Degradative Potential of Laccase and Manganese Peroxidase to Mycotoxins on Infected Maize Grains by Fungi with Docking Interaction Studies

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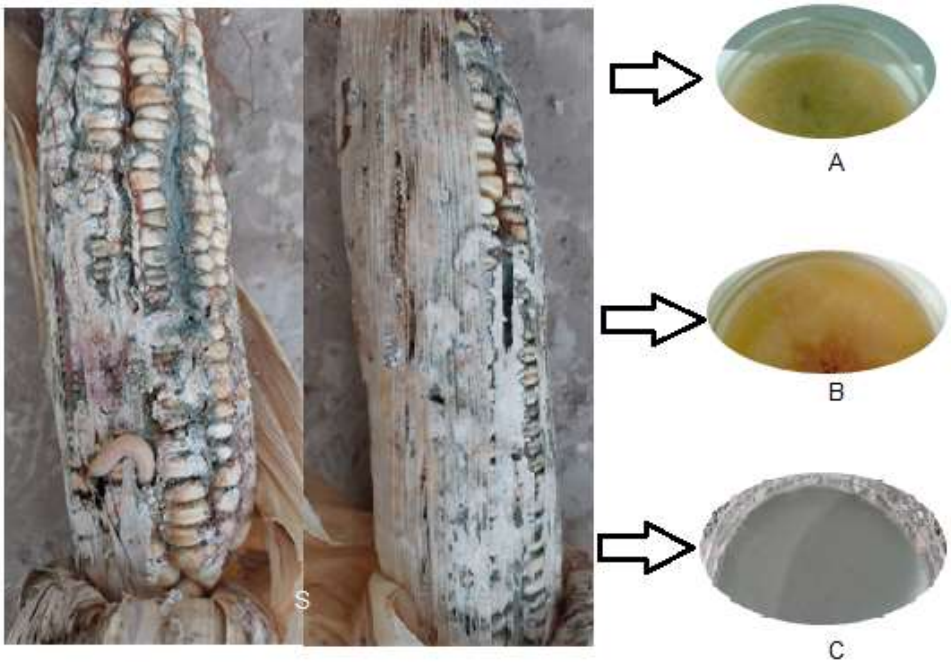
GRAPHICAL ABSTRACT



Degradative Potential of Laccase and Manganese Peroxidase to Mycotoxins on Infected Maize Grains by Fungi with Docking Interaction Studies

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Fungal infection in agricultural grains is a global problem, particularly if it is accompanied by mycotoxin production. In this study, the degradation of mycotoxins by laccase and manganese peroxidase was investigated. *Aspergillus flavus*, *Aspergillus fumigatus,* and *Fusarium graminearum* were recorded in infected maize grains. Aflatoxin B1 (AF B1) was detected (from 3.38 to 2.60 ppm) on the infected samples by fungi compared to other detected aflatoxins. Trichothecene (T-2) toxin and deoxynivalenol (DON) were recorded with concentrations ranging from 0.464 to 0.184 ppm and 0.370 to 0.214 ppm, respectively. The addition of laccase and manganese peroxidase to the inoculated medium with *A. flavus* and *F. graminearum* individually degraded the produced AF B1, B2, G1, G2, T-2 toxin, and DON from 5.0, 1.33, 0.76, 0.61, 0.63, and 0.38 ppm to 2.77, 0.66, 0.37, 0.15, 0.45, and 0.38 ppm using laccase, to 3.08, 1.25, 0.61, 0.39, 0.55, and 0.36 ppm using manganese peroxidase. The computational technique (docking) demonstrated the laccase and manganese peroxidase activities on aflatoxin and DON degradation. Consequently, the results suggested that laccase (PDB ID: 1HFU) and manganese peroxidase (PDB ID: 1MNP) promise innovative activity toward aflatoxin degradation, while 1HFU has more effect than 1MNP on DON degradation.

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Keywords: Enzymes; Aflatoxins; Trichothecene; Deoxynivalenol; Degradation; Docking interaction

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INTRODUCTION

In nature, fungi are found everywhere, in soil, irrigation water, and air. Their infections typically start in the field and spread to storage facilities. Grains and their products are commonly under unideal storage conditions. They may be contaminated with fungi besides its mycotoxins, which are secondary products of several fungi. Mainly the species belong to *Aspergillus*, *Alternaria, Penicillium*, and *Fusarium* (Abdelghany 2006; El-Taher *et al.* 2012; Abdelghany 2014). Depending on the kind and quantity of mycotoxin contamination, it can induce diverse unfavorable health consequences in people and animals. Today, more than 300 kinds of mycotoxins are identified, but some of them

represent a serious risk in the food sector; examples include aflatoxins (AFs) and ochratoxin A, fumonisins, HT-2, zearalenone, nivalenol, and T-2 toxins (Abdelghany *et al*. 2017; Sánchez-Zúñiga *et al.* 2024). Globally, maize is a critical source of food for humans and feed for animals. Its quality may be influenced by toxin-synthesizing fungi, particularly *Aspergillus flavus, Fusarium verticillioides* and *Fusarium graminearum* (Nyandi *et al*. 2024). Several kinds of Aflatoxins were recognized, including B1, B2, G1, and G2, that were created mainly *via Aspergillus flavus* (Abdelghany *et al*. 2020).

