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Chlorinated pesticide DDT degradation by the laccase-mediator system from newly isolated Ascomycete

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Wood rotting-fungi demonstrated to be good candidates for xenobiotic decontamination through biotechnological processes. Among those xenobiotic pollutants, pesticides are known to be toxic, carcinogenic, and persistent in the environment. In the present study, Wood-decay fungi were isolated from wood debris collected from the soil of Khulais (from Northwest of Jeddah, Saudi Arabia). Subsequently, lignin oxidative enzymatic systems, especially, laccases were determined by the test plate method. The purpose was to select those fungal isolates with the highest laccase potential for chlorinated pesticide DDT enzymatic degradation. The best laccase-producing fungal isolate (G3-MZ841818.1) was identified using both microscopic observation and Internal Transcribed spacer ITS sequence identification. Fungal laccase-mediator system extracted and semi-purified from a newly isolated fungus identified as *Chaetomium sp.* was successfully used for DDT biotransformation. Chromatograms of GC-MS showed a decrease in the intensity of DDT peaks after 2h of *Chaetomium sp.* laccase treatment compared with the control profile proposing the cleavage of the main compounds DDT.

Keywords: Laccase, Degradation, Pesticides

INTRODUCTION

Various pesticides have been extensively applied to enhance agricultural growth by an agrochemical control of unwanted species (Kumar and Singh, 2015). Though, the potential of pesticides in crops protection against target pests, their massive usage has damaged not only targeted pests but also other species which lead to serious dangers to the wildlife, beings, and human health as well (Nicolopoulou-Stamati et al. 2016; Kamal et al. 2021).

Typically, pesticides are synthetic chemical substances that produced to be poison towards reproductive or nervous systems of such undesirable pests and also may interfere with the endocrine hormonal system of organisms (animals, humans, and plants) by disrupting the physiological processes (Chattopadhyay and Chattopadhyay, 2015; Treviño et al. 2015). Consequently, inside humans' or animals' bodies, those chemical products are probably metabolized, stored, excreted, or bioaccumulated causing long-term negative impacts on environmental compartments' safety as well as on human health (Pirsaheb et al. 2015; Prabha et al. 2016).

Currently, many toxicologic tests conducted on animal models, have demonstrated the endocrine effects of organochlorine pesticides (OCPs) due to their bioaccumulation and neurotoxicity in human welfare (Lukaszewicz-Hussain, 2010). Thus, Knowledge about the endocrine effect of OCPs, and their capacity to make disorders on biological processes such as metabolism, synthesis, fertility, and reproduction, at extremely low doses, have been given attention as an environmental issue (Combarnous, 2017; Jabłońska-Trypuć, 2017). Since 1940, Dichlorodiphenyltrichloroethane (DDT) was one of the highly potent OCPs, persistent, semi-volatile molecules, and designed to be bioactive towards many arthropods. Due to the adverse impact on human and environmental safety, DDT usage was banned subsequently since 1970 by Sweden followed by others countries (Turusov et al. 2002). Nevertheless, DDT is still applied and is commonly persistent in food, water, and soil which require their removal from the contaminated sites.

Considering the adverse negative effects of OCPs and their toxicity, several strategies either Physico-chemical or biological were implemented to reduce or remove DDT from different ecological areas (Esplugas et al. 2007).

As an efficient, economic, and safe method for the natural environments, bioremediation by lignin-decomposing fungi has been recognized for the degradation and transformation of OCPs including Chlorinated pesticide DDT (Suhara et al. 2011). This biodegradative process has been assumed to be associated with the ligninolytic enzymatic system including laccases (Lac), lignin peroxidases (LiP), Manganese peroxidase (MnP), and versatile peroxidase (VP) (Zhao et al. 2010; Rodríguez-Delgado et al. 2016). Sometimes, laccases with low-redox potential cannot catalyze target xenobiotic compounds including chlorinated pesticides (Zeng et al. 2017). In this regard, mediators play the role of electron shuttle within laccase/subtract that increase the target pollutants catalyzed by laccase (Bilal et al. 2019). In enzymatic bioconversion, optimization of reactional conditions including pH, temperature, pollutant, enzyme, and redox mediator, is essential for successful remediation.

The objective of the present study was to investigate the enzymatic biodegradation of DDT by the laccase and the laccase-mediator catalyzed oxidative system extracted from a newly isolated fungus *Chaetomium sp.* The metabolites, resulting from the enzymatic oxidation systems of the DDT, were identified by Gas Chromatography-Mass Spectrometry (GC-MS).

