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NOTE

Effect of lignocellulosic materials and chlorpyrifos pesticide on secretion of ligninolytic enzymes by the white rot fungus – *Stereum ostrea*

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ABSTRACT

The present study compared the effect of chlorpyrifos on secretion of ligninolytic enzyme by the white rot fungus – *Stereum ostrea* grown in the presence and the absence of *Tectona grandis* cubes under submerged liquid conditions. Significantly higher yields of laccase and manganese peroxidase to the extent of 164.30 and 59.28 U/ml were registered in control containing only teak wood cubes than in the culture on combination of cubes and chlorpyrifos with 111.65 and 47.24 U/ml, respectively on 10th day of incubation. Reversed pattern in lignin peroxidase secretion with peak values on 6th day of incubation was noticed in the culture of *S. ostrea* immobilized on *Tectona grandis* cubes under influence of chlorpyrifos. Under free state conditions, significantly higher secretions of laccase (125.33 U/ml) and manganese peroxidase (53.60 U/ml) occurred in chlorpyrifos-amended medium than in the medium devoid of chlorpyrifos on 10th day incubation. Under moderate shaking conditions, supplementation of wheat bran alone at 3% level to the medium resulted in enhanced (3–5 folds) secretions of laccase, manganese peroxidase and lignin peroxidase by *S. ostrea* in medium free of wheat bran on 12th and 10th day of incubations. The presence of chlorpyrifos in addition to wheat bran in the medium further raised production of ligninolytic enzymes – laccase, MnP and LiP to the level of 311.78, 130 and 3 U/ml respectively on the 12th (higher LAC and MnP secretions) and 10th (higher LiP secretions) day of incubations. Our data in the present study suggest that secretion of ligninolytic enzymes by the culture is dependent on the nature of lignocellulosic material used in the study and secretion of higher titers of ligninolytic enzymes on cubes of *Tectona grandis* in the absence of chlorpyrifos rather in the presence of chlorpyrifos occurred. For executing microbial applications, it is critical to understand how lignocellulosic materials and toxic xenobiotic pesticides interact with white rot fungi and possible role of ligninolytic enzymes in the bioremediation process.

KEYWORDS

Chlorpyrifos; ligninolytic enzymes [Laccase, Lignin peroxidase, Manganese peroxidase]; *Stereum ostrea*; teak wood cubes; wheat bran; white rot fungi

Introduction

Lignocellulose is considered the most abundant natural organic material on the earth and composed of cellulose, hemicellulose and lignin (Dias et al. 2010). In lignocellulosic biomass lignin occupies around 10–30% (Asgher, Bashir, and Iqbal 2014, Pérez 2020, Kawaguchi et al. 2016). It is the natural aromatic polymer and the second most abundant element after cellulose of plant biomass (Rahman et al. 2013). It forms a complex matrix as a structural part of the plant cell wall and protects cellulose and hemicellulose chains in the plant cell wall from microbial attacks. Its degradation is an important aspect for the reuse of carbon in the ecosystem and is being explored for the industrial use of biomass (Saldarriaga-Hernández et al. 2020).

White rot fungi (WRF), primarily found in the Basidiomycota, are a physiological group with an extraordinary ability to degrade lignin and lignin-like substances giving the wood a bleached white appearance (Abdel-Hamid, Solbiati, and Cann

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2013, Zhuo and Fan 2021). Also, WRF have developed a rich collection of extracellular oxidative enzymes to degrade lignin by employing different types of heme-containing peroxidases – lignin peroxidases [LiP], manganese peroxidases [MnP], versatile peroxidases [VP] and dye-decolorizing peroxidases [DyP] (Lambertz et al. 2016) and Laccase [LAC] (Kumar and Chandra 2020, Kumar and Verma 2020). These enzymes are commonly known as lignin modifying enzymes [LMEs]. It has been documented that ligninolytic potential of certain WRF such as *Phanerochaete chrysosporium* (Kuppuraj et al. 2021), *Ganoderma sp.* (Padma and Sudha 2013, Kumar et al. 2015), *Trametes versicolor* (Zahmatkesh, Spanjers, and Van Lier 2018) was assessed to a greater extent. *Stereum ostrea,* considered as one of the WRF and has a greater ability to secrete three ligninolytic enzymes-LAC, MnP and LiP (Viswanath et al. 2008, Praveen et al. 2011). LAC is identified as a dominant enzyme in the ligninolytic system in *S. ostrea* (Usha, Praveen, and Reddy 2014, Shanthi Kumari et al. 2019).

