**Dynamics of microbial enzymes in the compost substrates during button mushroom production**

**ABSTRACT**

Mushrooms degrade lignocellulosic substrates through lignocellulosic enzyme production and utilize the degraded products to produce their fruiting bodies. Hydrolytic enzymes (cellulases and hemicellulases) are known to be responsible for polysaccharide degradation, while oxidative enzymes (ligninases) are responsible for lignin modification and degradation. Quantitative estimation of laccase, MnP, cellulase and xylanase enzyme was done at three different stages of button mushroom production *viz*., before spawning stage (BS), fruit body development stage (FBD) and after harvest (AH) stage. The observation depicted that laccase activity increased during growth of mushroom mycelium in compost and decreased afterwards. Before spawning stage and fruit body development stage treatment 3 (PS+ WS1:1) showed maximum laccase enzyme activity (0.43 IU and 0.37 IU) and after harvesting stage maximum in treatment 4 (only PS) (0.31 IU).TheMnP enzyme activity was found maximum in treatment 3 (2.92 IU), (8.14 IU and 7.42 IU) at all the stages of growth.Filter paperase activity in before spawning stage and fruit development stage was recorded maximum in treatment 3 (PS+ WS 1:1) 27.32 IU and 35.30 IU, after harvest stage, maximum in treatment 2 (PS+ WS 3:1) (18.95 IU). CMCase activity in before spawning stage, observed maximum in treatment 2 (PS+ WS 3:1) 43.21 IU. In fruit body development stage and after harvest stage, treatment 3 (PS+ WS 1:1) recorded the maximum enzyme activity 43.29 IU and 34.53 IU. β-Glucosidase enzyme activity in before spawning stage and fruit body development stage, was recorded maximum in treatment 2 (PS+ WS 3:1) 6.49 IU and 10.82 IU. After harvest stage, maximum in treatment 3 (PS+ WS 1:1) 5.59 IU. Xylanase enzyme activity, in before spawning stage was recorded maximum in treatment 1 (PS+ WS 1.5:1) 5.25 IU. In fruit body development stage and after harvest stage, maximum in treatment 3 (PS+ WS 1:1) 9.68 IU and 5.06 IU.

**Keywords:** Cellulase, xylanase, Laccase, MnP, quantitative estimation and white button mushroom compost.

**INTRODUCTION**

*“Agaricus bisporus* mushroom is a useful bio-factor for agrowaste recycling. It is environmental friendly, capable of converting the lignocellulosic waste materials into food, feed and fertilizers” (Jaradat, 2010). “Agro-industrial waste mainly consists of cellulose, hemicellulose and lignin, all of which are collectively defined as lignocellulosic materials that are hard to degrade” (Kumla et al., 2020). “Mushroom has secrete different enzymes that helps in compost degradation” (Dou et al., 2025). “Cellulose is the most abundant component, followed by hemicellulose and lignin. Cellulose and hemicellulose are sugar derivative macromolecules; whereas lignin is the aromatic polymers made from the phenylpropanoid precursors. Cellulose is (35–50%) followed by hemicellulose (20–35%) and lignin (10–25%)” (Rangabhashiyam and Balasubramanian, 2019). “Straws and stalks are the most abundant lignocellulosic residues/by-products of lignocellulosic crops. Majority of the consumable fungi has enzymatic frameworks that can break these complex substances. The button mushroom is grown on composted lignocellulosic substrates and a variety of raw materials have been used in composting all over the world. The raw materials can be wheat straw, paddy straw, reed plants, waste paper, oat straw, waste tea leaves and some water plants. Cellulose, hemicellulose and lignin are the major components of lignocellulosic materials. The bioconversion of lignocellulosic biomass by the mushroom industry to food and useful products is already a significant contribution to the management of agricultural and industrial wastes at regional and national levels. Mushrooms degrade lignocellulosic substrates through lignocellulosic enzyme production and utilize the degraded products to produce their fruiting bodies. This lignocellulose degrading ability of the fungi can be attributed to their highly well-organized enzymatic system. There are two types of extracellular enzyme system, one which produces hydrolases for the degradation of polysaccharides and another one a unique extracellular and oxidative liginolytic system, which cleaves open phenyl rings and thus degrades lignin” (Sanchez, 2009). “Hydrolytic enzymes (cellulases and hemicellulases) are known to be responsible for polysaccharide degradation, while oxidative enzymes (ligninases) are responsible for lignin modification and degradation. Cellulose and hemicellulose are carbohydrates that act as carbon sources. Lignin provides carbon that is used by mycelium. Ultimately, lignin is converted into a nitrogen-rich lignin–humus complex” (Wang et al., 2016).