MATERIALS AND METHODS

Chemicals

Dichlorodiphenyltrichloroethane (4,4′-DDT) (Cas. 50-29-3) was used in the present study as EDCs. Dichloromethane (Cas. N. 75-09-2) and Dimethyl sulfoxide (DMSO) (Cas. N. 67-68-5) was used to dissolve EDCs.

2,6-dimethoxyphenol (2,6-DMP) and 2-2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were used as laccase substrates. Sigma-Aldrich Chemical Company (St. Louis, MO, USA) was the source of the major chemicals used in the current study.

Selection and identification of laccase-producing fungi

Debris of decaying wood was collected from the soil of Khulais regions, Jeddah, Saudi Arabia during the month of Mars 2020. The isolation of fungal strains was carried on according to Daâssi et al. 2016.

Pure fungal strains were obtained by sub-culturing young mycelium developing on the initial medium (Malt extract agar; 30 g/L; pH 5.5) into fresh MEA plates. The purity of the isolated fungi was proofed by microscopic examination of the culture at 40× magnification using a light microscope.

Laccase-producers fungal isolates were screened using selective solid medium MEA supplemented with 0.15 mM copper sulfate (as Lac inducer) and 5 mM of 2,6-DMP/or 1mM ABTS (Lac substrates) then, incubated at 30 ºC.

Plates are shown yellow halos (with 2,6-DMP) and green halos (with ABTS) proofed that the fungal isolated was Laccase-producing and transferred into flasks of Malt extract broth (MEB) with 0.15 mM CuSO4, for further identification.

All the collected fungal strains were maintained at 4 °C on malt extract agar (MEA) (30 g/L; pH 5.5) Petri plates supplemented with ampicillin and streptomycin (0.01%) to inhibit bacterial proliferation.

Culture conditions

The selected strain was firstly cultured in 250-mL cotton-plugged Erlenmeyer flasks containing 100 mL of Malt Extract Broth (MEB) starting from six mycelial plugs (diameter, 3 mm), taken from a 5-day old fungal culture plate. After 5 days of growth at 30 °C under shaking (150 rpm), fungal mycelia were homogenized using sterilized beaded glasses, and 2% (v/v) aliquot of mycelial suspension were introduced in a fresh MEB medium as inocula for laccase production. On the first day of cultivation, 0.15 mM of $CuSO_4$ was added to all cultures as a laccase-inducer. The Erlenmeyer flasks were incubated for 15 days under shaking (30 °C; 150 rpm).

At the end of the culture, flasks contents were filtrated to separate biomass from extracellular juice. The crude enzyme was collected to be characterized, concentrated by salt precipitation, and used as a biocatalyst for DDT degradation.

Laccase assay

Laccase activity was determined spectrophotometrically based on the oxidation of 2,6-dimethoxyphenol (2,6-DMP) or 2-2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Rodríguez et al. 2008). The enzymatic assays were done at room temperature using a Shimadzu UV-Vis 2600-spectrophotometer.

Salt precipitation of *Chaetomium sp* laccase

Crude enzyme with high laccase activity was collected from MEB liquid culture then precipitated by ammonium sulfate at 4 °C in the range of (40%-100%). Bradford assay was used to estimate the total amount of protein (Bio-Rad Protein assay Kit).

Laccase activity was determined for all proteolytic precipitated fractions (40%, 60%,80%, and 100%). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied to estimate the molecular weight of the semi-purified laccases.

Properties of the crude enzyme

Enzyme thermostability was studied by calculating the remaining laccase activity after keeping the crude enzyme for 3 hours at a wide range of temperatures (40 ºC to 80 °C) in absence of substrate. The residual laccase activity was determined at the pH of 5.0

The effect of such solvents on *Chaetomium sp* laccase was performed by pre-incubated isopropanol, acetone, methanol, and DMSO in the presence of the semi-purified laccase for 1 hour. The reaction without solvent was taken as 100% activity to calculate the residual activities.

