

Core-Shell Nanoparticles Based on ZnO/yeast Cell Wall: Characterization, Antifungal Properties and Aflatoxin B₁ Binding

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Abstract:

A class of mycotoxins such as aflatoxins are harmful and significant concern for public health, food safety, and agricultural practices. Recent challenges are involve reducing and the eliminating aflatoxins from contaminated food and feed using bio-based strategies. The development of edible binders is a promising approach to mitigate aflatoxin as a health risk. This study aimed to develop and characterize of core-shell nanoparticles using yeast cell wall extracted from *Saccharomyces Cerevisiae* as a shell surrounding ZnO nanoparticle core (CW/ZnO NPs). The obtained CW/ZnO core-shell was characterized regarding physicochemical properties, antifungal effect and aflatoxin B₁ (AFB₁) binding capacity. FTIR confirmed the chemical composition of the synthesized nanoparticles. The size distribution and zeta potential of CW/ZnO NPs were in the acceptable range. Based on the microscopy techniques, a spherical shape (diameters of 21.36 – 42.54 nm) and a rough surface were observed, indicating a cell wall assigned to the shell of the ZnO core. According to the determined MIC and MFC, significant antifungal activity was obtained against *A. parasiticus* (500 µg/mL) and *C. albicans* (125 µg/mL). The CW/ZnO core-shell showed the highest AFB₁ adsorption (55.4%) compared to the cell wall and ZnO samples in PBS. This research introduces a bio-based nanoparticle with a core-shell structure as an AFB₁ binder. Considering its potential for reducing aflatoxin bioavailability could be recommended to apply in the food and feed industry.

Keywords: Cell wall; Core-shell; Aflatoxin; ZnO; *S. cerevisiae*; Binding; Nanoparticle

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1. Introduction

A group of mycotoxins named Aflatoxins (AFs) are difuranocoumarin-structured substances generated by a class of *Aspergillus* species. The main AF generators are *A. flavus*, *A. parasiticus*, and *A. nomius* via possible growth in crops, food, and feed during production processes [1–3]. Any inattention in pre and post-harvest stages could provide the desired conditions for fungi proliferation and AF generation. The neglected good manufacturing practice (GMP) is a route of AF contamination in edible products [4–6]. The incidence of AFs as serious contamination is related to con-

siderable economic losses in crop yield, animal productivity, and human health costs [7]. AFs are recorded as a potent carcinogenic, teratogenic, and motagenic compound that considered a risk to vertebrates worldwide [8]. From farm to fork, AF monitoring is crucial in aspects of food security, economic stability, and public health efforts [9, 10]. According to epidemiological studies, AFs are the most potent liver carcinogens and reaffirmed by FAO/WHO. Given the serious need to reduce aflatoxin incidence, the Codex Alimentarius Commission defined codes of practice for reducing AFs in food. These recommendations are practices

for aflatoxin reduction during pre- and post-harvest [11]. New attempts are based on urgent and comprehensive AF mitigation strategies using physical, chemical, and biological methods [8, 12–15]. Each practice that aimed to prevent *Aspergillus* strains from growing could directly result in reduced AFs. In this case, using antifungal agents is a way to control AF production. Among the innovative strategies emerging to manage AF contamination, using microorganisms presents a promising frontier. It has been proved that some microorganisms, such as lactic acid bacteria strains and *Saccharomyces* yeast, as viable and non-viable cells and their by-products can bind aflatoxin from the media. Besides, they are generally recognized as safe (GRAS) and offer a beneficial alternative for reducing aflatoxins in food/feed. There are several researches shown the potential of AFB₁ binding by *Saccharomyces cerevisiae* (SC) via physical adsorption on the glycoprotein section, mainly mannoprotein and beta-glucan of the cell wall structure. Therefore, the extracted SC cell wall could effectively be used in aflatoxin removal approaches. Ongoing studies focused on the use of antifungal and biological binders in novel structures with enhanced inhibitory/absorbance capacity [16, 17]. In recent years, nanoscale structures, meticulously engineered with distinct surfaces, have exhibited exceptional binding capabilities and have been successfully applied in biotechnology and for biological goals [18–21]. One of the noble and emerging microstructures is nanostructures developed using different materials [22, 23]. A series of nanostructures are core-shell structures in which a metal element or a bioactive agent is placed in the inner core surrounded by a shell from another material, creating a unique structure with desired functional properties. These novel nanoparticles have diverse compositions, including inorganic and organic components and biopolymers [24, 25]. They are utilized in various fields like drug delivery systems for tumor targeting, biological developers, molecular probes, and carriers [26]. They provide benefits like bio-compatibility, tunable physico-chemical properties, improved bio-permeability, and target-specific delivery [27, 28]. Developed core shells have been investigated as antimicrobial structures that showcase significant antibacterial activity [29, 30].

