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Cultivation and Production of Pleurotus Mushroom on Non-Traditional Growing Media

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ABSTRACT

The study was carried out to evaluation different substrate as growing media for two different species of pleurotus (*Pleurotus ostreatus* and *Pleurotus cystidiosu*) on growth and yield of spawn and their contents from elements. Three substrates mixed with agar were used potato dextrose agar, vermicompost tea agar and compost tea agar for spawn production. The same substrates without agar (broth) in addition to, wheat seeds were used as control media for propagation of spawn on rice straw as traditional media for mushroom production. The results indicated that various substrate formulas gave significant differences in weight of spawn, growth speed rate, total yield on rice straw and its content of nourishes. Vermicompost tea as growing media was more efficient in spawn weights than other growing media with the both types of pleurotus. The highest yield was obtained when its spawn grown on rice straw (9.95 and 7.5 Kg) with both *Pleurotus ostreatus* and *Pleurotus cystidiosus* respectively. Total carbohydrates and energy were very high when *Pleurotus ostreatus* has grown on wheat seed (55.3 and 312.9 kcal/100 g respectively). Percentages of total protein, fat and ash were also high when it has grown on vermicompost tea (27.3, 2.3 and 6.8 5 respectively).Vermicompost tea achieved the greatest productivity and biological efficiency (156.25) and *Pleurotus ostreatus*has higher total yield and biological efficiency than *Pleurotus cystidiosus*.

Keywords: mushroom, pleurotus. vermicompost, compost, tea, biological efficiency

1. Introduction

Pleurotus spp. agronomy has increased extremely in the world during the last few years (Royse, 2002). Pleurotus mushroom are considering as 14.2 % of the total world production of edible mushroom in 1997 (Chang, 1999). The fruiting body of the paddy straw mushroom is divided into six different developmental stages viz., pinhead, tiny button, button, egg, elongation and mature stage. Each stage has its own morphology and anatomy. Mushrooms were harvested when the mushroom cap surface were flat to slightly up-rolled at the cap margins. The harvested fruiting bodies in each bag were then counted and weighed. At the end of the harvest period, the accumulated data were used to calculate the biological efficiency and mushroom weight, (Pavlik, 2005).

Mushrooms are used as meat alternatives and flavorings. Edible mushrooms are low in fat and calories, rich in vitamin B and C, contain more protein than any other food of plant source. It is a good foundation of mineral nutrients (Bahl, 1998). Rates of protein mushrooms can differ from 10-40 % of dry weight. It contains all the essential amino acids, (Chang, 1991). In addition, mushroom has important role in medical field, where some polysaccharides were extracted from spore and mycelium and has a role in antitumor dehydrated submerged liquid culture (Rahman *et al.*, 2012). Mushrooms with their flavour, texture, nutritional value and high efficiency per unit area have been identified as an excellent nourishment source to improve malnutrition in developing countries (Eswaran and Ramabadran 2000). Pleurotus mushroom cultivation can play a vital role in management of organic wastes whose disposal

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has become a problem. Mushroom can use wide range of cellulosic materials and the C: N ratio needed is about 40 to 60, compared to other cultivated mushrooms. It can be grown quite quickly and easily on uncomposted substrates such as paddy straw and cotton waste or other cellulosic organic waste materials (Das and Mukherjee, 2007).

Compost and vermicompost tea contains high population of benefit microbes such as nitrogen fixers and phosphate solubilizes and also the secret growth regulators which have a role to enhance the growth and increase yield of different crops. Mushroom grow on different media such as compost and vermicompost which achieve the best significant yield potential and growth behavior with Pleurotus spp. (Sylvia, 2004; Antonio *et al.,* 2008).

In Egypt, Mushroom production is stilling limited and is progressed slowly as compared to other countries in the world. The causes for slow progressive of mushroom are shortage of technically trained people, unsaved fixe source and trust to produce spawn. It exposed to damage during transport and storage processes and low researches in this filed. Egypt import high quantity with highly cost although, it has territories with the various temperature and weathers which are suitable for mushroom growth. The needing became argent to save the production of spawn with high quantity and low cost.

