



Growth-enhanced Performance by *Pleurotus ostreatus* Cultivated on Salon Effluent and Spent Calcium-carbide Amended Substrates

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Authors' contribution:

This work was carried out in collaboration among all authors. Author JOO designed the study, wrote the protocol, managed the literature searches, set up the experiment, managed the analysis of the study and wrote the first draft. Author M-IA and GB contributed in setting up the experiment. Author A-AM contributed in designing the study and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the growth response of *Pleurotus ostreatus*, a wood-rotting fungus, to different growth substrates [Sawdust (SD), dry banana leaves (BL) and a combination of both BL and SD (BLSD)] amended with waste [salon effluent (SE) and spent calcium-carbide (SC)].

Place and duration of study: Department of Plant and Ecological Studies, University of Calabar, Cross River State, Nigeria, between May 2015 and August 2015.

Methodology: Amendments were applied to growth substrates at different levels of concentration as follows: 0 ml and 0 g, 5 ml and 5 g, 10 ml and 10 g, 15 ml and 15 g per kg substrate. Mature mushrooms were harvested and assessed on the following parameters; number of fruit bodies, fresh weight, dry weight, length of stipe, girth of stipe, pileus area using conventional method.

Results: Number of fruitbodies, fresh weight, dry weight and stipe length increased with increase in concentration of additives. Best performances of these growth parameters were obtained at 15 g/kg and 15 ml/kg concentration. The highest number of fruitbodies (with a peak mean value of 28.42 fruitbodies at 15 g/kg concentration), highest value of fresh weight and dry weight were observed in

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SD. The longest stipe length, largest stipe girth and pileus area were observed in BLSD, though it exhibited least performances in other growth parameters. BLSD amended with salon effluent produced mushrooms with the largest pileus area (with a peak mean value of 53.8 cm² at 15ml concentration) compared to the other substrates.

Conclusion: This study reveals that all growth parameters of *P. ostreatus* assessed were positively influenced by all the levels of amendments on the substrates used in this study. Therefore, these wastes could be used to increase the yield of *P. ostreatus* and possibly remediate sites polluted by these wastes.

Keywords: *Pleurotus ostreatus*; salon effluent; calcium-carbide; substrates; growth parameters; fruitbodies.

1. INTRODUCTION

Effective waste management is one of the greatest challenges facing humanity and the planet. The ongoing trend of industrialization and economic growth has resulted in increased waste production especially in cities with high population [1]. It is on record that of the wastes produced world over, the majority results from industrial, agricultural and domestic activities. Concerns exist worldwide about sound management of these wastes. If they are allowed to accumulate they become sources of severe environmental pollution [2]. There is a dire need for an adequate and safe waste management system that is inexpensive, easy to use, requiring no after storage liabilities for secondary waste, and even allows the treated materials to be reused thereafter [1].

The nutritional and medicinal values of mushrooms have long been known. However modern researchers have found that particular strains of fungi may be used to manage waste and remediate certain polluted sites. This rising curiosity in the use of mushroom for waste management stems from the numerous advantages it has over commercialized remediation technologies [3] [4]. Many mushroom species across the globe has been use to rid the environment of waste generated from industrial, household and agricultural activities. [5-10], [4].

Pleurotus commonly referred to as oyster mushroom, is a widespread edible mushroom, said to be first domesticated in Germany for basic consumption at the time of the First World War. However, today it is grown as food for marketing around the world and is listed as the third largest domesticated mushroom in the world [4]. Increasing interest in the cultivation and consumption of *Pleurotus ostreatus* is due to its easy and cheap production technology and higher biological efficiency. This mushroom has been cultivated on a lot of agricultural wastes

