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Research Article

The potential use of rice waste lignocellulose and its amendments as substrate for the cultivation of *Pleurotus eous* Strain P-31 in Ghana

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ABSTRACT

The use of mushroom for the bioconversion of low quality lignocellulosic agricultural waste into nutritional food and animal feed rich in protein and minerals offers an alternative food and feed supplement. The genus *Pleurotus* comprises of edible lignolytic mushrooms capable of selective delignification of lignocellulolytic crop residue converting into edible protein and mineral salts. The effect of raw untreated rice straw and pre-treated (composted for 0, 4, 8 and 12 days supplemented with either 5, 10 and 15% rice bran 1% $CaCO_3$ on growth rate, yield performance and Biological Efficiency (BE) of *Pleurotus eous* Strain P-31 was investigated using the conventional pasteurized bagged technique. The traditional "wawa" (*Triplochiton scleroxylon*) sawdust was used for comparison purposes only. The growth parameters (pH, moisture, spawn running time, fruit body formation, interval between flushes, total number of fruiting bodies during the cropping time and mushroom yield (Biological Efficiency) as well as the nutrient and mineral profiles of the fruiting bodies were determined by the conventional techniques. It was observed that amendment or supplementation of the substrate (rice straw) did not significantly ($p \leq 0.05$)enhance mushroom yield of *Pleurotus eous* such that growth on the composted substrates (4 - 8 days) gave the best yield with BE 67.1 – 75.1%. Yield on the unfermented rice straw was comparatively good (BE = 53.3 - 72.8)%. Both the pH and moisture content for all the substrates were within the optimum range of 6.0 - 8.0, 60 – 70% RH respectively. The standard substrate, "wawa" sawdust, currently in use for cultivation of *Pleurotus* spp. gave BE of 55.6 – 64.8%. The fruiting body contained high fat, protein, crude fibre and carbohydrates but differed depending on the type of substrate used. The carpophore contained mineral elements Ca, Mg, K, Na, and P in appreciable amounts useful for good health. Although heavy metals such as Cu, Fe, Mn, Pb and Zn were detected they were far below the WHO stipulated safe limits. The presence of high potassium over sodium makes *P. eous* fruiting body suitable for therapeutic treatment of hypertension.

KEYWORDS: Rice lignocellulose, Biological Efficiency, Nutritional and elemental contents, Fruiting body, and *Pleurotus eous*

INTRODUCTION

Rice is the third largest cereal crops among seven principal cereals grown worldwide. Paddy rice fields occupy 155 million hectares of global agricultural lands and provides 659 million tonnes of rice (i.e. 28% of global total cereal production)[1,2]. In Ghana large amount of lignocellulose wastes are generated through agro-industrial activities each year. These agro lignocellulosic wastes are underutilized and disposed of in the environment without any proper treatments. Sometimes, they are even burnt as fuel

leading to serious environmental pollution problems. In Ghana, there is an annual production of approximately 1,779,859.667 tonnes of paddy rice[3.] Such is the case that large amount of wastes (including straw, husk, bran which include the straw on the farm land, rice husk and bran) from the milling process are left at the vicissitudes of the environment. However, these agricultural wastes can be potentially bio-converted into value-added products such as food protein from mushroom, pulp, animal feed, and biofuel as well as bio-fertilizer through the action of lignin-degrading enzymes secreted by fungi such as member of the genus *Pleurotus* (oyster mushrooms).

The genus *Pleurotus* comprises of edible lignolytic mushrooms capable of selective delignification of lignocellulosic farm residues[4,5,6,7], as a result of which the cellulose is exposed and can be utilized by ruminants as well[8]. There are various parameters affecting the growth and fruiting of oyster mushrooms among which are substrate source, substrate quality, pH, spawn, compost, strain of the mushroom and supplementation[9,10]. Macro-fungi of the genus *Pleurotus* are preferred by many people worldwide for their delicate taste, mild and chewy texture and unique aroma. The world trade of these oyster mushrooms shows an increasing pattern and gives promising opportunity for traders[11]. For sustained cultivation to supply consumers it is necessary to explore cheap cultivation techniques. Currently, *Pleurotus* mushrooms are cultivated in large amounts using lignocellulose materials such as wheat straw, paddy straw, cotton and banana pseudostem, Bahia grass[12], bamboo leaves, lawn grass[13], wild grass (*Pennisetum* sp.) corn stover (*Zea mays*), oil palm (*Elaeis guineensis*), fruit fibres, cocoa shells[14, 15,5]. In Ghana the most preferred compost / substrate for the cultivation of oyster mushroom *Pleurotus* species is ‘‘wawa’’ sawdust (*Triplochiton scleroxylon*) composted for up to 24 – 28 days[15,5]. However, rice lignocellulose has not been tried as compost for cultivation of *P. eous* Strain P-31. In Ghana there are added nutritional and medicinal properties of *Pleurotus*[16] which necessitates increased commercial production. Nutritionally, the mushroom has been found to contain vitamins B1 (thiamine), B2 (riboflavin), B5 (niacin), B6 (pyridoxine) and B7 (biotin)[17]. Medically, the species of *Pleurotus* have been reported to decrease cholesterol levels[18]. The fruiting body of the mushroom is also a potential source of lignin and phenol degrading enzymes[19]. This mushroom is also used industrially as a bio-remediator[20, 21]. Recently, the species have also attracted great attention as a source of bioactive metabolites for the development of drugs and nutraceuticals[22, 23]. Some of them have also been found to be a source of some secondary metabolites such as flavonoids, terpernoids, sterol, phenolic compounds, β carotene, lycopene and antioxidants[24, 25, 26] and ability to ward off cancers, HIV, AIDS and other viral ailments; they are antimutagenic, antitumoral and can be used to manage cardiovascular disorders[27]. There was also significant ($p \leq 0.001$) difference among *Pleurotus* mushroom strains in their mineral element content such as Mg, Fe, Ca, Mn, Cu, Zn, Ni, Cd, Pb, Cr, Na, K[25, 26, 28].These mineral elements are also useful in promoting healthy living. The objective of this study was to report the use of rice lignocellulose and its amendments on the spawn running time, fruiting formation, yield, nutrient and mineral elements of the test mushroom *Pleurotus eous* cultivated under the Ghanaian tropic conditions.