WRF have a wide range of industrial and environmental applications in removal of xenobiotic chemicals such as pesticides, polyaromatic hydrocarbons [PAHs], biphenyls, halogenated aromatics, some dyes and other toxic chemicals (Baratto et al. 2015) because of participation of the nonspecific nature of their enzymes in degradation of some complex aromatic polymers with molecular structures similar to lignin (Tišma, Zelić, and Vasić-Rački 2010, Mahmood et al. 2021). In recent years a lot of interest in WRF has been generated for exploration due to several advantages, including low specificity and the elimination of need for pre-acclimatization to chemicals and nonspecific nature of action of their LMEs (Zhang et al. 2008).

It has been established that on a global level, organophosphates (OP) are the most widely used pesticides after organochlorine insecticides (Phung et al. 2012). Chlorpyrifos (CPF), O,O-diethyl O-[3,5,6-trichloro-2-pyridyl phosphorothioate] is an organophosphate (OP) insecticide that was launched in United States by Dow Chemicals in 1965 for the management of foliage insects in agricultural practices, domestic use, and soil-borne insect pests (John and Shaike 2015). The repeated application of this insecticide causes its accumulation in soil affecting soil microorganisms and also non-target ecologically important insects like beetles, bees, and wasps as well as aquatic organisms (Jabeen et al. 2016).

The effects of pesticides on WRF development, particularly on the production of ligninolytic enzymes by WRF, are less well understood. However, a few of the studies provided information on the influence of pesticide chemicals on WRF development and the production of ligninolytic enzymes. An organophosphorus insecticide – malathion at concentrations within a range of 25–100 µg/ml caused inhibited growth of *Pleurotus ostreatus* (Ganash, Abdel Ghany, and Reyad 2016). Growth of *Ganoderma lucidum* GL-2 strain on rice bran substrate at 30 °C and pH 5.6 in the presence of 4 ppm of lindane in liquid [SmF] as well as solid-state fermentation [SSF] induced production of ligninolytic enzymes (Kaur, Grewal, and Kocher 2003). Diuron enhanced only LiP activity of *P. chrysosporium* whereas MnP production was lowered in the same culture under the influence of diuron (Coelho-Moreira et al. 2013). Laccase was not at all detected in the culture filtrate of *P. chrysosporium* grown in the presence of diuron.

However, the interaction between an OP pesticide CPF and the WRF on growth and ligninolytic enzyme secretion was not evaluated. Furthermore, WRF have limited stability in a complex environment because of their weak anti-adversity, which can be a serious challenge for WRF systems in practical applications (Cao et al. 2019). To overcome the limitations, the immobilization technique should be considered as an efficient technology to improve the degradation efficiency of contaminants treatment owing to its high processing performance, reliability, wide environmental sensitivity and easy liquid-solid separation (Saha and Bhaskara Rao 2021). Immobilized fungal cells show apparent benefits compared to free fungal cells, as it can make the system more resistant to environmental disturbances and provide the comfort for regeneration. Entrapment and adsorption methods are commonly used for immobilization of microorganisms (Feng et al. 2021). There are many carriers successfully used up to now, such as sodium

alginate, polyvinyl alcohol [PVA] and some plant-derived materials for immobilization of fungal cells (Bilal et al. 2017). Plant tissue structure typically has a complex internal microstructure with relatively wide pores. Plant materials, as a result of their microstructure, offer the possibility of cell immobilization as carriers (Varjani 2017). Li et al. (2015) explored the utilization of WRF immobilized in wood chips to remove carbamazepine and naproxen.

Therefore, the present study was aimed at assessment of the extent of secretion of ligninolytic enzymes by the less characterized WRF like *S. ostrea* immobilized on natural plant material such as teak wood cubes and wheat bran under the influence of CPF.

Materials and methods

Fungal culture and chemicals

The culture of white rot fungus – *Stereum ostrea* used in this study was kindly supplied by Department of Microbiology, Kakatiya University, Telangana, India and was isolated from the wood logs (Shanthi Kumari et al. 2019). The culture was maintained on Koroljova-Skorobogat medium (Koroljova Skorobogat'ko et al. 1998).

Lignocellulosic substrate materials such as wheat bran and teak (*Tectona grandis*) wood cubes, selected for the present study, were procured from a local market (Anantapuramu, AP, India), and dried in air. Chlorpyrifos (CPF) stock solution [1000 ppm concentration] was prepared by adding sterilized distilled water to commercial formulation of 20% E.C. [emulsified concentrate] of CPF (Cheminova India Limited, Mumbai, India). Chlorpyrifos stock solution was filter-sterilized through Millipore membrane filter with a pore size of 0.45 µm for use in experiments for studying effect of CPF on secretion of ligninolytic enzymes.

