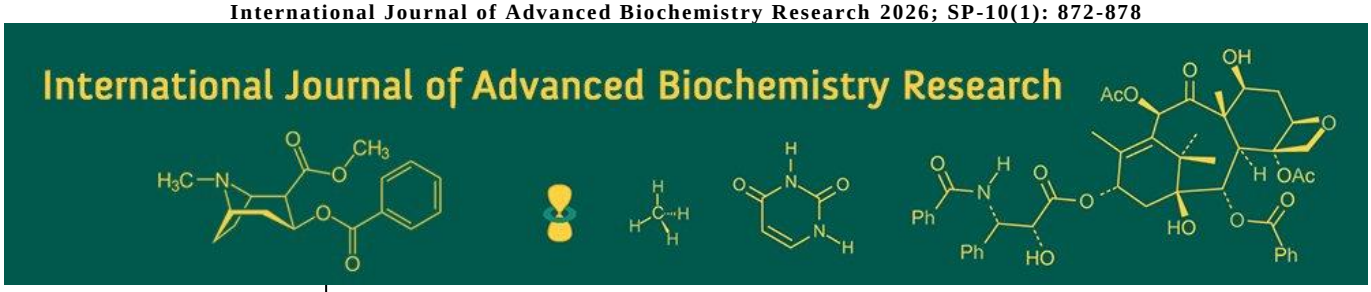

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(1): 872-878
www.biochemjournal.com
Received: 06-12-2025
Accepted: 08-01-2026

Bhavika Pandya
Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

Amee Taunk
Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

Susy Albert
Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

Corresponding Author:
Amee Taunk
Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

A novel approach of using Ash gourd as a substrate for enhancing lingo-cellulolytic enzyme production in mono and co-culture of two white rot fungi

Bhavika Pandya, Amee Taunk and Susy Albert

DOI: https://www.doi.org/10.33545/26174693.2026.v10.i1Sk.7108

Abstract
White rot fungi are known to degrade wood due to its ability to produce various lignocellulolytic enzymes. The synergistic effect of fungal coculture on lignocellulosic degradation is not much known. In the present study lignocellulolytic enzyme activity of *Irpex lacteus* and *Daedaleopsis confragosa* were compared in mono culture and coculture supplemented with various concentrations of Ash gourd (*Benincasa hispida* Cogn.). The result revealed that gradual increasing in the concentration of ash gourd from 1-10% leads to enhancement of enzyme activity but 5% concentration proved to be best for all the lignocellulolytic enzyme activity after that decline in the enzyme activity was noticed which was still higher than the activity in the control medium with no ash gourd. *I. lacteus* showed an absence of LiP enzyme activity throughout the present study. Both the monocultures and coculture showed an absence of cellulase enzyme activity in control as well as media supplemented with different concentrations of ash gourd pulp. LiP, MnP, Laccase, AAO and Xylanase activity enhanced in 5% ash gourd pulp in monoculture as well as in coculture of *I. lacteus* and *D. confragosa*.

Keywords: Ash gourd, lignocellulolytic enzymes, *Irpex lacteus* and *Daedaleopsis confragosa*

Introduction
It is already known that basidiomycetes fungal isolates with higher secretion of ligninolytic enzymes as well as enzymes having unique capacity to be used for their biotechnological, environmental and industrial application (Kiiskinen *et al*., 2004; Mikiashvili *et al*., 2004) [19, 27]. But implementing of these potential applications of enzymes at industrial scale needs large amount of these enzymes which should have been coast effective also. So, selection of the organisms having ability to produce such enzymes in greater amount needs to be carried out.

White-rot fungi (basidiomycetes) are having distinctive ability to degrade all components of wood due to their competency to produce cell wall degrading enzymes. Almost all the enzymes produced are extracellular. Which are oxidative and hydrolytic in nature. So, they are also known as lignocellulolytic enzymes which are responsible for degradation of lignocellulose (Liu *et al*., 2013a, van den Brink and de Vries, 2011, Bissaro *et al*., 2018) [2, 34, 5].

The fungi produce one or more of three extracellular enzymes that are important for lignin degradation: lignin peroxidase (EC 1.11.1.14), manganese-dependent peroxidase (EC1.11.1.13), and laccase (EC 1.10.3.2). Some white rot fungi secrets all these enzymes while others produce only few of them. The major hydrolytic enzymes are endo-1, 4-β-D-glucanase (EC 3.2.1.4), exo-1, 4- β -D- glucanase (EC 3.2.1.91), and xylanase (EC 3.2.1.8).

Several methodologies like use of mixed culture (co-culture) of organisms, genetically modified organisms, use of some lignocellulosic waste material or chemical enhancers can results in higher production of such enzymes. Lignocellulosic material is rich in soluble carbohydrates and chemical inducers. So, intermediate compounds of enzyme synthesis helps in enhancing production of these enzymes (Elisashvili *et al* 2006, Rosales *et al* 2005, Songulashvili *et al* 2006) [10, 31].

The selection of lignocellulosic materials and its concentration played an important role in promoting fungal growth and targeted enzyme synthesis (Kapich *et al* 2004 and Rosales *et al* 2007) [18, 32]. Several raw materials have been used successfully for enhancing lignocellulolytic enzyme production in submerged (SF) and solid-state (SSF) fermentation

(AboSiada *et al* 2017, Kachlishvili *et al*. 2006; Elisashvili *et al*. 2006, 2008; Rosales *et al*. 2007; Osma *et al*. 2007; Levin *et al*. 2007) [1, 15, 10, 32, 30, 21].

