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Effect of organic and inorganic supplementation on the yield and biological efficiency of two *Pleurotus* spp. growth on different agricultural wastes

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SUMMARY

Different concentration of carbon sources *i.e.* lactose, nitrogen sources *i.e.* peptone, inorganic chemicals *i.e.* $MgSO_4$ and $FeSO_4$, vitamin sources *i.e.* vit. B-complex, growth promoter *i.e.* EDTA and organic source *i.e.* wheat flour, besan flour, soya flour and rice flour were screened to determine the most suitable concentration for better yield of two *Pleurotus* species *i.e. P. sajor caju* and *P. Florida*. Two lignocellulosic agricultural waste material *i.e.* wheat straw and paddy straw were used for experimentation. It was found that organic source soya flour-3% gave maximum yield and biological efficiency of *P. sajor caju* in wheat straw (97.7 %), beasn flour-1% concentration in wheat straw in *P. sajor caju* (95.7%), inorganic source lactose-3% concentration in *P. sajor caju* (96.5%) in wheat straw. *P. florida* gave maximum yield and biological efficiency with soya flour-3% in wheat straw (94.1%).On that basis of Lactose and $FeSo_4$ were proved superior carbon source and inorganic chemical, respectively. Vitamin B-complex gave maximum yield and biological efficiency in *P. sajor caju* with 100 mg/l in wheat straw (94.0%). Among the two lignocellulosic agricultural waste material used as a substrate, best growth and high yield was found in *P. sajor caju* in wheat straw.

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Key words : Supplement, Biological efficiency, Lignocellulosic waste

The cultivation of edible fungi has been accepted as the easiest on farm biotechnology for profitable removal of various lignocellulosic agricultural, industrial and forestry by-products, specially in the developing countries. Although about 200 species of edible fungi have been reported from India, only three *viz.*, *Pleurotus* spp., *Agraricus bisporus* and *Volvariella* species are preferred for commercial cultivation. *Pleurotus* species constitute about 65% of the total mushrooms production in the country (Munjal, 1982).

Vast quantities of renewable lignocellulose wastes are generated every year in developing countries like India with economics, which are agricultural based. However, mushroom spp. have been reported to grow and yield successfully on many plant wastes. In special reference of Chhattisgarh, the agricultural waste material left after animal consumptions can be used as substrates for mushroom production. *Pleurotus* species show much diversity in their adaptation to the varying agro-climatic conditions and show difference in yield depending on the substrates and organic amendments used. Wide spread malnutrition with ever-increasing gap in developing countries has necessitated the search of alternative sources of protein because the production of pulses has not kept pace of our requirement due to production growth.

Different concentrations of carbon source (lactose), nitrogen source (peptone), Inorganic chemicals ($MgSO_4$ and $FeSO_4$), growth promoters (EDTA), vitamin source (B- Complex) and organic supplement wheat flour, besan flour, soya flour and rice flour were screened to determine the most suitable concentration for better yield, biological efficiency and suitable substrates of *Pleurotus sajor caju* and *Pleurotus florida*. The aim of various different organic and inorganic supplementations was to get maximum production of fruiting bodies but it is an intricate problem. Moreover, the knowledge, shall help to plan and prepare for the crop and equip one with a physiological tool to control the quality, quantity, timing and other characters of the mushroom production.

MATERIALS AND METHODS

This work was carried out in Biotech. Lab. training

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and demonstration centre, Ambikapur, Chhattisgarh during June 2008 to December 2008. Parental strains of *Pleurotus sajor caju* and *Pleurotus florida* were provided by Mushroom Biotechnology lab, Indira Gandhi Agriculture University, Raipur, Chhattisgarh. The cultures were maintained on Malt extract agar medium with regular sub-cultures at monthly intervals. Spawn was prepared on wheat grain following standard procedure. 15 days old spawn was used for experimentation.

Different concentrations of supplement *i.e.* carbon source, lactose (1.0%, 2.0% and 3.0%), nitrogen source, peptone (1.0%, 2.0% and 3.0%), inorganic chemicals *i.e.* $MgSO_4$ (1.0%, 2.0% and 3.0%), and $FeSO_4$ (1.0%, 2.0% and 3.0%), vitamin source *i.e.* vitamin B-complex (100, 200 and 300 mg/l), growth promoter *i.e.*EDTA (0.1%, 0.2% and 0.3%) and organic source *i.e.* wheat flour (1.0%, 2.0% and 3.0%), besan flour (1.0%, 2.0% and 3.0%), soya flour (1.0%, 2.0% and 3.0%) and rice flour (1.0%, 2.0% and 3.0%) were selected for the research purpose.