MATERIALS AND METHODS

Preparation of Stock Pure Cultures and spawns:

One-week old pure cultures of *Pleurotus eous* (Berk.) Sacc. Strain P-31obtained from the National Mycelium Bank at the CSIR-Food Research Institute was used for this study. Stock cultures of *P. eous* were grown on slants of Potato Dextrose Agar (PDA) in McCartney tubes and on Petri dishes and sub cultured subsequently spawns were prepared using in accordance to some researchers[29, 30].

Collection of rice straw, preparation and spawning:

Rice straw was collected from Dawhenya and Aveyime-Battor area on Rice Farms in the Volta Region of Ghana and prepared in accordance to Narh et al. and Obodai et al.[30, 31].The substrate was either supplemented with 1% $CaCO_3$ and 10% rice bran respectively or was used without amendment. The substrate was either allowed to undergo composting for 4, 8 and 12 days respectively or was used as unfermented (0 day) substrate.

Bagging and sterilization of substrates:

At the end of the fermentation process the mixture was either supplemented with different percentages of rice bran (5, 10 and 15%) as additional source of nitrogen or bagged without the addition of any supplements. Each substrate was thoroughly mixed after water was sprinkled on the mixture to obtain a moisture content of about 70% (w/w). The substrate was bagged into heat resistant transparent polyethylene bags. Bagged composted substrates were steam sterilized at a temperature of 90-100^{o}C for 3 hours[15, 5, 30, 31].These were then allowed to cool and inoculated with fully grown spawns. After which they were incubated and allowed to thicken for a period of 4 weeks.

Cropping and harvesting of fresh fruit bodies:
At the end of spawn run, the bags of thick mycelial colonisation were transferred to the cropping house and stacked on wooden shelves and opened. Matured mushrooms were then harvested.

Cultivation of *Pleurotus eous*:
Pretreated and untreated rice straw and husk with various combinations of 5, 10 and 15% of rice bran were used as the cultivation substrates. Parameters recorded included the spawn run period i.e. the number of days from inoculation to complete colonization of the compost bag by the mycelia, mycelial density (was done by direct observation), number of days taken till appearance of pinheads and the number of flushes per treatment. The days from bag opening to first flush, weight and number of carpophores per flush, weight and number of carpophores per bag, interval between flushes (the average number of days that lapses between consecutive flushes) and the BE values were also assessed. Biological Efficiency (BE) values were calculated in accordance to Royse et al. [9]
B.E. = [Weight of fresh mushrooms harvested / dry weight of substrate] x 100.

Analytical methods:
Estimation of lignin, cellulose, hemicellulose and crude protein and silica was done by standard methods of[32]. The fruiting bodies of *P. eous* were collected dried in an oven at 60°C to a constant weight and kept under refrigeration at 4°C. Samples of mushrooms were analysed for their proximate composition (crude protein, fibre, carbohydrate, moisture, and ash) and elemental composition using the procedures given by a report[32]. The nitrogen factor used for protein calculation was (N × 4.38); minerals such as P, Cu, Fe, Mg, Mn, Pb, and Zn were determined using Atomic Absorption Spectrophotometer (Perkin Elmer AAS Model PinAAcle 900T), whereas Na and K were determined using flame photometer method.

pH and moisture content determination:
The acidity of the sterilized substrates was measured using a pHM92 Lab pH meter (MeterLab™, Radiometer Analytical A/S, Copenhagen, Denmark). Moisture content of the sterilized substrates was determined using the conventional hot oven method (Gallenkamp oven, 300plus series, England) at 107ºC.

Statistical analysis:
Data analysis was conducted using Statistical Package for Social Sciences (SPSS,) version 16 by Analysis of variance (ANOVA) test along with Least Significant Difference (LSD $\alpha \leq 0.05$) and the separation of means was done by post-hoc comparisons with Duncan Multiple Range Test. Values reported are the means and standard errors of five replicates for each treatment.

RESULTS
pH and Moisture Content of Substrates:
The average pH and moisture content of all treated and untreated substrates during bagging and after sterilization ranged approximately from pH 7.5 - 8.2 and 66.3 - 76.1%respectively. For example, the following moisture 76.1, 70.8, 68.3 and 66.4% was recorded for unfermented (0 day) and fermented compost (4,8 and 12 days) respectively. The average pH values recorded were as follows 7.96, 8.13, 8.26 and 8.07 at 25.0, 24.8, 24.9 and 24.8°C for 0, 4, 8 and 12 days compost respectively. These values were not significantly different ($p \geq 0.05$) from all the other treatments with the exception of the moisture content recorded for unfermented substrate (76.1%) which was statistically significant ($p \leq 0.05$) from the 12 days compost substrate (67.8%). Both the pH and the moisture content for all the substrates were generally within the optimum range of pH 6.0-8.0 and 60-75% respectively.

Spawn running, primordia and fruit body formation:
The spawn run period, days till primordial formation and the days from bag opening to first flush behaved variably. For example, data obtained on spawn run period and bag opening to first flush was not significantly different ($p \geq 0.05$) among all the treatments (i.e. irrespective of the number of composting days and or the treatment applied to the compost in effect did not have any influence on these two parameters measured (Table 1). The average spawn run period (total colonization) recorded in this study was between 3 - 5 weeks (35 – 42) (Table1). The difference between the days till primordia formation (days from bag opening to first flush) indicated that on the average it takes 2-3 days for the fruit body of *P. eous* to mature from the primordial to the matured stage.
The mean interval between flushes ranged from 14 – 24 days with the modal interval between flushes of about 16 days for all treatments (Table 1). The average number of pinheads recorded in the unsupplemented rice husk substrate formulated for 0 (unfermented), 4, 8 and 12 days were 66, 82, 73 and 71 respectively (Table 1). Substrates which were supplemented with either 5, 10 or 15% rice bran and composted for 0, 4, 8 and 12 days produced pinheads ranging from 57 – 82. In all cases the unfermented rice husk compost supplemented with rice bran

recorded the highest number of pinheads (72 – 82) (Table 1) corresponding to total fruit bodies of 46-60 (Table 1) as compared to 37 – 52 on the supplemented substrates. The days from the opening of bags to first flush was shorter for the unfermented rice husk compost (2 – 4 days) as compared to 3 - 7 days for the rest of the treatments. The total number of fruit bodies formed on the unfermented (0 day) and fermented (4 – 12 days) were significantly ($p \leq 0.05$) higher (46 – 60 fruit bodies) than in the supplemented substrates (36 – 52 fruit bodies) (Table 1). Fruiting periods observed for the various treatments was about 6 weeks (42 days). Total fruit bodies recorded on the sawdust which served as control ranged from 39 – 49 fruits, although many pinheads were formed not all resulted in successful fruiting body formation.