Ash gourd (*Benincasa hispida* Cogn.) is cultivated as a vegetable throughout India, in other Southeast Asian countries, and Japan, China, and Australia. Ash gourd is known to have a high concentration of carbohydrates, especially xylan (Mazumdar *et al*., 2004) [25] and pectin (Mazumdar *et al*., 2005) [26].

The present study evaluates the effect of a co-culture of two compatible white rot fungi and various concentrations of ash gourd pulp on the lignocellulolytic enzyme production using submerged fermentation process.

Material and Methods

Fungal cultures and maintenance

Fungal isolates of *Irpex lacteus* and *Daedaleopsis confragosa* were procured from Forest Research Institute, Dehradun. On the basis of paired interaction tests performed in the laboratory *Irpex lacteus* and *Daedaleopsis confragosa* were compatible with each other and so selected for co culture experiment. Cultures were maintained on Malt Extract Agar (MEA) medium at 4 (±1)^{0}C in Seed Anatomy laboratory, Department of Botany, The Maharaja Sayajirao University of Baroda, Gujarat, India.

Lignocellulosic substrate preparation

Ash gourd was acquired from the local market of Vadodara, Gujarat. The peel and seeds of Ash gourd were removed. Only the pulp portion was cut in to small pieces and oven dried at 80˚ C.

Preparation of fungal enzyme sample

Enzyme assays were executed in 100 ml of 3% Malt extract broth (MEB) medium. The MEB medium was sterilized by autoclaving. After cooling, inoculation was inoculation was performed with 9 mm disc of 10 days old culture in aseptic condition and for co-culture/dual culture 9 mm agar disc of both the selected fungal culture were inoculated in the Erlenmeyer flask of MEB medium. To estimate the effect of various concentration of ash gourd the dried pieces of ash gourd were augmented in the 100 ml of above mentioned medium (1%, 2%, 3%, 4%, 5%, 6%, 8% and 10%). The culture was incubated at 25 ±1˚ C for the desired incubation period. After completion of incubation period the cultures were removed, mycelium was homogenized in a Waring laboratory blender and filtered through whatman paper No. 1 disc. This crude filtrate was used to evaluate the activity of enzymes.

Enzyme assay

Lignin peroxidase (LiP) and Manganese-dependent peroxidase (MnP)

Lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP) activities were estimated according to the procedure of (Castillo *et al*., 1997) [7] using 0.167 mM 3-methyl-2-benzothiazolinone hydrazone (MBTH) as substrate which interact with 2.37 mM 3-(dimethylamino) benzoic acid (DMAB) producing a purple coloured reaction. Substrates were prepared on 0.1 M succinic-lactic acid buffer at pH 4.5. Reaction mixture contained 417.5 µl MBTH, 417.5 µl DMAB, 100 µl $MnSO_4$, 50 µl supernatant (enzyme) and 15 µl H_2O_2 to measure the enzyme activity. To discriminate between magnesium dependent and independent peroxidases, reaction was performed in the presence and absence of 3mM $MnSO_4$. 4mM H_2O_2 respectively to initialise the reaction. Reactions were carried out at 37°C and monitored spectrophotometrically at 590 nm. The enzyme activity was calculated using extinction coefficient $\varepsilon = 53000\ M^{-1}cm^{-1}$.

Laccase

Laccase activity was examined using 2, 2'-azino-bis (3-ethylbenziazoline-6-sulphonic acid) (ABTS) as a substrate (Niku-paavola *et al*., 1988) [29]. Reactions were carried out with 500 µM ABTS in 50 mM Sodium acetate buffer at pH 4.5. A 20 µl aliquot of enzyme solution was added to 580 µl of the ABTS. Absorbance at 420 nm were observed and the enzyme activity was calculated using extinction coefficient ($\varepsilon = 36,000\ M^{-1}cm^{-1}$).

Aryl alcohol oxidase (AAO)

Aryl alcohol oxidase (AAO) activity was estimated using Veratryl alcohol as substrate (Guillen *et al*., 1990) [13]. The reaction mixture contained 5mM veratryl alcohol in a 0.1 mM sodium phosphate buffer pH 6 and 50 µl enzyme solution. Reaction was performed at 30°C and visualized spectrophotometrically at 310 nm and the enzyme activity was calculated using extinction coefficient ($\varepsilon = 9300\ M^{-1}cm^{-1}$).

Xylanase

The amount of xylanase produced was measured by using 1% birch wood xylan as the substrate (Bailey *et al*., 1992) [3]. Xylanase activity was assayed in 3.0 ml of a reaction mixture containing 0.1 ml of crude extracellular enzyme sample, 0.5 ml of 1% birch wood xylan (prepared in 0.05 M Na-citrate buffer, pH 5.3). The mixture was incubated at 55°C for 30 min. The reaction was stopped by the addition of 0.6 ml of 3, 5- dinitrosalicylic acid (DNSA) (Miller, 1959) [28] and 1.8 ml of distilled water was added to the tubes to make the reaction mixture 3 ml. The contents were boiled for 15 min (Miller, 1959) [28]. After cooling, the color developed was read at 540 nm. The amount of reducing sugars liberated was quantified using xylose as standard. One unit of enzyme activity is defined as the amount of enzyme which releases 1 µmol of xylose in 1 min under assay conditions (Khan *et al*., 1986) [20].

