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Biodegradation potential of indigenous litter dwelling ligninolytic fungi on agricultural wastes

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Abstract

Background: The present study was focused to study the efficiency of two indigenous litter dwelling ligninolytic fungi (such as *Mucor circinelloides* GL1 and *Fusarium verticillioides* GL5) in degrading the agricultural wastes (areca husk, coffee husk and paddy straw) through solid-state fermentation.

Results: After fermentation process, the lignocellulosic residues left over were evaluated for their physico-chemical studies and degradation pattern of cell wall constituents along with the activity of enzymes. In each substrate, the initial pH was found to change from near-neutral to acidic pH after fungal decomposition. Significantly increased loss of total organic matter and organic carbon content was observed in each substrate decomposed by the fungal strains selected. The total nitrogen, crude protein, total phosphorus and total potassium contents of the fungal decomposed substrates were significantly increased with the progress of time. The study indicated that the degradation patterns of lignin and holocellulose were more effective from 20 to 120 days after fungal inoculation with respect to their loss between the different harvesting intervals. During decomposition process, both the strains produced the ligninolytic enzymes [laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP)] and carboxymethyl cellulase (CMCase) on each substrate with their remarkably varied activities with respect to different harvesting times.

Conclusions: In concern with the present environmental problems, the present study suggested that these potential ligninolytic fungi can be utilized successfully for the management of agricultural wastes and reuse of their residues in the forest soil conservation system to eliminate the harmful effects of the crop residue burning.

Keywords: Agricultural wastes, Biodegradation, Fungi, Holocellulose, Lignin, Lignocelluloses

Background

Lignin protects the total polysaccharide fraction of plant cell wall that made up of a mixture of cellulose and hemicellulose. It is a large recalcitrant substrate to degrade due to its non-phenolic aromatic nature and heterogeneous structure which make the accumulation of lignocellulosic materials in the forest soils (Ruiz-Dueñas and Martínez 2009; Janusz et al. 2017). The accumulated lignocellulosic materials mainly consist of deadwood and leaf litter in the forest soil. In India, the removal or *in situ* combustion of forestry and agricultural crop wastes is being practiced by most subsistence farmers which seriously reduce the soil quality (Kumar et al. 2015; Bhuvaneshwari et al. 2019). The combustion of agricultural crop wastes emits the noxious gases, particulate matter and other reactive hydrocarbons thereby resulting in a significant disturbance in the global atmospheric chemistry (Jain et al. 2014).

Nowadays, it has attracted a lot of attention of scientists towards the lignocellulosic biomass and waste valorization (Arancon et al. 2013; Abdel-Shafy and Mansour 2018; Xu et al. 2019). Fungi are the best-known microorganisms that quickly invade the substrates and capable of

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degrading cellulose, hemicellulose and lignin efficiently by several enzymes. They can be effectively exploited for *in situ* decomposition of various crop wastes. Most of the extracellular ligninolytic enzymes involved in the degradation of plant litter have been attributed to the fungi which produce them by adapting their basic metabolism to nutrient availability in the natural habitats (Rineau et al. 2013; Lashermes et al. 2016; Janusz et al. 2017). An eco-friendly strategy has been developed for the enzyme production by solid-state fermentation process using various agricultural wastes as supporting substrates (Lizardi-Jiménez and Hernández-Martínez 2017; Sadh et al. 2018). The production of hydrolytic (cellulase and hemicellulase) and ligninolytic enzymes depends on the mode of fermentation process and cultivation of microorganisms, especially fungi.

Inevitably generated agro-industrial wastes are primarily composed of cellulose, hemicellulose and lignin, and found in lignocellulosic nature. They are exploited as solid materials to aid as the nutrients sources and physical supports for the cultivation of microbes during solid-state fermentation in absence of free-flowing liquid (Krishna 2005). This fermentation process provides the same conditions under which the fungi are being grown and making it an excellent method for the bioconversion of various agro-industrial wastes. The use of these wastes supports the excellent fungal growth and increases their enzyme activity by making the availability of rich energy nutrients to such fungi (Takó et al. 2015; Sadh et al. 2018). Besides, the fermentation process also offers the new promising possibility of successfully managing and reusing various agro-industrial wastes.

Generally, several fungi are being employed in the rapid degradation of various agricultural wastes by solid-state fermentation (Belewu and Babalola 2009; Mussatto et al. 2012; Yoon et al. 2014; Barrios-González and Tarragó-Castellanos 2017). However, there is a few numbers of reports on the degradation of agro-industrial wastes by the litter decomposing fungal species wherein their activity of ligninolytic enzymes was reported (Hättenschwiler et al. 2005). Therefore, in the present study, after successful screening of fifty-eight ligninolytic fungal strains isolated from litter, two potential strains (*M. circinelloides* GL1 and *F. verticillioides* GL5) were selected and used to study their efficiency in the decomposition of some agricultural wastes.

Methods

Microorganisms

Two potential ligninolytic fungi such as *Mucor circinelloides* GL1 (GenBank Accession No.: MF458974) and *Fusarium verticillioides* GL5 (GenBank Accession No.: MF458977) which were indigenous to the litter samples of Western Ghats of Karnataka (India) were selected (Unpublished data) and used to study their efficiency in decomposing some agricultural wastes in the laboratory.

Agricultural wastes

Three different agricultural wastes such as areca husk, coffee husk and paddy straw were used as the solid substrates to determine the efficiency of selected potential ligninolytic fungal strains to decompose them. The solid substrates were procured from the local agricultural farms of Kodagu district in Karnataka (India) during January, 2017. The substrates were brought to the research laboratory and oven dried at 80 °C. The dried substrates were pulverized and sieved through 20 mesh sieve size and then stored at room temperature in the polyethylene bags for further experiments.

Solid-state fermentation process of agricultural wastes by ligninolytic fungi

Solid-state fermentation process was performed to check the different substrates degradation efficiency of selected ligninolytic fungal strains (Takó et al. 2015). Briefly, 10 g dried substrate was moistened with 40 mL sterile water in 250-mL Erlenmeyer flask and autoclaved for 20 min at 121 °C. Five mycelial plugs (5 mm of diameter) of 7-day-old fungal culture actively growing on malt-extract agar (MEA) medium (2%) were inoculated onto the flasks separately containing three different sterilized solid substrates. The flasks separately containing different sterilized solid substrates without fungal inoculation were used as the controls. The flasks were then incubated under static conditions in dark at 28 ± 2 °C for 120 days. For better fungal growth, the moisture content was maintained at 70% throughout the incubation period of decomposition by sprinkling the sterile water regularly in each experiment. The decomposed samples were harvested at the periodic intervals of 0, 20, 40, 60, 80, 100 and 120 days after fungal inoculation and used for determining the various parameters as described below. After harvesting, the decomposed samples were washed twice with sterile water to remove the mycelia, dried and then used for further experiments.

Physico-chemical analyses

Determination of pH

The decomposed substrate was homogenized with deionized water at the ratio of 1:10 (w/v) by shaking gently for 30 min at 200 rpm. The mixture was filtered through a Whatman filter paper and centrifuged for 10 min at 6000 rpm. The pH of filtrate was recorded using a digital pH meter.

Determination of total organic matter and organic carbon content

The total organic matter of the decomposed substrate was assessed by using the loss on ignition (LOI) method of Salehi et al. (2011). The decomposed substrate was heated to 560 °C by placing it in a ceramic crucible until constant weight was achieved in the muffle furnace. Then, the dried sample was cooled in the desiccator and weighed. Total organic matter was calculated by using the following formula.

$$\text{Total organic matter (mg/g)} = \frac{\text{Initial weight of sample} - \text{Final weight of dried sample}}{\text{Initial weight of sample}}$$

The total organic carbon of the decomposed substrate was then calculated by dividing the total organic matter by the factor of 1.724.

$$\text{Total organic carbon (mg/g)} = \frac{\text{Total organic matter}}{1.724}$$

where 1.724 is the factor for converting the total organic matter to total organic carbon.