Interval between flushes, number of flush, total no. of fruiting bodies and cropping period:

Mean interval between flushes ranged from 13-17 days with modal mean interval between flushes as 16 days for all treatments (Table1). The modal flush number for the treatments ranged from 2-5 over the 7 weeks (42 days) of cropping. Data reported in this present study were based on three flushes due to the inability of *P. eous*to attain either the 4^{th} and or 5^{th} flushes on some of the other rice waste lignocellulose. The average number of pinheads per treatment for *Pleurotus eous* recorded were 66, 82, 73 and 71 for both unfermented and fermented (4, 8 and 12 days) compost respectively. These were not significantly different ($p \geq 0.05$) from each other with the exception of the mean number of pinheads recorded for 0 and 4 days composts. Compost substrates that were either amended with and or supplemented with additional nitrogen source (5, 10 and 15% rice bran) did not significantly ($p \geq 0.05$) increase the number of pinheads formed (Table 1). Sawdust substrate which was used purposely for comparison recorded the 78, 62, 65 and 57 number of pinheads with the corresponding total number 49, 39, 49 and 37 of fruiting bodies formed for 0, 4, 8 and 12 days respectively (Data not shown). These were not statistically different ($p \geq 0.05$) from results reported on ricewaste lignocellulose.

Total mushroom yield of *Pleurotus eous*:

P. eous grew variably on both fermented and unfermented rice straw used for this study. For example *P. eous* grown on non-supplemented 4 and 8 days fermented rice straw substrate recorded the highest yield of 219.5 and 221.5g with BE values of 75.6 and 76.4% respectively which were statistically ($p \leq 0.05$)higher as compared to unfermented (0 and 12 days composted substrates (Table 2). When it was grown on supplemented rice straw (1% $CaCO_3$ and 10% rice bran) the highest yield recorded were on 4 and 8 days fermented rice straw (209.8g and 217.9g with corresponding BE values of 72.3 and 75.1%) and was statistically significant ($p \leq 0.05$) from the 0 and 12 days compost as recorded on rice straw only (Table 3). The maximum yield obtained on amended rice straw substrate supplemented with different amounts of rice bran (5, 10 and 15%) recorded as 197.6g with BE = 68.1% was obtained on unfermented (0 day) rice straw supplemented with additional 5% rice bran (A_5). Further supplementation with rice bran above 5% did not statistically ($p \geq 0.05$) increase yield of *P. eous* above the non-supplemented batch (Table 4). The highest yield recorded on rice straw and rice husk (1:1w/w) combination was obtained on unfermented (0 day) compost substrate 211.0g with corresponding BE of 72.8% was statistically significance ($p \leq 0.05$) from all the other treatments (Table 5). It was observed that both the amendment of the rice straw with rice husk and with additional supplementation of rice bran to the substrates did not significantly ($p \geq 0.05$) increase yield and nutritional value of *P. eous*. The standard substrate ''wawa'' sawdust currently in use for *Pleurotus* spp. cultivation used for comparison purposes only yielded fruiting bodies ranged from 194.3 - 226.9g akin to what was obtained on the rice waste lignocellulose used for this present study. However, BE was higher rice straw 55.7 – 76.4% in contrast with 55.6 – 64.8% on the sawdust (Tables 2 - 6).

Proximate analyses and mineral content of fruiting bodies:

Tables 7 and 8 show the results obtained. There were no significant differences ($p \geq 0.05$) in the dry matter and total ash content of samples irrespective of the treatment. However, total fat ranged from 3.99 – 19.05% but was highest in the mushroom cultivated in the uncomposted samples (Table 7) ranging from 14.8 – 19.05%. Corresponding carbohydrates contents were also lower (9.35 – 11.5%) as compared to fruiting bodies grown on rice straw only and 'wawa' sawdust28.25 - 28.55% (Table 7) composted for 8 days. Crude protein contents ranged from 21.61 – 35.99% across all treatment.

Ten mineral elements were found were found in the mushroom. Mineral contents K (11.0 – 24.0 mg/kg), Na (6.0 – 14.0 mg/kg) and P (6.31 -10.07 mg/kg) were high (Table 8) although other minerals such as Ca, Mg could be detected in low quantities (Table 8). Heavy metals such as Fe, Mn, Pb, Cu and Zn were also detected albeit in very low levels (Table 8).

Biochemical analysis of raw straw and spent straw:

Figs 1&2 summarize results obtained. There was a general slight increase in crude protein and a decrease in cellulose, hemicellulose, lignin, silica and gross moisture. Fine dry weight remained nearly constant (Figs 1 & 2). The maximum increase in protein content of the spent straw among the different substrates was 4.67 – 8.71% and the maximum decrease in hemicellulose and cellulose was recorded as 24.75 – 36.64% (hemicellulose) and 1.95 – 24.8% (cellulose).

DISCUSSION

pH and moisture content of substrate at bagging:

Results from this paper show that the average pH and moisture content of the substrate ranged from pH 7.5 – 8.2 and 66.3 – 76.1% mc in all the treated and untreated substrates used in the cultivation of *P. eous* (data not shown). Some researchershave reported that generally, the optimum range of pH for growth of *Pleurotus* was 6.8 -8.0 and best moisture content was 60-75%[31,33-35]. Tesfaw etal. reported a similar pH (5.8-7.02) range when they worked on optimization of oyster (*Pleurotus ostreatus*) mushroom cultivation using locally available substrates and materials in DebreBerhan, Ethiopia[78]. In the same study, the team has recorded the moisture content of the substrates to range from 69.8% to 74.5% whereas the pH of the straw before addition of chalk and gypsum was 5.8. These investigators conjectured it might be because of the acids produced by microbes in substrates during soaking of substrates in water since it was soaked overnight. These previousresults agree with current data from this paper.

Spawn running, primordial and fruit body formation:

The mycelial growth through the bags was uniform and white in all treatments. However, the spawn run periods (weeks) varied from 3 – 5 weeks (21-35 days) depending on treatment (Table 1). Clearly, it took 2 – 7 days for the fruit body of *P. eous* to mature and was fastest in the uncomposted and unamended substrate. This agrees with the recent reports by various researchers on completed spawn running in 17-20 days on different substrates and time for pinheads formation was noted as 23-27 days whereas the pin heads developed in 26-31 days for *P. ostreatus*[36-38,78].