Therefore, mushroom cultivation can be considered a biotechnological process for the reduction and valorization of agro-industrial waste. Such waste is generated as a result of the eco-friendly conversion of low-value agri by-products into new resources that can be used to produce value-added products. Objective of this study was to estimation the different enzyme activity during button mushroom production in different compost formulations at different stages. This study helps farmers to know the role of enzymes in compost degradation, how much quantity they form at different stages, and their effect on mushroom production.

**MATERIALS AND METHODS**

The research work was carried out in Mushroom Section of Department of Plant Pathology, Dr.Y.S.Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.).

**Compost preparation steps and substrate used**

Five different formulations of compost were prepared by using different substrate for growing button mushroom (*Agaricus bisporus)* using short method of composting (Sinden and Hause 1950). The ingredients used in different formulations are given below (Table.1). Mushroom U3 strain of *Agaricus bisporus* was used which took from the Mushroom laboratory, Chambaghat, Dr.Y.S.Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). Spawn of U3 strain of *Agaricus bisporus* was prepared on wheat grains as per the standard procedure given by Sharma and Kumar (2011).

**Table .1 Ingredient of substrate used for compost preparation of button mushroom**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatments** | **T1 (kg)**  **PS:WS**  **(1.5:1)** | **T2 (kg)**  **PS: WS** (**3:1)** | **T3 (kg)**  **PS:WS**  **(1:1)** | **T4 (kg)**  **PS** | **T5 (kg)**  **WS**  **(Control)** |
| **Paddy straw (PS)** | 600 | 750 | 500 | 1000 | **-** |
| **Wheat straw (WS)** | 400 | 250 | 500 | **-** | 1000 |
| **Chicken manure** | 600 | 600 | 600 | 600 | 600 |
| **Wheat bran** | 100 | 100 | 100 | 100 | 100 |
| **Urea** | 15 | 15 | 15 | 15 | 15 |
| **Gypsum** | 30 | 30 | 30 | 30 | 30 |
| **Nitrogen (%)** | 1.68 | 1.69 | 1.66 | 1.72 | 1.60 |

**Experiment size:** - No. of treatments: 5, No. of replications: 4, No. of bags per treatment: 10, Bag size: 10Kg

**Statistical design**: CRD (Completely Randomized Design)

**Mushroom Cultivation**

1. **Compost preparation**

Compost preparation was completed in two phases i.e., Phase I (outdoor composting) and Phase II (Indoor composting).

**1) Phase I**

The raw material (ingredients) were mixed together, watered and periodically turned according to following schedule:

**i) -4 day:** Paddy Straw, wheat straw, chicken manure were mixed by trampling to encourage uptake of moisture and aerobic fermentation.

**ii) -2 day:** Whole of mass was turned and made into slightly smaller stacks and more water was added.

**iii) 0 day:** On this day, the stack was again broken and the entire quantities of other raw material like wheat bran and urea were added. Water was also added according to the requirement. On this day a high aerobic stack of 5’×5’ (L× B) was made with the help of iron boards.

**iv) +2 day:** At this stage, first turning was given to compost heap.

**v +4 day:** Second turning was given to heap.

**vi) +6 day:** Third turning was given to heap and gypsum was added.

**vii) +8 day:** At this stage, the compost was filled in the pasteurization chamber for Phase 2 (pasteurization).

**2) Phase II**

**Filling of compost in chamber:**

On +8 day, the compost was filled in pasteurization chamber. As soon as the compost in chamber was completely filled, the door and fresh air ventilator were closed. The blower was put on for circulation of air @ 150-250 cubic meter/1000 kg of compost/hour.

**The Phase II was completed in 3 stages as under:**

**a) Pre- peak heat stage:**

After about 12-15 hours of filling of compost in the chamber, the temperature started rising automatically. The temperature was maintained at 48-50°C for 36-40 hours with the manipulation of ventilation system. This temperature range was achieved by self generation of heat by the compost mass.

**b) Peak heat stage:**

Temperature was raised to 58-59oC by adjustment of fresh and recirculation air in the pasteurization chamber and it was maintained for 6-8 hours to ensure proper pasteurization.

