal properties, CS and its derivatives have an extensive range of uses.

Along with its unique qualities, such as biodegradability, high permeability, non-toxicity to humans, and cost efficiency, chitosan nanoparticles (CSNPs) can be employed in a variety of applications. The impact of CSNPs on a variety of microorganisms has been thoroughly researched (Ali, Joshi, & Rajendran, 2011). CSNPs have been shown to have antifungal action against the fungus *Macrophomina phaseolina*, *Pyricularia grisea*, *Alternaria solani*, and *F. oxysporum*, according to Saharan *et al*. (2013) and Sathiyabama and Parthasarathy (2016), respectively.

As a result, the study's main objectives were to isolate and identify fungi with grains of various species from local markets in the Kurdistan region, characterize morphologically and physically nanostructured chitosan with determine the formulations' in vitro antifungal activity on the treated fungus, and use UV light to detect the ability of *A. flavus* isolates to produce aflatoxin.

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2. Materials and methods

2.1 Samples collection

Fifty various types of local maize and nut samples were gathered from Kurdistan Region markets, including (peanuts, cashew, walnuts, pistachio, almond, sunflower) Raisin (black and white). Each sample was put in its own sterile plastic container before being aseptically delivered to the lab for analysis.

2.2 Isolation of fungi

Fifty grains from each maize and nut sample were surface-sterilized by immersing them in a 2 percent sodium hypochlorite solution in a 250 ml conical flask for 1 minute, then washing them three times with sterilized distilled water. The grains were then dried with sterilized filter paper in a laminar flow hood and placed on potato-dextrose agar medium containing chloramphenicol (125 mg/L) using four petri plates The fungus was extracted and sub-cultured to get pure culture after being incubated for 5 to 7 days at 25°C.

2.3 Identification of *Aspergillus flavus* isolates

Identification was performed using the keys and descriptions provided by after acquiring the pure culture of each fungus (Romero, Guerra, Paes, & MacÃªdo, 2001; Watanabe, 2002; Williams-Woodward, 2001).

2.4 Detecting the ability of *Aspergillus flavus* isolates for aflatoxin B production

2.4.1 Cultural screening for aflatoxin production

Aflatoxin synthesis was initially screened using coconut agar medium (CAM). For five minutes, 100 grams of coconut shreds were homogenized in 300 ml of hot distilled water. The homogenate was filtered through four layers of cheese cloth in all cases. The pH of the clear filtrate was then raised to pH 7 with 2 N NaOH before agar (20 g/liter) was added. After being autoclaved at 121 °C for 15 minutes to sanitize the mixture, it was cooled to between 40 and 45 °C and then poured onto Petri plates. The isolates were inoculated on a petri plate containing 10 ml of CAM and incubated for 7 days at 25°C in the dark. Under long-wave UV light (365 nm), cultures were examined for fluorescence with in 3, 5, and 7 days (Sukmawati et al., 2018).

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2.5 Chitosan preparation

Chitosan concentration of 1.0% was prepared by adding the equal amount (w/v 1:100) of acetic acid to chitosan. The chitosan-acetic acid mixture was added to the total volume of distilled water and stirred overnight at room temperature. The solution was adjusted to pH 5.5 with 1N NaOH solution. Then 0.1 ml of Tween 80 was added.

2.6 Chitosan nanoparticles preparation

Chitosan nanoparticles were synthesized according to the methodology proposed by Correa-Pacheco, Bautista-Baños, Valle-Marquina, and Hernández-López (2017). Medium molecular chitosan solution at a concentration of 0.05% (w/v) was dissolved in glacial acetic acid (1% v/v) and distilled water. 2.5 ml of this chitosan solution was dissolved in methanol (40 ml) by using a peristaltic pump under moderate stirring. The obtained solution was placed in a rotary evaporator at 40 ^{o}C and 50 rpm. The final volume of nanoparticles was 2 ml.

2.7 Characterization techniques of chitosan nanoparticles

The characterization of chitosan nanoparticles was done with a variety of methods and techniques. It includes UV–Visible spectrophotometer, X-ray diffraction (XRD), energy-dispersive X-ray (EDX) and scanning electron microscope (SEM).

2.8 Nanoformulations preparation

Different formulations were elaborated based on Correa-Pacheco *et al*. (2017) methodology by varying the percentage of the components such as chitosan solution and chitosan nanoparticles (Table 1). All formulations contained glycerol at 0.3% and canola oil at 0.1%. Control consisted in *Sabouraud Dextrose Agar* (SDA) medium.

Table 1: Formulations and percentage of concentrations.

Formulation	Concentration %						
Chitosan nanoparticles	0.01	0.05	0.1	0.5	1	1.5	2
Chitosan solution	1	5	10	15	20	-	-

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2.9 Application of formulations

After *sabouraud dextrose agar* culture medium solidification, 1.0 ml of each formulation treatment were uniformly dispersed on Petri plates.