Chemical, physical, and biological methods have been used to eliminate and degrade mycotoxins. Biological enzymatic approaches have been applied to destroy mycotoxins while releasing degradable products with little toxicity (Abdelghany *et al.* 2016; Al-Rajhi *et al.* 2022a). The approaches *via* biological enzymes are commonly more specific, ecofriendly, and efficient in mycotoxins degradation than other chemical and physical methods (Lyagin and Efremenko 2019). The utilization of full microorganism for mycotoxins degradation introduces some disadvantages such as production of other toxins and requiring specific growth conditions, while the utilizing of specific enzymes overcomes on these issues of microorganism's application (Adegoke *et al*. 2022; Sun *et al*. 2023; Abada *et al*. 2024). The detoxification by enzymes of natural origin or synthesized by microorganisms represent promising methods to decrease or eliminate mycotoxin in food (Wang *et al.* 2019; Nahle *et al*. 2022; Orozco-Cortés *et al.* 2023). The enzymes responsible for mycotoxin degradation have potential, but their degradation mechanisms and pathways need clarification.

Several investigations used the molecular docking skill to discover new active ingredients, develop drugs, and study the action mechanisms of active compounds with its target (Qanash *et al.* 2022; Al-Rajhi *et al.* 2023; Al-Rajhi and Abdelghany 2023a,b; Alghonaim *et al.* 2023; Alsalamah *et al.* 2023). Moreover, this skill is broadly employed as the consequence of toxic pathways of mycotoxin (Chen *et al.* 2019). The interaction among enzymes and mycotoxins *via* molecular docking was achieved to determine the activity of enzymes to degrade the mycotoxins. For instance, laccase (Liu *et al*. 2020), protease, and lipase (Al-Rajhi *et al.* 2024a) were docked with aflatoxins. The aim of the present work was to evaluate the fungal infection and mycotoxins in corn grains by studying the ability of laccase and manganese peroxidase to degrade mycotoxins.

EXPERIMENTAL

Source of Used Enzymes

Laccase and manganese peroxidase of *Aspergillus sp.* and white-rot fungus (*Phanerochaete chrysosporium*) origin respectively were taken from Sigma-Aldrich Saint Louis, USA.

Infected Samples Collection, Isolation and Identification of Fungi

Five fruits of corn cobs with infected grains were collected from farm fields at governorate of Jazan, Saudi Arabia. The fruits were stored in sterile plastic bags at 3 °C for further study. The infected grains of each sample were subjected to fungi isolation and mycotoxins detection. Potato Dextrose Agar (PDA) was utilized for growth of isolated fungi, where the infected grains were located on the PDA surface and incubated for 5 days at 25 °C. At the end of required period (5 days) to fungal development, the visualized fungi were transferred to PDA for purification with the appropriate conditions as mentioned in

the isolation process (Hamed *et al*. 2016). The purified fungi were identified based on published identification keys (Raper and Fennell 1973; Domsch *et al*. 1980; Nelson *et al*. 1983).

Mycotoxins Detection in Maize Grains

The mycotoxins were detected by microtitre plate enzyme-linked immunosorbent assay (ELISA) (Leszczynska *et al*. 2001). The infected grains of each sample (50 g) were ground and extracted with 100 mL of 70% methyl alcohol, and then agitated for 30 min using magnetic stirrer. The solution containing mycotoxins was passed through Whitman No.1 filter paper. The filtrate (10 mL) was diluted with 30 mL of H_2O and 0.5 mL of polysorbate 20 (Tween 20), then agitated for 5 min. The standard toxins including 50 µL of each AF B1, AF B2, AF G1, and AF G2 besides T-2 toxin and DON were diluted to different final concentrations (0.25, 0.5, 1, 2, 4, and 8 ppm). Then 50 µL aliquots of the prepared filtrate were injected in wells of the micro-titer plate. For 30 min at 25 °C, the micro-titer plates were incubated. Then from each well, the broth was removed, followed by washing each well by PBS-polysorbate-Buffer (250 µL with pH 7.2). Later, 50 µL of enzyme substrate and 50 µL of tetramethyl-benzidine as chromogen were transferred to each well. The reaction mixture was incubated without exposure to light at for 30 min at 25 °C. The reaction was stopped *via* addition of sulfuric acid (1M) (100 µL), followed by measuring the absorbance (450 nm) *via* ELISA reader.

