Evaluation of some locally available casing materials on production of two *Agaricus* (LANGE) strains

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ABSTRACT

Six different casing materials were investigated in this study (1) peat moss (100, control), (2) peat moss + spent mushroom (3:1), (3) peat moss + vermi-compost (3:1), (4) peat moss + spent mushroom + palm fronds (2:1:1), (5) peat moss + palm fronds (1:1) and (6) peat moss + vermicompost + palm fronds (2:1:1), on production and harvest quality of two *Agaricus bisporus* strains (S¹ and S²). These experiments were conducted at Tanta Mushroom Station, Gharbia Governorate in 2019. The results showed that the type of casing material had a significant effect on the yield potential and growth behavior of mushroom. Among the tested Casing materials, the best casing layer was the mixture of (peat moss + palm fronds + vermicompost) with both (S¹) and (S²) strains which exhibited in pinhead initiation (28 and 29.5 days); growth period of the three harvests (42 and 43 days) and total weight (1806.5 and 2542.5 g), respectively, compared to control (29 and 30 days, 43 and 43.5 days and 1467.5 and 1507 g, respectively). Total phenolic contents of mushroom insignificantly differed between casing layers with strain (S¹), while it was very clear with strain (S²) where the casing layer that composed of peat moss + vermin (3:1) recorded the highest content (252.93mg/100g). Flavonoid content was higher in case of peat moss + fronds (1:1) with both strains. The highest antioxidant activity in both strains was achieved when peat moss 100% was used as acasing layer.

Keywords: *Agaricus bisporus*, casing materials, flavonoid, phenols, antioxidants and yield potential.

1. Introduction

The white button mushroom (*Agaricus bisporus* Lange) is the extensively cultivated and consumed mushroom throughout the world and represented about 56% of total world mushroom production (Yadav et al., 2017). Mushrooms’ market is experiencing a big increase around the world, but the largest expansion is in China, which accounts for more than 85% of the total worldwide production amounting to 34 million tons produced in 2013; the annual crop income was 3.2 billions dollars worldwide in 2009 and is now more than 4 billion dollars (Sonnenberg et al., 2017). Huge quantities of farm yard manure, vermincompost, saw dust and other organic wastes are generated annually through the activities of agricultural, forest and food processing industries. Egypt is ranked third in the Middle East when it comes to the availability of agriculture palm fronds waste that amounts 71.5 million tons (dried weight) where only 10% is reused in traditional uses like cages and furniture, while the rest (90%) are burned every year. This causes environmental pollution and is a waste of sustainable materials (that include highly recyclable contents, rapidly renewable and biodegradable products) and local resources (Eldeeb 2017). The casing materials of mushroom provide suitable physical, chemical and biological conditions that stimulate initiation of fruiting body formation (Gerrits, 1974). Casing is the part of cultivation of white button mushroom and milky mushroom for fruit body formation. The casing layer is applied after competition of spawn running in mushroom beds. This casing layer prepared from different materials such as loam soil, farm yard manure, vermicompost, sandy soil, cotton waste, saw dust and jute coir pith, for its unique water holding and structural properties. Hence, mushroom cultivation can reduce the
environmental pollution by recycling the agricultural wastes. According to Kaur and Rampal, 2017, the highest total yield of *A. bisporus* (1066.97 g) was obtained from casing mixture, cocopeat + rice husk + formaline + red soil and the lowest yield (607.93 g) was obtained with farmyard manure + sandy soil + formaline. The second highest yield (646.57 g) was obtained from farmyard manure + sandy soil + rice husk + formaline casing mixture. Evaluation of seven different casing mixtures prepared from 5 materials, farm yard manure (FYM), spent compost, vermicompost (VC), coir pith and press mud, for their yield potential and the fruiting body of button mushroom, *Agaricus bisporus* was carried out by Bhatt et al. (2006) and Dhar et al., (2006). They found that FYM, VC were the best casing layers. The good quality of casing soil can be enhanced the yield of mushrooms via quantitative and qualitative attributes of mushroom such as number of fruit bodies, weight, and size of fruiting bodies. Therefore, the present investigation tested some casing materials prepared locally from available wastes for enhancing production of white button mushroom and its effect on some pharmaceutical substances content.

2. Materials and Methods

2.1. Mushroom strains

Two strains of button mushroom (*Agaricus bisporus*) were used in this study for growth and yield potential. S-1 strain of the hybrid Sylvan A15 was kindly provided from Microbiological Resources Centre (Cairo Mircen) Ain shams University, kalubia, Egypt whereas S-2 strain was developed by the Environment and Bio-Agriculture team on Petri dishes by hyphal fusion (anastomosis) method. The developed strain was cultured on fresh Potato Dextrose Agar (PDA) medium and maintained by periodic sub-culturing with incubation at 25°C for 15 days and kept thereafter in a refrigerator at 2 to 5°C for the following studies.

2.2. Compost preparation

The compost was prepared by long composting method which took 28-30 days and 8 turning (Mantel et al., 1972). The compost contained wheat straw only (1000 Kg) in addition to required nitrogen supplements as poultry manure (800 Kg); soybean flour (50 Kg) and urea (6 Kg), beside gypsum (80 Kg) to aid in pH control. The straw is thoroughly watered on clean concrete floor for 24 hr. and mixed with fertilizer separately. The substrate prepared was formed into a large heap of 5 feet width and 7 feet height to encourage intense microbial activities. Turning was carried out every 3-4 days after adding water to maintain the moisture around 75% to keep aerobic conditions. Gypsum is added at the 3rd turning and the compost was ready for spawning after the 8th turning. More turnings, if the compost heap was not free from ammonia were considered until it is completely ammonia free. polyetherin in box (Length 50× width 30× Height 10cm) that contained 10 kg of wheat straw compost were seeded with 75 g mushroom seeds and inoculated with *Pseudomonas putida* as a bio-fertilizer at a rate of 15 ml (that contained 1x10⁸ cfu) and mixed gently in compost under sterilized conditions (Kapoor, 2004).