Zinc oxide (ZnO) nanoparticles are a class of nanomaterials applied in different industrial fields. It has interesting properties, is cheap and nontoxic, and is a biodegradable and edible agent, making it worthwhile for cosmetic, drug, and food products [31]. Regarding the recorded antimicrobial potential of ZnO NPs, there is a raised interest in their use as an antimicrobial agent in different applications [32]. It has been used in many research studies to synthesize antimicrobial core-shell nanoparticles. Besides, the Food and Drug Administration (FDA) had confirmed the safety of ZnO for using to humans and animals [33]. In the present study, ZnO was selected as an antifungal agent in the core of the particle, and the extracted SC cell wall was used as an AF binder on the shell to evaluate the antifungal property and AFB₁ binding efficiency of the CW/ZnO core-shell nanoparticle.

2. Material and methods

2.1 Materials and strains

Zinc oxide (ZnO) nanoparticles (10 – 30 nm, 5.606 g/cm³, 20 – 60 m²/gr) were purchased from Fine-Nano Company (Tehran, Iran), with > 99% purity. Aflatoxin B₁ and all the chemicals used for cell wall extraction and making core-shell obtained from Sigma (USA). The reagents used for AF quantification were analytical grade and acquired from Merck (Darmstadt Germany). *S. cerevisiae* (PTCC 5052), *Aspergillus parasiticus* (ATCC 15517) and *Candida albicans* (ATCC 10231) were taken from Persian type culture collection of Iran. The SDA, SDB, and YM media were purchased from Merck (Darmstadt Germany).

2.2 Yeast cell wall extraction

The *S. cerevisiae* strain 5052 (PTCC 5052) first cultured in yeast mold (YM) agar at 24 °C for 48 h. The inoculation were done in five container containing 150 mL of sterilized standard YM broth grown for 24 h at 30 °C under shaking at 120 rpm. Then the medium was centrifuged (4500 for 10 min) and obtained pellet was washed 5 times to be clear. The yeast cells was mechanically disrupted using ice-cold lysis buffer (Sigma Aldrich, United States) and homogenization (BeadBug, Benchmark Scientific) at 15 cycles (1 min homogenization then 5 min cold rest). The cell lysate was washed two times with NaCl 1 M solution and centrifuged (4 °C for 10 min, 4000 g). The obtained pellet was extracted with phosphate buffer solution (PBS) and made hot for 10 min up to 100 °C, then centrifuged. The resultant pellet was washed with deionized water, which contained yeast cell wall isolate [34]. Finally, the whole cell wall extract was freeze-dried for further studies.

2.3 Synthesis of CW/ZnO core shell nanoparticles

To coat the ZnO nanoparticles with yeast cell wall extract, 0.5 g of ZnO nanoparticles were dissolved in acetic acid (1%) to change to zinc cations. Then 0.5 g of dried yeast cell wall extract was added and stirred on a stirrer for 1 hour. The sonication was done on mixture (30 min). Next, 1 M NaOH continuously was added up pH 10 on a magnetic stirrer. The solution warmed to 37 °C then centrifuging and washing performed to remove extra ZnO and cell walls. In the following, the remaining mixture was dried with a freeze-dryer. Finally, the CW/ZnO core shell was stored in the refrigerator [35, 36].

2.4 Physicochemical characterization

Using Fourier Transform Infrared Spectroscopy (FT-IR) the CW/ZnO NPs component interactions and attendance was determined. The AVATAR 370 FTIR Thermo Nicolet was used to capture FTIR spectra at 400 – 4000 cm⁻¹, and SigmaPlot software was used to plot them.

