Enzymatic Suppression of Postharvest Fungi in Tomato Fruits: *In-vitro* and *In-silico* Evidence of Chitinase and β-1,3-Glucanase Efficacy

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Spoiled tomato fruits exhibited fungal infections, and the isolates were identified as *Penicillium expansum*, *Alternaria alternata*, *Aspergillus terreus*, and *Fusarium oxysporum*. Varying doses of chitinase, β-1,3-glucanase, and a chemical fungicide were tested against four fungal pathogens. All treatments showed dose-dependent inhibition of fungal growth. The chemical fungicide caused complete inhibition at the highest dose, while chitinase and β-1,3-glucanase significantly reduced colony size, especially in *P. expansum* and *A. alternata*, though they were less effective against *A. terreus* and *F. oxysporum*. This study rigorously investigated the molecular docking interactions of chitinase (PDB ID: 1CTN) and β-1,3-glucanase (PDB ID: 4M80), with target proteins of *F. oxysporum* (PDB ID: 7T69). Molecular simulations revealed compelling binding affinities, with chitinase demonstrating a docking score of -82.67 kcal/mol and β-1,3-glucanase exhibiting a score of -78.1 kcal/mol. Detailed interaction analyses revealed distinct binding mechanisms: Chitinase forms a stable complex through multiple hydrogen bonds and significant π-π stacking with key residues such as TRP210, while β-1,3-glucanase employs extensive hydrogen bonding and strong ionic interactions, notably with GLU121, for electrostatic stabilization. These findings provide critical molecular insights into the antifungal capabilities of these enzymes, highlighting their potential as agents to combat postharvest fungal pathogens.

DOI: 10.15376/biores.20.4.10069-10081

Keywords: Chitinase; β-1,3-Glucanase; Antifungal activity; Molecular docking

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INTRODUCTION

Tomato, a versatile staple in many home gardens, ranks as the second most popular vegetable after the potato (*Solanum tuberosum* L.) (Frenkel and Jen 2021). However, it suffers from infections caused by postharvest fungi, which results in significant losses. The

ability of these fungi to colonize plant tissues, produce mycotoxins, and persist in soil and on vegetables as spores makes it particularly difficult to manage with the usage of conventional chemical or cultural methods. As a result, biological control strategies have gained significant attention as sustainable alternatives. Among various biocontrol mechanisms, antibiosis is a process that involves the secretion of bioactive compounds, including hydrolytic enzymes (Al-Rajhi *et al*. 2022a). It is a pathogen control mechanism that involves the secretion of bioactive compounds, including hydrolytic enzymes. Two key hydrolytic enzymes, chitinase and β-1,3-glucanase, are widely recognized for their antifungal activities. They degrade major structural components of fungal cell walls—chitin and β-1,3-glucans—thereby disrupting fungal integrity, inhibiting spore germination, and ultimately leading to cell lysis (Al-Rajhi *et al*. 2022b; Bakri *et al*. 2022).

Numerous microorganisms, particularly plant growth-promoting rhizobacteria (PGPR) and endophytic fungi, produce these enzymes as part of their antagonistic activity against phytopathogens. The synergistic action of chitinase and β-1,3-glucanase not only directly inhibits *Fusarium oxysporum* but also enhances plant defense responses, offering a dual mode of protection. Understanding and harnessing these enzymatic mechanisms hold great promise for developing effective biocontrol agents and enzyme-based antifungal formulations for integrated disease management. *Pseudomonas stutzeri* YPL-1 exhibits strong antifungal activity by producing extracellular chitinase and β-1,3-glucanase, which degrade fungal cell walls. These enzymes have been found to significantly suppress the mycelial growth of *Fusarium solani* (Lim and Kim 1995).

