Harnessing Chitosan and Chitosan Phosphate *in vitro* to Combat Fungal Spoilage in Vegetables through Molecular Docking Interaction Mechanisms

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Chitosan (CH) is a natural, biodegradable polymer derived from chitin. It is known for its broad-spectrum antimicrobial properties. Three fungal pathogens—*Mucor circinelloides*, *Cladosporium herbarum*, and *Aspergillus niger*—were isolated from decayed cucumbers. The CH and chitosan phosphate inhibited fungal growth in a concentration-dependent manner. The chitosan phosphate exhibited superior antifungal activity, achieving up to 84.2% inhibition at 2.75% (w/v). *M. circinelloides* was more sensitive than *C. herbarum* and *A. niger*. The CH was investigated for its antifungal potential *via* molecular docking against key protein targets from three pathogenic fungi: *M. circinelloides* (PDB: 6VRX), *C. herbarum* (PDB: 7KQV), and *A. niger* (PDB: 1GAL). Using MOE 2019 software, docking scores and interaction profiles were analyzed. Chitosan exhibited the most favorable binding affinity towards *M. circinelloides* with a docking score of -7.81 kcal/mol, followed by *C. herbarum* protein (PDB: 7KQV; -6.78 kcal/mol) and *A. niger* protein (PDB: 1GAL; -6.62 kcal/mol). Hydrogen bonding dominated the interactions, with critical residues including ASP 80 (6VRX), GLU 190 (7KQV), and ASP 416 (1GAL). These results suggest chitosan phosphate's potential as a broad-spectrum antifungal agent targeting essential fungal enzymes.

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INTRODUCTION

Postharvest fungal spoilage poses a significant threat to the quality, safety, and marketability of vegetables, resulting in substantial economic losses worldwide. The heavy reliance on synthetic fungicides to control these pathogens has led to increased concerns over toxic residues, human health risks, environmental pollution, and the rise of resistant fungal strains. In response, researchers and producers alike are shifting toward eco-friendly, biologically derived alternatives that are both safe and sustainable (Debnath *et al.* 2022).

Chitosan (CH), a natural biopolymer obtained by the deacetylation of chitin, has gained considerable attention due to its biodegradability, biocompatibility, and low toxicity

(Shih *et al.* 2019). Its antifungal properties have been demonstrated against a range of plant and human pathogens, including *Candida albicans*, *Fusarium solani*, and *Aspergillus niger* (Ing *et al.* 2012). The CH operates through a dual mechanism in postharvest protection: it inhibits fungal growth directly and simultaneously activates host defense responses. When applied as a coating, it forms a semi-permeable film on vegetable surfaces, reducing respiration, minimizing moisture loss, and ultimately prolonging shelf life (Debnath *et al.* 2022).

Chitosan phosphate's (CHP) enhanced water solubility and robust interaction with cellular membranes allow it to display a wide range of biological activities. Oxidative stress and bacterial contamination in tissues are lessened by its strong antibacterial, anti-inflammatory, and antioxidant qualities. Because it encourages cell adhesion, proliferation, and tissue regeneration, this substance is useful for bone and wound repair. Furthermore, by improving medication delivery and biocompatibility, chitosan phosphate permits therapeutic compounds to be released over an extended period of time. It has also been shown in studies to stimulate collagen synthesis and angiogenesis, which aids in tissue remodeling and healing (Egorov *et al.* 2023).

Phosphate groups can interact strongly with the chitosan's structure, modifying its physicochemical behavior and enhancing its dispersibility and biological activity. Rather than implying complete molecular dissolution, the presence of multivalent phosphate ions promotes the formation of stable chitosan–phosphate complexes that remain well-dispersed at physiological pH. This improved colloidal stability makes phosphorylated chitosan derivatives more suitable for biomedical applications than native chitosan, which is only soluble under acidic conditions. This alteration enhances its regenerative, anti-inflammatory, and antioxidant qualities. Comparing it to unmodified chitosan, it also improves tissue healing, cell adhesion, and development. Consequently, it is believed that chitosan phosphate is a more potent bioactive substance for tissue engineering and medicinal uses (Žigrayová *et al.* 2024).