In the present study, two types of Pleurotus were growing in different media to achieve a significant medium for the best growth of mushroom with low cost to reduce the duration period of growth.

2. Materials and Methods

The experiment was carried out for the possibility to use different Pleurotus spp. for spawn production in Mushroom Unite in Faculty of Agriculture in Cairo, Al- Azhar University, Egypt.

2.1. Mushroom Strains:

Two different strains of pleurotus spp. (*Pleurotus ostreatus* and *Pleurotus cystidiosus*) were used for the observation of their growth and yield potential. Strains were kindly obtained from Agricultural Microbiology Dept., Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt.

2.1.1. Wheat grain and rice straw were kindly obtained from Filed Crops Institute, ARC, Giza, Egypt. Rice straw analysis is presented in Table (1)

Table 1: Chemical analysis in rice straw

Organic matter %	Organic carbon %	Ash %	Carbon Nitrogen Ratio	Total Nitrogen %	Total Phosphorus %	Total Potassium %	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
80.18	46.5	19.82	95 :1	0.49	0.52	0.77	722	75	220	61

2.1.2 Mother culture has completely good characteristic (size and shape) from Pleurotus spp. It was 24 hours old on petri dish contained potato dextrose media to produce white fungimycelium.

2.3. Spawn preparation: mother culture was inoculated on wheat grain after incubation period.

2.3.1. Compost and compost tea (CT) Preparation: compost was prepared from mixture of plant and animal wastes (1:1) for three months until mature phase. Compost tea was prepared from compost completely submerged the respective inside cotton bags with ratio of 1:5 and soaked for 10 days with aerated system and then amended with 0.5 % (v/v) molasses.

2.3.2. Vermicompost and vermicompost tea (VT) preparation: vermicompost was prepared from previous compost after finishing the thermophilic phase using *Eisenia fetida* worms for two months. Vermicompost tea was prepared according the method described by Shrestha *et al.,* (2013).

2.4. Media preparation:

2.4.1. Compost tea agar (CTA) and vermicompost tea agar (VTA) media: twenty gram agar (20g/L) were added to 250ml of CT or VT in Erlenmeyer flasks to prepare CTA or VTA media. The pH was adjusted at pH 7 by diluted potassium hydroxide to the nutrient broth in both media then sterilized in autoclave 121^{o}C, for 20 minutes, (Stamets, 2005).

2.4.2. Potato dextrose agar media (PDA): Potato Infusion of 200 g, Dextrose 20g and agar 15g/L, pH was adjusted to 5.6 ± 0.2 at 25°C. 39 g of the medium were suspend in one liter of purified water, frequently agitated and boiled for one minute to completely dissolve, the medium was sterilized in autoclave at 121°C for 15 minutes (Vanderzant and Splittstoesser, 1992).

2.4.3. Liquid media inoculation: The liquid media of Potato dextrose (PD), vermicompost tea (VT) and compost tea (CT) were inoculated with one disk from the primary grown mycelium to get fungi spawn.