and has also been reported for used in mycoremediation purposes. *Pleurotus* has been cultivated on diverse agro wastes including cotton stalks, groundnut haulms, soybean straw, pigeon pea stalks and leaves, wheat straw, paddy straw, rice straw, sawdust, dry banana leaves, cereal straw, grass straw, cotton waste, corn cobs, sugarcane or sorghum bagasse, coffee waste, groundnut haulms, vinegar wastes, winery wastes, banana peel, palm kernel cake and groundnut cake and animal dung [11] [5] [12] [6-7] [13-16] [9] [17]. All agro wastes evaluated supported the growth of the mushroom, though with varied degree of efficiency thereby ensuring the production of healthy and safe food from waste for the population. Straw-bedded horse manure is said to be the most used substrate for cultivating mushrooms in Europe as well as in the USA and Canada [18]. Mandeel et al. [19] reported the use of untreated organic wastes such as chopped office papers, cardboard, sawdust and plant fibres in Bahrai or mushroom cultivation. Atikpo et al. [20] have reported the use of fresh fish waste, cooked fish waste, sawdust from *Tryplochyton scleroxylon* and rice bran as substrate in the cultivation of *Pleurotus ostreatus* in Ghana.

Mushrooms have also been extensively employ in the management of industrial waste especially in crude oil and spent engine oils contaminated soils. There have been several reports on the use of mushrooms to clean up oil spills which have become a regular occurrence. For example, several fungi like *Pleurotus ostreatus*, *Lentinus subnudus* and *Pleurotus tuber-regium* have been use to decontaminate soils contaminated with petroleum hydrocarbon [21] [3] [10]. Ogbo and Okhuoya [8] showed that *P. tuber regium* was able to decontaminate crude oil contaminated soils reducing the various petroleum hydrocarbons in crude oil to varying degrees. It was said that the contaminants improved the growth of the mushroom. In another experiment, Adenikepeun, 2008 noted that *Pleurotus tuber-*

regium is able to increase nutrient contents in soils polluted with 1 - 40% engine-oil concentration after six months of incubation. Solid sludge and effluent of both cardboard and handmade paper industries was composed for developing a mushroom growing method to accomplish zero waste discharges. Results from this study revealed that when 50% paper industries waste was mixed with 50% (w/w) wheat straw, there was significant increase (96.38%) in biological efficiency when compared to what was obtained in the wheat straw alone [22]. However, industrial waste is not limited to oil spillage alone.

Calcium carbide is a chemical represented with the formula CaC_2 . It is industrially used in the production of acetylene and calcium cyanamide. Acetylene with the formula C_2H_2 is a very valuable hydrocarbon owing to the energy that is confined in the triple-bond between carbon and hydrogen atoms. This energy is used in different ways for an array of industrial purposes [23]. Acetylene gas is mostly used as cylinder gas for metal construction. The gas is used to cut or bond various types of metals, clean them of surface deficiencies, and strengthen them by means of flame hardening. However, calcium carbide has a characteristic smell which is unpleasant to some. The spent calcium carbide also serves as a serious environmental pollutant if it is not treated properly. Calcium carbide is a rich source of the nitrification inhibitor, acetylene and plant hormone ethylene. [24] [23] [25] [26] Many research works support the use of CaC_2 as an effective inhibitor of oxidation of NH_4^+ into NO_3^- which results in increased availability of nitrogen to plants thereby improving the tolerance, growth and yield of crops [27-30].

Hair relaxer is used worldwide for hair care and beautification. However, the used hair relaxer water can become a source of air and land pollution if not properly disposed. A careful observation of the ingredients of most relaxers reveals its high nutritional content. Most hair relaxer ingredients include shea butter, olive oil, mink oil, tea-tree oil, jojoba oil, milk protein, egg protein, vitamin E, honey and silk amino acids. These ingredients have high nutrient content hence may be a good supplement for mushroom cultivation.

In the quest for effective but low cost waste management system, this research utilized two agro wastes (sawdust and dry banana leaves) and two industrial wastes (spent carbide and

salon effluent) in the cultivation of *Pleurotus ostreatus*.

2. MATERIAL AND METHODS

2.1 Location of Study

This research was carried out at the University of Calabar Staff quarters, University of Calabar, Cross River State. The University of Calabar lies between longitude $4^\circ 57' 0''$ North and $8^\circ 19' 0''$ East [5].