The antifungal efficiency of CH and CHP has been significantly enhanced through its conversion into chitosan–phosphate complexes. Despite the promising potential of CH and its nano-derivatives, the specific molecular mechanisms by which they exert antifungal effects remain poorly understood. Most studies have focused on *in vitro* efficacy, while the interaction between CH compounds and fungal target proteins at the molecular level has not been thoroughly explored (Shih *et al.* 2019; Alsolami *et al*. 2025). Understanding these interactions could provide critical insights into their mode of action and help optimize their use in postharvest disease management of vegetables. The antifungal activity of nanomaterials is attributed to several mechanisms, including the production of reactive oxygen species (ROS) that damage cellular components, disruption of fungal cell walls, and impairment of hyphal and spore integrity, as well as the activation of fungal defense responses (Slavin and Bach 2022). Among different nanomaterials, silver nanoparticles have been reported to inhibit pathogenic fungi such as *Candida* and *Aspergillus* by interfering with cell membrane integrity and altering metabolic processes (Thapliyal *et al*. 2025). Similarly, CH and its nanoparticle formulations (CHs) have demonstrated strong antifungal effects, particularly against *S. sclerotiorum*, where they cause severe plasma membrane disruption (Al-Rajhi *et al.* 2025; Desouky *et al.* 2025).

Molecular docking is a powerful computational technique widely employed to predict the interaction between a small molecule (ligand) and a biological target (typically a protein), providing insights into binding affinity and possible modes of interaction (Al

Abboud *et al.* 2022; Al-Rajhi *et al.* 2022). It is particularly useful in drug discovery, antimicrobial research, and natural product evaluation, where understanding the molecular basis of bioactivity is essential. In the context of antifungal research, molecular docking helps identify how bioactive compounds interact with key fungal proteins involved in pathogenicity, cell wall synthesis, or enzyme regulation (Al-Rajhi *et al.* 2023a; Alsalamah *et al.* 2023). Through simulation of these interactions, docking can reveal the binding conformation, interaction energies, hydrogen bonding patterns, and other critical molecular forces contributing to the compound's inhibitory effects. Based on the increasing demand for safe and natural antifungal agents, this study addressed some research questions, such as can chitosan and its nanoparticles inhibit the development of common vegetable-spoilage fungi? Also, can molecular docking provide mechanistic insights into their interactions with fungal proteins? The present work considers the hypothesis that CHP and its nano-form would show noteworthy antifungal activity, with nano-form showing superior efficacy due to their nanoscale properties, and that docking analysis would confirm energetically favorable interactions with tested fungal target proteins. Therefore, this study aims to evaluate the antifungal efficacy of chitosan phosphate CHP and its nanoparticles against major spoilage fungi of vegetables and to investigate their molecular docking interactions with selected fungal proteins. Through integrating experimental findings with computational modeling, this work seeks to bridge the gap between empirical efficacy and mechanistic understanding, paving the way for the development of efficient, eco-compatible antifungal strategies.

EXPERIMENTAL

Source of Chitosan (CH) and Chitosan phosphate (CHP)

Chitosan (CAS Number: 9012-76-4, low molecular weight, ≥ 75% deacetylated) was procured from Sigma-Aldrich (St. Louis, MO, USA) and chitosan phosphate (CAS Number: 9012-76-4, average particle size 80 to 100 nm) was procured from Nanochemazone Company (Alberta, Canada) and used without further modification.