Wheat straw and paddy straw were water-socked over night in 1% Bavistin and 2% formaldehyde solution. At the following day, the substrate was spread on clean floor to drain off excess of water. Polythene beg was prepared by layering methods. All the supplements of lactose, peptone, inorganic chemicals $MgSO_4$ and $FeSO_4$, and organic source *i.e.* wheat flour, besan flour, soya flour and rice flour were supplemented into the substrate just before the spawning in the case of growth promoters EDTA (0.1%, 0.2% and 0.3%) and vitamin source vitamin B-complex (100, 200 and 300 mg/l) were sprayed on the surface of begs and at the time of pinhead initiation and after the harvesting fruiting bodies.

The bags were incubated in cultivation room at 25^0-30^0C for spawn run after completion of spawn run, time of pinhead initiation, yield and biological efficiency were separately recorded for each treatment at the time of each flush.

RESULTS AND DISCUSSION

The results obtained during the present investigation are presented in the Tables 1 to 10 and Fig. 1. All the sets supplemented with carbon source (lactose) Table 1 took shorter time for spawn run and primordial initiation than control (13 and 17 days). The yield and biological efficiency was observed maximum in carbon source (lactose-3%) 965 g, 96.5% in wheat straw in *Pleurotus sajor caju* compared with control 729 g and 72.9% biological efficiency, in the same supplement *Pleurotus florida* was observed maximum (lactose-2%) 889 g, 88.9% in wheat straw. In case of paddy straw, maximum yield was recorded by lactose-3% in *Pleurotus florida* 843 g and 84.3% biological efficiency. Minimum yield was recorded in carbon source *i.e.* 776 g in lactose-1% concentration in *Pleurotus sajor caju* in paddy straw. These are the evidences that increase in carbohydrate beyond an optimum point results in an absolute as well as relative decrease in growth of fungus (Ward *et al.,*1935).

The sets treated with peptone took equal time for spawn run, *Pleurotus sajor caju* wheat straw (17 days), paddy straw (18 days) and *Pleurotus florida* wheat straw (18 days), paddy straw (21 days) compared with control (19 days).They showed variation in day of pin initiation (Table 2). It was observed maximum in peptone-1% concentration of paddy straw (24 days) in *Pleurotus florida*. The increasing concentrations caused positive effect on yield and biological efficiency within a certain limits, *i.e.* peptone-1, 2 and 3% (83.0%, 84.4% and 85.9%) *Pleurotus florida* in wheat straw. The set treated with peptone was observed the maximum biological efficiency in peptone 3% in wheat straw (85.9 g and 85.9 %) in *Pleurotus florida*. This was due to peptone that served additional available nitrogen to fungus and thus stimulated fungal growth. It also helped in maintaining high cellulose activity and cell mass synthesis. Various workers have also reported that addition of organic source of nitrogen enhances the yield of *Pleurotus sajor caju*. In the present investigation, it was found that beyond certain concentration, yield and biological efficiency decreased and recorded to the minimum peptone 1 % in paddy straw (711 g and 71.1%) in *Pleurotus florida*

The sets supplemented with inorganic chemicals $MgSO_4$ was found to exhibit maximum yield and biological efficiency in 1.0% concentration in wheat straw (881 g and 88.1%) in *Pleurotus sajor caju* (Table 3). These results are similar to findings of Verma (2005) who observed higher yield and biological efficiency in 0.5% concentration while working with *Volvariella volvacea* as a test fungus. He also stated that the use of inorganic chemicals are capable of interacting with phenolics that can protect the side chains of extra cellular enzymes, important during the fruiting process as well as substrate utilization. The use of micronutrient at low concentrations may intervene the importance of in enzymatic reaction. Several workers also used inorganic sources in the supplementation of various substrates, increasing *Pleurotus* spp. productivity. The yield and biological efficiency was observed maximum in inorganic chemicals $FeSO_4$ ($FeSO_4$-3%) 929 g, 92.9% in wheat straw in *Pleurotus florida* compared with control 689 g and 68.9% biological efficiency, in the same supplement *Pleurotus sajor caju* was observed maximum ($FeSO_4$-2%) 841 g, 84.1% in wheat straw (Table 4).

