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Original Article

Cultivation of *Pleurotus* HK-37 and *Pleurotus sapidus* (oyster mushrooms) on cattail weed (*Typha domingesis*) substrate in Tanzania

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Abstract

The suitability cattails weed botanical fractions namely, stems and leaves (SL), spikes (SP), rhizomes and roots (RR) and whole cattail weed (WCW) as substrates for growing two oyster mushrooms was investigated at three spawn rates 1%, 1.5% and 4% on wet substrate weight basis using plastic bag technology cultivation under local conditions. Amongst data collected were yield, biological efficiency (BE), crop period, mushroom number and size. Maximum mushroom yield (297g/kg wet substrate), biological yield (826 g/kg dry substrate) were obtained in WCW at spawn rate of 4% for *Pleurotus* HK-37. In *Pleurotus sapidus* maximum mushroom yield (239 g/kg wet substrate) and biological yield (1027 g/kg dry substrate) were obtained from WCW and RR, respectively at 1.5% spawn rate. A maximum BE (101%) was obtained when *Pleurotus* HK-37 was grown on RR at spawn rate of 4%. Conversely, maximum BE (103%) for *Pleurotus sapidus* was obtained in RR at spawn rate of 1.5%, followed by (66%) in WCW at same spawn rate. The study recommends WCW as a new substrate for cultivation of *Pleurotus* HK 37 at spawn rate of 4% and *Pleurotus sapidus*, at spawn rate of 1.5% under local conditions, which is being reported for the first time in Tanzania.

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Keywords: Spawn run time, Time to production, Yield, Biological efficiency, Cropping period, Mushroom size

1. Introduction

Cattails are plants in *Typha* genus, which has about 11 species. They are emergent weeds with very high rapid growth rate. Therefore they become monotypic cultures and eliminate other native plant species when hydrology, salinity, or fertility changes. They are found in wetlands such as ponds, lakes, streams, rivers and in depression areas. Cattails (*Typha* spp) are best of the wild edible plants with a variety of parts that are edible, including the rhizomes, underground stems, the young shoots and the flower spike. The young shoots can be cooked like vegetables and pollens are used in baked and roasted products. The cattail core can be ground into flour and this flour contains approximately 80% carbohydrates and 6-8% protein [1]. Even cattails have been desired as a potential source of foods but in Tanzania this plant is considered an undesirable weed. Currently, in Northen Tanzania's wetlands, lake Jipe in particular face degradation due to spread of invasive cattail, *Typha domingesis* Pers [2]. The weed has resulted into ecological, environmental and economic problems including shrinkage of the lake [3]. Suitable utilization of weeds is a subject of interest as most weeds are not used even as fodder due to the presence of lignin and anti-metabolites like phenolics, glycosides, Xavonoides and other compounds [4]. Physical harvesting (manually or mechanically) is highly preferred method towards utilization of weeds for value addition since the biomass harvested could be dried and used to grow nutritious protein rich food in the form of edible mushrooms [5,6].

Cultivation of the oyster mushroom, *Pleurotus* spp has increased greatly throughout the world during the last few decades and consitute the second largest variety of mushrooms produced in the world. Its popularity has been increasing due to its ease of cultivation on various unfermented cellulose and lignin containing wastes, high yield potential, high nutritional value and purpoted to have

medicinal properties [7,8]. Although *Pleurotus* spp (oyster mushrooms) are efficient decomposers and its multilateral enzyme system can biodegrades a large range of lignocelluloses wastes. But all the wastes are not suitable for every species of *Pleurotus* [6]. There has been reports on oyster mushrooms cultivation on terrestrial weed plants such as, *Leonotis* sp, *Sida acuta*, *Parthenium argentatum*, *Ageratum conyzoides*, *Cassia sophera*, *Tephrosia purpurea* and *Lantana camara* [5] and aquatic weeds such a water hyacinth (*Eichhornia crassipes* [9,10], papyrus, fern (*Azolla*), reed (*Phragmites*) [11], cattails (*Typha latifolia*) [12,13]. However, the main problem of oyster mushroom cultivation on most weed substrate is low yields, the problem, which could be overcome by blending weed plants with other lignocelluloses wastes such as rice straw, saw dust etc. For example *Leonotis* sp. was found to be the best substrate in fruit body production of *Pleurotus ostreatus* when it was mixed with rice straw (1:1, wet wt/wet wt) for mushroom cultivation [5]. Nevertheless, cattail weed (*Typha spp*) is one of the biomass with little published information on its utilization as a substrate for oyster mushrooms cultivation. Utilization of cattails (*Typha latifolia*) as substrate for *P. ostreatus* cultivation has only been recently reported Vetayasuporn [12, 13] in Thailand. The studies aimed at cultivation of *P. ostreatus* on various combination of saw dust and cattail. It was found that even though cattail weed provided economic acceptable substrates but with less yields, low biological efficiency (BE) in the range of 46-53% and more time consuming in mushroom growing processes. Therefore the substrate combination of sawdust and cattail alone has shown low potential for use as raw material for *P. ostreatus* cultivation. Therefore research on oyster mushroom production from cattails using other innovative approaches is imperative. To produce *Pleurotus sapidus* and *Pleurotus* HK-37 it is important to identify suitable substrate for better yield and higher bioconversion. Combination of two or more substrates has been reported to increase the yields of *Pleurots* spp [14]. In this paper the study was undertaken investigate a new nonconventional approach of blending various botanical fractions of cattails (*Typha domingesis* Pers) namely spike, leaves, stem, rhizome and roots to assess possibilities of increasing the mushroom yield of the two oyster mushrooms *Pleurotus sapidus* and *Pleurotus* HK-37. Also this work reported for the first time the influence of spawn rates on the productivity of the two oyster mushrooms on cattail weed.

