ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com
Journal DOI: 10.21474/IJAR01

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

BIODEGRADATION OF DICHLOROPHENOLS BY BJERKANDERA ADUSTA AND LENTINUS SQUARROSULUS AND EXTRACELLULAR LIGNINOLYTIC ENZYME ACTIVITIES.

Astha Tripathi[1], R.C. Upadhyay[2], Surendra Singh[3] and Saurabh Dixit[4]

1. Assistant professor, Shoolini University of Biotechnology and Management Sciences, Bajhol, Solan (H.P.)-173229 india.
2. Principal Scientist, Directorate of Mushroom Research, Chambaghat, Solan (H.P.)-173213 india.
3. Professor, Department of Botany, Banaras Hindu University, Varanasi (U.P.)-221005 india.
4. Reder, MNDAV Dental College and Hospital, Tatul, Solan (H.P.)- 173229

Manuscript Info

Manuscript History:

Received: 19 March 2016
Final Accepted: 26 April 2016
Published Online: May 2016

Key words:
Dichlorophenols/ white rot fungi/ ligninolytic enzymes/ biodegradation.

Corresponding Author
Astha Tripathi.

Abstract

Dichlorophenols are a group of toxic compounds that have been widely used as biocides. Fifty fungal strains were screened for their potential to tolerance with 2,3-dichlorophenol, 2,5-dichlorophenol and 3,4-dichlorophenol on solid medium supplemented with 2% malt extract (MEA). Growth rate ($mmday^{-1}$) was determined at three concentrations (0.10, 0.15 and 0.2 mM) of all the three dichlorophenols. From the fifty fungal strains only Bjerkandera adusta and Lentinus squarrosulus were able to tolerate all the three dichlorophenols (DCPs). These white-rot fungi (WRF) were chosen for liquid medium studies for the biodegradation of DCPs and ligninolytic enzyme activity at 0.10 mM concentration over the period of 20 days. Both varieties completely degrade all the three DCPs. Aryl Alcohol Oxidase (AAO) is the main oxidase enzyme in B. adusta while laccase plays important role in L. squarrosulus. MnP (Manganese peroxidase) showed good activity with both varieties. The DCPs biodegradation potential of the WRF positively correlated with their potential to express ligninolytic enzymes such as laccase, AAO, MnP and lignin peroxidase (LiP). These fungal strains were capable to degrade DCPs and could be used for bioremediation applications on large scale.

Abbreviations:- AAO- Aryl alcohol oxidase, DCP- dichlorophenol, HPLC- high performance liquid chromatography, LiP- lignin peroxidase, MnP- manganese peroxidase, NPM- nutrient poor medium, NRM- nutrient rich medium, VA- veratryl alcohol, WRF- white rot fungi

Introduction:-

Chloroorganic contaminants are present in the environment due to their extensive use in agriculture and industry. Chlorophenols (CPs) are a group of toxic compounds that have been widely used as biocides to control bacteria, fungi, algae, mollusks, insects, slime and other biota. CPs that enter non-target upland, wetland, and aquatic environments associate with colloidal and particulate matter and, if not photodegraded, eventually settle onto surface soils (Shiu et al. 1994). The chlorinated phenols have been recognized one of the major environmental problems because they have been widely found in surface water and in wastewater, in particular, in pulp mill effluents (Schellenberg et al. 1984). Chlorinated phenols are considered to be relative persistent in the environment and they are highly toxic. The number of chloro substituents on the phenolic ring affects the optimum pH for peroxidases (Dec and Bollag 1990). There are 19 compounds of chlorophenols of three monochlorophenol, six isomeric substances each of dichlorophenol, trichlorophenol, as well as three isomeric substances tetrachlorophenol, and one fully chlorinated pentachlorophenol.

Chlorophenols are introduced into the environment through various human activities such as waste incineration, uncontrolled use of wood preservatives, pesticides, fungicides and herbicides, as well as via bleaching of pulp with chlorine and the chlorination of drinking water (Contrerasa et al. 2003). CPs are one of the most dangerous classes of environmental pollutants have been produced in thousands of tons annually by the pulp and paper and agrochemical industries (Steiert et al. 1985). CPs with few chlorine atoms are used mainly as starting materials for the synthesis of higher chlorinated phenols or chlorophenol-derived products, such as the chlorophenoxyacetic acid herbicides. Because of the toxicity of CPs, techniques for their removal are urgently needed. Currently, many physical and chemical methods are available for decontaminating CPs. But, considering the high costs of these procedures, attention has been focused on oxidative coupling of CPs. The oxidative process gives rise to less soluble, higher molecular weight polymers; these can consequently be removed by sedimentation or filtration (Bollag et al. 2003).

Biodegradation of chlorophenols is dependent on number and position of chlorine substituents; the rate of biodegradation decreases with increase in number of chlorine substituents. Metabolism of CPs is not unusual in fungi. WRF are a group of filamentous wood decay fungi which have ability to metabolize lignin and the polysaccharide components of wood. WRF are also effective in destroying xenobiotics. Azo- and other textile dyes, polyaromatic hydrocarbons, amino-, nitro- and chloroorganic compounds, including chlorophenols, and other xenobiotics, were found to be substrates for these fungi and their extracellular enzymes (Field et al. 1993; Cameron et al. 2000; Pointing 2001). Extracellular production of ligninolytic enzymes is responsible for the biodegradation of CPs by fungi (Mileski et al. 1988; Reddy et al. 1988; Valli and Gold 1991). WRF capable of degrading CPs attracts considerable attention as an effective biotreatment tool in aqueous effluents (Michizae et al. 2001). The degradation pathways of several CPs by the WRF P. chrysosporium have been determined in vitro (Valli and Gold 1991; Joshi and Gold 1993; Reddy and Gold 2000). The bioremediation method depends on selecting the right microbes in the right place with the right environmental factors for degradation to occur.

Several studies are currently focused in the feasibility of using WRF for treatment of phenolic compounds which represent toxicity and an environmental concern. WRF have been shown to be potent degraders of a broad variety of recalcitrant xenobiotic compounds including polycyclic aromatic hydrocarbons (Aranda et al. 2010), polychlorinated biphenyls (PCBs), dioxins, DDT (Reddy and Mathew 2001) and industrial dyes (Faraco et al. 2009). Among xenobiotic environ mental concern, several monoaromatic compounds have been shown to be degraded by the Phanerochaete chrysosporium. 2,4-DCP (Valli and Gold 1991), 2,4,5-trichlorophenol (Joshi and Gold 1993) and pentachlorophenol (Mileski et al. 1988) were shown to be efficiently degraded by P. chrysosporium, where the fungus produce lignin peroxidase and manganese peroxidase (Grey et al. 1998). Extracellular ligninolytic enzymes are secreted from WRF and produced during a fermentation process and possess the ability to break down bonds within organic compounds and catalyze their transformation into less toxic and more biodegradable forms (Hatakka 2001). The main extracellular enzymes participating in lignin degradation and biodegradation of xenobiotic compounds are heme-containing lignin peroxidase (LiP, EC 1.11.1.14), MnP (EC 1.11.1.13) and Cu-containing laccase (EC 1.10.3.2) (Hatakka 2001). In addition, enzyme involved in hydrogen peroxide production such as AAO (EC 1.1.3.7) is considered to belong to the ligninolytic system (Hatakka 2001).

The objectives of this study were to accelerate the biodegradation of DCPs by two WRF. The ligninolytic enzyme pattern secreted during degradation of DCPs has been determined.

Materials and methods:-

Cultures:-

Mycelial cultures of various saprophytic, ligninolytic and mycorrhizic fleshy fungi were selected from the "Mushroom Gene Bank" of Directorate of Mushroom Research, Solan (Himachal Pradesh, India). Name of fifty different mycelial cultures are given in Table-1.

Chemicals:-

Analytical grade 2,3-DCP, 2,4-DCP, 3,4-DCP (MW 163.0 $gmol^{-1}$), Methanol (MW 32.04 $gmol^{-1}$) and Ortho Phosphoric Acid (OPA, MW 98 $gmol^{-1}$) were purchased from Merck. 2,2'-azinobis(3-ethylbenzthiazoline-6-sulphonate) (ABTS, MW 548.7 $gmol^{-1}$) was obtained from Sigma. Veratryl alcohol (VA, MW 168.19 $gmol^{-1}$) was purchased from Aldrich. All other chemicals used were of analytical grade.

Screening of fungi:
2,3-DCP, 2,4-DCP, 3,4-DCP (0.1, 0.15, 0.2 mM) were individually added in 2% MEA containing 20 gl^{-1} of malt extract and 20 gl^{-1} of agar powder after autoclaving. Plates were inoculated with 8-10 days old mycelial culture bit (5 mm in diameter) in the centre and incubated at 25ºC±1ºC except Auricularia mesentrica, Volvariella spp., Calocybe indica, Lentinus squarrosulus, Ganoderma sp., Bjerkandera adusta and Phanerochaete chrysosporium were incubated at higher temperature (at 30ºC±2ºC). Radial mycelial growth was taken after every 48 h and average radial growth was calculated. In control mycelium spread was complete, no further observations were recorded. The percentage inhibition in mycelial growth was calculated by comparing mycelial growth with DCPs treated plates and control. Reduction (%) in mycelial growth was calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Growth (mm/day) control} - \text{Growth (mm/day) in treated plates}}{\text{Growth (mm/day) control}} \times 100$$

High Performance Liquid Chromatography (HPLC) analysis of DCPs:-
The biodegradation studies of DCPs were carried out in the nutrient rich medium (NRM) containing 2 g of ammonium tartrate, 10 g of glucose, 1 g of KH_2PO_4, 1 g of yeast extract, 0.5 g of $MgSO_4.7H_2O$, 5 g of KCl, 1 ml of solution containing trace elements per liter of medium and nutrient poor medium (NPM) containing 10 g of glucose, 2 g of KH_2PO_4, 0.2 g of yeast extract, 0.1 g of peptone, 1 ml of solution containing trace elements per liter of medium. Solution of trace elements containing 10 mg of $Na_2B_4O_7.10H_2O$, 7 mg of $ZnSO_4.7H_2O$, 5 mg of $FeSO_4.7H_2O$, 1 mg of $CuSO_4.5H_2O$, 1 mg of $(NH_4)^6Mo_7O_{24}.4H_2O$, 1 mg of $MnSO_4$ dissolved in 100 ml of water (Espindola et al. 2007). The pH was adjusted to 7 before autoclaving at 120°C for 20 min. and 0.1 mM (16.3 μgml^{-1}) of 2,3-DCP, 2,5-DCP and 3,4-DCP were individually added after autoclaving. 150 ml Erlenmeyer flasks containing 50 ml of the liquid medium were inoculated with 5-8 days old 5 mycelium bits (5 mm in diameter). Three replicates of both media were incubated in static condition in BOD incubator and shaking condition in a rotatory shaker at 150 rpm at 30°C. The cultures were harvested on 5^{th}, 10^{th}, 15^{th} and 20^{th} day of incubation. Each sample was centrifuged (10,000 x g for 15 min) at 4°C. The supernatant of liquid culture was used for quantify biodegradation of DCPs. Quantitative estimation of DCPs was done by reverse phase lichrosphere 5 µM, RP-18 columns (125 x 4mm ID). The apparatus consists of one LC-200 series pump, one UV/VIS detector LC-295 and one sample injector L-7350. All components are from Perkin Elmer 1022. Methanol, water and OPA in different concentrations (40:60:05, 50:50:05, 60:40:05) were used as mobile phase and finally 60:40:05 mobile phase was selected for HPLC. The flow rate was 1ml/minute. The wavelength for DCPs was 280 nm and 10 µl sample was injected in column after cleaning with syringe filters (0.45 µm). The concentration of DCPs was quantified using standard curve from the known substances against the area. Control was served without culture and with DCPs for calculated actual amount degraded by selected varieties.