Interval between flushes, number of flush, total no. of fruiting bodies and cropping period and Biological Efficiency :

The mean interval between flushes ranged from 14 – 24 days with a modal mean interval of 16 days for all treatments. This is slightly higher than the 7 -14 days recorded by Stamets et al.[34] for *P. ostreatus*. The modal flush number for the treatments ranged from 2 – 5 over 7 weeks of cropping which corresponds with 2 – 6 flushes recorded by Mandeel et al.[39] on papers, cardboards, fibre and sawdust by *P. ostreatus*. The unfermented rice husk either supplemented or unsupplemented could produce higher numbers of pinheads resulting in higher successful fruiting. The days from the opening of bags to first flush was shorter for the unsupplemented rice husk (2 – 4 days) as compared to 3 – 7 days for the rest of the treatment. This is an added advantage given the fact that the total number of successful fruit bodies from the unfermented (0 day) and fermented (4 – 12 days) were significantly higher (40 - 60 fruits) than in the supplemented substrates (36 – 52 fruits). Interestingly, the total successful fruiting bodies recorded on the sawdust compost which served as control and is usually used for mushroom production in Ghana, ranged from 39 – 49 fruits. Although many more pinheads were formed they aborted leading to low successful fruit formation.

Mushroom yield and Biological Efficiency:

Growth and yield of *P. eous* on both fermented and unfermented rice straw and its amendments was variable. Generally yield was best on unsupplemented and 4 – 8 days fermented rice straw substrate (221.5g) and supplementation with $CaCO_3$ and rice bran was not advantageous. The high Biological Efficiency of *P. eous* (67.1 – 76.4%) on 0 – 8 days composted straw only is being recorded for the first time. A wide range of enzymes such as laccase and xylanases[40, 41, 42]produced by mushroom mycelia are capable of utilizing complex organic compounds. However, *P. eous* seem to have utilized uncomposted rice straw substrates efficiently presumably because of the possible ‘‘*de novo*’’ induction of some enzymes by the substrates responsible for the efficient utilization by *P. eous*. This is a virtual factor in mushroom cultivation[43]. Yildiz et al. stated that rice straw provides a reservoir of cellulose, hemicellulose and lignin which is utilized by *P. eous* during growth and fructification[44]. Indeed recent studies has shown that rice straw used in this study contains cellulose (29.71 – 38.82%), hemicellulose (20.81 – 24.99%), lignin (5.38 – 8.95%) and silica (11.74 -19.18%)[28].

Proximate analysis and mineral content of fruiting bodies:

The effect of the different combinations and substrate supplementation of rice straws on the mineral

nutrient composition of the *P. eous* was variable. There was no significant ($p \geq 0.05$) difference in dry matter (83.44 – 88.98%) and total ash content (6.53 - 10.54%) irrespective of treatment. Total fat was higher in the mushroom cultivated in the uncomposted samples (14.87 – 19.05%) which is in excess of the reported lipids content of 1.6 – 5.0% on dry weight basis for *Pleurotus* species by various researchers[45-48]. Total carbohydrate which includes polysaccharides of glucan, mono and disaccharides, sugar alcohols, glycogen and chitin ranged from 9.35 – 28.6% on dry wt. basis depending on treatment (Table 7). [49] found that the carbohydrate content of *P. ostreatus* was 62.5% and 69.9% for *Lentinusedodes* on dry matter basis. This may be attributed to the different substrates used. However, data from this study were similar and within the results reported by various authors [47, 48, 50.] Crude protein content obtained for *P. eous* across the treatments ranged from 21.63 - 35.99%. Previous studies by many researchers have reported crude content of 23.1 - 34.8% for some *Pleurotus* species when cultivated on agro-waste such rice straw, wheat straw, rice and paper combination, sugarcane bagasse[51,47,48]. It is has been demonstrated that different formulation and combination of substrate would influence yield and quality of mushroom produced[52, 53, 54]. Recently, Sueli et al. investigated the effects of various sawdust substrates namely Fig tree (T2), Rain Tree (T3), Mahogany tree (T4), Ipilipil tree (T5), Eucalyptus tree (T6) and mixture of all sawdust (T1), supplemented with 30% wheat bran and 1% lime on the nutritional composition of oyster mushroom (*Pleurotus ostreatus*)[77]. These researchers recorded the highest amount of carbohydrate (42.36%) in the T4 sawdust substrate, highest amount of dry matter (10.53%), lipid (4.46%) on T2 substrate treatment, and the maximum amount of crude fiber (20.53%) on T6 whereas as the highest content of protein (27.30%) was obtained on T5[77]. Interestingly, the results obtained in this current paper agree with the data reported by these investigators.

Mineral content:

Minerals in diet are essential for metabolic reactions, healthy bone formation, transmission of nerve impulses, and regulation of water and salt balances[55].The mineral content of *P. eous* varied with the different substrates and their combination as expected (Table 8)[56]. The preponderance of K in the sporophore tissues may be due to the enhance absorption of this element from the substrate. Potassium content of *Pleurotus* spp. ranged from 182 to 395mg/100g (0.0182 to 0.0395mg/kg)[44], far below what was detected in *P. eous* in this present study. The Recommended Daily Intake RDI of potassium is 3100mg/Day[57]. Sodium concentration in *P. eous* fruit bodies varied with the different formulation of the substrate (6.0 – 14.0mg/kg) (Table 8). A balance of high potassium and low sodium content in *P. eous* is obvious in this present study. Patil and his team also reported a balance between high potassium and low sodium content in *P. ostreatus* cultivated on different substrates[46]. Among the different substrates, paddy rice straw showed maximum K:Na ratio (7.79), followed by a combination of soybean and wheat straw (7.69) while the least ratio was recorded on wheat straw (6.88) [46]. Various other research teams also reported a high potassium and low sodium concentration in mushroom fruiting body[49,58]. The presence of high potassium content over sodium in diet is associated with the therapeutic effectiveness of mushroom like *P. eous* against hypertension. Phosphorus content of the mushrooms cultivated on the variously formulated substrate ranged from 6.3 – 10.07mg/kg (Table 8). Patil SS and his team showed that out of six substrates used in the cultivation of *P. ostreatus*, the recorded phosphorus content ranged from 790 – 1000mg/100g (0.079 – 0.100mg/kg)[46]. The Recommended Daily Intake RDI of phosphorus is 0.7g. Thus *P. ostreatu*s and *P. eous* are high in phosphorus content to contribute to human nutrition as a good source of phosphorus [59]. Although calcium and magnesium were detected in low quantities (0.125 – 3.95mg/kg and 0.116 – 0.86mg/kg) respectively, they could play an essential role for a balanced human nutrition.