2.2 Source of Materials

The additives (spent carbide and hair relaxer water) were sourced within Cross River State. The spent carbide was obtained from an automobile workshop at Charmley Street and salon effluent was obtained from a hair dressing salon at Eyo-ita Street both in Calabar, Cross River State. Materials for substrate composition (dry banana leaves and sawdust) were obtained from the University of Calabar farms and government owned timber market at MCC road Calabar, respectively. The spawn of *P. ostreatus* were obtained from Royal farms at Ikot-Effanga, Calabar. Rice bran was obtained from rice mill in Ugep, Yakurr L.G.A of Cross River State and lime (gypsum) was purchased from Watt market in Calabar.

2.3 Preparation of Substrate

The dry banana leaves substrates, sawdust substrates and the combination (dry banana leaves and sawdust) substrates were prepared as described by [5] [19-20]. Pasteurization was also carried out as reported by [5] [19-20] [31].

2.4 Spawning

The pasteurized substrates were allowed to cool for 12 hours, then each substrate was divided into 1.0 kg portions and each of these portions was treated with 0 g, 5 g, 10 g or 15 g of spent carbide or 0 ml, 5 ml, 10 ml or 15 ml of salon effluent before being dispensed into plastic bags measuring 30 cm x 12 cm. Each of these bags was inoculated with spawns of *Pleurotus ostreatus*. The open bags were secured with PVC pipes 2 cm in diameter and length wrapped with rubber band and plugged with cotton wool [4] [6-7]. Each experimental unit was replicated thrice.

2.5 Spawn Running

The bags were hung with ropes serially from the roof down with each line carrying a maximum of eight bags. Room temperature was in the range of 25°C – 30°C and relative humidity between 60 % and 75 % achieved by spraying the compost bags and walls of the spawn running room 2 to 3 times daily with clean water, light penetration was limited [31] [6-7].

2.6 Cropping and Harvesting

At the end of the spawn run (when the mycelium completely colonized the substrate) the bags were moved to the cropping room and cropping was carried out as described by Markson et al, 2017a. Mature mushrooms (mushrooms with fully opened pileus) were harvested and assessed on the following parameters; number of fruit bodies, fresh weight, dry weight, length of stipe, girth of stipe, pileus area using conventional method [11] [4].

2.7 Data Analysis

The experimental design was a complete randomized design (CRD) with three replicates. Data collected were analyzed using SPSS version 21.0. Means were separated using Fisher's Least Significant Difference (LSD) test.

3. RESULTS AND DISCUSSION

All growth parameters were positively influenced by the treatments. The number of fruitbodies produced by spent carbide amended substrates was significantly higher ($P \leq 0.05$) than those produced by salon effluent in all the substrates, across all the flushes (Table 1). Fig. (1) reveals that the highest numbers of fruitbodies were obtained at 15 (g/kg and ml/kg) concentration in SD and BL whereas in BLSD the number of fruitbodies obtained at 10 and 15 (g/kg and ml/kg) concentrations were comparable ($P \leq 0.05$).

Fresh and dry weights of fruitbodies produced on substrates treated to spent carbide were significantly higher (with peak mean values of 54.63g and 6.45g respectively both on BLSD) than those produced on substrates treated to salon effluent (Tables 2 and 3). The least value of .00g was observed in BL and BLSD substrates treated to salon effluent. Figs. 2 and 3 shows that best performances of fresh and dry weights were observed at 15 (g/kg and ml/kg) followed by 10 (g/kg and ml/kg).

Mean values for stipe length, dry weight and pileus area on BLSD and SD treated to salon effluent were significantly higher ($P \leq 0.05$) than those produced on BLSD and SD treated to spent carbide in the first four flushes (Tables 4-6). However, the reverse was observed in BL where the mean values for stipe length, dry weight and pileus area of mushroom produced by spent carbide were significantly higher ($P \leq 0.05$) than those produced on BL treated to salon effluent across all the flushes. Figs 4 – 6 indicates that best performances of stipe length, stipe girth and pileus area for BL and BLSD were observed at 15 (g/kg and ml/kg) whereas for SD substrates stipe girth and pileus area were significantly higher ($P \leq 0.05$) at 10 (g/kg and ml/kg) but stipe length was significantly higher ($P \leq 0.05$) at 15 (g/kg and ml/kg).