Table 1 : Effect of different concentrations of carbon source (lactose) on yield and biological efficiency of *P. sajor caju* and *P. florida* in different substrates

Species	Substrates	Supplement concentration	Mode of application	SRT (Days)	Pin initiation (Days)	Total yield (g)	BE (%)
Pleurotus sajor caju	Wheat straw	Lactose-1%	Supplement	13	17	874	87.4
		Lactose-2%	Supplement	13	17	922	92.2
		Lactose-3%	Supplement	13	17	965	96.5
	Paddy straw	Lactose-1%	Supplement	15	19	776	77.6
		Lactose-2%	Supplement	15	19	802	80.2
		Lactose-3%	Supplement	15	19	831	83.1
Pleurotus florida	Wheat straw	Lactose-1%	Supplement	14	18	847	84.7
		Lactose-2%	Supplement	14	18	889	88.9
		Lactose-3%	Supplement	14	18	867	86.7
	Paddy straw	Lactose-1%	Supplement	16	21	790	79.0
		Lactose-2%	Supplement	16	21	798	79.8
		Lactose-3%	Supplement	16	21	843	84.3
		Control		16	21	729	72.9

Table 2 : Effect of different concentrations of nitrogen source (peptone) on yield and biological efficiency of *P. sajor caju* and *P. florida* in different substrates

Species	Substrates	Supplement concentration	Mode of application	SRT (Days)	Pin initiation (Days)	Total yield (g)	BE (%)
Pleurotus sajor caju	Wheat straw	Peptone-1%	Supplement	17	21	789	78.9
		Peptone-2%	Supplement	17	21	767	76.7
		Peptone-3%	Supplement	17	21	799	79.9
	Paddy straw	Peptone-1%	Supplement	18	21	730	73.0
		Peptone-2%	Supplement	18	20	839	83.9
		Peptone-3%	Supplement	18	20	785	78.5
Pleurotus florida	Wheat straw	Peptone-1%	Supplement	18	23	830	83.0
		Peptone-2%	Supplement	18	23	844	84.4
		Peptone-3%	Supplement	18	23	859	85.9
	Paddy straw	Peptone-1%	Supplement	21	24	711	71.1
		Peptone-2%	Supplement	21	23	788	78.8
		Peptone-3%	Supplement	21	23	750	75.0
		Control		19	23	698	69.8

Table 3 : Effect of different concentrations of inorganic chemical ($MgSO_4$) on yield and Biological efficiency of *P. sajor caju* amd *P. florida* in different substrates

Species	Substrates	Supplement concentration	Mode of application	SRT (Days)	Pin initiation (Days)	Total yield (g)	BE (%)
Pleurotus sajor		$MgSO_4$-1%	Supplement	14	17	881	88.1
caju	Wheat straw	$MgSO_4$-2%	Supplement	14	17	864	86.4
		$MgSO_4$-3%	Supplement	14	17	798	79.8
		$MgSO_4$-1%	Supplement	14	19	771	77.1
	Paddy straw	$MgSO_4$-2%	Supplement	13	19	812	81.2
		$MgSO_4$-3%	Supplement	14	19	798	79.8
Pleurotus		$MgSO_4$-1%	Supplement	15	18	789	78.9
florida	Wheat straw	$MgSO_4$-2%	Supplement	15	18	830	83.0
		$MgSO_4$-3%	Supplement	15	18	844	84.0
		$MgSO_4$-1%	Supplement	17	19	737	73.7
	Paddy straw	$MgSO_4$-2%	Supplement	16	19	832	83.2
		$MgSO_4$-3%	Supplement	17	19	776	77.6
		Control		17	20	709	70.9

Table 4 : Effect of different concentrations of inorganic chemical ($FeSO_4$) on yield and biological efficiency of *P. sajor caju* & *P. Florida* in different substrates