4,4-DDT pesticide metabolic degrading capacity of *Chaetomium sp* laccase

4,4-DTT (2 mM) was incubated in a reaction mixture containing 1.5 U/mL of *Chaetomium sp* laccase activity in 100 mM sodium acetate buffer (pH 5.0) at 50°C in complete darkness under shaking (90 rpm). The enzymatic reaction was occurring for 2h, then stopped by increasing the pH to 8.0. Reactions were performed both in the presence and the absence of laccase-mediator 1-hydroxy benzotriazole (HBT) at 0.2 mM. (Daâssi et al. 2016). These reactions were previously optimized in our laboratory.

The reaction that doesn't contain enzyme was prepared as a control under the same conditions for the possible abiotic oxidative degradation. A heat-killed enzyme was used in control reactions, while blanks contained the same elements of the reaction mixture except for the 4,4-DDT. All reactions were performed in triplicate.

Quantitative and qualitative analysis of 4,4-DDT by Gas chromatography-mass spectrometry GC-MS

Controls and tests samples were extracted by dichloromethane using a separating funnel. The organic part completely evaporated using IKA rotary evaporator.

Before GC-MS analysis, concentrated extracted samples were trimethylsilylated (TMS) as suggested by (Daâssi et al. 2016).

After derivatization, the degradation products resulting from the laccase oxidation of the 4,4-DTT were identified using GC-MS equipment (GC-2010 Plus coupled to a GCMS-QP 2010 plus mass spectrometer (Shimadzu)).

The GC column was (Rtx 5ms). The injector and detector were programmed at 305 °C for 1 µL volume, splitless per 1 min. The carrier gas used was Helium at 100 kPa pressure. Temperature programming analysis was started at 120 °C per min with a rate of 10 °C/min and finished at 300 °C per 6 min (total 30 min). The MS analysis was operated in selected ion monitoring (SIM) mode with electron impact (EI) ionization for quantitation. EI experiments were performed using 70 eV as electron energy, temperature 200 °C, scan 45-800 m/z, or SIM.

The residual DDT after enzymatic degradation and the metabolites extracted from controls were identified and compared with the WILEY mass spectra database.

RESULTS AND DISCUSSION

Selection and identification of the laccase-producer fungus

Ten fungal strains were isolated from wood debris in the soil of Khulais. Laccase-positive fungal strains were isolated from wood debris by plate agar assay with 2,6-DMP and 0.15 mM CuSO4. The fungal isolate G3 showed a higher intensity of the orange color that proved the oxidation of 2,6-DMP to quinones (Fig.1).

Figure 1: *Chaetomium sp G3* (accession number, MZ841818) growth in MEA plate supplemented with 5 mM 2,6-DMP

As can be seen in fig. 2a, G3 cultural growth on MEA plate showed dark-gray color, globular perithecia, and cylindrical asci. Ascocarp aspects often found in Ascomycetes were detected under a microscope (Fig.2b-d). Similar microscopic characteristics were reported by Soytong and Quimio (1989), Bell (2005), and Tongon & Kasem, 2016).

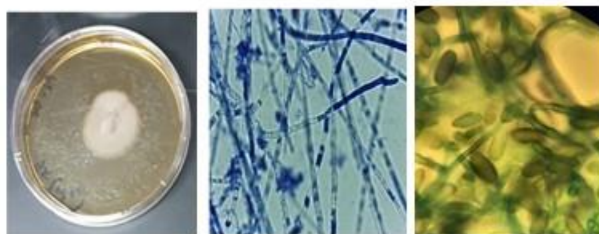

Figure 2: (a) : *Chaetomium sp.* (b): Haphae , (c): ascocarps

Based on the apparent morphological aspects of the fungal isolate (Fig .2a) and the microscopic characteristics observed under a microscope (Fig.2 b-c) in parallel to the molecular identification by analyzing the nuclear ribosomal ITS1-5.8-ITS4 sequence's similarity at 99% with the strains of *Chaetomium sp.* and *Ovatospora brasiliensis* appeared in the GenBank database. The isolated strain G3 was affiliated as *Chaetomium brasiliensis.* Its corresponding accession number: was MZ841818 received recently from GenBank.

Based on ClustalW multiple alignments of fungal ITS sequence with their homolog subject sequences selected from GenBank, a phylogenetic tree was established using the neighbor-joining method (Fig.3).