2. Materials and Methods

2.1 *Site description and sampling*

The *Typha domingesis* Pers used in this study were collected from lake Jipe, Mwanga district, Kilimanjaro region in Tanzania. The details of the site and collection of cattail has been previously reported by Mshandete [3]. All the cultivation mushroom processes of the two oyster mushrooms on dried cattail weeds ordered as follows; substrate preparation, transfer substrate to polypropylene bags, pasteurization, inoculation (spawning), spawn run, initiation of pinheads (primordia), and harvesting of fruiting bodies (mushrooms). The experiments were conducted out in mushroom growing laboratory at the Department of Molecular Biology and Biotechnology, University of Dar es Salaam, Tanzania. The temperature, relative humidity and ventilation were not controlled to mimic the expected mushroom growing rooms for small-scale mushroom growers, whom will relay on manipulating ambient prevailing environmental conditions.

2.2 *Fungal materials source and maintenance of culture*

Pleurotus HK-37 and *Pleurotus sapidus* were obtained from strain bank of Department of Molecular Biology and Biotechnology, University of Dar es Salaam in Tanzania. The oyster mushroom *Pleurotus* HK-37 strain originated from South Africa is among the oyster mushrooms grown in Tanzania [15]. Tanzania Industrial Research Development Organization (TIRDO) has previously generously provided *Pleurotus sapidus* strain. All stock fungi cultures were maintained on potato dextrose agar (PDA) medium. The medium consisted of 0.4% potato extract, 2% dextrose, and 2% agar. Cultures on slants and plates were incubated at ambient temperature, which ranged from 25-30°C for 5-7 days. Maintenance of pure mycelial cultures is a necessity for ultimate spawn preparation and spawn production. Cultures were stored for short time at -20°C in a solar freezer/refrigerator (AECA, Solar Refrigerator/Freezer Super A, Pentan, Austria). Long-term storage of the culture was done in liquid nitrogen (LN_2) at -196°C in a LN_2 unit (Thermolyne (Bio-Cane ^{TM} 20, CAN and CANE SYSTEM, USA). Subcultures were made routinely every one-month.

2.3 Spawn preparation

Spawn of the two edible oyster mushrooms (*Pleurotus sapidus* and *Pleurotus* HK-37) were prepared with intact sorghum grains, which were bought from Kariakoo market in Dar es Salaam, Tanzania. The grains were washed thoroughly, and 1 kilogram of sorghum grains was boiled in 2 liters of water until the grains were semi-soft. The grains were left for 15 minutes in hot water to allow them to absorb moisture. Then the grains were decanted over a sieve to remove excess water and allowed cool to ambient tropical temperature. They were mixed with 1% (w/w) $CaSO_4$ and 2% (w/w) $CaCO_3$ to prevent sticking and adjusting pH, respectively. One hundred gram wet weight grains were put in 500 ml wide mouth bottles which were later on covered with aluminium foil. Bottles were sterilized at 121°C and 1 atmosphere for 15 minutes. Thereafter was aseptically inoculated with three 1 cm^2 pieces of mycelia taken from 5-day-old cultures of one of the two oyster mushroom used. The mycelia inoculated grains were incubated in darkness at (27 ± 2) °C until the mycelia fully colonized the grains.

2.4 Substrate preparation and characterization

Fresh whole cattail weeds were harvested manually and were divided into four cattail weeds botanical fractions,

namely: shoots (comprised of stem and leaves), rhizomes (comprised of rhizomes and roots), spikes and whole cattail (Mixture of shoots, rhizomes and spikes). These fractions were chopped into 3-7 cm long bits and were sun-dried for five days. Cattail weeds botanical fractions before growing oyster mushrooms were characterized, as follows: Total solids (TS), volatile solids (VS) and the ash content of the substrate were determined by the oven-drying and ignition method, respectively according to standard methods APHA [16]. Total carbon was determined by the dry combustion method previously described by Allen [17]. The organic matter content was done by the dry combustion method as described by Lyimo *et al.* [18]. The total fibres were determined by ignition of previously oven-dried sample at 800 ºC for 45 minutes. There after allowed to cool to room temperature before further ignition at 400 ºC for 30 minutes. Total nitrogen was determined by the Indophenol-blue method according to APHA [16].