2.3. Spawn preparation

The spawn was prepared using sorghum (*Sorghum bicolor*) grains. The grains were washed and then soaked in water for 24 hours for maximum absorption of water and then boiled for 30 minutes as excessive moisture was removed using blotting paper. Three replicates of polyethylene bags (12 x 25 cm) each contained 500 g of sorghum grains were separately prepared for each treatment. The grains were supplemented with 4% gypsum (CaSO₄) and 2% lime (CaOH₂) and then the polyethylene bags were sterilized at 121 °C for 30 min. Following sterilization, each polyethylene bag was inoculated under sterilized conditions with mycelial disc (9 mm diameter) of either S-1 or S-2 strains. The sealed inoculated bags were incubated at 25°C for 20 days (Ishaq et al., 2017).

2.4. Procedure

Six different casing materials (treamnts) were prepared in different ratios and used as a coat soil, (1) control, 100% peat moss ; (2) peat moss + spent mushroom (3:1); (3) peat moss + vermicompost (3:1); (4) peat moss + spent mushroom + vermicompost (2:1:1); (5) peat moss + palm fronds (1:1) and
(6) peat moss + palm fronds + vermicompost (2:1:1). The pH was adjusted at 7.0 by adding lime stone (30 kg per cubic meter of casing layer). The casing layer is moistened to 60% and left to rest for 20 days. Then 30 % wood charcoal (1-2 cm) was added and pasteurized at 62 °C for 8 hours. The casing layer was spread on the surface of the compost by hand. Each polyesterin box (10 Kg capacity) received 250 g casing material that formed a layer of about 4 cm thickness. The medium was covered with plastic and incubated for 15 days at 28 ± 1 °C. Each treatment was treplicated. After colonizing the cover soil, the boxes were randomly arranged in shelves within a greenhouse with a semi-controlled environment, micro-sprinklers with a flow rate of 7 L/hr were used to control the relative humidity between 60-90%. The temperature ranged from 20 to 34 °C. White button mushrooms were harvested on the basis of maturity rather than size. In general, button mushroom was harvested at a stage when cap diameter is twice the length of stipe (Kohli, 1984). Mushroom fruits were have a very kept on refrigerator lower shelves unit used to study the different morphological characteristic. Total yield was the sum of 1st, 2nd and 3rd flushes.

**Determination of total phenolics and, flavonoids contents and antioxidant activity**

I. **Extract preparation**

One gram dry matter of mushroom was mixed with 15 ml of 70 % methanol and stored at room temperature. After 48 h, infusions were filtered through Whatman No. 1 filter paper (Singleton et al., 1999) as the extract was received in 100 mL Erlenmeyer Flasks.

II. **Determination of total phenolic content**

Total phenolic content (mg/100 g) of each treatment were determined in the representative dry mushroom samples according to Singleton et al., (1999) where the reaction mixture was prepared by mixing 0.5 ml of methanolic extract, 2.5 ml of 10% Folin-Ciocalteu’s reagent dissolved in water and 2.5 ml of 7.5% Na₂CO₃. Blank was concomitantly prepared by replacing the methanolic extract by methanol. The samples were thereafter incubated at room temperature for 45 min. The absorbance was determined using spectrophotometer at λ max = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in the extracts was expressed in terms of gallic acid equivalent (mg of GA/g of mushroom).

III. **Determination of flavonoids content**

Flavonoids content (mg/100g) of each treatment were determined in representative dry mushroom samples according to Quettier et al., (2000) as the reaction mixture was prepared by mixing 1 ml of methanolic extract and 1 ml of 2% AlCl₃ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λ max= 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution rutin and the calibration line was construed. Based on the measured absorbance, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU/g of mushroom).

IV. **Antioxidant activity**

The ability of the plant extract to scavenge 1,1-Diphenyl-2-picrylhydrazyl (DPPH), free radicals was assessed by the standard method (Tekao et al., 1994), adopted with suitable modifications (Kumarasamy et al., 2007) as DPPH was prepared by dissolving 10 mg sample in 100 ml 70% methanol. A known volume (50 µl) of the methanolic extract was dissolved in 450 µl 70% methanol then mixed with DPPH (0.5 ml). The mixtures were well shaken and then placed in darkness at room temperature for 30 min. The absorbance was recorded at 517 nm. The control samples contained all the reagents except the extract. Methanol was used to zero the spectrophotometer. The inhibition percentage of the DPPH radical was calculated according to the formula:

\[ I\% = \left(\frac{(AB - AS)}{AB}\right) \times 100 \]  

(1)
Where I = DPPH inhibition %, AB = absorbance of control sample and AS = absorbance of a tested sample at the end of the reaction. Each assay was carried out in triplicate.

2.5. Statistical analyzed
The data were analyzed using SPSS version 14 software. A one-way analysis of variance (ANOVA) was used to test for significance of variation in yield and yield attributes of casing media on different flushes. Means were compared using Duncan test, when F-test from ANOVA was significant at p<0.05.

3. Results and Discussion
The casing layer influences yield, quality and uniformity of cropping of the button mushroom. Thus, mushroom productivity, size and mass are directly affected by the casing layer. The casing soil is reported to possess certain physical, chemical and microbiological properties having stimulatory role in Agaricus fruiting (Ahlawat 2002). Shrivastava (2012) stated that any organic material with a high water holding capacity is suitable as a casing material, such as peat moss and perlite mixture (Ergun et al., 2007); farm yard manure, spent mushroom substrate and vermi compost (Choudhary, 2011) and garden loam soil, farm yard manure, waste tea and vermicompost (Kumar et al., 2018).