Growth of fungus and induction of ligninolytic enzymes

Culture of the white rot fungus *S. ostrea* has been grown on solid Koroljova (Peptone 3 g/L, Glucose 10 g/L, K_2HPO_4 0.4 g/L, KH_2PO_4 0.6 g/L, $MgSO_4$ 0.5 g/L, $MnSO_4$ 0.05 g/L, $ZnSO_4$ 0.001 g/L, $FeSO_4$ 0.0005 g/L, Agar 20 g/L, Distilled water 1000 ml, pH of the culture medium 5.5) slants. The fungal mycelial suspension was prepared after 6 days of incubation by adding 5 ml of sterile distilled water to the slant (Shanthi Kumari et al. 2017). The homogenized fungal culture suspension has been used as a source of inoculum for growing *S. ostrea* culture in various experiments.

Secretions of ligninolytic enzymes on plant materials by S. ostrea in the presence of CPF

For comparison of secretion of ligninolytic enzymes by *S. ostrea* grown on teak wood cubes and in free state (devoid of cubes) under the influence of CPF, sterilized Koroljova liquid medium was distributed into sterilized 250 ml Erlenmeyer conical flasks at the rate of 50 ml medium per flask. These flasks were divided into two groups. Each flask of the first group received three dried cubes of teak (*Tectona grandis)* wood, together equivalent to 1.5 g whereas flasks of the second group were devoid of wood cubes. Filter-sterilized CPF of commercial grade was added at 20 ppm level to one half of flasks of both the first and second groups. The other half of flasks of both groups received sterile distilled water of corresponding volume. All flasks were inoculated with the culture of *S. ostrea* and were incubated at 30 °C in an incubator cum shaker (Scigenics Orbitek, Chennai, India) at 160 rpm. Fungal culture in flasks devoid of CPF served as controls to fungal culture grown in the presence of CPF either on wood cubes or in free state devoid of wood cubes. All the flasks with growing culture of *S. ostrea* were withdrawn at different time intervals for measurement of fungal biomass and extracellular protein content and activities of ligninolytic enzymes in the culture filtrate (Section-Analytical methods).

Another experiment was conducted with only wheat bran at 3% level in Koroljova liquid broth in the same manner except replacement of teak wood cubes by wheat bran and moderate shaking conditions (75 rpm) with focus on the same parameters in the previous experiment.

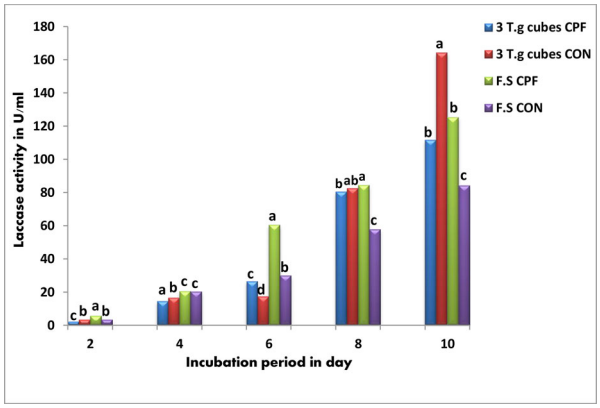

Figure 1. Secretion of laccase on 3 *Tectona grandis* cubes under influence of CPF.
Data presented in figure are means of 3 replicates. Mean bars for each sampling interval, followed by the same letter are not significantly different ($P > 0.05$) from each other according to Duncan's Multiple Range (DMR) test.
CON: Control devoid of the pesticide-chlorpyrifos; 3 T.g cubes: *Tectona grandis* (teak wood) cubes; F.S: Free state conditions with absence of teak wood cubes; CPF: Chlorpyrifos.

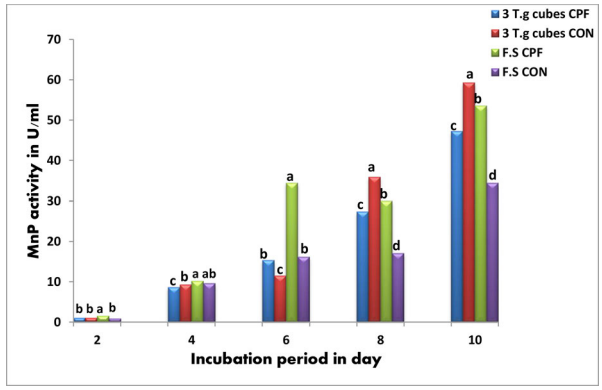

Figure 2. Secretion of manganese peroxidase on 3 *Tectona grandis* cubes under influence of CPF.
Data presented in figure are means of 3 replicates. Mean bars for each sampling interval, followed by the same letter are not significantly different ($P > 0.05$) from each other according to Duncan's Multiple Range (DMR) test.
CON: Control devoid of the pesticide-chlorpyrifos; 3 T.g cubes: *Tectona grandis* (teak wood) cubes; F.S: Free state conditions with absence of teak wood cubes; CPF: Chlorpyrifos.