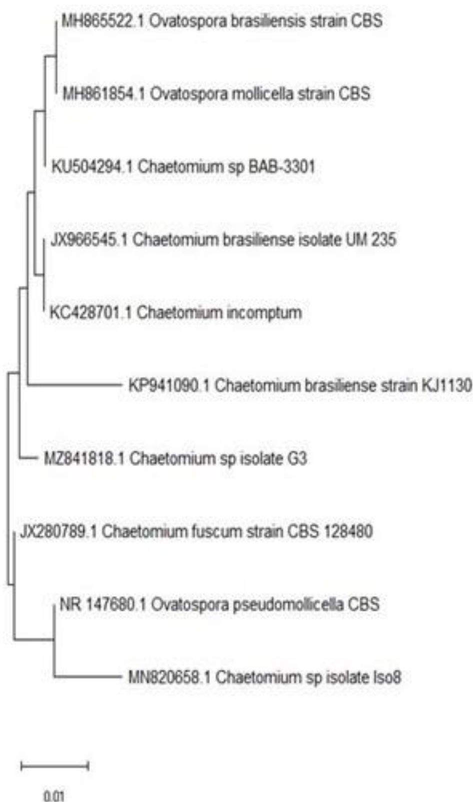

Figure 3: Neighbor-joining phylogenetic tree constructed based on the ClustralW alignment of ITS sequences of the isolated fungus G3, with homolog sequences obtained from the NCBI GenBank.

Phylogeny analysis revealed that the fungus, designated as G3, is closed to the genus *Chaetomium* sp. This fungus G3 was selected as laccase-producer and used for laccase production in a liquid medium.

Production and Semi-purification of G3 laccases

The kinetic of laccase production by *Chaetomium sp.* G3 was performed in the MEB production medium.

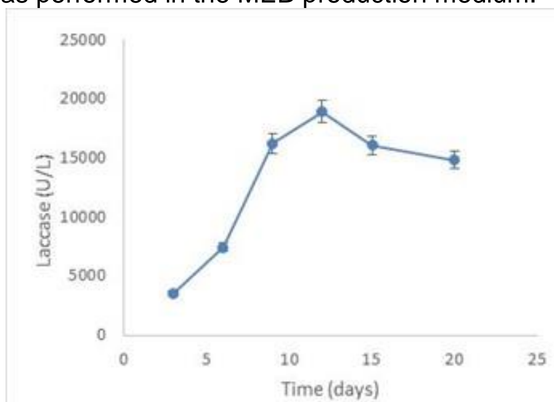

Figure 4: Profile of laccase production by *Chaetomium sp G3* in Malt Extract Broth MEB medium at 30 °C on a rotary shaker at 150 rpm

As shown in Fig. 4, laccase activity was detected after 3 days of culture incubation and the laccase activity reached the maximum rate of 20000 U/L on the 12th day. The extracellular laccase secreted by *Chaetomium brasiliensis* G3 was concentrated by ammonium sulfate precipitation at different fractions (40-60-80-100%). All factions were tested for Laccase activity. The fraction 60% of ammonium salt showed the highest laccase activity and further characterization assays were performed using this fraction. The presence of enzyme activity was visualized by a native zymogram in non-denaturing conditions using the 2,6-DMP as laccase substrate (Fig. 5).

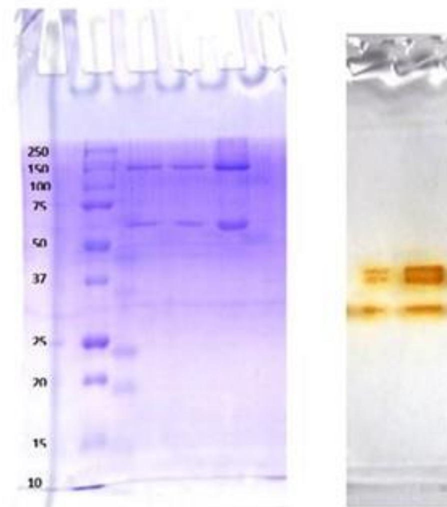

Figure 5: (A) SDS-PAGE (10%) with thermic treatment of samples analysis of laccase isozymes of *Chaetomium sp G3* by Coomassie blue R-250 staining.
(B) laccase zymograms in native conditions (10%) with activity staining by 2,6 Dimethoxyphenol .1-Crude enzymes, 2-fraction 60% $(NH4)_2SO_4$ precipitation.

The partially purified laccase showed a 1.6-fold increase in the activity with a final recovery yield of 78.2% (Table 1).