Dynamic Light Scattering (DLS) and zeta potential technique (Zetasizer Nano-ZS, Malvern Instruments, United Kingdom) were used to measure the average hydrodynamic particle size and distribution of ZnO and synthesized CW/ZnO NPs.

The surface structure was evaluated using a Field Emission Scanning Electronic Microscope (FESEM) (Sigma 300- HV,

Zeiss, Germany). The samples were prepared by a platinum sputter coater. The Energy Dispersive X-ray (EDX) microscopy (EDXRF EDX-8000 SHIMADZU) was used to analyze the elements present in the synthesized nanoparticle.

The surface topography of the synthesized core-shell sample was characterized using an Atomic Force Microscope (multi-mode ARA-AFM, Iran).

2.5 Antifungal assay

Antifungal effects of the CW, ZnO NP and CW/ZnO, against the *Aspergillus parasiticus* (ATCC 15517) and *Candida albicans* (ATCC 10231) were assessed based on serial dilution technique in a 96-wells. The proper strain was grown overnight at 25 °C to 37 °C in related media. 100 µL of prepared media in the plates inoculated with 100 µL of fungi to obtain 1×10^5 Spore/mL for *A. parasiticus* and 1×10^5 cfu/mL for *C. albicans*. The incubation was done at 25 °C for 72 h and to calculate the minimum inhibitory concentrations (MICs). Nystatin and Fluconazole were considered as positive controls. The minimum fungicidal concentrations (MFC), were assayed on 20 µL of samples of each compound and subsequent treatment in sabora dextrose agar media (SDA) via incubation at 37 °C, 24 h. All the experiments were done triplicate [37].

2.6 Aflatoxin binding assay

AF binding capacity was tested in phosphate buffer saline (PBS), which spiked by AF standard solution (100 µg/L). The microtubes were inoculated by three types of particles: ZnO, extracted cell wall, CW/ZnO NPs, and a tube was considered as blank with no inoculation and kept at 37 °C for 12 h. After centrifugation for 15 minutes at 3000 g, the obtained supernatants stored at 4 °C until AF analysis. The AFB₁ and AFT (µg AFB₁/mg of particle) was determined regarding the concentration of remained aflatoxin.

2.7 Aflatoxin analysis

The AFB₁ concentrations were determined by a high-performance liquid chromatography (HPLC) (Waters e2695, Blue series, USA). The water/methanol (60:40) used as mobile phase at 2 mL/min, the excitation and emission

wavelengths were adjusted at 365 nm and 435 nm. In this procedure the limit of detection (LOD) and limit of quantification (LOQ) were 0.3 and 1 µg/mL.

2.8 Statistical analysis

The result of experiments was measured triplicate and depicted as the mean and standard deviation. One-way analysis of variance (ANOVA) and Duncan's test was considered to differences determination. The significance level was $P < 0.05$.

3. Result and discussion

3.1 Characterization of CW/ZnO NPs

The FT-IR absorption spectrum of ZnO, cell wall, and CW/ZnO nanoparticles, are shown in Fig. 1, respectively. A peak at 472 cm^{-1} is the distinctive absorption of the Zn-O bond and the two significant peaks at 3380 and 1625 cm^{-1} is related to water. In the spectrum of CW, the strong absorption bond at 3396 cm^{-1} attributed to a several -OH stretching vibrations that are a characteristic peak of hydroxyl group in the peptidoglycan structure in the yeast cell wall. The other sharp peak at 2925 cm^{-1} is ascribed to C-H stretching. The obtained peaks at 1650 cm^{-1} , as shown in Fig. 1, typically related to the C-O also angular vibration of C-H was at 1403. The spectrum of CW/ZnO NPs shows the characteristic absorption peaks compatible with both CW and ZnO spectrum. As it can be seen in Fig. 1 the peak attributed to Zn-O at 470 cm^{-1} , also the notable peaks at 3396, 2925, 1650, and 1403 cm^{-1} , which refer to O-H, C-H, and C-O stretching were observed in CW/ZnO NPs spectrum. Therefore we can infer that cell wall as shells originated from *S. cerevisiae* successfully coated onto the ZnO as the core.