3.1. Effect of casing materials on growth period, weight and shelf-life
Pinhead initiation, growth period of the three harvests, total mushroom weight and shelf-life period of (S-1) and (S-2) strains are presented in Tables (1) and (2), respectively. The present findings indicated that, the best casing layer for (S-1) and (S-2) strains was peat moss + palm fronds + vermicompost which exhibited for pinhead initiation (28 and 29 days); growth period of the 3rd harvests (42.5 and 39 days) and total weight (2466.5 and 2542.5 g), respectively, compared to control (29 and 30 days, 43 and 43.5 days and 1467.5 and 1507 g, respectively). As regard to shelf-life period, (S-1) and (S-2) fruits grown on (peat moss + palm fronds + vermicompost) treatment remained valid for a longer time (5 and 4.5 days as fresh fruits and 13 and 9 days when refrigerated, respectively) compared to other treatments. The lowest mushroom total weight was recorded when (peat moss + vermicompost) was used as (S-1) and (S-2) recorded 1375.5 and 1302 g, respectively. Highly significant differences were observed between all treatments in the pinhead initiation, growth period of the three harvests, total weight and shelf-life period of the two strains. Only the differences between treatments for pinhead initiation and growth period of the first harvest of strain (S-1) were insignificant. The casing mixture of peat moss + palm fronds + vermicompost produced higher total yields than other treatments due to improving the aeration palm fronds a result of addition to peat moss + vermicompost. Vermicompost has high water holding capacity as its moisture holding capacity is such that it can be watered without sealing off the compost. Carbon dioxide (and other gases), formed in the compost during spawn running and fruiting, must be able to escape through the casing. Effects of high carbon dioxide concentration include production of small caps and elongation of stipe. The low yields recorded in peat moss + vermicompost, may be attributed not only to its inability to hold sufficient moisture but also the rapid rate at which it loses it by infiltration and evaporation. Water instead of just wetting the casing, may have been draining down into the compost thereby causing sogginess, which interferes with mycelia development and performance. Similar results are recorded by Chandra et al., (2014) that revealed that casing mixture CCP + VC + FYM + SD + Sand recorded the highest yield (320 g) whereas CCP + FYM ( 250 g) showed lowest yield in the harvesting of second flush. The highest total yield (1112.26 g) was obtained from casing mixture, CCP + VC + FYM + SD + Sand and lowest yield (736.67g) from CCP + FYM. Casing mixture of CCP + FYM + SD recorded second highest yield (1033.67 g). Singh et al., (2000) found that casing mixture prepared using FYM + spent compost (2:1) performed the best giving a yield of Agaricus bisporus in the first and second crop (18.83 kg and 17.21 kg/ quintal compost).

3.2. Effect of casing materials on total fruits number
The average maximum total number of mushroom fruits of strain (S-1) was achieved when the treatment covered with peat moss + palm fronds + vermicompost mixture recording 93 fruits while it recorded 70 fruits when the treatment covered with peat moss + vermicompost as presented in Table (3). Number of mushroom fruits in the control treatment was 81.5. The average maximum number of
Table 1: Effect of casing materials on growth period, weight and shelf life of *A. bisporus* (S-1) strain.

| Casing media                           | Growth period in days after spawning (Mean±SE) | Weight (g) (Mean±SE) | Total Weight (g) | Shelf life (days) (Mean±SE) |
|----------------------------------------|-----------------------------------------------|----------------------|------------------|---------------------------|
|                                        | Pinhead initiation | 1st Harv. | 2nd Harv. | 3rd Harv. | 1st Harv. | 2nd Harv. | 3rd Harv. | Fresh air | Refrig. |
| 100% Peat moss (Control)               | 29.0 ±0.87<sup>ab</sup> | 33.50 ±0.29<sup>a</sup> | 36.5 ±0.29<sup>c</sup> | 43.0 ±0.29<sup>c</sup> | 539.5 ±83.42<sup>b</sup> | 503.0 ±15.59<sup>bc</sup> | 425.0 ±15.59<sup>c</sup> | 1467.5 | 3.00 ±0.00<sup>d</sup><sup>cd</sup> | 8.50 ±0.29<sup>bc</sup> |
| 75% Peat moss + 25% Spent mushroom     | 30.5 ±0.29<sup>ab</sup> | 33.50 ±0.29<sup>a</sup> | 39.5 ±0.29<sup>ab</sup> | 45.5 ±0.29<sup>ab</sup> | 537.5 ±99.59<sup>b</sup> | 611.0 ±45.61<sup>b</sup> | 553.50 ±6.64<sup>cd</sup> | 1702.0 | 2.50 ±0.87<sup>d</sup> | 9.50 ±0.29<sup>b</sup> |
| 75% Peat moss + 25% Vermi              | 30.0 ±0.58<sup>ab</sup> | 33.0 ±0.29<sup>a</sup> | 38.0 ±0.58<sup>cd</sup> | 44.5 ±0.29<sup>b</sup> | 379.0 ±12.12<sup>bc</sup> | 504.0 ±34.01<sup>bc</sup> | 492.5 ±63.79<sup>de</sup> | 1375.5 | 4.00 ±0.58<sup>abc</sup> | 9.50 ±0.29<sup>b</sup> |
| 50% Peat moss + 25% Spent mushroom + 25% Vermi | 31.0 ±0.58<sup>a</sup> | 33.5 ±0.29<sup>a</sup> | 38.5 ±0.58<sup>bc</sup> | 45.5 ±0.29<sup>b</sup> | 550.0 ±54.85<sup>b</sup> | 526.0 ±45.61<sup>bc</sup> | 677.0 ±39.25<sup>b</sup> | 1753.0 | 4.00 ±0.58<sup>abcd</sup> | 8.50 ±0.29<sup>b</sup> |
| 50% Peat moss + 50% fronds             | 30.0 ±0.58<sup>ab</sup> | 33.5 ±0.29<sup>a</sup> | 40.0 ±0.58<sup>a</sup> | 46.0 ±0.58<sup>a</sup> | 733.5 ±32.04<sup>a</sup> | 658.5 ±105.94<sup>ab</sup> | 599.50 ±26.84<sup>bc</sup> | 1991.5 | 4.50 ±0.29<sup>ab</sup> | 9.50 ±0.29<sup>b</sup> |
| 50% Peat moss + 25% fronds + 25% Vermi | 28.0 ±0.58<sup>c</sup> | 32.0 ±0.58<sup>a</sup> | 37.00 ±0.58<sup>de</sup> | 42.5 ±0.58<sup>a</sup> | 880.5 ±3.17<sup>a</sup> | 776.0 ±7.50<sup>a</sup> | 810.00 ±17.32<sup>a</sup> | 2466.5 | 5.00 ±0.58<sup>a</sup> | 13.00 ±0.00<sup>a</sup> |
| F. value                               | 1.885 | 2.100 | 17.200 | 32.500 | 10.960 | 6.476 | 16.219 | - | 4.591 | 424.500 |