**c) Post- peak heat stage:**

The temperature was gradually lowered down and maintained at 45-55oC till no traces of ammonia were detected in the compost. It took 3-4 days for ammonia to cease off. When the compost was free from ammonia, fresh air was introduced by opening the damper to the maximum capacity till the compost cooled down to ambient temperature for spawning.

**2 Spawning:**

Spawn was mixed with compost in layers. In compost bags, about 3–4 layers of spawn and compost were layered and finally, one layer of spawn spread on the top of the compost. The spawning rate was kept at 0.5–0.7 per cent, i.e. 50-70 g/10 kg bag of compost. The bags were placed in cropping rooms for spawn run at 20–25 °C in dark conditions.

**3 Preparation of casing soil**

Casing material was steam pasteurized at 65 ± 1oC for 6-7 hours. Hydrogen ion concentration (pH) of casing soil was adjusted to around 8.0 by adding calcium carbonate before pasteurization. The casing material was used to case the spawn run bags after a period of 2 weeks from the day of spawning. The moisture level of casing was adjusted to 60 per cent before placing it on the spawn run compost. After 10-12 days of casing, the bags were shifted to cropping room having 14-18oC temperature with 80-90 per cent relative humidity, which was maintained till the crop was over. For maintaining the humidity, water was sprayed on the walls and floor of the growing chamber and cropping bags were also sprayed with water whenever required.

**4 Fruiting**

White button mushroom requires 22-25°C temperature in cropping room for vegetative growth (spawn run) and 14-18°C for reproductive growth. Besides, it requires relative humidity of 80-90 per cent and CO2 concentration (0.08-0.15 %) for fruit body initials to form. During cropping, ventilation was provided to pinheads, which gradually developed into button stages.

**5 Harvesting**

Mushrooms were harvested in the button stage. Harvesting was done by holding the cap with forefingers and thumb by slightly pressing against the soil and twisting it off. The soil particles and mycelial threads clinging to the base of the stalk were chopped off. The studies were conducted to select best compost formulation to study their quality and effect on growth and yield of white button mushroom in most economical manner.

**Compost sampling for estimation of enzyme activity**

Compost Samples for estimation of enzyme activity was tookat three different stages of button mushroom production *viz*., before spawning stage (BS), fruit body development stage (FBD) and after harvest (AH) stage. Samples were collected from the five selective bags from each treatment at 5cm depth of the mushroom bags from the top.

**Estimation of enzymes activity in different compost formulations**

1. **Laccase assay (Rehan et al., 2016)**

The laccase assay was performed by the following method described by Rehan et al. (2016).

**2. Manganese dependent peroxidiseassay (MnP) (Paszczynski et al. (1986); Mata and Savoie, (1998)**

The activity of Manganese dependent peroxidise (MnP) was estimated according to method of Paszczynski et al. (1986); Mata and Savoie, (1998), based on the rate of oxidation of Mn (II) to Mn (III) and hydrogen peroxide ions produced by fungi to degrade lignin substrate.

**3.Cellulase assay**

3.1 Carboxymethyl Cellulase (CMC) assay (Reese and Mandel, 1963)

3.2 FPase assay (Reese and Mandel, 1963)

3.3 β glycosidase assay (Bergheim and Patterson, 1973)

**4. Xylanase assay (Miller, 1959)**

Xylanase activity was determined by measuring the amount of reducing sugar released from xylan using (DNS) method (Miller, 959).

**RESULTS AND DISCUSSION**

**Estimation of degaradative enzyme activities in different compost formulations**

Quantitative estimation of enzymes like laccase, xylanase, cellulase and manganese dependent peroxides s(MnP) was done at three different stages of button mushroom production i.e., before spawning (BS), fruit body development (FBD) and after harvest (AH) stage. Enzymes activities of the five different compost formulations were assayed.

**Quantitative estimation of ligninases in different compost formulations at different stages of mushroom production**

In the process of composting, the production of extracellular hydrolytic enzymes by microbes plays an importanst role in starting stage of degradation and crop production. These hydrolytic enzymes degrade the lignocellulosic substrate to simple monomers.