Extracellular ligninolytic enzyme essay:-
The production of extracellular enzymes with or without DCPs, were carried out in NRM and NPM, same medium and procedure used for HPLC. Medium without DCPs used as control. Each sample was centrifuged (10,000 x g for 10 min) at 4°C. The enzymatic reactions were determined using a double beam Perkin Elmer Lambda 12 UV/VIS spectrophotometer. All the enzyme activities were measured at room temperature (20±2ºC). The enzymatic activity was expressed as International units (U) defined as the amount of enzyme required to produce 1 µmol product min^{-1} and expressed as Ul^{-1}.

Protein concentration was determined following Bradford method (Bradford 1976). Protein content in the sample was determined from standard curve and the amount of protein μgml^{-1} was calculated. Laccase activity was measured following the oxidation of ABTS ($\varepsilon_{420,}$ 36,000 M^{-1} cm^{-1}). The assay mixture contained 100 mM sodium acetate buffer pH 5 and 5 mM ABTS (Han et al. 2005). AAO activity was assayed as the oxidation of Veratryl alcohol to veratraldehyde at 310 nm (9,300 M^{-1} cm^{-1}) and activity was measured with 5 mM VA in 100 mM sodium phosphate buffer at pH 6 (Varela et al.1999). LiP activity was measured by the oxidation of 2 mM VA to veratraldehyde (ε_{310}, 9,300 M^{-1} cm^{-1}) in 100 mM sodium tartrate buffer (pH 3) in the presence of 0.4 mM H_2O_2 (30%) (Camarero et al. 1999). MnP activity was determined by the production of a Mn^{3+} tartrate complex (ε_{238}, 6,500 M^{-1} cm^{-1}) from 0.1 mM $MnSO_4$ in 100 mM sodium tartrate buffer pH 4.5 with 0.1 mM H_2O_2 (30%) (Cohen et al. 2001).

Statistical analysis:-
All the experimental analysis was carried out in triplicates. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test using SAV v.9.1.3 program. Differences at $p<0.05$ were considered to be significant.

Results:-

Screening of fungi for DCPs tolerance on solid medium:- Fifty fungal strains of various, saprophytic, ligninolytic and mycorrhizic fleshy fungi representing different group of fungi were selected to screen for their potential to tolerate DCPs on solid medium. From the fifty fungal strains only B. adusta, L. squarrosulus and P. chrysosporium were showed best tolerance with all DCPs. P. citrinopileatus, P. djamor, P. playtypus, G. flaccossus and T. hirsutus also showed good mycelia growth with all three DCPs at all three concentrations. However, A. bisporus, C. comatus, C. hortanse, Descolia sp., Marasmius sp. and Tremella sp. gave very poor tolerance. Mycelial growth was drastically reduced at lower to higher concentration. 3,4-DCP was more toxic than 2,3-DCP and 2,5-DCP. B. adusta showed better tolerance in comparison to L. squarrosulus. Reduction (%) in mycelia growth by DCPs is shown in Table-1.

B. adusta and L. squrrosulus were selected on the basis of initial screening. Both varieties proved to be the most effective among the tested fungi in tolerance of DCPs. B. adusta is non-edible while L. squarrosulus is edible mushroom grow extensively on saw dust. B. adusta is known their ability to degrade many xenobiotic compounds. Both varieties are WRF and produced ligninolytic enzymes (Tripathi et al. 2012).

Biodegradation of DCPs by B. adusta and L. squarrosulus:-
Three isomers of DCP (2,3-, 2,5-, 3,4-) were selected for this study and 0.1 mM (16.3 μgml^{-1}) concentration was used for all DCPs. NPM gave better results in comparison to NRM and under shaking condition, DCPs were fast degrade than static condition. 3,4-DCP was more toxic than 2,3-DCP and 2,5-DCP. The toxicity of the chlorinated phenols is dependent on the number and position of the chlorine substituents in the benzene ring (Kishino and Kobayashi 1994). The toxicity decreases as the number of the chlorine substituents decreases. The PCP was found to be the most toxic of chlorinated phenols. In the PCP all five available substituent positions are occupied by chlorine atom. Both varieties were fully degraded all DCPs in 20 days in both media and condition. 2,3-DCP and 3,4-DCP were fast degraded by B. adusta while 2,5-DCP was fast degraded by L. squarrosulus (Table-2). Biodegradation or mineralization of chlorophenols is also dependent on number and position of chlorine substituents; the rate of biodegradation decreases with increase in number of chlorine substituents. However the scientists have not found agreement on the effect of substituent position on biodegradation (Annachhatre and Gheewala 1996). Fig.1 shows the time course of all three DCPs biodegradation by B. adusta and L. squarrosulus.

dichlorophenol (2,3-DCP):- 2,3-DCP was easily degrade by both varieties in both media and conditions. Retention time (RT) of 2,3-DCP is 7.31 min.

In B. adusta one metabolite was common in both media and conditions (RT-4.12 min). One another metabolite was found in NRM shaking condition (RT-2.36 min) on 10^{th} and 15^{th} day. In NPM one metabolite was formed under both conditions (RT-5.71 min) and one another minor metabolite was formed under shaking condition (RT-3.31 min). Except one common metabolite (RT-4.12 min) rest metabolites were not detected on 20^{th} day.

In L. squarrosulus NPM was better than NRM. Metabolites were not detected in both media and conditions up to 20 days (Fig.2).

dichlorophenol (2,5-DCP):- 2,5-DCP was not observed by both varieties on 20^{th} day (RT-8.51 min). In NRM static condition rate of biodegradation was slow. L. squarrosulus fast degrade 2,5-DCP in comparison to B. adusta.

In B. adusta metabolites were not detected in NRM under both conditions and NPM under static condition. Two metabolites were observed in NPM shaking condition on 15^{th} day and these metabolites has a RT of 3.52 and 5.49 min respectively. Metabolites were degraded with 2,5-DCP on 20^{th} day.

In L. squarrosulus one metabolite was detected in NRM shaking and NPM both conditions on 15^{th} day (RT-8.23 min). This metabolite was degrade with 2,5-DCP on 20^{th} day (Fig.3).

dichlorophenol (3,4-DCP):- 3,4-DCP was degraded by both varieties, media and conditions in 20 days. Biodegradation was noticeably slow in NRM under static condition in comparison to other medium and condition. B. adusta could faster degrade 3,4-DCP than L. squarrosulus. RT of 3,4-DCP is 9.32 min. One metabolite was common in both varieties, media and conditions except in L. squarrosulus in NRM static condition this metabolite was not detected. This metabolite had a RT of 2.23 min.

In B. adusta four metabolites were detected in both media and conditions and RT of these metabolites are 2.23, 2.89, 4.91 and 5.42 min respectively. All metabolites were degraded up to 20 days in NRM under both conditions while in NPM minor peck of one metabolite (2.23 min) was observed in both conditions and rest metabolites were degraded with 3,4-DCP.

In L. squarrosulus metabolites were not detected in NRM under static condition. Two metabolites were detected in static condition of NPM and shaking condition of both media one was common (2.23 min) and another had a RT of 8.01 min. One more metabolite was detected in NPM static condition and this metabolite was also detected in B. adusta (2.89 min). Selected HPLC chromatograms are shown in Fig.4.

Extracellular ligninolytic enzymes in the presence of DCPs:- Ligninolytic enzymes are believed to be responsible for degradation or biodegradation of xenobiotic compounds due to their non specific nature for the substrate.

Extracellular protein in B. adusta, was not observed on 5^{th} and 10^{th} day in NRM shaking condition and NPM both conditions but on 20^{th} day, this media and conditions gave highest protein, 34.5, 30.9, 31.9 μgml^{-1} respectively in control. Extracellular protein was best with 3,4-DCP in NRM static condition (24.6 μgml^{-1}) on 10^{th} day followed by with 2,5-DCP in NPM static condition and 2,3-DCP in NRM static condition respectively on 15^{th} day (20.3, 19.0 μgml^{-1}). In L. squarrosulus, amount of protein was best with 3,4-DCP in NRM shaking condition on 5^{th} day (21.1 μgml^{-1}). It was seen that the amount of protein was different with DCPs in both varieties (Table-3). The extracellular protein was obtained from both the strains; however the crude protein content of L. squarrosulus was comparable to L. tigrinus (Adejumo and Awosanya 2005).

Laccase activity was very less in B. adusta with all DCPs. In control activity was observed only in NRM static condition. Laccase activity reached a significant level only with 3,4-DCP (5.7 Ul^{-1}) and 2,5-DCP (4.2 Ul^{-1}) in NRM static condition on 20^{th} day. It indicates that laccase activity is inducible in B. adusta and it requires nutrient rich condition for its secretion. In L. squarrosulus laccase activity was higher in comparison to B. adusta. With 2,3-DCP and 2,5-DCP laccase activity was best under static condition of both media on 10^{th} day. In the presence of 3,4-DCP laccase activity was best in NRM static condition (179.0 Ul^{-1}) on 10^{th} day followed by in NPM and NRM shaking condition respectively on 5^{th} day (169.2, 152.7 Ul^{-1}). 3,4-DCP showed best laccase activity in comparison to 2,3-DCP and 2,5-DCP. It was seen that presence of DCPs increase the activity of laccase (Table-4).

AAO activity in B. adusta was better under shaking condition of both media. 2,3-DCP, 2,5-DCP and in control AAO activity was best in NRM under shaking condition while 3,4-DCP showed better activity in NPM under shaking condition on 15^{th} and 20^{th} day. It indicates that AAO activity is directly related to nutritional and incubation conditions. L. squarrosulus gave very poor AAO activity in comparison to B. adusta. Significant activity was found in control on 15^{th} day in NRM shaking condition (19.8 Ul^{-1}) followed by with 2,3-DCP on 20^{th} day and in control on 10^{th} day in NPM shaking condition respectively (18.3, 16.7 Ul^{-1}; Table-5).

LiP activity in B. adusta was good with 2,5-DCP on 15^{th} day in NRM shaking condition (18.2 Ul^{-1}) followed by in control on 10^{th} day in NRM static condition (16.5 Ul^{-1}). LiP activity was not more significant and some times LiP activity could not be detected in the culture supernatant, but it appeared during enzyme purification (Heinfling et al. 1998a). In L. squarrosulus LiP activity was better than B. adusta and best activity was observed with 2,5-DCP in NPM shaking condition on 5^{th} day (20.4 Ul^{-1}; Table-6).

MnP activity in B. adusta was higher in control in NRM static condition on 15^{th} day (215.9 Ul^{-1}). In all selected DCPs 2,3-DCP gave best MnP activity in NPM shaking condition on 15^{th} day (118.3 Ul^{-1}). L. squarrosulus gave higher MnP activity in comparison to B. adusta. In the presence of DCPs best activity was observed with 2,3-DCP on 5^{th} day under shaking condition and on 15^{th} day under static condition of NRM. MnP from both varieties suggested that a corresponding enzymatic activity might be responsible for the biodegradation of DCPs (Table-7).