The number of fruitbodies produced following each treatment in all substrates was concentration dependent. This explains the higher number of fruitbodies obtained at 15 (g or ml) concentrations in the first three flushes whereas in the fourth and fifth flushes the number of fruitbodies at concentrations 10 and 15 were comparable ($P \leq 0.05$) suggesting that at higher flushes the nutrient level required for the development of the mushroom fruitbodies declines with the declining growth vigor of the mycelial. The least number of fruitbodies were produced at the fourth and fifth flushes. This is a clear result of nutrient depletion from the substrates. From this observation, it is expedient to state that the number of mushroom flushes obtained in any mushroom culture at any time depends not only on the substrate type but also on the nutrient status of the substrate. SD substrates gave significantly ($P \leq 0.05$) higher number of fruitbodies at 10 and 15 concentrations compared to BL and BLSD substrates indicating that the compatibility between the treatment and the substrate is higher in SD substrate than in BL and BLSD. Comparing the effect of the two additives, spent carbide amended substrates produced mushrooms with significantly ($P \leq 0.05$) higher number of fruitbodies across all the flushes and in all substrates. This may imply that the quality or type of nutrient component necessary for mushroom growth is higher in spent carbide than in salon effluent and that certain component(s) of carbide is very essential for mushroom growth. Calcium carbide (CaC_2) is an effective nitrification inhibitor [26] [32] [33] A report by Banerjee et al. [34] asserted that CaC_2 inhibits *Nitrosomonas* activity hence prolongs the stay of

N in soil as NH_4^+ ion which makes nitrogen available for organism to use. The work of many researchers also supported the use of CaC_2 as an effective inhibitor of oxidation of NH_4^+ into NO_3^- under both flooded and non-flooded soil conditions [29] [30]; [27]. Oei [35] reported that for mushroom cultivation, a nitrogen source such as rice bran should be supplemented in the substrate. The nitrogen is usually converted to ammonium nitrate which is available for use by the mushrooms for growth and development. The

fresh and dry weight of the fruitbodies produced by *Pleurotus ostreatus* followed the same pattern, with highest value at 15 g / kg or ml / kg concentration across the flushes. The trend was similar to number of fruitbodies produced which implies that the availability of higher nutrient at higher treatment levels (10 g / kg and 15 g / kg) promoted better growth hence higher dry weight, fresh weight and number of fruitbodies recorded.

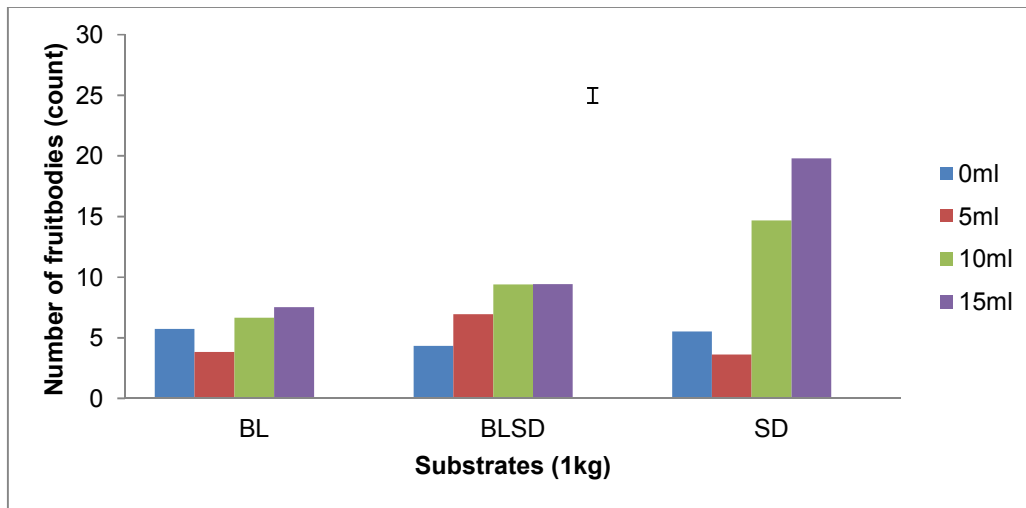


Fig. 1. Effect of substrate type and additive level on the number of fruitbodies produced per substrate