**Laccase enzyme**

The quantitative estimation of laccase enzyme was done at three different stages of button mushroom production *viz*., before spawning stage (BS), fruit body development stage (FBD) and after harvest (AH) stage. The observation depicted that laccase activity increased during growth of mushroom mycelium in compost and decreased afterwards. Laccase enzyme activity during before spawning stage, recorded maximum in treatment 3 (0.43 IU) followed by treatment 1 (0.41 IU), which were statistically at par to each other whereas, minimum enzyme activity was recorded in treatment 5 (0.32 IU). During fruit body development stage, maximum enzyme activity recorded in treatment 3 (0.37 IU) followed by treatment 1 (0.34IU). Minimum enzyme activity observed in treatment 2 (0.28IU). After harvesting stage, the laccase activity was reduced and the maximum enzyme activity was found in treatment 4 (0.31IU) followed by treatment 3 and 5 (0.29IU). Minimum enzyme activity was recorded in treatment 2 (0.22 IU). Overall, it was observed that treatment 3 had maximum laccase enzyme activity in all three (Table.2). Observation depicted that enzymes activity increased from before spawning stage to during fruiting bodies development stage and decreased in after harvesting stage. Lignin degradation is the primary step in lignocellulose degradation enabling the accessibility of cellulose and hemicellulose (Anderson et al., 2008 and Jurak et al., 2015). Ligninolytic microorganisms can degrade lignins via the secretion of oxidative enzymes, such as peroxidases and laccases or by producing a source of heterogeneous aromatics. Ligninolytic enzymes or ligninases are mainly comprised of laccases, (Lac), lignin peroxidases (LiPs,) manganese peroxidases (MnPs), versatile peroxidases (VPs) and dye decolorizing peroxidases (DyPs) (Scharf and Tartar, 2008; Familoni et al., 2018) These enzymes display less substrate specificity than cellulases and hemicellulases (Scharf and Tartar, 2008; Pollegioni et al., 2015 and Liang et al., 2019). Additionally, Lac, LiP and MnP and many other enzymes, such as aromatic acid reductase, aryl alcohol dehydrogenase, catalase aromatic aldehyde oxidase, dioxygenase, quinone oxidoreductase, vanillate hydroxylase, veratryl alcohol oxidase and versatile peroxidase, are also involved in lignin digestion (Pollegioni et al., 2015). Mushroom species are most frequently reported as Lac and MnP producers.

**Table .2 Quantitative estimation of laccase enzyme in different button mushroom compost formulation at different stages of mushroom production**

|  |  |  |  |
| --- | --- | --- | --- |
| **Laccase enzyme activity (IU)** | | | |
| **Treatments** | **Before**  **Spawning** | **Fruit body development** | **After**  **Harvesting** |
| **T1**  **Paddy straw+ Wheat straw (1.5:1)** | 0.41 | 0.34 | 0.25 |
| **T2**  **Paddy straw+ Wheat straw (3:1)** | 0.34 | 0.28 | 0.22 |
| **T3**  **Paddy straw+ Wheat straw (1:1)** | 0.43 | 0.37 | 0.29 |
| **T4**  **Paddy straw** | 0.38 | 0.31 | 0.31 |
| **T5**  **Wheat straw (Control)** | 0.32 | 0.30 | 0.29 |
| **Mean** | 0.38 | 0.32 | 0.27 |
| **C.D. (0.05)** | 0.05 | 0.02 | 0.02 |
| **SE** | 0.02 | 0.01 | 0.01 |

**IU (International unit =** µM/min/ml**)**

**Mangnese peroxidase enzyme**

MnP enzyme activity was found to increase from before spawning stage to fruit body development stage and decreased afterwards (Table .3). In before spawning stage, MnP enzyme activity (2.92 IU) was maximum in treatment 1 followed by treatment 2 (2.00IU). Minimum MnP enzyme activity was shown by treatment 5 (0.24IU). In fruit body development stage, maximum enzyme activity recorded in treatment 3 (8.14IU) followed by treatment 5 (7.31IU) while minimum enzyme activity was observed in treatment 2 (2.55IU). The MnP activity in post harvest stage was maximum in treatment 3 (7.42IU) followed by treatment 5 (6.44IU) while minimum enzyme activity was recorded in treatment 1 (0.30IU ). Overall, treatment 3 showed maximum MnP enzyme activity.