Some heavy metals (Cu, Fe, Mn, Pb and Zn) were detected in the sporophore of *P. eous* albeit in small quantities. Heavy metal concentration in mushroom is considerably higher than those in agricultural crops such as vegetables and fruits. This connotes that mushrooms have an effective mechanism which enables them to readily take up some heavy metals from the environment[60] due to their dense mycelia system which ramify the substrate[61,62]. The maximum Fe detected in the *P. eous* fruiting body was 0.350mg/kg (Table 8). This is far below the limit of 15mg/kg set by Senesse et al.[63]. Iron deficiency anaemia affects one third of the world's population but excessive intake of iron is associated with an increased risk of cancer [64].

Copper was either not detected from the sporophore of *P. eous* grown on the sawdust / rice straw: rice husk combination or low in the remaining substrate combinations (0.0020 – 0.015mg/kg). These values were far below the stipulated safe limit of 40.0mg/kg in foods set by Senesse et al[63]. Copper levels reported in mushrooms in the pertinent literature are 4.71 – 51.0mg/kg [65]; 13.4 – 50.6mg/kg [66]and 12.0 – 181.0mg/kg [67]. Recently Obodai and his team showed that there was a significant difference ($p \leq 0.05$)

between the concentrations of Cu in the sporophore of *P. ostreatus* Strain EM-1 cultivated on composted cassava peel (6.96±1.33mg/kg) than in the uncomposted cassava peel (3.7±0.08mg/kg)[25]. The accumulation of heavy metals in mushroom has been found to be affected by environmental factors such as organic matter content of substrate, pH, metal concentration in soil as well as species morphology, development of carpophore, age of the mycelium, intervals between fructifications and biological composition of substrate [68].

Lead concentration of *P. eous* fruiting body varied from 0.0134 – 0.2020mg/kg (Table 8). These values were far below the 10.0mg/kg safe limit set by Senesse et al for lead in raw plant material[63]. Lead levels in mushrooms reported in the literature are 0.75 – 7.77mg/kg[65]; 0.40 – 2.80mg/kg[69]; 1.43 – 4.17mg/kg [67]. Lead is toxic even in trace levels [70]and the impairment of human functions related to Pb toxicity include abnormal size and haemoglobin content of erythrocytes, hyper stimulation of erythroporesis and inhibition of haem synthesis of haemoglobin[71].

Manganese is an essential metal as it plays an important role in biological systems such as its presence in metalloproteins[72]. The highest and lowest Mn concentrations in the fruiting body of *P. eous* cultivated on different substrates were 0.116 and 0.886mg/kg respectively. These values fall far below the toxicity limit of 400 -1000mg/kg of Mn in plant tissue[63]. Interestingly, varying ranges of Mn in mushrooms have been documented as 14.5 - 63.5mg/kg[73]; 12.9 – 93.3mg/kg[67]; 14.2 – 69.7mg/kg [66]. Zinc is also an essential mineral and is a component of a wide variety of enzymes and co-enzymes. This mineral performs catalytic, structural and regulatory roles in protein synthesis and enzyme metabolism[76]. The minimum and maximum levels of Zn in *P. eous* obtained in this study were 0.018mg/kg and 0.190mg/kg respectively. The WHO recommended permissible limit of Zn in foods is 60mg/kg[63]. Value obtained in this present study fall well below the permissible level. One can say that *P. eous* is a good bio-accumulator of Fe, K, Na, P and Mg. Ramirez et al. stated that Fe, Mg and P were elements which were present in high amounts in rice straw ranged from 582 to 1,302mg/kg but found no connection between extractable soil Fe and Mn content of rice straw also used in this present study[75]. Recent a study by Obodai et al using oyster mushrooms (*Pleurotus pulmonarius, P. ostreatus, P. sapidus* and *P. cintrinopileatus* have shown that Mg, Ca, Cu, Zn, Ni, Cd, Pb, Cr were present in appreciable concentrations[25].

Cultivation of *Pleurotus eous* on different substrates essentially needs an understanding of the methods of cultivation as well as the chemical composition of both the substrate and the fruiting body. Data from this paper provides wealth of information on both the micro and macro-elements needed for fruiting which are similar to that of the higher plants. Apart from P, K, Mg and S which are necessary for fungal growth others like Na, Mg, Ca are required for the fruiting body[77]. Wang et al [76]stated that the widely studied micro elements for growth of many fungal species are Fe, Zn, Al, Mn, Cu, Cr and Mo some of which have been detected in the fruiting body of *P. eous* in this present study and in *P. ostreatus*[28]. Currently some research workers[77] investigated the effects of various sawdust substrates namely Fig tree (T2), Rain Tree (T3), Mahogany tree (T4), Ipilipil tree (T5), Eucalyptus tree (T6) and mixture of all sawdust (T1), supplemented with 30% wheat bran and 1% lime on the nutritional composition of oyster mushroom (*Pleurotus ostreatus*). They reported that the highest calcium (31.98 mg/100g) and magnesium (19.85 mg/100g) were found in the T4 sawdust substrate treated mushrooms whereas phosphorous (0.91%) and molybdenum (14.76 mg/100g) were highest for the T1 substrate treated mushrooms. The maximum concentration of iron (42.55 mg/100g), zinc(27.65 mg/100g) and selenium (6.77 mg/100g) were obtained for T2 substrate treatment. However, the highest level of cobalt (22.40 mg/100g) was found for T3 substrate treatment[77].Whilst cobalt, molybdenum, selenium were not analysed for in the fruiting body in this present study, the concentrations of the remaining minerals detected in the fruit body of *P. eous* was similar to that obtained for *P. ostreatus* reported by[77].This previous finding showed that the best nutritional composition containing mushroom was grown on T2 (Fig tree) sawdust substrate, followed by T1, T5, T6, T3 and T4[77]. It corroborates that different substrates, supplementation and composting of substrates influence the nutritional and mineral composition of the fruiting body. This present finding confirms to the reports recorded by these previous researchers.

Biochemical analysis of raw straw and spent straw:

There was a general slight increase in crude protein and a decrease in cellulose, hemicellulose, lignin, silica and gross moisture whilst fine dry weight remained almost constant. There was no significant different ($p \geq 0.05$) in the biochemical content of the raw, fermented and spent compost irrespective of composting duration and supplementation of substrates. Although there was no statistical difference ($p \geq 0.05$) in the biochemical analysis of non-supplemented rice straw or supplemented rice straw and spent rice straw was numerically different.