Stability of the laccase

Data in table 2 showed that the crude enzyme from G3 retained 97.6%, 95.02%, and 80.3% of the laccase activity at 30 °C, 40 °C, and 50 °C, respectively after 3hs. In literature, previous studies reported that commonly fungal laccases are stable within the temperature range 30°C to 50 °C and certainly lose activity above 60 °C. (Baldrian, 2006, Daâssi et al. 2013; Mtibaa et al. 2017).

As seen in table 2, the residual activities of laccase were reduced by about 59.4%, 79.9%, and 88.7% in parallel to the temperature increase to 60 °C, 70 °C, and 80 °C after 3hs of incubation. Moreover, the crude enzyme retained about 50% of its initial activity at 60 °C for 120 min which is defined as T_{50}. Similar T_{50} of the laccase purified from the genus Chaetomium at 60 °C after 90 min of incubation, was recorded by Mtibaa et al. (2017). The

T_{50} at 60 °C was reported in many fungal thermostable laccases (Baldrian 2006; Chakroun et al. 2010; Yang et al. 2014). Meanwhile, certain studies recorded not stable laccases above 50 °C such as a purified laccase from *Streptomyces Grammaticus* (Niladevi et al. 2008). This decline in enzymatic activity can be associated with the denaturation of the protein 's shape responsible for the catalytic function. In the case of laccase, a release of copper ions may happen at high temperatures making a loss of activity (Rich et al. 2013). These findings demonstrated that the studied Laccase from *Chaetomium sp.* G3 is seen to be thermostable, so a promising enzyme for further industrial applications at high-temperature conditions.

Many studies reported that laccases` substrates are organic pollutants highly soluble in such solvents (Wu al. 2019). So, laccases with cosolvent tolerance may be highly recommended for further biodegradation processes of such organic pollutants.

The effect of organic solvent on the crude enzyme of *Chaetomium sp.* was illustrated in table 3. the pre-incubation of the enzyme with 10% (v/v) isopropanol and acetonitrile increased the laccase activity by about 15% and 21%, respectively. However, the residual measured laccase activities were about 56%, 67%, and 70.2% in the presence of 40% (v/v) methanol, 25% (v/v) acetone, and 10% (v/v) dichloromethane, respectively. While the activity declined to 64.5% in the presence of 5% of DMSO.

Similar results were reported by Farnet et al. 2008 in studying the effect of 40% (v/v) methanol and acetone on laccase from *Marasmius quercophilus* which remained 50% of its initial activity. Accordingly, Yan et al. 2015 reported that 5% (v/v) of DMSO causes a 41.3% decline of laccase activity from the genus *Trametes tragic LK13.* Accordingly, Wu et al. 2019 demonstrated that solvent pre-incubation enhances effective 1.5- to the 4.0-fold improvement of fungal laccase activities.

DDT metabolic degrading capacity of *Chaetomium sp* laccase

Fungal laccases are known to be efficient and specific for several organic pollutants compounds (OPCs) degradation such as (textile dyes, Polycyclic aromatic hydrocarbons, pesticides (Manai et al. 2016; Bilal et al. 2019; Daâssi et al. 2021).

Several studies have shown the biotransformation of chlorinated pesticides using fungal purified laccases (Suhara et al. 2011; Jin et al. 2016; Bilal et al. 2019).

In the current study, the semi-purified *Chaetomium sp.* laccase showed a potential ability to degrade or transform the chlorinated pesticide DDT.

The removal of DDT by a concentrated crude laccase was performed in the absence and presence of a redox mediator (HBT) at pH 5.0 and 50 °C. The remained DDT were extracted from the reaction mixture of control and test.

Fig. 6 shows the time course of DDT (2 mM) removal in the presence of 1.5 U/mL laccases and 0.2 mM redox mediator.

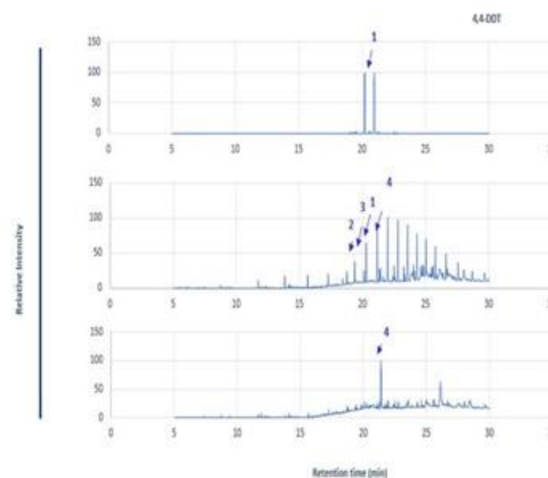

Figure 6: GC-MS chromatogram of the remainder 4,4-DDT in the control (a), test (b), and laccase-HBT catalyzed reactions (c).