2.10 Effect of formulations on *A. flavus* mycelia growth

10 µl of the *A. flavus* (10^5) conidia concentration were added to the middle of the Petri plates after the formulations had dried and were then incubated at 20 ^oC until the fungus had achieved their optimum development (7 days). Each day, radial fungal growth in each treatment was measured in a Petri dish, during 7 days of incubation. Data were evaluated as the mycelial rate of growth, and percentage inhibition. The following formula was used to calculate the proportion of mycelial growth that was inhibited in comparison to the control:

% Inhibition of mycelial growth = (A – B) / A * 100 (Oh, Chun, & Chandrasekaran, 2019).

where **A** is the mycelial growth of the pathogen control and **B** is the mycelial growth of the pathogen in the formulation.

2.11 Detecting the inhibition rate of aflatoxin B production of *Aspergillus flavus* isolates treated with chitosan nanoparticles by UV light

After CAM solidification, 1.0 ml of chitosan nanoparticles at concentration 0.5 % was uniformly dispersed on Petri plates. After dried, 10 µl of the conidia concentration of *A. flavus* (10^5) were spreaded in the surface of the petri plates and control was done without nanoparticles only *A. flavus* on media. Aflatoxins production was detected via UV fluorescent light, light blue greenish circle closed to fungal colonies was appeared after one-week incubation at 25 °C.

3. Results and discussion

3.1 Morphological characterization of *Aspergillus flavus*

3.1.1 Macroscopic characteristics of *Aspergillus flavus* on PDA

Figure 1 depicts the colony form of *A. flavus* on PDA. *A. flavus* had a whitish mycelium at first. The *A. flavus* colony developed olive-green conidia after three days of

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incubation, which dominated the colony's appearance. The colonies were generally flat on the edges and rising in the center.

Morphological characterization was used in this study to underscore the need of such fundamental identification procedures for quick screening of isolates in most developing countries, where access to modern technology is still a problem. In this investigation, a valid identification of *A. flavus* was achieved with the use of taxonomic key descriptors. In culture media, the isolates' cultural properties were investigated, including colony color, texture, and edges, as well as the presence of exudates and sclerotia.

A. flavus had olive-green colonies surrounded by a white circle that was eventually covered by conidia. The textures of the colonies had been regularly fuzzy or velvety with a floccose core. Sclerotia production has been recorded as a rare feature of *A. flavus*, despite the fact that it is one of its distinguishing features. These traits, as illustrated in figure 1, were consistent with those previously described for *A. flavus* (Diba, Kordbacheh, Mirhendi, Rezaie, & Mahmoudi, 2007; Rodrigues et al., 2007).

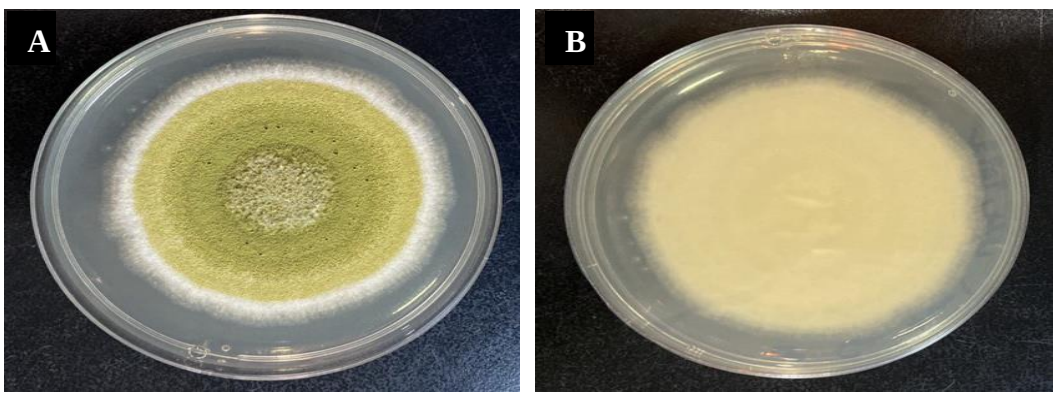

Figure 1. Colony morphology of *Aspergillus flavus* on the potato dextrose agar (PDA); **A** = obverse, **B** = reverse.

3.1.2 Microscopic characteristics of *A. flavus*

Figure 2 depicts the microscopic properties of *A. flavus*. The conidiophores of *A. flavus* isolates were colorless, thick-walled, roughed, and vesicular under the microscope. The conidiophores' diameters varied from 800 to 1200 µm. *A. flavus* isolates' vesicles

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were globose to subglobose in form. The vesicles measured between 1800 and 2000 µm in diameter. The cells were uniseriate or biseriate. The phialides grew on the metulae in biseriate cells, but not on the vesicles in uniseriate cells. The metulae enclosed the vesicles' surface and emitted in all directions. The conidia were globose, thin-walled, slightly roughed.

Microscopical characteristics like as metulae and phialides, according to Rodrigues *et al.* (2007), can be employed as fundamental keys to distinguish *A. flavus* from *A. parasiticus*. Seriation among *A. flavus* and *A. parasiticus* tend to be vary in a way that *A. parasiticus* is mostly observed having uniserate conidial head. However, *A. flavus* shows biseriate conidial.