For example %moisture content (92.32-93.28); hemicellulose (20.81-24.79) %; cellulose (34.36-38.82) %;crude protein (4.41 – 5.36) % and silica (11.74-14.29)% for raw rice straw whereas the following range were recorded for spent rice straw (84.56-92.66; 7.11-8.71; 2.13-3.05; 28.55-35.24 and 3.96-6.45) % for moisture content, crude protein, hemicellulose, cellulose and lignin respectively. Obodai et al. reported a similar findings where they observed significant increases of cellulose, hemicellulose and fat contents were observed up to day 12 (10.37, 16.1 and 9.39%, respectively) after which there were gradual declines of 15.4, 57.6 and 56.12%[25]. The increase in cellulose, hemicellulose and lignin during the first 12 days will likely due to the consumption of starch by microorganisms. The decrease of these compounds (cellulose and hemicelluloses) in subsequent days indicate that when starch is removed mostly, microorganisms start to degrade also the (hemi) cellulose. Lignin, protein and crude fibre values showed a gradual increase from day 0 to 28, with a maximum value of 23.73, 49 and 73%, respectively (Figures 5, 6 and 7). These changes could be due to the type of microorganisms present in the substrate. Presumably, antibiosis was at play in the composting cassava peel byproduct as the composting process involves microbial activity[25]. This present finding is similar to this previous study.

CONCLUSION

This paper contains data showing that the use of *P. eous* for bioconversion of low quality rice lignocellulose and its amendments into edible food is feasible and is being recorded for the first time in Ghana. The substrates used for the cultivation of *P. eous* had the suitable pH and moisture for the cultivation of the species. Although the spawn run period varied from 3 – 5 weeks depending on treatment, it took 2 - 7 days for the fruit body to mature and was fastest on the uncomposted and unamended substrate. The unfermented rice husk either supplemented or unsupplemented produced higher numbers of pinheads resulting in higher successful fruiting. The days from opening of bags to first flush was also shorter for the unsupplemented rice husk substrate (2 - 4 days) as compared to 3 - 7 days for the rest of the treatment. This is an added advantage given the fact that the total number of successful fruiting bodies from the unfermented (0 day) and fermented rice straw (4 -12 days) were (40 – 60 fruits) significantly ($p \leq 0.05$) higher than in the supplemented substrates. The total successful fruiting bodies on sawdust composted usually used for the cultivation of oyster mushroom in Ghana ranged from 39 – 49 fruits. Supplementation of rice straw with $CaCO_3$and rice bran had no advantage. The high Biological Efficiency of *Pleurotus eous* (67.1 – 76.4%) on 0 – 8 days composted rice straw only is economically profitable to the cultivator. The level of total fat, total carbohydrate, crude protein etc makes the mushroom nutritionally useful to human health not excepting the content of K, Na, P, P, Ca, Mg and heavy metals Fe, Zn, Mn, which were all below the recommended safe limits by[64]. The presence of high potassium over sodium makes *P. eous* suitable mushroom candidate for therapeutic treatment of hypertension.

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Table 1

Days for completion of spawn running, pinhead and fruit body formation and cropping period of *P. eous* strain P-31 grown on rice with different indicated treatments

Treatment	Spawn run period (weeks)	Average mycelia growth rate (cm)	Mycelial density	Days from bag opening to 1st flush	Average Interval between flushes (days)	Total no. of pinheads	Total no. of fruit bodies
0	3	9.0	+++	2	14	66	46
4	3	9.4	+++	3	15	82	60
8	4	9.8	+++	4	16	73	49
12	4	8.3	+++	5	17	71	47
A_5	4	8.9	+++	3	15	82	52
A_{10}	4	8.4	+++	5	16	72	44
A_{15}	3	8.1	+++	3	17	82	57
B_5	4	7.7	+++	5	20	62	40
B_{10}	4	7.2	+++	5	21	62	40
B_{15}	4	5.6	+++	4	19	67	41
C_5	4	6.2	+++	7	23	60	38
C_{10}	4	6.2	+++	7	23	55	36
C_{15}	4	7.0	+++	6	23	62	41
D_5	5	6.7	+++	7	24	61	38
D_{10}	5	6.4	+++	5	23	59	38
D_{15}	5	5.7	+++	6	24	57	37

Degree of mycelial density when mycelia fully colonize the substrate

+++ Mycelium totally grows through the bag and is uniformly white. 0, 4, 8 and 12 days represent unfermented and fermented compost which were not supplemented with additional nitrogen source (rice bran) before bagging with corresponding composting periods. A, B, C and D represent initial day (0), 4th, 8th and 12th days compost respectively which were supplemented with either 5%, 10% and 15% rice bran respectively before bagging

Table 2

Total yield and Biological Efficiency (BE) of *P. eous* strain P-31 grown on rice straw without supplementation (additives)

Period of composting / day(s)	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	111.6 ± 5.6	27.3 ± 4.1	22.6 ± 5.4	161.5[a]	55.7
4	126. 4 ± 3.5	55.8 ± 3.8	37.1 ± 3.8	219.3[b]	75.6
8	127.9 ± 3.9	55.6 ± 9.1	38.0 ± 5.8	221.5[b]	76.4
12	88.2 ± 7.1	68.4 ± 8.5	28.9 ± 2.7	185.5[c]	64.0

The letters indicate significant differences to 95% in accordance with one way ANOVA Test.
Values in the same column followed by a different letter do differ significantly from each other
All values are means of five replicates

Table 3

Total yield and Biological efficiency of *P. eous* strain P-31 grown on rice straw substrate supplemented with 1% $CaCO_3$ and 10% and rice bran and composted for the varying periods

Period of composting / day(s)	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	134.1 ± 1.8	37.3 ± 3.5	18.0 ± 1.2	189.4[a]	67.3
4	124.8 ± 2.4	56.3 ± 1.8	28.7 ± 3.8	209.8[b]	72.3
8	125.5 ± 3.8	55.2 ± 2.9	37.2 ± 4.7	217.9[b]	75.1
12	108.8 ± 7.5	56.0 ± 8.7	29.7 ± 6.9	194.5[a]	67.1

The letters indicate significant differences to 95% in accordance with one way ANOVA Test.
Values in the same column followed by a different letter do differ significantly from each other
All values are means of five replicates