Species	Substrates	Supplement concentration	Mode of application	SRT (Days)	Pin initiation (Days)	Total yield (g)	BE (%)
Pleurotus sajor caju	Wheat straw	$FeSO_4$-1%	Supplement	17	21	827	82.7
		$FeSO_4$-2%	Supplement	17	21	841	84.1
		$FeSO_4$-3%	Supplement	16	21	802	80.2
	Paddy straw	$FeSO_4$-1%	Supplement	18	24	770	77.0
		$FeSO_4$-2%	Supplement	18	22	808	80.8
		$FeSO_4$-3%	Supplement	18	20	744	74.4
Pleurotus florida	Wheat straw	$FeSO_4$-1%	Supplement	14	19	909	90.9
		$FeSO_4$-2%	Supplement	14	18	923	92.3
		$FeSO_4$-3%	Supplement	14	18	929	92.9
	Paddy straw	$FeSO_4$-1%	Supplement	17	23	811	81.1
		$FeSO_4$-2%	Supplement	17	23	798	79.8
		$FeSO_4$-3%	Supplement	19	22	777	77.7
		Control		19	23	689	68.9

Table 5 : Effect of different concentrations of vitamin source (B-Complex) on yield and biological efficiency of *P. sajor caju* and *P. florida* in different substrates

Species	Substrates	Supplement concentration	Mode of application	SRT (Days)	Pin initiation (Days)	Total yield (g)	BE (%)
Pleurotus sajor caju	Wheat straw	Vit.B-100 mg/l	Spray	14	16	940	94.0
		Vit.B-200 mg/l	Spray	14	16	933	93.3
		Vit.B-300 mg/l	Spray	14	16	911	91.1
	Paddy straw	Vit.B-100 mg/l	Spray	15	18	766	76.6
		Vit.B-200 mg/l	Spray	15	19	782	78.2
		Vit.B-300 mg/l	Spray	15	18	797	79.7
Pleurotus florida	Wheat straw	Vit.B-100 mg/l	Spray	14	16	891	89.1
		Vit.B-200 mg/l	Spray	14	17	834	83.4
		Vit.B-300 mg/l	Spray	14	18	866	86.6
	Paddy straw	Vit.B-100 mg/l	Spray	17	20	766	76.6
		Vit.B-200 mg/l	Spray	17	19	811	81.1
		Vit.B-300 mg/l	Spray	17	22	809	80.9
		Control		18	21	678	67.8

Table 6 : Effect of different concentrations of growth promoter (EDTA) on yield and biological efficiency of *P. sajor caju* and *P. florida* in different substrates

Species	Substrates	Supplement concentration	Mode of application	SRT (Days)	Pin initiation (Days)	Total yield (g)	BE (%)
Pleurotus sajor caju	Wheat straw	EDTA-0.1%	Spray	14	17	775	77.5
		EDTA-0.2%	Spray	13	19	890	89.0
		EDTA-0.3%	Spray	13	19	865	86.5
	Paddy straw	EDTA-0.1%	Spray	17	21	699	69.9
		EDTA-0.2%	Spray	17	22	771	77.1
		EDTA-0.3%	Spray	17	22	689	68.9
Pleurotus florida	Wheat straw	EDTA-0.1%	Spray	16	18	705	70.5
		EDTA-0.2%	Spray	15	19	716	71.6
		EDTA-0.3%	Spray	16	19	709	70.9
	Paddy straw	EDTA-0.1%	Spray	19	23	733	73.3
		EDTA-0.2%	Spray	19	22	721	72.1
		EDTA-0.3%	Spray	21	22	765	76.5
		Control		19	23	705	70.5

Table 7 : Effect of different concentrations of wheat flour on yield and biological efficiency of *P. sajor caju* and *P. florida* in different substrates

Species	Substrates	Supplement concentration	Mode of application	SRT (Days)	Pin initiation (Days)	Total yield (g)	BE (%)
Pleurotus sajor caju	Wheat straw	Wheat flour-1%	Supplement	16	19	910	91.0
		Wheat flour-2%	Supplement	16	19	930	93.0
		Wheat flour-3%	Supplement	16	19	909	90.9
	Paddy straw	Wheat flour-1%	Supplement	16	22	822	82.2
		Wheat flour-2%	Supplement	18	22	883	88.3
		Wheat flour-3%	Supplement	18	21	825	82.5
Pleurotus florida	Wheat straw	Wheat flour-1%	Supplement	17	21	876	87.6
		Wheat flour-2%	Supplement	19	22	843	84.3
		Wheat flour-3%	Supplement	19	23	900	90.0
	Paddy straw	Wheat flour-1%	Supplement	20	24	771	77.1
		Wheat flour-2%	Supplement	20	24	803	80.3
		Wheat flour-3%	Supplement	19	24	780	78.0
		Control		19	24	731	73.1