Analytical methods

At regular intervals, culture broth of two experiments was filtered through Whatmann filter No 1 filter paper for collection of fungal biomass on the pre-weighed filter and culture filtrate in a suitable container. Fungal biomass recovered on the filter was dried in a hot air oven at 60°C and growth of *S. ostrea* was expressed in terms of dry weight of fungal biomass per flask.

Culture filtrate derived after separation of fungal biomass in the experiments was first checked for pH with a pH meter. For finding out the extent of secretion of proteins including ligninolytic enzymes such as laccase, manganese peroxidase and lignin peroxidase in extracellular medium, enzyme titers in culture filtrate were determined following standard protocol. The culture filtrate was centrifuged at 4 °C in a refrigerated centrifuge (Remi C-24, Mumbai) at 1500 rpm for 15 min. Supernatant was used as a source of extracellular protein content/ligninolytic enzymes. Appropriate dilution of culture filtrate derived from different experiments was used for the estimation of soluble protein content according to the method of Lowry et al. (1951). Laccase enzyme assay was determined with use of an aliquot of enzyme source (culture filtrate) according to the method of Das, Sengupta, and Mukherjee (1997). Manganese peroxidase (MnP) activity was assayed as specified by Bonnen, Anton, and Orth (1994). Lignin peroxidase (LiP) enzyme assay was based on the method of Tien and Kirk (1988). Enzyme activities were expressed in terms of IU where one unit of enzyme is defined as the amount of enzyme that oxidized one micromole of substrate per min. All data presented are the means of three replicates and statistically analyzed by Duncan's Multiple Range (DMR) test.

Results and discussion

Activities of ligninolytic enzymes of S. ostrea on teak wood cubes in the presence of CPF

Generally, fungal cultures colonize and grow on solid matrices under natural conditions. Therefore, impact of CPF on secretion of three ligninolytic enzymes by *S. ostrea* immobilized on 3 teak wood (*Tectona grandis*) cubes suspended in the medium or the free state (devoid of cubes) was assessed. Inclusion of teak wood cubes in the liquid medium for growth of *S. ostrea* under shaking conditions supported the secretion of three ligninolytic enzymes – LAC, MnP and LiP (Figures 1–3). Among the four treatments, significantly peak titers of LAC and MnP were registered in control (devoid of CPF) containing teak wood cubes. Yields of LAC and MnP in the control medium with cubes on the 10th day of incubation were 164.30 and 59.29 U/ml,

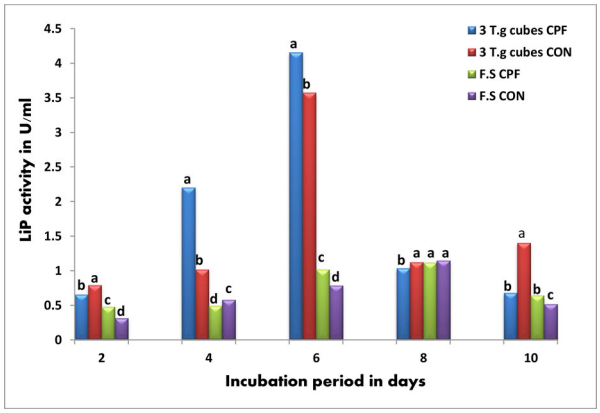

Figure 3. Secretion of lignin peroxidase on 3 *Tectona grandis* cubes under influence of CPF by *S. ostrea*.
Data presented in figure are means of 3 replicates. Mean bars for each sampling interval, followed by the same letter are not significantly different ($P > 0.05$) from each other according to Duncan's Multiple Range (DMR) test.
CON: Control devoid of the pesticide-chlorpyrifos; 3 T.g cubes: *Tectona grandis* (teak wood) cubes; F.S: Free state conditions with absence of teak wood cubes; CPF: Chlorpyrifos.

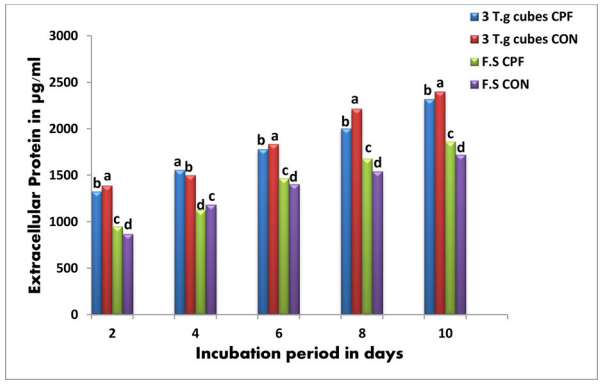

Figure 4. Secretion of extracellular protein by *S. ostrea* on 3 *Tectona grandis* cubes under influence of CPF.
Data presented in figure are means of 3 replicates. Mean bars for each sampling interval, followed by the same letter are not significantly different ($P > 0.05$) from each other according to Duncan's Multiple Range (DMR) test.
CON: Control devoid of the pesticide-chlorpyrifos; 3 T.g cubes: *Tectona grandis* (teak wood) cubes; F.S: Free state conditions with absence of teak wood cubes; CPF: Chlorpyrifos.