Table 4

Total yield and Biological Efficiency (BE) of *P. eous* strain P-31 grown on rice straw substrate amended with 1% $CaCO_3$ and 10% rice bran and composted for varying periods (0-12 days) prior to supplementation with different amounts of nitrogen source (5, 10 and 15% rice bran) at bagging before sterilization

Period of composting (day(s))	Treatments	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
		1st Flush	2nd Flush	3rd Flush		
0	A_5	125.0±6.7	41.7±4.4	30.9±3.5	197.6[a]	68.1
	A_{10}	95.1±10.8	39.1± 7.0	31.0 ± 6.0	165.2[b]	57.0
	A_{15}	91.4 ± 12.3	44.8± 7.0	23.4 ± 1.6	159.6[c]	55.0
4	B_5	101.1±20.0	48.7± 10.3	34.7 ±4.6	184.5[d]	63.6
	B_{10}	92.7± 11.8	46.1± 6.4	41.0 ± 6.4	179.8[e]	62.0
	B_{15}	78.7 ± 4.6	60.9± 3.6	35.1 ± 3.3	174.7[e]	60.2
8	C_5	63.7 ± 2.4	49.3± 4.4	42.5 ±7.5	155.5[c]	53.6
	C_{10}	80.2 ± 5.4	36.5± 2.8	52.8 ± 4.3	169.5[b]	58.4
	C_{15}	70.3 ± 8.3	49.3± 4.4	34.3 ± 5.4	153.9[c]	53.1
12	D_5	85.7 ± 12.4	59.6± 8.1	40.1 ± 4.6	185.4[d]	63.9
	D_{10}	94.0 ±7.5	46.6± 6.2	35.0 ± 7.4	175.6[e]	60.6
	D_{15}	67.5 ± 12.4	40.4± 6.5	32.4 ± 4.5	140.3[f]	48.4

The letters indicate significant differences to 95%, in accordance with one way ANOVA Test.
Values in the same column followed by a common letter do not differ significantly. All values are means of five replicates.
Keys:A,B,CandD, represent initial day (0), 4th, 8th and 12th day respectively
5%, 10% and 15% rice bran respectively

Table 5

Total yield per flush and biological efficiency (BE) of *P. eous* strain P-31 grown on rice on rice straw and rice husk (1:1 w/w) amended with 1% $CaCO_3$ and 10% rice bran and composted for 0-12 days prior supplementation with different amounts of nitrogen source (5, 10 and 15% rice bran) at bagging before sterilization

Period of composting (day(s))	Treatments	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
		1st Flush	2nd Flush	3rd Flush		
0	A5	110.8 ± 3.0	66.3 ± 9.8	33.9 ± 5.0	211.0a	72.8
	A10	80.7 ± 1.9	51.2 ± 6.0	32.5 ± 4.6	164.4b	56.7
	A15	78.6 ± 5.2	49.8 ± 3.7	26.3 ± 2.5	154.7c	53.3
4	B5	65.1 ± 12.0	38.5 ± 4.5	29.9 ± 4.5	133.5d	45.9
	B10	64.0 ± 9.6	41.9 ± 3.0	20.5 ± 1.9	126.4e	43.4
	B15	60.8 ± 2.2	42.3 ± 3.5	24.1 ± 0.9	127.2e	43.9
8	C5	63.5 ± 4.6	38.4 ± 4.2	20.2 ± 3.5	122.1e	42.1
	C10	56.5 ± 6.2	34.9± 3.3	20.4 ± 3.6	111.8f	38.6
	C15	58.8 ± 4.2	42.2 ± 2.8	20.2 ± 3.5	121.2e	41.8
12	D5	53.8 ± 3.7	38.8 ± 5.1	21.3 ± 4.3	113.9f	39.3
	D10	53.6 ± 3.1	37.4 ± 4.4	22.8 ± 3.7	113.4f	39.1
	D15	52.6 ± 4.3	36.3 ± 5.8	19.3 ± 2.7	108.4g	37.4

The letters indicate significant differences to 95%, in accordance with one way ANOVA Test.
Values in the same column followed by a common letter do not differ significantly. All values are means of five replicates.
Keys: A, B, C and D represent initial day (0), 4th, 8th and 12th day respectively. 5%, 10% and 15% rice bran respectively

Table 6

Total yield per flush of*P. eous* strain P-31on on ‘‘wawa’’ sawdust (*Triplochitonscleroxylon*) amended with 1% $CaCO_3$ and 10% rice bran and composted for varying periods (0-12days) prior to bagging for sterilization

eriod of composting (day(s))	Yield / Flush (g)			Total Yield (g)	Biological Efficiency (%)
	1st Flush	2nd Flush	3rd Flush		
0	113.4±6.9	52.1±4.0	28.8±6.0	194.3a	55.6
4	104.7±6.1	56.0±2.6	36.2±8.0	196.7a	56.2
8	122.3±5.4	62.1±9.0	42.5±4.3	226.9b	64.8
12	119.8±2.2	57.2±4.7	41.4±7.6	218.4b	62.4

The letters indicate significant differences to 95% in accordance with one way ANOVA Test.
Values in the same column followed by a different letter do differ significantly ($p \leq 0.05$) from each other. All values are means of five replicates.

Table 7
Proximate Analysis of *Pleurotus eous* strain P-31 mushroom grown on different agro-lignocellulosics materials (rice straw, husk and sawdust) on dry matter basis (DMB)

Composting period (days) treatment	Treatment code	% Dry Matter	% Fat	% Crude Fibre	% Crude Protein	% Total Ash	% Carbohydrate	% NDF
8 days RS	RS	85.55	3.99	24.54	21.63	5.26	28.25	47.91
8 days RS	RS+L+RB	86.73	5.91	21.78	23.28	4.97	18.11	54.00
0day D_5RS	RS+L+%RB	88.44	14.87	16.37	28.83	5.73	11.54	45.41
0 day RSD_5	RS+H+%RB	85.14	19.05	12.19	35.99	5.63	9.35	39.55
8 days Sawdust	S+L+RB	85.50	5.56	17.25	27.82	4.89	28.55	44.60

Key
RS –rice straw only (without additives)
RS+L+RB- rice straw with1% lime and 10% rice bran
RS+L+%RB- rice straw supplemented with 5% rice bran
RS+H+%RB- rice straw and rice husk combination (1:1) substrate supplemented with5% rice bran
S+L+RB- sawdust with 1% $CaCO_3$ and 10% rice bran

Table 8
Total mineral content of *Pleurotus eous* strain P-31 fruit bodies grown on different rice straw and sawdust lignocellulose materials.