Determination of total nitrogen and crude protein

The total nitrogen of the decomposed substrate was analyzed by using the micro-Kjeldahl acid digestion method of Rhee (2001). The decomposed substrate was digested with concentrated sulfuric acid and distilled into 4% boric acid solution using the micro-Kjeldahl distillation apparatus. Then, the aliquot was titrated against diluted sulfuric acid with the blank. The total nitrogen was calculated from the volume of sulfuric acid consumption using the following formula.

$$\text{Total nitrogen content}\left(\text{mg/g}\right) = \frac{\text{Titer value} \times \text{N of } H_2SO_4 \times 14.007}{1000 \times \text{Weight of the sample in g}}$$

where the titer value is the difference between the volume of consumption of sulfuric acid in the test sample and blank, 14.007 is the molecular weight of nitrogen (g/mol), and 1000 is the conversion factor (mL to L).

The crude protein of each substrate was calculated by using the following formula.

$$\text{Crude protein content (mg/g)} = \text{Total nitrogen content} \times 6.25$$

Determination of total phosphorus

The total phosphorus of the decomposed substrate was estimated by using the molybdenum-blue spectrophotometric method after ascorbic acid reduction (Singh et al. 2015). The decomposed substrate was incinerated at 550 °C for about 3 h in muffle furnace, powdered and digested with the mixture of tri-acids (sulfuric acid, perchloric acid and nitric acid) at 300 °C. After digestion, the digest was filtered through a Whatman filter paper and reacted with fresh mixed reagent (ammonium molybdate, antimony potassium tartrate and 50% sulfuric acid). The reaction mixture was reduced by the acidified ascorbic acid solution (as a reducing agent) to form the molybdenum-blue colored complex. The blue color intensity was measured at 880 nm using spectrophotometer. The total phosphorus content was estimated by using the standard curve plotted with the absorbance against the known concentration of standard phosphorous. The content was expressed as mg/g of the decomposed substrate.

Determination of total potassium

The total potassium of the decomposed substrate was estimated by using the flame emission spectrophotometric method (Néel et al. 2014). The decomposed substrate was incinerated at 550 °C for about 3 h in muffle furnace, powdered and digested with the mixture of tri-acids (sulfuric acid, perchloric acid and nitric acid) at 300 °C. After digestion, the digest was extracted with deionized water and filtered through a Whatman filter paper. Then, the filtrate was fed into the flame emission spectrophotometer and read the absorbance at 766 nm. The total potassium was calculated by using the standard curve plotted with the absorbance against the known concentration of standard potassium. The content was expressed as mg/g of the decomposed substrate.

Determination of carbon to nutrient supply (N, P and K) ratio

Carbon to nutrient supply (N, P and K) ratio of the decomposed substrate was calculated by dividing the total organic carbon content with each nutrient (such as total nitrogen, total phosphorus and total potassium).

Determination of lignocelluloses content and loss

Determination of lignin content and loss

The lignin content before and after fungal treatment was evaluated by measuring Klason lignin and acid soluble lignin of the substrate. The gravimetric measurement of

Klason lignin content of the substrate was performed as per the modified Klason method using hot sulfuric acid digestion (Fagerstedt et al. 2015). Briefly, the substrate was treated successfully with sulfuric acid (72% v/v) to depolymerize the crude cellulose and hemicellulose for 1 h with stirring frequently to assure the complete solution at 30 °C. The acid was then diluted with deionized water to make 3% (v/v) sulfuric acid for the hydrolysis of dissolved polysaccharides. The suspension was subsequently autoclaved for about 1 h at 121 °C and cooled to room temperature. The precipitate was filtered and dried for 4 h at 525 °C in the muffle furnace. After cooling, the dried sample was weighed to determine the Klason lignin content, acid insoluble residue and ash content present. Then, Klason lignin is calculated by subtracting the acid insoluble ash from the acid insoluble residue. The acid soluble lignin was estimated by reading the absorbance spectrophotometrically at 205 nm using the absorption coefficient ($\varepsilon = 110\ M^{-1}\ cm^{-1}$). The total lignin content was determined by the addition of Klason lignin with the acid soluble lignin. The change in lignin content was measured to calculate % of lignin loss by using the following formula.

$$\text{Lignin loss}\ (\%) = \frac{\text{Initial lignin content} - \text{Final lignin content}}{\text{Initial lignin content}} \times 100$$

Determination of holocellulose content and loss

The holocellulose (i.e., the total carbohydrate fraction containing both cellulose and hemicellulose) content of the substrate before and after fungal treatment was determined (Rabemanolontsoa and Saka 2012). The sample was ground and extracted with the mixture of alcohol:benzene (1:1 v/v) at 70 °C for 8 h to remove the extractives. One gram of the sample was suspended in 150 mL of sterile water. The solution was mechanically stirred. Ten drops of glacial acetic acid and sodium chlorite (1.5 g) were added to the solution with vigorous shaking. The mixture was then incubated in a water bath for about 1 h at 70 °C. After incubation, the same quantity of reagent was added and the same process was repeated until the total reaction time was 4 h. The solution was cooled to room temperature, filtered and washed with sterile water until free of acid, then followed by washing with acetone. The residue was oven dried at 105 °C for 4 h, cooled in the desiccator and weighed. Holocellulose content was calculated by using the following formula.

$$\text{Holocellulose content}\ (\%) = \frac{\text{Oven dried weight of holocellulose}}{\text{Oven dried weight of initial sample}} \times 100$$

The change in holocellulose content was measured to calculate % of holocellulose loss by using the following formula.

$$\text{Holocellulose loss}\ (\%) = \frac{\text{Initial holocellulose content} - \text{Final holocellulose content}}{\text{Initial holocellulose content}} \times 100$$

Enzyme activity

Extraction of enzymes

The enzymes were extracted from fermented and unfermented solid substrates according to the procedure of Leite et al. (2019) with slight modification. The decomposed substrate (10 g) was suspended in 50 mL of sodium acetate buffer (50 mM pH 5.0) and gently shaken for 30 min at 150 rpm. The content was transferred to a muslin cloth and filtered. The supernatant was then centrifuged at 10,000 rpm for 15 min at 4 °C. The resultant filtrate was served as a source of crude enzyme for the estimation of protein content and the activity of ligninolytic enzymes and carboxymethyl cellulase.

Estimation of protein content

The protein content was estimated at the wavelength of 595 nm by Bradford method (He 2011) using bovine serum albumin (BSA) as a standard. The protein content was expressed as mg/mL.

Estimation of activity of enzymes

The activity of ligninolytic enzymes [laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP)] and carboxymethyl cellulase (CMCase) was determined. Laccase activity was determined by monitoring the oxidation of guaiacol as per the method described by Arora and Sandhu (1985). The MnP activity was determined by monitoring the oxidation of phenol red as per the method described by Orth et al. (1993). The LiP activity

was determined by monitoring the oxidation of dye azure B as per the methods described by Archibald (1992), and Arora and Gill (2001). The CMCase activity was determined as per the method described by Miller (1959) using 1% (w/v) carboxymethyl cellulose (CMC) as a substrate and 3,5-dinitrosalicylic acid (DNS) as a coupling reagent. The enzyme activity was expressed as International Units (IU) per mL.

Statistical analysis

For each solid substrate, all the assays were performed by using the randomized complete block design (RCBD) with triplicates. Each experiment was repeated three times. The experimental data were analyzed statistically and subjected to one-way ANOVA by using IBM SPSS Statistics, version 23 (Wagner 2016). The significant differences observed between the treatment means were determined by the highest significant difference which was calculated by using Tukey's test at level $p \leq 0.05$.