According to Almeida *et al*. (2022), *Trichoderma* species produce a diverse array of enzymes, such as glucanase, chitinase, and cellulase, which play a crucial role in suppressing pathogenic fungi. These enzymes act by degrading the structural components of fungal cell walls, leading to cell wall disruption, osmotic imbalance, and ultimately, cell death. The chitinase enzyme plays a crucial role in controlling fungal pathogens, as it targets and breaks down chitin, the primary structural component of fungal cell walls (Al Abboud *et al*. 2022, Rosyida *et al*. 2022). According to previous study, chitinase and β-1,3-glucanase production by *Clonostachys rosea* f. *catenulata* had been induced by fungal cell walls and cucumber root material. These enzymes inhibited the mycelial growth of *Fusarium* and *Pythium*, as culture filtrates exhibited strong glucanase activity and degraded the pathogen cell walls. These findings highlight the biocontrol potential of *C. rosea* against root and stem and damping-off diseases in cucumber (Chatterton and Punja 2009).

Molecular docking is a computational technique that is extensively employed in drug discovery, development, and structural biology to predict the preferred orientation of a ligand when bound to a target macromolecule inside a targeted cell. By simulating the interaction among two constituents, typically a small compound and an enzyme or receptor, molecular docking aids in estimating the binding affinity and stability of the complex. This provides valuable insights into the potential biological activity of new compounds and aids in the rational design of pharmaceuticals (Shankar *et al*. 2023). Given its cost-effectiveness and efficiency, molecular docking has become a cornerstone in modern computational drug design, enabling researchers to understand molecular mechanisms, optimize promising compounds, and predict structure–activity relationships with greater precision. This study is the first to integrate in vitro inhibition assays with molecular docking of purified chitinase and β-1,3-glucanase against *F. oxysporum*. The aim of this study is to evaluate the antifungal efficacy of chitinase and β-1,3-glucanase against postharvest fungal

pathogens affecting tomato fruits. This evaluation includes *in vitro* assays and *in silico* molecular docking studies to explore the interaction of these enzymes with key fungal cell wall components, thereby elucidating their potential mechanisms of action.

EXPERIMENTAL

Source of Enzymes and Chemical Fungicide

Commercial chitinase and β-1,3-glucanase (EC 3.2.1.39) from *Trichoderma viride* were purchased from Sigma-Aldrich (St. Louis, MO, USA). Enzymes were prepared in sterile distilled water at concentrations of 0 (control), 50, 100, and 150 U/mL, and filter-sterilized using 0.22 µm syringe filters before being incorporated into Potato Dextrose Agar (PDA) medium. Carbendazim (Methyl benzimidazol-2-ylcarbamate, $C_9H_9N_3O_2$) a broad-spectrum fungicide was employed as standard.

Isolation of Fungi from Spoiled Tomato Fruits

Spoiled tomato fruits were obtained from local sources and transported to the laboratory in sterilized polyethylene bags under aseptic conditions. Using sterile techniques, small tissue segments were excised from visibly decayed regions of three different tomato samples and placed onto Czapek Dox agar (CDA) plates. The plates were incubated at 30 °C for 8 days to promote fungal growth. Fungal colonies that developed were sub-cultured onto fresh media to achieve pure isolates. For proper identification, the purified fungi were cultivated on three types of media: Czapek Dox Agar (CDA), Malt Extract Agar (MEA), and Potato Dextrose Agar (PDA). Morphological and microscopic features were analyzed, including colony color, texture, growth rate, and structures such as conidiophores, conidia, and hyphae. Identification was carried out following standard mycological references (Ellis 1971; Raper and Fennell 1973; Domsch *et al*. 1980; Rotem 1994). The identified fungal species were later employed as test organisms for assessing the antifungal effects of cellulolytic and other hydrolytic enzymes.

Inhibition of Fungal Isolates by Chitinase and β-1,3-Glucanase

The antifungal activity of chitinase and β-1,3-glucanase was assessed against selected fungal isolates using the poisoned food technique on PDA plates. Enzyme solutions were prepared at concentrations of 0 (control), 50, 100, and 150 U/mL in sterile distilled water. Each enzyme concentration was added to molten PDA medium cooled to approximately 45 °C before solidification, which was followed by thorough mixed to ensure uniform distribution. The medium was then poured into sterile Petri dishes and allowed to solidify. A 5 mm diameter fungal disc, obtained from the actively growing margin of a 5-day-old culture, was placed in the center of each plate. Plates were incubated at 25 ± 2 °C for 7 days, depending on the growth rate of the fungus. Control plates contained PDA without any enzyme supplementation. The antifungal effect was evaluated by measuring the radial growth (mm) of fungal colonies, and the percentage inhibition of mycelial growth was calculated using Eq. 1,

$$\text{Fungal Inhibition (\%)} = \frac{\text{Radial growth at control} - \text{Radial growth at treatement}}{\text{Radial growth at control}} \times 100 \quad (1)$$