2.5 Mushroom cultivation experiments

After characterization, the sun dried cattail weeds botanical fractions were then soaked in tap water for 3 hours for moisture absorption. Excess water was allowed to drain until no water was dripping from the substrates. Then palm squeeze test was performed to ensure right amount of moisture in the substrates. No water dripping between palm fingers confirmed that moisture in the substrate was correct to sustain mushroom cultivation. One kilogram wet weight of each cattail weed fractions investigated was packed into transparent polypropylene bags each bag was kept open on both ends but tied loosely by sisal rope. Each one kilogram wet weight corresponded to 329.95 g dry weight of stems and leaves, 200.64 g dry weight of rhizomes and roots, 145.69 g dry weight of spikes and 358.23 g dry weight of whole cattail weed. The bags were steam pasteurized at 97 ºC for 3 hours in a fabricated 200 liters steel drum. The cultivation experiments were conducted in March to July 2010 under local environmental conditions.

2.6 Substrate inoculation and spawn running

The effects of spawn rates (1%, 1.5% and 4% wet substrate weight) and cattail weeds botanical fractions (spikes, shoots, rhizomes and whole mixed cattail weeds) on two oyster mushrooms (*Pleurotus sapidus* and *Pleurotus* HK-37) productivity were investigated in polypropylene plastic bags containers. The bags were inoculated by above rate of inocula by layer spawning method. After inoculation the bags were placed horizontally on wooden shelves disinfected by 3% of house hold disinfectant (JIK, Reckitt Benckiser East Africa Ltd, Nairobi, Kenya) in a spawning running room. The room had a concrete floor pre-disinfected with 3% JIK. The windows and the door frame were covered with wire gauze to block insects and rodents; they were hung with black cotton curtains to create darkness and to limit fresh air circulation as recommended by Stamets and Chilton [19]. The spawning room was kept humid by pouring 15 liters tap water on the floor per day. However, no artificial lighting was applied to enhance darkness. Vegetative development was followed by direct observation of the inoculated substrates until the substrates were fully colonized by mycelia of the two oyster mushroom species. The temperature and humidity of the room was 28±1ºC and 78±2%, respectively.

2.7 Fructification: fruit body development

The fruiting body formation was triggered by shifting the environmental variables namely moisture, air exchange, temperature and light in the cropping room [20]. When the mycelia of the oyster mushrooms had colonized throughout the whole substrate, some holes were made on the bags with a needle to increase air exchange. Curtains on the windows were removed to allow ventilation and more light. These were done to initiate pinheads formation while at the same time lowering carbon dioxide concentration in the cropping room. Relative humidity and the temperature in the room were increased to 82±2% and temperature decreased to 25±2 °C, respectively by pouring 20 litres of tap water per day on the floor and on the walls. The bags were also sprayed with tap water twice a day using a hand sprayer to keep them moisturized.

2.8 Mushroom harvesting and yield

Fruit primordia (pinhead) were allowed to develop to a complete fruiting bodies and were picked. Mushrooms were harvested by removing them by hand from the substrate, then were taken away and weighed the same day. The oyster mushrooms were harvested when the in–rolled margins of the basidiomes began to flatten [21]. The length of stipe and cap diameter of the harvested mushrooms were measured. Dates of each harvest were also recorded. Total number of flushes (flush number) produced per each bag was noted. The distribution of the yield per flush was tabulated to observe changes in yield over the course of multiple flushes. Duration of time from inoculation to final harvest was calculated. Seven aspects of crop yield were evaluated according to Morais *et al.* [22] and Tisdale *et al.* [21] and Amin *et al.* [23] as follows: (i) Mushroom size (MS). (ii) Biological efficiency (BE) (iii) Biological yield (BY) (iv) mushroom yield (MY) (v) flush number (vi) Crop period (sum of incubation and fruiting periods) (vii) Fresh weight. BE values were calculated according to Stamets [20], Royse *et al.* [24]. BE = [Weight of fresh mushrooms harvested (g) /dry substrate weight (g)] x100. On the other hand MY values were calculated as previous reported by Morais *et al.* [22]. MY= [Weight of fresh mushrooms harvested (g) per fresh substrate weight]. The average MS was calculated as total fresh weight of mushrooms harvested divided by their total number of mushrooms. BY= [Weight of fresh mushrooms harvested (g) per dry substrate weight] and was expressed as g fresh mushrooms/kg dry substrate weight according to Amin *et al.* [23] Average weight of individual mushrooms was determined as quotient of the total fresh weight mushrooms harvested by their total numbers according to Phillipoussis *et al.* [25].

Table-1. Proximate compositions of cattail weed botanical fractions used as substrates for oyster mushrooms cultivation (Values are Mean ± standard deviation (SD), n=3).

Parameters	WCW	SL	SP	RR
Total Solids (% fresh sample)	16.01±0.5	12.68±1	28.62±0.9	8.34±0.5
Moisture Content (%)	83.99±1.2	87.32±0.8	71.38±1.1	91.66±0.9
Volatile Solids (% of TS)	81.73±0.7	80.27±0.5	94.47±0.6	62.02±0.8
Ash content (% of TS)	18.27±0.3	19.73±0.5	5.53±0.4	37.98±0.2
VS/TS ratio	5.10	6.33	3.30	7.43
Total Carbon	45.93±1	45.56±0.8	52.04±1	35.74±1.5
Total Organic Matter	86.63±0.9	80.05±1	92.41±0.9	58.19±1.2
Crude fiber	19.05±1.1	15.63±0.9	6.14±0.3	23.96±0.9
Total Nitrogen (TN) (% TS)	4.05±0.4	2.03±0.5	1.97±0.2	4.74±0.6
Crude protein (TN%x6.25)	25	12.5	12.31	29.62
Carbon: Nitrogen ratio	11.48	24.44	26.41	7.5

WCW: whole cattail weed; SL:stem and leaves, SP: spikes; RR: rhizomes and roots

Table-2. Growth characteristics of *Pleurotus* HK-37 grown on whole cattail weed and its botanical fractions substrates at different spawn rates. Values recorded are (mean ± SD, n=3).