Cellulase

Cellulase (CMCase) activity was determined by mixing 0.5 ml of 1% (w/v) CMC (prepared in 0.05 M acetate buffer pH 5.3) with 0.1 ml of crude extracellular enzyme solution incubating at 50°C for 30 min (Casimir *et al*., 1996) [6]. The reaction was stopped by the addition of 0.6 ml of 3, 5-dinitrosalicylic acid (DNSA) (Miller, 1959) [28] and 1.8 ml of distilled water was added to the tubes to make the reaction mixture 3 ml. The contents were boiled for 15 min. The colour developed was read at 540 nm. The amount of reducing sugar liberated was quantified using glucose as standard. One unit of cellulase is defined as the amount of enzyme that liberates 1 µmol of glucose equivalents per minute under the assay conditions (Mandels *et al*., 1981) [24].

Results and Discussion

In the present study assessment and comparison of the ligninolytic enzymes Lignin Peroxidase (LiP), Manganese Peroxidase (MnP), Laccase, Aryl alcohol oxidase (AAO),

Xylanase and Cellulase in monocultures and co cultures of I. *lacteus* and *D. confragosa* at diverse incubation period were carried out and the results obtained are represented in Table 1. The optimum days for maximum production of the enzyme was also observed. Further effect of various concentration of ash gourd pulp was also evaluated on the day when highest enzyme activity was obtained. There was a variation in the enzyme activity of mono culture and co culture. Graphical representation of the enzymatic activity is represented in Fig 1.

The gradual increase in the enzyme activity was observed with increase in incubation time and once the maximum enzyme activity was achieved a gradual decries in the enzyme activity was observed. The greatest enzyme activity of all the enzymes except xylanase was observed on 25th day and 30th day in mono cultures of *D. confragosa* and *I. lacteus* respectively. The maximum xylanase activity was noticed on 20th day after inoculation in both the monoculture.

It was also observed that in co-culture of *D. confragosa* and *I. lacteus* the enzymes produced were more in amount, compared to its monoculture and also at less incubation days. The maximum secretion of all the lignin cellulolytic enzymes were noted on 20th day whereas xylanase produced highest amount on 15th day. Cellulase enzyme activity was observed to be absent throughout the experimental set up and *I. lacteus* did not showed LiP activity.

Table 1: Lignocellulolytic enzyme activity from crude extracts of fungi in mono and co culture

Days		3	5	10	15	20	25	30
LiP	IL	0	0	0	0	0	0	0
	DC	0	0	0.94±0.12	1.69±0.06	1.76±0.06	2.47±0.03	1.82±0.06
	IL+DC	0.77±0.09	1.13±0.062	1.46±0.07	2.03±0.09	2.74±0.09	1.73±0.04	1.17±0.09
MnP	IL	0.15±0.04	0.27±0.04	0.33±0.03	0.39±0.04	0.50±0.06	0.54±0.04	0.69±0.06
	DC	0	0.08±0.03	0.40±0.03	0.77±0.03	1.00±0.06	1.75±0.06	0.58±0.09
	IL+DC	0.39±0.09	0.92±0.09	1.30±0.13	1.53±0.09	1.97±0.09	1.74±0.03	1.00±0.06
Lacc	IL	0.94±0.013	0.99±0.015	1.06±0.015	1.15±0.026	1.26±0.010	1.57±0.02	1.94±0.012
	DC	0.59±0.02	0.76±0.017	0.89±0.017	0.93±0.014	0.99±0.019	1.07±0.017	0.87±0.018
	IL+DC	1.34±0.067	1.48±0.018	2.78±0.049	3.12±0.042	5.45±0.030	1.51±0.019	1.11±0.022
AAO	IL	20.70±0.21	24.96±0.10	26.41±0.14	29.89±0.14	32.41±0.15	32.98±0.15	36.95±0.12
	DC	13.27±0.14	15.62±0.11	19.64±0.10	20.60±0.10	22.53±0.10	24.42±0.11	23.50±0.10
	IL+DC	24.99±0.16	26.82±0.13	28.40±0.10	30.90±0.10	49.12±0.12	26.77±0.12	24.31±0.16
Xyl	IL	1.32±0.66	4.59±1.12	8.98±0.80	9.57±1.37	16.87±0.43	6.05±0.46	1.96±0.49
	DC	2.23±0.74	4.77±0.73	5.06±1.16	9.46±1.33	13.86±1.32	12.83±0.93	1.65±0.72
	IL+DC	4.75±1.02	8.23±0.72	5.94±0.73	22.68±0.39	10.20±0.49	3.72±0.87	3.37±0.52
Cell	IL	0	0	0	0	0	0	0
	DC	0	0	0	0	0	0	0
	IL+DC	0	0	0	0	0	0	0

Average enzyme activity determined from three replicates after respective incubation period in ±S.D.