Discussion:-

Different approaches have been used to study fungal biodegradation of chlorinated phenols: intact cultures of fungi (Alleman et al. 1992; Mileski et al. 1988; Reddy et al. 1998; Valli and Gold 1991), separation of mycelium and culture filtrate separately (Armenante et al. 1994), free and immobilized enzymes (Iimurya et al. 1996; Roy-Arcand and Archibald 1991; Ruggiero et al. 1989; Ruttiman-Johnson and Lamar 1996; Shuttleworth and Bollag 1986). These studies revealed that lignin-degrading enzyme systems are usually responsible for the CP transformation or degradation. The most extensive and diverse research was conducted with the Phanerochaete chrysosporium, chosen because it has been the most studied model of ligninolytic fungi. Temperate fungal species offer more advantages, provided they have extracellular enzymes capable of DCPs transformation. 2,4-DCPl (Valli and Gold 1991), 2,4,5-trichlorophenol (Joshi and Gold 1993) and pentachlorophenol (Mileski et al. 1988) were shown to be efficiently mineralized by P. chrysosporium under conditions, where the fungus produces LiP and MnP. These enzymes have originally evolved for degradation of lignin and the reactions they perform are non-specific.

All selected DCPs showed variations in enzymatic activity in both varieties, media and conditions. In B. adusta laccase activity was not significant and some studies have been reported that laccase is not present (Heinfling et al. 1998a; Belcarz et al. 2005). Supplementation and incubation conditions were affected the enzymatic activity and activity was varying with compound to compound in both media and conditions. Some WRF like Volvariella volvacea gave good laccase activity under high nitrogen condition when medium was supplemented with Cu but in the absence of Cu activity was not found and under low nitrogen condition laccase activity was very poor (Chen et al. 2003). Laccase was main oxidase enzyme in L. squarrosulus. Laccase is the most active enzyme for CP transformation in some other WRF like Coriolus versicolor (Ullah et al., 2000a). In cultures of T. versicolor, laccase is reported to be the dominant ligninolytic enzyme involved in CPs degradation (Kadhim et al. 1999; Leontievsky et al. 2002b; Roy-Arcand and Archibald 1991; Ullah et al. 2002b). 3,4-DCP gave best laccase activity in comparison to 2,3-DCP and 2,5-DCP in L. squarrosulus. Production of laccase depends on appropriate growth media for the fungus, with different isomers of enzyme being expressed under different growth conditions. The reactivity of CPs with laccase depends on the number and position of substituted chlorines on the aromatic ring. The lower degree of substitution of the CPs, the more reactive molecule with ortho and para substituted CPs being transformed by laccase more readily than those with meta substitution (Kadhim et al. 1999). AAO activity was better in B. adusta in comparison to L. squarrosulus. In B. adusta, 2,3-DCP and 2,5-DCP gave best AAO activity in NRM shaking condition while 3,4-DCP gave best activity in NPM shaking condition and MnP activity was good with 2,5-DCP in comparison to 2,3-DCP and 3,4-DCP in NRM under both conditions and NPM static condition. Bjerkandera spp. biosynthesize veratryl alcohol and veratraldehyde de-novo from glucose, together with anisaldehyde, 3-chloro-anisaldehyde and 3,5-dichloro-anisaldehyde and their respective aryl alcohols (de Jong et al. 1994). It is interesting to note that the AAO of Bjerkandera spp. has a much lower affinity for VA than the AAO of the Pleurotus spp. Thus VA is well protected against unwanted AAO mediated oxidation (de Jong et al. 1994). AAO is the main oxidases enzyme in B. adusta while laccase plays important role in L. squarrosulus for biodegradation of DCPs. In some WRF both enzymes were observed during lignin degradation like P. ostreatus has shown that the concerted action of laccase and AAO, produces significant reduction in the molecular mass of soluble lignosulphonates (Marzullo et al. 1995). AAO is H_2O_2-generating enzyme in basidiomycetes, such as P. sajor-caju, P. ostreatus, P. eryngii, B. adusta and P. chrysosporium which has been purified and their properties characterized (Okamoto and Yanase 2002). LiP was better in L. squarrosulus in comparison to B. adusta. LiP is a true peroxidase and the kinetic of enzyme intermediates have been studied in detail and a heme peroxidase which is secreted extracellularly at the onset of secondary metabolism, triggered by nitrogen limitation, in P. chrysosporium, but it can also be produced in nitrogen-sufficient conditions by other WRF (Collins et al. 1997). Under ligninolytic conditions, degradation of chlorophenols is initiated by oxidative dechlorination of the substrate to its corresponding p-quinone, catalyzed by two extracellular peroxidases, LiP and MnP (Reddy and Gold 2000; Duran et al. 2002). In both selected varieties MnP was very important enzyme for DCPs biodegradation. MnP was found to be responsible for transformation of chlorophenols (Leontievsky et al. 2002). CPs are also substrates for MnP and LiP that requires hydrogen peroxide and Mn(II) and for reaction of MnP (Milstein et al. 1992) and veratryl alcohol for reaction of LiP (Reddy and D'souza 1994). This peroxidase oxidizing Mn^{2+} (MnP) is described for the first time in B. adusta (Heinfling et al. 1998b). MnP catalyzed polymerization of aromatic compounds was reported in pentachlorophenol and ferulic acid and synthesis of DHP by MnP from B. adusta in 70% aqueous acetone (Iwahara et al. 2000). MnP activity was higher in L. squarrosulus in comparison to B. adusta. Purified or crude MnP has been used in cell-free systems (in vitro) and shown to oxidize not only lignin, chlorolignins and synthetic lignin compounds, but also Humic Substances (HS) from brown coal, and HS synthesized from catechol, nylon, PAH, chlorophenols, nitroaromatic compounds and arsenic-containing warfare agents (Steffen 2003).

Metabolism of CPs is not unusual in fungi. Many WRF such as Ganoderma lucidum and Polyporus can synthesize chlorophenols, and some can release large quantities of organic chlorides, including halomethanes, which are thought to be utilized as substrate cofactors for ligninolytic pathways (Beynon et al., 1981; Oberg et al., 1997; Teunissen and Field, 1998; Watling and Harper, 1998). The ligninolytic enzymes secreted by WRF may be involved in the first oxidative dechlorination step in the degradation of several chlorinated phenol. Several chlorophenols have also been shown to be oxidized by laccases (Roy-Arcand and Archibald, 1991). The present study describes a procedure to produce high amounts of extracellular MnP activity with mycelium of the B. adusta and L. squarrosulus. Both varieties gave good activity of MnP. Activity varies with DCPs, medium and their incubation conditions. Among the four ligninolytic enzymes, AAO in B. adusta, laccase in L. squarrosulus and MnP in both varieties, seemed to be responsible for the biodegradation of DCPs. These results show that enzymatic activity of lignininolytic enzymes were affected by the presence of DCPs biodegradation which can be important in the elimination of the toxicity of different xenobiotics. Further investigation will have to identify metabolites and their role as well as the metabolic pathway of DCPs biodegradation.

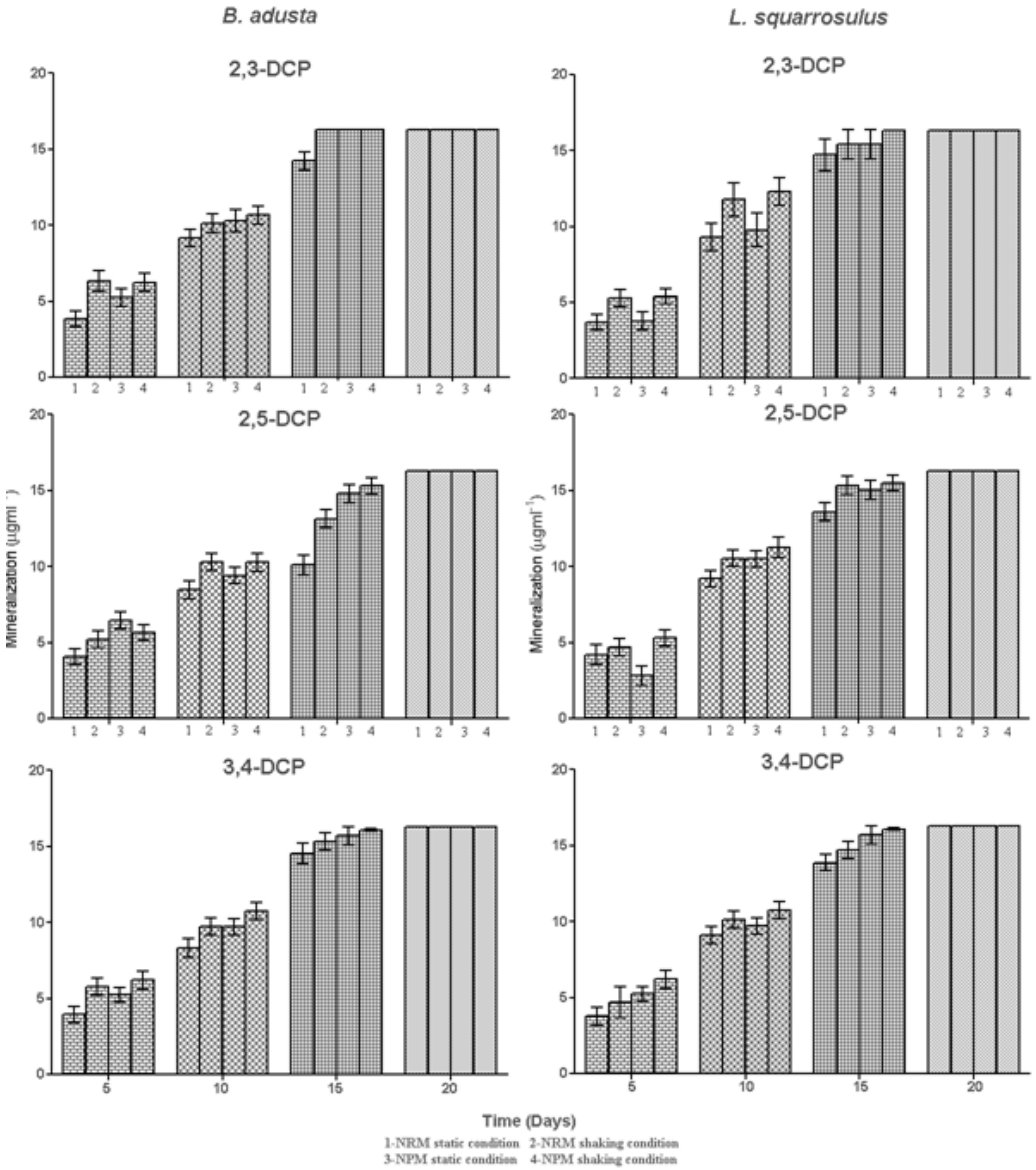

Fig.1:- Time course removal of DCPs by B. adusta and L. squarrosulus. Columns represents mean of three replicates with SD