Results

Growth of ligninolytic fungi on the solid substrates

All the three substrates (such as areca husk, coffee husk and paddy straw) used were provided the first glimpse of primary mechanism of spread of selected ligninolytic fungal strains from 2 days after inoculation. Both *M. circinelloides* GL1 and *F. verticillioides* GL5 were able to grow extensively on all the three tested substrates. The extensive mycelial growth and hyphal infiltration of both the fungal strains into the substrates were clearly evident from the bottom of the inoculated flasks from 5 days after inoculation which indicates the suitable environmental conditions for the growth of inoculated fungi. However, paddy straw was well supported the fastest mycelial growth and spread of both the fungal strains compared to other substrates.

Physico-chemical analyses

Determination of pH

The initial pH of each substrate was changed from near-neutral to acidic pH at the end of 120 days fermentation period (Table 1), which supports the extensive fungal growth and their abundance. The initial pH was changed from 6.2 to 5.0, 5.8 to 4.2 and 6.5 to 4.4 in areca husk, coffee husk and paddy straw, respectively, decomposed by *M. circinelloides* GL1. The treatment with *F. verticillioides* GL5 was also changed the initial pH to 4.5, 4.3 and 4.8 in areca husk, coffee husk and paddy straw, respectively.

Determination of total organic matter and organic carbon content

Decomposing time had a significant reducing effect on both the total organic matter and organic carbon contents in all the substrates by the selected fungal strains, but the reducing effect was varied depending on the substrate type (Table 1). There was the higher loss of initial total organic matter of 65.1 to 21.5, 72.7 to 18.3 and 68.2 to 16.5 mg/g in areca husk, coffee husk and paddy straw, respectively, decomposed by *M. circinelloides* GL1 at the end of fermentation period. Also, when treated with *F. verticillioides* GL5, the initial total organic matter was reduced to 39.5, 37.5 and 35.8 mg/g in areca husk, coffee husk and paddy straw, respectively. A similar trend of increase in loss of initial total organic carbon was observed from 37.7 to 12.4, 42.1 to 10.6 and 39.5 to 9.5 mg/g in areca husk, coffee husk and paddy straw, respectively, decomposed by *M. circinelloides* GL1. The reduction in initial organic carbon content to 22.9, 21.7 and 20.8 mg/g was also observed in areca husk, coffee husk and paddy straw, respectively, when treated them with *F. verticillioides* GL5.

Determination of total nitrogen and crude protein content

An improvement in the total nitrogen and crude protein contents of all three substrates decomposed by both the fungal strains was observed as the fermentation proceeds when compared to the un-inoculated control (Table 1). The initial total nitrogen content was increased from 0.58 to 1.63 mg/g (areca husk), 0.59 to 1.92 mg/g (coffee husk) and 0.56 to 1.25 mg/g (paddy straw) which caused by the inoculation with *M. circinelloides* GL1 compared to *F. verticillioides* GL5 and control. In addition, the initial crude protein of areca husk (3.6 mg/g), coffee husk (3.7 mg/g) and paddy straw (3.5 mg/g) was increased to 10.1, 12.0 and 7.8 mg/g, respectively, in treatment with *M. circinelloides* GL1 and followed by *F. verticillioides* GL5.

Determination of total phosphorus and potassium content

The periodical changes of both phosphorus and potassium contents during the fermentation of the substrates indicated the increased total phosphorus and potassium contents were varied significantly over that of initial contents by the treatment with selected fungi (Table 1). The initial total phosphorus content was increased from 0.49 to 1.5 mg/g (areca husk), 0.52 to 1.49 mg/g (coffee husk) and 0.5 to 1.21 mg/g (paddy straw) in the treatment with *M. circinelloides* GL1 compared to *F. verticillioides* GL5 and control. Also, the initial total potassium content of areca husk (0.68 mg/g), coffee husk (0.93 mg/g) and paddy straw (0.88 mg/g) was significantly increased to 6.85, 3.55 and 5.3 mg/g, respectively, in treatment with *M. circinelloides* GL1 and followed by *F. verticillioides* GL5.

Table 1 Changes in physico-chemical properties of solid substrates treated with ligninolytic fungi during solid-state fermentation in different time intervals