Table 8 : Effect of different concentrations of besan flour on yield and biological efficiency of *P. sajor caju* and *P. florida* in different substrates

Species	Substrates	Supplement concentration	Mode of application	SRT (Days)	Pin initiation (Days)	Total yield (g)	BE (%)
Pleurotus sajor caju	Wheat straw	Besan flour-1%	Supplement	14	17	957	95.7
		Besan flour-2%	Supplement	14	18	944	94.4
		Besan flour-3%	Supplement	14	18	931	93.1
	Paddy straw	Besan flour-1%	Supplement	16	19	872	87.2
		Besan flour-2%	Supplement	15	18	760	76.0
		Besan flour-3%	Supplement	16	18	829	82.9
Pleurotus florida	Wheat straw	Besan flour-1%	Supplement	14	16	765	76.5
		Besan flour-2%	Supplement	14	16	780	78.0
		Besan flour-3%	Supplement	15	17	862	86.2
	Paddy straw	Besan flour-1%	Supplement	18	22	761	76.1
		Besan flour-2%	Supplement	18	22	765	76.5
		Besan flour-3%	Supplement	17	21	715	71.5
		Control		17	22	711	71.1

Table 9 : Effect of different concentrations of soya flour on yield and biological efficiency of *P. sajor caju* and *P. florida* in different substrates

Species	Substrates	Supplement concentration	Mode of application	SRT (Days)	Pin initiation (Days)	Total yield (g)	BE (%)
Pleurotus sajor caju	Wheat straw	Soya flour-1%	Supplement	13	16	944	94.4
		Soya flour-2%	Supplement	13	16	951	95.1
		Soya flour-3%	Supplement	13	16	977	97.7
	Paddy straw	Soya flour-1%	Supplement	15	18	889	88.9
		Soya flour-2%	Supplement	16	21	872	87.2
		Soya flour-3%	Supplement	16	19	844	84.4
Pleurotus florida	Wheat straw	Soya flour-1%	Supplement	14	18	933	93.3
		Soya flour-2%	Supplement	14	18	939	93.9
		Soya flour-3%	Supplement	14	18	941	94.1
	Paddy straw	Soya flour-1%	Supplement	16	19	776	77.6
		Soya flour-2%	Supplement	16	21	731	73.1
		Soya flour-3%	Supplement	15	21	783	78.3
		Control		17	22	711	71.1

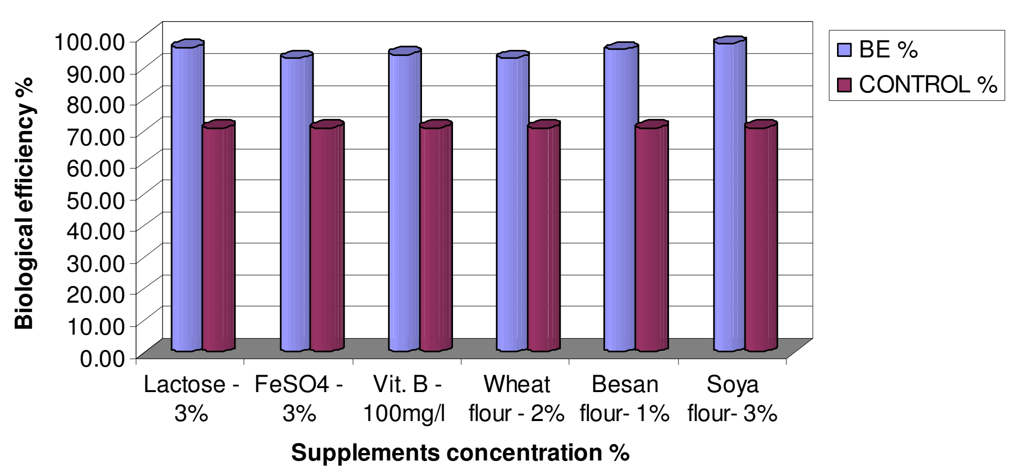
The sets treated with vitamin B-complex showed the maximum yield and biological efficiency in 100mg/ltr *i.e. Pleurotus sajor caju* 940 g and 94.0% in wheat straw. The yield and biological efficiency decreased with increasing order of concentration (Table 5). All the sets took equal time for spawn run, *Pleurotus sajor caju* (14 days wheat straw, 15 days paddy straw) and *Pleurotus florida* (14 days wheat straw, 17 days paddy straw). In case of EDTA supplementation they showed lesser time for harvesting of crop when treated with growth promoter EDTA (Table 6). Yield and biological efficiency found that 0.2% concentration in wheat straw in *Pleurotus sajor caju* (890 g and 89.0%).