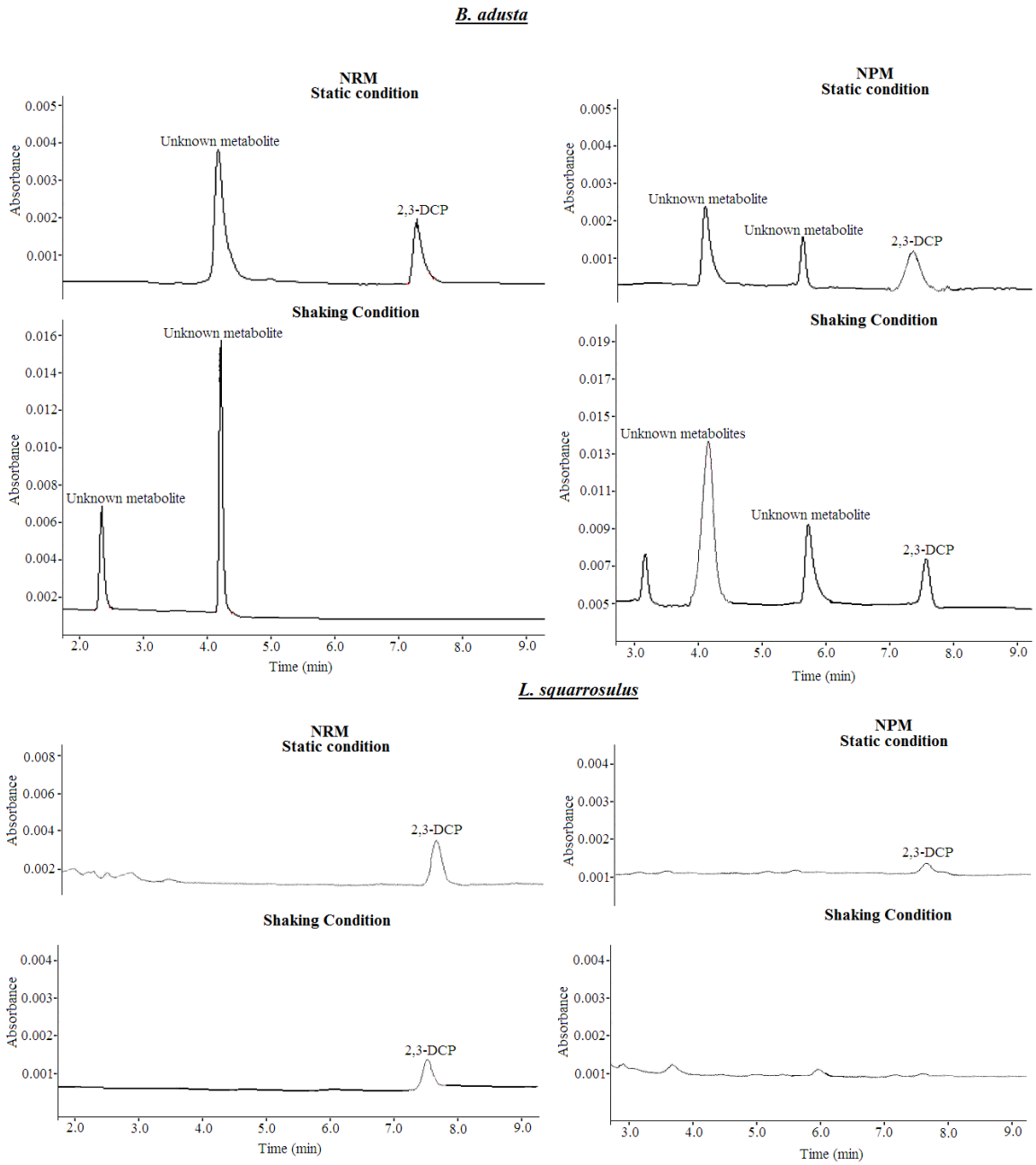

Fig.2:- HPLC chromatogram of 2,3-DCP on 10th day in B. adusta and on 15th day in L. squarrosulus and further peaks are unknown metabolites, which generated during biodegradation of 2,3-DCP by both varieties in both medium and conditions.

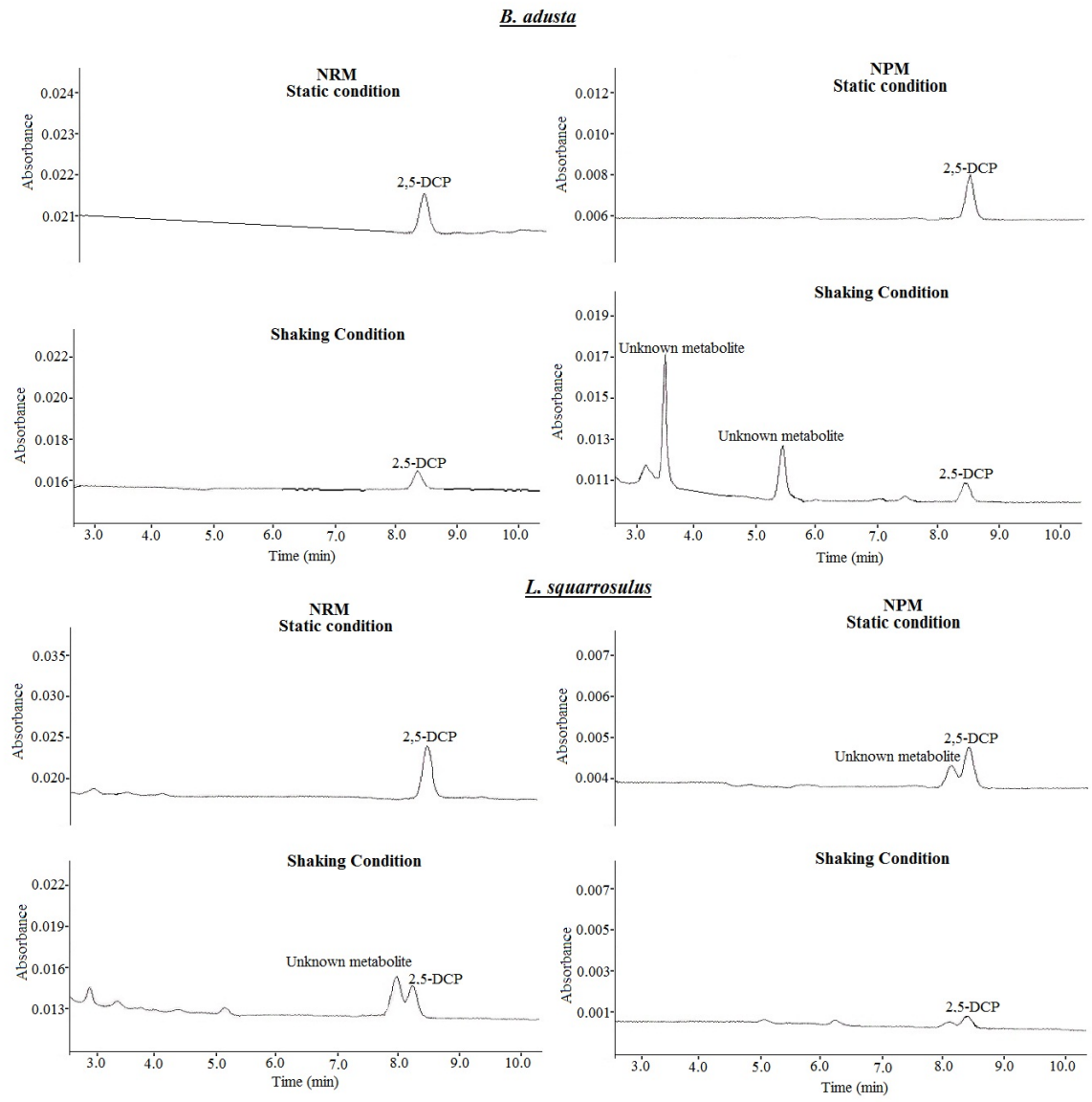

Fig.3:- HPLC chromatogram of 2,5-DCP on on 15th day and further peaks are unknown metabolites, which generated during biodegradation of 2,5-DCP by both varieties in both medium and conditions.

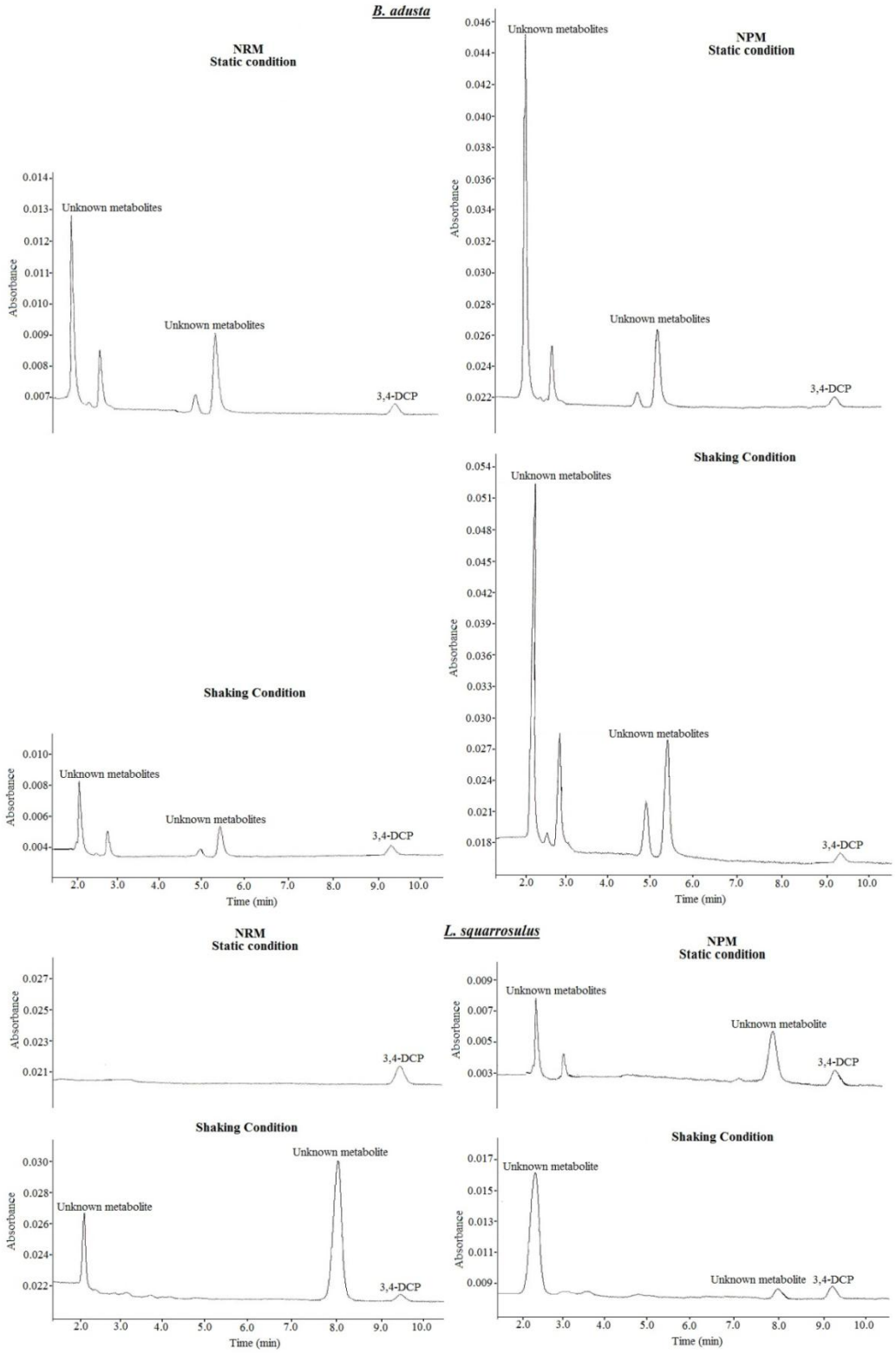

Fig.4:- HPLC chromatogram of 3,4-DCP on 15th day and further peaks are unknown metabolites, which generated during biodegradation of 3,4-DCP by both varieties in both medium and conditions.