Isolation and Identification of Fungal Isolates

Spoiled cucumbers were surface sterilized utilizing 70% ethanol for 30 s, followed by 1% sodium hypochlorite for 1 min. After rinsing with sterile distilled water, small segments from visibly infected tissues were aseptically transferred onto Potato Dextrose Agar (PDA) plates amended with chloramphenicol (100 mg/L) to prevent bacterial contamination. The plates were incubated at 25 °C for 7 days. Distinct fungal colonies were then sub-cultured onto fresh PDA plates for purification and identification. Fungal isolates were identified based on their macroscopic and microscopic characteristics. Colony morphology—including color, pigmentation, texture, edge, and growth rate—was recorded. For microscopic examination, lactophenol cotton blue staining was employed. A small portion of fungal mycelium was mounted on a glass slide, stained, and observed under a light microscope (400× magnification). Identification was performed by comparing morphological features including spore type, arrangement, hyphal structure, and conidial morphology with standard identification keys and literature (Ellis 1971; Raper and Fennell 1973; Domsch *et al.* 1980).

Inhibitory Effect Assessment of CHP and CHP on Fungal Mycelial Growth

To evaluate the antifungal efficacy of CH and CHP, a series of concentrations (ranging from 0.25% to 2.75% w/v) after dispersion (CHP was dispersed in water using 0.5 % (v/v) acetic acid under continuous stirring for 24 h at room temperature to form a stable colloidal suspension. The CHP was prepared employing the ionic gelation technique with sodium tripolyphosphate (TPP) as a crosslinking agent, which enables the formation of nanoparticles through electrostatic interactions among the positively charged amino groups of chitosan and the negatively charged phosphate groups of TPP) were incorporated into potato dextrose broth (PDB). Fungal cultures (*Mucor circinelloides*, *Cladosporium herbarum*, and *Aspergillus niger* were selected because they are causing spoilage of cucumbers as a postharvest pathogens). The cultures were inoculated into the treated media and incubated (Incubator: Memmert IN30, Memmert GmbH, Germany) under static conditions for a period of 8 days at 25 ºC. Following incubation, the fungal biomass was collected, dried to constant weight, and the dry weight (DW) was recorded (Al-Rajhi *et al*. 2023b). The percentage of mycelial growth inhibition was calculated by comparing the biomass produced in treated samples with that of the control (untreated) using the following Eq. 1,

$$\text{Inhibition } (\%) = \frac{\text{DW1} - \text{DW2}}{\text{DW1}} \times 10 \quad (1)$$

where DW1 refers to the dry weight (mg/100 mL) of fungal mycelia in control flasks without CHP, and DW2 represents the dry weight (mg/100 mL) obtained from flasks containing CHP or its nanoparticles.

Molecular Docking Interaction

Chitosan, a natural polysaccharide with known antimicrobial properties, was investigated for its antifungal potential *via* molecular docking against key protein targets from three pathogenic fungi: *M. circinelloides* (PDB: 6VRX), *C. herbarum* (PDB: 7KQV), and *A. niger* (PDB: 1GAL). Using MOE 2019 software, docking scores and interaction profiles were analyzed. The general docking scenario was run for 100 ns on the stiff receptor atoms. The protein targets were chosen based on their essential roles in fungal pathogenicity and cell wall integrity, including chitinase and β-1,3-glucanase, which are widely reported as critical enzymes for fungal growth and survival.

Statistical Analysis

The analysis results were performed as mean ± standard deviation (SD) and estimated employing Microsoft Excel 365.

RESULTS AND DISCUSSION

In this study, three fungal species—*Mucor circinelloides*, *Cladosporium herbarum*, and *Aspergillus niger*—were successfully isolated from visibly decayed cucumber samples. These fungi are among the most commonly reported spoilage organisms associated with postharvest deterioration of vegetables, particularly cucumbers, which are highly perishable due to their high moisture content and delicate skin. The presence of *M. circinelloides* in cucumber spoilage is consistent with previous studies highlighting *Mucor* spp. as primary agents of soft rot in vegetables stored under humid conditions. *C. herbarum*