**Table. 3 Quantitative estimation of MnP enzyme from the button mushroom different compost formulations**

|  |  |  |  |
| --- | --- | --- | --- |
| **MnP enzyme activity (IU)** | | | |
| **Treatments** | **Before**  **spawning** | **Fruit body development** | **After**  **Harvesting** |
| **T1**  **Paddy straw+ Wheat straw (1.5:1)** | 2.92 | 4.62 | 0.30 |
| **T2**  **Paddy straw+ Wheat straw (3:1)** | 2.00 | 2.55 | 2.16 |
| **T3**  **Paddy straw+ Wheat straw (1:1)** | 0.68 | 8.14 | 7.42 |
| **T4**  **Paddy straw** | 0.12 | 4.92 | 1.40 |
| **T5**  **Wheat straw (Control)** | 0.24 | 7.31 | 6.44 |
| **Mean** | 1.19 | 5.51 | 3.54 |
| **C.D. (0.05)** | 0.20 | 0.12 | 0.31 |
| **SE** | 0.07 | 0.04 | 0.10 |

**IU (International unit =** µM/min/ml**)**

Agricultural wastes are comprised of the raw and processed agricultural products. They are mainly obtained from the plants under field conditions and from industries during processing. They are mainly composed of 35 – 50% cellulose, 25 – 35% hemicellulose, 10 – 25% lignin and rest with ash and others (Kumla et al., 2020). The degradation of lignocellulosic biomass is achieved through cooperative activities of hydrolytic and oxidative enzymes (Lombard et al., 2013; López et al., 2016 and Madeira et al., 2017). The hydrolytic system is responsible for cellulose and hemicellulose degradations, whereas the oxidative system is known to participate in lignin degradation. Laccase is the key enzyme belongs to the group of oxidative enzymes and involved behind the lignin degradation. Laccase is one of the major lignolytic enzyme produced by the Basidiomycota fungus, which can be determined using Guaiacol as substrate. Oxidation of guaiacol by laccase produces red color which is an indicator for production of laccase enzyme (Monssef et al., 2016). Laccase can be used for lignin removal in prehydrolysis of lignocellulosicbiomass (Shi et al., 2014).

Manganese peroxidase is an important enzyme associated with the lignin and organic pollutant degradation systems, for instance bioremediation (Khanongnuch, et al., 2006). Manganese peroxidase (MnP) belongs to the family of oxidoreductases and cannot react directly with the lignin structure (Ardon et al., 1998). There are two groups: (1) Manganese dependent peroxidase is an extracellular enzyme that requires both H2O2 for lignin oxidation, Mn2+as a co-factor and (2) Manganese independent peroxidase is an extracellular enzyme that requires H2O2  in lignin oxidation but does not need Mn2+ (Zhao et al., 2015).

**Quantitative estimation of celluloses and hemicellulase in different button mushroom compost formulation at different stages of mushroom production**

Cellulase consists of three enzymes: β-glucosidase, endo-1,4-β-D-glucanase (CMCase) and exo-1,4-β-D-glucanase (filter paperase). These three enzymes are involved in the hydrolysis of cellulose by synergetic action for accomplished and effective hydrolysis of cellulose (Patel et al., 2019). These enzymes convert cellulose in order to oligosaccharides, cellobiose, and glucose (Horn et al., 2012 and Ritota et al., 2019).

**Filter paperase enzyme**

Filter paperase is also known as C1 cellulase catalyzing the degradation of crystalline cellulose at carbon 1 of the glucose chain. During the present studies, it was observed that cellulase enzyme activity increased from before spawning stage to fruit body development stage and decreased afterward (Table.4).

In before spawning stage, treatment 3 showed maximum filter paperase activity (27.32IU) followed by treatment 5 (19.57IU) while minimum was recorded in treatment 4 (17.25IU). In fruit body development stage, maximum enzyme activity was recorded in treatment 3 (35.30IU) followed by treatment 1 (27.17 IU). The minimum enzyme activity was observed in treatment 4 (22.25IU). After harvest stage, maximum enzyme activity was found in treatment 2 (18.95IU) while minimum enzyme activity was observed in treatment 3 (15.84IU). Overall, it was observed that treatment 3 was showed maximum C1 cellulase enzyme activity. Exoglucanase hydrolyzes non-reducing ends of crystalline cellulose and forms cellobiose or glucose as the major end products. Exoglucanases (Filter paperase) release cellobiose from the reducing or the non-reducing end of the cellulose chain, facilitating the production of mostly cellobiose (Zhang et al., 2006; Yeoman et al., 2010 and Madeira et al., 2017).