Bar represents LSD ($P \leq 0.05$)

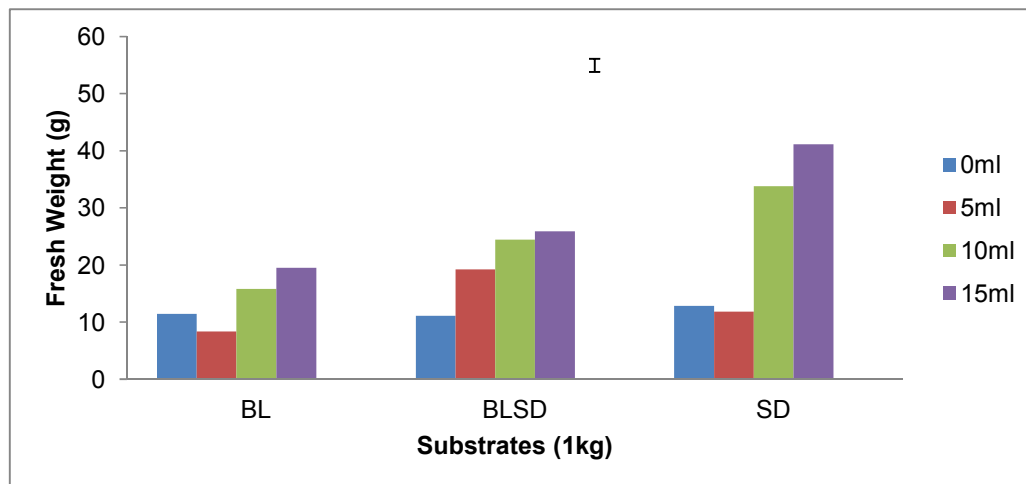


Fig. 2. Effect of substrate type and additive level on the fresh weight of fruitbodies produced per treatment concentration level

Bar represents LSD ($P \leq 0.05$)

Table 1. Influence of the additives on the number of fruitbodies produced per flush

number of fruitbodies per substrate (1 kg)															
	F1			F2			F3			F4			F5		
Additives	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD
SE	6.33	20.50	13.42	5.67	7.50	13.00	3.75	4.33	9.67	0.83	1.17	4.58	0.00	0.00	1.25
SC	13.25	24.00	28.42	12.50	8.33	16.75	10.83	5.42	12.00	4.50	2.83	6.25	1.75	1.17	3.67

*Values are means of three replicate. LSD = 0.78. SE: salon effluent. SC: Spent calcium-carbide. BL: Dry banana leave substrate. SD: sawdust substrate. BLSD: dry banana leaves/sawdust substrate. F1 – F5: Flushes

Table 2. Effect of additives on the fresh weight of fruitbodies produced per flush

Fresh weight (g) per substrate (kg)															
	F1			F2			F3			F4			F5		
Additives	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD
SE	18.29	37.78	39.50	13.94	23.05	34.46	10.03	17.48	28.68	3.18	6.84	15.35	0.00	0.00	2.99
SC	31.52	54.63	47.70	25.56	24.86	32.94	21.06	19.44	24.59	9.79	11.70	14.68	4.53	5.93	8.49

Values are means of three replicate. LSD = 1.44. SE: salon effluent. SC: Spent calcium-carbide. BL: Dry banana leave substrate. SD: sawdust substrate. BLSD: dry banana leaves/sawdust substrate. F1–F5: Flushes

Table 3. Influence of additives on dry weight of fruitbodies produced per flush

Dry weight (g) per substrate (kg)															
	F1			F2			F3			F4			F5		
Additives	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD
SE	2.27	4.97	4.95	2.05	3.19	4.24	1.64	2.43	3.55	0.39	1.17	2.45	0.00	0.00	0.45
SC	3.61	6.45	5.27	2.92	3.32	3.91	2.83	2.48	3.05	1.46	1.73	1.80	0.72	0.93	1.23

