



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>

INTERNATIONAL JOURNAL
OF ADVANCED RESEARCH

RESEARCH ARTICLE

CULTIVATION OF SELECTED *Pleurotus* SPECIES USING SUGARCANE BAGASSE, WASTE PAPER AND LEAVES OF *Prosopis juliflora* (Sw.) DC.

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Manuscript Info

Manuscript History:

Received: 15 December 2014
Final Accepted: 22 January 2015
Published Online: February 2015

Key words:

Edible mushrooms, *Pleurotus*
species, *Prosopis Juliflora*,
proximate composition, Biological
efficiency

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Abstract

Cultivation of mushrooms using agricultural and industrial residues provides a very cheap and ecofriendly alternative for producing foods with high nutritional value. Currently, about 40% of sugarcane bagasse from sugar factories and a large quantity of wastepaper as well as biomass from lignocellulosic plants, which could have been used as cheap source of growth substrate for mushrooms. Thus, an experiment was conducted to cultivate three selected species of *Pleurotus* (*Pleurotus florida*, *Pleurotus ostreatus* and *Pleurotus sajor-caju*) using three different substrates and their combinations at Metahara Sugar Factory Research Station and to determine their biological efficiency and nutritional compositions. The results showed that the highest crude protein (35.93%) was obtained from *Pleurotus ostreatus* grown on sugarcane bagasse alone. The least crude protein (21.25%) was obtained from *Pleurotus sajor-caju* grown on the substrate combination of 50%waste paper +50%Leaves of *Prosopis juliflora*. Moisture content, crude fat, total ash, crude fiber, carbohydrate and biological efficiencies obtained from this experiment generally ranged from 77.5% - 85.5%, 1.33%-2.07%, 6.46%-8.06%, 8.8%-12.39%, 34-26.6% and 44.6%-70.5%, respectively. In this study, the highest biological efficiency for the studied mushroom species was recorded from sugarcane bagasse (70.5%) followed by wastepaper (68.9%). In contrast, mushrooms grown on leaves of *Prosopis juliflora* alone showed the least (44.6%) biological efficiency. Negative results were recorded for *Pleurotus florida* and *Pleurotus Sajor-caju* when grown on LPJ alone. The nutritive values of the studied mushrooms were quite very high, and the selected growth substrates were reasonably as good as sugarcane bagasse when used in mixtures.

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INTRODUCTION

One of the world's biggest challenges is food insecurity. This problem is largely common in low and middle-income countries that mainly have poor food production systems and suffer from serious malnutrition. Mushroom cultivation could be a possible option to alleviate poverty and develop the life style of the vulnerable people (Diriba et al., 2013). Mushrooms include edible, medicinal and poisonous species and are nature's gift as they are protein rich foods for human beings. The edible mushrooms are excellent food that can be incorporated into well balanced diets due to their low content of fat and energy, and high content of dietary fiber and functional compounds (Bismita, 2011). According to edible mushroom the *Pleurotus* species have received considerable attention for their nutritional value, medicinal properties and biodegradation abilities. They are efficient colonizers and bioconverters of lignocellulosic agro-industrial residues into pleasant human food with medicinal properties, with the productivity of the conversion being expressed by biological efficiency (Singh et al., 2005).

The cultivation of oyster mushroom requires the use of cellulosic materials or residues. These residues and by-products can be recovered and upgraded to higher value and useful products by using them as growth substrates