2.5. Procedures

Good characterized (size and shape) of Pleurotus spp. (24 hours old), surface was washed with sodium hypochlorite (0.5%) for 5 minutes (Booth 1971), submerged in distilled water for two minutes to remove the residue of sterilized solution and dry by filter paper (Stamets and Chilton, 1983). The cap was removed and the fungi stem was vertical cut off to cubic pieces by sterilized gift then submerged in sterilize central petri dish contents PDA media (Oei, 2003), incubated at 25C^{o} for 5 days, ten petri dishes were replicated for each strain. After fishing the incubation period, white fungi mycelium was appeared and measured its growth period, the fast growth called mother culture. Mother culture was cut down in to disks by cork borer, then inoculated one disk to each solid media CTA, VTA and PDA. Cooked wheat grain, compost and vermicompost of each solid media included five plastic bags and three replicate of each contented 500grams of solid media. The liquid media (PD, VT and CT) were inoculated with one disk from primary mycelium to get fungi spawn. Each treatment has five flasks (1000 ml) with three replicates. Both solid and liquid media were incubated at 25C^{o}. To produce spawn, wheat grain soaked in water for 24 hours for cleaning and removing broken and damaged seeds then submerged in boiling water for ten minutes, filtered and left to dry until the moisture content reached to 48-50% and fifteen gram of calcium carbonate, 10gram hydrate calcium sulfate were added to one kilogram of seeds on the bases of fresh weight and good mixed then. 400g of the transported into poly ethylene bags closed and sterilized in autoclave at 121C^{o} for 45 minutes. After sterilization each bag was inoculated with one disk (5Mm) from the previously prepared mother culture and incubated at 25C^{o} in darkness (Wayne, 2001). Spawn bag was stored at 4C^{o} until using (Zadrazil, 1976).Previous spawn grown on CT, VT, PD media was cultivate on rice straw which previous by serialized by steam to evaluated end product.

Treatments

- *Pleurotusostreatus* grows on compost tea cultivated on rice straw media
- *Pleurotus ostreatus* grows on vermicompost tea cultivated on rice straw media.
- *Pleurotus ostreatus* grows on potato dextrose cultivated on rice straw media.
- *Pleurotus cystidiosus* grows on compost tea cultivated on rice straw media.
- *Pleurotus cystidiosus* grows on vermicompost tea cultivated on rice straw media.
- *Pleurotus cystidiosus* grows on potato dextrose cultivated on rice straw media.
- Spawn growing on wheat grain seed cultivated on rice straw media (control).

2.6. Measurements

Physico-chemical determinations of compost and vermicompost and their tea were analyzed to determine the extent of stabilization according method of AOAC (2000).

Organic matter and organic carbon were determined after igniting of sample in a muffle furnace at 550°c for 60 minutes (Nelson and Sommers, 1996). The method described by John (1970) was used for measuring total phosphorus using spectrophotometer (Junway 2202) and total potassium by using flame photometer-128 after digesting the samples. Microbiological assays were run according to Difico manual (1985). Parasitics were determined according to Jirillo *et al*. (2014), seed germination percentage

was estimated according to Yu *et al.* (2010) and nematodes were examined as mentioned by to Lenore *et al.* (1999). Humification parameters for assessment of organic matter stabilization in compost were also calculated:

- Humification Rate (HR) % = C (HA + FA)/ TOC x100, humification index (HI) = TC extract-C (HA +FA) /C (HA + FA) and humification degree (HD) % = C (HA + FA) / TC extract x100 (Ciavatta *et al*., 1990).
- Growth speed rate = Colony diameter (mm) / Number of days from the start of growth.
- Pleurotus mushroom samples were determined for chemical composition asused by AOAC methods (AOAC, 1995).
- Protein content was determined according to the method of Leco Manuel (thermal conductivity) by the Kjeldahl method. The nitrogen factor used for protein calculation was total nitrogen x 6.25. (Ezeibekwe *et al*., 2009).
- Total Energy, fat and carbohydrate levels were determined by the method of Watt and Merrill, (1975).
- Total energy calculated as the following equation:
- Total Energy (kcal/100g) =4×Protein+4×Carbohydrate+9×Fat.
- The mushroom yield was calculated according to Morais *et al.*, (2000), using the equation: Mushroom yield = (Weight of fresh mushroom harvested (g) per fresh substrate weight).
- Biological efficiency percentage (BE %) was determined according to yang *et al*., (2013), Stamets, (2000) and Royse *et al.,* (2004), where, Biological efficiency % = weight of fresh mushrooms harvested per bag / weight of dry substrate per bag × 100.