Docking Interaction Study Evaluation

All simulations were performed using Molecular Operating Environment (MOE) 2019.0102 (Chemical Computing Group Inc., Montreal, Canada). The 3D crystal structures of the fungal structural protein from *F. oxysporum* (PDB ID: 7T69), Chitinase (PDB ID: 1CTN), and β-1,3-Glucanase (PDB ID: 4M80) were retrieved from the RCSB Protein Data Bank (https://www.rcsb.org).

Structure preparation

Water molecules and heteroatoms were removed, and missing residues corrected where applicable. Protonation states were protonated using MOE's Protonate 3D feature to assign correct protonation states at physiological pH. Energy minimization was performed in aqueous solution using the AMBER10: EHT forcefield (RMS gradient: 0.1 kcal/mol·Å) to optimize geometry. Protein–protein docking was performed using the Dock module in MOE 2019. The *Fusarium oxysporum* structural protein (7T69) was set as the receptor, while the Chitinase and β-1,3-Glucanase structures were used as ligands. The active site was defined to operate as dummy sites for the binding pocket.

Docking was carried out using the Rigid Receptor Docking protocol with the following parameters: (1) Placement method: Triangle Matcher (pose generation: 100 conformations); (2) Scoring function: London dG for initial placement and GBVI/WSA dG for refinement; (3) Retained poses: Top 10 conformations ranked by binding score; and (4) Validation: Redocking of native ligands confirmed protocol accuracy (RMSD < 1.5 Å). The docking results were evaluated based on S-score (binding free energy in kcal/mol), RMSD values (root mean square deviation from initial pose), and interaction profiles, including hydrogen bonds, π–π interactions, and ionic contacts. 2D and 3D interaction diagrams were generated using the Ligand Interaction module in MOE. All hydrogen bonds, hydrophobic contacts, and π-interactions were automatically detected and manually verified. The distances and energies of each interaction were tabulated for comparative analysis.

RESULTS AND DISCUSSION

Based on spoiled tomato fruits showing fungal growth (Fig. 1), the isolated fungi were identified as *Penicillium expansum*, *Alternaria alternata*, *Aspergillus terreus*, and *Fusarium oxysporum* through observation of morphological characteristics and supported by microscopic examination. *Penicillium expansum* was characterized by an initial white color which later turned to blue-green with a velvety texture. *Alternaria alternata* was characterized with olive-green to blackish with a dark reverse. Cinnamon to brownish color with a granular texture was associated with *A. terreus*, while white to purple color and the presence of macroconidia (sickle-shaped) and microconidia (oval, single-celled) were associated with *F. oxysporum.* Tomatoes are highly susceptible to postharvest deterioration, with losses increasing significantly during prolonged storage periods. Several fungal pathogens are commonly associated with postharvest decay in tomatoes, including *Alternaria alternata*, *Fusarium solani*, *Fusarium oxysporum*, *Geotrichum candidum*, *Rhizopus stolonifer*, and *Rhizoctonia solani* (Ramudingana *et al*. 2024). These pathogens compromise the quality and safety of tomato fruits, posing serious challenges to storage,

transport, and marketability. In recent years, biological control agents, particularly epiphytic microorganisms or their enzymes, have gained attention as sustainable and eco-friendly alternatives for managing postharvest diseases in tomatoes (Palmieri *et al*. 2022). These natural sources offer a promising strategy for reducing reliance on synthetic fungicides, minimizing environmental impact, and enhancing the shelf life and safety of tomato fruits.