Table-1:- Reduction (%) in mycelial growth of mushrooms by three different concentrations (mM) of DCPs (mean ± SD; n = 3)

Varieties	2,3-DCP			2,5-DCP			3,4-DCP		
	0.1	0.15	0.2	0.1	0.15	0.2	0.1	0.15	0.2
Agaricus bisporus	81.8 ± 0.13	100	100	81.8 ± 0.48	100	100	72.7 ± 0.21	100	100
Agrocybe aegerita	20.8 ± 0.21	37.5 ± 0.17	58.3 ± 0.32	33.3 ± 0.34	50.0 ± 0.47	62.5 ± 0.15	58.3 ± 0.36	87.5 ± 0.16	100
A. badia	23.1 ± 0.04	42.3 ± 0.05	61.5 ± 0.13	23.1 ± 0.91	34.6 ± 0.86	46.2 ± 0.63	69.2 ± 0.52	100	100
Agrocybe sp.	31.8 ± 0.17	54.5 ± 0.07	63.6 ± 0.75	40.9 ± 0.35	59.1 ± 0.92	72.7 ± 0.72	36.4 ± 0.37	45.4 ± 0.43	68.2 ± 0.26
Amanita caesarea	28.5 ± 0.42	54.3 ± 0.15	68.6 ± 0.91	48.6 ± 0.75	62.8 ± 0.45	82.8 ± 0.58	65.7 ± 0.29	77.1 ± 0.52	82.8 ± 0.52
Auricularia mesentrica	43.6 ± 0.06	59.5 ± 0.11	100	48.4 ± 0.05	56.3 ± 0.61	76.2 ± 0.11	64.3 ± 0.15	96.0 ± 0.67	100
Bjerkandera adusta	7.2 ± 0.32	26.9 ± 0.24	35.8 ± 0.43	7.6 ± 0.17	23.8 ± 0.65	32.6 ± 0.85	12.7 ± 0.63	25.0 ± 0.42	38.9 ± 0.74
Calocybe indica	33.3 ± 0.11	72.4 ± 0.06	100	9.5 ± 0.39	52.4 ± 0.57	71.4 ± 0.06	52.4 ± 0.85	66.6 ± 0.19	100
Chlorophyllum hortense	72.2 ± 0.32	100	100	72.2 ± 0.88	100	100	83.3 ± 0.99	100	100
Coprinus comatus	90.0 ± 0.07	100	100	90.0 ± 0.25	100	100	100	100	100
Coprinus sp.	50.0 ± 0.26	80.0 ± 0.03	100	30.0 ± 0.31	60.0 ± 0.87	70.0 ± 0.36	70.0 ± 0.74	90.0 ± 0.55	100
Descolia sp.	100	100	100	100	100	100	100	100	100
Flammulina sp.	26.5 ± 0.08	94.1 ± 0.78	100	47.1 ± 0.54	70.5 ± 0.90	88.2 ± 0.44	41.1 ± 0.43	94.1 ± 1.32	100
F. velutipes	90.6 ± 0.17	100	100	6.3 ± 0.63	68.7 ± 0.43	87.5 ± 0.96	56.2 ± 0.48	100	100
Ganoderma sp.	38.9 ± 0.24	52.8 ± 0.24	62.5 ± 0.17	12.2 ± 0.74	53.7 ± 0.48	68.7 ± 0.16	43.7 ± 0.29	68.7 ± 0.72	100
Gleophyllum sp.	42.8 ± 0.17	57.1 ± 0.37	71.4 ± 0.88	20.0 ± 0.86	31.4 ± 0.60	54.2 ± 0.26	25.7 ± 0.74	48.6 ± 0.47	65.7 ± 0.38
Gomphus flaccossus	11.1 ± 0.01	46.7 ± 0.64	100	11.1 ± 0.30	16.7 ± 0.37	33.3 ± 0.77	11.1 ± 0.53	46.7 ± 0.59	100
Hypholoma fasciculare	30.0 ± 0.04	55.0 ± 0.76	75.0 ± 0.64	50.0 ± 0.66	65.0 ± 0.76	100	50.0 ± 0.25	70.0 ± 0.11	100
Kuehneromyces sp.	22.2 ± 0.09	44.4 ± 0.09	88.9 ± 0.32	16.7 ± 0.34	33.3 ± 0.84	55.5 ± 0.61	72.2 ± 0.19	100	100
Lentinula edodes	51.1 ± 0.55	94.4 ± 1.21	100	38.9 ± 0.18	55.6 ± 0.29	72.2 ± 0.84	50.0 ± 0.72	72.2 ± 0.45	100
Lentinus squarrosulus	16.7 ± 0.21	25.0 ± 0.76	58.3 ± 0.48	16.7 ± 0.77	33.3 ± 0.64	44.4 ± 0.32	11.1 ± 0.16	27.8 ± 0.92	55.6 ± 0.92
Macrolepiota procera	62.5 ± 0.17	100	100	37.5 ± 0.04	100	100	100	100	100
M. rhacoides	46.7 ± 0.13	60.0 ± 0.25	66.7 ± 0.51	13.3 ± 0.16	33.3 ± 0.85	66.7 ± 0.47	66.7 ± 0.25	100	100
Marasmius sp.	100	100	100	20.0 ± 0.74	100	100	100	100	100
Mycena sp.	18.7 ± 0.03	50.0 ± 0.43	100	12.5 ± 0.36	31.2 ± 0.17	50.0 ± 0.76	18.7 ± 0.49	100	100
Pleurotus florida	53.3 ± 0.18	100	100	16.7 ± 0.52	33.3 ± 0.11	48.7 ± 0.59	20.0 ± 0.74	23.3 ± 0.60	100

P. citrinopileatus	16.1 ± 0.71	32.2 ± 0.17	51.6 ± 0.29	12.9 ± 0.75	51.6 ± 0.51	61.3 ± 0.93	29.0 ± 0.92	51.6 ± 0.58	61.3 ± 0.26
P. coremia sp. 1	40.0 ± 0.05	93.3 ± 0.89	100	20.0 ± 0.39	53.3 ± 0.87	80.0 ± 0.17	66.7 ± 0.15	80.0 ± 0.97	100
P. coremia sp. 2	41.8 ± 0.07	100	100	27.3 ± 0.30	33.3 ± 1.17	45.5 ± 0.25	24.8 ± 0.73	51.5 ± 0.77	100
P. coremia sp. 3	38.1 ± 0.15	60.0 ± 0.34	76.2 ± 0.71	38.1 ± 0.75	52.4 ± 0.36	66.7 ± 0.36	47.6 ± 0.22	69.5 ± 0.51	95.2 ± 0.79
P. cornucopiae	53.6 ± 0.04	61.5 ± 0.48	87.2 ± 0.74	38.5 ± 0.27	61.5 ± 0.83	74.4 ± 0.72	35.9 ± 0.50	48.7 ± 0.48	97.4 ± 0.46
P. cystidiosus sub sp. abalonus	17.6 ± 0.48	29.4 ± 0.22	41.2 ± 0.43	23.5 ± 0.54	35.3 ± 0.52	94.1 ± 0.58	52.9 ± 0.36	64.7 ± 0.66	82.4 ± 0.53
P. cystidiosus sub sp. cystidiosus	8.3 ± 0.03	45.8 ± 0.83	50.0 ± 0.27	37.5 ± 0.17	58.3 ± 0.64	70.8 ± 0.47	50.0 ± 0.73	66.7 ± 0.13	83.3 ± 0.36
P. djamor	7.9 ± 0.14	21.0 ± 0.26	36.8 ± 0.85	15.8 ± 0.11	34.2 ± 0.71	60.5 ± 0.49	28.9 ± 0.87	52.6 ± 0.19	100
P. flabellatus	21.1 ± 0.23	35.6 ± 0.74	100	7.8 ± 0.38	25.0 ± 0.69	47.2 ± 0.02	15.0 ± 0.33	46.7 ± 0.57	100
P. membranaceus 1	33.3 ± 0.17	55.6 ± 0.08	66.7 ± 0.94	22.2 ± 0.20	52.8 ± 0.77	72.2 ± 0.79	30.6 ± 0.29	52.8 ± 0.48	75.0 ± 0.91
P. membranaceus 2	50.0 ± 0.09	62.5 ± 0.29	72.5 ± 0.58	45.0 ± 0.16	57.5 ± 0.46	67.5 ± 0.53	40.0 ± 0.25	52.5 ± 0.32	100
P. opuntiae	40.0 ± 0.16	48.6 ± 0.53	60.0 ± 0.72	22.9 ± 0.38	54.3 ± 0.91	65.7 ± 0.83	37.1 ± 0.19	54.3 ± 0.79	65.7 ± 0.27
P. ostreatus	27.1 ± 0.73	59.6 ± 0.47	100	17.5 ± 0.33	24.7 ± 0.53	37.9 ± 0.96	27.7 ± 0.05	48.8 ± 0.63	100
P. platypus	10.0 ± 0.15	23.3 ± 0.88	40.0 ± 0.47	13.3 ± 0.29	36.7 ± 0.37	63.3 ± 0.58	26.7 ± 0.14	40.0 ± 0.47	50.0 ± 0.74
P. sajor-caju	41.8 ± 0.27	100	100	27.3 ± 0.74	33.3 ± 0.33	45.5 ± 0.61	24.8 ± 0.73	51.5 ± 0.72	100
P. sapidus	22.4 ± 0.13	56.9 ± 0.95	100	4.7 ± 0.09	29.4 ± 0.74	38.2 ± 0.49	22.9 ± 0.25	44.1 ± 0.22	100
Phanerochaete chrysosporium	8.2 ± 0.26	28.5 ± 0.36	42.3 ± 0.34	10.0 ± 0.28	30.7 ± 0.81	43.6 ± 0.61	16.6 ± 0.22	28.9 ± 0.18	40.5 ± 0.39
Phellinus badius	12.5 ± 0.05	43.7 ± 0.72	62.5 ± 0.25	25.0 ± 0.57	50.0 ± 0.65	75.0 ± 0.27	50.0 ± 0.84	75.0 ± 0.15	100
Stropharia rugoso annulata	38.1 ± 0.08	61.9 ± 0.48	90.5 ± 1.56	38.1 ± 0.38	52.4 ± 0.73	61.9 ± 0.09	57.1 ± 0.43	76.2 ± 0.09	100
Trametes cinnabarinus	33.3 ± 0.19	55.5 ± 0.46	100	44.4 ± 0.86	55.5 ± 0.48	88.9 ± 1.19	38.9 ± 0.12	100	100
Trametes hirsutus	13.8 ± 0.36	42.8 ± 0.17	57.1 ± 0.47	25.7 ± 0.39	40.0 ± 0.93	54.3 ± 0.48	22.8 ± 0.74	47.1 ± 0.82	65.7 ± 0.57
Tremella sp.	72.7 ± 0.14	100	100	70.9 ± 0.90	94.5 ± 0.25	100	100	100	100
Volvariella sp.	20.0 ± 0.04	50.0 ± 0.49	100	22.5 ± 0.73	37.5 ± 0.19	100	50.0 ± 0.48	87.5 ± 0.51	100
Volvariella volvacea	46.6 ± 0.57	69.9 ± 0.71	100	15.2 ± 0.26	48.3 ± 0.45	57.6 ± 0.32	44.9 ± 0.22	76.7 ± 0.68	100

Table-2:- Biodegradation of DCPs (16.3 μgml^{-1}) followed by HPLC (mean ± SD; n = 3)