(Dawit, 1998). In this regard paper is almost 100% cellulosic in composition. At a global level, about 40-65% of paper is wasted and disposed off to the environment (Prognos, 2010). *Prosopis juliflora* is xerophytic and has now invaded most of the Ethiopian pastoral areas in Afar Regional State and some parts of Oromia Regional state including Fentale Woreda, which is found in the Rift Valley (Mehari, 2008) and also sugarcane bagasse is the matted cellulose fiber residue from sugarcane that has been processed in a sugar mill. The cane remaining after milling is bagasse (Moghtaderi et al., 2006). Sugar factories use most of this sugarcane bagasse as fuel source and dispose some of it to the environment. Thus to reduce environmental pollution and alleviate malnutrition cultivation of edible mushrooms may be the only currently economical biotechnology for lignocellulosic organic waste recycling that combines the production of protein rich food. Hence this study was conducted to examine the suitable substrate to cultivate three edible mushroom species: *Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Pleurotus florida* using sugarcane bagasse, wastepaper and leaves of *Prosopis juliflora* as growth substrate. In addition to this the biological efficiency and proximate composition were also investigated.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at Metahara Sugar Factory Research Station, which is located at 200 Km East of Addis Ababa, Ethiopia, in East Shoa, Oromia Regional State and 10 km to the south of Metehara town. Metahara is geographically located in the Middle Awash Valley, central rift valley of Ethiopia at a latitude and longitude of 8°53'N and 39°30'E, respectively, and an altitude 950 meters above sea level. The mean annual maximum and minimum temperatures of the area are 31°C and 17.5°C, respectively, while the humidity ranges between 85.4% and 30.3%. Average bagasse produced per year from this sugar factory was around 312,115 tones. Approximately around 60% of this biomass was used as a fuel source by the factory while the remaining 40% was dumped as waste in to the environment.

3.2. Experimental Design

The experiment was designed in a Completely Randomized Design (CRD) with three replications involving a 21 X 3 factorial arrangement with seven preparations of growth substrates (Bagasse, Waste paper, Leaves of *Prosopis juliflora* and their four different mixtures) and three selected *Pleurotus* namely: *Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Pleurotus florida*.

3.3. Sources of Experimental Materials

3.3.1. Source of spawn

Pure cultures of edible *Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Pleurotus florida* spawn were obtained from Mushroom Research, Production and Training Laboratory of Haramaya University, Ethiopia.

3.3.2. Source of growth substrates

Leaves of *Prosopis juliflora* were collected from Metahara Town and its surroundings, while sugarcane bagasse and wastepaper were obtained from Metahara Sugar Factory and waste containers of Government Offices of Metahara (Schools, Government Offices in Metahara Town and Metahara Sugar Factory), respectively. Leaves of *Prosopis juliflora* were chopped, dried and grinded while wastepaper was soaked in water, dried and grinded. These substrates were then transported to Metahara Sugar Factory Research Station Laboratory and used for cultivation of the selected mushroom species.

3.4. Mushroom Cultivation

3.4.1. Preparation of substrates for Inoculation

Seven different substrates and substrate combination preparations were made for each of the three mushroom species (*Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida*) by mixing wastepaper, *Prosopis juliflora* leaves, and bagasse in varying proportions as shown in Table 6. The substrates were spread and allowed to dry for about one week at a regular turning interval of 3 days. They were then sterilized for 2 hours at a temperature of 121°C in a dry fire-heated drum to avoid contamination. The sterilized substrates were kept in a clean room and allowed to cool down overnight (for 12 hours) (Atikpo et al., 2008).

After cooling, the three substrates (Waste paper, leaves of *Prosopis juliflora* and sugarcane bagasse) were soaked in 80 liters of water and 110ml of 2% formalin for about 24 hours for further sterilization (Diriba et al., 2013). Sixty

three (63) transparent plastic bags were arranged corresponding to the three sets of seven substrate preparations in triplicates. Among these, seven substrate preparations in triplicates were used (7x3) for *Pleurotus ostreatus*, seven substrate preparations in triplicates (7x3) for *Pleurotus sajor-caju*, and seven substrate preparations in triplicates (7x3) for *Pleurotus florida*. The bags were labeled or arranged according to substrate type and mushroom species they contained.