is another dominant isolate and is typically associated with surface discoloration and dark green to blackish patches on the cucumber skin. This species is commonly found in both field and storage environments and is considered a ubiquitous air- and soil-borne fungus. *A. niger* is frequently isolated from advanced spoilage stages, particularly in samples showing signs of black mold rot. Its strong enzymatic potential enables the degradation of plant cell walls, leading to softening, water leakage, and collapse of tissue integrity. The isolation of these species from cucumber suggests that spoilage is the result of a complex interaction of environmental conditions (moisture, temperature, and storage hygiene) and the physiological status of the vegetable. The findings of this investigation not only confirm the role of these fungi as key spoilage organisms but also provide a basis for further testing of bio-based antifungal treatments, including CH and CHP and its nanoparticles, to inhibit their growth and extend the shelf life of fresh produce.

The findings in Table 1 demonstrate the inhibitory effects of CH and CHP on the mycelial growth of three fungal species (*M. circinelloides*, *C. herbarum*, and *A. niger*) as measured by dry weight (mg/100 mL). The results clearly indicate a concentration-dependent antifungal activity for both treatments. All fungal species at 0.0% concentration (without treatment), exhibited maximum growth, serving as the control. As the concentration of CH and CHP increased, there was a progressive reduction in fungal biomass across all tested species. Notably, CHP consistently exhibited stronger antifungal effects than bulk CH at every concentration. For example, at 2.75%, CHP achieved inhibition degrees of 84.2%, 83.3%, and 78.3% against *M. circinelloides*, *C. herbarum*, and *A. niger*, respectively, compared to 79.6%, 77.0%, and 71.8% inhibition by regular CH. Similar trends were observed by El-Araby *et al.* (2024), who reported that at 3% CH, inhibition of mycelial growth among several postharvest spoilage fungi, such as *A. niger* and *F. oxysporum*, ranged from 81.4% to 92.7%, along with substantial suppression of spore germination. This enhanced efficacy of CHP is attributed to their superior interaction with fungal cell membranes, which allows for enhanced penetration and disruption of cellular functions. The results also reveal species-specific responses to the treatments. *C. herbarum* and *A. niger* showed lower sensitivity. In contrast, *M. circinelloides* indicated a comparatively higher susceptibility. At low concentrations (0.25 to 0.75%), the antifungal effect was already apparent with CHP, where even 0.25% led to 13.3% inhibition of *M. circinelloides* and 11.3% of *A. niger*, while CH at the same dose achieved only 5.5% and 2.9%, respectively. This study's results were supported by previous studies that have demonstrated the potent antifungal potential of CH. While metallic nanoparticles such as silver, copper, magnesium, and zinc oxide NPs have demonstrated potent antifungal properties through mechanisms such as reactive oxygen species generation and membrane disruption, their potential cytotoxicity and environmental persistence raise safety concerns (Abdelghany *et al.* 2023; Al Abboud *et al.* 2024). In contrast, chitosan-based phosphate, being biodegradable and biocompatible, represent a safer alternative with comparable antifungal efficacy (Choi *et al.* 2022; Poznanski *et al*. 2023), as supported by the present findings. This comparison situates this work within the broader nanotechnology field and underscores the unique advantages of biopolymer-based nanoparticles.

Kanawi *et al.* (2021) demonstrated that the antifungal efficiency of CH and chitin against pathogenic fungi, such as *Macrophomina* sp., reached over 70% at just 4 mg/mL, while lower concentrations still maintained measurable inhibition, echoing the present findings at lower doses. The present study also confirmed species-specific sensitivity, a phenomenon explained by the variability in fungal plasma membrane composition.