Dynamic Light Scattering (DLS) results showed that the diameters of ZnO and CW/ZnO NPs (core-shell structure) were 105.7 and 458.7 nm, respectively (Fig. 2 (a)). The zeta potentials were -9.18, and -10.1 mV for ZnO and CW/ZnO NPs (core-shell) respectively (Fig. 2 (b)). The data revealed that a thin layer of cell wall particles seems be covered around ZnO core and create core-shell particles properly.

X-ray diffraction was utilized to analyze synthesized

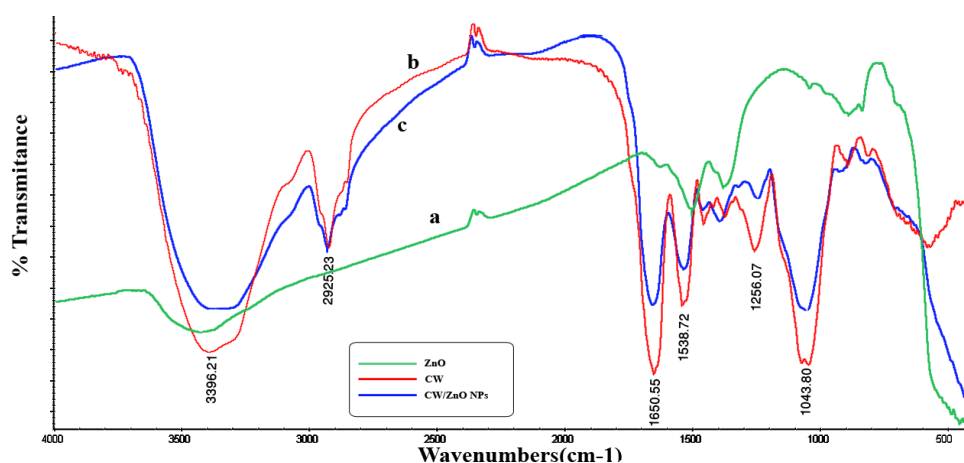


Figure 1. The FTIR spectrum of (a) ZnO nano particle, (b) cell wall particles, (c) cell wall-ZnO nanoparticle (core-shell).