	Time interval (day)	pH (1–14)	Total OM (mg/g)	Total OC (mg/g)	Total N (mg/g)	Crude protein (mg/g)	Total P (mg/g)	Total K (mg/g)
Areca husk								
Control		*6.2 ± 0.115^{a}*	*65.1 ± 1.66^{a}*	*37.7 ± 0.96^{a}*	0.58 ± 0.014^{g}	3.6 ± 0.08^{g}	0.49 ± 0.034^{i}	0.68 ± 0.09^{e}
M. circinelloides GL1	20	6.0 ± 0.115^{ab}	62.8 ± 1.14^{a}	36.4 ± 0.66^{a}	0.72 ± 0.020^{f}	4.4 ± 0.12^{f}	0.67 ± 0.020^{gh}	1.20 ± 0.11^{e}
	40	5.8 ± 0.173^{abc}	55.9 ± 1.49^{bc}	32.4 ± 0.86^{bc}	0.89 ± 0.020^{e}	5.5 ± 0.12^{e}	0.93 ± 0.013^{e}	2.85 ± 0.13^{d}
	60	5.6 ± 0.115^{bcd}	45.0 ± 1.15^{ef}	26.1 ± 0.66^{ef}	0.93 ± 0.026^{e}	5.8 ± 0.16^{e}	1.07 ± 0.020^{d}	4.75 ± 0.14^{c}
	80	5.4 ± 0.057^{cde}	31.0 ± 0.86^{g}	17.9 ± 0.50^{g}	1.13 ± 0.013^{d}	7.0 ± 0.08^{d}	1.23 ± 0.017^{c}	5.74 ± 0.15^{b}
	100	5.2 ± 0.115^{def}	27.5 ± 1.60^{g}	15.9 ± 0.93^{g}	1.38 ± 0.011^{b}	8.6 ± 0.07^{b}	1.38 ± 0.044^{b}	6.09 ± 0.23^{b}
	120	5.0 ± 0.173^{efg}	21.5 ± 1.04^{h}	12.4 ± 0.60^{h}	*1.63 ± 0.023^{a}*	*10.1 ± 0.14^{a}*	*1.50 ± 0.031^{a}*	*6.85 ± 0.18^{a}*
F. verticillioides GL5	20	5.8 ± 0.115^{abc}	63.2 ± 1.60^{a}	36.6 ± 0.93^{a}	0.69 ± 0.02^{f}	4.3 ± 0.13^{f}	0.56 ± 0.026^{hi}	0.83 ± 0.12^{e}
	40	5.6 ± 0.057^{bcd}	56.8 ± 0.62^{b}	32.9 ± 0.36^{b}	0.74 ± 0.017^{f}	4.6 ± 0.10^{f}	0.68 ± 0.043^{g}	1.18 ± 0.15^{e}
	60	5.4 ± 0.100^{cde}	50.8 ± 1.66^{cd}	29.4 ± 0.96^{cd}	0.88 ± 0.017^{e}	5.4 ± 0.10^{e}	0.80 ± 0.026^{f}	2.78 ± 0.17^{d}
	80	5.1 ± 0.115^{def}	46.7 ± 1.15^{de}	27.0 ± 0.66^{de}	0.96 ± 0.023^{e}	6.0 ± 0.14^{e}	0.94 ± 0.020^{e}	4.60 ± 0.10^{c}
	100	4.8 ± 0.173^{fg}	43.0 ± 1.60^{ef}	24.9 ± 0.93^{ef}	1.25 ± 0.028^{c}	7.8 ± 0.17^{c}	1.08 ± 0.017^{d}	4.99 ± 0.22^{c}
	120	4.5 ± 0.115^{g}	39.5 ± 0.86^{f}	22.9 ± 0.50^{f}	1.36 ± 0.023^{b}	8.5 ± 0.14^{b}	1.21 ± 0.026^{c}	5.94 ± 0.18^{b}
Coffee husk								
Control		*5.8 ± 0.115^{a}*	*72.7 ± 1.87^{a}*	*42.1 ± 1.08^{a}*	0.59 ± 0.014^{i}	3.7 ± 0.09^{i}	0.52 ± 0.037^{h}	0.93 ± 0.04^{e}
M. circinelloides GL1	20	5.5 ± 0.208^{ab}	68.7 ± 2.22^{a}	39.8 ± 1.29^{a}	0.72 ± 0.018^{gh}	4.5 ± 0.11^{gh}	0.70 ± 0.041^{fg}	1.14 ± 0.06^{de}
	40	5.3 ± 0.115^{abc}	54.9 ± 1.26^{bc}	31.8 ± 0.72^{bc}	0.85 ± 0.020^{fg}	5.3 ± 0.12^{fg}	0.82 ± 0.049^{ef}	1.64 ± 0.15^{cd}
	60	5.0 ± 0.057^{bcd}	41.6 ± 1.71^{ef}	24.1 ± 0.99^{ef}	1.08 ± 0.037^{e}	6.7 ± 0.23^{e}	0.97 ± 0.043^{de}	1.86 ± 0.13^{c}
	80	5.0 ± 0.152^{bcd}	30.9 ± 1.70^{g}	17.9 ± 0.98^{g}	1.45 ± 0.026^{c}	9.1 ± 0.16^{c}	1.16 ± 0.026^{bc}	2.18 ± 0.10^{c}
	100	4.6 ± 0.100^{de}	24.5 ± 1.35^{h}	14.2 ± 0.78^{h}	1.72 ± 0.027^{b}	10.7 ± 0.17^{b}	1.28 ± 0.044^{b}	2.85 ± 0.15^{b}
	120	4.2 ± 0.115^{e}	18.3 ± 1.32^{h}	10.6 ± 0.76^{i}	*1.92 ± 0.023^{a}*	*12.0 ± 0.14^{a}*	*1.49 ± 0.031^{a}*	*3.55 ± 0.18^{a}*
F. verticillioides GL5	20	5.4 ± 0.173^{abc}	69.3 ± 1.48^{a}	40.2 ± 0.85^{a}	0.68 ± 0.046^{hi}	4.2 ± 0.28^{hi}	0.62 ± 0.026^{gh}	1.10 ± 0.08^{de}
	40	5.1 ± 0.100^{bcd}	59.2 ± 1.54^{b}	34.3 ± 0.89^{b}	0.78 ± 0.017^{gh}	4.9 ± 0.10^{gh}	0.70 ± 0.026^{fg}	1.14 ± 0.11^{de}
	60	4.9 ± 0.057^{cd}	54.4 ± 1.28^{bc}	31.5 ± 0.74^{bc}	0.92 ± 0.015^{f}	5.7 ± 0.09^{f}	0.83 ± 0.020^{ef}	1.67 ± 0.14^{cd}
	80	4.7 ± 0.100^{de}	50.4 ± 1.21^{cd}	29.2 ± 0.70^{cd}	1.28 ± 0.043^{d}	8.0 ± 0.27^{d}	0.97 ± 0.029^{de}	1.82 ± 0.14^{c}
	100	4.6 ± 0.115^{de}	45.3 ± 1.66^{de}	26.3 ± 0.96^{de}	1.33 ± 0.040^{cd}	8.3 ± 0.25^{cd}	1.07 ± 0.034^{cd}	2.06 ± 0.20^{c}
	120	4.3 ± 0.057^{e}	37.5 ± 0.93^{f}	21.7 ± 0.54^{f}	1.45 ± 0.028^{c}	9.0 ± 0.17^{c}	1.17 ± 0.027^{bc}	2.95 ± 0.16^{b}
Paddy straw								
Control		*6.5 ± 0.115^{a}*	*68.2 ± 1.12^{a}*	*39.5 ± 0.65^{a}*	0.56 ± 0.023^{i}	3.5 ± 0.14^{h}	0.50 ± 0.037^{h}	0.88 ± 0.08^{e}
M. circinelloides GL1	20	6.2 ± 0.152^{ab}	61.3 ± 1.18^{b}	35.5 ± 0.68^{b}	0.68 ± 0.026^{fg}	4.2 ± 0.16^{fg}	0.69 ± 0.026^{fg}	1.36 ± 0.15^{e}
	40	6.0 ± 0.115^{abc}	53.8 ± 1.09^{c}	31.2 ± 0.63^{c}	0.83 ± 0.029e	5.2 ± 0.18^{e}	0.84 ± 0.023^{de}	2.62 ± 0.15^{d}
	60	5.8 ± 0.057^{bcd}	48.3 ± 1.72^{c}	28.0 ± 0.99^{c}	1.04 ± 0.037^{cd}	6.4 ± 0.23^{cd}	0.91 ± 0.035^{cd}	3.24 ± 0.14^{cd}
	80	5.4 ± 0.173^{de}	35.8 ± 1.41^{d}	20.7 ± 0.82^{d}	1.11 ± 0.020^{bc}	6.9 ± 0.12^{bc}	1.00 ± 0.023^{bc}	3.98 ± 0.26^{bc}
	100	5.1 ± 0.100^{ef}	26.0 ± 1.61^{e}	15.0 ± 0.93^{e}	1.19 ± 0.034^{ab}	7.4 ± 0.21^{ab}	1.10 ± 0.029^{ab}	4.66 ± 0.17^{ab}
	120	4.4 ± 0.115^{g}	16.5 ± 1.32^{e}	9.5 ± 0.76^{f}	*1.25 ± 0.028^{a}*	*7.8 ± 0.17^{a}*	*1.21 ± 0.028^{a}*	*5.30 ± 0.17^{a}*
F. verticillioides GL5	20	6.3 ± 0.115^{ab}	66.5 ± 0.85^{ab}	38.5 ± 0.49^{ab}	0.63 ± 0.026^{gh}	3.9 ± 0.16^{gh}	0.60 ± 0.029^{gh}	1.04 ± 0.18^{e}
	40	6.1 ± 0.057^{ab}	63.5 ± 1.65^{ab}	36.8 ± 0.95^{ab}	0.76 ± 0.020^{ef}	4.7 ± 0.12^{ef}	0.76 ± 0.023^{ef}	1.26 ± 0.19^{e}
	60	5.9 ± 0.100^{bcd}	61.2 ± 1.58^{b}	35.5 ± 0.92^{b}	0.83 ± 0.034^{e}	5.2 ± 0.21^{e}	0.85 ± 0.028^{de}	2.52 ± 0.22^{d}
	80	5.5 ± 0.115^{cde}	53.8 ± 1.09^{c}	31.2 ± 0.63^{c}	0.98 ± 0.034^{d}	6.1 ± 0.21^{d}	0.91 ± 0.040^{cd}	3.15 ± 0.35^{cd}
	100	5.2 ± 0.115^{ef}	48.3 ± 1.41^{c}	28.0 ± 0.82^{c}	1.05 ± 0.017^{cd}	6.5 ± 0.10^{cd}	1.03 ± 0.040^{bc}	3.96 ± 0.31^{bc}
	120	4.8 ± 0.173^{fg}	35.8 ± 1.25^{d}	20.8 ± 0.72^{d}	1.18 ± 0.015^{ab}	7.3 ± 0.09^{ab}	1.09 ± 0.034^{ab}	5.21 ± 0.17^{a}

'±' indicates standard error (SE) calculated by using triplicates

Values (mean of triplicates) with different letters within the same vertical column indicate the significant difference at $p \leq 0.05$

A italicized cell represents the maximum value

Total OM total organic matter, *Total OC* total organic carbon, *Total N* total nitrogen, *Total P* total phosphorus, *Total K* total potassium

Table 2 Changes in carbon to nutrient supply ratios in solid substrates treated with ligninolytic fungi during solid-state fermentation in different time intervals