respectively. Yields of LAC and MnP from the culture immobilized on cubes in CPF-amended medium touched peak on 10th day of incubation with 111.65 and 47.24 U/ml, respectively but were significantly lower. Nevertheless, under free state conditions, LAC production in CPF-amended medium on 10th day incubation was 125.33 U/ml as against 84.19 U/ml in the control (Figure 1). Higher secretion of MnP (53.60 U/ml) in CPF-amended medium on the 10th day of incubation as against 34.39 U/ml in control devoid of CPF under free state conditions was also recorded. Synergistic effects of both CPF and teak wood cubes on toxicity may be responsible for reduction in secretions of LAC and MnP in medium amended with both CPF and cubes.

The pattern of secretion of LiP was different from laccase and MnP. Unlike LAC and MnP, LiP secretion was higher in culture broth of *S. ostrea* grown on wood block cubes in the presence of CPF in medium on 4th and 6th day of incubation (Figure 3). LiP yield from the culture broth of *S. ostrea* on wood block cubes under influence of CPF was 4.15 U/ml as against 3.57 U/ml yielded by the same culture grown on CPF-free medium with only wood block cubes (control) on 6th day of incubation. There was no clear-cut difference in LiP yield on the 6th day onwards. In free state conditions, LiP productions from CPF-amended medium and CPF-free medium on the 8th day of incubation were limited to 1.11–1.14 U/ml.

Extracellular protein (2400 µg/ml) was obtained from the culture of *S. ostrea* grown on the medium amended with only 3 cubes of *Tectona grandis* on 10th day of incubation as against 2319.3 µg/ml from medium amended with both 3 *Tectona grandis cubes* and CPF (Figure 4). A very low amount of extracellular protein content was recovered from free state culture of control on the 10th day of incubation. The pattern of secretion of extracellular protein in the culture of *S. ostrea* grown in free state and immobilized on blocks was parallel to the pattern of secretion of ligninolytic enzymes by the same culture under respective conditions.

Comparison of fungal biomat derived from growth of *S. ostrea* indicated that *S. ostrea* grew better on wood block cubes in the absence of CPF with registering maximum biomass of 26.43 mg/ml than in the presence of CPF (20.12 mg/ml) on 10th day of incubation (Table 1). Uninoculated wood blocks and growth on wood block cubes in liquid medium with and without CPF on the 6th day and 10th day of incubation are shown in Photo S_1. Biomass of *S. ostrea* was relatively higher on wood block cubes than in free state under similar conditions. Biomass of 26.43 mg/ml from wood block cubes

Table 1. Growth of *S. ostrea* on teak wood cubes under the influence of CPF.

	Biomass (in mg/ml) after incubation period of				
Treatment	2 days	4 days	6 days	8 days	10 days
3 T.g cubes CPF	4.99[b]	7.44[b]	10.88[b]	18.61[b]	20.12[b]
3 T.g cubes CON	5.31[a]	7.69[a]	13.05[a]	22.36[a]	26.43[a]
F.S CPF	3.68[d]	4.35[c]	8.56[c]	13.48[c]	16.81[c]
F.S CON	3.74[c]	3.91[d]	7.01[d]	10.27[d]	15.43[c]

Data presented in table are means of 3 replicates. Means in each column followed by the same superscript letter are not significantly different ($P \leq 0.05$) from each other according to the Duncan's Multiple Range (DMR) test.

CON: Control devoid of the pesticide-chlorpyrifos; 3 T.g cubes: *Tectona grandis* (teak wood) cubes; F.S: Free state conditions with absence of teak wood cubes; CPF: Chlorpyrifos.

Table 2. Changes in pH of the culture broth of *S. ostrea* grown on teak wood cubes.

	pH of the broth culture of *S. ostrea* measured after growth of				
Treatment	2 days	4 days	6 days	8 days	10 days
3 T.g cubes CPF	5.60[b]	5.53[a]	5.00[a]	4.80[a]	4.65[a]
3 T.g cubes CON	5.61[a]	5.50[b]	4.83[b]	4.60[b]	4.40[b]
F.S CPF	5.42[d]	5.34[c]	4.40[c]	4.14[d]	3.70[d]
F.S CON	5.46[c]	5.28[d]	4.32[d]	4.16[c]	3.92[c]

Data presented in table are means of 3 replicates. Means in each column followed by the same superscript letter are not significantly different ($P \leq 0.05$) from each other according to the Duncan's Multiple Range (DMR) test.