Treatment code	Mineral content (mg/kg)									
	Ca	Cu	Fe	K	Mg	Mn	Na	P	Pb	Zn
RS	0.3950	0.0020	0.2340	11.7000	0.7650	0.0180	6.0000	7.5900	0.0500	0.1900
RS+L+RB	0.3690	0.0130	0.2310	12.1500	0.7580	0.0160	6.0010	7.5950	0.1015	0.1870
RS+L+%RB	0.3780	0.0150	0.2680	12.2500	0.8600	0.0060	6.0000	10.0750	0.2020	0.1760
RS+H+%RB	0.1301	0.0000	0.3200	24.0000	0.1163	0.0011	13.000	8.2025	0.0134	0.0184
S+L+RB	0.1250	0.0000	0.3500	16.1020	0.1289	0.0598	14.0000	6.3150	0.0000	0.0130

Key
RS –rice straw only (without additives), RS+L+RB- rice straw with1% lime and 10% rice bran, RS+L+%RB- rice straw supplemented with 5% , ice bran, RS+H+%RB- rice straw and rice husk combination (1:1) substrate supplemented with5% rice bran, S+L+RB- sawdust with 1% $CaCO_3$ and 10% rice bran

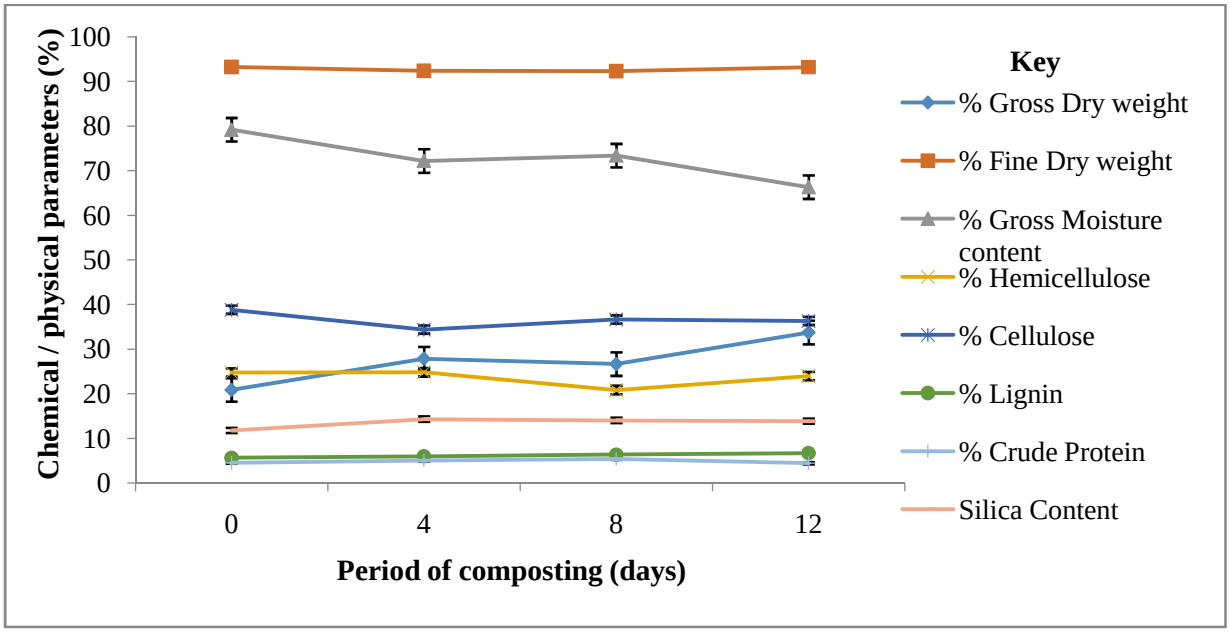

Fig 1
Chemical analysis of unfermentedand fermented rice straw only during the indicated period of composting in days

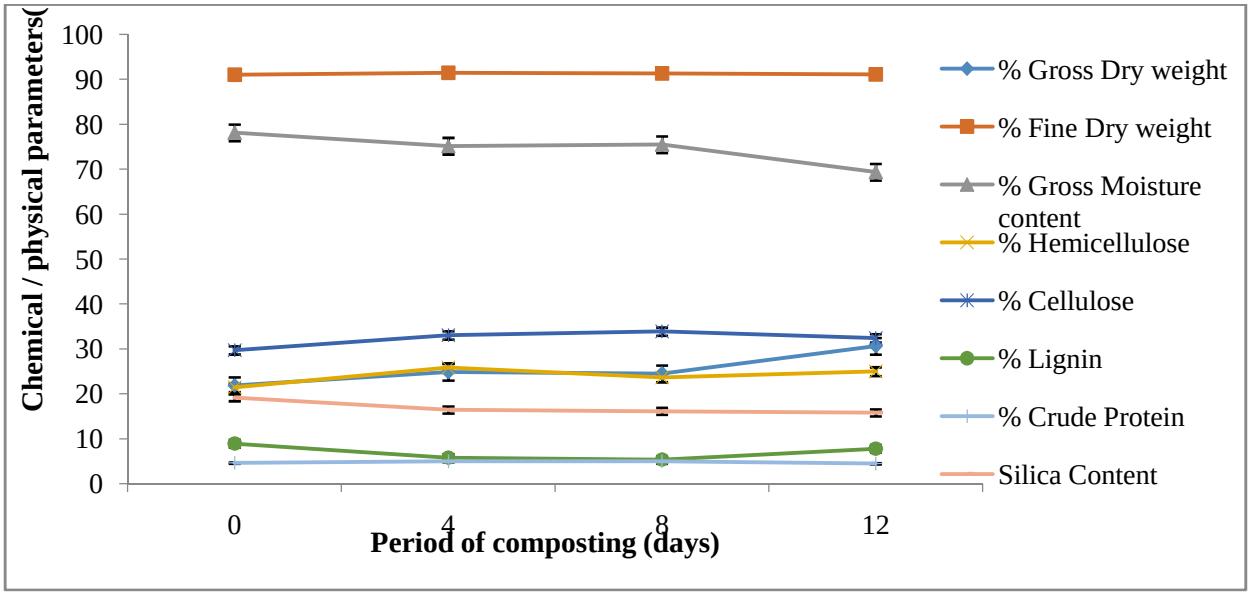

Fig 2
Chemical analysis of unfermentedand fermented rice straw amended with 1% $CaCO_3$ and 10% rice bran during the indicated period of composting in days

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