In mono culture the highest LiP activity was obtained by DC 2.47U/L whereas IL showed lack of Lip activity and their coculture showed 2.74U/L LiP activity which was slightly elevated compared to monocultures (Fig.1 A). MnP activity was observed in the range of 0.15 U/L to 1.97 U/L. In mono culture the optimum MnP activity was produced by DC (1.75U/L) followed by IL (0.69U/L) while coculture produced 1.97 U/L (Fig.1 B). This result designates that in coculture more than two fold increase was noted in comparison with IL. Highest Laccase activity was secreted by IL (1.94 U/L) followed by DC 1.07U/L and coculture showed 5.45 U/L (Fig.1 C) representing fivefold increase in laccase enzyme activity in coculture. Highest AAO activity between the monoculture was found in IL (36.95 U/L) followed by DC (24.42U/L) and coculture exhibited higher amount of enzyme activity (49.12 U/L) (Fig.1 D).

The maximum xylanase enzyme activity was observed in IL (16.87U/ml) followed by DC (13.86 U/ml) and co-culture produced (22.68U/L) (Fig.1 E). Cellulase enzyme activity was found to be absent in monoculture and coculture (Fig.1 F).

In the experimental studies of Hong *et al* 2012 on Lip activity it was observed that four different white rot fungi (*Pleurotus ostreatus*, *Coriolus versicolor* , *Tyromyces albidus* and *Trametes gallica*) in static submerged fermentation using peat as a substrate indicating some white rot fungi may not be producing LiP activities. Kamitsuji *et al*., 2004 [17] also demonstrated that *Pleurotus* species have been documented to produce no typical LiP activity. Absence of LiP activity was detected in the *C. versicolor* cultured medium using Olive mill wastewater as the substrate (Ergul *et al*., 2009) [12]. For ligninolytic enzymes, some white rot fungi produce all of these enzymes while others produce only one or two of them. Present study also demonstrated that IL showed absence of LiP activity.

Maximum MnP and Laccase activity was detected in the co culture of IL+DC where in Laccase activity 5.45 U/L while in mono culture IL and DC produced 1.94 and 1.07 U/L respectively. MnP enzyme activity observed in co cultures of IL+DC was 1.97 and in mono cultures of IL and DC showed 0.75 and 1.75 U/L respectively which is in agreement with the observations of Chi *et al*. (2007) [8] who has reported that enzyme laccase and MnP secretion was enhanced in co-cultures of *Pleurotus ostreatus* and *Ceriporiopsis subvermispora* / *Physisporinus rivulosus*. Baldrian, 2004 also observed enhancement of Laccase enzyme in the co culture of *Trametes versicolor* and *Pleurotus ostreatus.* In Malt extract medium maximum lingocellulolytic enzymes like laccase and MnP production is attributed as malt extract provides the complete pool of amino acids required for enzyme synthesis (Arora & Sandhu 1985) [2].

Ma *et al* 2011 described effect of co culture treatment of *Irpex lacteus* and *Auricularia polytricha* on lignocellulosic

degradation which showed highest activity on the 20th day which was higher than observed in the mono cultures of both fungi which was exactly similar as the present study.