The sets supplemented with organic supplement wheat flour (Table 7) was found that gave maximum yield and biological efficiency in 2% concentration in wheat straw (930 g and 93.0%) in *Pleurotus sajor caju* compared with control 731 g and 73.1% biological efficiency, in the same supplement *Pleurotus florida* was observed maximum 843 g, 84.3% in wheat straw. *Pleurotus sajor caju* treated with wheat flour in wheat straw took equal time for spawn run (16 days) and pin initiation (19 days) compared with control (19 and 24 days). The increasing concentrations caused negative effect on yield and biological efficiency with in a certain limits, *i.e.* besan flour-1, 2 and 3% (957, 944 and 931 g) *Pleurotus sajor caju* in wheat straw (Table 8).

Sets treated with soya flour (Table 9) shows maximum yield and biological efficiency in 3% *i.e. Pleurotus sajor caju* 977 g and 97.7% in wheat straw. The yield and biological efficiency increasing order of concentrations in soya flour, wheat straw in *Pleurotus*

Table 10 : Effect of different concentrations of rice flour on yield and biological efficiency of *P. sajor caju* and *P. florida* in different substrates

Species	Substrates	Supplement concentration	Mode of application	SRT (Days)	Pin initiation (Days)	Total yield (g)	BE (%)
Pleurotus sajor caju	Wheat straw	Rice flour-1%	Supplement	17	22	891	89.1
		Rice flour-2%	Supplement	18	22	822	82.2
		Rice flour-3%	Supplement	17	22	860	86.0
	Paddy straw	Rice flour-1%	Supplement	20	24	734	73.4
		Rice flour-2%	Supplement	20	23	749	74.9
		Rice flour-3%	Supplement	19	24	772	77.2
Pleurotus florida	Wheat straw	Rice flour-1%	Supplement	16	22	890	89.0
		Rice flour-2%	Supplement	17	21	877	87.7
		Rice flour-3%	Supplement	17	22	881	88.1
	Paddy straw	Rice flour-1%	Supplement	18	21	766	76.6
		Rice flour-2%	Supplement	17	21	812	81.2
		Rice flour-3%	Supplement	17	21	786	78.6
		Control		18	21	732	73.2



Fi.g 1 : The biological efficiency of different supplementation of *Plurotus* species

sajor caju (94.4%, 95.1% and 97.7%) and (93.3%, 93.9% and 94.1%) in *Pleurotus florida* compared with control (71.1%). The set treated with rice flour (Table 10) was observed the maximum biological efficiency rice flour-1% in wheat straw (891 g and 89.1 %) in *Pleurotus sajor caju*.

From the foregoing experiment it was found that organic source soya flour-3% gave maximum yield and biological efficiency of *P. sajor caju* in wheat straw (97.7 %), beasn flour-1% concentration in wheat straw in *P. sajor caju* (95.7%), inorganic source lactose-3% concentration in *P. sajor caju* (96.5%) in wheat straw. In case of *P. florida* was found that gave maximum yield and biological efficiency of soya flour-3% in wheat straw (94.1%).On that basis, lactose and $FeSO_4$ were proved superior carbon source and inorganic chemical, respectively. Vitamin B-complex gave maximum yield and biological efficiency in *P. sajor caju* in 100-mg/l in wheat straw (94.0%). Among the two lignocellulosic agricultural waste material used as a substrate, best growth and high yield of *Pleurotus* species found in *P. sajor caju* was obtained in wheat straw.

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