	Time interval (day)	C/N ratio	C/P ratio	C/K ratio
Areca husk				
Control		*64.4 ± 0.26*^{a}	*77.6 ± 3.56*^{a}	*57.2 ± 6.59*^{a}
M. circinelloides GL1	20	50.7 ± 2.25^{b}	54.5 ± 2.42^{c}	30.7 ± 3.19^{b}
	40	36.3 ± 0.36^{d}	34.6 ± 0.46^{de}	11.4 ± 0.81^{c}
	60	27.9 ± 1.24^{e}	24.2 ± 0.51^{fg}	5.5 ± 0.27^{c}
	80	15.8 ± 0.60^{fg}	14.6 ± 0.61^{hi}	3.1 ± 0.08^{c}
	100	11.5 ± 0.72^{gh}	11.5 ± 0.95^{hi}	2.6 ± 0.25^{c}
	120	7.6 ± 0.30^{i}	8.2 ± 0.31^{i}	1.8 ± 0.13^{c}
F. verticillioides GL5	20	53.1 ± 1.75^{b}	65.6 ± 2.21^{b}	46.1 ± 6.35^{a}
	40	44.5 ± 1.24^{c}	48.6 ± 3.22^{c}	28.9 ± 4.07^{b}
	60	33.4 ± 1.04^{d}	36.9 ± 1.82^{d}	10.6 ± 0.31^{c}
	80	28.2 ± 1.37^{e}	28.8 ± 1.30^{ef}	5.8 ± 0.23^{c}
	100	19.9 ± 1.07^{f}	22.9 ± 0.76^{fg}	5.0 ± 0.24^{c}
	120	16.8 ± 0.33^{f}	18.9 ± 0.73^{gh}	3.8 ± 0.17^{c}
Coffee husk				
Control		*71.2 ± 3.52*^{a}	*82.2 ± 7.86*^{a}	*45.4 ± 2.81*^{a}
M. circinelloides GL1	20	54.8 ± 0.68^{b}	57.1 ± 2.55^{bc}	35.2 ± 2.61^{b}
	40	37.3 ± 0.31^{d}	38.9 ± 3.13^{de}	19.8 ± 2.40^{c}
	60	22.2 ± 0.17^{e}	25.0 ± 1.83^{fg}	13.1 ± 1.28^{cde}
	80	12.3 ± 0.90^{gh}	15.3 ± 0.50^{gh}	8.3 ± 0.86^{def}
	100	8.2 ± 0.49^{hi}	11.1 ± 0.94^{h}	5.0 ± 0.56^{ef}
	120	5.5 ± 0.35^{i}	7.0 ± 0.36^{h}	3.0 ± 0.37^{f}
F. verticillioides GL5	20	59.2 ± 3.25^{b}	64.8 ± 4.05^{b}	37.0 ± 3.38^{ab}
	40	43.6 ± 0.16^{c}	49.1 ± 1.77^{cd}	30.8 ± 4.04^{b}
	60	34.2 ± 0.41^{d}	38.0 ± 0.09^{de}	19.1 ± 2.04^{c}
	80	22.8 ± 1.19^{e}	30.0 ± 0.94^{ef}	16.2 ± 1.28^{cd}
	100	19.7 ± 1.16^{ef}	24.6 ± 1.70^{fg}	12.8 ± 0.80^{cde}
	120	15.0 ± 0.59^{fg}	18.5 ± 0.07^{fgh}	7.4 ± 0.57^{def}
Paddy straw				
Control		*71.0 ± 4.11*^{a}	*80.3 ± 7.65*^{a}	*45.5 ± 3.64*^{a}
M. circinelloides GL1	20	52.2 ± 2.98^{c}	51.2 ± 2.69^{c}	26.7 ± 3.50^{c}
	40	37.4 ± 1.41^{ef}	37.2 ± 1.78^{def}	11.9 ± 0.48^{d}
	60	27.0 ± 1.97^{gh}	30.8 ± 2.23^{efg}	8.6 ± 0.08^{d}
	80	18.6 ± 0.65^{hi}	20.6 ± 0.36^{gh}	5.2 ± 0.48^{d}
	100	12.6 ± 0.42^{ij}	13.6 ± 1.20^{hi}	3.2 ± 0.32^{d}
	120	7.6 ± 0.52^{j}	7.9 ± 0.73^{i}	1.8 ± 0.20^{d}
F. verticillioides GL5	20	61.4 ± 3.29^{b}	64.1 ± 2.55^{b}	39.4 ± 6.80^{ab}
	40	48.1 ± 2.14^{cd}	48.5 ± 1.52^{cd}	30.4 ± 4.47^{bc}
	60	42.8 ± 2.40^{de}	41.9 ± 2.24^{cde}	14.3 ± 1.63^{d}
	80	31.6 ± 0.93^{fg}	34.1 ± 1.29^{ef}	10.1 ± 1.26^{d}
	100	26.7 ± 0.65^{gh}	27.3 ± 1.70^{fg}	7.1 ± 0.67^{d}
	120	17.6 ± 0.82^{i}	19.0 ± 1.20^{ghi}	4.0 ± 0.27^{d}

'±' indicates standard error (SE) calculated by using triplicates

Values (mean of triplicates) with different letters within the same vertical column indicate the significant difference at $p \leq 0.05$

A italicized cell represents the maximum value

C/N carbon/nitrogen, *C/P* carbon/phosphorus, *C/K* carbon/potassium

Determination of carbon to nutrient (N, P and K) ratios

After solid-state fermentation, the selected fungal strains reduced the initial carbon to nutrient (N, P and K) ratios compared to untreated control (Table 2). *Mucor circinelloides* GL1 rapidly decreased the initial C/N ratio of areca husk (64.4), coffee husk (71.2) and paddy straw (71.0) to 7.6, 5.5 and 7.6, respectively, compared to *F. verticillioides* GL5 and control. Likewise, the treatment of *M. circinelloides* GL1 significantly decreased the initial C/P ratio of areca husk (77.6), coffee husk (82.2) and paddy straw (80.3) to 8.2, 7.0 and 7.9, respectively, when compared with the treatment of *F. verticillioides* GL5 and control. It was also observed the reduction in initial C/K ratio of areca husk (57.2), coffee husk (45.4) and paddy straw (45.5) to 1.8, 3.0 and 1.8, respectively, which offered by *M. circinelloides* GL1 treatment.

Determination of the content and loss of lignin and holocellulose

Both the fungal strains were able to effectively degrade the lignin and holocellulose in all three substrates tested in the study. The degradation pattern of lignin and holocellulose was more effective after fungal inoculation with respect to their loss between the different harvesting times (Table 3). Inoculation with *M. circinelloides* GL1 was offered a significant reduction in initial lignin content of areca husk (19.9%), coffee husk (18.7%) and paddy husk (16.0%) to 6.5, 6.4 and 3.0%, respectively. Thus, *M. circinelloides* GL1 was causing a significant higher lignin loss of 67.1, 65.7 and 80.8% in areca husk, coffee husk and paddy husk, respectively.

Similarly, the inoculation with *M. circinelloides* GL1 also exhibited a significant reduction in initial holocellulose content of areca husk (55.1%), coffee husk (58.8%) and paddy husk (57.5%) to 28.0, 26.3 and 15.2%, respectively. Besides, *M. circinelloides* GL1 caused a significantly higher degree holocellulose loss of 49.0, 55.2 and 73.5% in areca husk, coffee husk and paddy husk, respectively.

Estimation of activity of enzymes

The fermentation profiles of the selected fungal strains had shown significant differences in the studied enzymes (laccase, MnP, LiP and CMCase) activity in decomposition of agricultural wastes. Both strains produced all enzymes, but their activities were remarkably varied with respect to fermentation time (Figs. 1, 2, 3, 4). The distinguished higher activity of laccase (9.4 IU/mL), MnP (1.45 IU/mL) and LiP (3.5 IU/mL) recorded by *M. circinelloides* GL1 on 80, 60 and 60 day of cultivation, respectively, in areca husk compared to *F. verticillioides* GL5 and untreated control (Fig. 1a–c), while *M. circinelloides* GL1 produced the maximum activity of laccase (12.15 IU/mL), MnP (0.98 IU/mL) and LiP (2.47 IU/mL) on above-mentioned cultivation periods in coffee husk (Fig. 2a–c). In case of paddy straw, *M. circinelloides* GL1 inoculation showed the significantly maximum activity of laccase (15.83 IU/mL), MnP (0.75 IU/mL) and LiP (4.5 IU/mL) on above-mentioned cultivation periods (Fig. 3a–c). However, it was also observed that *M. circinelloides* GL1 offered the maximum CMCase activity of 9.95, 8.9 and 11.73 IU/mL on 20 days of incubation in areca husk, coffee husk and paddy straw, respectively (Fig. 4a–c). The enzyme activity decreased gradually as the fermentation time increased.