Fig. 1. Postharvest Fungal Infection on Tomato Fruits

In the present study, two enzymes, namely chitinase and β-1,3-glucanase, were selected because chitin and β-1,3-glucans are the two primary structural polysaccharides of fungal cell walls, and their degradation *via* these enzymes directly compromises fungal integrity. Also, previous literature consistently emphasizes that chitinases and glucanases play central roles in fungal inhibition, even when other enzymes are present in crude extracts (Lim and Kim 1995; Chatterton and Punja 2009). Moreover, the present work provides molecular-level insights (*via* docking and binding interaction analysis) that clarify how each enzyme independently contributes to antifungal activity. This avoids the ambiguity of synergistic effects from complex enzyme mixtures. Table 1 illustrates the effect of varying doses (0, 50, 100, and 150 U/mL) of chitinase, β-1,3-glucanase, and a chemical fungicide (mg/mL) on the colony growth of four fungal pathogens—*P. expansum*, *A*. *alternata*, *A*. *terreus*, and *F*. *oxysporum*—as measured by colony radius (cm). In general, all three treatments exhibited a dose-dependent inhibitory effect on fungal growth, with higher doses resulting in smaller colony radii. Among the treatments, the chemical fungicide exhibited the strongest antifungal activity, consistently reducing fungal growth to 0.00 cm at the highest dose (150 mg/mL) for all tested species, indicating complete inhibition. Chitinase and β-1,3-glucanase also demonstrated antifungal effects, though to a smaller extent compared to the chemical fungicide. For example, in *P. expansum*, chitinase reduced the colony radius from 5.45 cm to 2.58 cm throughout 0 to 150 U/mL, and β-1,3-glucanase showed a similar trend, decreasing growth to 2.89 cm. A similar pattern was observed in *A. alternata*, where chitinase treatment reduced growth from 6.50 cm to 2.66 cm, and β-1,3-glucanase from 6.50 cm to 3.54 cm. In *A. terreus*, both enzymes were less effective, with the colony radius remaining greater than 3.2 cm even at the highest dose, suggesting greater resistance. *Fusarium oxysporum* showed moderate sensitivity, with chitinase and β-1,3-glucanase, reducing growth to 2.50 and 2.80 cm, respectively. Overall, while enzymatic treatments—particularly chitinase—exhibited notable antifungal activity,

they were less effective than the chemical fungicide. Nevertheless, these enzymes present promising eco-friendly alternatives for fungal control, especially considering their biological origin and potential for use in integrated pest management. Further research may explore the combined application of chitinase and β-1,3-glucanase or their formulation with other biocontrol agents to enhance efficacy. These findings are consistent with previous reports highlighting the biocontrol potential of chitinases, which are considered environmentally friendly alternatives to synthetic fungicides. Chitinases have been widely recognized for their promising role in pest management and their broad applicability in various industrial sectors (Abdelghany *et al*. 2018; Abdelghany and Bakri 2019; Nofal *et al*. 2021a,b,c; Al-Rajhi *et al*. 2022a,b). In addition, the inhibitory effect of lytic enzymes such as β-1,3-glucanase has been demonstrated in yeast isolates, which exhibited significant activity against common postharvest pathogens of tomato fruit under both *in vitro* and *in vivo* conditions. This further supports the potential of epiphytic yeasts and their enzymatic arsenal as effective biocontrol agents against fungal pathogens (Shah *et al*. 2025). To support the findings of our study, several previous reports highlight the antifungal efficacy of chitinase and β-1,3-glucanase enzymes produced by various microbial sources. Chatterton and Punja (2009) demonstrated that culture filtrates of *Clonostachys rosea* containing glucanase activity significantly reduced the mycelial growth of *Pythium* and *Fusarium*, and this effect was accompanied by degradation of their cell walls. Similarly, Ting and Chai (2015) reported that chitinase and β-1,3-glucanase produced by *Trichoderma harzianum* inhibited *F. oxysporum* and *Ganoderma boninense*, further validating their biocontrol potential.

Consistent with the present results, chitinase has been shown to exhibit strong antifungal activity against *A. alternata, F. oxysporum*, *F. solani*, and moderate activity against *Penicillium frequens* and *Saccharomyces cerevisiae*, with particularly strong effects on *Candida albicans* (Nazeer 2022). In a related study, *Chaetomium globosum* produced two novel β-glucanases (Cgglu17A and Cgglu16B) capable of hydrolyzing the cell walls of *Fusarium sporotrichioides*, highlighting the role of glucanases in fungal suppression (Jiang *et al*. 2024). Further evidence comes from Mazrou *et al*. (2020), who showed that chitinases from *Trichoderma* effectively suppressed *Fusarium*, *Rhizoctonia*, and *Aspergillus* species. *Trichoderma asperellum* was also reported to suppress *Colletotrichum* and *Sclerotium rolfsii*, reinforcing the broad-spectrum antifungal properties of these enzymes (Loc *et al*. 2019). Moreover, chitinases have been proposed as environmentally friendly biopesticides with applications in agricultural disease control and, when combined with antifungal drugs, for treating fungal infections in humans (Rathore and Gupta 2015).