2.7. Statistical analysis:

The randomized complete block design was used. The data were subjected to analysis of variance (ANOVA) using WASP statistical analysis software. The treatment means were separated using a Fisher's least significant difference (LSD) test. All analyses were conducted at a significance value of $P \leq 0.05$ (Snedecor and Cochran, 1980.).

3. Results and Discussion

Compost and vermicompost are suitable for tea extract in which, their maturation degree reached to high content of organic matter (34.82 and 38.98% respectively). Both compost and Vermicompost were free of pathogenic bacteria, parasitic, nematode and weed seeds. Vermicompost was more effecting in maturation and germination percentage (83%), their content of humic substances, humic and fulvic acids were more than compost. The humifiction parameters in vermicompost was more higher than compost where, HI was 0.85, humification rate 59% and humification degree 61% comprised with compost (1.1, 52 and 49.5% respectively) (Table 2).

Such trend of growth on mature compost was obtained by Afifi, *et al.* (2012) in which, the compost is mature when HI less than 1 and HR and HD% more than 50%.

Data in table (3) clears the overall results of biochemical composition analysis for both compost and vermicompost tea where, pH and electric conductivity were higher in compost tea than vermicompost tea. On the other hand, organic matter (12.6%) in vermicompost tea was higher than compost tea as well as the content of total nitrogen, phosphorus and potassium. Proportion of total coliform, fecal coliform and salmonella and shigella bacteria were not detected either in compost or vermicompost tea.This meansthat raw compostmaterials and vermicompost are well manufactured andreached to their maturity.

The quality of compost or vermicompost tea is depended on microbial food sources, compost to water ratio, levels of aeration, degree of maturation, compost age, duration of incubation, and the quality of water used. It seems also that a good, compost tea compost tea depends on sugar or molasses to be commonly used as amendments for compost or vermicompost tea to increase its quality (Ingham, 2005).

Table 2: Physical, chemical and biological analysis of compost and vermicompost.

Type of analysis	Compost	Vermi.		Compost	Vermi.
Physical and chemical analysis					
Density (kg/ m^3)	536	400	Total nitrogen (%)	1.3	1.6
Moisture content (%)	18	27	Organic matter (%)	34.82	38.98
Dry matter (%)	82	73	Organic carbon (%)	20.19	22.61
pH (1:10)	7.5	7.2	Ash (%)	65.18	61.02
EC dS/m (1:10)	5.2	3.7	C/N ratio	16: 1	14 :1
Ammonia (ppm)	90	58	Total phosphorus (%)	0.69	1.2
Nitrate (ppm)	277	402	Total potassium (%)	0.58	0.89
Biological analysis					
Total bacterial count (cfu/g x10^6)	60	120	Total coliform (cfu/g x10^3)	nd	nd
Total actinomycets (cfu/g x10^4)	12	10	Faecal coliform (10^3)	nd	nd
Total fungi (cfu/g x10^4)	9	13	Salmonella & Shigella (cfu/gx10^3)	nd	nd
Nematode (larva/200g)	nd	nd	Weed Seeds	nd	nd
Parasitic	nd	nd	Germination percentage	78	83
Humifiation parameters					
Humic index (HI)	1.1	0.85	Humic substances%	19	27
Humification rate % (HR)	52	59	Humic acid %	10.5	18.6
Humification degree % (HD)	49.5	61	Fulvic acid %	8.1	8.2

Vermini. : Vermicompost; nd: not detected; C/N: Carbon / Nitrogen ratio; cfu: colony forming unit

Table 3: Chemical and biological determinations on compost and vermicompost tea

Type of analysis	Compost tea	Vermicompost tea
Chemical analysis		
pH	6.96	8.05
EC(dS/m)	4.96	2.70
Organic matter (%)	8.8	12.6
Organic-Carbon (%)	5.14	7.3
N-NH^+_4 (ppm)	93	8.0
N-NO^-_3 (ppm)	115	36
Total-N(ppm)	369	810
Available-P(ppm)	88	11200
Available-K(ppm)	118	13200
Biological determination (cfu/ml)		
Total coliform	nd	nd
Fecal coliform	nd	nd
Salmonella and shigella	nd	nd

nd: not detected. organic matter and total nitrogen determined as dry basis.