After 2hs of the enzymatic treatment with *Chaetomium* laccase, more than 80% of the DDT was degraded in the enzymatic reaction without a mediator. A decrease of 87% in DDT concentration was achieved by adding 0.2 mM of HBT. The profiles of DDT and its metabolites were examined in the degrading reaction by GC–MS (Fig. 6a-c).

Table 1: Precipitation of crude laccase secreted by *Chaetomium sp G3*

Steps	Proteins (mg)	Lac activity (U)	Special activity (U/mg)	Purification (fold)	Yield (%)
The crude enzyme (100 mL)	25.45	2205	86.64	1	100
Ammonium Salt precipitation 60% (100 mL)	12.41	1724	138.92	1.6	78.2

Table 2: Stability of the semi-purified laccase from *Chaetomium sp.* at different temperatures during 3hs.

Temperature (°C)	Residual activity (%)				
	60 min	90 min	120 min	150 min	180 min
4 °C	100	100	100	100	100
30	99.4±0.2	98.7±0.1	98.5±0.2	97.8±0.5	97.6±0.7
40	98.42±0.8	97.7 ±1.6	96.2±2.4	95.3±0.7	95.02±0.2
50	94.8±1.4	89.5 ±2.4	86.7±1.2	83.1±2.2	80.3±1.7
60	77.3±2.5	62.8 ±2.09	54.2±0.9	47.4±1.4	40.6±0.8
70	61.8±0.9	39.7±3.8	33.4±3.2	24.2±0.9	20.1±2.3
80	35.6±1.7	27.2±1.1	18.3±2.6	14.4±1.8	11.3±0.4

Table 3: Effect of some organic solvents on the crude laccase from *Chaetomium sp. G3*

Organic solvent	Concentration (% v/v)	Residual activity (%)
Control	-	100
Acetonitrile	10	121.7± 4.6
Isopropanol	10	115.2± 2.2
Methanol	40	56.4 ± 1.7
dichloromethane	10	70.2± 0.8
Acetone	25	67.3 ± 1.3
DMSO	5	64.5± 3.2

Table4: Metabolites detected during the oxidative degradation of 4 Dichlorodiphenyltrichloroethane (4,4′-DDT) by *Chaetomium sp* laccase.

Peak	Name	Linear Formula	MI[a]	RT[b] (min)
Dichlorodiphenyltrichloroethane (4,4′-DDT)				
1	4,4′-DDT	(ClC6H4)2CHCCl3	235	20.973
				20.209
2	Octadecanoic acid, 2-oxo-, methyl ester	C19H36O3	253	18.5
3	2-Butenedioic acid (E)-, bis(2-Ethylhexyl) ester	C20H36O4	70	19.6
4	Phenol, 2,2'-methylene-bis [6-(1,1-dimethyl ethyl)-4-methyl	C23H32O2	177	21.38

Chromatograms of GC-MS showed a decrease in the intensity of DDT peaks after 2hs of Chaetomium sp. laccase treatment compared with the control profile proposing the cleavage of the main compounds; meanwhile, the appearance of new peaks in the enzymatic reaction represented the degrading products or presumed metabolites. *Chaetomium sp laccase* was effective in degrading the chlorinated pesticide DDT.

of DDT (20.97 min and 20.209 min), Octadecanoic acid, 2-oxo-, methyl ester (18.5 min), 2-Butenedioic acid (E)-, bis(2-ethylhexyl) ester (19.6 min), and Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl (21.38 min). Data presented in both Fig.6 and table 4 revealed that *Chaetomium* sp. laccase can oxidize the DDT via a meta-cleavage pathway **(**Fig.7)

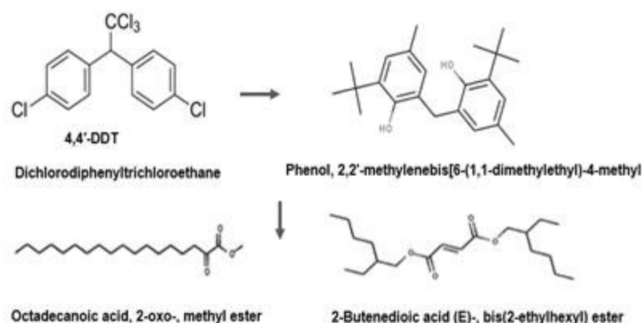

Figure 7: Degradation products of 4,4'-DDT by semi-purified laccases from *Chaetomium sp.* strain