Table 1. Arrangement of growth substrates and substrate combinations for cultivation of the three selected species of mushrooms

Mushroom Species	Growth Substrates						
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇
<i>Pleurotus ostreatus</i>	50% WP + 50% LPJ	50% WP + 50% SCB	50%LPJ + 50% SCB	100% SCB	100% LPJ	100% WP	33%SCB +33%WP +33% LPJ
<i>Pleurotus sajor-caju</i>	50% WP + 50% LPJ	50% WP + 50% SCB	50%LPJ + 50% SCB	100% SCB	100% LPJ	100% WP	33%SCB +33%WP +33% LPJ
<i>Pleurotus florida</i>	50% WP + 50% LPJ	50% WP + 50% SCB	50%LPJ + 50% SCB	100% SCB	100% LPJ	100% WP	33%SCB +33%WP +33% LPJ

SCB=Sugarcane Bagasse WP= Waste Paper LPJ= Leaves of *Prosopis juliflora* S= Substrate

3.4.2. Spawning and spawn running

After the substrate preparation was over, the wet substrate was spread on a clean alcohol swabbed polyethylene sheet. Then after, 50 g (which was equal to 10% of the weight of the substrate mixed) of the edible mushroom (*Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Pleurotus florida*) spawns were added and thoroughly mixed with the substrate kept in the polyethylene bags using sterile spoons under the laminar flow hood. Then, rubber bands tied the open ends of the bags and nine small holes were made using sterile needle to allow air exchange of bags (Dawit, 2008).

3.4.3. Incubation, control of the environment and cropping

All inoculated bags were incubated for 18 days at 28-31°C on shelves 15cm apart in a completely randomized design in a clean and disinfected dark room according to (Dawit, 1998). Fresh air exchange between the dark room and the outside environment was allowed by opening windows at night and closing during the daytime to enhance the quick colonization of the substrate. After fully colonization, the bags were transferred to the cropping room, whose environment was kept illuminated by sunlight through the improvised windows and a temperature and humidity of 29°C and 75-85%, respectively, were maintained by sprinkling the bag with water twice a day.

The humidity and temperature ranges were maintained by spraying water to the walls and floors of the cropping room. Formation of a complete mushroom occurs one week after the colonized substrates were transferred to the cropping room (Oei, 2005). After 32 days of incubation, fully matured mushroom species on each substrate were collected and analyzed for their proximate composition (moisture, crude protein, crude fat, crude fiber, ash and carbohydrate content) and biological efficiency.

3.5. Data Collection

3.5.1. Determination of proximate composition (Analysis)

Proximate composition of the selected edible mushrooms (*Pleurotus ostreatus*, *Pleurotus sajor-caju* and *Pleurotus florida*) was determined according to AOAC (1995).

3.5.1.1. Determination of moisture content

The moisture content (MC) of the harvested mushroom was determined by the gravimetric method (AOAC, 1995). The percentage MC would be generally calculated using the following formula;

$$\% \text{ MC} = \frac{\text{Wet Weight} - \text{Dry Mass}}{\text{Wet Weight}} \times 100$$

3.5.1.2. Determination of crude protein

The mushroom species were dried and grinded using a mortar and pestle and, was analyzed for crude protein content. The Kjeldahl method as described in James (1995) and Chang (2003) in which the nitrogen content was first determined and multiplied with 6.25 to obtain the protein content of the sample. The percentage of nitrogen was calculated using appropriate formula and the value was converted to percentage protein by multiplying with 6.25 (AOAC, 1995).

3.5.1.3. Determination of total ash

The organic matters were burned off as CO₂, oxides of Nitrogen and H₂O vapor and the remaining matter was determined as ash (AOAC, 1995).

3.5.1.4. Determination of crude fat

The Soxhlet solvent extraction method of James (1995) was employed for determination of fat content.

3.5.1.5. Determination of crude fiber

The fiber content in percentage was calculated using the formula of (AOAC, 1990).