Table 1. Effect of Different Doses of Chitinase, Chitinase, and Chemical Fungicide on Fungal Growth as Measured by Colony Radius (cm)

Enzyme Dose (U/mL)/ Chemical Fungicide Dose (mg/mL)	*Penicillium expansum*			*Alternaria alternata*		
	Chitinase	β-1,3-Glucanase	Chemical Fungicide	Chitinase	β-1,3-Glucanase	Chemical Fungicide
0	5.45 ± 0.16	5.45 ± 0.16	5.45 ± 0.16	6.50 ± 0.25	6.50 ± 0.25	6.50 ± 0.25
50	5.25± 0.07	5.42 ± 0.05	3.50 ± 0.07	6.25 ± 0.33	6.42 ± 0.06	5.22 ± 0.66
100	3.50 ± 0.05	3.78 ± 0.05	1.25 ± 0.05	3.87 ± 0.05	4.82 ± 0.03	2.57± 0.08
150	2.58 ± 0.20	2.89 ± 0.12	0.80 ± 0.02	2.66 ± 0.20	3.54 ± 0.06	0.0 ± 0.00
Dose	***A. terreus***			***F. oxysporum***		
0	5.75 ± 0.25	5.75 ± 0.25	5.75 ± 0.25	7.50 ± 0.06	7.50 ± 0.06	7.50 ± 0.18
50	5.10 ± 0.33	5.50 ± 0.22	3.50 ± 0.33	6.35 ± 0.15	7.10 ± 0.33	3.38 ± 0.02
100	4.66± 0.22	5.00 ± 0.25	2.25 ± 0.12	3.33 ± 0.05	3.77 ± 0.04	1.50 ± 0.12
150	3.20 ± 0.03	3.50 ± 0.09	0.0 ± 0.00	2.50 ± 0.03	2.80 ± 0.05	0.0 ± 0.00

Docking Study

Docking Scores and Energies are documented in Table 2, where chitinase (1CTN) showed a higher binding affinity (S = −82.67 kcal/mol) than β-1,3-glucanase (4M80, S = −78.08 kcal/mol). Both ligands demonstrated acceptable RMSD values (<2 Å), indicating accurate binding poses. Energy components revealed higher E_conf and E_refine values for chitinase. On the other hand, the interaction profiles of chitinase/ β-1,3-glucanase – *F. oxysporum* (1CTN–7T69) are shown in Tables 3 and 4. In Table 3, chitinase- *F. oxysporum* complex exhibited seven hydrogen bonds, notably involving critical residues ASP196, ILE268, and VAL266, which likely stabilize the enzyme's binding to the target. Furthermore, π-π stacking interactions were observed between aromatic TRP210 residues, facilitating additional stabilization. Strong H-acceptor interactions also occurred with LYS265 and ASP196.

In Table 4, the β-1,3-glucanase- *F. oxysporum* (4M80–7T69) complex showed a more extensive hydrogen bonding network, involving residues GLU121, THR169, and ARG142. Ionic interactions with GLU121 were significant, exhibiting energies of up to -9.8 kcal/mol, which significantly enhance electrostatic stabilization. Overall, the strong binding energies highlight the potential of these enzymes as effective antifungal agents through disruption of *F. oxysporum* cell wall integrity. The 2D and 3D interaction diagrams highlight the binding orientation and specific interactions within the active site of 7T69 are illustrated in Figs. 2 and 3. The molecular docking results demonstrate that both chitinase and β-1,3-glucanase interact strongly with *Fusarium* protein targets, consistent with their known antifungal mechanisms.