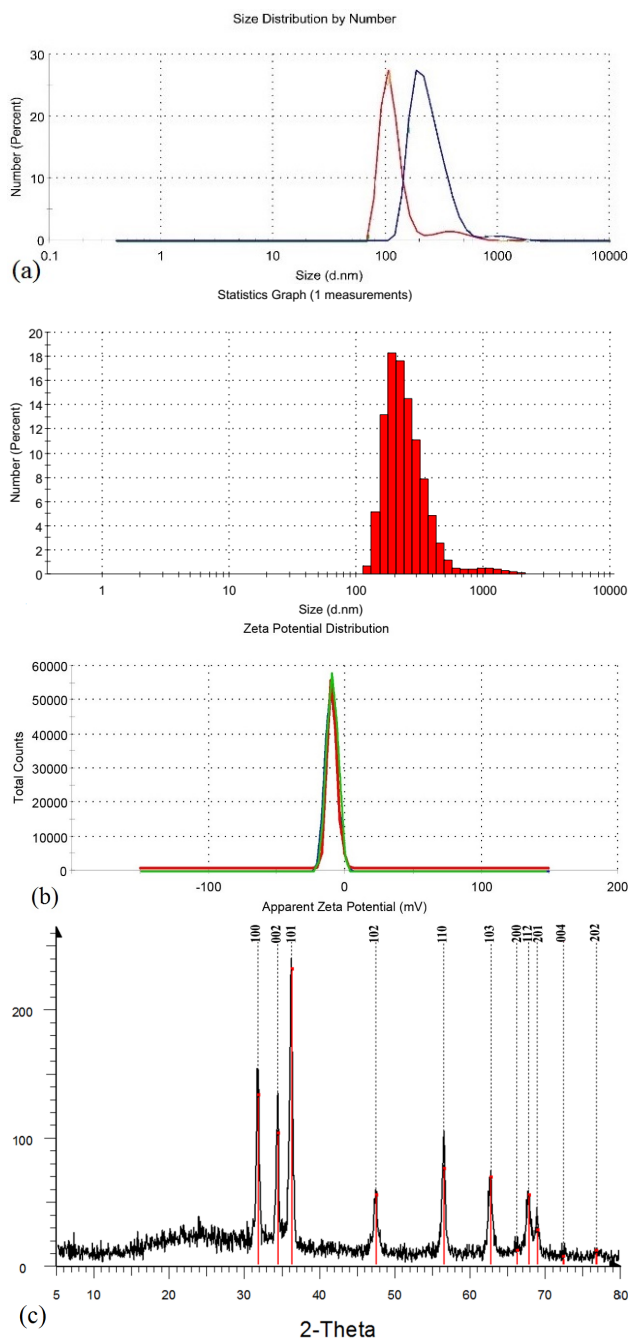


Figure 2. The DLS (a) zeta potentials (b) of ZnO and CW/ZnO NPs (core-shell structure), and XRD spectra of CW/ZnO NPs (c).

nanoparticle samples composition and crystal structure. The X-ray diffraction (XRD) spectra of CW/ZnO NPs (core-shell structure) is shown in Fig. 2 (c), a series of characteristic peaks: 2.814 (100), 2.608 (002), 2.475 (101), 1.911 (102), 1.624 (110) and 1.478 (103) are visible, which are by the zinc phase of ZnO. XRD pattern that was derived indicates that the yeast cell wall does not influence the crystalline structure of ZnO. The cell wall in new structure keeps the ZnO nature.

Field Emission Scanning Electronic Microscopy (FESEM) was used to evaluate the structure of CW/ZnO NPs core-shell. As shown in Fig. 3 (a) the synthesized core-shell nanoparticles have spherical shapes and their diameters are 21.36 – 42.54 nm. The obtained microstructure indicated

that the yeast cell wall as a shell surrounded a core of ZnO nanoparticles. Besides, it indicate a regular morphologies and no aggregation pattern, as given in Fig. 3 (a).

EDX spectrum of CW/ZnO NPs signals is shown in Fig. 3 (b) indicate the existence of Zn, O, and C elements in the CW/ZnO core-shell NPs. These elements are the most considered items in synthesized CW/ZnO NPs. The existence of ZnO in the new structure of the core shell is well understood from the obtained spectrum.

The surface physical properties of synthesized CW/ZnO NPs were evaluated using AFM. As shown in the obtained topographic image in Fig. 3 (c), synthesized particles have a spherical shape and a rough surface. The observed surface roughness indicated the yeast cell wall assigned as a coat at the surface of particles. The visualized microstructure confirmed the successful formation of target core-shell particles at desired nano size. The AFM image also shows a monodispersed spherical of particles with no aggregation or agglutination of core-shell particles.