Because several *Aspergillus* species have similar morphological characteristics, distinguishing them from *A. flavus* might be challenging. It has a colony color that is similar to *A. oryzae*. For accurate identification and categorization, this necessitates a thorough examination employing both microscopic and macroscopic criteria. However, there is agreement that several traits are shared by all *Aspergillus* isolates, even when seen under a microscope. This research looked at the properties of conidiophores, vesicles, sterigmata, and conidia. The globose vesicles with radiating sterigmata and rough conidiophore walls were distinctive features of *A. flavus*. The fine to moderately rough walls of the globose conidia, which were another crucial diagnostic trait, were rather thin. Figures 1 and 2 demonstrate traits that were similar to those described by Rodrigues *et al*. (2007) and Diba *et al*. (2007) for *A. flavus*.

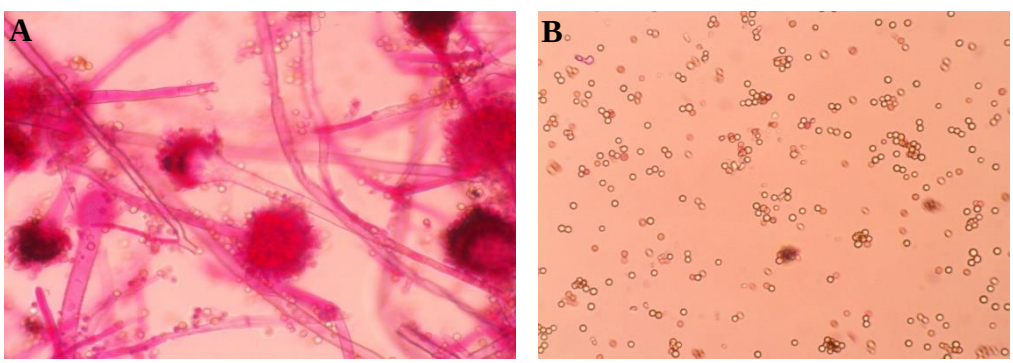

Figure 2. Microscopic characteristics of *Aspergillus flavus*; **A)** conidiophore and conidia under the 40x objective; **B)** conidia under the 40x objective.

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3.2 Detection of fungi from different grains species by using agar plate method

The results of the current investigation demonstrate that multiple fungus species were isolated from various types of nuts (Table 2). *Rhizopus* sp., *Penicillium* sp., *Aspergillus parasiticus*, *Penicillium* spp., *Aspergillus niger*, and *Aspergillus flavus* were the most prevalent fungal genera found in maize, pistachio, walnut, sunflower, cashew, almond, raisin, and peanut by using the agar plate method.

Aspergillus spp. was the most prevalent genus. *A. niger*, *A. flavus*, *Penicillium* spp. and *Rhizopus* spp. most fungi appear in more type of nuts while *A. parasiticus* the less fungi effect of nuts.

These findings are consistent with those of Abbas, Naz, Shafique, Jabeen, and Abbas (2019), who found *A. niger* and *A. flavus* to be the most common nut contaminants, Alhussaini (2012) shown in his result *A. niger* and *A. flavus* were isolated from all nuts samples (corn, Almond, Cashew and Pistachio), while Murad and Abdul-Rahim (2016) record *Aspergillus* spp. most isolates contamination of nuts.

The varying levels of contamination for food items are determined by the nature of the substance that is favorable for fungal development, as well as the ambient conditions of hydrogen function and temperature (Rostami, Nadafi, Aghamohammadi, NAJAFI, & FAZLZADEH, 2009) *Aspergillus* spp. can grow on a wide range of surfaces and adapt to a wide range of environmental conditions. They can also generate a large number of conidia with great sensitivity to tolerate harsh environmental conditions (Hagiwara *et al*., 2017).

Table 2: Several fungal species that isolated from different types of nuts.

Fungi	Corn	Pistachio	Walnut	Sunflower	Cashew	Almond	Raisin	Peanut
Aspergillus flavus	+	+		+			+	+
Aspergillus parasiticus					+			+
Aspergillus niger	+	+	+	+		+	+	
***Penicillium* sp.**	+	+	+	+		+		
***Rhizopus* sp.**	+	+			+	+	+	