CON: Control devoid of the pesticide-chlorpyrifos; 3 T.g cubes: *Tectona grandis* (teak wood) cubes; F.S: Free state conditions with absence of teak wood cubes; CPF: Chlorpyrifos.

without CPF was recorded as 15.4 mg/ml from free state without CPF. Utilization of nutrients in the medium by the culture and formation of new cells could lead to increase in biomass during the course of incubation in the present study. pH of culture broth of *S. ostrea* grown either in free state or on wood block cubes initially rose from 5.0 by 0.4 to 0.6 units within 4 days of incubation (Table 2) and declined below 4.0 in later intervals of incubation. Decrease in pH in culture broth of *S. ostrea* in the presence or absence of chlorpyrifos under both conditions during growth could be attributed to formation of acidic metabolites.

Many experimental studies have been conducted with WRF immobilized on inert synthetic substrates and natural lignocellulosic substrates in the form of pellets/biobeds for production of ligninolytic enzymes. For instance, adsorption of mycelia of the fungal culture onto sponge cubes resulted in immobilization of the fungal culture and functioned in the normal way of secretion of extracellular proteins including ligninolytic enzymes (Pallavi 2011, Babič, Likozar, and Pavko 2012). According to Pallavi (2011), *S. ostrea* immobilized on sponge cubes gave significantly higher yields of laccase than the same culture in the free state in the presence of dye. Among different carriers tested for immobilization of *P. chrysosporium* (Urek and Pazarlioğlu 2004), polystyrene was found the best carrier based on production of MnP and biomass. The white rot fungus *P. chrysosporium*, immobilized to the mineral kissiris, produced LiP of 174 U/l and 500 U/l within 7–9 days (Ghasemzadeh, Kargar, and Lotfi 2011). Ligninolytic enzymatic potential of *P. chrysosporium* immobilized on polyurethane foam under the influence of reactive dye K-2BP showed better performance in comparison with the control [freely suspended culture]. The peak levels of production of MnP and LAC by the immobilized culture were approximately 5 and 48 times higher than those of the suspended culture and reached earlier (Gao et al. 2008). In a comparative study on 3 supports of natural lignocellulosic materials – wood chips, wheat straw and wheat grains, a most rapid growth of *Anthracophyllum discolor* with fast colonization of wheat grains along with maximum MnP production was recorded (Rubilar et al. 2011). Ligninolytic activity during the incubation of the three types of pellets – complex, coated and simple pellets of *A. discolor* in liquid medium for 15 days was due to mainly MnP followed by MiP (Elgueta et al. 2012). Above studies reported growth of different fungal cultures on different solid substrates in the absence of xenobiotics. Nonetheless, the present study evaluated secretion of extracellular protein including ligninolytic enzymes by *S. ostrea* on wood block cubes under the influence of CPF. In the present study, growth, and secretion of ligninolytic enzymes by *S. ostrea* on wood block cubes in the presence of CPF were significantly low in comparison to control.

Activities of ligninolytic enzymes of S. ostrea on wheat bran in the presence of CPF

Under moderate shaking conditions of 75 rpm level, growth of *S. ostrea* on wheat bran in the presence of CPF was significant as evident from photos S_2 and S_3 of growing culture taken on 10th and 12th day of incubation. The production

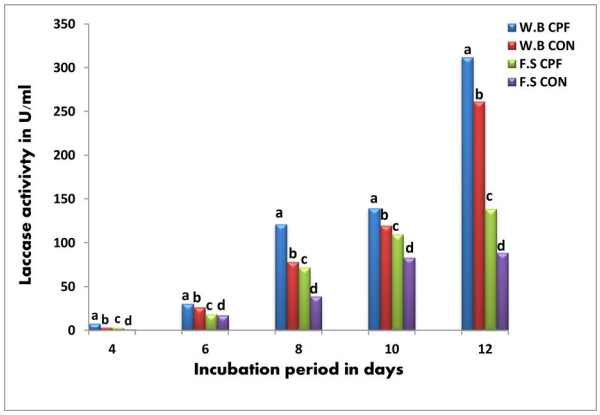

Figure 5. Secretion of laccase by *S. ostrea* on wheat bran under influence of CPF and moderate shaking.
Data presented in figure are means of 3 replicates. Mean bars for each sampling interval, followed by the same letter are not significantly different ($P > 0.05$) from each other according to Duncan's Multiple Range (DMR) test.
CON: Control devoid of the pesticide-chlorpyrifos; W.B: Wheat bran; F.S: Free state conditions with absence of wheat bran; CPF: Chlorpyrifos.