Substrate	Treatment	Parameters			
	Spawn rate (%)	Days to complete mycelium running	Days taken till appearance of pinheads	Days taken for first flush	Interval between flush (days)
WCW	1	26±1	32±1	34±2	10.67±1.5
	1.5	26±1	31±1	33±1	5±1
	4	24±1	28±1	30±1	4±1
SL	1	27±1	33±1	35±1	10.33±0.57
	1.5	27±1	28±1	30±1	6.33±0.57
	4	24±1	28±1	30±1	4.33±0.57
SP	1	30±1	58±1	61±1	7.33±0.57
	1.5	29±1	57±1	60±1	6±1
	4	28±1	56±1	58±1	5±1
RR	1	30±1	39±1	43±2	10±1
	1.5	28±1	44±1	47±1	9±1
	4	28±1	51±1	52.33±1.53	8.7±0.57

2.9 Data analysis

All experiments were performed in triplicates. The experiments were laid out in a completely randomized design (CRD) with the four substrates, three spawn rates, two oyster mushrooms (*Pleurotus sapidus* and *Pleurotus* HK-37). Data analysis was done primarily using descriptive statistics such as the comparison of treatment means and percentages. The collected data were also subjected to ANOVA to ascertain significant differences between treatments (0.05 levels).

3. Results and Discussion

3.1 Chemical composition of whole cattail and botanical fractions used as substrates for oyster mushroom cultivation

The cultivation of mushrooms using different organic biowastes substrates is a value added bioprocess as it gives base for the growth of mycelium. The chemical constituents of cattails and its botanical fractions are listed in

Table-3. Growth characteristics of *Pleurotus sapidus* grown on whole cattail weed and its botanical fractions substrates at different spawn rates. Values recorded are (mean ± SD, n=3).

Substrate	Treatment	Parameters			
	Spawn rate (%)	Days to complete mycelium running	Days taken till appearance of pinheads	Days taken for first flush	Interval between flush (days)
WCW	1	26±1	88±1	91±1	7±1
	1.5	26±1	88±1	90±2	7.33±0.57
	4	24±1	69±1	73±3	7±1
SL	1	27±1	88±1	92±3	7.33±0.57
	1.5	27±1	88.33±0.57	92±3	7.33±0.57
	4	24±1	88±1	91±3	7.67±1.15
SP	1	30±1	65±1	69±2	8.67±0.57
	1.5	29±1	58±1	60±1	9.67±0.57
	4	28.33±0.57	56±1	59±1	10±1
RR	1	30±1	69±1	72.33±2.08	9±1
	1.5	28±1	65.67±1.52	69±1	9±1
	4	28±1	76±1	78.67±1.52	8.33±0.57

Table-4. Pattern of fruiting and productivity evaluation of *Pleurotus* HK-37 on WCW, SL, SP and RR substrates at three different spawn rates

Substrate	Spawn rate (%)	Total weight of fresh mushroom harvested (g)	Total number effective of fruiting bodies	Average weight of fruiting body (g)	Stipe height (mm)	Cap diameter (mm)	Mushroom size	MY (g/kg wet substrate)	BY (g/kg)dry substrate	% BE	Cropping period (days)
WCW	1.0	205.42±0.50	79.93±1	2.57±0.19	18.60±0.53	49.8±0.10	2.57	205.42±0.50	572.63±0.23	57.56±0.27	50±1
	1.5	289.67±0.20	132.87±1	2.18±0.05	10.47±0.72	44.23±0.20	2.18	289.67±0.20	808.35±0.31	80.85±0.04	54±1
	4.0	296.65±0.10	175.5±1	1.69±0.01	21.63±0.55	44.36±0.15	1.69	296.65±0.10	826.24±2.80	82.82 ±0.05	58±1
SL	1.0	161.77±0.25	60.81±1.53	2.66±0.01	17.1±0.1	42.6±0.20	2.66	161.77±0.25	490.33±0.24	49.28±0.26	45±1
	1.5	184.92±0.02	70.85±1.53	2.61±0.053	15.1±0.1	46±0.10	2.61	184.92±0.02	560.63±0.163	56.24±0.17	53±1
	4.0	218.92±0.64	99.51±1	2.20±0.015	20.9±0.1	43.33±0.35	2.20	218.92±0.64	663.69±0.23	66.45±0.11	52.61±1.5
SP	1.0	8.7±0.015	4.9±1	1.77±0.01	21.10±0.1	31.53±1.0	1.77	8.7±0.015	59.96±0.04	6.02±0.05	68±1
	1.5	45.17±0.2	21.51±1.5	2.1±0.05	22.26±0.15	31.7±0.2	2.1	45.17±0.2	309.9±0.06	30.99±0.01	65±1
	4.0	60.7±0.1	26.74±1	2.27±0.03	23.9±0.1	42.9±0.72	2.27	60.7±0.1	415.96±0.04	41.61±0.015	70.33±1.5
RR	1.0	11.35±0.21	3.01±1	3.76±0.025	32.9±0.1	33.07±0.15	3.76	11.35±0.21	56.49±0.21	5.60±0.04.	69±1
	1.5	78.53±0.33	85.35±1	0.92±0.015	29.6±0.1	34.67±0.20	0.92	78.53±0.33	392.14±0.05	39.19±0.13	70.33±1.53
	4.0	201.69±0.29	91.68±1.52	2.2±0.10	27±0.1	36.83±0.20	2.2	201.69±0.29	1007±2	100.57±0.07	70±1