CONCLUSION

The newly isolated strain *Chaetomium sp.* G3 and its laccase-mediator system showed efficiency in the biotransformation of DDT as a persistent and toxic pollutant in the environment.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

DD designed and conducted the experiments, contributed to the data analysis, and also wrote the manuscript. SRA performed the isolation of the fungal strain and the DDT enzymatic treatments. SAZ performed and designed the semi-purification and stability experiments of the fungal laccases. All authors read and approved the final version.

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REFERENCES

Baldrian P, 2006. Fungal laccases-occurrence and properties. FEMS Microbiol Rev 30:215-242.

Bell A, 2005. An illustrated guide to the coprophilous ascomycetes of Australia. CBS Biodiversity Series 3, 1–172.

Bilal M, Iqbal HMN, Barceló D, 2019. Persistence of pesticides-based contaminants in the environment and their effective degradation using laccase-assisted biocatalytic systems. Sci Total Environ 10; 695:133896.

Booij P, Holoubek I, Klánová J, Kohoutek J, Dvorská A, Magulová K, Al-Zadjali S, Čupr P, 2016. Current implications of past DDT indoor spraying in Oman. Sci Total Environ 550:231-240.

Chakroun H, Mechichi T, Martinez MJ, Dhouib A, Sayadi S, 2010. Purification and characterization of a novel laccase from the ascomycete *Trichoderma atroviride*: application on bioremediation of phenolic compounds. Process Biochem 45:507–513.

Chattopadhyay S, and Chattopadhyay D, 2015. Remediation of DDT and Its Metabolites in Contaminated Sediment. Curr Pollution Rep 1:248–264. DOI 10.1007/s40726-015-0023-z.

Combarnous Y, 2017. Endocrine Disruptor Compounds (EDCs) and agriculture: The case of pesticides. C R Biol. 340:406-409.

Daâssi D, Prieto A, Zouari-Mechichi H, Jesús Martinez M, Nasri M, Mechichi T 2016.Degradation of bisphenol A by different fungal laccases and identification of its degradation products. Int Biodeterior Biodegrad 110: 181-188.

Daâssi D, Zouari-Mechichi H, Belbahri L, Barriuso J, Martınez MJ, Nasri M, Mechichi T, 2016. Phylogenetic and metabolic diversity of Tunisian forest wood-degrading fungi: a wealth of novelties and opportunities for biotechnology. 3 Biotech 6:46. DOI: 10.1007/s13205-015-0356-8.

Daâssi D, Zouari-Mechichi H, Prieto A, Martınez MJ, Nasri M, Mechichi T 2013. Purification and biochemical characterization of a new alkali-stable laccase from *Trametes sp.* isolated in Tunisia: role of the enzyme in olive mill wastewater treatment. World J Microbiol Biotechnol 29:2145-2155.

Esplugas S, Bila DM, Krause LG, Dezotti M, 2007. Ozonation and advanced oxidation technologies to remove endocrine-disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) in water effluents. J Hazard Mater. 149: 631-642.

Farnet AM, Gil G, Ferre E, 2008. Effects of pollutants on laccase activities of *Marasmius quercophilus*, a white-rot fungus isolated from a Mediterranean *schlerophyllous litter*. Chemosphere 70:895-900.

Jabłońska-Trypuć A, 2017. Pesticides as inducers of oxidative stress. React Oxygen Species. 3:96–110.

Jin X, Yu X, Zhu G, Zheng Z, Feng F, Zhang Z, 2016. Conditions Optimizing and Application of Laccase-mediator System (LMS) for the Laccase-catalyzed Pesticide Degradation. Scientific Reports 6, 35787.

Kamal S, Junaid M, Bibi I, Rehman S, Rehman K, Sajid M, Akash H, 2021. Chapter 17: Role of Pesticides as EDCs in Metabolic Disorders. Akash M. S. H. et al. (eds.), Endocrine Disrupting Chemicals-induced Metabolic Disorders and Treatment Strategies, Emerging Contaminants and Associated Treatment Technologies, https://doi.org/10.1007/978-3-030-45923-9_17.