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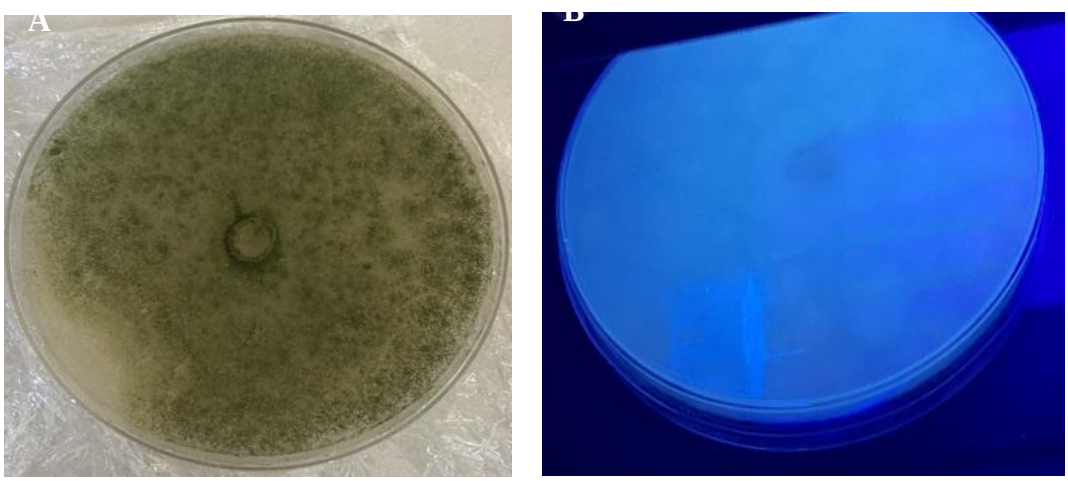
3.3 Fluorescence screening of aflatoxins

The coconut agar plates were examined under ultraviolet light after they had been incubated. Which can be seen in figure 3, there was a greenish-blue colored luminescence. The presence of aflatoxins dispersed into the agar might explain the greenish-blue fluorescence. This finding is comparable to that of Saito and Machida (1999) as well as Yazdani, Zainal, Tan, and Kamaruzaman (2010). While (Riba, Matmoura, Mokrane, Mathieu, & Sabaou, 2013) verified that the cultures of aflatoxigenic Aspergillus were examined for 365 nm UV light fluorescence and for strong orange-yellow colony reverse pigmentation, there was disagreement with them.

The growth and generation of aflatoxin by *A. flavus* and *A. parasiticus* have been shown to be efficient on coconut agar medium. In the context of the current study, the coconut agar medium is unquestionably favorable as a plating media. In the context of the current experiment, using coconut agar medium as a plating media is unquestionably favorable. The whiteness of this medium's surface makes it ideal for evaluating early initiation and intensity of sporulation across colonies, as well as discerning small changes in spore coloring. The surface of the coconut agar medium absorbs a lot of UV light, thus it's a good backdrop for spotting fluorescent aflatoxins zones around micro colonies.



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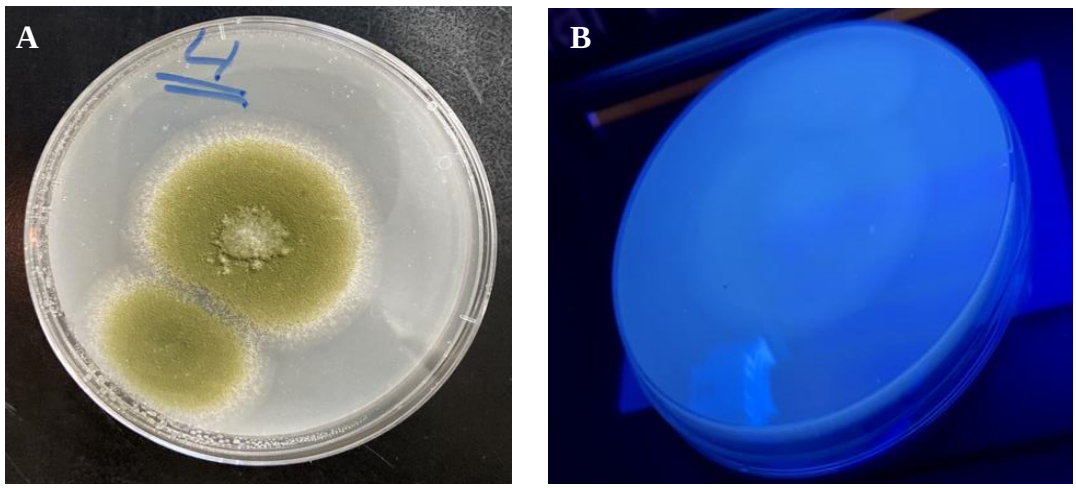

Figure 3. Colony of *Aspergillus flavus* after seven days of growth on (CAM); A = colony surface; B = colony reverse under UV -light 365 nm.

3.4 Characterization of CSNPs and effect of formulations on *A. flavus* mycelia growth

Figure 4 illustrates the UV-vis, SEM, XRD, and EDX characterization of chitosan nanoparticles. UV-Vis spectroscopy was used to characterize the synthesized CSNPs, the formed chitosan nanoparticles in the solution were scanned and detected in the range of 200–900 nm in a spectrophotometer to verify the formation of nanoparticles, which revealed a peak formed at 270 nm as shown in figure 4, which agrees with (Vaezifar *et al*., 2013) findings.