**Table.4 Quantitative estimation of C-1 cellulase enzyme from the different button mushroom compost formulation at different stages of mushroom production**

|  |  |  |  |
| --- | --- | --- | --- |
| **Filter paperaseenzyme activity (IU)** | | | |
| **Treatments** | **Before**  **Spawning** | **Fruit body development** | **After**  **Harvesting** |
| **T1**  **Paddy straw+ Wheat straw (1.5:1)** | 19.41 | 27.17 | 18.51 |
| **T2**  **Paddy straw+ Wheat straw (3:1)** | 18.18 | 23.65 | 18.95 |
| **T3**  **Paddy straw+ Wheat straw (1:1)** | 27.32 | 35.30 | 15.84 |
| **T4**  **Paddy straw** | 17.25 | 22.25 | 16.20 |
| **T5**  **Wheat straw (Control)** | 19.57 | 24.80 | 16.93 |
| **Mean** | 20.34 | 26.63 | 17.29 |
| **C.D. (0.05)** | 0.07 | 0.14 | 0.19 |
| **SE** | 0.02 | 0.05 | 0.06 |

**IU (International unit =** µM/min/ml**)**

**Carboxymethyl cellulase enzyme (CMCase)**

CMCase enzymes are type of cellulases catalyzing the degradation of cellulose at random places in the glucose chain producing oligosaccharides and called as endocellulases. During the study, the CMCase enzyme activity increased from before spawning stage to fruit body development stage and decreased in after harvesting stage (Table. 5).

In before spawning stage, treatment 2 showed maximum CMCaseactivity (43.21 IU) while minimum enzyme activity was recorded in treatment 5 (27.92IU). In fruit body development stage, treatment 3 recorded the maximum enzyme activity (43.29IU) followed by treatment 5 (41.55IU). The minimum enzyme activity was observed in treatment 4 (34.74IU). After the harvest of crop, maximum enzyme activity was found in treatment 3 (34.53IU) followed by treatment 5 (33.76IU) and minimum was in treatment 2 (28.29IU).Overall, it was observed that treatment 3 was shown maximum CMCase enzyme activity. Carboxymethylcellulase (CMCase) activity was found to be higher till 60 days and then declined in all the treatments till 90 days. The highest value of CMCase (0.43 IU/g) was observed till 60 days, whereas the highest activity of FPase (0.47 IU/g) was observed till 90 days in the treatment supplemented with compost inoculants (CI) and efficient microorganism (EM). β-1,4 Endoglucanase (CMCase) acts upon the native cellulose which provide reactive sites for the action of exoglucanase (FPase); so this could be a possible reason for the high activity of CMCase till 60 days and FPase till 90 days. Ohmiya et al. (1997) reported that endoglucanase acts on inner sites of oligosaccharides found in carboxymethyl cellulose, cello-oligosaccharides or amorphous cellulose. Endoglucanases (CMCase enzymes) preferentially hydrolyze internal β-1,4-glucosidic linkages in the cellulose chains, generating a number of reducing ends (Horn et al., 2012 and Sajith, et al., 2016). This enzyme also acts on cellodextrins, which are the intermediate product of cellulose hydrolysis, and converts them to cellobiose and glucose.

**Table. 5 Quantitative estimation of CMCase enzyme from the different button mushroom compost formulation at different stages of mushroom production**

|  |  |  |  |
| --- | --- | --- | --- |
| **CMCaseenzyme activity (IU)** | | | |
| **Treatments** | **Before**  **Spawning** | **Fruit body development** | **After**  **Harvesting** |
| **T1**  **Paddy straw+ Wheat straw (1.5:1)** | 32.71 | 39.21 | 32.37 |
| **T2**  **Paddy straw+ Wheat straw (3:1)** | 43.21 | 35.21 | 28.29 |
| **T3**  **Paddy straw+ Wheat straw (1:1)** | 34.42 | 43.29 | 34.53 |
| **T4**  **Paddy straw** | 32.67 | 34.74 | 28.50 |
| **T5**  **Wheat straw (Control)** | 27.92 | 41.55 | 33.76 |
| **Mean** | 34.91 | 38.80 | 31.49 |
| **C.D. (0.05)** | 0.51 | 1.23 | 0.90 |
| **SE** | 0.17 | 0.41 | 0.30 |