Discussion

The success of fungal decomposition process primarily depends on the selection of an adequate solid substrate (as a supporting material) for performing the solid-state fermentation. It is a traditional cultivation practice to use the agro-industrial lignocellulosic wastes for small and larger scale production of major lignin-degrading enzymes. The responsible use of indigenous microbes for the degradation of agro-industrial wastes at a faster rate leads to improve soil organic matter and nutrients availability (Anwar et al. 2014). In the present study, three agricultural wastes (such as areca husk, coffee husk and paddy straw) as the lignocellulosic substrates were used to study the patterns of production of major ligninolytic and cellulolytic enzymes under fermentation. Ligninolytic fungi have been most extensively studied due to their apparent ligninolytic properties as well as their faster growth and more easily handling in the fields (Levin et al. 2004; Singh and Singh 2014).

The substrates used in this study were well supported for the extensive mycelial growth and hyphal infiltration of selected fungal strains. However, paddy straw supported the fastest mycelial extension of both strains, followed by coffee husk and areca husk. The rapid colonization of fungi on substrates may be due to their high competitive saprophytic nature. This remarkable result was in agreement with the studies of Jonathan et al. (2008) and Adejoye and Fasidi (2009). In the solid-state fermentation process, it is very much essential to optimize the initial moisture content as it greatly affects the substrate utilization and enzyme production. Therefore, approximately about 70% moisture content was maintained throughout the experimental periods. The moisture content varies between 30 and 85% for the optimum fungal growth and their nature of utilization of substrates. However, it depends upon the organisms and types of substrates used for the cultivation (Raimbault 1998). An increase in moisture content decreases the substrate porosity and limits oxygen and mass transfer.

Table 3 Changes in lignocellulose content and loss of solid substrates treated with ligninolytic fungi during solid-state fermentation in different time intervals

	Time interval (day)	Lignin content (%)	Lignin loss (%)	Holocellulose content (%)	Holocellulose loss (%)
Areca husk					
Control		*19.9 ± 0.58*^{a}	–	*55.1 ± 1.24*^{a}	–
M. circinelloides GL1	20	17.0 ± 0.64^{b}	14.5 ± 3.23^{e}	48.4 ± 1.27^{bc}	12.1 ± 2.30^{ef}
	40	15.0 ± 0.63^{bc}	24.2 ± 3.19^{de}	47.8 ± 1.33^{bc}	13.1 ± 2.42^{ef}
	60	13.0 ± 0.76^{cd}	34.6 ± 3.83^{cd}	44.6 ± 1.32^{cd}	18.9 ± 2.41^{de}
	80	11.9 ± 0.59^{d}	40.0 ± 2.97^{c}	41.0 ± 1.58^{de}	25.5 ± 2.88^{cd}
	100	8.7 ± 0.52^{ef}	56.2 ± 2.66^{ab}	32.0 ± 1.60^{fg}	41.9 ± 2.91^{ab}
	120	6.5 ± 0.44^{f}	*67.1 ± 2.21*^{a}	28.0 ± 1.73^{g}	*49.0 ± 3.14*^{a}
F. verticillioides GL5	20	17.5 ± 0.32^{b}	12.0 ± 1.61^{e}	53.9 ± 0.74^{ab}	2.0 ± 1.34^{f}
	40	15.5 ± 0.54^{bc}	21.9 ± 2.75^{de}	49.0 ± 1.32^{bc}	11.0 ± 2.41^{ef}
	60	13.7 ± 0.66^{cd}	31.1 ± 3.34^{cd}	47.8 ± 1.30^{bc}	13.1 ± 2.36^{ef}
	80	12.3 ± 0.49^{d}	38.1 ± 2.48^{c}	45.1 ± 1.81^{cd}	18.1 ± 3.30^{de}
	100	9.2 ± 0.55^{e}	53.7 ± 2.76^{b}	37.5 ± 1.44^{ef}	31.9 ± 2.61^{bc}
	120	7.2 ± 0.52^{ef}	63.8 ± 2.66^{ab}	32.9 ± 1.21^{fg}	40.1 ± 2.20^{ab}
Coffee husk					
Control		*18.7 ± 0.51*^{a}	–	*58.8 ± 1.27*^{a}	–
M. circinelloides GL1	20	16.2 ± 0.40^{bc}	13.1 ± 2.16^{ef}	52.3 ± 1.15^{bc}	11.0 ± 1.96^{fg}
	40	15.5 ± 0.51^{c}	17.1 ± 2.77^{e}	47.4 ± 1.35^{cd}	19.2 ± 2.31^{ef}
	60	11.5 ± 0.58^{de}	38.3 ± 3.10^{cd}	41.1 ± 1.61^{ef}	30.1 ± 2.75^{cd}
	80	9.8 ± 0.40^{ef}	47.2 ± 2.18^{bc}	36.9 ± 1.45^{fg}	37.1 ± 2.46^{bc}
	100	8.3 ± 0.46^{fg}	55.6 ± 2.47^{ab}	33.0 ± 1.04^{g}	43.8 ± 1.76^{b}
	120	6.4 ± 0.69^{g}	*65.7 ± 3.70*^{a}	26.3 ± 1.08^{h}	*55.2 ± 1.84*^{a}
F. verticillioides GL5	20	18.1 ± 0.32^{ab}	3.2 ± 1.71^{f}	56.5 ± 1.16^{ab}	3.9 ± 1.98^{g}
	40	16.0 ± 0.57^{bc}	14.4 ± 3.08^{ef}	51.2 ± 1.72^{bc}	12.9 ± 2.92^{fg}
	60	12.6 ± 0.92^{d}	32.6 ± 4.93^{d}	46.9 ± 1.24^{cd}	20.1 ± 2.11^{ef}
	80	11.5 ± 0.72^{de}	38.4 ± 3.86^{cd}	43.1 ± 1.34^{de}	26.6 ± 2.28^{de}
	100	11.0 ± 0.40^{de}	41.1 ± 2.15^{cd}	36.5 ± 1.24^{fg}	37.8 ± 2.11^{bc}
	120	9.1 ± 0.58^{ef}	51.3 ± 3.13^{bc}	32.6 ± 1.48^{g}	44.4 ± 2.51^{b}
Paddy straw					
Control		*16.0 ± 0.55*^{a}	–	*57.5 ± 1.12*^{a}	–
M. circinelloides GL1	20	14.3 ± 0.31^{bc}	10.4 ± 1.98^{gh}	52.5 ± 1.24^{ab}	8.6 ± 2.16^{gh}
	40	12.3 ± 0.38^{d}	22.7 ± 2.40^{f}	43.6 ± 1.55^{cd}	24.1 ± 2.71^{ef}
	60	9.7 ± 0.43^{ef}	39.3 ± 2.72^{de}	41.8 ± 1.42^{cd}	27.1 ± 2.47^{ef}
	80	7.6 ± 0.37^{g}	52.2 ± 2.34^{c}	32.1 ± 1.27^{e}	44.1 ± 2.21^{d}
	100	5.2 ± 0.34^{h}	67.5 ± 2.16^{b}	21.2 ± 1.21^{g}	63.1 ± 2.11^{b}
	120	3.0 ± 0.34^{i}	*80.8 ± 2.17*^{a}	15.2 ± 0.98^{h}	*73.5 ± 1.70*^{a}
F. verticillioides GL5	20	15.6 ± 0.17^{ab}	2.2 ± 1.10^{h}	54.4 ± 1.51^{a}	5.3 ± 2.63^{h}
	40	13.2 ± 0.46^{cd}	17.5 ± 2.88^{fg}	47.5 ± 1.07^{bc}	17.3 ± 1.86^{fg}
	60	10.4 ± 0.31^{e}	34.5 ± 1.98^{e}	44.7 ± 1.53^{c}	22.1 ± 2.66^{f}
	80	8.7 ± 0.40^{fg}	45.6 ± 2.52^{cd}	38.1 ± 1.64^{d}	33.7 ± 2.86^{e}
	100	7.5 ± 0.43^{g}	53.1 ± 2.72^{c}	30.0 ± 1.32^{ef}	47.7 ± 2.31^{cd}
	120	5.4 ± 0.32^{h}	66.2 ± 2.01^{b}	25.0 ± 1.26^{fg}	56.5 ± 2.20^{bc}