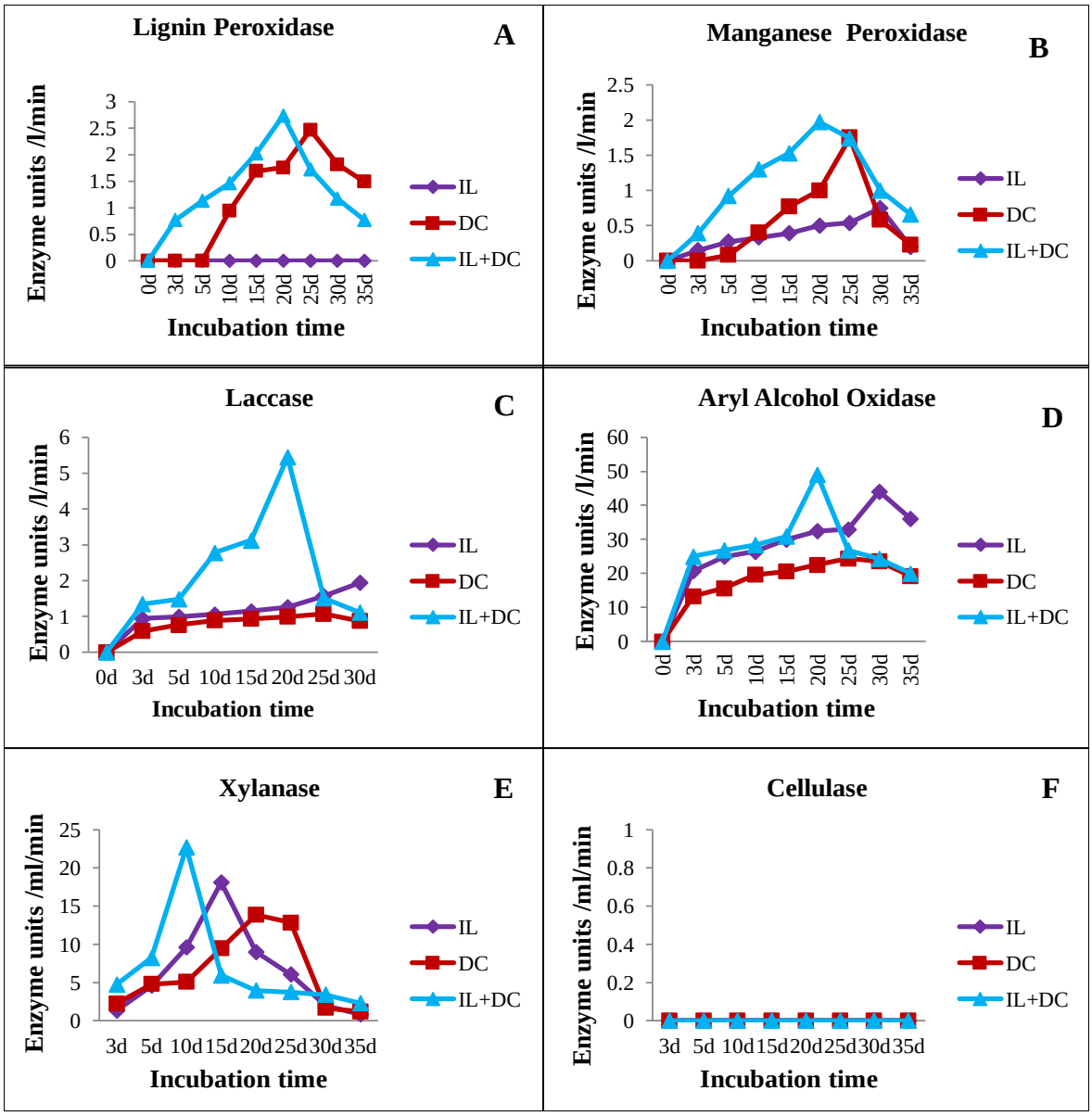

Fig 1: Enzymes produced by monoculture and coculture of fungi at carious incubation period IL-*I. lacteus* DC-*D. confragosa*

In the present study both the fungi in monoculture and coculture showed variable amount of xylanase production and an absence of cellulase enzyme secretion. Comparable results were also obtained by Kamble *et al* 2010 in which six fungal isolates secreting cellulase-free xylanase under submerged culture conditions were screened amongst which *Rhizopus oryzae* (205.0 U ml-1) and *Rhizopus microsporus* CB-10 (126.0 U ml-1) were the most prominent cellulase-free xylanase producers while *Hamigera insecticola* (66.0 U mL-1), *Rhizopus* sp. (75.10 U ml-1), *Aspergillus niger* (76.0 U ml-1), *Aspergillus flavus* (48.0 U ml-1), *Penicillium citrinum* (63.0 U ml-1) and *Penicillium sp.* (45.0 U ml-1) showed absence of cellulase in submerged fermentation.

To explain the role of different concentration of Ash gourd on the lignocellulolytic enzyme production MEB medium was augmented with 1%, 2%, 3%, 4%, 5%, 6%, 8% and 10% ash gourd and after completion desired incubation period the culture filtrates were collected and enzyme activity was analyzed. The maximal enhancement in the lignocellulolytic enzyme activity was recorded in 5% after which decrease in the enzyme production was observed up to 10% which was still greater than control which was not augmented with ash gourd (Table 2). Graphical representation of enhanced enzyme activity was represented in Fig.2.

Maximum LiP, MnP and laccase activity was revealed when media augmented with 5% ash gourd used in monoculture and co culture. *I. lacteus* showed an absence of LiP activity throughout the experiment whereas *D. confragosa* displayed maximum accumulation of LiP in media containing 5% ash gourd concentration after which a decline in the activity was noticed and in co culture of *I. lacteus* and *D. confragosa* also 5% ash gourd resulted in the maximum production of LiP enzyme (Fig. 2 A).

In mono culture of *I. lacteus* MnP activity was enhanced from 0.94 U/L to 1.63 U/L in the range of 1% to 5% concentration of ash gourd after that MnP activity was dropped up to 1.13 U/L in 10% ash gourd. *D. confragosa* showed maximum MnP enzyme production 2.45 U/L in media supplemented with 5% ash gourd which was

decreased up to 2.02 U/L in 10% but greater than control 1.75U/L. The co culture of *I.lacteus* and *D. confragosa* favoured the highest MnP activity in 5% 2.89 U/L which further decline in 10% ash gourd up to 2.13 U/L. (Fig 2 B).

Table 2: lignocellulolytic enzymes activity in the media supplemented with Ash gourd pulp