The existence of C and O in the chitosan nanoparticles was revealed by EDX, whereas the other peaks corresponded to the element gold (Au), which was present because a gold coating was applied to boost the clarity of SEM pictures. The existence of an impurity was also verified by EDX. Figure 4 shows the elemental values for CSNPs: carbon 58.95 percent, oxygen 41.04 percent. SEM analysis was used to investigate the surface morphological structure further. Figure 4 shows a SEM picture of the formulation. The morphological structure of chitosan nanoparticles reveals their large particle size and agglomerated condition. The formation of these nanoparticles in the range 97 nm (Agarwal *et al*., 2018).

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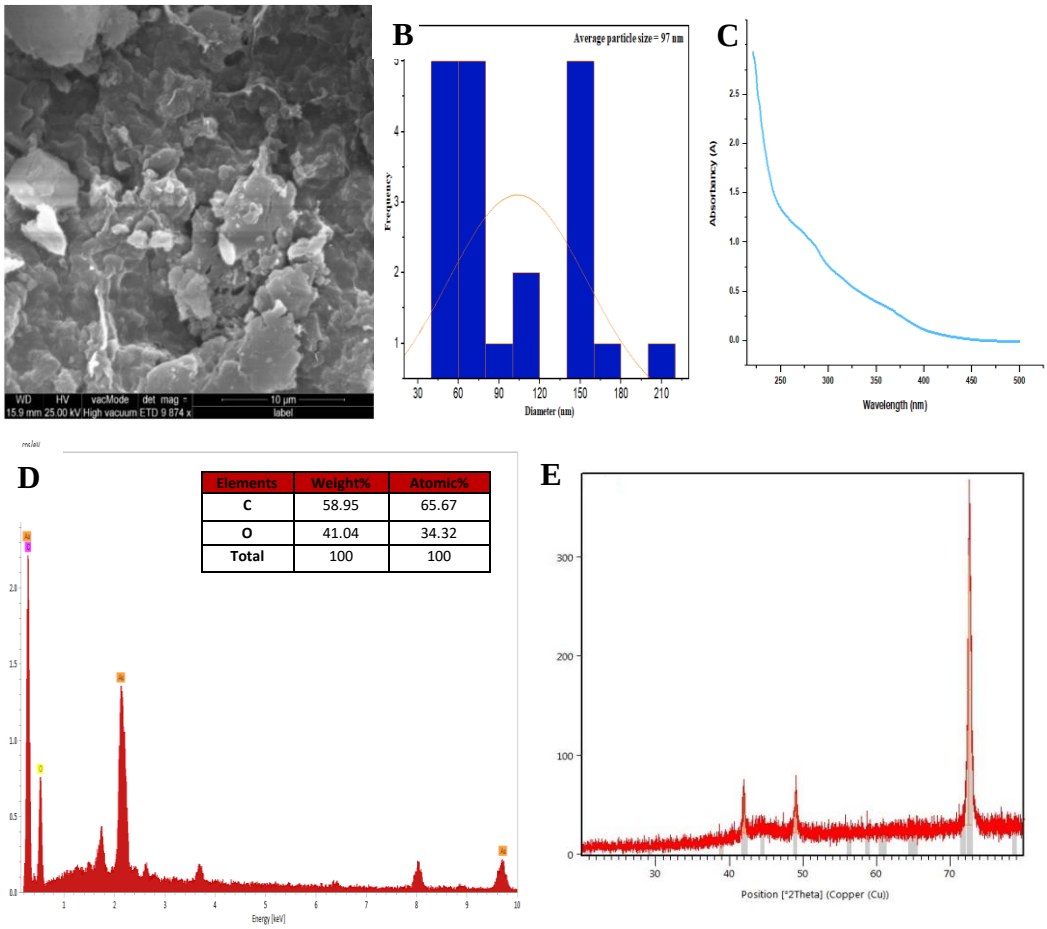

Elements	Weight%	Atomic%
C	58.95	65.67
O	41.04	34.32
Total	100	100

Figure 4. Characterization of CSNPs spectra: **(A)** SEM; **(B)** Size histogram of nanoparticles; **(C)** UV–Visible spectrum; **(D)** EDX; **(E)** XRD.

The effects of CS solution and CSNPs on fungal growth *A. flavus* during a 7 days incubation were studied in this work (Table 3). In comparison to the control, however, the development of *A. flavus* was slowed. The inhibitory effects on *A. flavus* mycelial growth were measured in centimeters. Effect of CS solution on *A. flavus* at different concentration 1, 5, 10, 15, and 20 % were 56, 58, 60, 73, 69 % respectively. Effect of CSNPs on mycelial growth of *A. flavus* at different concentration 0.01, 0.05, 0.1, 0.5, 1, 1.5 and 2 % were 67, 75, 66, 79, 79, 81 and 100 % respectively. In comparison to CS solution and control, CSNPs had a greater inhibitory impact on *A. flavus* mycelial

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development figure 5. *A. flavus* growth was inhibited by 68.8% when chitosan solution was used at a concentration of 15%. *A. flavus* is totally inhibited by CSNPs at a concentration of 2%. The antifungal efficacy of CSNPs was proven against two aflatoxin producers, *A. flavus* and *A. parasiticus* (Mekawey, 2018), and CSNPs were successful in lowering total aflatoxin generation and suppressing fungal growth.