'±' indicates standard error (SE) calculated by using triplicates

Values (mean of triplicates) with different letters within the same vertical column indicate the significant difference at $p \leq 0.05$

A italicized cell represents the maximum value

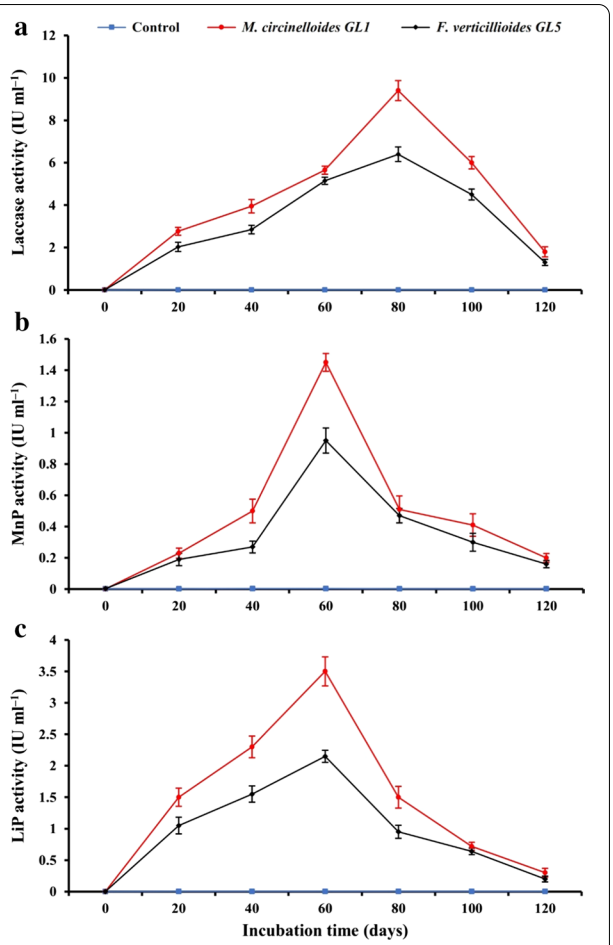

Fig. 1 Changes in ligninolytic enzyme activity of areca husk treated with ligninolytic fungi during solid-state fermentation in different time intervals. **a** laccase, **b** MnP and **c** LiP

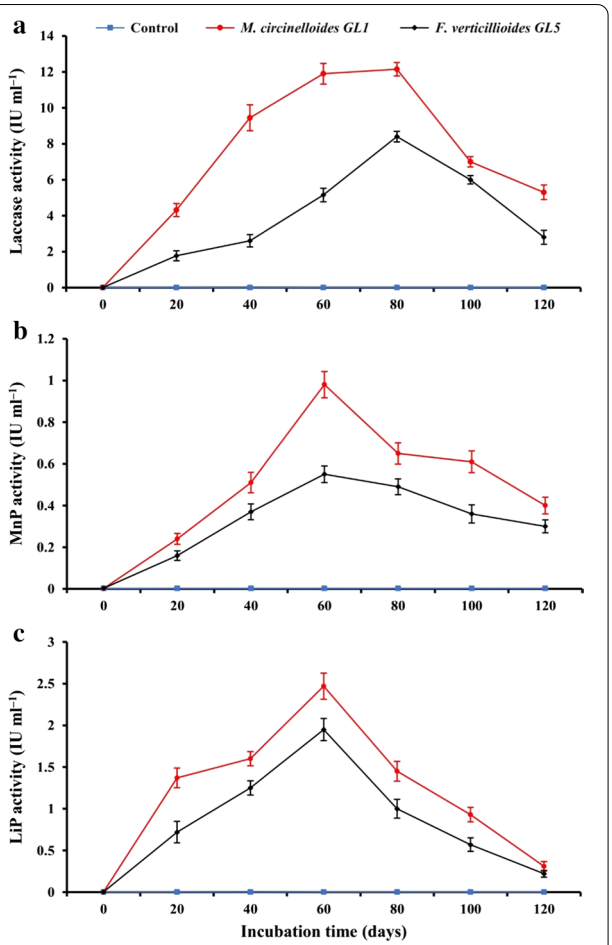

Fig. 2 Changes in ligninolytic enzyme activity of coffee husk treated with ligninolytic fungi during solid-state fermentation in different time intervals. **a** laccase, **b** MnP and **c** LiP

The relatively high moisture content may also result in limited fungal growth within the substrate and their surface growth.

The fungal growth favors at lower pH and resulting in 50-fold increase in their relative abundance between high pH (7.4) and low pH (3.3) (Rousk et al. 2011). We have observed the reduction in initial pH from neutral to acidic pH in all substrates decomposed by both strains for the period of 120 days after inoculation. The change in initial pH in the fungal decomposed agricultural wastes as the period of fermentation increased may be linked to the increase in metabolic products within the substrates. Initial pH of the straw-mycelium mixture was considerably changed during the growth of fungi, and the pH change was directly correlated with the fungal decomposition (Zadražil 1977).

The loss of initial total organic matter was significantly increased in all the substrates decomposed by selected strains. This result was in agreement with Sharma and Arora (2010) wherein the loss of total organic matter could be ascribed to use as the energy and available carbon source during the degradation of lignocellulosic materials by white-rot fungi. Study on the effect of carbon (C) and nitrogen (N) source on the production of ligninolytic enzymes is getting much importance. The C and N sources have proved as the powerful nutritional factors that regulating the production of ligninolytic enzymes by wood rotting basidiomycetes (Mikiashvili et al. 2006). The release of glucose from the lignocelluloses has promoted the rapid fungal growth within the raw materials as well as fungi need readily metabolizable carbon sources (such as glucose) for lignin degradation (Wu et al. 2005). The organic carbon is converted into carbon dioxide (CO_2) and energy as the metabolic end products; subsequently, the carbon content of substrate reduces as the degradation process progresses. We have also noticed a significantly decreasing trend in initial organic carbon during decomposition of substrates

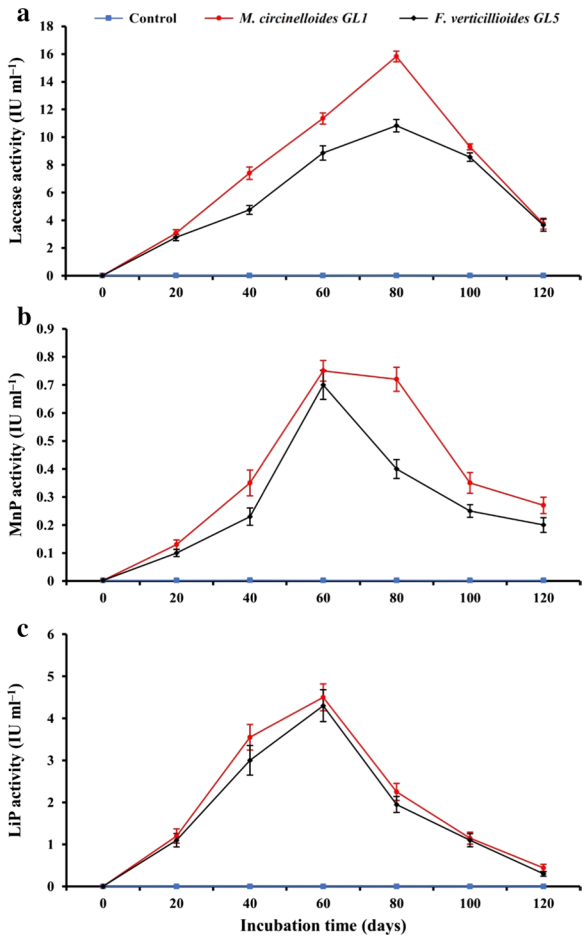

Fig. 3 Changes in ligninolytic enzyme activity of paddy straw treated with ligninolytic fungi during solid-state fermentation in different time intervals. **a** laccase, **b** MnP and **c** LiP