3.5.1.6. Determination of total carbohydrate

The available carbohydrate content was determined using the following equation (Raghuramulu et al., 2003):

3.5.2. Determination of Biological Efficiency (Yield of mushroom/bag) (%)

The biological efficiency (BE) of the mushroom species was calculated using the formula recommended by Chang and Miles (1989) as follows:

$\% \text{ BE} = \frac{W_2}{W_1} \times 100$ Where: W₂= Fresh weight of harvested mushroom, W₁= Dry weight of substrate before inoculation.

3.6. Data Analysis

The collected data on proximal compositions were subjected to Analysis of Variance (ANOVA) Gomez (1984) with three replications using Statistical Analysis System (SAS Institute and Cary NC) Version 9.0. Means were compared for significant difference using Fisher's LSD (FLSD) at P<0.05.

4. RESULTS AND DISCUSSION

4.1. Effect of Growth Substrate and Substrate Combinations on Proximate Compositions of Mushroom (Pleurotus Species)

4.1.1. Comparative analysis of the effect of growth substrate and substrate combination on proximate composition of the three mushroom species

4.1.1.1. Moisture content

The interaction between the types of growth substrate or substrate combinations and types of species had significant (p<0.05) effect on moisture contents of mushrooms. The results indicate that the moisture contents of the mushrooms ranged from 77.5%-85.5%. The highest values of moisture contents (85.50 %) for *Pleurotus ostreatus* grown on 50% WP+50 SCB, 100% SCB, 100% WP; *Pleurotus florida* grown on 50% WP+50 SCB, *Pleurotus sajor-caju* grown on 100% SCB and 100% WP, respectively. The least moisture contents were 77.5% obtained from *Pleurotus ostreatus* grown on LPJ alone. These results are closely related to the finding of Mckeller and Khorma (1990) in which oyster mushroom moisture contents of 70-90% reported

Pleurotus ostreatus and Pleurotus sajor caju had the higher moisture content than Pleurotus florida when grown on SCB alone. Among substrates, SCB alone provided higher moisture content than others, whereas LPJ alone provided the least moisture content only for Pleurotus ostreatus. Pleurotus florida and Pleurotus sajor-caju were unable to grow on LPJ probably because of their inability to decompose and utilize the substrate.

4.1.1.2. Crude protein

Pleurotus ostreatus grown on 50% WP+50% SCB, Pleurotus ostreatus grown on 33% WP+33% SCB+33% LPJ and Pleurotus florida grown on 100% SCB were not significant ($p>0.05$) different from one another. The highest crude protein (35.93%) was recorded from Pleurotus ostreatus grown on SCB alone followed by Pleurotus florida grown on SCB alone (31.66%), Pleurotus ostreatus grown on 33%SCB+33%WP+33%LPJ (30.62%) and Pleurotus ostreatus grown on 50% SCB+50% LPJ (30.25%). Whereas the least crude proteins were obtained from Pleurotus sajor-caju grown on 50% WP+50% LPJ (21.25%), Pleurotus florida grown on 50% WP+50% LPJ (21.56%), Pleurotus florida grown on 50% SCB+50% LPJ (21.64%) and Pleurotus sajor-caju grown on 50% SCB+50% LPJ (22.81%). The results are in agreement with those of Breene (1990) who reported values of crude protein content ranging from 19-39%. From these crude protein content values, it is possible to rank growth substrates or substrate combinations and mushrooms used in the current study. Pleurotus ostreatus had higher crude protein content than Pleurotus florida and Pleurotus sajor-caju when grown on SCB alone. Among substrates, SCB alone provided higher crude protein content than others, whereas 50% WP+50%LPJ and 50% SCB+50% LPJ provided the least crude protein content in Pleurotus florida and Pleurotus sajor-caju. The protein contents of mushrooms are dependent on biological, chemical differences and the C: N ratio of substrates (Sangwan and Saini, 1995; Ragunathan and Swaminathan, 2003). The maximum or minimum in nitrogen content during growth of the mushroom might be because of its ability to utilize nitrogen (Singh and Pandey, 2011).