-Sig. | .131 | .097 | .000 | .000 | .001 | .000 | - | .004 | .000 |

-Means with different letters in the same columns are significantly different (P<0.05) by Duncan multiple test.
Table 2: Effect of casing materials on growth period, weight and shelf life of *A. bisporus* (S-2) strain.

| Casing media                     | Growth period in days after spawning (Mean±SE) | Weight (g) (Mean±SE) | Total Weight (g) | Shelf life (days) (Mean±SE) |
|----------------------------------|-----------------------------------------------|----------------------|------------------|-----------------------------|
|                                  | Pinhead initiate | 1st Harv. | 2nd Harv. | 3rd Harv. | 1st Harv. | 2nd Harv. | 3rd Harv. | Fresh air | Refriger. |
| 100% Peat moss (Control)         | 30.00 ±0.29<sup>cd</sup> | 27.00 ±2.31<sup>b</sup> | 37.00 ±0.00<sup>bc</sup> | 43.50 ±0.29<sup>ab</sup> | 334.00 ±25.40<sup>g</sup> | 607.50 ±21.65<sup>b</sup> | 565.50 ±40.12<sup>bc</sup> | 1507.0 | 2.50 ±0.29<sup>b</sup> | 10.00 ±0.58<sup>b</sup> |
| 75% Peat moss + 25% Spent mushroom substrate | 30.00 ±0.58<sup>ab</sup> | 33.00 ±0.58<sup>a</sup> | 38.00 ±0.58<sup>ab</sup> | 44.00 ±0.58<sup>ab</sup> | 561.00 ±60.62<sup>de</sup> | 540.50 ±46.47<sup>b</sup> | 427.50 ±102.48<sup>c</sup> | 1529.0 | 3.50 ±0.29<sup>b</sup> | 9.500 ±0.29<sup>bc</sup> |
| 75% Peat moss + 25% Vermi        | 31.00 ±0.58<sup>a</sup> | 33.50 ±0.29<sup>a</sup> | 38.50 ±0.29<sup>a</sup> | 44.50 ±0.29<sup>a</sup> | 481.50 ±47.05<sup>bc</sup> | 397.50 ±1.44<sup>ad</sup> | 423.00 ±19.63<sup>e</sup> | 1302.0 | 5.00 ±0.00<sup>a</sup> | 11.500 ±0.29<sup>a</sup> |
| 50% Peat moss + 25% Spent mushroom + 25% Vermi | 29.50 ±0.29<sup>bc</sup> | 32.50 ±0.29<sup>a</sup> | 36.00 ±0.58<sup>cd</sup> | 42.50 ±0.29<sup>a</sup> | 383.00 ±34.64<sup>r</sup> | 481.50 ±33.77<sup>bc</sup> | 629.50 ±42.43<sup>b</sup> | 1494.0 | 2.50 ±0.29<sup>b</sup> | 8.500 ±0.29<sup>ed</sup> |
| 50% Peat moss + 50% fronds       | 29.50 ±0.29<sup>bc</sup> | 32.50 ±0.29<sup>a</sup> | 37.00 ±0.58<sup>bc</sup> | 43.00 ±0.29<sup>a</sup> | 698.00 ±23.09<sup>b</sup> | 498.50 ±89.78<sup>bc</sup> | 619.00 ±37.52<sup>b</sup> | 1806.5 | 2.50 ±0.29<sup>b</sup> | 8.00 ±0.58<sup>d</sup> |
| 50% Peat moss + 25% fronds + 25% Vermi | 29.00 ±0.58<sup>b</sup> | 31.00 ±0.58<sup>a</sup> | 34.00 ±0.58<sup>c</sup> | 39.00 ±0.58<sup>d</sup> | 900.50 ±33.19<sup>de</sup> | 823.00 ±17.32<sup>bc</sup> | 819.00 ±17.89<sup>bc</sup> | 2542.5 | 4.50 ±0.29<sup>a</sup> | 9.00 ±0.00<sup>cd</sup> |
| F. value                         | 5.000 | 5.421 | 11.056 | 15.952 | 23.825 | 16.696 | 8.311 | - | 9.889 | 8.800 |

**Sig.** | .003 | .002 | .000 | .000 | .000 | .000 | .000 | - | .000 | .000 |

-Means with different letters in the same columns are significantly different (P<0.05) by Duncan multiple test.
Table 3: Effect of casing materials on total fruits number of two A. bisporus tested strains.