Day Int.	2,3-DCP							
	B. adusta				L. squarrosulus			
	NRM		NPM		NRM		NPM	
	Static	Shaking	Static	Shaking	Static	Shaking	Static	Shaking
5	3.8 ± 0.90 b	6.3 ± 1.17 a	5.2 ± 1.04 ab	6.2 ± 1.05 a	3.7 ± 0.92 b	5.3 ± 1.05 ab	3.8 ± 1.08 b	5.4 ± 0.92 ab
10	9.2 ± 0.95 c	10.1 ± 1.05 bc	10.3 ± 1.25 bc	10.7 ± 1.06 bc	9.3 ± 0.90 c	11.8 ± 1.11 b	9.8 ± 1.11 c	12.3 ± 0.90 a
15	14.2 ± 1.01 b	16.3 ± 0.00 a	16.3 ± 0.00 a	16.3 ± 0.00 a	14.7 ± 1.05 ab	15.4 ± 0.95 ab	15.4 ± 0.95 ab	16.3 ± 0.00 a
20	16.3 ± 0.00*	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00
	2,5-DCP							
5	4.1 ± 0.91 bc	5.2 ± 0.95 ab	6.5 ± 0.97 ab	5.7 ± 0.91 ab	4.2 ± 1.10 bc	4.7 ± 0.95 ab	2.8 ± 1.12 c	5.3 ± 0.95 ab
10	8.5 ± 1.05 c	10.3 ± 0.98 bc	9.4 ± 0.92 bc	10.3 ± 1.05 bc	9.2 ± 0.95 bc	10.5 ± 0.91 b	10.5 ± 0.95 b	11.4 ± 1.15 a
15	10.1 ± 1.11 c	13.1 ± 1.05 b	14.8 ± 1.05 ab	15.3 ± 0.95 a	13.6 ± 1.05 ab	15.3 ± 1.05 a	15.0 ± 1.11 ab	15.5 ± 0.92 a
20	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00
	3,4-DCP							
5	3.9 ± 0.91 b	4.8 ± 1.07 ab	5.2 ± 0.81 ab	6.2 ± 1.05 a	3.8 ± 1.05 b	4.7 ± 1.73 ab	5.4 ± 0.92 ab	5.6 ± 1.05 a
10	8.3 ± 1.08 c	9.7 ± 1.00 bc	9.7 ± 0.90 bc	10.7 ± 1.00 b	9.1 ± 0.95 bc	10.1 ± 0.95 bc	9.8 ± 1.13 bc	10.5 ± 1.00 a
15	14.5 ± 1.22 ab	15.3 ± 0.95 ab	15.7 ± 1.05 ab	16.1 ± 0.15 a	13.9 ± 0.95 b	14.7 ± 0.95 ab	15.4 ± 1.07 ab	15.9 ± 1.12 a
20	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00	16.3 ± 0.00

Note: In each row different letters means significant difference ($p<0.05$)

* Compound was not detected through HPLC

Table 3:- Extracellular protein (μgml^{-1}) in B. adusta and L. squarrosulus with or without DCPs (mean ± SD; n = 3)

S.No.	Medium	Condition	B. adusta				L. squarrosulus			
			Day Interval				Day Interval			
			5	10	15	20	5	10	15	20
2,3-DCP	NRM	Static	2.3 ± 0.44 de	10.7 ± 0.77 e	19.0 ± 0.15 de	18.8 ± 0.40 cd	18.9 ± 0.15 b	19.9 ± 0.74 a	14.1 ± 0.68 b	8.7 ± 0.87 d
		Shaking	1.6 ± 0.34 ef	5.0 ± 0.82 h	12.0 ± 0.87 ef	7.0 ± 0.38 h	12.7 ± 0.79 c	18.4 ± 0.39 ab	11.2 ± 0.71 cd	6.6 ± 0.32 f
	NPM	Static	2.3 ± 0.73 de	8.4 ± 0.70 fg	10.6 ± 0.88 de	8.2 ± 0.65 gh	2.8 ± 0.24 g	7.3 ± 0.61 fg	8.0 ± 0.89 efg	7.6 ± 0.70 def
		Shaking	1.6 ± 0.60 ef	6.7 ± 0.53gh	14.4 ± 0.79 ef	9.0 ± 0.46 gh	3.7 ± 0.10 fg	9.0 ± 0.64 ef	13.3 ± 0.36 b	3.9 ± 0.46 g
2,5-DCP	NRM	Static	12.3 ± 1.02 a	16.1 ± 0.80 c	18.7 ± 0.84 a	12.5 ± 0.71 e	5.9 ± 0.43 e	5.0 ± 0.50 hi	12.7 ± 0.74 bc	11.7 ± 0.67 bc
		Shaking	4.1 ± 0.88 cd	13.6 ± 0.67 d	14.2 ± 0.83 cd	19.3 ± 0.54 c	5.1 ± 0.58 ef	8.6 ± 0.39 f	9.1 ± 0.72 efg	16.3 ± 0.43 a
	NPM	Static	12.1 ± 0.59 a	18.5 ± 0.50 b	20.3 ± 0.68 a	11.8 ± 0.47 ef	3.2 ± 0.44 g	6.6 ± 0.18 gh	7.3 ± 0.28 g	8.5 ± 0.50 de
		Shaking	7.7 ± 0.68 b	9.8 ± 0.96 ef	9.8 ± 0.47 b	9.9 ± 1.01 fg	3.7 ± 0.80 fg	4.5 ± 0.14 i	8.0 ± 0.76 efg	12.8 ± 0.22 b
3,4-DCP	NRM	Static	9.0 ± 0.59 b	24.6 ± 0.84 a	18.1 ± 0.86 b	16.8 ± 0.52 d	8.2 ± 0.55 d	7.8 ± 0.61 fg	19.1 ± 0.76 a	11.9 ± 0.87 bc
		Shaking	3.4 ± 0.78 cde	9.2 ± 0.64 ef	6.8 ± 0.56 cde	16.7 ± 0.78 d	21.1 ± 0.51 a	17.4 ± 0.78 bc	9.5 ± 0.43 de	10.9 ± 0.55 c
	NPM	Static	1.5 ± 0.54 ef	7.8 ± 0.80 fg	14.1 ± 0.98 ef	9.4 ± 1.08 g	9.3 ± 0.72 d	8.3 ± 0.49 fg	9.1 ± 0.25 efg	7.5 ± 0.37 def
		Shaking	4.1 ± 1.20 cd	6.8 ± 0.90 gh	8.0 ± 1.44 cd	12.6 ± 0.81 e	9.4 ± 0.49 d	13.5 ± 0.29 d	8.0 ± 0.52 efg	6.7 ± 0.98 ef
Control	NRM	Static	4.4 ± 0.88 c	7.9 ± 0.44 fg	12.1 ± 0.35 c	16.9 ± 0.62 d	3.9 ± 0.46 fg	10.7 ± 1.11 e	11.2 ± 0.77 efg	11.4 ± 0.36 bc
		Shaking	nd	nd	12.1 ± 0.42 f	34.5 ± 0.49 a	8.3 ± 0.38 d	16.2 ± 0.82 c	19.4 ± 0.44 a	12.6 ± 0.54 bc
	NPM	Static	nd	nd	3.4 ± 0.35 f	30.9 ± 0.15 b	3.3 ± 0.52 g	4.6 ± 1.00 i	7.5 ± 0.23 fg	6.1 ± 0.64 f
		Shaking	nd	nd	5.8 ± 0.19 f	31.9 ± 1.13 b	3.4 ± 0.96 g	7.4 ± 0.43 fg	9.3 ± 0.54 def	6.3 ± 0.61 f

Note: In each column different letters means significant difference ($p<0.05$)
nd- not detected

Table-4:- Extracellular laccase production (Ul^{-1}) in B. adusta and L. squarrosulus with or without DCPs (mean ± SD; n = 3)

S.No.	Medium	Condition	B. adusta				L. squarrosulus			
			Day Interval				Day Interval			
			5	10	15	20	5	10	15	20
2,3-DCP	NRM	Static	nd	nd	nd	nd	21.6 ± 0.31 j	108.5 ± 0.35 d	59.1 ± 0.35 d	34.6 ± 0.15 de
		Shaking	nd	nd	nd	nd	96.7 ± 0.69 c	47.7 ± 0.89 g	4.9 ± 0.74 l	3.7 ± 2.63 i
	NPM	Static	nd	nd	nd	2.6 ± 0.82 bcd	28.3 ± 1.02 i	129.9 ± 2.37 c	88.3 ± 0.92 b	83.7 ± 0.78 a
		Shaking	nd	nd	nd	3.2 ± 0.50 bc	61.7 ± 1.07 e	28.0 ± 0.81 i	24.9 ± 0.93 h	17.8 ± 1.44 g
2,5-DCP	NRM	Static	nd	nd	1.9 ± 0.70 b	4.2 ± 0.71 ab	17.6 ± 0.40 k	75.4 ± 0.15 e	43.1 ± 0.29 f	18.6 ± 0.21 g
		Shaking	nd	nd	nd	2.5 ± 0.96 cd	44.7 ± 1.55 f	6.6 ± 0.30 l	nd	nd
	NPM	Static	nd	nd	nd	nd	23.4 ± 0.46 j	63.3 ± 0.74 f	55.0 ± 1.55 e	46.6 ± 0.96 c
		Shaking	nd	1.9 ± 0.20 b	nd	nd	36.5 ± 0.15 g	18.6 ± 0.91 jk	22.0 ± 1.72 i	33.9 ± 0.49 e
3,4-DCP	NRM	Static	nd	nd	nd	5.7 ± 0.62a	78.7 ± 0.53 d	179.0 ± 0.62 a	82.6 ± 0.26 c	20.4 ± 0.15 fg
		Shaking	nd	nd	nd	3.6 ± 1.21 bc	152.7 ± 0.08 b	20.6 ± 1.17 j	8.5 ± 0.65 k	19.2 ± 0.49 fg
	NPM	Static	nd	nd	nd	nd	76.9 ± 1.36 d	144.9 ± 0.91 b	98.9 ± 0.90 a	85.0 ± 1.16 a
		Shaking	nd	nd	nd	1.2 ± 0.50 de	169.2 ± 0.92 a	37.6 ± 1.82 h	32.9 ± 1.65 g	22.7 ± 1.74 f
Control	NRM	Static	3.3 ± 1.30 a	5.4 ± 0.70 a	2.3 ± 0.21 a	1.5 ± 0.57 de	16.6 ± 0.32 k	29.1 ± 0.29 i	18.8 ± 0.47 j	12.7 ± 0.76 h
		Shaking	nd	nd	nd	nd	44.0 ± 1.04 f	28.1 ± 1.81 i	16.9 ± 0.55 j	10.6 ± 0.32 h
	NPM	Static	nd	nd	nd	nd	28.0 ± 0.81 i	45.0 ± 1.04 g	57.2 ± 0.51 de	64.0 ± 1.65 b
		Shaking	nd	nd	nd	nd	32.2 ± 0.84 h	16.6 ± 1.28 k	32.7 ± 0.82 g	37.7 ± 2.18 d

Note: In each column different letters means significant difference ($p<0.05$)
nd- not detected

Table-5:- AAO activity (Ul^{-1}) in B. adusta and L. squarrosulus with or without DCPs (mean ± SD; n = 3)

