Estimation of enzymes at different stages of Button mushroom production by using different compost formulations

**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 showed maximum laccase enzyme activity (0.43 IU and 0.37 IU) and after harvesting stage maximum in treatment 4 (0.31 IU). The MnP 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 (27.32 IU and 35.30 IU), after harvest stage, maximum in treatment 2 (18.95 IU). CMCase activity in before spawning stage, observed maximum in treatment 2 (43.21 IU). In fruit body development stage and after harvest stage, treatment 3 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 (6.49 IU and 10.82 IU). After harvest stage, maximum in treatment 3 (5.59 IUl). Xylanase enzyme activity, in before spawning stage was recorded maximum in treatment 1 (5.25 IU). In fruit body development stage and after harvest stage, maximum in treatment 3 (9.68 IU and 5.06 IU).

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

**INTRODUCTION**

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). 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. 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.

 **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.).

**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).

**Reagent**

1. 2mM Guaiacol
2. 10mM Sodium acetate buffer (pH 5.5)

**Procedure**

 Reaction mixture contained 1ml of 2mM of guaiacol in 3ml sodium acetate buffer (pH5.5) and 1ml of culture supernatant. The blank was also prepared with all components except the enzyme (culture supernatant). The reaction mixture was incubated at 30ºC for 15 min. The absorbance was read at 450nm using UV spectrophotometer. The enzyme activity was expressed in terms of International Unit (IU). One International Unit (IU) of enzyme activity represents 1 µmoles guaiacol released/min/ml of enzyme.

The activity of laccase assay was calculated by using this formula:

$$Laccase activity =\frac{A ×V}{t× e× v}$$

Where,

|  |  |  |
| --- | --- | --- |
| A | = | Absorbance (O.D.) |
| V  | = | Total volume of reaction mixture |
| T | = | Incubation time |
| E | = | Extinction coefficient of guaiacol at 450nm (0.6740 µM/cm) |
| V | = | Volume of culture supernatant |

**2. Manganese dependent peroxidise assay (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.

**Reagent**

1. 0.5M Sodium Tartrate buffer (pH 5.0)
2. 1mM Guaiacol
3. 1Mm Manganese sulphate (MnSO4)
4. 1Mm Hydrogen Peroxide (H2O2)

**Procedure**

 Reaction mixture was contained 0.2ml of 0.5M Sodium Tartrate buffer, 0.25ml of 1mM Guaiacol, 0.1ml of 1Mm MnSO4, 0.35ml of enzyme dilution (culture supernatant) and 0.1ml f 1mM H2O2 in test tube. After adding all these reagents to the test tube, the initial reading was taken at 465nm by using a spectrophotometer. The final reading was taken after 5 minutes of incubation at room temperature. The blank was also prepared with all components except the enzyme (culture supernatant). One International Unit (IU) of enzyme activity represents 1 µmoles guaiacol released/min/ml of enzyme.

 MnP activity calculated by using formula given below:

$$MnP activity (U/L) =\frac{\left(∆ Absorbance\right)×10}{e× R× t}$$

Where,

|  |  |  |
| --- | --- | --- |
| ∆ Absorbance | = | Final absorbance- initial absorbance |
| e  | = | Extinction coefficient of guaiacol substrate (26.6 mM-1 cm-1.) |
| R | = | Amount of enzyme (reaction mixture) in broth (1ml) |
| T | = | Reaction Time (5min) |

**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)

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

**Reagents**

i) 1 % CMC in citrate buffer (0.05M, pH 5.0)

ii) Dinitrosalicylic acid (DNSA) Reagent: NaOH: 1.0 g, Phenol: 0.2 g, Sodium potassium tartarate : 20.0 g, Sodium sulphate : 0.05g, DNSA reagent : 1.0g, Distilled water : 100ml

1. Standard solution of glucose ( 0.4mg/ml)

**Procedure**

 The reaction mixture contained 0.5ml of 1% CMC in citrate buffer (0.05M, pH-5.0) and 0.5ml of culture supernatant. Reaction mixture was incubated at 50°C for 30 min. After incubation 3ml of DNSA reagent was added. Tubes were immersed in boiling water bath and removed after 15 min when colour development was complete. Control was run with all the components except the enzyme. Tubes were cooled at room temperature and O.D was read at 540nm in spectrophotometer against the reagent blank i.e. 1ml of distilled water and 3ml of DNSA reagent. The standard curve was made from the stock solution of glucose (0.4 mg/ml) with concentration i.e. 100µl, 200µl, 300µl, 400µl, 500µl, 600µl, 700µl, 800µl, 900µl, 1000µl. The enzyme activity was expressed in terms of International Unit (IU). One International Unit (IU) of enzyme activity represents µ moles of glucose released/min/ml of enzyme.