(Table 1). The overall composition varied considerably among four cattail weed botanical fractions. A significant difference ($p<0.05$) was found on four substrate cattail weed botanical fractions. The main reason being that the distribution of nutrients resources in plants is allocated differently in botanical fractions. Similar observation has also been reported for chemical composition of entire and botanical fractions of the same plant, *Typha domingesis* Pers by Mshandete [3] and in water hyacinth, *Eicchornia crassipes* by Aboud *et al.*[26]. The moisture content of the fresh entire cattail and botanical fractions obtained (Table 1) which ranged between 71-92% were within the ranges of 76-90% reported previous for 19 emergent species including *T. latifolia linn* [27]. The entire cattails VS (% of TS) 81.73% was close to the range of 88.8-93.5% reported for *T. latifolia linn* [28]. The range values of 5-38% ash content recorded for the entire cattail and its botanical fractions were within or slightly higher when compared to 5-25% and 8-28% ash content ranges reported for water hyacinth and cattail aquatic plants [3, 26]. The total carbon and organic matter obtained

Table -5. Pattern of fruiting and productivity evaluation of *Pleurotus sapidus* on WCW, SL, SP and RR substrates at three different spawn rates

Substrate	Spawn rate (%)	Total weight of fresh mushroom harvested (g)	Total number of effective fruiting bodies	Average weight of fruiting body (g)	Stipe height (mm)	Cap diameter (mm)	Mushroom size	MY (g/kg wet substrate)	BY (g/kg dry substrate)	% BE	Cropping period (days)
WCW	1.0	233.83±0.21	132.10±1	1.77±0.19	16.57±0.51	53.27±2.21	1.77	233.83±0.21	652.6 ±0.48	65.23±0.06	109±1
	1.5	239.48±0.21	94.66±1	2.53±0.41	25.03±3.69	52.13±2.06	2.53	239.48±0.21	663.8±0.15	66.38±0.16	110±1
	4.0	116.93±0.06	43.79±1	2.67±0.16	35.67±6.33	55.67±2.21	2.67	116.93±0.06	326.43±0.51	32.63±0.03	98±1
SL	1.0	163.87±0.15	60.6±1	2.70±0.1	35.2±2.80	46.33±0.75	2.70	164.23±0.42	496.40±0.42	49.67±0.05	113.33±1.53
	1.5	217.17±0.45	94.83±1	2.29±0.4	27.8±0.36	54.37±0.55	2.29	217.69±0.23	658.43±0.23	65.68±0.11	113.67±1.53
	4.0	128.05±0.45	55.67±1	2.30±0.13	20.57±0.66	39.43±0.15	2.30	128.61±0.31	388.4±0.05	38.84±0.05	113±1
SP	1.0	11.60±0.40	9.9±1	1.17±0.04	23.10±2.27	38.10±0.50	1.17	11.49±0.4	76.87±0.13	7.65±0.04	89±1`
	1.5	82.64±0.35	31.30±1	2.64±0.10	30.33±1.90	48.90±1	2.64	83.09±0.8	564.47±0.20	56.49±0.03	98±1
	4.0	74.64±0.30	23.54±1	3.17±0.07	33.67±2.90	43.50±0.40	3.17	74.70±0.33	510.45±0.28	51.20±0.21	90.33±1.53
RR	1.0	13.84±0.20	5.94±1	2.33±0.01	35.23±3.44.	52.87±1.70	2.33	13.84±0.20	69.46±0.49	6.95±0.04	91.67±1.53
	1.5	205.84±0.20	73.51±1	2.8±0.1	37.13±1.09	49.5±0.45	2.8	205.84±0.20	1026.66±0.34	102.67±0.04	96±1
	4.0	35.92±0.11	13.81±1	2.6±0.07	33.87±0.77	46.0±1	2.6	35.92±0.11	179.63±0.34	17.91±0.07	93±1

was significantly high in the spike cattail weed fraction, while the rhizome-roots fraction had the slightly lowest values as demonstrated in (Table 1). The range reported was 36-52% for total carbon and 58-92% for total organic matter. On the other hand, the rhizome-roots fraction showed highest nitrogen content of 4.7%. The low total crude fiber and high organic matter and total carbon as well as total nitrogen indicated that cattail weeds represent an abundant resource for bioconversion into value added bioproducts such as such as mushrooms and animal feed. Although substrate composition analysis is important but does not always correlate with growth or yield of mushrooms but should be monitored to determine trends in substrate preparation for increased mushroom productivity. The laboratory analysis should be used as guidelines and establishing trends from crop to crop of mushrooms.