Effect of Laccase and Manganese Peroxidase on Mycotoxins Degradation

Two fungi (*A. flavus* and *Fusarium graminearum*) were selected from the isolated fungi based on the available mycotoxins (AF B1, AF B2, AF G1, and AF G2 for *A. flavus*, T-2 toxin and DON for *F. graminearum*). *A. flavus* and *F. graminearum* were inoculated in autoclaved medium containing 2% powder of grinded corn grains (healthy without fungal infections) and amended with 2% sucrose, and incubated for 10 days at 25 °C. Then under aseptic conditions, the enzymes were added at two doses (0.25 and 0.5 U/mL) separately to each inoculated medium and completed to 15 days of incubation. After that, the fungal mycelia were removed and 20 mL of broth medium were mixed with 40 mL of methyl alcohol (70%). Then the procedures were completed to detect the mycotoxins as mentioned in maize grains procedures (Wang *et al*. 2011).

Docking Studies of Laccase and Manganese Peroxidase with Mycotoxins

Molecular docking, an internationally recognized and versatile in silico approach, makes it possible to select physiologically favorable models from a database of molecules earlier they are generated. It predicts how the optimal conformers for various ligands interact with the receptor protein, which aids in the specification of synthetic targets (Al-Rajhi *et al*. 2024a). Employing the Molecular Operational Environment (MOE) tool, the molecular docking investigations of AF B1 and DON was done to examine the binding mechanisms of the ligands and their proposed enzymes laccase (PDB ID: 1HFU) and manganese peroxidase (PDB ID:1MNP). ChemDraw Ultra 15.0 software was used to create the structures of each chemical, which were then saved as MDL files (".sdf") for MOE to view. The studied enzymes were subjected to energy minimization. Next the enzyme's configuration and formal charges on atoms were checked employing 2D representation. By default, partial charges were created. The chemical to be considered was entered into the database and transferred as an MDB file for docking calculations with target proteins. Crystal structures of enzymes were obtained from the Bank of Protein Data

(http://www.rcsb.org/pdb). Subsequently the water molecules around the protein were removed, and atoms of hydrogen were introduced. The MMFF94x force field was utilised to give the parameters and charges. Subsequently generating alpha-site spheres with MOE's site finder module, the compounds were docked in the active site utilizing the DOCK module. The dock scoring for the MOE program was recognized utilizing the London dG scoring approach, which included placement: triangle matcher, retain 10, and refinement: force field. The leading conformations of the docked ligands were determined using the values of RMSD, binding of both energies, and modes with the specified residues.

RESULTS AND DISCUSSION

Maize is a vital crop in all countries – developed or non-developed – and contains nutrients that are necessary and support the fungal growth. The growth of several fungi is accompanied with production of various mycotoxins depending on species, environmental conditions, and nutritional conditions. Several problems arising from the contamination by fungi and their toxins such as low quality and quantity of crop yields, pose risks to crop consumers of human and animal. In this study, three fungi were isolated from the infected samples by fungi. As shown in Fig. 1, these fungi were *Aspergillus flavus*, *Aspergillus fumigatus*, and *Fusarium graminearum*. These results are consistent with other reports (Olugbenga and Chongs 2024; Price *et al*. 2024) which informed the occurrence of *Aspergillus* and *Fusarium* in maize grains. On maize grains in the present investigation, the co-existence of three fungal species was documented. The quantitative and qualitative production of mycotoxins differed according to the co-existing species of fungi (Giorni *et al*. 2019). Avoiding toxin production depends on the prevention of fungi growth. The search for safe compounds is operating particularly after the crops harvest. Based on the available mycotoxins in the authors' labs, the infected corn grains were subjected to examine the presence of mycotoxins namely AF B1, AF B2, AF G1, AF G2, T-2 Toxin, and deoxynivalenol (DON). Previously, on maize grains according to Giorni *et al*. (2019), the identified AF B1 and DON, respectively were associated to *A. flavus* and *F. graminearum.*

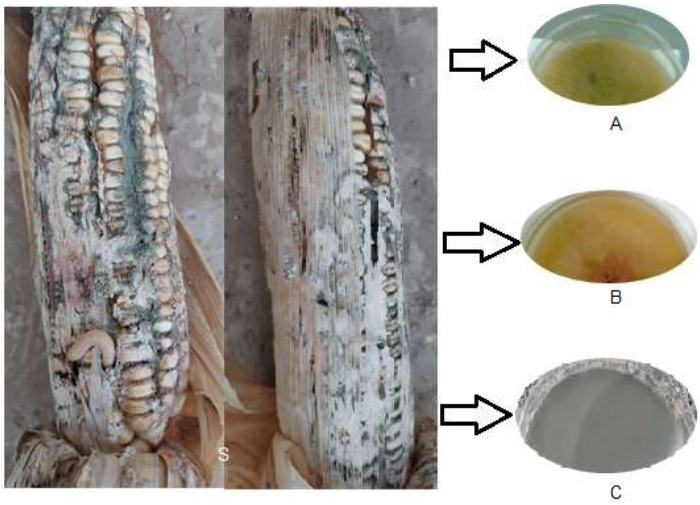

Fig. 1. Infected maize grain samples (S) and isolated fungi *Aspergillus flavus* (A), *Aspergillus fumigatus* (B), and *Fusarium graminearum* (C)