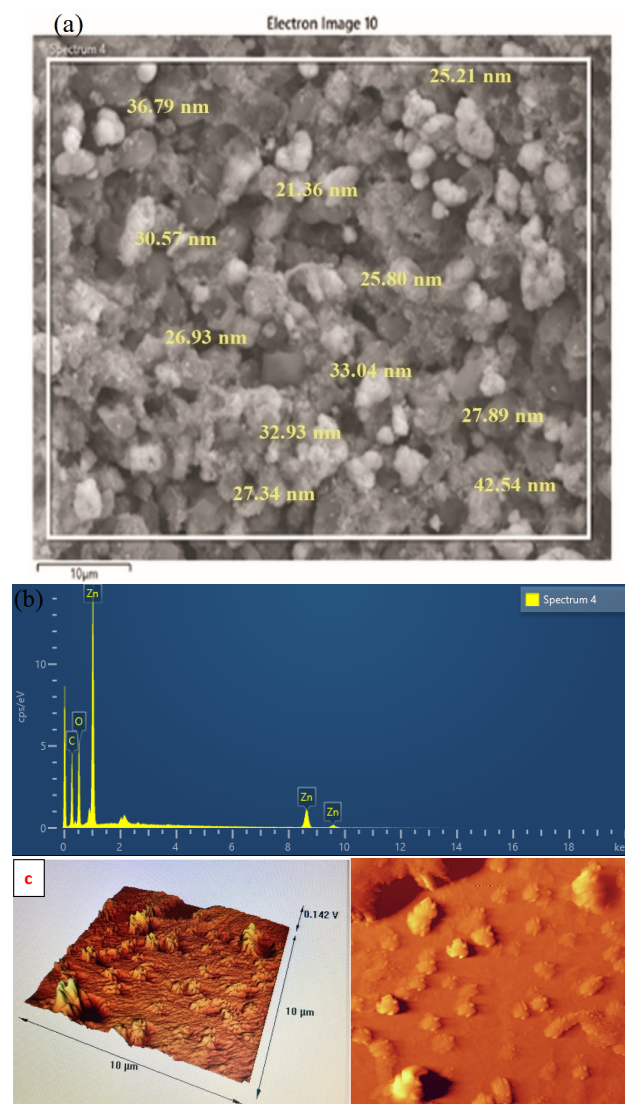


Figure 3. Field Emission Scanning Electronic Microscopy (FESEM) of CW/ZnO NPs (core-shell structure) (a), and EDX spectrum of CW/ZnO NPs (core-shell structure) (b), topographic image of CW/ZnO NPs obtained by Atomic Force Microscope (AFM) (c).

3.2 Antifungal activity of CW/ZnO NPs

In antimicrobial assay it was observed that CW has no antifungal effects. Still the antifungal property of the ZnO NP (62.5 µg/mL) were higher than the CW/ZnO core shell (500 µg/mL) at low concentration against *A. parasiticus*. According to determined MIC, both ZnO NP and CW/ZnO NPs were more effective against *C. albicans* than *A. parasiticus*. Fungicidal activity (MFC) of ZnO NP against *A. parasiticus* and *C. albicans* was significantly more than CW/ZnO ($p < 0.05$). ZnO NPs are widely reported has potential to the inhibition of pathogen bacteria, yeasts, and fungi. The unique biological properties of ZnO NPs introduce it for use as an antifungal agent. The antifungal mechanisms of ZnO NPs are associated to the capability to damage the cell wall and DNA of microorganism, the disruption of membrane [38, 39]. In this study the used ZnO NPs showed a significant antifungal activity against aflatoxin producer strain and also pathogen strain. Besides, the use of ZnO NPs in the core of developed core shell surrounded by yeast cell wall resulted in antifungal properties. However, the fungi growth inhibition observed by core shell was lower than ZnO NPs. It seems ZnO NPs multifaceted antimicrobial mechanisms is more effective to fungi inhibition as pure nanoparticle comparing when is in contact with cell wall in core shell structure. The results are given in Table 1 as Nystatin and Fluconazole antibiotics considered as positive controls.

3.3 AFB₁ binding capacity

The capacity of AFB₁ reduction by the samples including ZnO, cell wall and CW/ZnO NPs (core-shell structure) is presented in Fig. 4. Based on the results, different samples were able to decrease AFB₁ content from 2.66% to 55.04%. ZnO particles showed a low binding efficiency, whereas cell wall and CW/ZnO NPs had a remarkable AFB₁ adsorption. As it can be found from the results, the produced core-shell structure resulted in a higher binding. The binding sites in yeast cell-wall seem to have more capacity at the new structure and size distribution. Some yeast and bacteria strains have been recorded as mycotoxin adsorbents due to their cell wall components. Furthermore, the studies indicated that non-viable cells and microorganisms by-products such as cell-wall or purified cell-wall components such as mannan and betaglukan are more efficient in aflatoxins binding comparing viable cells. Recently, it has been confirmed that peptidoglycan part of cell wall is the most possible carbohydrate involved in aflatoxin binding mechanism. The adsorption of aflatoxin on the surface of yeast cell wall is a physical mechanism that occur between aflatoxin molecule and the cell wall component.