Conc of Ash gourd	Fungal isolate	Enzyme					
		LiP	MnP	Lac	AAO	Xyl	Cellulase
Cont	IL	0	0.75±0.06	1.94±0.012	44.12±0.13	18.11±1.03	0
	DC	2.47±0.03	1.75±0.06	1.07±0.017	24.42±0.11	13.86±1.32	0
	IL+DC	2.74±0.096	1.97±0.09	5.45±0.030	49.12±0.12	22.68±0.39	0
1%	IL	0	0.94±0.06	2.44±0.05	44.10±0.13	21.13±0.11	0
	DC	2.49±0.04	1.87±0.06	2.19±0.06	34.81±0.14	15.28±0.10	0
	IL+DC	3.05±0.09	2.08±0.06	7.03±0.06	56.24±0.11	27.13±0.16	0
2%	IL	0	1.07±0.06	2.69±0.05	60.56±0.11	23.19±0.11	0
	DC	2.59±0.09	2.08±0.06	2.45±0.08	59.21±0.11	16.67±0.14	0
	IL+DC	3.20±0.06	2.17±0.04	7.92±0.05	60.72±0.18	29.24±0.11	0
3%	IL	0	1.26±0.06	3.04±0.09	66.31±0.13	25.39±0.12	0
	DC	2.71±0.06	2.14±0.06	4.48±0.08	72.28±0.18	19.90±0.12	0
	IL+DC	3.31±0.04	2.39±0.06	8.46±0.07	73.05±0.15	32.10±0.17	0
4%	IL	0	1.45±0.06	4.19±0.08	93.61±0.13	28.36±0.17	0
	DC	2.87±0.06	2.19±0.06	6.80±0.05	88.0±0.11	24.17±0.16	0
	IL+DC	3.37±0.09	2.71±0.06	10.11±0.05	97.80±0.16	34.98±0.18	0
5%	IL	0	1.63±0.06	4.94±0.05	125.02±0.10	34.66±0.13	0
	DC	3.05±0.07	2.45±0.06	7.32±0.05	85.53±0.11	26.20±0.11	0
	IL+DC	3.51±0.06	2.89±0.06	12.74±0.10	143.06±0.10	40.96±0.11	0
6%	IL	0	1.44±0.06	4.74±0.06	107.23±0.15	34.12±0.14	0
	DC	2.76±0.06	2.32±0.06	7.04±0.05	84.15±0.15	25.30±0.21	0
	IL+DC	3.29±0.09	2.64±0.06	11.36±0.08	112.19±0.12	36.34±0.17	0
8%	IL	0	1.25±0.06	4.24±0.04	79.78±0.12	29.66±0.13	0
	DC	2.61±0.04	2.19±0.06	6.16±0.04	78.42±0.11	24.29±0.16	0
	IL+DC	3.14±0.06	2.38±0.06	10.77±0.10	109.33±0.11	29.25±0.19	0
10%	IL	0	1.13±0.06	3.59±0.05	76.29±0.11	28.41±0.12	0
	DC	2.57±0.06	2.02±0.06	5.57±0.06	74.85±0.12	22.39±0.11	0
	IL+DC	2.89±0.06	2.13±0.06	10.17±0.07	99.96±0.10	27.72±0.17	0

I. lacteus, *D. confragosa* and co culture of both indicated the highest level of laccase activity 4.94, 7.32 and 12.74 U/L respectively in 5% concentration (Fig. 2 C). In 5% concentration of ash gourd AAO activity indicated by *I. lacteus*, *D. confragosa* and co culture of *I. lacteus* and *D. confragosa* were 125.02, 85.53 and 143.06 U/L respectively which denoted the highest activity amongst all other concentrations (Fig.2 D). The results of xylanase also revealed that 5% ash gourd was the most ideal concentration for xylanase enxyme production after that decline in the xylanase activity was observed up to 10% but it was higher as compared to control. *I. lacteus*, *D. confragosa* and co culture of *I. lacteus* and *D. confragosa* were producing 34.66, 26.20 and 40.96 U/ml respectively in 5% of ash gourd which was the greatest activity observed amongst all other concentrations (Fig. 2 E). Cellulase enzyme was also seen to be absent even if the media was augmented with ash gourd (Fig. 2 F).

The activity of LiP, MnP and laccase, AAO and xylanase enzyme reduced even after the concentration of ash gourd increased in the medium up to 10%. However the enzyme activity showed a decline it was more than the control (ie without ash gourd supplementation).

Compared to *D. confragosa*, *I. lacteus* showed higher xylanase and AAO enzyme activity and the coculture showed higher enzyme production than monoculture with ash gourd, maximum activity is observed with 5% concentration which is fourfold increase. 10% concentration showed a decline in the activity, but still it is four fold more than the control in which ash gourd is not supplemented to the medium.

Ash gourd have higher amount of carbohydrates, specially xylan and pectin (Mazumdar *et al* 2004, 2005) [25, 26]. In the present study Ash gourd pulp was used as a substrate for the first time. In monocultures all the enzymes showed enhancement. When ash gourd was used as a substrate and in co culture LiP and MnP were moderately enhanced but laccase and xylanase were enhanced significantly when the medium was supplemented with ash gourd pulp.

As a outcome of present study to use the Ash gourd pulp for enhancing enzyme activity both the oxidative as well as hydrolytic enzymes enhanced significantly when ash gourd pulp without any other chemical enhancer in monocultures and coculture. Utilization of such low-cost lignocellulosic materials afford an opportunity to obtain high yield of lignocellulolytic enzymes in the simple medium which played major role in the degradation of lignin without augmenting it with expensive enhancers to the culture medium. The selection of lignocellulosic material adequate for the growth of fungus and target enzyme synthesis may lead to develop an efficient technology for scaling up the lignocellulolytic enzyme production.