AF B1 represents the main detected aflatoxins on the examined five collected maize samples. They ranged from 3.38 to 2.60 ppm followed by AF B2 (0.90 to 0.69 ppm) and AF G1 (0.51 to 0.39 ppm), while AF G2 was detected on three samples only with lower concentrations (0.41 to 0.32 ppm). The presence of these mycotoxins was associated with *A. flavus*, as mentioned in several studies (Abdelghany 2014; Abdelghany *et al.* 2020). Other toxins were detected in infected five maize grains samples including T-2 toxin and DON with different concentrations as shown in Table 1. T-2 toxin and DON were produced by *Fusarium* spp. For instance DON was produced by *F. graminearum* (Sherif *et al.* 2023).

Table 1. Detected Mycotoxins (PPM) in Infected Maize Grain Samples (1-5)

Sample type	AF B1	AF B2	AF G1	AF G2	T-2 toxin (ppm)	DON
Control (uninfected)	0.00	0.00	0.00	0.00	0.00	0.00
Infected 1	2.60	0.69	0.39	0.32	0.184	0.290
Infected 2	3.38	0.90	0.51	0.41	0.300	0.230
Infected 3	2.92	0.78	0.44	0.36	0.310	0.214
Infected 4	2.99	0.79	0.45	0.00	0.346	0.280
Infected 5	3.06	0.73	0.41	0.00	0.464	0.370

Laccase and manganese peroxidase were applied at two different concentrations to degrade the present detected mycotoxins. From the obtained results, laccase was more effective than manganese peroxidase in the degradation of detected mycotoxins. For instance the detected concentrations of AF B1, AF B2, AF G1, AF G2, T-2 toxin, and DON were 2.77, 0.66, 0.37, 0.15, 0.45, and 0.38 ppm using laccase; while it were 3.08, 1.25, 0.61, 0.39, 0.55, and 0.36 ppm using manganese peroxidase compared with its concentrations 5.0, 1.33, 0.76, 0.61, 0.63, and 0.38 ppm at control, respectively (Fig. 2). At the same time, manganese peroxidase less effective on the degradation of T-2 toxin, and DON compared to aflatoxins. The different levels of mycotoxins degradation may be due to its different chemical structures, as shown in Fig. 3. Slight differences in the degradation of mycotoxins exposed to the two different concentrations of enzymes (0.25 and 0.5 U/mL). Detoxification of AF B1 was recorded previously *via* manganese peroxidase of *Phanerochaete sordida* origin (Wang *et al.* 2011) and laccase of *Trametes versicolor* origin (Zeinvand-Lorestani *et al.* 2015). Wang *et al.* (2011) discussed the mechanisms of AF B1degradation by manganese peroxidase, which include the oxidation of AF B1 to epoxy-derivative and then to 8,9-dihydrodiol AF B1 through de-epoxidation in the presence of H_2O_2. Another enzyme, namely oxidoreductase of *Bacillus subtilis* origin according to Afsharmanesh *et al.* (2018), detoxified AF B *via* decomposition of its lactone ring. Also, DON was degraded *via Aspergillus niger* lipase (Yang *et al*. 2017). In numerous studies mycotoxins are degraded into other compounds *via* action of enzymes. For instance AF B1 was degraded into AF Q1 *via* laccase, the generated metabolite was nontoxic where it failed to cause apoptosis and cell death of epithelial cells (Hao *et al.* 2023). In another study AF B1 was degraded to AF B1-8,9-dihydrodiol (much fewer toxic than AFB1) *via* microbial enzymes (Sun *et al.* 2023). Previous study mentioned that microbial enzymes can detoxify the aflatoxin rather than its absorption or binding into the microbial cell wall (Fan *et al.* 2013). Also, DON was transformed into low toxic compounds *via* several enzymes such as dehydrogenase and peroxidase which break the certain constructions of this toxin DON (Qin *et al.* 2021).