There is a lot of interest in developing more effective and environmentally friendly anti-toxigenic fungicides with little or no mycotoxin residues that don't impair plant development or crop yield of critical agriculture components (Rai & Abd-Elsalam, 2019). The present study's main goal is to see how antifungal bio-polymers like chitosan work against aflatoxin-producing *A. flavus* strains.

The antibacterial activity is most likely generated from the electrostatic interaction between the microorganism's negatively charged cell membrane and positively charged nanoparticles. It shows that CSNPs have a great antibacterial and antifungal impact, as well as the ability to reduce bacterial and fungal development in practical applications. CSNPs offer a more scalable and non-toxic way of nanoparticle synthesis. Applications of CSNPs based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

A series of research have been carried out to limit the synthesis of aflatoxins and to inhibit the proliferation of fungi that produce them because of their harmful effects. However, the situation is exacerbated by the fact that the poisons cannot be removed from foods or animal feeds using standard processing methods. Because fungicides cannot be used on food or animal feed, essential oils and plant extracts were the most appealing option (Anfossi, Giovannoli, & Baggiani, 2016). Also Amal (2010) employed a variety of honeys that showed a significant decrease in aflatoxins generation. Aflatoxin B_1 and B_2 were created by *A. flavus*, while aflatoxin B_1, B_2, G_1 and G_2 were produced by *A. parasiticus*. Foodborne mycotoxin contamination is the most common cause of contamination in manufactured goods. The assessment of this problem, which is widespread in many developing nations such as Egypt, will aid in the development of control solutions.

In prior studies (Beyki *et al*., 2014; Khalili *et al*., 2015), various nanogels have been used for the encapsulation of antifungal compounds against fungi. For instance,

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Zhaveh *et al*. (2015) employed a chitosan-caffeic acid nanogel for the encapsulation of *Cuminum cyminum* essential oils to enhance the antimicrobial activity toward *A. flavus*.

Table 3: Rate of colony growth (cm/day)

Day	Concentration of Chitosan solution %					
	1	5	10	15	20	Control
1	-	-	-	-	-	0.5
2	-	-	-	-	-	1
3	-	-	-	-	-	2.3
4	0.3	0.3	0.3	0.3	0.3	3
5	0.5	1.2	0.8	0.8	0.6	3.5
6	1.2	1.8	1.4	1	1.3	4
7	2.3	2.2	2.1	1.4	1.6	4.5

Day	Concentration of Chitosan nanoparticles %							
	0.01	0.05	0.1	0.5	1	1.5	2	Control
1	-	-	-	-	-	-	-	0.5
2	-	-	-	-	-	-	-	1
3	0.3	0.2	0.2	0.3	0.3	0.3	-	2.3
4	0.7	0.5	0.5	0.6	0.7	0.7	-	3
5	0.9	1	0.9	0.9	0.8	0.7	-	3.5
6	1.3	1.2	1.3	1	0.9	0.8	-	4
7	1.7	1.3	1.8	1.1	1	1	-	4.5

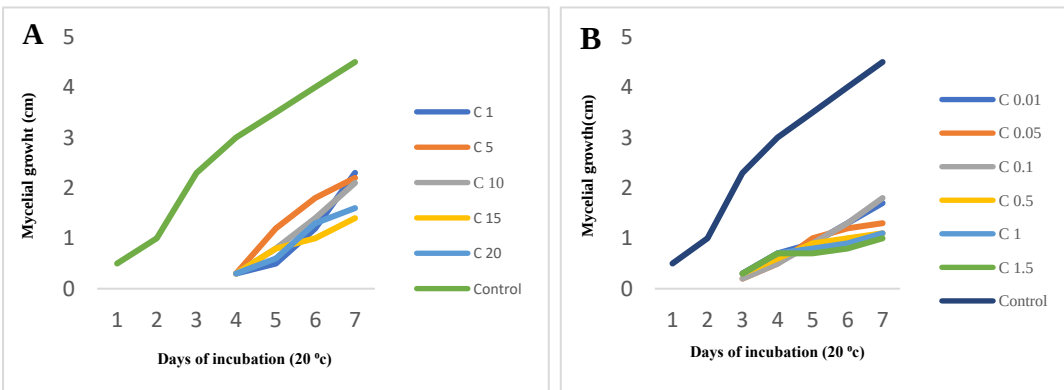

Figure 5. Mycelial growth of *Aspergillus flavus* incubated in different concentration **(A)** chitosan solution; **(B)** chitosan nanoparticles.