Comparing the growth weight (mycelium) (Table. 4) *Pleurotus ostreatus* was greater than *Pleurotus cystidiosus* in all treatments. Vermicompost tea agar media has as an significant value compared to other media where, it obtained 23.7 and 13.3g with both species after incubation periods (21 days) respectively, Meanwhile, it revealed that the lowest effect of colony diameter (4.9 mm) with *Pleurotus ostreatus*. On the other hand, CTA media presented the lowest weight with *Pleurotus ostreatus* (8.3g), whereas, PDA in media was not the same value. Also, the growth of *Pleurotus cystidiosus* species (7.7g) was not pronounced in PDA medium. The spread of colonies (colony diameter) of *Pleurotus cystidiosus* on petri-dishes in PDA media produced for growth gave only 8.2g, while CTA media was the lowest (6.7g). The result in Tab. 4 revealed that the growth speed rate with both *Pleurotus ostreatus* and *Pleurotus cystidiosus* has high rate in PDA media (0.38 and 0.39 respectively).

Figures 1 and 2 showed that both pleurotus species were well grown in differentliquid media (potato dextrose, vermicompost tea and compost tea) before the propagation of two types on rice straw.

Table 4: Weight, colony diameter and growth spread rate for two species of pleurotus after the incubation period (21 days).

Mean ± SE	*Pleurotus ostreatus*			*Pleurotus cystidiosus*		
Types of media	Weight (g)	Colony Diameter (Mm)	Growth Speed Rate	Weight (g)	Colony Diameter (mm)	Growth Speed Rate
Potato dextrose Agar	13.6b	7.9a	0.38	7.7c	8.2a	0.39
Vermicompost tea agar	23.7a	4.9c	0.23	13.3a	7.9ab	0.38
Compost tea agar	8.3c	7.3ab	0.35	8b	6.7c	0.32
LSD (0.05)	0.501	0.227		0.2978	0.2599	

Means followed by the same letter (s) within a column in each block are not significantly different (P≤ 0.05).

Potato dextrose **Vermicompost tea** **Compost tea**

Fig. 1: Show *Pleurotus ostreatus* grows on different liquid media.

Potato dextrose **Vermicompost tea** **Compost tea**

Fig 2: Show *Pleurotus cystidiosu* grows on different liquid media.

Various liquid media inoculated on rice straw as traditional media to evaluate the best growth for pleurouts production. The production was achieved from numbers of picks (four picks). The first pick of *Pleurouts cystidiosus* was after 21 days of incubation period on rice straw media also fifteen days were followed between each pick (second, third and fourth pick). In these respect *Pleurotusostreatus* gave total yield more than *Plourotus cystidiosus* with all media, vermicompost tea media was more superior for all treatments where, achieved 9.95 Kg more than control. Moreover, the *Pleurotus ostreatus* which has grown on wheat seeds was in the second rank (8.6 Kg). Meanwhile, compost tea of the third rank obtained 6.75 Kg, while, *Pleurotus ostreatus* which grown on potato dextrose media, produced about (5Kg) (Table 5) and figure (3).

Table 5: Weight (Kg) of *Pleurotus ostreatus* during incubation period (3 months)

Numbers of picks Types of media	Pick (1)	Pick (2)	Pick (3)	Pick (4)	Total weights
Wheat seeds (control)	2.25	2.5	2.3	1.5	8.55
Vermicompost tea	2.5	2.6	2.6	2.25	9.95
Compost tea	2	2	1.5	1.25	6.75
Potato dextrose	1.7	1.5	1.3	0.5	5