Other authors Singh and Kumar (2012) also noted and suggested that maximum or minimum in nitrogen content of the Pleurotus species. The difference in crude protein content is attributed to the differences in nitrogen content of growth substrate and efficiency of the mushroom species for nitrogen utilization and nitrogen fixation by Pleurotus species (Ortega et al., 1992; Sturion & Oetterer, 1995; Patrabansh & Madan, 1997).

4.1.1.3. Crude fat

The Pleurotus ostreatus grown on 50% WP+50% SCB and Pleurotus sajor-caju grown on 50% WP+50% LPJ were not statistically different from each other. The highest (2.17%) crude fat was recorded from Pleurotus ostreatus grown on 50% SCB+50% WP, followed by 2.07, 2.02 and 2.0% for Pleurotus sajor-caju grown on 50% WP+50% SCB, Pleurotus ostreatus grown on 50% SCB+50% LPJ and Pleurotus ostreatus grown on WP alone, and Pleurotus sajor-caju grown on WP alone, respectively.

These results were in agreement with Anthony (2007) who reported that the crude fat content of mushrooms contain 0.6–3.1%. Fat analysis has been vary in mushrooms and factors that might influence fat contents have not been completely described (Kurtzman, 1997).

4.1.1.4. Total ash

The total ash contents of the selected Pleurotus species obtained were found in the range of 6.46%-8.06%. The highest total ash contents were 8.06, 8.05 and 8.04% for Pleurotus sajor-caju grown on SCB alone, Pleurotus ostreatus grown on LPJ alone and Pleurotus ostreatus grown on WP alone, while the least total ash contents were 6.46 and 6.51% obtained from Pleurotus ostreatus grown on 50% SCB+50% LPJ and 50% WP+50% LPJ, respectively.

These results are related and in line with Rai and Crison (1978) in which total ash, contents of 6.1-9.8% were reported. Nevertheless, 6.51% and 6.46 values obtained from Pleurotus ostreatus grown on 50% SCB+50% WP and 50% SCB+50% LPJ are less than Oei (2003) and Dawit (1998) who reported ash content of 8.8% and 7.2% for Pleurotus species mushrooms, respectively.

4.1.1.5. Crude Fiber

The highest crude fiber values 12.39, 11.96 and 11.91% were obtained from Pleurotus ostreatus grown on SCB alone, Pleurotus sajor-caju grown on substrate combinations of 50% WP+50% SCB and Pleurotus ostreatus grown on WP alone, respectively. The least fiber contents 9.55, 9.40 and 8.80% were obtained from the Pleurotus ostreatus grown on 100% LPJ, Pleurotus florida grown on 50% SCB+50% LPJ and Pleurotus sajor-caju grown on 50% SCB+50% LPJ, respectively. The crude fiber results obtained from the selected Pleurotus species grown on different substrates and substrate combinations are in agreement with Obodai (1992) who reported the crude fiber contents of oyster mushrooms (7.5-16.5%).

4.1.1.6. Total Carbohydrate

The value of total carbohydrate in *Pleurotus sajor-caju* grown on 100%SCB was significantly ($p<0.05$) higher than the values of total carbohydrate found in the other *Pleurotus* species grown on different substrates and substrate combinations investigated. However, the least total carbohydrate contents were 26.67, 26.88, 27.64 and 27.73% found in *Pleurotus florida* grown on 50% SCB+50% LPJ, *Pleurotus ostreatus* grown on 100% LPJ, *Pleurotus ostreatus* grown on 50% LPJ+50% WP and *Pleurotus sajor-caju* grown on 50% SCB+50% LPJ, respectively. The total carbohydrate contents in this finding are in agreement with the results reported by Bernas et al. (2006) in *Pleurotus* mushrooms (16-85%).

From these carbohydrate content values, it is possible to compare growth substrates or substrate combinations and mushrooms used in the current finding.