| Casing media                                    | Strain-1 | Strain-2 |
|------------------------------------------------|----------|----------|
|                                                | 1st Flush | 2nd flush | 3rd flush | Total No. | 1st flush | 2nd flush | 3rd flush | Total No. |
| 100% Peat moss (Control)                       | 25.00 ±2.31<sup>bc</sup> | 27.50 ±1.44<sup>ab</sup> | 27.50 ±2.02<sup>abc</sup> | 81.5 | 28.50 ±0.87<sup>b</sup> | 28.50 ±0.87<sup>ab</sup> | 24.50 ±2.02<sup>bc</sup> | 80.0 |
| 75% Peat moss + 25% Spent mushroom             | 25.50 ±2.56<sup>bc</sup> | 23.50 ±0.87<sup>b</sup> | 28.00 ±2.31<sup>ab</sup> | 77.0 | 26.00 ±0.00<sup>c</sup> | 24.50 ±0.87<sup>bcd</sup> | 29.00 ±0.58<sup>ab</sup> | 79.5 |
| 75% Peat moss + 25% Vermi                      | 22.50 ±0.87<sup>a</sup> | 23.50 ±2.02<sup>b</sup> | 24.00 ±0.58<sup>bc</sup> | 70.0 | 29.00 ±0.58<sup>b</sup> | 26.00 ±1.73<sup>abc</sup> | 28.50 ±1.44<sup>abc</sup> | 83.5 |
| 50% Peat moss + 25% Spent mushroom + 25% Vermi | 24.00 ±0.58<sup>bc</sup> | 27.00 ±0.58<sup>ab</sup> | 23.50 ±0.29<sup>c</sup> | 74.5 | 25.00 ±1.15<sup>c</sup> | 21.50 ±0.87<sup>d</sup> | 27.00 ±0.58<sup>abc</sup> | 73.5 |
| 50% Peat moss + 50% fronds                    | 27.00 ±2.89<sup>bc</sup> | 25.50 ±0.29<sup>b</sup> | 27.50 ±0.29<sup>abc</sup> | 80.0 | 33.00 ±0.58<sup>a</sup> | 23.50 ±2.59<sup>cde</sup> | 29.50 ±2.02<sup>b</sup> | 86.0 |
| 50% Peat moss + 25% fronds + 25% Vermi        | 33.50 ±0.29<sup>a</sup> | 30.50 ±0.29<sup>a</sup> | 29.00 ±0.00<sup>a</sup> | 93.0 | 29.50 ±0.87<sup>b</sup> | 29.50 ±0.29<sup>a</sup> | 29.50 ±0.87<sup>a</sup> | 88.5 |

| F. value                                      | 4.116 | 4.504 | 2.516 | - | 30.884 | 5.500 | 2.602 | - |

| Sig.                                           | 0.007 | 0.004 | 0.054 | - | 0.000 | 0.001 | 0.048 | - |

Means with different letters in the same columns are significantly different (P<0.05) by Duncan multiple test
Table 4: Effect of casing materials on fruit weight of two *A. bisporus* tested strains.

| Casing media                          | Fruit weight (g) of *Agaricus bisporus* (Mean±SE) |
|---------------------------------------|----------------------------------------------------|
|                                       | 1st flush 2nd flush 3rd flush Total wt. 1st flush 2nd flush 3rd flush Total wt. |
| 100% Peat moss (Control)              | 19.20±0.94c 21.15±1.09b 22.10±0.97ab 62.8 21.70±0.50b 19.80±0.74a 21.30±0.50bc 62.8 |
| 75% Peat moss+ 25% Spent mushroom     | 21.55±0.38ab 21.50±0.45b 18.90±0.93c 61.9 18.20±0.63de 20.10±0.75b 20.55±0.42bc 58.8 |
| 75% Peat moss + 25% Vermi             | 20.80±0.70abc 18.40±0.73b 18.80±0.36c 58.0 20.15±1.05bcd 19.20±0.60a 19.95±0.78c 59.3 |
| 50% Peat moss + 25% Spent mushroom + 25% Vermi | 19.40±0.87bc 18.25±0.71c 19.85±0.77bc 57.5 19.60±0.44cde 12.73±4.15b 20.65±0.64bc 52.9 |
| 50% Peat moss + 50% fronds            | 20.15±0.58bc 19.95±1.07bc 19.10±0.52c 59.2 20.70±0.78bc 19.85±0.63bc 20.45±0.78bc 61.0 |
| 50% Peat moss + 25% fronds + 25% Vermi| 22.70±0.25a 24.30±0.45a 23.30±0.46a 70.3 24.00±0.18a 24.00±0.24a 24.45±0.25a 72.4 |
| F. value                              | 3.395 6.292 4.184 - 10.555 3.823 7.110 - |
| Sig.                                  | 0.017 0.001 0.006 - 0.000 0.010 0.000 - |

Means with different letters in the same columns are significantly different (P<0.05) by Duncan multiple test.
mushroom fruits of strain (S-2) was 88.5 in the treatment covered with peat moss + palm fronds + vermicompost while the control casing of strain (S-2) recorded 80 fruits. Both strains showed significant differences between treatments in the first and second flush while the differences between treatments at the third flush were insignificant.

3.3. Effect of casing materials on fruit weight of two *A. bisporus* tested strains.

Data presented in Table (4) showed that the maximum weight of mushroom fruits of strain (S-1) and (S-2) were 70.3 and 72.49 g, respectively, obtained in the treatment covered with peat moss + palm fronds + vermicompost, while the minimum weight was 57.5 and 52.9 g obtained in the treatment covered with peat moss + spent mushroom + vermicompost. When the casing of both strains was peat moss only, the average total weight gave 62.8 g. In general, weight of mushroom fruits in treatments covered with peat moss + palm fronds + vermicompost for both strains was significantly higher compared to other casing layers.

Kumar *et al.*, (2020) studied Physical parameters of casing materials on yield parameter of white button mushroom, they found that, fresh and dry matter were increased on fruiting body of mushroom with maximum in FYM + Soil + Sand (2:1:1), representing 1192.41 and 173.42 g, against 763.89 g and 82.8 g in case of control.

3.4. Effect of casing materials on stipe length and stipe breadth

Fruit stipe length of both strains was generally longer for the first harvest compared to those of the second and third harvests. However, it was noticed that breadth of stipe was decreased with increasing length (Tables 5 and 6). Fruit stipe length of (S-1) and (S-2) strains in treatment of peat moss + palm fronds + vermicompost was (4.61, 2.35 and 2.23 cm) and (3.47, 3.26 and 3.10 cm) in 1st, 2nd and 3rd flushes, respectively. Also fruit stipe breadth of the (S-1) and (S-2) in the same treatment was (1.46, 1.77 and 1.78 cm) and (1.56, 1.6 and 2.02 cm), respectively. In general all, both two strains showed insignificant differences compared with other treatments in the first, second and third flushes for fruit stipe length and breadth of stipe. However, in terms of fruit stipe length, S-1 strain showed significant differences (p<0.05) at the 2nd and 3rd flushes. In a similar experiment, Kumar *et al.*, (2020) found that, the maximum length of white button mushroom stalk (cm) in FYM + Soil + Sand (2:1:1), followed by 2.97 cm in FYM + Soil + burnt rice husk (Ash) (2:1:1) and 2.94 cm in FYM + Sand + Ash (3:1:1/2). They found also the maximum diameter and thickness of pileus (cm) in FYM + Soil + Sand (2:1:1), representing 5.49 cm and 1.89 cm. against 4.01 and 1.14 cm in case of control.