S.No.	Medium	Condition	B. adusta				L. squarrosulus			
			Day Interval				Day Interval			
			5	10	15	20	5	10	15	20
2,3-DCP	NRM	Static	nd	14.5 ± 0.10 f	13.8 ± 0.30 i	16.9 ± 0.56 l	3.3 ± 0.06 d	9.0 ± 0.41 c	12.0 ± 0.78 b	5.8 ± 0.57 b
		Shaking	22.2 ± 0.86 a	75.4 ± 1.00 a	117.6 ± 1.84 b	146.9 ± 0.43 b	nd	nd	nd	4.8 ± 1.66 bc
	NPM	Static	nd	4.2 ± 0.06 k	14.3 ± 0.95 i	21.9 ± 0.23 k	6.9 ± 0.79 a	2.0 ± 0.62 fg	nd	nd
		Shaking	nd	15.2 ± 0.87 ef	33.9 ± 0.70 f	38.4 ± 0.61 g	4.8 ± 0.76 bc	4.7 ± 0.95 e	8.7 ± 0.32 c	18.3 ± 0.74 a
2,5-DCP	NRM	Static	3.9 ± 0.76 fg	6.7 ± 0.50 j	9.9 ± 0.66 j	4.8 ± 0.71 n	nd	3.7 ± 0.55 ef	2.5 ± 0.36 ef	nd
		Shaking	22.7 ± 0.81 a	48.0 ± 0.75 b	154.7 ± 2.17 a	131.9 ± 1.82 c	2.5 ± 0.85 de	4.6 ± 0.58 e	2.1 ± 0.11 f	1.6 ± 0.10 d
	NPM	Static	nd	5.5 ± 0.87 jk	9.0 ± 0.60 jk	27.9 ± 0.50 j	1.3 ± 0.21 ef	2.4 ± 0.26 f	2.7 ± 0.15 ef	nd
		Shaking	5.1 ± 0.61 f	11.0 ± 0.25 hi	16.1 ± 0.29 hi	35.8 ± 0.72 h	5.6 ± 0.42 ab	13.5 ± 1.19 b	9.4 ± 0.32 c	5.4 ± 0.87 b
3,4-DCP	NRM	Static	7.5 ± 0.66 e	11.1 ± 0.66 hi	22.8 ± 0.40 g	8.4 ± 0.96 m	nd	nd	nd	nd
		Shaking	7.2 ± 0.65 e	17.0 ± 0.58 e	37.5 ± 0.75 e	69.5 ± 0.80 e	nd	3.5 ± 0.44 ef	6.8 ± 0.74 d	nd
	NPM	Static	2.0 ± 0.36 h	13.2 ± 0.68 fg	22.8 ± 0.84 g	8.5 ± 0.52 m	1.5 ± 0.38 e	2.4 ± 0.43 f	nd	nd
		Shaking	10.3 ± 0.99 d	48.2 ± 0.59 b	112.4 ± 0.73 c	88.3 ± 0.66 d	nd	7.2 ± 0.87 cd	5.6 ± 0.10 d	3.6 ± 0.49 c
Control	NRM	Static	2.3 ± 0.40 gh	11.9 ± 0.70 gh	18.1 ± 0.50 h	33.0 ± 0.65 i	6.2 ± 0.65 ab	8.2 ± 0.45 c	3.6 ± 0.55 e	nd
		Shaking	4.3 ± 0.25 f	44.4 ± 0.74 c	111.7 ± 1.36 c	163.1 ± 1.46 a	nd	5.6 ± 0.51 de	19.8 ± 0.92 a	nd
	NPM	Static	15.5 ± 1.05 c	9.7 ± 0.47 i	6.1 ± 0.38 k	4.5 ± 0.25 n	3.8 ± 0.79 cd	4.7 ± 1.44 e	nd	nd
		Shaking	18.9 ± 0.64 b	25.5 ± 0.99 d	41.6 ± 0.45 d	57.5 ± 0.95 f	3.6 ± 0.72 cd	16.7 ± 1.00 a	11.8 ± 0.50 b	5.6 ± 0.61 b

Note: In each column different letters means significant difference ($p<0.05$)
nd- not detected

Table-6:- LiP activity (Ul^{-1}) in B. adusta and L. squarrosulus with or without DCPs (mean ± SD; n = 3)

S.No.	Medium	Condition	B. adusta				L. squarrosulus			
			Day Interval				Day Interval			
			5	10	15	20	5	10	15	20
2,3-DCP	NRM	Static	nd	4.8 ± 0.96 d	nd	nd	nd	nd	nd	nd
		Shaking	nd	2.3 ± 0.57 e	10.3 ± 0.85 c	7.6 ± 0.67 c	nd	7.4 ± 0.81 d	18.4 ± 1.10 a	nd
	NPM	Static	nd	4.8 ± 0.68 d	nd	nd	nd	10.0 ± 0.50 c	6.0 ± 0.61 d	4.8 ± 0.91 cd
		Shaking	nd	4.8 ± 0.41 d	6.7 ± 0.93 de	4.2 ± 0.90 d	14.2 ± 0.91 b	18.5 ± 0.61 a	11.7 ± 0.57 b	5.9 ± 2.20 bc
2,5-DCP	NRM	Static	nd	1.4 ± 0.61 ef	2.9 ± 0.75 f	15.8 ± 1.31 a	nd	1.3 ± 0.21 f	nd	nd
		Shaking	nd	10.4 ± 0.84 b	18.2 ± 0.52 a	9.6 ± 1.12 b	nd	nd	nd	9.4 ± 0.58 a
	NPM	Static	nd	nd	nd	2.4 ± 0.76 e	7.8 ± 0.46 c	13.5 ± 0.70 b	7.5 ± 0.89 cd	5.5 ± 0.49 c
		Shaking	5.1 ± 0.61 b	4.3 ± 0.67 d	nd	nd	20.4 ± 1.10 a	nd	8.6 ± 0.20 c	nd
3,4-DCP	NRM	Static	nd	1.9 ± 0.31 e	7.1 ± 0.35 de	15.0 ± 0.74 a	3.4 ± 0.67 d	nd	nd	nd
		Shaking	nd	nd	8.3 ± 0.92 d	15.5 ± 0.81 a	nd	nd	8.3 ± 1.00 c	2.9 ± 0.21 de
	NPM	Static	nd	9.8 ± 0.12 bc	7.5 ± 0.90 de	nd	7.3 ± 0.81 c	12.4 ± 1.21 b	7.6 ± 0.83 cd	3.4 ± 0.10 d
		Shaking	nd	8.5 ± 0.20 c	14.1 ± 0.81 c	5.2 ± 0.1 d	nd	8.0 ± 0.74 d	19.7 ± 0.55 a	nd
Control	NRM	Static	nd	16.5 ± 0.60 a	nd	nd	nd	nd	nd	6.2 ± 0.50 bc
		Shaking	nd	nd	6.3 ± 0.95 e	nd	nd	3.9 ± 0.70 e	11.9 ± 1.41 b	7.6 ± 0.45 ab
	NPM	Static	10.0 ± 0.85 a	1.1 ± 0.62 ef	nd	nd	nd	7.3 ± 0.66 d	5.8 ± 0.75 d	2.9 ± 0.30 de
		Shaking	5.6 ± 0.81 b	4.5 ± 0.15 d	nd	nd	nd	10.6 ± 0.53 c	7.0 ± 0.67 cd	1.4 ± 0.21 ef

Note: In each column different letters means significant difference ($p<0.05$)
nd- not detected

Table-7:- MnP (Ul^{-1}) in B. adusta and L. squarrosulus with or without DCPs (mean ± SD; n = 3)

S.No.	Medium	Condition	B. adusta				L. squarrosulus			
			Day Interval				Day Interval			
			5	10	15	20	5	10	15	20
2,3-DCP	NRM	Static	nd	33.8 ± 0.81f	14.8 ± 0.81 h	nd	34.6 ± 2.37 b	37.6 ± 2.25 hi	339.6 ± 6.27 a	86.6 ± 2.97 h
		Shaking	nd	nd	nd	3.9 ± 0.74 f	381.7 ± 7.05 a	180.7 ± 7.91 e	108.7 ± 4.00 g	64.1 ± 1.95 i
	NPM	Static	nd	nd	10.9 ± 0.75 i	18.8 ± 1.27 e	36.5 ± 2.66 b	249.1 ± 5.03 a	289.1 ± 4.75 d	235.1 ± 4.07 b
		Shaking	nd	25.1 ± 0.95 g	118.3 ± 1.05 b	34.9 ± 1.33 c	34.1 ± 2.95 b	nd	nd	192.8 ± 5.20 c
2,5-DCP	NRM	Static	12.3 ± 0.95 e	48.5 ± 0.60 c	34.6 ± 0.84 f	26.6 ± 1.76 d	34.0 ± 1.75 b	36.2 ± 1.05 hi	207.6 ± 2.65 f	160.3 ± 2.15 e
		Shaking	16.1 ± 0.78 cd	37.0 ± 0.85 e	nd	nd	33.6 ± 1.61 b	nd	nd	nd
	NPM	Static	7.3 ± 0.66 f	17.3 ± 1.79 h	9.2 ± 0.15 i	nd	34.8 ± 1.44 b	197.9 ± 5.82 d	253.8 ± 5.12 l	309.6 ± 7.46 a
		Shaking	9.8 ± 0.56 e	13.9 ± 0.47 ij	17.8 ± 0.65 h	nd	35.0 ± 0.82 b	7.7 ± 0.46 j	nd	nd
3,4-DCP	NRM	Static	nd	37.2 ± 1.27 e	44.6 ± 0.06 e	17.9 ± 1.01 e	35.9 ± 2.00 b	44.6 ± 1.13 hi	306.5 ± 3.65 c	138.4 ± 1.50 f
		Shaking	nd	11.8 ± 0.55 j	3 4.9 ± 1.46 f	26.7 ± 1.46 d	25.6 ± 0.80 cd	34.7 ± 1.82 i	53.2 ± 2.50 h	37.7 ± 2.20 k
	NPM	Static	nd	4.2 ± 0.45 k	8.2 ± 0.55 i	nd	32.9 ± 1.95 bc	47.2 ± 1.78 h	251.5 ± 3.84 e	171.8 ± 3.53 d
		Shaking	nd	15.4 ± 1.64 hi	33.1 ± 0.62 fg	33.9 ± 2.21 c	nd	nd	nd	48.6 ± 1.04 j
Control	NRM	Static	45.0 ± 1.30 a	105.7 ± 0.81 a	215.9 ± 3.02 a	215.7 ± 3.35 a	24.0 ± 1.31 d	225.7 ± 5.57 b	326.9 ± 5.86 b	194.9 ± 3.81 c
		Shaking	17.6 ± 0.66 c	66.6 ± 0.70 b	75.1 ± 0.26 d	47.9 ± 0.60 b	37.8 ± 1.88 b	209.8 ± 4.70 c	350.6 ± 4.80 a	104.3 ± 1.87 g
	NPM	Static	25.8 ± 0.30 b	43.4 ± 1.07 d	106.8 ± 0.64 c	48.7 ± 1.13 b	32.8 ± 2.26 bc	165.4 ± 5.42 f	253.9 ± 4.76 e	303.8 ± 2.54 a
		Shaking	15.1 ± 2.51 d	37.4 ± 1.70 e	31.2 ± 0.87 g	33.9 ± 2.47 c	nd	86.5 ± 0.86 g	31.8 ± 2.89 i	36.9 ± 1.70 k

Note: In each column different letters means significant difference ($p<0.05$)
nd- not detected

Acknowledgement:-

The authors are thankful to the Director, Directorate of Mushroom Research, Solan (H.P.) for their support. Thanks are also given to ICAR for providing Application of Microorganisms in Agriculture and Allied Sectors (AMAAS) funded project.