Pleurotus sajor-caju had higher carbohydrate content than *Pleurotus florida* and *Pleurotus ostreatus* when grown on SCB alone. Among substrates, SCB alone provided higher carbohydrate content than others, whereas LPJ alone and 50% SCB+50% LPJ provided the least carbohydrate content in *Pleurotus ostreatus* and *Pleurotus florida* respectively.

The nutrient composition of the substrate is one of the factors that can affect quantitative and qualitative yield of cultivated mushrooms (Philippoussis et al., 2001), supplements containing sugars and starch (easily available carbohydrates). The difference in carbohydrate content of *Pleurotus* species grown on different substrates could be due to the difference in carbon content of substrate and, substrate combinations and variation in selected mushroom species.

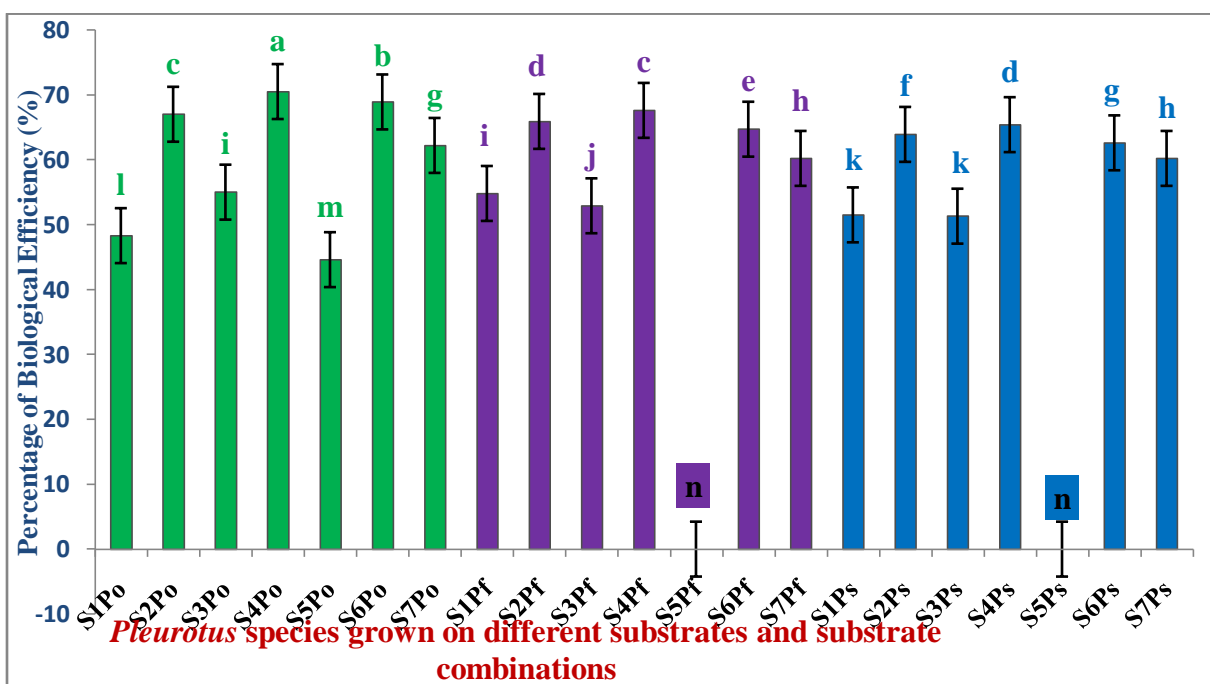
Table. 2. Comparative effect of growth substrate and substrate combinations on Proximate Composition of selected Pleurotus species