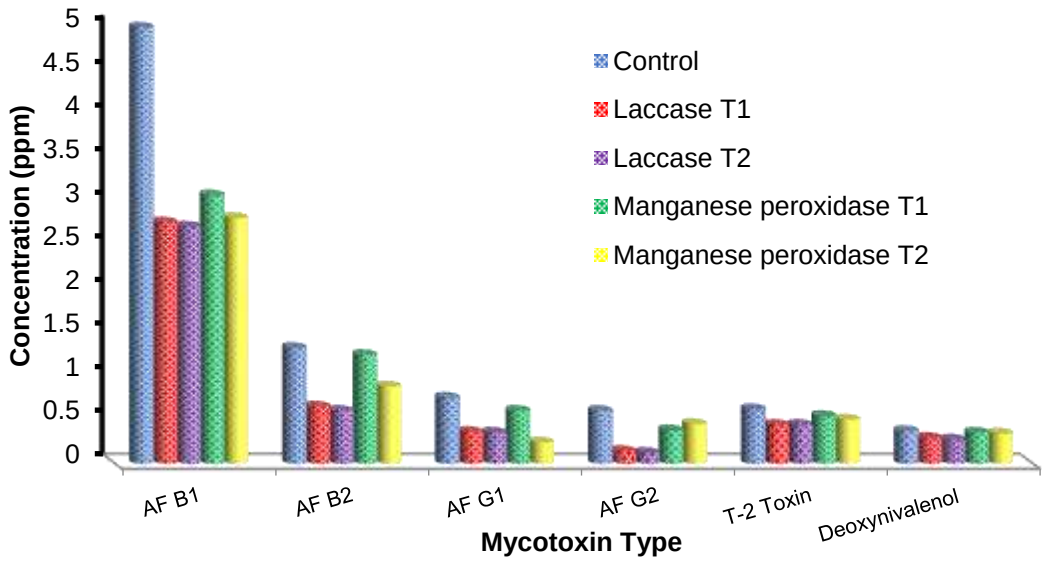

Fig. 2. Degradative effect of laccase and manganese peroxidase at two different doses (T1, 0.25 U/mL and T2, 0.5 U/mL) on mycotoxins

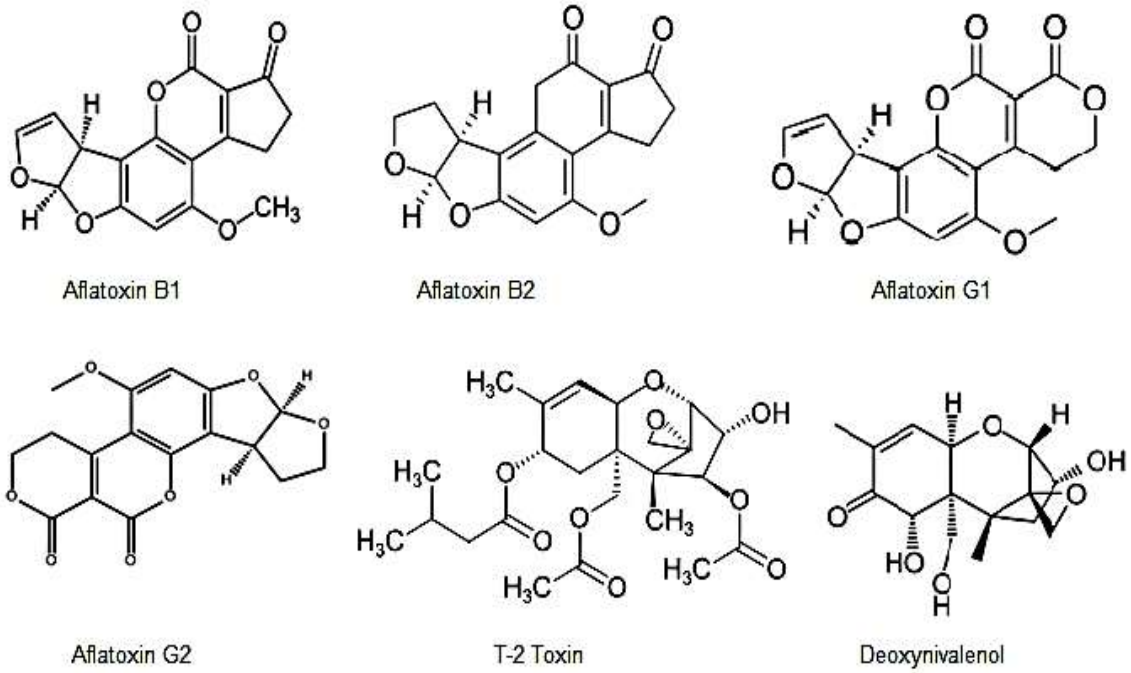

Fig. 3. Chemical configuration on examined mycotoxins

As shown in Figs. 4 through 7 and Tables 2 through 5, aflatoxin and DON were exposed to molecular docking tests with laccase (PDB ID: 1HFU) and manganese peroxidase (PDB ID:1MNP) as further assistance for biological degradation examinations. The findings, reported in Tables 1 and 2, demonstrated the docking and laboratory findings had a very great level of concordance. Both ligands exhibit high binding affinities of -5.52 and -5.18 kcal/mol respectively, using laccase (PDB ID: 1HFU). AF B1 interacts toward the protein receptors pocket 1HFU through LYS 40 via O 19 atom, whereas DON connects with 1HFU by ASP 128, GLY 101, and LYS 40 using O 31 and O 40 atoms. On the other

hand, manganese peroxidase (PDB ID:1MNP) showed a clear biological activity against AF B1 with a strong negative docking score of -6.66 kcal/mol, unlike DON, which showed no effect by 1MNP receptors, giving a docking score of -0.0304 kcal/mol.