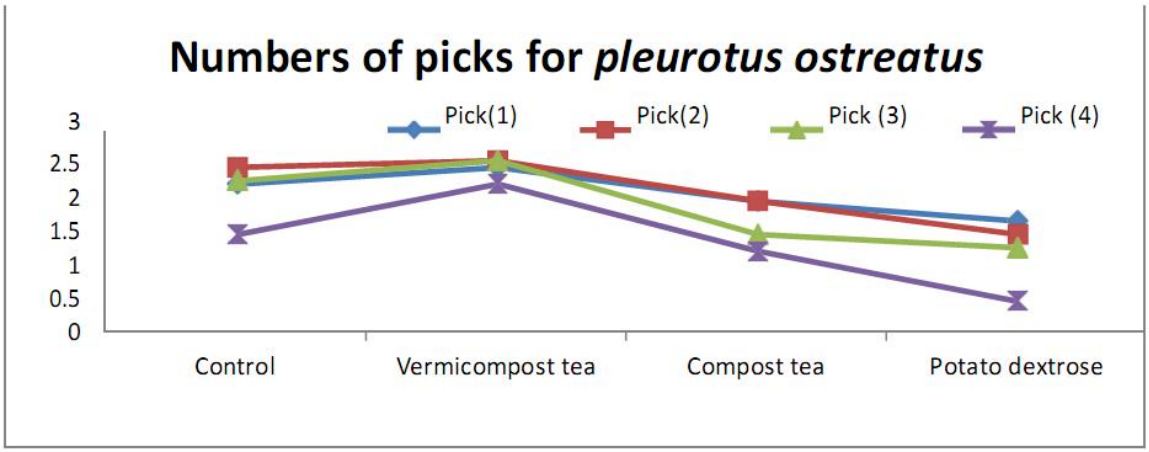

Fig. 3: Number of picks for *pleurotus ostreatus*

It worth to mention, that the period of picks continues from three to four days from the beginning to get the primary fruits until all growth was picked. Also, it should be noted that, the *Plourotus cystidiosus* which has grown on vermicompost tea media was successfully proportioned than other treatments in total picks after three months (7.5 Kg).The content of organic matter, total nitrogen, phosphorus and potassium in vermicompost tea media were greater than the other media. Meanwhile, the growth of *Pleurotus cystidiosus* has on wheat seed media (control) followed by vermicompost tea media where the total weight reached to7kg. On the other hand, the pleurouts which grown on potato dextrose produced only 3.5kg (Table (6) and figure (4).

Table 6: Weight (Kg) of *Pleurotus cystidiosus* during incubation period (3 months)

Number of picks Types of media	Pick (1)	Pick (2)	Pick (3)	Pick (4)	Total weights
Wheat seeds (control)	2.5	2.5	1.5	0.5	7.0
Vermicompost tea	2.25	2.0	2.0	1.25	7.5
Compost tea	1.5	1.5	1.0	0.5	4.5
Potato dextrose	1.5	1	1	--	3.5