Varieties	Substrates	Proximate Compositions (%)					
		Moisture	Crude protein(db)	Crude fat (db)	Crude fiber(db)	Ash(db)	Carbohydrate(db)
Pleurotus ostreatus	50% WP+50LPJ	79.08±0.36 ⁱ	24.37±0.36 ^h	1.86±0.02 ^{de}	10.65±0.08 ^{efg}	6.51±0.22 ^{cd}	27.64±0.30 ^{gh}
	50% WP+50SCB	84.50±0.29 ^{ab}	30.25±0.54 ^b	2.17±0.03 ^a	11.51±0.22 ^{bc}	7.26±0.19 ^b	32.91±0.43 ^b
	50% SCB+50LPJ	80.00±0.29 ^{ghi}	25.62±0.72 ^{fgh}	2.02±0.02 ^{bc}	10.29±0.13 ^{gh}	6.46±0.14 ^d	29.81±0.52 ^f
	100% SCB	85.50±0.28 ^a	35.93±0.78 ^a	1.95±0.02 ^{bcd}	12.39±0.20 ^a	7.95±0.12 ^a	30.59 ±0.62 ^{def}
	100% LPJ	77.50±0.28 ^j	25.17±0.42 ^{gh}	1.33±0.02 ⁱ	9.55±0.10 ⁱ	8.05±0.13 ^a	26.88±0.41 ^{gh}
	100% WP	84.85±0.17 ^a	28.65±0.25 ^c	2.02±0.05 ^{bc}	11.91±0.09 ^{ab}	8.04±0.13 ^a	31.35±0.74 ^{cd}
	33% LPJ+33% SCB+33% WP	80.33±0.44 ^{fgh}	30.62±0.72 ^b	1.93±0.05 ^{cde}	11.26±0.25 ^{cd}	7.36±0.24 ^b	30.83 ±0.24 ^{def}
	50% WP+50LPJ	81.00±0.58 ^{efg}	21.56±0.24 ^{ij}	1.33±0.01 ⁱ	10.31±0.24 ^{gh}	7.30±0.19 ^b	30.56±0.34 ^{def}
Pleurotus florida	50% WP+50SCB	84.50±0.29 ^{ab}	26.66±0.90 ^{efg}	1.69±0.06 ^{gh}	10.52±0.07 ^{fgh}	7.18±0.09 ^b	31.42±0.21 ^{cd}
	50% SCB+50LPJ	81.17±0.33 ^{ef}	21.64±0.33 ^{ij}	1.41±0.04 ⁱ	9.40±0.33 ⁱ	6.95±0.10 ^b	26.67±0.23 ^h
	100% SCB	83.42±0.36 ^c	31.66±0.41 ^b	1.71±0.04 ^{fg}	11.49±0.15 ^{bc}	7.95±0.10 ^a	31.95±0.26 ^{bc}
	100% LPJ	0.00 ±0.00 ^k	0.00 ±0.00 ^k	0.00 ±0.00 ^j	0.00±0.00 ^k	0.00±0.00 ^e	0.00 ±0.00 ⁱ
	100% WP	82.67±0.17 ^{cd}	24.65±0.34 ^h	1.57±0.04 ^h	11.18±0.11 ^{cd}	7.36±0.14 ^b	30.98 ±0.32 ^{cde}
	33% LPJ+33% SCB+33% WP	81.67±0.73 ^{de}	27.07±0.17 ^{def}	1.36±0.01 ⁱ	10.69±0.17 ^{efg}	7.17±0.13 ^b	29.92 ±0.09 ^f
	50% WP+50LPJ	79.45±0.33 ^{hi}	21.25±0.97 ^j	2.07±0.06 ^{ab}	10.07±0.25 ^h	7.15±0.13 ^b	30.53±0.45 ^{def}
	50% WP+50SCB	82.83±0.09 ^c	28.16±0.39 ^{cde}	1.90±0.01 ^{cde}	11.96±0.07 ^{ab}	7.32±0.17 ^b	31.54±0.29 ^{cd}
Pleurotus caju	50% SCB+50LPJ	79.68±0.44 ^{hi}	22.81±0.54 ⁱ	1.82±0.08 ^{ef}	8.80±0.21 ^j	7.09±0.12 ^b	27.73±0.54 ^g
	100% SCB	85.50±0.58 ^a	28.54±0.55 ^{cd}	1.92±0.04 ^{cde}	11.11±0.09 ^{cde}	8.06±0.17 ^a	34.00±0.62 ^a
	100% LPJ	0.00 ±0.00 ^k	0.00 ±0.00 ^k	0.00 ±0.00 ^j	0.00 ±0.00 ^k	0.00±0.00 ^e	0.00 ±0.00 ⁱ
	100% WP	84.57±0.24 ^{ab}	26.53±0.59 ^{fg}	2.00±0.03 ^{bc}	11.08±0.08 ^{cde}	7.83±0.10 ^a	31.04±0.15 ^{cde}
	33% LPJ+33% SCB+33% WP	83.63±0.07 ^{bc}	26.45±0.75 ^{fg}	1.99±0.05 ^{bc}	10.92±0.21 ^{def}	7.30±0.29 ^b	29.99±0.28 ^{ef}
	CV	0.822	3.914	4.574	3.014	4.025	2.335
	LSD	1.01	1.56	0.12	0.48	0.44	1.06