$$Enzyme activity =\frac{ Product concentration × Total ml}{Molecular weight × ml of enzyme × Incubation time}×Dilution factor$$

**3.2 Filter Paperase (FPase) assay (Reese and Mandel 1963)**

**Reagents**

i) Strips of filter paper (Whatman no.1)

ii) 0.05M citrate buffer (pH 5.0)

iii) Dinitrosalicylic acid (DNSA) Reagent

iv) Standard solution of glucose (0.4 mg/ml)

**Procedure**

 To 50 mg of filter paper strips (Whatman no. 1), 0.5 ml of citrate buffer (0.05M, pH-5) and 0.5ml of culture supernatant was added. Reaction mixture was incubated at 50°C for 30 min. After incubation 3ml of DNSA reagent was added. Tubes were immersed in boiling water bath and removed after 15 min when colour development was complete. Control was run with all the components except the enzyme. Tubes were cooled at room temperature and O.D was read at 540nm in spectrophotometer against a reagent blank i.e. 1ml of distilled water and 3ml of DNSA reagent. The standard curve was prepared from the stock solution of glucose (0.4mg/ml) with concentration i.e 100µl, 200µl, 300µl, 400µl, 500µl, 600µl, 700µl, 800µl, 900µl, 1000µl. The enzyme activity was expressed in terms of International Unit (IU). One International Unit (IU) of enzyme activity represents µmoles of glucose released/min/ml of enzyme.

$$Enzyme activity =\frac{ Product concentration × Total ml}{Molecular weight × ml of enzyme × Incubation time}×Dilution factor$$

**3.3 β-Glucosidase assay (Berghem and Petterson 1973)**

**Reagents**

1. 1mM ρ-nitrophenyl β-D-glucopyranoside in 0.05M sodium acetate buffer of pH 5.0
2. 1M sodium carbonate solution
3. Standard solution of ρ-nitrophenol (80 µg/ml)

**Procedure**

 Reaction mixture contained 1ml of 1mM of ρ-nitrophenyl β-D**-**glucopyranoside in 0.05M sodium acetate buffer (pH 5.0) and 100 µl of culture supernatant. After incubation at 40°C for 10 min, 2ml of 1M of Na2CO3 was added to the reaction mixture to stop the reaction. Reaction mixture was heated in boiling water bath for 15 min and after heating it was diluted to 10ml with distilled water. ρ-nitrophenol liberated was determined as the absorbance at 400nm against reagent blank i.e. 1ml of distilled water and 2ml of 1M of sodium carbonate. The standard curve was prepared from the stock solution of ρ-nitrophenol (80 µg/ml) with concentration i.e 100µl, 200µl, 300µl, 400µl, 500µl, 600µl, 700µl, 800µl, 900µl, 1000µl. The enzyme activity was expressed in terms of International Unit (IU). One International Unit (IU) of enzyme activity represents µmoles of nitrophenyl ß-D- glucopyranoside released/min/ml of enzyme.

$$Enzyme activity =\frac{ Product concentration × Total ml}{Molecular weight × ml of enzyme × Incubation time}×Dilution factor$$

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

 Xylanase activity was determined by measuring the amount of reducing sugar released from xylan using (DNS) method (Miller 1959).

**Reagents**

i) 1.0 g Oatspelt xylan in 100 ml of 0.05 M citrate buffer, pH 4.0

ii) DNSA reagent

iii) Standard solution of xylose (0.4 mg/ml)

**Procedure**

 0.8 ml of xylan solution (which was incubated overnight at 37°C) and 0.2 ml of culture supernatant was taken in a test tube. The control was run with 0.2 ml distilled water and 0.8 ml xylan solution except the enzyme (culture supernatant). The reaction mixture was incubated at 45°C for 10 min. After the incubation, 3 ml of DNSA reagent was added and the mixture was then heated in boiling water bath for 30 min. After cooling down at room temperature, absorbance of reaction mixture was recorded at 540 nm. The enzyme activity was expressed in terms of International Unit (IU). One International Unit of enzyme activity represents µmoles of glucose released/min/ml of enzyme.

$$Enzyme activity =\frac{ Product concentration × Total ml}{Molecular weight × ml of enzyme × Incubation time}×Dilution factor$$

**RESULTS AND DISCUSSION**

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

Quantitative estimation of enzymes like laccase, xylanase, cellulase and manganese dependent peroxides (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**

**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 (0.34 IU). Minimum enzyme activity observed in treatment 2 (0.28 IU). After harvesting stage, the laccase activity was reduced and the maximum enzyme activity was found in treatment 4 (0.31 IU) followed by treatment 3 and 5 (0.29 IU). 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.1).