3.5. Effect of casing materials on pileus diameter and thickness

Fruit pileus diameter and thickness of both strains were generally larger for the first harvest compared to those of the second and third harvests (Tables 7 and 8). In the 1st harvest, fruit pileus diameter and thickness of peat moss + palm fronds + vermicompost treatment exhibited the highest values (3.78 and 1.99 cm for S-1 strain and 3.90 and 1.92 cm for S-2 strain, respectively) followed by those of peat moss + palm fronds treatment. Both two strains showed insignificant differences between treatments in the 1st, 2nd and 3rd flushes for fruit pileus diameter and thickness except treatments at first flush for fruit pileus diameter of strain (S-1) and third flush for fruit pileus diameter of strain (S-2), the differences were significant (*P*<0.05).

3.6. Effect of casing materials on phenolics, flavonoids and antioxidants

Phenolic compounds belong to bioactive compounds, although they are non-essential dietary components. Their biological function is related to free radical scavenging activity, metal chelation ability and inhibition of lipid oxidation (Cheung *et al.*, 2003). There are many diseases such as heart disease, cancer, arthritis, and the aging process itself, in which free radicals are implicated. To combat these free radicals the body needs antioxidants (Harman, 1997) and in this respect, flavonoids and other phenols have shown to possess an important antioxidant activity towards these radicals, which is principally based on the redox properties of their phenolic hydroxyl groups and the structural relationships between different parts of their chemical structure (Bors and Saran, 1987). Antioxidant compounds can increase shelf life by retarding the process of lipid peroxidation, which is also one of the major reasons for deterioration of food products during processing and storage (Halliwell, 1997). Thus a need for identifying sources of antioxidants has been created, and the search for natural
Table 5: Impact of casing materials of *A. bisporus* (S-1) strain on stipe length and stipe breadth.

| Casing media                                      | 1<sup>st</sup> flush | 2<sup>nd</sup> flush | 3<sup>rd</sup> flush | 1<sup>st</sup> flush | 2<sup>nd</sup> flush | 3<sup>rd</sup> flush |
|--------------------------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| 100% Peat moss (Control)                         | 2.77±0.18<sup>b</sup> | 2.64±0.25<sup>b</sup> | 2.25±0.47<sup>ab</sup> | 1.77±0.14<sup>ab</sup> | 1.82±0.11<sup>a</sup> | 1.84±0.09<sup>a</sup> |
| 75% Peat moss + 25% Spent mushroom               | 2.91±0.15<sup>ab</sup> | 2.44±0.12<sup>b</sup> | 2.34±0.18<sup>b</sup> | 1.80±0.11<sup>a</sup> | 1.82±0.12<sup>a</sup> | 1.82±0.11<sup>a</sup> |
| 75% Peat moss + 25% Vermi                        | 3.25±0.31<sup>a</sup> | 3.21±0.17<sup>a</sup> | 3.20±0.16<sup>a</sup> | 1.60±0.09<sup>ab</sup> | 1.82±0.08<sup>a</sup> | 1.88±0.11<sup>a</sup> |
| 50% Peat moss + 25% Spent mushroom + 25% Vermi   | 3.40±0.21<sup>a</sup> | 3.28±0.03<sup>a</sup> | 3.23±0.17<sup>ab</sup> | 1.69±0.02<sup>ab</sup> | 1.74±0.02<sup>a</sup> | 1.79±0.02<sup>a</sup> |
| 50% Peat moss + 50% fronds                      | 3.42±0.13<sup>a</sup> | 3.41±0.14<sup>a</sup> | 3.23±0.16<sup>a</sup> | 1.58±0.06<sup>ab</sup> | 1.63±0.05<sup>a</sup> | 1.68±0.09<sup>a</sup> |
| F. value                                         | 2.198                 | 7.308                 | 3.263                 | 1.948                 | 0.559                 | 1.381                 |
| Sig.                                             | 0.084                 | 0.000                 | 0.020                 | 0.120                 | 0.758                 | 0.268                 |

*Means with different letters in the same columns are significantly different (P<0.05) by Duncan multiple test.

Table 6: Impact of casing materials of *A. bisporus* (S-2) strain on stipe length and stipe breadth.

| Casing media                                      | 1<sup>st</sup> flush | 2<sup>nd</sup> flush | 3<sup>rd</sup> flush | 1<sup>st</sup> flush | 2<sup>nd</sup> flush | 3<sup>rd</sup> flush |
|--------------------------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| 100% Peat moss (Control)                         | 3.08±0.47<sup>a</sup> | 2.78±0.20<sup>ab</sup> | 2.73±0.51<sup>a</sup> | 1.82±0.11<sup>a</sup> | 1.82±0.11<sup>a</sup> | 1.87±0.13<sup>a</sup> |
| 75% Peat moss + 25% Spent mushroom               | 3.23±0.04<sup>a</sup> | 3.22±0.26<sup>b</sup> | 2.69±0.06<sup>a</sup> | 1.54±0.05<sup>a</sup> | 1.61±0.08<sup>a</sup> | 1.88±0.03<sup>a</sup> |
| 75% Peat moss + 25% Vermi                        | 3.33±0.17<sup>a</sup> | 3.23±0.11<sup>ab</sup> | 3.16±0.13<sup>a</sup> | 1.64±0.05<sup>ab</sup> | 1.70±0.04<sup>a</sup> | 1.83±0.04<sup>a</sup> |
| 50% Peat moss + 25% Spent mushroom + 25% Vermi   | 3.39±0.07<sup>a</sup> | 3.11±0.17<sup>a</sup> | 3.02±0.31<sup>a</sup> | 1.67±0.01<sup>ab</sup> | 1.70±0.02<sup>ab</sup> | 1.75±0.07<sup>a</sup> |
| 50% Peat moss + 50% fronds                      | 3.43±0.16<sup>a</sup> | 3.26±0.08<sup>ab</sup> | 2.80±1.42<sup>a</sup> | 1.57±0.06<sup>ab</sup> | 1.58±0.06<sup>b</sup> | 1.89±0.49<sup>a</sup> |
| 50% Peat moss + 25% fronds + 25% Vermi           | 3.47±0.19<sup>a</sup> | 3.26±0.48<sup>ab</sup> | 3.10±0.12<sup>a</sup> | 1.56±0.10<sup>b</sup> | 1.60±0.07<sup>a</sup> | 2.02±0.07<sup>a</sup> |
| F. value                                         | 0.624                 | 1.486                 | 1.029                 | 2.023                 | 2.020                 | 0.595                 |
| Sig.                                             | 0.709                 | 0.231                 | 0.434                 | 0.108                 | 0.108                 | 0.731                 |