The H-acceptor relationship among O 11 of AF B1 and the SER 172 residue of the 1MNP enzyme was discovered, according to the two-dimensional maps of this ligand. The docking interaction investigations were performed to study the interaction between discovered or developed or screened compounds and target protein to document its activities or mechanisms (Qanash *et al*. 2023a&b; Yahya *et al*. 2022; Al-Rajhi *et al*. 2022b and 2024b). Numerous proteins of enzymes were docked with the patulin to detect its ability to degrade this mycotoxin, YKL069W from the investigated proteins give a highest binding affinity of -7.5 kcal/mol according to study of Yang *et al.* (2024). Compared with our findings, the docking interaction of laccase from *Bacillus amyloliquefaciens* with AF B1, AF B2, AF G1, and AF G2 was resulted binding affinities of −5.60, −6.82, −6.58, and −5.31 kcal/mol, respectively (Xiong *et al.* 2022).

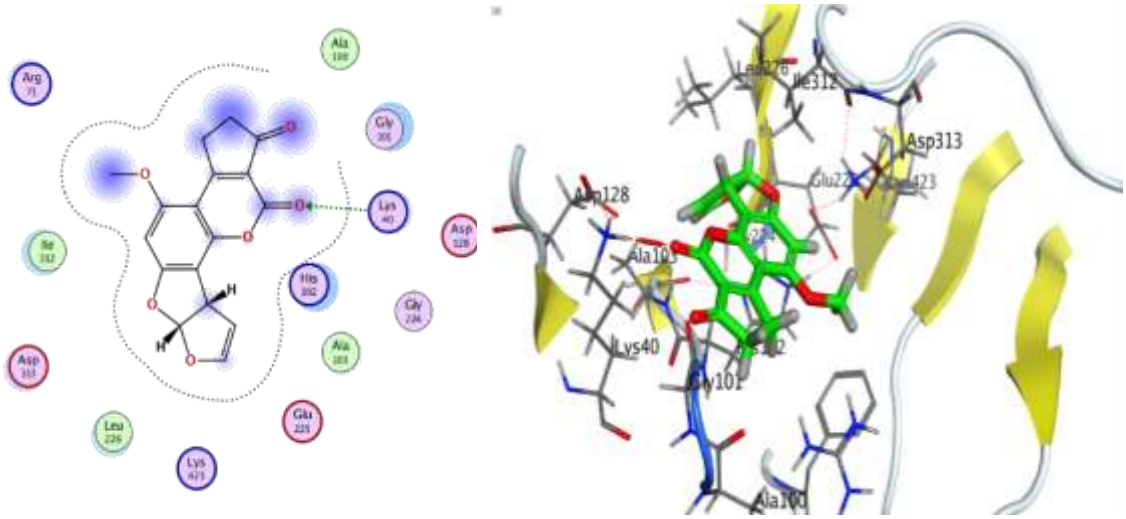

Fig. 4. Graphs of 2D and 3D show the interaction between AF B1 and active sites of laccase 1HFU protein

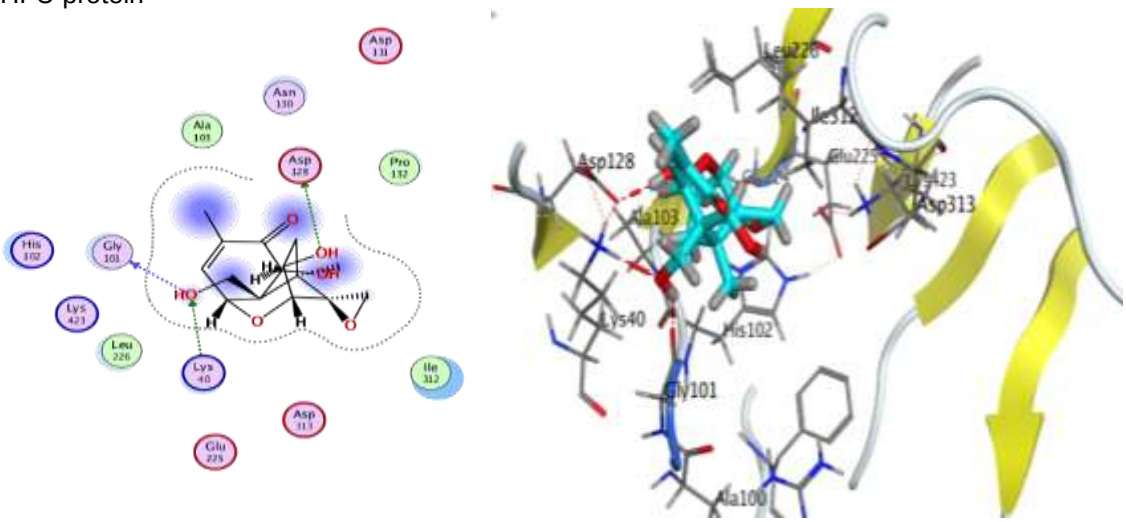

Fig. 5. Graphs of 2D and 3D show the interaction between DON and active sites of laccase 1HFU protein