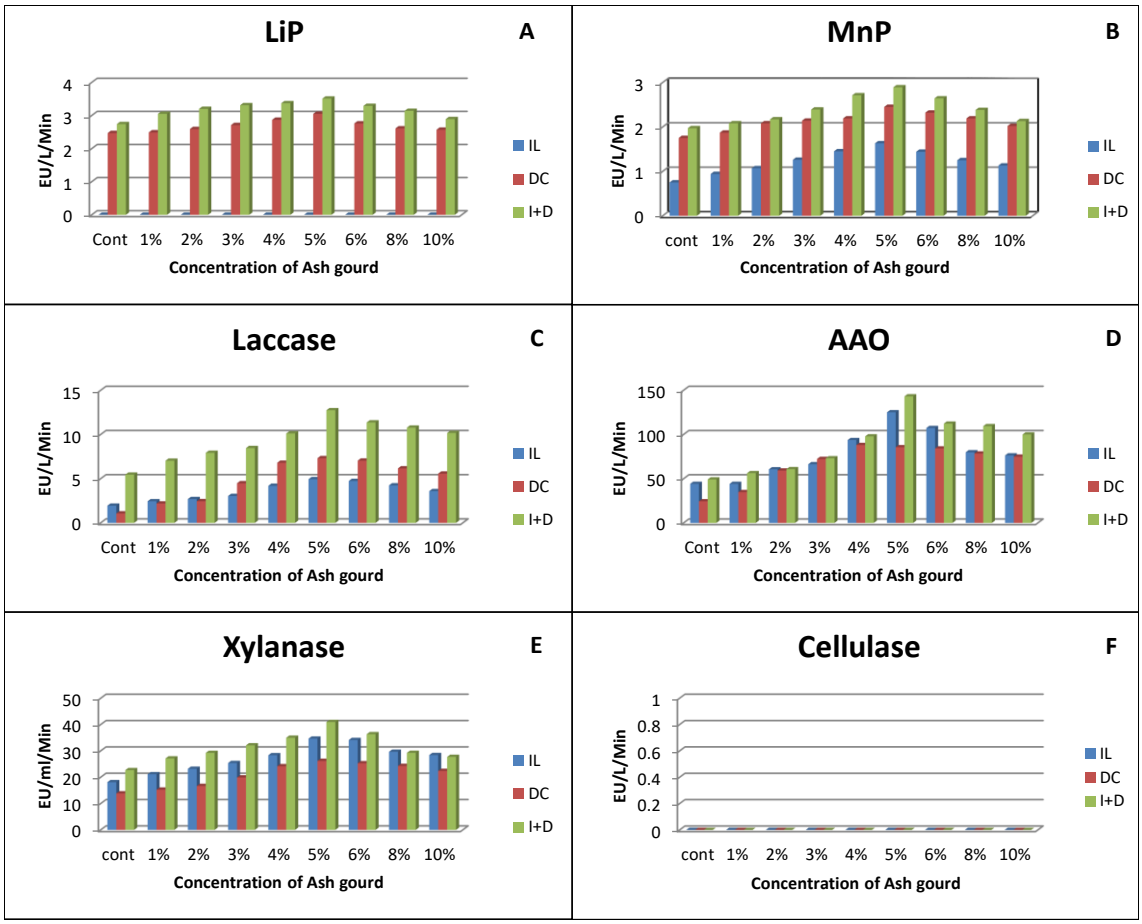

Fig 2: Effect of various concentrations of Ash gourd pulp on enzyme activity

References

1. AboSiada O, Negm M, Basiouny M, Fouad M, Elagroudy S. Nutrient enrichment of agro-industrial waste using solid state fermentation. Microbiology Research Journal International. 2017;22(1):1-11.
2. Arora DS, Sandhu DK. Laccase production and wood degradation by a white rot fungus *Daedalea flavida*. Enzyme and Microbial Technology. 1985;7:405-408.
3. Bailey MJ, Biely P, Poutanen K. Interlaboratory testing of methods for assay of xylanase activity. Journal of Biotechnology. 1992;23(3):257-270.
4. Baldrian P. Increase of laccase activity during interspecific interactions of white-rot fungi. FEMS Microbiology Ecology. 2004;50:245-253.
5. Bissaro B, Várnai A, Røhr ÅK, Eijsink VGH. Oxidoreductases and reactive oxygen species in conversion of lignocellulosic biomass. Microbiology and Molecular Biology Reviews. 2018;82(4):10-1128.
6. Casimir SJ, Davis S, Fiechter A, Gysin B, Murray E, Perrolaz JJ, *et al*. Pulp bleaching with thermostable xylanase of *Thermomonospora fusca*. US patent; 1996.
7. Castillo del Pilar M, Ander P, Stenstrom J. Lignin and manganese peroxidase activity in extracts from straw solid substrate fermentations. Biotechnology Techniques. 1997;11(9):701-706.
8. Chi Y, Hatakka A, Maijala P. Can co-culturing of two white-rot fungi increase lignin degradation and the production of lignin-degrading enzymes. International Biodeterioration and Biodegradation. 2007;59:32-39.
9. Elisashvili V, Penninckx M, Kachlishvili E, Tsiklauri N, Metreveli E, Khardziani T, *et al*. *Lentinus edodes* and *Pleurotus* species lignocellulolytic enzyme activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition. Bioresource Technology. 2008;99:457-462.
10. Elisashvili V, Penninckx M, Kachlishvili E, Asatiani M, Kvesitadze G. Use of *Pleurotus dryinus* for lignocellulolytic enzyme production in submerged fermentation of mandarin peels and tree leaves. Enzyme and Microbial Technology. 2006;38:998-1004.
11. Elisashvili V, Penninckx M, Kachlishvili E, Tsiklauri N, Metreveli E, Khardziani T. *Lentinus edodes* and *Pleurotus* species lignocellulolytic enzyme activity in submerged and solid-state fermentation of lignocellulosic wastes of different composition. Bioresource Technology. 2008;99:457-462.
12. Ergul FE, Sargin S, Ongen G, Sukan FV. Dephenolisation of olive mill wastewater using adapted *Trametes versicolor*. International Biodeterioration and Biodegradation. 2009;63:1-6.
13. Guillen F, Martinez AT, Martinez MJ. Production of hydrogen peroxide by aryl alcohol oxidase from the ligninolytic fungus *Pleurotus eryngii*. Applied Microbiology and Biotechnology. 1990;32:465-469.
14. Hong Y, Dashtban M, Chen S, Song R, Qin W. Enzyme production and lignin degradation by four basidiomycetous fungi in submerged fermentation of peat-containing medium. International Journal of Biology. 2012;4(1):172-180.
15. Kachlishvili E, Penninckx MJ, Tsiklauri N, Elisashvili V. Effect of nitrogen source on lignocellulolytic enzyme production by white-rot basidiomycetes under solid-state cultivation. World Journal of Microbiology and Biotechnology. 2006;22:391-397.