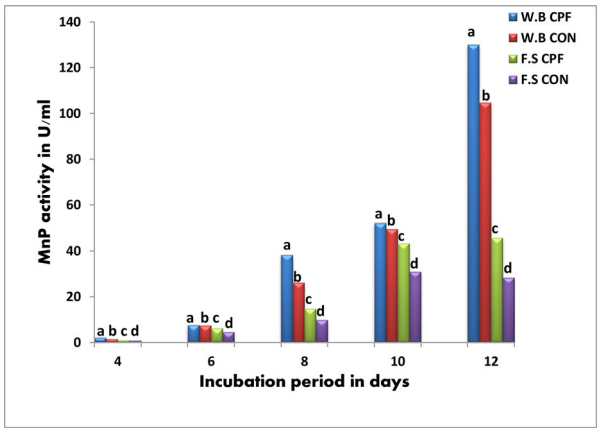

Figure 6. Secretion of manganese peroxidase by *S. ostrea* on wheat bran under influence of CPF and moderate shaking.
Data presented in figure are means of 3 replicates. Mean bars for each sampling interval, followed by the same letter are not significantly different ($P > 0.05$) from each other according to Duncan's Multiple Range (DMR) test.
CON: Control devoid of the pesticide-chlorpyrifos; W.B: Wheat bran; F.S: Free state conditions with absence of wheat bran; CPF: Chlorpyrifos.

of ligninolytic enzymes on wheat bran under influence of CPF was improved (Figures 5–7). Maximum production of laccase (311.78 U/ml) on medium amended with wheat bran and CPF was obtained against 261.29 U/ml in control with only wheat bran on 12th day of incubation (Figure 5). Corresponding yields of LAC by *S. ostrea* in the presence of CPF and in absence of CPF in free state on the 12th day of incubation were138.27 and 88.39 U/ml respectively.

Twelve-day old culture broth of *S. ostrea* grown on medium supplemented with wheat bran and CPF yielded MnP to the extent of 130 U/ml as against 104.69 U/ml (control) (Figure 6). MnP production from culture broth of *S. ostrea* grown on medium with CPF in free state on 12th day of incubation was 45.69 U/ml alongside 28.30 U/ml in the culture broth devoid of CPF after growth for 12 days. On the 10th day of incubation, LiP production by *S. ostrea* on medium supplemented with wheat bran in presence and absence of CPF was limited to 2-3 U/ml (Figure 7). LiP yield by *S. ostrea* on medium with and without CPF in free state were comparatively low.

S. ostrea secreted higher yield of extracellular protein content of 2403.6 µg/ml on medium amended with both wheat bran and CPF during 12 days of incubation whereas the culture broth of the same culture grown on control medium with only wheat bran contained extracellular protein content of 2147.3 µg/ml at the end of 12-day incubation (Figure 8). Free state cultures of *S. ostrea* in the presence of CPF liberated extracellular protein content to the extent of 1683.4 µg/ml as against 1443.5 µg/ml in free state cultures in the absence of CPF over a period of 12 days.

pH changes occurred in the culture broth of *S. ostrea* grown on wheat bran and under free state conditions in the presence and absence of CPF (Table 3). There was an increase in pH from an initial set value of 5.0 by 0.5–1.0 unit within 4 days of incubation followed by drop in pH at later intervals.

Under moderate conditions of shaking in the present study, wheat bran supported better growth and higher production of ligninolytic enzymes by *S. ostrea* in comparison to growth and secretions of ligninolytic enzymes by the same culture in free state. Maximum production of ligninolytic enzymes on wheat bran by *S. ostrea* is in agreement with earlier reports of higher production of ligninolytic enzymes by the same culture in solid state fermentation (Usha, Praveen, and Reddy 2014). Wheat bran served as the best substrate in production of other enzymes such as cellulolytic enzymes (Hanif, Yasmeen,

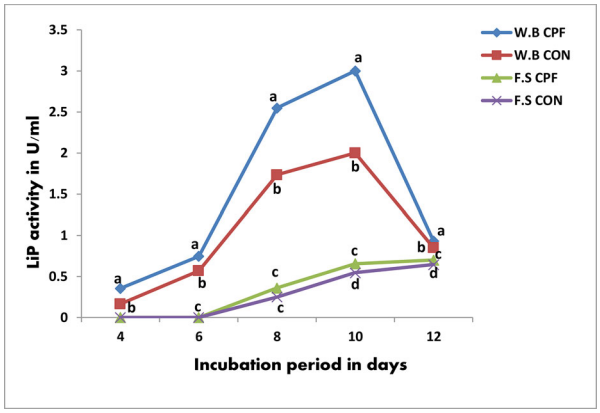

Figure 7. Secretion of lignin peroxidase by *S. ostrea* on wheat bran under influence of CPF and moderate shaking.
Data presented in figure are means of 3 replicates. Mean for each sampling interval followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to the DMR test.
CON: Control devoid of the pesticide-chlorpyrifos; W.B: Wheat bran; F.S: Free state conditions with absence of wheat bran; CPF: Chlorpyrifos.