Kumar S, and Singh A, 2015. Biopesticides: Present status and the future prospects. J. Fertilizers Pesticides, 6: 1-2.

Lukaszewicz-Hussain A, 2010. Role of oxidative stress in organophosphate insecticide toxicity–short review. Pestic Biochem Physiol. 98(2) :145–50

Manai I, Miladi B, El Mselmi A, Smaali I, Ben Hassen A,

Hamdi M, Bouallagui H (2016) Industrial textile effluent decolourization in stirred and static batch cultures of a new fungal strain *Chaetomium globosum* IMA1 KJ472923. J Environ Manage 170:8–14

Mtibaa` R, de-Eugenio L, Ghariani B, Louati I, Belbahri L, Nasri M, Mechichi T, 2017. A halotolerant laccase from Chaetomium strain isolated from desert soil and its ability for dye decolourization. 3 Biotech 7:329.

Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, and Hens L, 2016. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Front Public Health 4:148.

Niladevi KN, Jacob N, Prema P, 2008. Evidence for a halotolerant-alkaline laccase in *Streptomyces psammoticus*: purification and characterization. Process Biochem 43:654–660.

Pirsaheb M, Limoee M, Namdari F, Khamutian R, 2015. Organochlorine pesticides residue in breast milk: a systematic review. Med J Islam Repub Iran 29:228.

Prabha S, Yadav A, Kumar A, Yadav A, Yadav HK, Kumar S, Yadav RS, Kumar R, 2016. Biopesticides - an alternative and eco-friendly source for the control of pests in agricultural crops. Plant Archives, 16: 902-906.

Rich JO, Leathers TD, Anderson AM, Bischoff KM, Manitchotpisit P, 2013. Laccases from Aureobasidium pullulans. Enzyme Microb Technol 53:33–37.

Rodríguez E, Ruiz-Duenas FJ, Kooistra R, Ram A, Martínez AT, Martínez MJ, 2008. Isolation of two laccase genes from the white-rot fungus *Pleurotus eryngii* and heterologous expression of the pel3 encoded protein. J Biotechnol 134:9-19.

Rodríguez-Delgado M, Orona-Navar C, García-Morales R, Hernandez-Luna C, Parra R, Mahlknecht J, Ornelas-Soto N, 2016. Biotransformation kinetics of pharmaceutical and industrial micropollutants in groundwaters by a laccase cocktail from *Pycnoporus sanguineus CS43* fungi. Int Biodeterior Biodegrad 108: 34-41.

Soytong K. and Quimio TH, 1989. A taxonomic study on the Philippine species of *Chaetomium*. Philip Agric 72(1):59-72.

Suhara H, Adachi A, Kamei I, Maekawa N, 2011. Degradation of chlorinated pesticide DDT by litter-decomposing basidiomycetes. Biodegradation 22:1075-86.

Tongon R. and Kasem S, 2016. Fungal Metabolites from *Chaetomium brasiliense* to Inhibit *Fusarium solani*. Int J Agricultur Technol 12:1463-1472.

Treviño LS, Wang Q, Walker CL, 2015. Phosphorylation of epigenetic “readers, writers and erasers”: implications for developmental reprogramming and the epigenetic basis for health and disease. Prog Biophys Mol Biol 118:8–13.

Turusov V, Rakitsky V, Tomatis L, 2002. Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. Environ Health Perspect 110:125–128.

Wu M-H, Lin M-C, Lee C-C, Yu S-M, Wang A.H.-J. and Ho T-H. D, 2019. Enhancement of laccase activity by pre-incubation with organic solvents. 9:9754.

Yan J, Chen Y, Niu J, Chen D, Chagan I, 2015. Laccase produced by a thermotolerant strain of *Trametes trogii* LK13. Braz. J. Microbiol. 46 (1): 59-65.

Yang J, Lin Q, Ng TB, Ye X, Lin J, 2014. Purification and characterization of a novel laccase from *Cerrena sp.* HYB07 with dye decolorizing ability. PLoS One 9: 110834.

Zeng S, Qin X, Xia L, 2017. Degradation of the herbicide isoproturon by laccase-mediator systems. Biochem Eng J 119: 92–100.

Zhao Y, Yi X, Li M, Liu L, Ma W, 2010. Biodegradation Kinetics of DDT in Soil under Different Environmental Conditions by Laccase Extract from White Rot Fungi. Chin J Chem Eng18(3);486-492.