3.2 Spawn running (mycelia development or vegetative growth), pin-head and fruit body development

Though variation exists among different *Pleurotus* mushroom species, composition of the substrate does play a role on vegetative growth. Substrate is one of the important parameter in mushroom production as mushrooms depend on substrates for nutrition to support mycelia growth and development into mushroom fruiting bodies. Also substrate structure is an important factor for the growth of mycelia as it should be suitable for the penetration of the mycelium in the basal substrate, which ultimately influences fruiting of mushrooms. The data of three phases namely, spawn running, pin-heads appearance and fruit body development are displayed in (Table 2 and 3) for *Pleurotus* HK 37 and *Pleurotus sapidus*, respectively. The data were statistically analyzed by repeated measures of ANOVA. A post-test was performed using Tukey-Kramer Multiple comparisons test. It was found that growth characteristics of *Pleurotus* HK-37 grown on four cattail weeds botanical fractions substrates at three different spawn rates were statistically significantly different at ($p<0.0001$), while that of *Pleurotus sapidus* were statistically significantly different at ($p<0.0037$). Similarly other previous investigators have observed that the duration of different growth stages of cultivated mushrooms are affected by several factors which would include, but not limited to, type of substrates and supplements used, the type of species and/or the strain employed, spawn type and the rate of inocula/spawn applied, spawning method, spawning/cropping containers as well as on the prevailing mushroom growing conditions etc [23, 25, 29, 30].

During spawn running (mycelia colonization of the substrate), the growing hyphen interconnects and intertwines with pieces of the substrate to form the base for the production of fruit bodies. Increasing the amount of spawn used has resulted in increased yield, thus, the increased level of spawn provide more energy for mycelial growth. It is evident from (Table 2 and Table 3) that spawn running took three to four weeks for both oyster mushrooms regardless of cattail weed fractions substrates. All cattail botanical fractions were inoculated on the same day and were incubated in darkness and in the same incubation room. These results agree with the findings of Adebayo *et al.* [31] who reported three weeks of mycelial colonization of *P. pulmonarius* on cotton waste. Mycelia of *Pleurotus* species are well known to colonize various lignocellulosic materials due to their extensive enzyme systems capable of utilizing complex organic compounds, which occurs in organic matter residues [21,32]. Thus a range of 12-41 days has been reported as time periods of spawn running for various *Pleurotus* species on lignocellulosic substrates [19,20, 24,32]. Thus, the results from this study concur with the previous spawn running periods reported by others on *Pleurotus* species.

Pinhead formation is the second stage of mycelial growth during cultivation of mushrooms. After completion of the spawn run, the bags were exposed to light. Tiny knots of mycelia were formed and later differentiated into tiny mushroom like structures called pinheads or primodia. The pinheads lengthened and enlarged to form mature fruit. As shown in (Table 2 and Table 3), the time taken for pinheads to appear after the spawn run period differed from each of the two mushroom species cultivated. *Pleurotus* HK-37 had the

shortest time for pinhead formation, which was between 4-8 weeks. Comparable results has been reported by Royse *et al.* [24], Quimio *et al.* [33], Baysal *et al.* [34] and Shah *et al.* [35] and on *Pleurotus* species grown on different substrates, which took 2-4 weeks for fruiting bodies to be formed after inoculation of the spawn. These pinheads grew into mushrooms, which were harvested 2-3 days later. On the other hand, results for *P. sapidus* showed that pinheads appearance took about 8-13 weeks in almost all spawn rates investigated and the cattail weed botanical fractions used, and thereafter, it took 2-4 days to develop to harvestable mushrooms. The present findings are similar with those of [12] who reported on *Pleurotus ostreatus* cultivation on narrow leaf cattail. The experiment found that spawn run took 40±2 days, primordial initiation was after 66±13.75 days while fruit body formation was realized after 71±13.25 days. Furthermore, Zadrazil [36] had also reported first fruiting bodies appearance after 55 days in the case of *Pleurotus sajor caju* (Fr.) Sing cultivated on straw substrate supplemented with inorganic and organic nitrogen supplements. The results in (Table 2 and 3) also indicated that regardless of the rate of spawn and cattail weed substrate fraction interval between flushes average ranged between 4 to 11 days. In practice flush or break or bloom are applied to crops of mushrooms that mature and picked within four to five days. Mushrooms are harvested usually in 7-10 days interval. Nevertheless, such an interval is influenced by various factors such as level of spawn, quantity of substrate and crop management [23,37]. It has been observed that rapid growth and colonization of mycelia may increase number of flushes during the fructification stage, which could be reflected on mushroom yield [12]. The present experiment findings together with those of other investigators both suggest that different species of oyster mushrooms have different optimum period for complete substrate colonization as well as the pinheads formation. It illustrates that each mushroom species and correspondent mushroom substrate singly or in combination need to be optimized separately for increased mushroom productivity.