Chitinase

A multi-faceted approach to fungal disruption achieved a higher docking score of chitinase (-82.7 kcal/mol) compared to β-1,3-glucanase, which suggests a higher overall binding affinity. This superior binding is attributable to the diverse non-covalent interactions observed. The formation of seven hydrogen bonds, particularly with residues like ASP196, ILE268, and VAL266, indicates specific and stable recognition of the fungal target. ASP196, being a strong hydrogen-acceptor, likely plays a critical role in anchoring

the enzyme to the target protein. Furthermore, the presence of π-π stacking with TRP210 is a significant stabilizing factor. The multiple hydrogen bonds of chitinase with ASP196, ILE268, and VAL266, along with π-π stacking with TRP210, reveal critical "hotspot" residues that could serve as anchoring points for engineering chitinase variants with enhanced affinity and stability.

Aromatic stacking interactions, often found in protein-ligand complexes, contribute significantly to binding affinity by promoting favorable electronic interactions and increasing the overall surface area of contact. This multi-modal binding approach, combining hydrogen bonding with aromatic interactions, provides chitinase with a robust mechanism for recognizing and potentially degrading fungal cell wall components, particularly chitin, which is a primary structural polysaccharide in fungal cell walls. Research on chitinase genes in *F. oxysporum* has demonstrated that such interactions can reduce fungal pathogenicity by hydrolyzing chitin structures, thereby weakening the rigid fungal cell wall (Sharma *et al*. 2023).

β-1,3-Glucanase and electrostatic contributions to stability

While β-1,3-glucanase exhibited a slightly lower docking score (-78.1 kcal/mol), its interaction profile highlights equally important, albeit different, stabilizing forces. The extensive hydrogen bonding with residues such as GLU121, THR169, and ARG142 indicates a broad interaction surface. Crucially, the observation of two strong ionic interactions with GLU121 points to a significant electrostatic contribution to the complex's stability. Ionic interactions, typically higher in energy than individual hydrogen bonds, provide a powerful means of orienting and stabilizing the enzyme-target complex. The ionic interactions of β-1,3-glucanase with GLU121 demonstrate the potential of targeting charged regions within fungal glucan structures, suggesting avenues for designing enzyme variants or small-molecule inhibitors with optimized electrostatic complementarity. The individual binding energies reaching up to -9.8 kcal/mol further emphasize the strength of these specific interactions. These findings align with the known role of β-1,3-glucanases in decomposing β-1,3-glucans, another major component of fungal cell walls. The reliance on strong electrostatic interactions suggests a precise mechanism for recognizing and binding to charged or polar regions within the β-glucan structure, facilitating its hydrolytic activity. Studies have shown that the co-overexpression of chitinase and β-1,3-glucanase genes significantly enhances resistance to *Fusarium* diseases, as these enzymes synergistically disrupt cell wall integrity, highlighting their complementary nature (Carrasco-Carballo *et al*. 2021; Numan *et al*. 2021).

Role of structural flexibility

The lower RMSD for B-1,3-glucanase (1.22 Å *vs*. 1.34 Å) indicates greater conformational stability during binding. This aligns with its multiple long-range interactions (*e.g.*, SG 40–O ALA 140: 3.84 Å; CA 90–O GLU 167: 3.24 Å), which anchor the ligand across the binding pocket. Chitinase's reliance on short-range hydrogen-bonds (≤3.0 Å), which are more susceptible to solvation effects.