According to Palma-Guerrero *et al.* (2009), CH-susceptible fungi tend to have membranes rich in unsaturated fatty acids, increasing membrane fluidity and vulnerability. Conversely, resistant strains may exhibit low membrane fluidity due to saturated fatty acids, as noted by Lopez-Moya *et al.* (2019). The enhanced activity observed with CHP in the present data may be attributed to their improved ability to penetrate fungal cells, potentially disrupting genetic processes and causing cell death—a mechanism proposed by Ing *et al.* (2012) in investigations on *Candida albicans*. Furthermore, the varied responses among fungi in the current study align with observations by Poznanski *et al.* (2023), who emphasized that the developmental stage and type of fungus significantly influence the response to CH-based treatments. Supporting this finding, previously Li *et al.* (2012) also demonstrated the inhibitory effect of CH against multiple plant pathogens at both 200 and 400 mg/mL concentrations. In postharvest utilizations, Martínez-Batista *et al.* (2024) demonstrated that CH effectively inhibited fungi such as *A. niger* and *F. verticillioides* in maize kernels, while Cuong *et al.* (2022) reported complete protection of citrus fruits treated with CHNPs against several storage fungi. These outcomes collectively reinforce the authors' conclusion that CHP are a highly effective, broad-spectrum antifungal agent. They act *via* several mechanisms, such as nutrient deprivation, disruption of cell membranes, and interference with genetic material, as summarized by Li *et al.* (2022).

Overall, the data highlight the superior performance of CHP over their conventional form in suppressing fungal growth. The improved efficacy at both low and high concentrations underscores their potential as powerful biofungicides for agricultural, food safety, and biomedical applications. These findings support the further development of CHP-based formulations as efficient, eco-friendly alternatives to synthetic antifungal agents. Future studies incorporating advanced imaging techniques are recommended to directly visualize CHP–fungi interactions and confirm the structural alterations underlying the observed antifungal activity.

Table 1. Effect of Chitosan and Chitosan Phosphate on Fungal Growth (Dry Weight mg/100 mL)

Conc. (%) w/v	Chitosan			Chitosan Phosphate		
	M. circinelloides	*C. herbarum*	*A. niger*	*M. circinelloides*	*C. herbarum*	*A. niger*
0.0	152.33 ± 2.52	188.0 ± 1.73	264.67 ± 2.52	152.33 ± 2.52	188.0 ± 1.73	264.67 ± 2.52
0.25	144.0 ± 1.41 (5.47%)*	186.67 ± 1.53 (0.71%)*	257.0 ± 2.65 (2.90%)*	132.0 ± 1.73 (13.33%)*	184.0 ± 4.58 (2.13%)*	234.75 ± 1.50 (11.31%)*
0.75	127.0 ± 2.0 (16.63%)*	179.0 ± 9.54 (4.79%)*	247.33 ± 1.15 (6.58%)*	120.0 ± 1.73 (21.24%)*	171.0 ± 5.57 (9.04%)*	224.25 ± 1.26 (15.27%)*
1.25	123.33 ± 4.04 (19.04%)*	162.0 ± 3.46 (13.83%)*	201.67 ± 2.89 (23.83%)*	111.33 ± 1.15 (26.91%)*	144.33 ± 1.15 (23.28%)*	172.0 ± 2.0 (35.01%)*
1.75	70.67 ± 3.79 (53.61%)*	94.0 ± 1.73 50.00%)*	149.67 ± 0.58 43.45%)*	55.33 ± 0.58 (63.66%)*	69.75 ± 1.26 (62.90%)*	126.0 ± 1.0 (52.39%)*
2.25	37.33 ± 1.15 (75.49%)*	77.33 ± 2.08 (58.87%)	103.0 ± 2.65 61.07%)*	31.67 ± 1.15 (79.21%)*	60.33 ± 1.53 (67.91%)*	88.33 ± 1.15 (66.64%)*
2.75	31.0 ± 1.73 (79.65%)*	43.33 ± 1.15 (76.95%)*	74.67 ± 2.31 (71.78%)*	24.0 ± 2.0 (84.24%)*	31.33 ± 1.53 (83.34%)*	57.33 ± 1.15 (78.34%)*