3.3 Yield attributes and mushroom yield of oyster mushrooms grown on cattail weeds substrates

The yield attributes and mushroom yield namely, mushroom fresh weight harvest, effective numbers of fruiting bodies, mushroom yield, biological yield, biological efficiency, stipe and cap dimensions, mushroom size and crop cycle for oyster mushrooms cultivated on four cattail weeds fractions at three different spawn rates are represented in (Table 4 and Table 5). The data on yield attributes and mushroom yield on four cattail weed substrate and three different inoculation rates were statistically analyzed by repeated measures of ANOVA. A post-test was performed using Tukey-Kramer Multiple comparisons test. It was found that yield attributes and mushroom yield were statistically significantly different at ($p<0.0001$) and ($p<0.02$) for *Pleurotus* HK-37 and *Pleurotus sapidus*, respectively.

The numbers of effective fruiting bodies for the two oyster mushrooms recorded from 1000 g of wet substrate but with different corresponding dry weights were as follows, for *Pleurotus* HK 37, the lowest numbers of mushrooms (3.01) was recorded for RR in (200.64 g dry weight) at 1% spawn rate, followed by (4.9) recorded for SP in 145.69 g dry weight at 1% spawn rate. The highest numbers (175.5) was recorded for WCW in 358.23g dry weight at 4% spawn rate followed by (132.87) which was recorded in MCW at 1.5% spawn rate. On the other hand, a similar trend observation was recorded for *Pleurotus sapidus*. Lowest numbers of mushrooms (5.94) were recorded from RR followed by (9.9) obtained from SP at 1% spawn rate. The highest numbers of mushrooms (132.10) were recorded from WCW at 1% spawn rate followed by (94.66) at 1.5 % spawn rate. SL in 329.95 g dry weight at 1.5% spawn rate recorded similar results as with those of WCW at 1.5 % spawn rate. Comparable results with the present experiment findings were not readily available in the literature. Nevertheless, recently Amin *et al.* [23] have reported direct proportional relation ship between the number of fruiting bodies and quantity of substrate dry weight and spawn rate in the case of *Pleurotus ostreatus* cultivated on rice straw.

The results of mushroom yields and biological yields are important both on scientific and growers perspectives. Yields results represented the quantity of fresh oyster mushrooms which can obtained from wet or dry weight cattail weeds botanical fractions. Mushroom yield and biological yield for *Pleurotus* HK 37 were found in the range of 8.7 to 296.65 g/kg wet substrate and 56.96 g to 826.24 g/kg dry weight substrate, respectively. Lowest mushroom yield (8.7 g) and biological yield (56.49 g) were harvested in SP at 1 % spawn rate and in RR at 1% spawn rate, respectively, which were statistically not significant at ($p>0.05$). Maximum mushroom yield (296.65 g/kg wet weight) and biological yield (826.24 g/kg dry weight) were both observed in WCW at 4% spawn rate, which were statistically significant different to those of SP at ($p<0.001$) and RR at ($p<0.05$). On the other hand, mushroom yield and biological yield for *Pleurotus sapidus* were found in the range of 11.49 to 239.48 g/kg wet substrate and 69.46 g to 1026. 66 g/kg dry weight substrate, respectively. Lowest mushroom yield (11.49 g/kg wet weight) and biological yield (69.46 g/dry weight) were harvested in SP at 1% spawn rate and in RR at 1% spawn rate, respectively, which were statistically not significant at ($p>0.05$). Maximum mushroom yield (239.48 g/kg wet weight) and biological yield (1026.66 g/kg dry weight) were observed in WCW and in RR, respectively both at 1.5 % spawn rate. The yield results were statistically significant different at ($p<0.05$). Generally mushroom yield and biological yield results of both oyster mushrooms showed that were direct proportionality with increasing spawn rate regardless of the cattail weeds substrates fractions employed. Similar trends of increasing yield with increasing rate of inocula to a certain optima have

been reported recently on rice straw substrate inoculated with different rates of inocula [23]. The yields results (Table 4 and 5) demonstrated that each cattail botanical fraction supported the growth of the two oyster mushrooms differently. This furthermore indicated that the mycelia of the two oyster mushrooms have different colonizing potentials for the substrates in which they are grown, which ultimately, corresponded to the yield obtained. With this observation in mind, different researchers had reported different mushroom yields for different mushroom species and growing substrates. However, mushroom yield and biological yield results in this work fall within the ranges reported previously on *Pleurotus* species cultivated on various non-composted substrates, with and without supplements [13, 21, 23, 32]