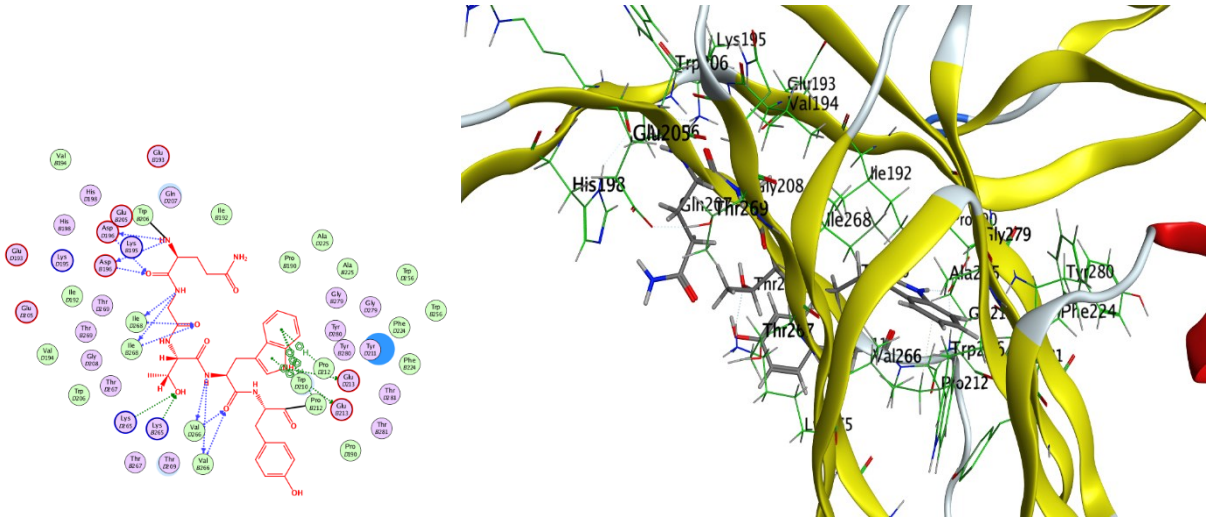

Fig. 2. 2D and 3D diagrams show the interaction between Chitinase(1CTN) and active sites of *F. oxysporum* 7T69 protein

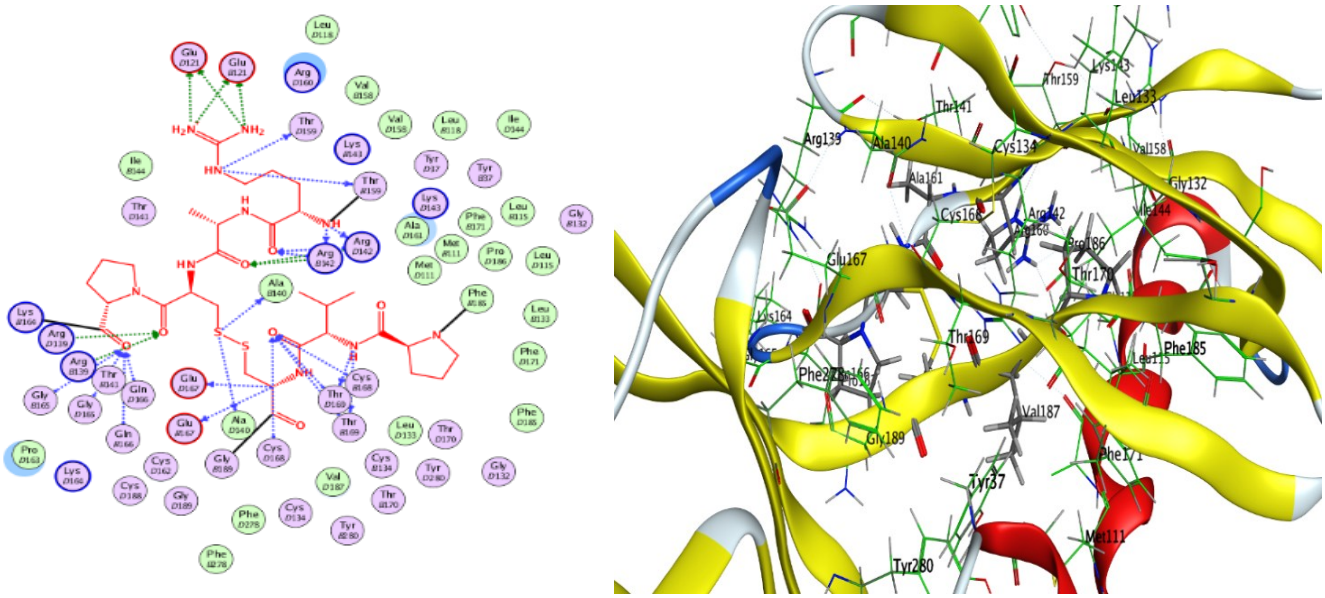

Fig. 3. 2D and 3D diagrams show the interaction between B-1,3-Glucanase (4M80) and active sites of *F. oxysporum* 7T69 protein