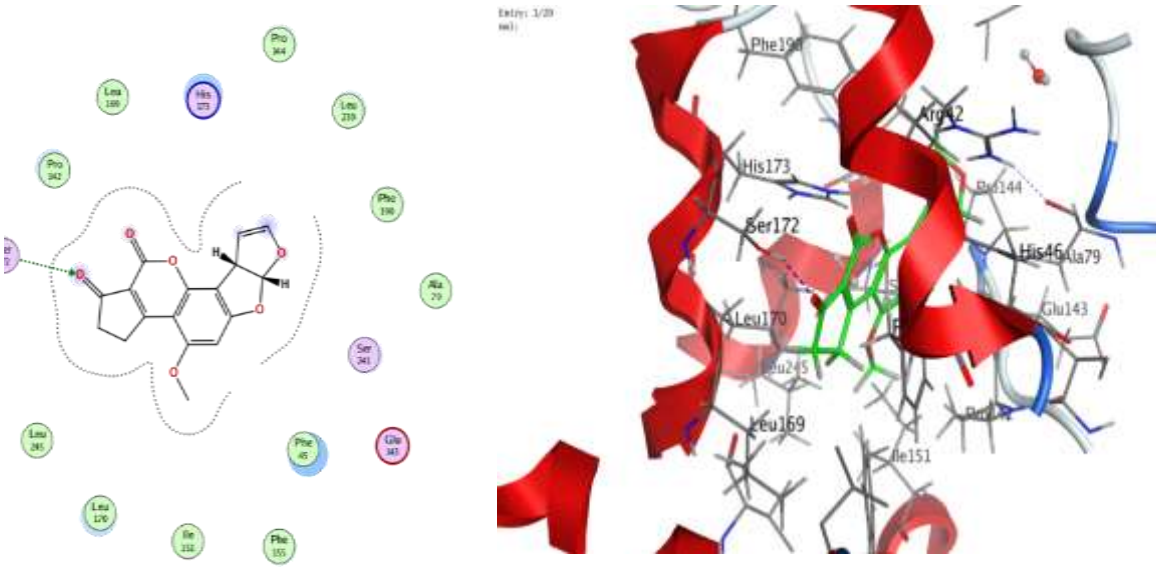

Fig. 6. Graphs of 2D and 3D show the interaction between AF B1 and active sites of manganese peroxidase 1MNP protein

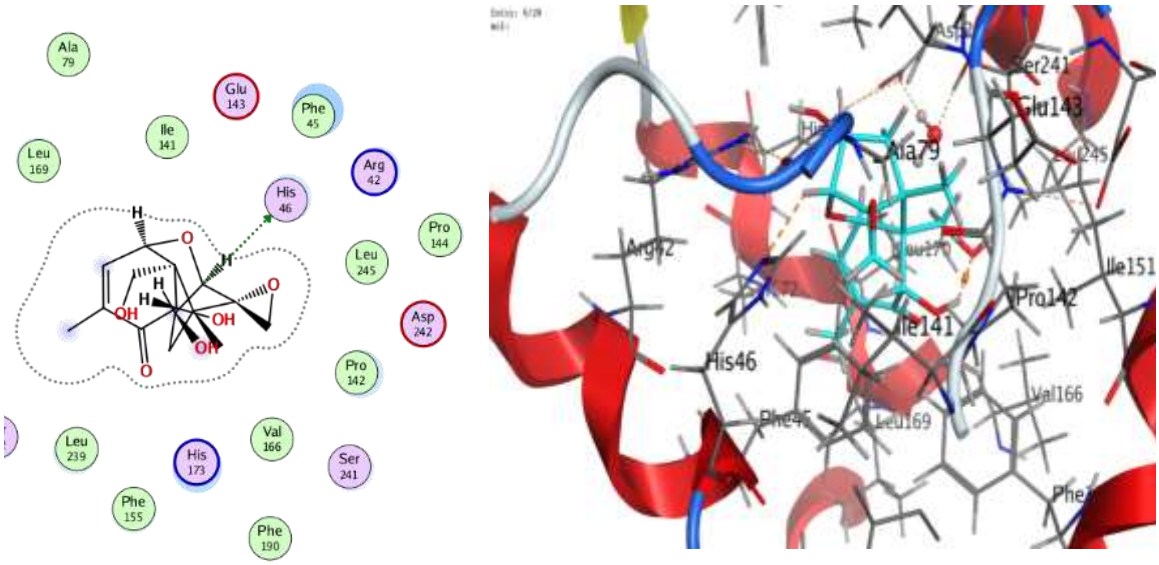

Fig. 7. Graphs of 2D and 3D show the interaction between DON and active sites of manganese peroxidase 1MNP protein