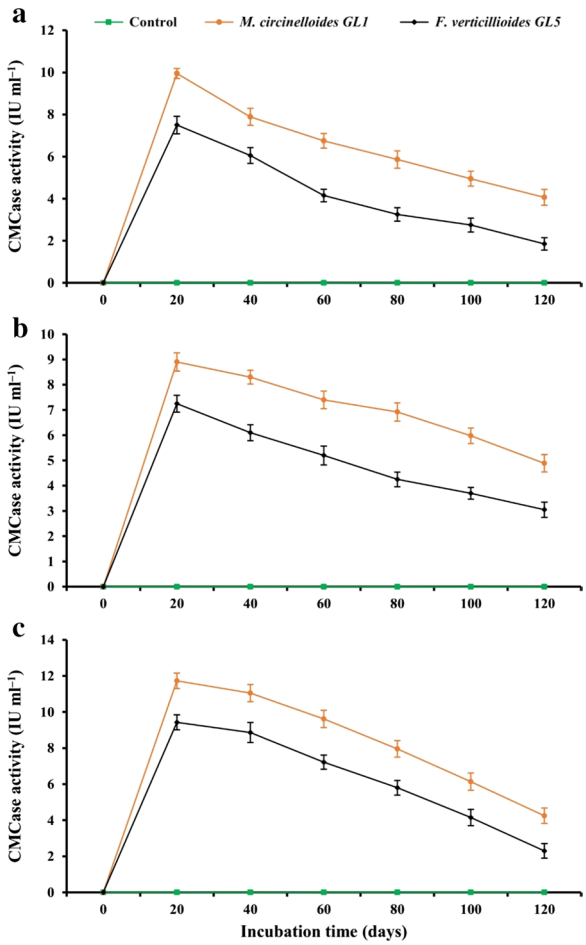

Fig. 4 Changes in CMCase enzyme activity of different solid substrates treated with ligninolytic fungi during solid-state fermentation in different time intervals. **a** areca husk, **b** coffee husk and **c** paddy straw

by the selected fungi. Pramanik (2010) has reported that the initial organic carbon content of organic wastes was found to decrease as CO_2 during vermicomposting by the ligninolytic fungi.

Our results showed that the total nitrogen content was in an upward trend with decomposing time which caused by the loss of total organic carbon associated with the mineralization of total organic matter. The increase in nitrogen content might be due to the formation of new cell structures, enzymes and hormones by the soil microbes (Zhu 2007). However, there was a raise in the crude protein content of areca husk, coffee husk and paddy straw inoculated with the fungal strains with increasing the incubation period. It may be due to the non-proliferation of fungi on the solid substrates during the decomposition process. Belewu and Babalola (2009) have reported that an increase of 16% in crude protein content of rice husk in an in vitro digestibility experiment conducted with *Rhizopus oligosporus* for 7 day of incubation. An increment of 51.6% in crude protein was reported in another study where the fermentation of rice husk was conducted by *Trichoderma viridii* for the period of 10 days (Zaid and Ganiyat 2009). In addition, it was observed that there was an increase in initial total phosphorus and potassium contents of substrates after decomposition by the selected fungi. The results were similar to the findings of Pramanik (2010) who reported that the total nitrogen, phosphorus and potassium contents in organic wastes were consistently found to increase during the vermicomposting by the ligninolytic fungi.

The elemental carbon to nutrient supply (N, P and K) ratio is used to estimate the availability of associated nutrients in the agricultural crop residues. As the plant litter decomposes, the labile organic carbon substrates decrease and decline in the C/N and C/P ratios as N and P compounds are incorporated to form the humic

substances (Grandy and Neff 2008). The data showed that a sharp decrease in the C/N, C/P and C/K ratios was observed in the decomposition of the tested substrates by the selected fungi with respect to the initial ratios. The C/N ratio is playing an important role in bioconversion and establishment of the compost maturity level of agricultural wastes (Bernal et al. 1998). The C/N ratio was declined rapidly in the composted substrates, as the C content was lost in the form of CO_2 through the microbial respiration and the N content was recycled during fermentation (Ryckeboer et al. 2003). Osono and Takeda (2001) have reported the decrease in the initial C/P and C/K ratios during the leaf litter decomposition by fungi.

The agro-industrial wastes are principally composed of a complex mixture of cellulose, hemicellulose and lignin which are used for the production of biofuels and other value-added products (Sadh et al. 2018; Tsegaye et al. 2019). Several fungal species have the ability to decompose lignocellulosic wastes by the successive production of a mixture of extracellular hydrolytic and oxidative (ligninolytic) enzymes (Navarro et al. 2014). During the degradation process, cellulose and other labile substrates are first degraded easily and then followed by lignin depolymerization with increased ligninolytic enzyme activity (Baldrian 2009). In our study, the selected potential fungal strains reduced the lignin and holocellulose contents of the substrates with respect to increase in incubation time. Li et al. (2008) have reported that an efficient ligninolytic fungus *F. concolor* was significantly degraded and removed 13.07% of lignin and 7.62% of holocellulose contents of wheat straw.

Moreover, all the three substrates used were well supported for the production of ligninolytic and cellulolytic enzymes by the selected fungi. The enzyme activities presented have shown that the enzyme production patterns are dependent on fungi and type of substrates. The fungal enzyme production levels could be ascribed to the different chemical compositions of the substrates. The statement agreed with the results reported by Shah et al. (2005), who reported the increased ligninolytic and cellulolytic enzymes production patterns in the bioprocessing of banana waste by *Aspergillus* spp. and *Phylosticta* spp.

Conclusion

The fungal decomposition process resulted in higher degradation pattern of lignocellulosic substances of agricultural wastes as evident from their loss of cell wall constituents as well as increase in fungal enzyme activities. Here, we noticed that the selected fungal strains (*M. circinelloides* GL1 and *F. verticillioides* GL5) were found to be very efficient producers of ligninolytic and cellulolytic enzymes in the solid-state fermentation of agricultural wastes. Therefore, the present study suggested that these potential ligninolytic fungal strains can be successfully used for managing and reusing the agricultural crop residues in the conservation system of forest soils to eliminate the harsh effects of the crop residue burning.

Abbreviations

BSA: Bovine serum albumin; *M. circinelloides*: *Mucor circinelloides*; *F. verticillioides*: *Fusarium verticillioides*; MEA: Malt-extract agar; LOI: Loss on ignition; CMC: Carboxymethyl cellulose; DNS: 3,5-Dinitrosalicylic acid; IU: International Units; MnP: Manganese peroxidase; LiP: Lignin peroxidase; CMCase: Carboxymethyl cellulose; OM: Organic matter; OC: Organic carbon; C: Carbon; N: Nitrogen; P: Phosphorus; K: Potassium; CO_2: Carbon dioxide; RCBD: Randomized complete block design.

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Authors' contributions

GPA and JM conceptualized and designed the research work. GPA and GHG performed the experiments, analyzed and interpreted the data. All authors read and approved the final manuscript.

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Competing interests

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