Values are means of three replicates. LSD = 0.16. SE: salon effluent. SC: Spent calcium-carbide. BL: Dry banana leave substrate. SD: sawdust substrate. BLSD: dry banana leaves/sawdust substrate. F1–F5: Flushes

Table 4. Influence of additives on the stipe girth produced per flush

Stipe girth (cm) per substrate (kg)															
	F1			F2			F3			F4			F5		
Additives	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD
S.E.	3.13	3.89	4.21	2.85	4.81	3.85	2.56	4.59	4.16	1.20	3.43	3.53	0.00	0.00	1.19
S.C.	4.18	3.83	2.15	3.64	3.98	2.11	3.72	3.87	1.84	3.08	3.28	1.32	2.08	1.83	1.37

Values are means of three replicates. LSD = 0.24. SE: salon effluent. SC: Spent calcium-carbide. BL: Dry banana leave substrate. SD: sawdust substrate. BLSD: dry banana leaves/sawdust substrate. F1–F5: Flushes

Table 5. Influence of additives on the stipe length produced per flush

Stipe length (cm) per substrate (kg)															
	F1			F2			F3			F4			F5		
Additives	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD
SE	3.53	4.79	4.12	3.33	5.63	3.94	3.50	5.30	4.02	1.48	3.00	3.65	0.00	0.00	1.15
SC	4.25	4.48	2.61	4.00	4.30	2.63	4.53	4.63	2.73	3.99	3.45	1.95	2.29	2.37	2.05

Values are means of three replicates. LSD = 0.16. SE: salon effluent. SC: Spent calcium-carbide. BL: Dry banana leave substrate. SD: sawdust substrate. BLSD: dry banana leaves/sawdust substrate. F1–F5: Flushes

Table 6. Influence of additive on the pileus area per flush

Pileus area (cm) per substrate (kg)															
	F1			F2			F3			F4			F5		
Additive	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD	BL	BLSD	SD
SE	27.03	40.64	38.98	27.77	47.14	45.73	25.76	53.88	46.39	11.95	39.58	34.71	0.00	0.00	13.38
SC	37.07	35.56	19.01	34.97	39.81	21.19	35.44	41.97	22.36	29.26	35.21	16.32	20.91	21.06	17.50

Values are means of three replicates. LSD = 0.16. SE: salon effluent. SC: Spent calcium-carbide. BL: Dry banana leave substrate. SD: sawdust substrate. BLSD: dry banana leaves/sawdust substrate. F1–F5: Flushes

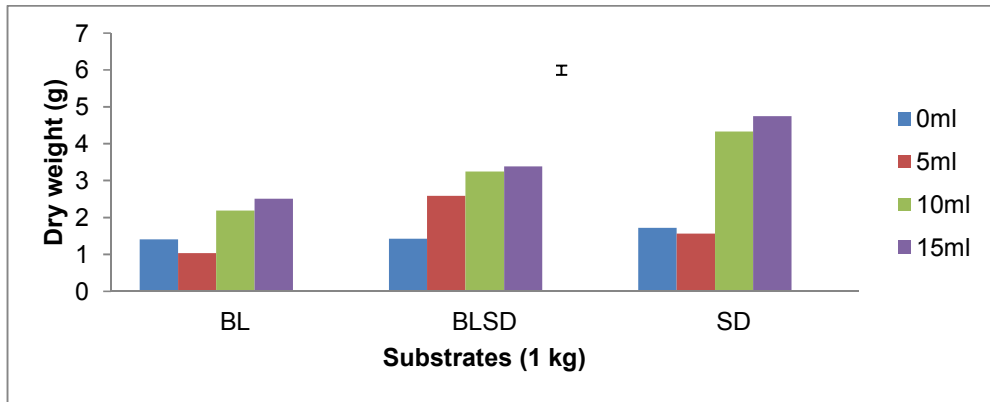


Fig. 3. Effect of substrate type and additive level on the dry weight of fruitbodies produced
 Bar represents LSD ($P \leq 0.05$)