CV= Coefficient Variation, LSD= Least Significant Difference, db= dry based WP= Waste paper, LPJ= Leaves of Prosopis juliflora SCB=Sugar cane bagasse. Means with the same letter within column are not significantly different (p≤0.05)

4.2. Effect of growth substrates and substrate combination on the BE of the selected *Pleurotus* species

The substrates and mushroom types significantly ($p < 0.05$) affected the biological efficiencies (BE). High values of BE and were recorded for *Pleurotus ostreatus* grown on SCB alone (70.5%) and *Pleurotus ostreatus* grown on WP alone (68.9%) followed by *Pleurotus florida* grown on SCB alone (67.6%) and *Pleurotus ostreatus* grown on 50% WP+50% SCB (67%). On the other hand, the least BE (0%) was observed from the *Pleurotus florida* and *Pleurotus sajor-caju* grown on LPJ alone followed by the relatively lower values of *Pleurotus ostreatus* grown on LPJ alone (44.6%) and *Pleurotus ostreatus* grown on 50% WP+50% LPJ (48.3%). The current study is closely related to the finding of Patra and Pani (1995), who reported the biological efficiency (50-75%) of *Pleurotus* species grown on most of agro-industrial residues, namely; corncobs, various grasses and reed stems, vine shoots, cottonseed hulls and sugarcane bagasse. In this finding, biological efficiency was indicated for comparison between substrate and mushroom species in which, the most effective substrate in bioconversion to fresh fruiting bodies for cultivation of *Pleurotus* species was SCB alone followed by WP alone; in contrast, LPJ which was not effective to be decomposed and not suitable for cultivation of *Pleurotus florida* and *Pleurotus sajor-caju* mushrooms. The mushroom species that provided the highest biological efficiency was *Pleurotus ostreatus*, while the mushroom that exhibited the minimum capability, in utilization and colonization of substrates was *Pleurotus sajor-caju*. Sugarcane bagasse supported the fast mycelial growth during cultivation of *Pleurotus* species.



BE= Biological Efficiency, S₁= 50% WP+50% LPJ, S₂=50% WP+50%SCB, S₃= 50% SCB +50% LPJ, S₄= 100% SCB S₅= 100% LPJ, S₆= 100% WP, S₇= 33% WP+33%LPJ+33%SCB, (Green color) Po= *Pleurotus ostreatus*, (Pink color) Pf= *Pleurotus florida*, (Blue color)Ps= *Pleurotus sajor-caju* WP= Waste paper, SCB= Sugarcane bagasse and LPJ= Leaves of *Prosopis juliflora*

CONCLUSIONS

The growth substrates or substrate combinations, type of species, can affect nutritional value of mushrooms. Despite their differences in the nutritional composition, the overall nutritional potential of the mushrooms were quite good. Moreover, the agricultural and industrial wastes such as sugar cane bagasse, waste paper and plants leaves can be used for cultivation of *Pleurotus* species.

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