References:-

1. Adejumo TO, Awosanya OB (2005) Proximate and mineral composition of four edible mushroom species from South Western Nigeria. Afr J Biotechnol 4(10):1084-1088.
2. Annachhatre AP, Gheewala SH (1996) Biodegradation of chlorinated phenolic compounds. Biotechnol. advances, 14(1):35-56.
3. Aranda E, Ullrich R, Hofrichter M (2010) Conversion of polycyclic aromatic hydrocarbons, methyl naphthalenes and dibenzofuran by two fungal peroxygenases. Biodegrad 21:267-281.
4. Belcarz A, Ginalska G, Kornillowicz-Kowalska T (2005) Extracellular enzyme activities of Bjerkandera adusta R59 soil strain, capable of daunomycin and humic acids degradation. Appl Microbiol Biotechnol 68:686-694.
5. Beynon KI, Crosby DG, Korte F, Still GG, Vonk JW, Greve PA (1981) Environmental chemistry of pentachlorophenol: a special report on pentachlorophenol in the environment. Pure Appl Chem 53: 1052-1080.
6. Bollag JM, Horong LC, Rao MA, Gianfreda L (2003) Enzymatic oxidative transformation of chlorophenol mixtures. J Environ Qual 32:63-69.
7. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein dye binding. Analyt Biochem 72:248-254.
8. Camarero S, Sarkar S, Ruiz-Duenas FJ, Martínez MJ, Martínez, AT (1999) Description of a versatile peroxidase involved in natural degradation of lignin that has both Mn peroxidase and lignin-peroxidase substrate binding sites. J Biol Chem 274:10324-10330.
9. Cameron MD, Timofeevski S, Aust SD (2000) Enzymology of Phanerochaete chrysosporium with respect to the degradation of recalcitrant compounds and xenobiotics. App Microbiol Biotechnol 54:751-758.
10. Chen DM, Bastias BA, Taylor AFS, Cairney JWG (2003) Identification of laccase-like genes in ectomycorrhizal basidiomycetes and transcriptional regulation by nitrogen in Piloderma byssinum. New Phytol 157:547-554.
11. Cohen R, Hadar Y, Yarden O (2001) Transcript and activity levels of different Pleurotus ostreatus peroxidases are differentially affected by Mn^{2+}. Environ Microbiol 3(5):312-322.
12. Collins PJ, Field JA, Teunissen P, Dobson AW (1997) Stabilization of Lignin Peroxidases in white-rot fungi by Tryptophan. Appl Environ Microbiol 63(7):2543-2548.
13. Contrerasa S, Rodriguez M, Al Momania F, Sansa C, Esplugasa S (2003) Contribution of the ozonation pretreatment to the biodegradation of aqueous solutions of 2,4-dichlorophenol. Water Res 37:3164-3171.
14. de Jong E, Cazemier AE, Field JA, de Bont JAM (1994) Physiological role of chlorinated aryl alcohols biosynthesized De Novo by the white-rot fungus Bjerkandera sp. strain BOS55. Appl Environ Microbiol 60:271-277.
15. Dec J, Bollag JM (1990) Detoxification of substituted phenols by oxidoreductase enzymes through polymerization reactions. Arch Environ Contam Toxicol 19:543-550.
16. Duran R, Deschler C, Precigou S, Goulas P (2002) Degradation of chlorophenols by Phanerochaete chrysosporium: effect of 3,4-dichlorophenol on extracellular peroxidase activities. Appl Microbiol Biotechnol 59:284-288.
17. Espindola LHS, Espindola FS, Freitas GR, Brandeburgo MAM (2007) Biodegradtion of Red 40 dye by the Pleurotus sp. Florida Biosci J 23(3):90-93.
18. Faraco V, Pezzella C, Giardina P, Sannia G (2009) Bioremediation of colored industrial wastewaters by the white-rot fungi Phanerochaete chrysosporium and Pleurotus ostreatus and their enzymes. Biodegrad 20:209-220.
19. Field JA, de Jong E, Costa GF, de Bont JAM (1993) Screening of ligninolytic fungi applicable to biodegradation of xenobiotics. Trends Biotechnol 11:44-49.
20. Grey R, Hofer C, Schlosser D (1998) Degradation of 2-chlorophenol and formation of 2-chloro-1,4-benzoquinone by mycelia and cell free crude culture liquids of Trametes versicolor in relation to extracellular laccase activity. J Basic Microbiol 38(5-6): 371-382.
21. Han M-J, Chai H-T, Song H-G (2005) Purification and characterization of laccase from the white-rot fungus Trametes versicolour. The J Microbiol 46(6):555-560.
22. Hatakka A (2001) Biodegradation of lignin. In Hofrichter, M., Steinbüchel, A. (Eds.) Lignin, Humic Substances and Coal, Vol. 1, Wiley-VCH, Weinheim, Germany, pp 129-180.
23. Heinfling A, Martínez MJ, Martínez AT, Bergbauer M, Szewzyk U (1998a) Purification and characterization of peroxidases from the dye decolorizing fungus Bjerkandera adusta. FEMS Microbiol Lett 165:43-50.
24. Heinfling A, Ruiz-Dueñas FJ, Martínez MJ, Bergbauer M, Szewzyk U, Martínez AT (1998b) A study on reducing substrates of manganese-oxidizing peroxidases from Pleurotus eryngii and Bjerkandera adusta. FEBS Lett 428:141-146.
25. Iwahara K, Honda Y, Watanabe T, Kuwahara M (2000) Polymerization of guaiacol by lignin-degrading manganese peroxidase from Bjerkandera adusta in aqueous organic solvents. Appl Microbiol Biotechnol 54:104-111.
26. Joshi DK, Gold MH (1993) Degradation of 2,4,5-trichlorophenol by the lignin degrading basidiomycete Phanerochaete chrysosporium. Appl Environ Microbiol 59(6):1779-1785.
27. Kadhim H, Graham C, Barrat P, Evans CS, Rastall RA (1999) Removal of phenolic compounds in water using Coriolus versicolor grown on wheat bran. Enzyme Microb Technol 24:303-307.

28. Kishino T, Kobayashi K (1994) Relation between the chemical structures of chlorophenols and their dissociation constants and partition coefficients in several solvent-water systems. Water Res 28(7):1547-1552.
29. Leontievsky AA, Myasoedova NM, Golovleva LA, Sedaraty M, Evans CS (2002) Adaptation of the white rot basidiomycete Panus tigrinus for transformation of high concentrations of chlorophenols. Appl Microbiol Biotechnol 59(4):599-604.
30. Marzullo L, Cannio R, Giardina P, Santini MT, Sannia G (1995) Veratryl alcohol oxidase from Pleurotus ostreatus participates in lignin biodegradation and prevents polymerization of laccase-oxidized substrates. J Biol Chem 270:3823-3827.
31. Michizoe J, Gato M, Furusaki S (2001) Catalytic activity of laccase hosted in reversed micelles. J Biosci Bioengin 92(1):67-71.
32. Mileski GJ, Bumpus JA, Jurek MA, Aust SD (1988) Biodegradation of pentachlorophenol by the white rot fungus Phanerochaete chrysosporium. Appl Environ Microbiol 54:2885-2889.
33. Milstein O, Gersonde R, Huttermann A, Chen M-J, Meister JJ (1992) Fungal biodegradation of lignopolystyrene graft copolymers. Appl Environ Microbiol 58:3225-3232.
34. Oberg G, Brunberg H, Hjelm O (1997) Production of organically bound chlorine during degradation of birch wood by common white-rot fungi. Soil bio Biochem 29:191-197.
35. Okamoto K, Yanase H (2002) Aryl alcohol oxidases from the white-rot basidiomycete Pleurotus ostreatus. Mycosci 43:391-395.
36. Pointing SB (2001) Feasibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 57:20-33.
37. Reddy GVB, Gelpke MDS, Gold M (1998) Degradation of 2,4,6-Trichlorophenol by Phanerochaete chrysosporium: Involvement of Reductive Dechlorination. J Bacteriol 180(19):5159-5164.
38. Reddy CA, D'souza TM (1994) Physiology and molecular biology of the lignin peroxidases of Phanerochaete chrysosporium. FEMS Microbiol Rev 13:137-152.
39. Reddy CA, Mathew Z (2001) Bioremediation potential of white-rot fungi. In: Gadd, G.M. (Ed.) Fungi in bioremediation, Cambridge University Press, pp. 52-78.
40. Reddy GVB, Gold MH (2000) Degradation of pentachlorophenol by Phanerochaete chrysosporium: intermediates and reactions involved. Microbiol 146:405-414.
41. Roy-Arcand L, Archibald FS (1991) Direct dechlorination of chlorophenolic compounds by laccases from Trametes (Coriolus) versicolor. Enzyme Microb Technol 13:194-203.
42. Ruggiero P, Sarkar JM, Bollag J-M (1989) Detoxification of 2,4-dichlorophenol by a laccase immobilized on soil or clay. Soil Sci 147:361-370.
43. Ruttimann-Johnson C, Lamar RT (1996) Polymerization of pentachlorophenol and ferulic acid by fungal extracellular lignindegrading enzymes. Appl Environ Microbiol 62:3890-3893.
44. Schellenberg K, Leuenberger C, Schwarzenbach RP (1984) Sorption of Chlorinated Phenols by Natural Sediments and Aquifer Materials. Environ. Science Tech 18:652-657.
45. Shiu WY, Ma KC, Varhanickova D, Mackay D (1994) Chlorophenols and alkylphenols: A review and correlation of environmentally relevant properties and fate in an evaluative environment. Chemosp 29:1155-1224.
46. Shuttleworth KL, Bollag JM (1986) Soluble and immobilized laccase as catalysts for the transformation of substituted phenols. Enzyme Microb Technol 8:171-177.
47. Steffen KT (2003) Degradation of recalcitrant biopolymers and polycyclic aromatic hydrocarbons by litter-decomposing basidiomycetous fungi. Dissertationes Biocentri Viikki Universitatis Helsingiensis
48. Steiert JG, Crawford RL (1985) Microbial degradation of chlorinated phenols. Trends in Biotechnol 12:300-306.
49. Teunissen PJM, Field JA (1998) 2-chloro-1,4-dimethoxybenzene as a novel catalytic cofactor for oxidation of anisyl alcohol by lignin peroxidase. Appl Environ Microbiol 64:830-835.
50. Tripathi A, Upadhyay RC, Singh S (2012) Extracellular ligninolytic enzymes in Bjerkandera adusta and Lentinus squarrosulus. Ind J Microbiol 52(3):381-387.
51. Ullah MA, Bedford CT, Evans CS (2000a) Reactions of pentachlorophenol with laccase from Coriolus versicolor. Appl Microbiol Biotechnol 53:230-234.
52. Ullah MA, Kadhim H, Rastall RA, Evans CS (2000b) Evaluation of solid substrates for enzyme production by Coriolus versicolor, for use in bioremediation of chlorophenols in aqueous effluents. Appl Microbiol Biotechnol 54(6):832-837.
53. Valli K, Gold MH (1991) Degradation of 2,4-dichlorophenol by the lignin-degrading fungus Phanerochaete chrysosporium. J Bacteriol 173:345-352.
54. Varela E, Martínez AT, Martínez MJ (1999) Molecular cloning of aryl alcohol oxidase from the fungus Pleurotus eryngii, an enzyme involved in lignin degradation. Biochem J 341:113-117.
55. Watling R, Harper DB (1998) Chloromethane production by wood rotting fungi and an estimate of the global flux to the atmosphere. Mycol Res 102:769-787.