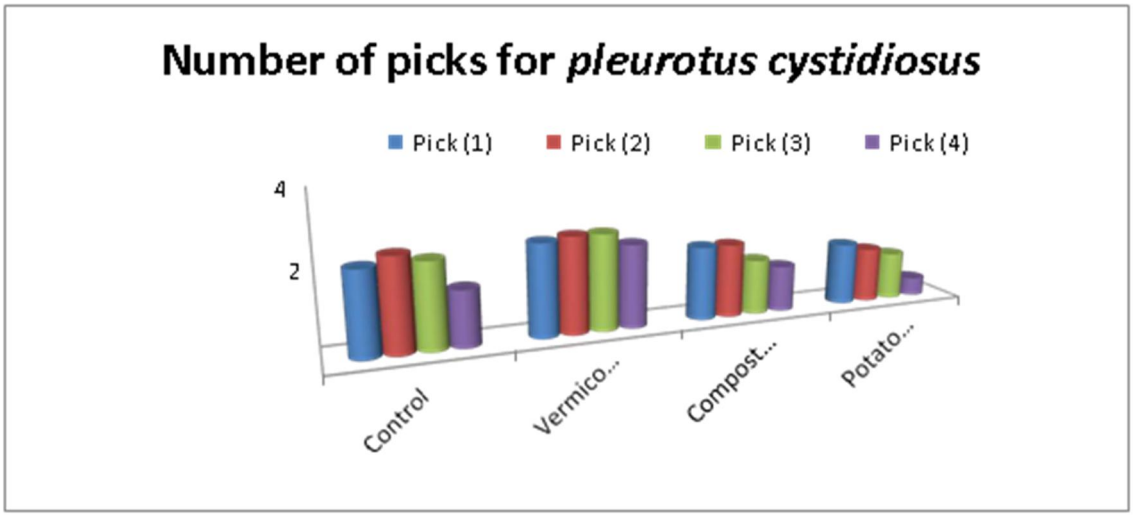

Fig. 4: Numbers of picks for *pleurotus cystidiosus*

Generally, the result revealed that mushroom weight of two species (*pleurotus cystidiosus* and *ostreatus* grown on various substrate types was significantly different. It is obvious to note that the Pleurotus species which grown on vermicompost with cotton waste and soil has the lowest yield and poor quality of mushroom. (Yadav*et al*., 2017).

Spawn production (Tab. 7) of *pleurotus ostreatus* cultivated in VT on rice straw achieved the

highest content of total protein, fat and ash 27.3, 2.3 and 6.8 5 respectively. This may be due to the high content of different macro elements as well as organic matter was in vermicompost with compost tea. While spawn which grown on wheat seed media (control) contained more total carbohydrates and energy (55.3 and 312.9 kcal/100 g respectively). Such results were also found when different species of mushroom were grown on various media (NurAziera *et al.,* 2015). In these respect, different biochemical values were overall trend to produce low carbohydrate content.

Table 7: Effect of different substrate formulas on nutritional composition of *Pleurotus ostreatus* mushroom.

Type of media	Macronutrients (%)			Total (%)				
	Nitrogen	phosphorus	Potassium	Carbohydrate	Proteins	Fat	Ash	Energy (kcal/100 g)
Wheat seeds	2.9	0.95	1.5	55.3	18.65	1.9	5.3	312.9
Compost tea	4.0	1.5	2.3	32.4	25.30	2.1	6.2	249.7
Vermicompost tea	4.4	1.9	2.8	39.2	27.30	2.3	6.8	286.7
Potato dextrose	3.4	1.3	2.1	33.5	21.3	2.0	5.8	237.2

Total yield and biological efficiency (BE) were used to measure the efficiency of mushroom (*Pleurotus cystidiosus* and *Pleurotus ostreatus*) to produce their yield from the given substrate (Tajdeen *et al.,* 2012). Results in table (8) reveled that *Pleurotus ostreatus* grown on vermicompost tea media gave higher yield of mushroom and BE % than other treatments and the wheat seeds (control) where it achieved 9950 g and 156.25 % respectively. The results indicated also that potato dextrose media produced low total yield and BE% (5000g and 72.91% respectively).

Table 8: Total yield and biological efficiency (BE) of *Pleurotus ostreatus* on different media.

Types of media	Total yield of mushrooms (g)	Biological efficiency (BE %)
wheat seeds (control)	8550	145.83
Vermicompost tea	9950	156.25
Compost tea	6750	93.75
Potato dextrose	5000	72.91

The growth of *Pleurotus cystidiosus* on vermicompost tea media was more pronounced in total yield (8200g) and biological efficiency (150%) compared with other treatments as well as the control treatment (Table 9). Similar results were obtained by Obodai and Vowotor (2003), but higher than some other report, which has also relatively well yields and BE (Zhang *et al.,* 2002).

T**able 9:** Total yield of and biological efficiency of *Pleurotus cystidiosus* on different media

Types of media	Total yield of mushrooms (g)	Biological efficiency (BE %)
Wheat seeds (control)	7440	122.70
Vermicompost tea	8200	150
Compost tea	6750	90.62
Potato dextrose	4500	59.20

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