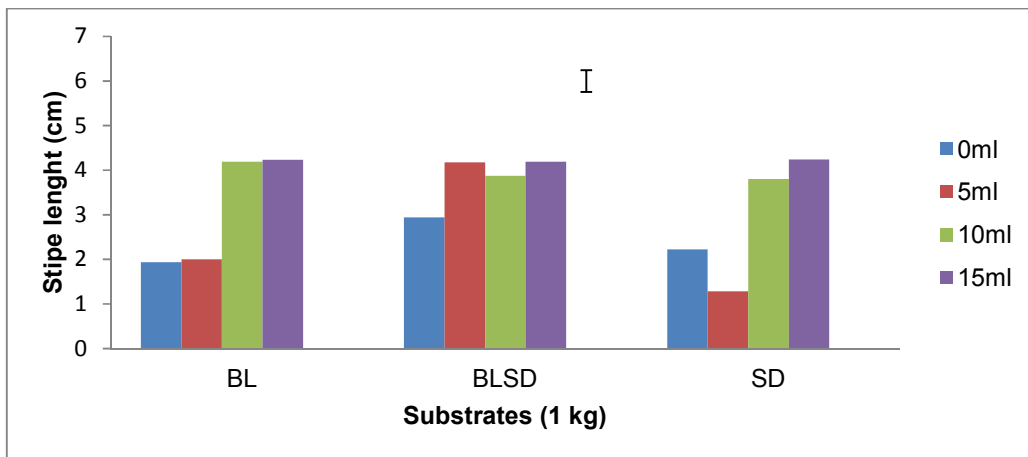


Fig. 4. Effect of substrate type and additive level on the length of stipe produced.
 Bar represents LSD ($P \leq 0.05$)

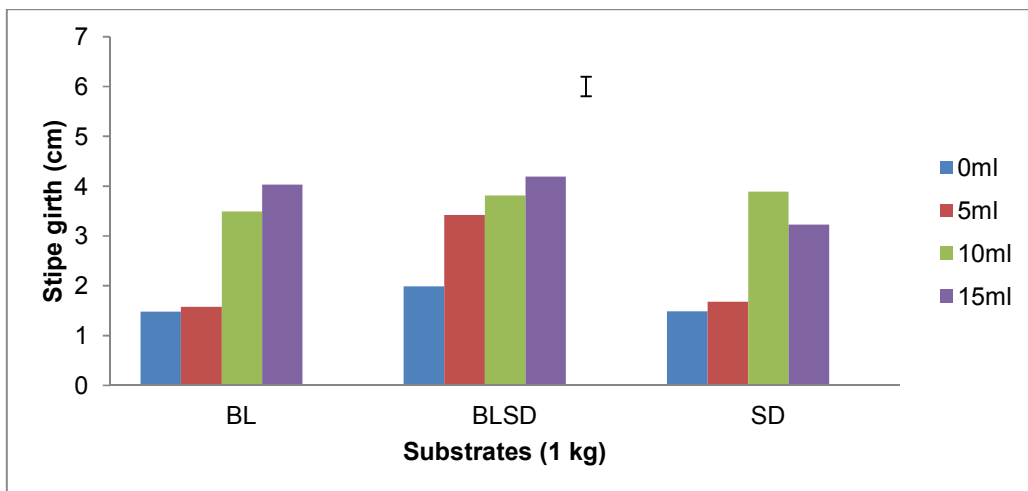


Fig. 5. Effect of substrate type and additive level on the girth of stipe produced
 Bar represents LSD ($P \leq 0.05$)