**IU (International unit =** µM/min/ml**)**

**β –Glucosidase enzyme**

β-Glucosidase is also a type of cellulase enzyme catalyzing the degradation of oligosaccharides releasing monomers of glucose. The observation depicted that β-Glucosidase enzyme activity increased from before spawning stage to fruit body development stage and decreased in after harvesting stage (Table.6). In before spawning stage, treatment 2 showed maximum β-Glucosidase enzyme activity (6.49 IU) followed by treatment 3 (6.32 IU) while minimum enzyme activity was recorded in treatment 1 (4.90IU). In during fruit body development stage, maximum enzyme activity recorded in treatment 2 (10.82IU) followed by treatment 3 (8.73IU). The minimum enzyme activity observed in treatment 1 (5.99IU). After harvest stage, maximum enzyme activity was found in treatment 3 (5.59IUl) followed by treatment 2 (5.55IU) and minimum enzyme activity was observed by treatment 1 (4.60IU). β-1,4 exoglucanase (FPase) enzyme activity increased during the later phase (90 days) of composting in all the treatments (Gaind et al., 2008 and Pandey et al., 2009). β-glucosidase acts on non-reducing ends of cellobiose and cellodextrin. Oligosaccharides released are converted to glucose by the action of cellodextrinases, whereas the cellobiose released mainly by the action of cellobiohydrolases is converted to glucose by β-glucosidases (Zhang et al., 2006; Yeoman et al., 2010; Sajith, et al., 2016 and Madeira et al., 2017).

**Table.6 Quantitative estimation of β-Glucosidase enzyme from the different button mushroom compost formulation at different stages of mushroom production**

|  |  |  |  |
| --- | --- | --- | --- |
| **β -Glucosidase enzyme activity (IU)** | | | |
| **Treatments** | **Before**  **spawning** | **Fruit body development** | **After**  **Harvesting** |
| **T1**  **Paddy straw+ Wheat straw (1.5:1)** | 4.90 | 5.99 | 4.60 |
| **T2**  **Paddy straw+ Wheat straw (3:1)** | 6.49 | 10.82 | 5.55 |
| **T3**  **Paddy straw+ Wheat straw (1:1)** | 6.32 | 8.73 | 5.59 |
| **T4**  **Paddy straw** | 5.22 | 6.49 | 5.02 |
| **T5**  **Wheat straw (Control)** | 5.11 | 6.09 | 5.03 |
| **Mean** | 5.61 | 7.62 | 5.16 |
| **C.D. (0.05)** | 0.22 | 0.05 | 0.26 |
| **SE** | 0.07 | 0.02 | 0.09 |

**IU (International unit =** µM/min/ml**)**

**Xylanase enzyme**

The observation depicted that xylanase enzyme activity was increased from before spawning stage to during fruit body development stage after that decreased in after harvesting stage (Table.7). In before spawning stage, treatment 1 showed maximum xylanase enzyme activity (5.25 IU) whereas, minimum enzyme activity was recorded in treatment 5 (3.33IU). In fruit body development stage, maximum enzyme activity was recorded in treatment 3 (9.68 IU) followed by treatment 2 (6.64IU) while the minimum enzyme activity was observed in treatment 5 (5.17 IU). After harvest stage, maximum enzyme activity was found in treatment 3 (5.06 IU) followed by treatment 4 (4.26IU) and minimum enzyme activity was shown by treatment 5 (3.49IU).Overall, it was observed that treatment 3 was shown maximum xylanase enzyme activity. The activity of xylanase enzyme was highest in the initial stage of composting; then, a decline was observed in all the treatments at a later stage of composting. Pandey et al. (2009) also observed the same pattern of high activity of xylanase in the initial stages of composting.

**Table.7 Quantitative estimation of xylanase enzyme from the different button mushroom compost formulation at different stages of mushroom production**

|  |  |  |  |
| --- | --- | --- | --- |
| **Xylanase enzyme activity (IU)** | | | |
| **Treatments** | **Before**  **Spawning** | **Fruit body development** | **After**  **Harvesting** |
| **T1**  **Paddy straw+ Wheat straw (1.5:1)** | 5.25 | 5.84 | 3.73 |
| **T2**  **Paddy straw+ Wheat straw (3:1)** | 4.20 | 6.64 | 4.06 |
| **T3**  **Paddy straw+ Wheat straw (1:1)** | 4.20 | 9.68 | 5.06 |
| **T4**  **Paddy straw** | 3.65 | 5.87 | 4.26 |
| **T5**  **Wheat straw (Control)** | 3.33 | 5.17 | 3.49 |
| **Mean** | 4.13 | 6.64 | 4.12 |
| **C.D. (0.05)** | 0.12 | 0.10 | 0.08 |
| **SE** | 0.04 | 0.03 | 0.03 |

**IU (International unit =** µM/min/ml**)**

.