*Means with different letters in the same columns are significantly different (P<0.05) by Duncan multiple test.
Table 7: Impact of casing materials of *A. bisporus* (S-1) strain on pileus diameter and pileus thickness

| Casing media                              | Fruit pileus (Mean±SE) | Pileus diameter (cm) | Pileus thickness (cm) |
|-------------------------------------------|-------------------------|----------------------|-----------------------|
|                                           |                         | 1<sup>st</sup> flush | 2<sup>nd</sup> flush | 3<sup>rd</sup> flush | 1<sup>st</sup> flush | 2<sup>nd</sup> flush | 3<sup>rd</sup> flush |
| 100% Peat moss (Control)                  |                         | 2.99±0.08<sup>b</sup> | 2.87±0.16<sup>b</sup> | 2.62±0.27<sup>b</sup> | 1.97±0.11<sup>a</sup> | 1.92±0.16<sup>a</sup> | 1.84±0.12<sup>a</sup> |
| 75% Peat moss + 25% Spent mushroom        |                         | 2.76±0.19<sup>a</sup> | 2.73±0.21<sup>ab</sup>| 2.72±0.19<sup>a</sup> | 1.99±0.07<sup>a</sup> | 1.88±0.09<sup>a</sup> | 1.75±0.14<sup>a</sup> |
| 75% Peat moss + 25% Vermi                 |                         | 3.37±0.16<sup>b</sup>| 3.12±0.35<sup>ab</sup>| 2.99±0.10<sup>a</sup> | 1.97±0.09<sup>a</sup> | 1.91±0.11<sup>a</sup> | 1.74±0.12<sup>a</sup> |
| 50% Peat moss + 25% Spent mushroom + 25% Vermi |                     | 3.37±0.14<sup>ab</sup>| 3.26±0.11<sup>a</sup> | 2.69±0.22<sup>a</sup> | 1.76±0.06<sup>a</sup> | 1.76±0.06<sup>a</sup> | 1.75±0.08<sup>ab</sup> |
| 50% Peat moss + 50% fronds               |                         | 3.57±0.08<sup>ab</sup>| 3.27±0.09<sup>a</sup> | 3.17±0.09<sup>a</sup> | 1.72±0.05<sup>a</sup> | 1.72±0.03<sup>a</sup> | 1.64±0.06<sup>ab</sup> |
| 50% Peat moss + 25% fronds + 25% Vermi   |                         | 3.78±0.03<sup>ab</sup>| 2.98±0.15<sup>b</sup> | 2.64±0.33<sup>a</sup> | 1.99±0.15<sup>a</sup> | 1.99±0.09<sup>a</sup> | 1.76±0.09<sup>a</sup> |
| F. value                                 |                         | 3.575                 | 2.139                 | 1.278                 | 0.258                 | 1.374                 | 2.665                 |
| Sig.                                      |                         | 0.013                 | 0.091                 | 0.310                 | 0.951                 | 0.270                 | 0.0440                |

-Means with different letters in the same columns are significantly different (P<0.05) by Duncan multiple test.

Table 8: Effect of casing materials of *A. bisporus* (S-2) strain on pileus diameter and pileus thickness.

| Casing media                              | Fruit pileus (Mean±SE) | Pileus diameter (cm) | Pileus thickness (cm) |
|-------------------------------------------|-------------------------|----------------------|-----------------------|
|                                           |                         | 1<sup>st</sup> flush | 2<sup>nd</sup> flush | 3<sup>rd</sup> flush | 1<sup>st</sup> flush | 2<sup>nd</sup> flush | 3<sup>rd</sup> flush |
| 100% Peat moss (Control)                  |                         | 2.95±0.31<sup>a</sup>| 2.87±0.28<sup>a</sup> | 2.77±0.22<sup>c</sup> | 1.90±0.13<sup>a</sup> | 1.90±0.16<sup>a</sup> | 1.87±0.14<sup>a</sup> |
| 75% Peat moss + 25% Spent mushroom        |                         | 3.44±0.28<sup>a</sup>| 2.86±0.25<sup>a</sup> | 2.82±0.10<sup>a</sup> | 1.72±0.09<sup>a</sup> | 1.70±0.09<sup>a</sup> | 1.65±0.03<sup>a</sup> |
| 75% Peat moss + 25% Vermi                 |                         | 3.43±0.16<sup>a</sup>| 3.12±0.13<sup>a</sup> | 3.08±0.06<sup>abc</sup> | 1.75±0.07<sup>a</sup> | 1.73±0.06<sup>a</sup> | 1.67±0.07<sup>a</sup> |
| 50% Peat moss + 25% Spent mushroom + 25% Vermi |                     | 3.42±0.14<sup>a</sup>| 3.25±0.16<sup>a</sup> | 3.19±0.12<sup>a</sup> | 1.77±0.07<sup>a</sup> | 1.71±0.06<sup>a</sup> | 1.63±0.05<sup>a</sup> |
| 50% Peat moss + 50% fronds               |                         | 3.50±0.16<sup>a</sup>| 3.40±0.19<sup>a</sup> | 3.18±0.18<sup>ab</sup> | 1.77±0.06<sup>a</sup> | 1.66±0.05<sup>a</sup> | 1.65±0.06<sup>a</sup> |
| 50% Peat moss + 25% fronds + 25% Vermi   |                         | 3.90±1.53<sup>a</sup>| 3.41±0.23<sup>a</sup> | 2.79±0.09<sup>a</sup> | 1.92±0.16<sup>a</sup> | 1.84±0.12<sup>a</sup> | 1.68±0.09<sup>a</sup> |
| F. value                                 |                         | 0.568                 | 1.842                 | 3.031                 | 1.299                 | 1.263                 | 0.663                 |
| Sig.                                      |                         | 0.751                 | 0.139                 | 0.027                 | 0.300                 | 0.316                 | 0.680                 |