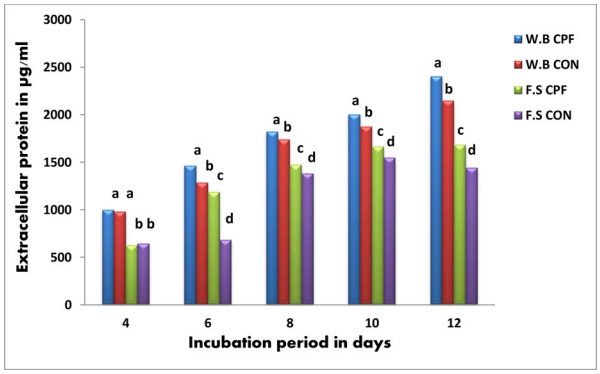

Figure 8. Secretion of extracellular protein by *S. ostrea* on wheat bran under influence of CPF and moderate shaking.
Data presented in figure are means of 3 replicates. Mean bars for each sampling interval, followed by the same letter are not significantly different ($P > 0.05$) from each other according to Duncan's Multiple Range (DMR) test.
CON: Control devoid of the pesticide-chlorpyrifos; W.B: Wheat bran; F.S: Free state conditions with absence of wheat bran; CPF: Chlorpyrifos.

and Rajoka 2004, Subhosh Chandra and Rajasekhar Reddy 2013) and amylases (Kaur, Grewal, and Kocher 2003) by different fungal organisms in submerged and solid state fermentations. The presence of CPF in the medium with wheat bran even further improved production of ligninolytic enzymes in the present study. Similarly, enhancement in secretion of ligninolytic enzymes by the fungal organisms under influence of a variety of xenobiotics such as pesticides – malathion, lindane, chlorpyrifos, trinitrotoluene, dyes, hydrocarbons especially at lower concentrations was reported in the absence of any lignocellulosic materials (Kaur, Kapoor, and Kaur 2016; Ganash, Abdel Ghany, and Reyad 2016, Kawaguchi et al. 2016).Wheat bran was demonstrated to be the best solid support and also provides nutrients without any toxic ingredients for growth of a variety of organisms including WRF.

Conclusion

In this study an impact of chlorpyrifos pesticide on secretion of three ligninolytic enzymes by white rot fungi *S. ostrea* immobilized on teak wood (*Tectona grandis* wood) cubes was assessed. Maximum laccase and MnP production were observed in control (devoid of CPF) containing teak wood cubes. Higher yields of laccase, MnP and LiP from the fungal culture immobilized on cubes in chlorpyrifos-amended medium touched peak on 10th day and 6th day of incubation respectively. Similarly, higher titers of laccase and MnP in medium with CPF and wheat bran was recorded on 12th of incubation whereas peak secretion of LiP was recorded on 6th day of incubation. Productions of the three ligninolytic enzymes were improved upon cultivation of *S. ostrea* on wheat bran in liquid medium under moderate shaking conditions. Knowledge of incubation conditions of the white rot fungi and their interaction with lignocelluloses and other materials is essential for enhancing production of LME for biotechnological applications. The

Table 3. Change in pH of the culture broth of *S. ostrea* grown on wheat bran.

	pH of the broth culture of *S. ostrea* measured after growth of				
Treatment (5ml mycelial suspension in 50ml media)	2 days	4 days	6 days	10 days	12 days
W.B CPF	5.66^{a}	5.58^{a}	5.43^{a}	5.32^{a}	5.48^{a}
W.B CON	5.56^{b}	5.46^{b}	5.35^{b}	5.26^{b}	5.40^{b}
F.S CPF	5.44^{d}	5.37^{c}	4.95^{c}	4.65^{c}	4.38^{c}
F.S CON	5.48^{c}	5.19^{d}	4.77^{d}	4.50^{d}	4.24^{d}

Data presented in table are means of 3 replicates. Means in each column followed by the same superscript letter are not significantly different ($P \leq 0.05$) from each other according to the Duncan's Multiple Range (DMR) test.
CON: Control devoid of the pesticide-chlorpyrifos; W.B: Wheat bran; F.S: Free state conditions with absence of wheat bran; CPF: Chlorpyrifos.

present study is an attempt to understand interaction among toxic and nontoxic lignocellulosic materials, toxic xenobiotic pesticides, and the white rot fungus – *S. ostrea* in production of ligninolytic enzymes for bioremediation of environment pollutants.

Conflict of interest

Authors declare no conflict of interest.

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