16. Kamble RD, Jadhav AR. Isolation, identification and screening of potential cellulase-free xylanase producing fungi and its production. African Journal of Biotechnology. 2010;11(77):14175-14181.
17. Kamitsuji H, Honda Y, Watanabe T, Kuwahara M. Production and induction of manganese peroxidase isozymes in a white-rot fungus *Pleurotus ostreatus*. Applied Microbiology and Biotechnology. 2004;65(3):287-294.
18. Kapich AN, Prior BA, Botha A, Galkin S, Lundell T, Hatakka A. Effect of lignocellulose-containing substrate on production of ligninolytic peroxidases in submerged cultures of *Phanerochaete chrysosporium* ME-446. Enzyme and Microbial Technology. 2004;34:187-195.
19. Kiiskinen LL, Ratto M, Kruus K. Screening for novel laccase-producing microbes. Journal of Applied Microbiology. 2004;97(3):640-646.
20. Khan AW, Tremblay D, LeDuy A. Assay of xylanase and xylosidase activities in bacterial and fungal cultures. Enzyme and Microbial Technology. 1986;8(6):373-377.
21. Levin L, Villalba L, Da Rea V, Forchiassin F, Papinutti L. Comparative studies of loblolly pine biodegradation and enzyme production by Argentinean white-rot fungi focused on biopulping processes. Process Biochemistry. 2007;42:995-1002.
22. Liu G, Qin Y, Li Z, Qu Y. Development of highly efficient, low-cost lignocellulolytic enzyme systems in the post-genomic era. Biotechnology Advances. 2013;31(6):962-975.
23. Ma F, Wang J, Zeng Y, Yu H, Yang Y, Zhang X. Influence of the co-fungal treatment with two white rot fungi on the lignocellulosic degradation and thermogravimetry of corn stover. Process Biochemistry. 2011;46(9):1767-1773.
24. Mandels M, Medeiros JE, Andreotti RE, Bissett FH. Enzymatic hydrolysis of cellulose: evaluation of cellulase culture filtrates under use conditions. Biotechnology and Bioengineering. 1981;23(9):2009-2026.
25. Mazumdar S, Morvan C, Thakur S, Ray B. Cell wall polysaccharides from chalkumra (*Benincasa hispida*) fruit. Part I. Isolation and characterization of pectins. Journal of Agricultural and Food Chemistry. 2004;52:3556-3562.
26. Mazumdar S, Lerouge P, Loutelier-Bourhis C, Driouich A, Ray B. Structural characterization of hemicellulosic polysaccharides from *Benincasa hispida* using specific enzyme hydrolysis, ion exchange chromatography and MALDI-TOF mass spectroscopy. Carbohydrate Polymers. 2005;59:231-238.
27. Mikiashvili N, Wasser S, Nevo E, Chichua D, Elisashvili V. Lignocellulolytic enzyme activities of medicinally important basidiomycetes from different ecological niches. International Journal of Medicinal Mushrooms. 2004;6:63-71.
28. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959;31(3):426-428.
29. Niku-Paavola ML, Karhunen E, Salola P, Raunio V. Ligninolytic enzymes of the white rot fungus *Phlebia radiata*. Biochemical Journal. 1988;254:877-884.
30. Osma JF, Saravia V, Herrera JLT, Couto SR. Mandarin peelings: the best carbon source to produce laccase by static cultures of *Trametes pubescens*. Chemosphere. 2007;67:1677-1680.
31. Rosales E, Rodriguez Couto S, Sanromán MA. Reutilisation of food processing wastes for production of relevant metabolites: application to laccase production by *Trametes hirsuta*. Journal of Food Engineering. 2005;66:419-423.
32. Rosales E, Rodriguez SC, Sanromán MA. Increased laccase production by *Trametes hirsuta* grown on ground orange peelings. Enzyme and Microbial Technology. 2007;40:1286-1290.
33. Songulashvili G, Elisashvili V, Wasser S, Nevo E, Hadar Y. Laccase and manganese peroxidase activities of *Phellinus robustus* and *Ganoderma adspersum* grown on food industry wastes in submerged fermentation. Biotechnology Letters. 2006;28:1425-1429.
34. Van den Brink J, de Vries RP. Fungal enzyme sets for plant polysaccharide degradation. Applied Microbiology and Biotechnology. 2011;91(6):1477-1492.