3.5 Detecting the inhibition rate of aflatoxin B_1 production of *Aspergillus flavus* isolates treated with chitosan nanoparticles by UV light

The capacity of toxigenic isolates to produce toxin was tested using CAM. In aflatoxigenic fungal cultures, beige rings can be visible in the absence of light. The blue fluorescence ring that surrounds the aflatoxigenic colony may also be seen when

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exposed to UV light. Under UV, the diameter of the observed beige ring and the brightness of its fluorescence emission increased with time, with the maximum observation towards the end of the week figure 6. *A. flavus* was unable to grow on CAM with CSNPs, but was able to grow on control. However, we discovered that the CAM screening method was considerably more straightforward, quick, and affordable than any of the other methods we looked at for the majority of objectives (Sukmawati et al., 2018). For fast finding of aflatoxin formation by *Aspergillus* spp., coconut agar medium (CAM) is commonly utilized (Carvajal-Campos et al., 2017). Cultivation on CAM is usually used as a screening method to see if aflatoxin formation is occurring (Alkhersan, Khudor, & Abbas, 2016). A microplate fluorescence reader-based test uses a liquid formulation of CAM to detect aflatoxins (Degola, Berni, & Restivo, 2011). In cultures of aflatoxigenic fungus, a distinct beige ring may be seen under UV light figure 6. Under UV light, a blue fluorescence around aflatoxigenic colonies could also be visible on the back of the plates (Almoammar, Bahkali, & Abd-Elsalam, 2013). In the presence of appropriate circumstances, atoxigenic isolates generated toxins in CAM (Hoeltz, 2005). However, CAM is an unsatisfactory approach for identifying the toxigenic potential of *A. flavus* and *A. parasiticus* due to the recurrent false negative findings (Taniwaki, 1996). Coconut milk agar (CMA), coconut extract agar, and coconut cream agar are some of the other coconut culture mediums used to discover toxigenic microorganisms (Iram, Anjum, Jabeen, & Abbas, 2018).

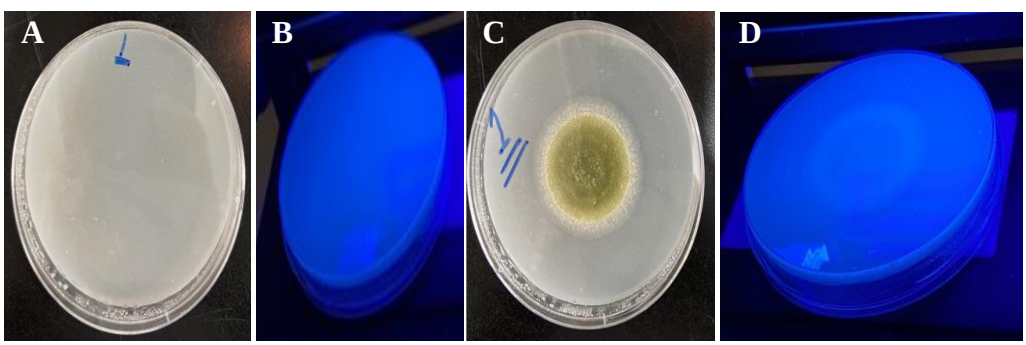

Figure 6. **(A)** *A. flavus* no growth on (CAM) with nanoparticles; **(B,D)** plates under UV light **(C)** control.

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4. Conclusion

A. flavus may be identified quickly using culture medium and morphology. These isolates had morphological traits that matched those described in published identification guides. The detection of aflatoxin in *A. flavus* isolates under UV light (365 nm) provides a quick and accurate way to differentiate between aflatoxigenic and non-aflatoxigenic isolates. Although culture-based procedures are easy and inexpensive, a more sophisticated technology such as high-performance liquid chromatography with fluorescence detection (HPLC-FLD) might be advantageous for the exact measurement of aflatoxin. The antifungal impact of chitosan was shown to be greatly enhanced when it was formulated into nanoparticles. As a result, chitosan nanoparticles are thought to have the potential to become an effective and safe natural antifungal agent. The results provided in this research reveal that chitosan inhibits *A. flavus* radial growth effectively.

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بایۆسێنتێزی و تایبەتمەندی نانۆگەردیلەکانی کیتۆسان و دیاریکردنی دژە کەڕوو و دژە ژەهراویبوونی لە دژی جیاکراوەکانی *Aspergillus flavus*

پـوخـتـه:

ئەفلاتۆکسین ژەهرێکە کە زیاتر لە کەڕووی *Aspergillus flavus* دروست دەبێت، کە لەوانەیە لە خۆراکدا هەبێت. ژەهرێکی شێرپەنجەییە هەم بۆ مرۆڤ و هەم بۆ ئاژەڵ. ئەم توێژینەوەیە بۆ هەڵسەنگاندنی ڕووداوی جۆرە ژەهراویەکانی *A. flavus* لەسەر چەرەزات لە هەرێمی کوردستان ئەنجامدراوە. بەگشتی پەنجا نمونەی چەرەزات لە چەندین بازاڕی ناوخۆیی کوردستان وەرگیراون، لەوانە گەنمەشامی، فستق، گوێز، گوڵەبەڕۆژە، کاجو، بادەم، کشمشی و فستق. بەهۆی لێکچوونی سەرنجڕاکێشی لەگەڵ جۆرە نزیکەکانی بۆ نموونە *A. parasiticus* ، جیاکردنەوەی *A. flavus* زەحمەتە. ڕەنگ و پێکهاتەی کۆلۆنی بەکارهێنران بۆ دیاریکردنی کوالیتییە ماکرۆمۆرفۆلۆژییەکان، لە کاتێکدا ڕەنگی سپۆرەکان، قەبارە، پێکهاتە، پێکهاتەی کۆنیدیۆفۆر، و شێوەی کیسەڵەکان بەکارهێنران بۆ هەڵسەنگاندنی تایبەتمەندییە وردە مۆرفۆلۆژییەکان. ڕۆشنایی سەروو بنەوشەیی بۆ کۆلۆنیەکانی *A. flavus* لەسەر coconut agar media (CAM) بەکارهێنرا بۆ دیاریکردنی دروستبوونی ئەفلاتۆکسین. پێکهاتەی سروشتی کیتۆسان وەک ڕێگەیەک بۆ ڕێگریکردن لە پیسبوونی کەڕوو پێشنیار کراوە بەهۆی کارکردنی دژە بەکتریایەکی بەرفراوان و ناسراویان دژی جۆرەها وردە زیندەوەر. ئامانجی ئەم لێکۆلینەوەیە بریتی بوو لە جیاکردنەوەو ناسینەوەی *A. Flavus*،دیاریکردنی تایبەتمەندی نانۆگەردیلەکان بە بەکارهێنانی شیکارییەکانیUV-visible ، پەرشوبڵاوی تیشکی ئێکس(XRD) ، تیشکی ئێکس وزە-پەرشوبڵاو(EDX) ، و شیکارییەکانی مایکرۆسکۆپی ئەلکترۆنی سکانکردن .(SEM) ، هەروەها چالاکیی دژە کەڕووەکان لەسەرکەڕووی چارەسەرکراو. چڕیی جیاوازی ڕێژەی سەدی پێکهاتەکانی وەک گیراوەی کیتۆسان، نانۆگەردیلەکانی کیتۆسان، گلیسیرۆل و زەیتی کانۆلا دروستکرا. چڕیی کۆتایی گیراوەی کیتۆسان و نانۆگەردیلەکانی کیتۆسان ٢٠% و ٢% بوو

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التخليق الحيوي وتوصيف جزيئات الكيتوسان النانوية وتحديد مضادات الفطريات ومضادات السمية ضد عزلات *Aspergillus flavus*

الملخص:

الأفلاتوكسين هو سم فطري ينتج في الغالب عن فطر *Aspergillus flavus* ، والذي يمكن العثور عليه في الطعام والأعلاف. وهو مادة مسرطنة للإنسان والحيوان. كان الهدف من هذا الدراسة هو معرفة ما إذا كان يمكن العثور على سلالات *A. flavus* السامة في المكسرات في إقليم كردستان. تم الحصول على ما مجموعه خمسون عينة من المكسرات والحبوب من عدة أسواق محلية في كردستان ، بما في ذلك الذرة والفستق والجوز وعباد الشمس والكاجو واللوز والزبيب والفول السوداني. بسبب تشابهها الكبير مع الأنواع ذات الصلة الوثيقة مثل *A. parasiticus* ، لذلك يصعب تمييز *A. flavus*. استخدم لون وملمس المستعمرة لتحديد الصفات المظهرية ، في حين تم استخدام لون البوغ وحجمها وبنيتها وتركيب كونيديوفور وشكل الحويصلة لتقييم الصفات المجهرية. استخدم الاسعة التصوير المباشر للاشعة فوق البنفسجي(UV) لمستعمرات *A. flavus* على وسط coconut agar media (CAM) للكشف عن تكوين الأفلاتوكسين. تم اقتراح المركبات الطبيعية ، مثل الكيتوسان ، كطريقة لمنع التلوث الفطري بسبب تأثيرها المضاد للبكتيريا والفطريات واسع النطاق والمعروف ضد مجموعة متنوعة من الكائنات الحية الدقيقة. كان الهدف من هذه الدراسة هو توصيف وتحديد عزلات *A. flavus*شكليًا والمنتجة للافلاتوكسينت،وكذلك تحضير وتوصيف الجسيمات كيتوسان النانوية باستخدام الأشعة فوق البنفسجية المرئية ، وحيود الأشعة السينية (XRD) ، والأشعة السينية المشتتة للطاقة (EDX) ، ومسح تحليلات المجهر الإلكتروني (SEM) ، وتحديد النشاط المضادة للفطريات في الاطباق على الفطريات المعزولة. حضرت تراكيز مختلفة عن طريق تغيير النسب المئوية للمكونات مثل محلول الكيتوسان وجسيمات الكيتوسان النانوية والجلسرين وزيت الكانولا. كانت التركيزات النهائية لمحلول الكيتوسانان والجسيمات النانوية 20٪ و 2٪ على التوالي.