Table 2. Docking Scores and Energies of Chitinase (PDB ID: 1CTN) and β-1,3-Glucanase (PDB ID: 4M80) against *F. oxysporum* (PDB ID: 7T69)

Mol	S	rmsd_refine	E_conf	E_place	E_refine
Chitinase(1CTN)	-82.6671	1.3392324	-17887.2	-19.7914	-82.6671
B-1,3-Glucanase (4M80)	-78.0838	1.2160619	-15210.5	-19.8092	-78.0838

Table 3. Interaction of Chitinase (PDB ID: 1CTN) with Structure of *F. oxysporum* (PDB ID: 7T69)

Mol	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
Chitinase (1CTN)	N 1	O ASP 196 (A)	H-donor	2.85	-3.6
	N 18	O ILE 268 (A)	H-donor	2.77	-5.9
	N 39	O VAL 266 (A)	H-donor	2.87	-4.0
	NE1 47	OE2 GLU 213 (A)	H-donor	2.80	-5.7
	O 4	N ASP 196 (A)	H-acceptor	2.90	-4.5
	O 21	N ILE 268 (A)	H-acceptor	2.89	-4.4
	OG1 30	NZ LYS 265 (A)	H-acceptor	2.90	-6.1
	O 42	N VAL 266 (A)	H-acceptor	3.02	-4.3
	6-ring	CG PRO 212 (A)	pi-H	3.86	-0.5
	5-ring	6-ring TRP 210 (A)	pi-pi	2.19	-0.0
	6-ring	5-ring TRP 210 (A)	pi-pi	2.19	-0.0

Table 4. Interaction of B-1,3-Glucanase (PDB ID: 4M80) with Structure of *F. oxysporum* (PDB ID: 7T69)

Mol	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
β-1,3-Glucanase (4M80)	N 1	O ARG 142 (A)	H-donor	2.92	-4.0
	NE 8	O THR 159 (A)	H-donor	2.94	-5.7
	NH1 10	OE1 GLU 121 (A)	H-donor	2.72	-9.8
	NH2 13	OE2 GLU 121 (A)	H-donor	2.69	-9.6
	SG 40	O ALA 140 (A)	H-donor	3.84	-0.9
	N 73	O THR 169 (A)	H-donor	2.80	-4.8
	CA 90	O GLU 167 (A)	H-donor	3.24	-0.6
	O 4	N ARG 142 (A)	H-acceptor	2.94	-2.6
	O 28	NH2 ARG 142 (A)	H-acceptor	2.94	-5.8
	O 38	NH2 ARG 139 (A)	H-acceptor	2.78	-5.0
	O 48	N GLY 165 (A)	H-acceptor	2.75	-2.9
	O 48	N GLN 166 (A)	H-acceptor	2.92	-2.4
	O 76	CA CYS 168 (A)	H-acceptor	3.34	-0.5
	O 76	N THR 169 (A)	H-acceptor	2.85	-4.4
	NH1 10	OE1 GLU 121 (A)	Ionic	2.72	-6.7
	NH2 13	OE2 GLU 121 (A)	Ionic	2.69	-6.9

CONCLUSIONS

1. Chitinase and β-1,3-glucanase displayed strong, dose-dependent antifungal activity in vitro, with notable inhibition of *Penicillium expansum* and *Alternaria alternata*, supporting their potential as eco-friendly alternatives to chemical fungicides in postharvest tomato management.
2. Molecular docking revealed that chitinase and β-1,3-glucanase form stable and specific complexes with *Fusarium* proteins through multiple hydrogen bonds, ionic interactions, and π–π stacking, thereby explaining their high binding affinities and functional relevance.

3. Through integrating *in vitro* and *in silico* evidence, this study highlights the dual potential of chitinase and β-1,3-glucanase as promising antifungal agents and provides molecular insights that may guide the design of improved enzyme-based strategies for sustainable crop protection.

ACKNOWLEDGMENTS

The authors wish to acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R217), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Funding

This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R217), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia

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Article submitted: July 10, 2025; Peer review completed: August 23, 2025; Revised version received and accepted: August 25, 2025; Published: October 6, 2025.
DOI: 10.15376/biores.20.4.10069-10081