Several studies have shown that cell-wall in small parti-

cle sizes have been developed surface area and presented more available adsorption sites. Therefore, the higher AFB₁ reduction capacity of synthesized core-shell nanoparticle structure containing *S. cerevisiae* cell-wall as the wall is probably is related to lower size and greater surface compared to cell-wall samples. In agreement with our study, Vahidimehr et al. (2020) showed that *S. cerevisiae* cell wall immobilized on nano-silica, which was entrapped in alginate able to reduce more aflatoxin M₁ (AFM₁) 87% compared to free cell wall (53%) [40]. A similar study by Zeinab et al. (2018) revealed enhanced AFB₁ binding ability for glucan mannan from *S. cerevisiae* encapsulated humic acid nanoparticles. The synthesized bio-hybrid nanoparticle seems synergistically to improve the AFB₁ absorption of each used material [30]. Novel metal oxide nanoparticles in core-shell structures has been illustrated as efficient materials in biological studies. The core-shell particles of TiO₂ and SiO₂ on the Ag surface showed better antimicrobial activity [41]. The silver/magnetite core-shell nanoparticles reported to have antibacterial effect against numerous microorganisms [42]. In another study, Ag@TiO₂ and Ag@SeO₂ core/shell nanoparticles synthesized by Beta

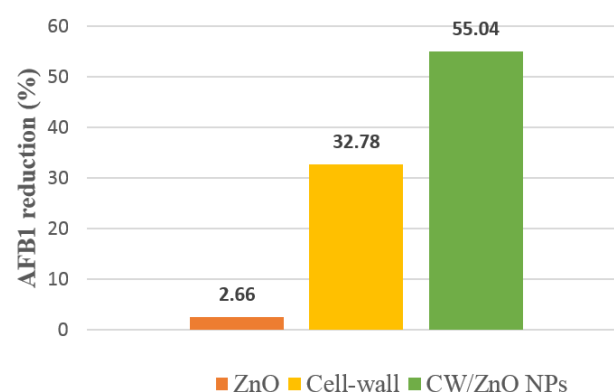


Figure 4. AFB₁ reduction by ZnO, cell wall, and CW/ZnO NPs (core-shell structure).

vulgaris L. extract showed antifungal properties against *Rhizoctonia solani* and *Sclerotia sclerotium* [43].

The developed nanoparticle in the present study is synthesized using edible component which are available and cost-effective materials. Also the production process of nanoparticle is simple and these aspects makes the particles unique and novel in terms of application in food and feed industry. This bio-based solution offers a promising, safer alternative for reducing aflatoxin levels in food and feed, enhancing agricultural safety. Further safety assessments are recommended before widespread application.

Table 1. The result of MICs and MFCs of CW, ZnO NP and CW/ZnO.

Microorganism	Means of MIC/ MFC for 3 independent tests (µg/mL)				
	CW	ZnO NP	CW/ZnO	Nys	Flu
<i>A. parasiticus</i>	> 1000	62.5/125	500/500	7.8/7.8	500/ > 1000
<i>C. abicans</i>	500/ > 1000	15.6625/31.25	125/500	0.95/0.95	62.50/500

4. Conclusion

In the present study, we developed a core-shell structure system using *S. cerevisiae* cell wall surrounded ZnO nanoparticles as antifungal and aflatoxin B₁ binder material. The antifungal activity, binding capacity, and the physicochemical characteristics of the developed particle were analyzed. The results indicated the core-shell particles were well synthesized and were showing the functional features assigned to the core and shell. A significant AFB₁ binding ability and also antifungal activity against *A. parasiticus* and *C. albicans* were obtained by synthesized CW/ZnO NPs core-shell. As all the used materials in developed core shell are edible, it is recommended to apply as a binder for AFB₁ detoxification in food and feed. However further cytotoxicity and *in vivo* tests are needed to guarantee the safety of this novel core shell particles.

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Ethical approval

The project had approved by the research ethics committee of Semnan University of Medical Sciences (approval ID: IR.SEMUMS.REC.1401.222).

Authors Contribution

Conceptualization, A. A.; Methodology, M. B, H. M and E. F; formal analysis, E. F; investigation, A. A.; resources, M. B and A. A.; Writing original draft preparation, A. A, N. S and H. M; writing review and editing, M. B.; supervision, M. B; project administration, A. A and M. B; funding acquisition, A. A. All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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