**CONCLUSION**

Agro-industrial waste mainly consists of cellulose, hemicellulose and lignin, all of which are collectively defined as lignocellulosic materials that are hard to degrade. *Agaricus bisporus* mushroom is a useful bio-factor for agrowaste recycling. It is environmental friendly, capable of converting the lignocellulosic waste materials into food, feed and fertilizers. In thus study we done the quantitative estimation of laccase, MnP, cellulase and xylanase enzyme was done at three different stages of button mushroom production *viz*., before spawning stage (BS), fruit body development stage (FBD) and after harvest (AH) stage to evaluate enzyme production, their role in degradation and in mushroom production. The observation revealed that irrespective of treatment, laccase activity increased during growth of mushroom mycelium and decreased afterwards. Maximum laccase enzyme activity was recorded in treatment 3 during spawn run and case run stage while it reduced after harvesting stage. MnP enzyme activity was also found to increase from before spawning stage to fruit body development stage and decreased afterwards. Maximum MnP enzyme activity was observed in treatment 3 and it followed the same trend as in case of laccase enzyme. Treatment 3 was shown maximum CMCase enzyme activity. Carboxymethylcellulase (CMCase) activity was found to be higher till 60 days and then declined in all the treatments till 90 days. The highest value of CMCase (0.43 IU/g) was observed till 60 days, whereas the highest activity of FPase (0.47 IU/g) was observed till 90 days. β-1,4 exoglucanase (FPase) enzyme activity increased during the later phase (90 days) of composting in all the treatments. Treatment 3 was shown maximum xylanase enzyme activity. The activity of xylanase enzyme was highest in the initial stage of composting; then, a decline was observed in all the treatments at a later stage of composting. Enzyme activity increased from before spawning to the fruit body development stage, and afterward started to decrease. Overall findings showed that maximum enzyme activity was recorded in treatment 3 (Paddy straw+ wheat straw, 1:1), which helps in fast degradation of compost, minimum days for fruiting bodies formation, also helps in improvement of mushroom quality and yield.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

**REFERENCES**

Berghem, L.E.R., and Pettersson, L.G. (1973). Mechanism of enzymatic cellulose degradation and purification of a cellulolytic enzyme from *T. viride* active on highly ordered cellulose. Journal of Biochemistry*,* 37, 21-30.

Dou, T., Zhang, K., Shi, X., Liu, W., Yu, F., Liu, D. (2025). Crop mushroom rotation: A comprehensive Review of its multifaceted impacts on soil quality, agricultural sustainability and ecosystem health. Agronomy, 15(3), 563

Kumla, J., Suwannarach, N., Sujarit, K., Penkhrue, W., Kakumyan, P., Jatuwong, K., Vadthanarat, S., and Lumyong, S. (2020). Cultivation of Mushrooms and Their Lignocellulolytic Enzyme Production through the Utilization of Agro-Industrial Waste. Molecules, 25(12), 2811.

Mata, G., and Savoie, J.M. (1998). Extracellular enzyme activities in six Lentinulaedodes strains during cultivation in wheat straw.World J.Microbiol.Biotechnol.14, 513 - 519.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31, 426–428

Paszczynski, A., Huyn, B,V., and Crawford, R.(1986). Comparison of ligninase I and peroxidase- M2 from the white rot fungus *Phanerochaetechrysosporium.* Arch. Biochem. Biophys, 244, 750.

Rangabhashiyam,S., and Balasubramanian, P. (2019). The potential of lignocellulosic biomass precursors for biochar production: Performance , mechanism and wastewater application — A review. Industrial Crops & Products, 405-23.

Reese, E.T., Mandel, M. (1963). Enzymatic hydrolysis of cellulose and its derivatives In: Method Carbohydrate Chemistry (Whistles R L eds.). Academic Press London, 139-143.

Rehan, A.M., Hassan, E., and Ramadan, E. (2016). Production of laccase enzyme for their potential application to decolorize fungal pigments on aging paper and parchment. Annals of Agricultural Sciences, 61, 1-11.

Sanchez, C. (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. Biotechnology Advance, 27, 185-194.

Wang, L., Mao, J., Zhao, H., Li, M., Wei, Q., Zhou, Y., and Shao, H. (2016). Comparison of characterization and microbial communities in rice straw- and wheat straw-based compost for *Agaricus bisporus* production. J IndMicrobiolBiotechnol, 43, 1249-60.