*: Inhibition % of fungal growth

The CH exhibited the highest binding affinity for *M. circinelloides* fumarase (6VRX) with a docking score of -7.81 kcal/mol and low RMSD (1.18 Å), indicating stable binding (Table 2). Lower affinity was observed for *C. herbarum* (7KQV; -6.78 kcal/mol) and *A. niger* (1GAL; -6.62 kcal/mol). Energy decomposition revealed dominant conformational energy contributions (E_conf: -249.9 to -265.9 kcal/mol). From the data in Table 2, hydrogen-donor and hydrogen-acceptor indicated that hydrogen bond interactions were critical for ligand–protein stability. Furthermore, possible π–π/π–cation interactions with aromatic residues and hydrophobic or van der Waals contacts further stabilize the complex and may explain the stronger binding of chitosan phosphate to *M. circinelloides* compared with the other tested fungi.

Residue-Specific Interactions

6VRX (Table 3): Five hydrogen bonds formed with residues ASP 80(A), GLY 54(A), and ALA 82(A) (Binding energy: -0.5 to -2.3 kcal/mol).

7KQV (Table 4): Key interactions with GLU 190(A) (H-donor; -2.1 kcal/mol) and LYS 187(A) (H-acceptor).

1GAL (Table 5): Dual H-donor bonds with ASP 416(A) (-1.4 and -0.7 kcal/mol).

2D/3D diagrams (Figs. 1 through 3) confirmed hydrogen bonding and hydrophobic contacts between CH and active sites of all three proteins, as well as key residues involved in each fungal protein target.

The molecular docking study demonstrated that CH has a promising antifungal interaction profile, particularly against *M. circinelloides*. The superior binding score (−7.81 kcal/mol) and stable interactions (*e.g.*, ASP80, GLY54, LYS58) suggest strong affinity toward the protein target 6VRX, which suggests relevant virulence or metabolic protein. Some studies have shown that proteins within this structural family are involved in key metabolic and virulence-associated processes in *Mucor* species, including growth regulation and stress adaptation, making them promising antifungal targets (Gobeil *et al*. 2021; Srinivasan *et al*. 2025). Therefore, the observed strong interaction of CH with 6VRX supports its potential to interfere with essential fungal pathways. Chitosan's affinity for conserved acidic residues (ASP/GLU) across targets highlights its role as a hydrogen bond donor. Hydrogen bond formation was the dominant interaction mechanism, critical for stabilizing ligand-receptor complexes. The interaction distances (< 3.3 Å) indicate valid and energetically favorable hydrogen bonds, aligning with literature reports emphasizing hydrogen bonding in chitosan bioactivity (Rabea *et al.* 2003; Kong *et al.* 2010; Qanash *et al.* 2023a).

Comparatively, the weaker scores and fewer interactions with *A. niger* and *C. herbarum* suggest lower affinity or less optimal binding sites, though still within the antifungal range (Badawy and Rabea 2011; Qanash *et al.* 2023b). These findings align with prior studies indicating chitosan's ability to inhibit fungal growth *via* membrane disruption and intracellular interaction. Docking thus provides molecular insight that supports experimental observations. Beyond laboratory efficacy of CHPNPs, its scalability poses practical challenges, as reproducibility and process optimization must be addressed for industrial application. Moreover, long-term safety, and compliance with international food safety regulations are essential considerations to ensure safe and sustainable use in real-world food systems.

Table 2. Docking Scores and Energies of CHP Against *M. circinelloides* (PDB ID: 6VRX), *C. herbarum* (PDB ID: 7KQV), and *A. niger* (PDB ID: 1GAL)

Molecule	Protein	Docking Score (S)	rmsd_ refine	E_conf	E_place	E_score1	E_refine	E_score2
CHP	6VRX	-7.81	1.18	-249.92	-68.57	-9.71	-51.56	-7.81
CHP	7KQV	-6.78	1.86	-257.99	-112.24	-12.26	-30.48	-6.78
CHP	1GAL	-6.62	2.53	-265.90	-100.74	-13.08	-39.30	-6.62

Table 3. Interaction of CHP with Structure of *M. circinelloides* (PDB ID: 6VRX)