Table 2. Docking Scores and Energies of Aflatoxin and DON with Laccase (PDB ID: 1HFU) Receptors

Mol	Protein	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2
AF B1	1HFU	-5.52416	1.8202698	30.7788	-56.6575	-10.0682	-31.3164	-5.52416
AF B1	1HFU	-5.51395	1.3185956	30.93103	-55.2812	-9.54235	-29.3633	-5.51395
AF B1	1HFU	-5.51273	1.1387398	38.61315	-69.0789	-11.0506	-28.3338	-5.51273
AF B1	1HFU	-5.38386	2.0482345	30.66612	-60.7002	-9.44125	-28.2547	-5.38386
AF B1	1HFU	-5.14735	3.0377085	32.22635	-58.9441	-10.3006	-28.4415	-5.14735
DON	1HFU	-5.17935	2.3638072	238.1926	-56.8203	-9.68297	-26.1865	-5.17935
DON	1HFU	-4.94039	1.1026493	239.9842	-69.139	-9.79632	-15.8295	-4.94039
DON	1HFU	-4.8217	2.1598337	223.5475	-102.576	-9.98635	-22.987	-4.8217
DON	1HFU	-4.72563	2.676589	226.552	-62.219	-10.6844	-21.8021	-4.72563
DON	1HFU	-4.70355	2.0609455	228.4663	-71.3962	-9.40482	-14.5203	-4.70355

Table 3. Docking Scores and Energies of Aflatoxin and DON with Manganese Peroxidase (PDB ID:1MNP) Receptors

Mol	Protein	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	E_score2
AF B1	1MNP	-6.6599	1.5384908	40.9162	-87.4948	-12.8948	-24.7885	-6.6599
AF B1	1MNP	-6.55354	1.2442065	38.68508	-77.5233	-12.838	-23.8269	-6.55354
AF B1	1MNP	-6.55182	0.63226599	35.90326	-77.2592	-12.712	-21.1416	-6.55182
AF B1	1MNP	-6.45181	1.584542	37.24467	-66.3989	-15.1262	-22.6591	-6.45181
AF B1	1MNP	-6.2919	0.79337341	37.23357	-69.6233	-13.3897	-19.1703	-6.2919
DON	1MNP	-0.03039	0.99937254	297.1387	-67.2708	-9.16881	61.03642	-0.03039
DON	1MNP	0.232494	1.2830188	370.4063	-36.9399	-9.20924	60.67991	0.232494
DON	1MNP	0.352779	0.85445637	319.0915	-44.2688	-10.3762	57.8454	0.352779
DON	1MNP	0.636702	1.4174296	317.5525	-48.9408	-8.96909	66.30833	0.636702
DON	1MNP	0.884563	1.6266948	290.7398	-71.2393	-9.77732	68.45197	0.884563

Table 4. Interaction of Aflatoxin and DON with Laccase (PDB ID: 1HFU) Receptors

Mol	Protein	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
AF B1	1HFU	O 19	NZ LYS 40 (A)	H-acceptor	3.11	-6.1
DON	1HFU	O 31	OD2 ASP 128 (A)	H-donor	2.72	-1.7
		O 40	O GLY 101 (A)	H-donor	2.86	-1.6
		O 40	NZ LYS 40 (A)	H-acceptor	2.79	-5.7

Table 5. Interaction of AF B1 and DON with Manganese peroxidase (PDB ID:1MNP) Receptors

Mol	Protein	Ligand	Receptor	Interaction	Distance	E (kcal/mol)
AF B1	1HFU	O 11	OG SER 172 (A)	H-acceptor	2.91	-0.5
DON	1HFU	C 23	NE2 HIS 46 (A)	H-donor	3.12	-0.5

CONCLUSIONS

1. Maize grains were infected with serious fungi including *Aspergillus flavus, Aspergillus fumigatus,* and *Fusarium graminearum*.
2. Laccase and manganese peroxidase played a vital role for aflatoxins, T-2 toxin, and DON degradation but with different levels of degradation depending on the kind of toxin and enzyme concentration.
3. *In silico* molecular docking analyses estimated 1HUF and 1MNP to be potential degraders to AF B1 and DON, proposing degradation of these mycotoxins as a possible mechanism for the *in vitro* treatment and focusing on the critical need for other options against mycotoxins, despite 1HFU being exceedingly active compared to 1MNP against DON, increasing the chance of its degradation mechanisms.

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