-Means with different letters in the same columns are significantly different (P<0.05) by Duncan multiple test.
antioxidants, especially of natural origin, has notably increased in recent years (Skerget et al., 2005). Mushrooms have become attractive as a functional food and as source for the development of drugs and nutraceuticals, namely for antioxidant compounds (Lo and Cheung, 2005).

In our results, phenolic compounds contents ranged from 237.1 to 218.47 mg dry weigh and the maximum content of total phenolics in (S-1) strain was observed in treatment of 100% peat moss (237.1 mg /g), while the minimum was recorded with peat moss + spent mushroom (218.47 mg /g) treatment (Table 9). The differences between all casing media of A. bisporus (S-1) for total phenolics content were insignificant. Flavonoids content ranged from 57.21 to 50.39 mg/g dry weigh and showed insignificant values between casing layer treatments except treatment peat moss + frond which was significantly higher compared to other treatments. Flavonoids content ranged between 50.39 and 57.21 mg/g for peat moss + spent mushroom+ vermicompost and peat moss with frond, respectively. The highest content of antioxidant activity was observed in control treatment (72.88 %) while the lowest activity (50.20 %) was observed in peat moss + frond + vermicompost treatment. The differences between all treatments and control were significant. On the other hand, in case of (S-2) strain, the highest content of phenolics (252.93 mg /g dry weigh) was recorded by the treatment That contained Peat moss + vermicompost whereas peat moss + spent mushroom recorded the lowest (208.70 mg /g dry weigh). The highest flavonoids content (54.79 mg/ dry weigh) and antioxidant (69.47 %) values in strain (S-2) were observed in the control treatment where the casing layer contained peat moss only (Table 10). Also the same treatment Peat moss + spent mushroom recorded the lowest values of both contents (46.81 mg/l dry weigh and 42.80 %, respectively). In general, total phenolic compounds, flavonoids content and antioxidant activity showed high significance between treatments at \( P<0.05 \). In another study by Mami et al., (2013), they found that total phenol and antioxidant were increased in the compost of A. bisporus when supplemented with ground corn and soybean seed as a casing layer.

### Table 9: Effect of casing materials of A. bisporus (S-1) strain for phenolics, flavonoids and antioxidant.

| Casing media                        | Phenolics (mg/g dry weight) (Mean±SE) | Flavonoids (mg/g dry weight) (Mean±SE) | Antioxidant (%) (Mean±SE) |
|-------------------------------------|--------------------------------------|---------------------------------------|--------------------------|
| 100% Peat moss (Control)            | 237.10±4.80a                         | 51.36±0.71b                          | 72.88±0.57a              |
| 75% Peat moss +25% Spent mushroom   | 218.47±9.47a                         | 53.32±0.79b                          | 58.06±0.88a              |
| 75% Peat moss + 25% Vermi           | 218.60±7.07a                         | 52.37±1.53b                          | 70.13±1.00b              |
| 50% Peat moss + 25% Spent mushroom + 25% Vermi | 223.53±0.43a | 50.39±1.39b | 67.58±0.29c |
| 50% Peat moss + 50% fronds         | 227.20±3.67a                         | 57.21±0.95a                          | 72.22±0.32a              |
| 50% Peat moss + 25% fronds + 25% Vermi | 227.70±9.90a | 51.62±0.95b | 50.20±1.18f |

Means with different letters in the same columns are significantly different (\( P<0.05 \)) by Duncan multiple test

### Table 10: Effect of casing materials of A. bisporus (S-2) strain for phenolics, flavonoids and antioxidant.

| Casing media                        | Phenolics (mg/g dry weight) (Mean±SE) | Flavonoids (mg/g dry weight) (Mean±SE) | Antioxidant (Mean±SE) (%) |
|-------------------------------------|--------------------------------------|---------------------------------------|--------------------------|
| 100% Peat moss (Control)            | 243.03±2.40ab                        | 54.79±1.24a                          | 69.47±0.82a              |
| 75% Peat moss +25% Spent mushroom   | 208.70±1.82c                         | 46.81±1.04c                          | 42.80±0.90d              |
| 75% Peat moss + 25% Vermi           | 252.93±9.10a                         | 52.53±0.38ab                         | 54.09±1.06c              |
| 50% Peat moss + 25% Spent mushroom + 25% Vermi | 244.03±4.40ab | 51.56±0.60b | 60.48±0.76b |
| 50% Peat moss + 50% fronds         | 219.47±9.14c                         | 53.97±0.90ab                         | 52.05±1.02c              |
| 50% Peat moss + 25% fronds + 25% Vermi | 209.00±6.14c | 47.91±1.46c | 43.08±0.94d |

Means with different letters in the same columns are significantly different (\( P<0.05 \)) by Duncan multiple test
4. Conclusion

In the present study, six different casing layers were tested which prepared locally from available wastes for enhancing production of two strains of white button mushroom *Agaricus bisporus* (S-1 and S-2) and evaluated pharmaceutical substances content. Tested casing layers were 100% peat moss (control), peat moss + spent mushroom (3:1), peat moss + vermicompost (3:1), peat moss + spent mushroom + palm fronds (2:1:1), peat moss + palm fronds (1:1) and peat moss + vermicompost + palm fronds (2:1:1). All casing layers gave high mushroom production as well as high contents of total phenol, flavonoids and antioxidant activity. The results showed that, casing layer peat moss + palm fronds + vermicompost was the best layer for production and harvest quality of the two strains.

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