Molecule	Atom Involved	Residue	Interaction Type	Distance (Å)	Binding Energy (kcal/mol)
CHP	O 14	O ASP 80 (A)	H-donor	2.94	-0.9
	O 16	O GLY 54 (A)	H-donor	2.86	-2.3
	N 28	O ASP 80 (A)	H-donor	3.12	-0.9
	C 41	O ALA 82 (A)	H-donor	3.27	-0.5
	O 8	N LYS 58 (A)	H-acceptor	3.17	-0.5

Table 4. Interaction of CHP with Structure of *C. herbarum* (PDB ID: 7KQV)

Molecule	Atom Involved	Residue	Interaction Type	Distance (Å)	Binding Energy (kcal/mol)
CHP	O 14	OE2 GLU 190 (A)	H-donor	3.05	-2.1
	N 25	O GLU 394 (A)	H-donor	3.13	-0.7
	C 53	OE1 GLU 190 (A)	H-donor	3.28	-0.6
	O 14	CE LYS 187 (A)	H-acceptor	3.24	-0.7

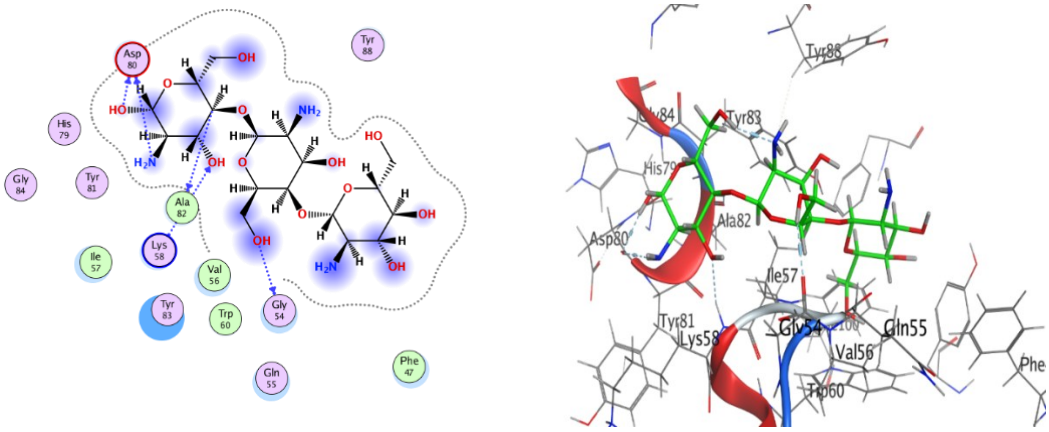

Fig. 1. The binding of CHP to the active sites of *M. circinelloides* 6VRX protein is represented in two- and three-dimensional diagrams.