From the experiment it was found that in all cattail weed fractions substrates for the two oyster mushrooms (*Pleurotus* HK 37 and *Pleurotus sapidus*), the percentages of biological efficiency (BE) ranged between 5.6-103% (Table 4 and 5). In the case of *Pleurotus* HK 37 the lowest BE were obtained at 1% rate of spawn from RR (5.6%) followed closely by (6.02%) obtained from SP also at spawn rate of 1% (Table 4). Those BE's were significant different at a confidence level of 95% to those found at spawn rate of 1.5% in WCW (80.85%), at spawn rate of 4% in RR (100.57%) also at spawn rate of 4% in WCW (82.82%). Even if the highest percentage of biological efficiency was obtained from RR at spawn rate of 4% but this percentage was insignificant different at a confidence level of 95% to that found in WCW (82.82%) at the same spawn rate of 4%. Again the BE of (82.82%) obtained from WCW at spawn rate of 4% was insignificantly different at a confidence level of 95% to that found in WCW (80.85%) at spawn rate of 1.5%. The BE data trend in the case of *Pleurotus* HK 37 was that BE increased with increasing rate of spawn i.e. the highest was obtained from 4% spawn rate. Contrarily, in the case of *Pleurotus sapidus* the highest BE were obtained at spawn rate of 1.5% in all four cattail weed fractions utilized as substrates (Table 5). The BE of (102.67%) obtained in RR was significant different at a confidence level of 95% to those found from SP (56.49%), WCW (66.38%) and SL (65.68%). On the other hand lowest BE (6.95%) in RR and (7.65%) in SP were both obtained from 1% spawn rate. The observed BE trend in *Pleurotus sapidus* obtained in four substrates clearly demonstrated BE increased progressively with rate of spawn from 1% to 1.5% then decreased relatively at 4% spawn rate. Best BE results in this case were obtained at 1.5 % spawn in all four substrates. Even though the high BE (102.67%) obtained in RR statistically was significantly higher than that of (66.38%) found in WCW, in real practical application the BE obtained from WCW at 1.5% spawn rate could be adopted in order to utilize the entire cattail weed. The overall trend observed in this study was that BE increased with increasing rate of spawn. Nevertheless, it was evident that regardless of the four substrates utilized each mushroom had optima spawn rate, which supported high BE. While the best spawn rate for *Pleurotus* HK 37 was 4%, in the case of *Pleurotus sapidus* was 1.5%. These trends in BE results in respect to rate of spawn inoculated on the mushroom substrate are comparable to other previous studies reporting increase in BE when spawn levels are increased to a certain optimum levels. Depending on the mushroom species, type and quantity of substrate, nutrient status of the substrate, blending of substrates, extend of spread of mycelium within the substrate [20, 23, 24, 38]. Although the influence of spawn rate on BE depends on many factors, often it may indicate the nutritional and moisture level of the substrate. Also the spawn strain varies in their inherent capacity for rapid or slow growth. This variation is a genetic characteristic; therefore, growers should be familiar with the characteristics of the different strains and the suppliers of spawn could be a good source of this information.

The overall cropping period (sum of incubation period and fruiting) of the two oyster mushroom cultivated on four substrate, at three different spawn rates are presented in (Table 4 and 5). The cropping period for two oyster mushrooms studied on average varied between 45 to 114 days. The cropping period for *Pleurotus* HK 37 was relatively shorter (45-70 days) than that of *Pleurotus sapidus*, which ranged between 89-114 days. These results were similar to previous reported findings, which established that oyster mushrooms grow repeatedly for about 3 to over 5 total crops cycles, over a period of about 100 days [12,13,20,21]. However, the authors also found that the crop period is influenced by many factors such methods (indoor or out door) and facilities used for growing oyster mushrooms, type of oyster mushroom grown, substrates utilized, type and amount of spawn, crop management etc. They also observed that since crop period indicates time invested per crop it is important to keep it low as possible without negatively affecting the overall yield. Since the amount of spawn used depends on the crop cycle and cost, cultivation of *Pleurotus* HK 37 on WCW at a spawn rate of 1.5 and 4% could be recommended for Tanzania growers. Specifically spawn rate of 1.5% could be adopted to maximize profit since the BE and cropping periods were at par with those obtained from 4% spawn rate.

Conclusions

It was technically feasible to cultivate *Pleurotus* HK 37 *and Pleurotus sapidus* on whole cattail weed and its botanical fractions namely stem and leaves, spike and rhizomes and roots at three various spawn rates. Considering the mushroom yield, biological yield, biological efficiencies and cropping period overall best results were obtained at spawn rate of 4% for *Pleurotus* HK 37 on WCW substrate and at 1.5% for *Pleurotus sapidus* on RR substrate. Nevertheless, in view of practical applicability of the results in the real situation and considering spawn costs, spawn rate of 1.5% could be adopted in production of *Pleurotus* HK 37 on WCW. On the other hand, 1.5% spawn rate could be adopted in order to utilize the entire cattail weed for *Pleurotus sapidus*, although

statistically results obtained from RR were significantly higher than those obtained from in WCW. It can be concluded that cattail weeds are potential alternative substrates for production of oyster mushrooms. The latter could be a new high-value cash crop, which could serve as a means for generating income and creating wealth in a developing country like Tanzania as well as form part of cattail weed sustainable management.

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Abbreviations
SL- stem and leaves
SP- spikes
RR- rhizomes and roots
WCW- whole cattail weed
ANOVA- one way analysis of variance
TIRDO-Tanzania Industrial Research Organization
PDA-potato dextrose agar
TS- total solids
VS-volatile solids
BE-biological efficiency
BY-biological yield
MY-mushroom yield
MS-mushroom size
CRD-completetly randomized design
SD-standard deviation
TN- total nitrogen

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