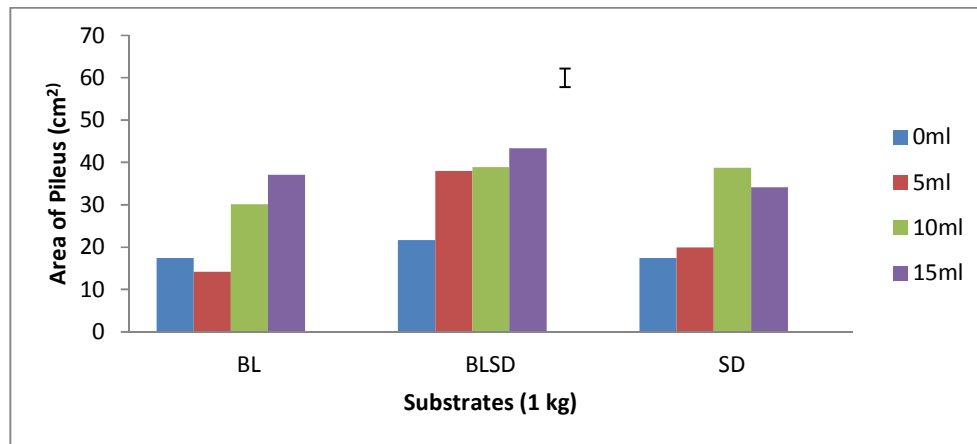


Fig. 6. Effect of substrate type and additive level on the area of pileus produced
Bar represents LSD ($P \leq 0.05$)

Stipe length was longest at concentration 15 g / kg or ml / kg. Considering the treatments, BL treated with spent carbide produced mushrooms with significantly ($P \leq 0.05$) longer stipe length than those produced in BL treated with salon effluent in all the flushes, which suggests the role of nitrogen in promoting stipe elongation. However, in BLSD and SD substrates, the stipe length of fruitbodies produced by spent carbide treated substrate were significantly ($P \leq 0.05$) shorter than those produced by BLSD and SD treated with salon effluent. This negative impact on the stipe length in BLSD and SD substrate is probably contributed by the presence of various growth inhibitors in sawdust which have been reported in wood. It is likely that such substances may have negatively impacted on the growth elongation of stipe in this case [6].

The stipe girth was highest at 15 g / kg or ml / kg in all the flushes except in the third flush where the highest value was at 10 g / kg or ml / kg concentration. BLSD substrate produced fruitbodies with significantly ($P \leq 0.05$) larger stipe girth than in other substrates at all concentrations. This could be as a result of BLSD having least number of fruitbodies.

Pileus size was also treatment dependent. At 10 g / kg and 15 g / kg concentrations, the mean values of pileus area were comparable ($P \leq 0.05$) but significantly ($P \leq 0.05$) higher than the pileus area produced at other concentration levels. However, at other treatment levels, BLSD produced fruitbodies with pileus area significantly ($P \leq 0.05$) larger than those of the other two substrates. Spent carbide in BL substrate enhanced the production of fruitbodies with

significantly ($P \leq 0.05$) larger pileus area than when salon effluent was added whereas the pileus area of fruitbodies produced in BLSD and SD treated with spent carbide were smaller than those produced in BLSD and SD treated with salon effluent. There seem to be compatibility between spent carbide and BL substrates than salon effluent and sawdust substrates. The synergy between spent carbide and BL substrates resulting in larger *Pleurotus* pileus area is likely a function of the ease of hydrolyses of the soft banana leaves tissues by the fungal enzymes coupled with the nitrogen made available by the denitrification inhibitory action of spent carbide. These two factors appear to make nutrients available to the mushroom for expansion of its pileus.

The affinity of salon effluent with sawdust substrates is likely a function of the nutrient (especially the protein, enzymes and hair growth promoting ingredients) in the hair relaxer that promotes and stimulates fungal growth vigor and possibly its ability to produce more hydrolytic enzymes necessary for the degradation of sawdust to release nutrients for the mushroom which are then converted to tissues (pileus).

4. CONCLUSION

This work utilized two agro wastes (sawdust and dry banana leaves) and two industrial wastes (spent carbide and salon effluent) in the cultivation of *Pleurotus ostreatus*. Results from this study reveal that all growth parameters of *P. ostreatus* assessed were positively influenced by all the levels of the amendments (salon effluent and spent carbide) on the substrates (BL, BLSD

and SD) used in this study. Sawdust substrate was the best in supporting the number of fruitbodies, high fresh and dry weights whereas best results of stipe length, stipe girth and pileus area were identified in BLSD. The performances of these growth parameters were observed to be best at 15 ml and 15 g per kg substrate. Though both amendments positively influenced the growth parameters, the performance of spent calcium-carbide was found to be the best. Hence, these wastes could be used to increase the yield of *P. ostreatus* which in turn result in proper management of these wastes and possibly remediate sites polluted by them.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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