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

**Table. 2 Quantitative estimation of MnP enzyme from the button mushroom different compost formulations**

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| --- |
| **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 lignocellulosic biomass (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**

**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.3 ).

In before spawning stage, treatment 3 showed maximum filter paperase activity (27.32 IU) followed by treatment 5 (19.57 IU) while minimum was recorded in treatment 4 (17.25 IU). In fruit body development stage, maximum enzyme activity was recorded in treatment 3 (35.30 IU) followed by treatment 1 (27.17 IU). The minimum enzyme activity was observed in treatment 4 (22.25 IU). After harvest stage, maximum enzyme activity was found in treatment 2 (18.95 IU) while minimum enzyme activity was observed in treatment 3 (15.84 IU). Overall, it was observed that treatment 3 was showed maximum C1 cellulase enzyme activity.

**Table.3 Quantitative estimation of C-1 cellulase enzyme from the different button mushroom compost formulation at different stages of mushroom production**

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| --- |
| **Filter paperase enzyme 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. 4).

In before spawning stage, treatment 2 showed maximum CMCase activity (43.21 IU) while minimum enzyme activity was recorded in treatment 5 (27.92 IU). In fruit body development stage, treatment 3 recorded the maximum enzyme activity (43.29 IU) followed by treatment 5 (41.55 IU). The minimum enzyme activity was observed in treatment 4 (34.74 IU). After the harvest of crop, maximum enzyme activity was found in treatment 3 (34.53 IU) followed by treatment 5 (33.76 IU) and minimum was in treatment 2 (28.29 IU). Overall, it was observed that treatment 3 was shown maximum CMCase enzyme activity.

**Table. 4 Quantitative estimation of CMCase enzyme from the different button mushroom compost formulation at different stages of mushroom production**

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| --- |
| **CMCase enzyme 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.5). 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 (4.90 IU). In during fruit body development stage, maximum enzyme activity recorded in treatment 2 (10.82 IU) followed by treatment 3 (8.73 IU). The minimum enzyme activity observed in treatment 1 (5.99 IU). After harvest stage, maximum enzyme activity was found in treatment 3 (5.59 IUl) followed by treatment 2 (5.55 IU) and minimum enzyme activity was observed by treatment 1 (4.60 IU).

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

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| --- |
| **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.6). In before spawning stage, treatment 1 showed maximum xylanase enzyme activity (5.25 IU) whereas, minimum enzyme activity was recorded in treatment 5 (3.33 IU). In fruit body development stage, maximum enzyme activity was recorded in treatment 3 (9.68 IU) followed by treatment 2 (6.64 IU) 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 (4.26 IU) and minimum enzyme activity was shown by treatment 5 (3.49 IU). Overall, it was observed that treatment 3 was shown maximum xylanase enzyme activity.

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

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| **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**)**

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 enzymesconvert cellulose in order to oligosaccharides, cellobiose, and glucose (Horn et al. 2012 and Ritota et al. 2019). Endoglucanases 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. Exoglucanases release cellobiose from the reducing or the non-reducing end of the cellulose chain, facilitating the production of mostly cellobiose, which can readily be converted to glucose by β-glucosidases (Zhang et al. 2006; Yeoman et al. 2010 and Madeira et al. 2017). These enzymes may also act on cellodextrins and larger cello-oligosaccharides and are commonly named cellodextrinases (Saini et al. 2015). Oligosaccharides released as a result of these activities 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 (Sajith, et al. 2016). Also Ohmiya et al. (1997) reported that endoglucanase acts on inner sites of oligosaccharides found in carboxymethyl cellulose, cello-oligosaccharides or amorphous cellulose. Exoglucanase hydrolyzes non-reducing ends of crystalline cellulose and forms cellobiose or glucose as the major end products. β-glucosidase acts on non-reducing ends of cellobiose and cellodextrin.

In the process of composting, the production of extracellular hydrolytic enzymes by microbes plays an important role in starting stage of degradation and crop production. These hydrolytic enzymes degrade the lignocellulosic substrate to simple monomers. Carboxymethyl cellulase (CMCase) activity was found to be higher till 60 days and then declined in all the treatments till 90 days. 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). 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. 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.

**CONCLUSION**

Laccase, manganese peroxides, cellulase, and xylanase, were performed for all the treatments at three stages of white button mushroom production (before spawning, fruit body development, and after harvesting). Enzyme activity increased from before spawning to the fruit body development stage, and afterward started to decrease. Maximum enzyme activity was also recorded in treatment 3 (Paddy straw+ wheat straw, 1:1). Most agro-industrial waste is disposed of in landfills or burned, leading to various environmental problems and pose potential harm to the health of humans and wildlife. However, agro-industrial waste can potentially be converted into different high-value products, including biofuels, value-added fine chemicals and cheap energy sources for microbial fermentation and enzyme. These waste products can represent a source of energy, as well as sources of carbon. Additionally, this form of waste is a source of the nutrients that are required for mushroom growth and lignocellulolytic enzyme production via solid state fermentation.

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