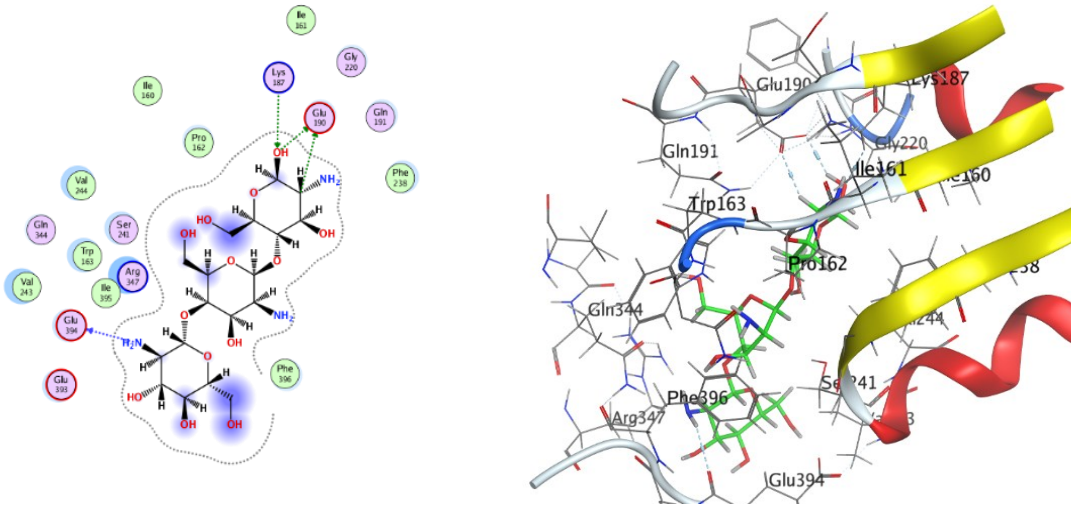

Fig. 2. The binding interactions of CHP with the active sites of *C. herbarum* 7KQV protein are shown through 2D and 3D diagrams.

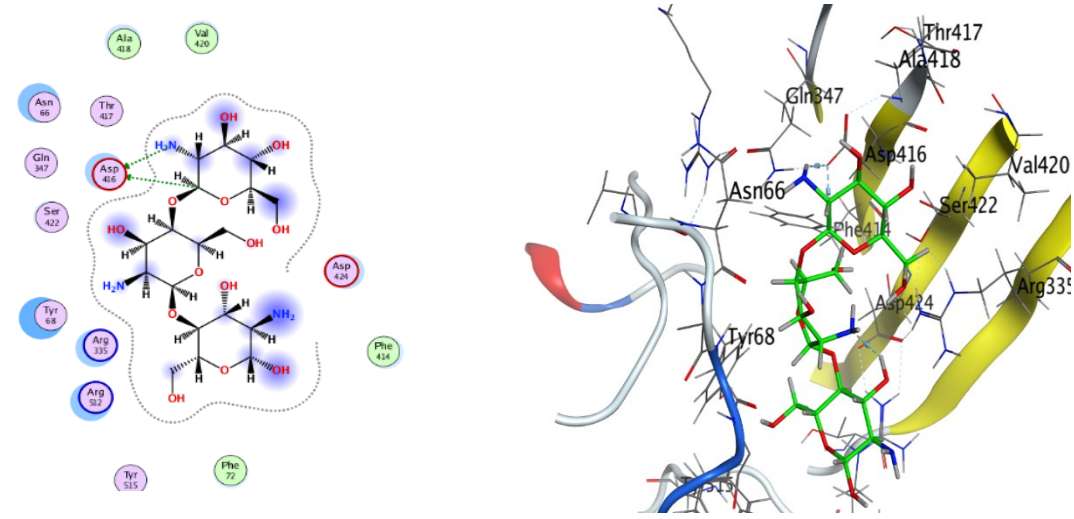

Fig. 3. The interaction between CHP and the active regions of the *A. niger* 1GAL protein is shown using 2D and 3D structural models.

Table 5. Interaction of CHP with Structure of *A. niger* (PDB ID: 1GAL)

Molecule	Atom Involved	Residue	Interaction Type	Distance (Å)	Binding Energy (kcal/mol)
CHP	N 25	OD2 ASP 416 (A)	H-donor	3.04	-1.4
	C 43	OD2 ASP 416 (A)	H-donor	3.18	-0.7

CONCLUSIONS

1. Chitosan and chitosan phosphate effectively inhibited the mycelial growth of *M. circinelloides*, *C. herbarum*, and *A. niger* in a concentration-dependent manner, with chitosan phosphate showing superior antifungal activity.

2. The enhanced efficacy of chitosan phosphate highlights their potential as a natural, eco-friendly alternative for controlling postharvest fungal spoilage and extending the shelf life of fresh vegetables like cucumber.
3. Through molecular docking investigation, this study confirmed the antifungal potential of chitosan, particularly against *M. circinelloides*, through favorable binding affinity and multiple stable interactions. The targeting of catalytic sites (*e.g.*, ASP 80, GLU 190) implies functional inhibition.

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