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COMPREHENSIVE ASSESSMENT OF QUALITY PARAMETERS IN OYSTER MUSHROOMS (*PLEUROTUS* SPP.) UNDER VARIOUS SUBSTRATES AND ENVIRONMENTAL CONDITIONS

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A B S T R A C T

The quality characteristics of Oyster mushrooms (*Pleurotus* spp.) under various environmental conditions and substrate compositions are thoroughly evaluated in this study. The objective of this successful research was to focus on investigating the role of synergistic relation of temperature ranges of 10-15°C, 15-25°C, and 25-35°C, along with humidity levels of 65% and 85% on various substrates named Substrate A, a combination of wheat straw and sawdust, cardboard, leaves, wheat straw, and sawdust along with the addition of 25 g of Plant fibers, lime and chicken dung in all substrates. The key parameters including First Primordial Initiation (DPI), Number of Effective Fruiting Bodies (NFB), Biological Efficiency (BE), and Yield (in grams) per 500g of substrate dry weight, Stem Diameter, Total Days of cultivation, were employed to check mushroom development. Overall, findings reveal that the optimal conditions for Oyster Mushroom growth are observed at 15-25°C with 85% relative humidity, resulting in the highest Biological Efficiency (BE) at 56% on Substrate A as compared to other environmental ranges, but 65 % RH gives more yield with higher temperatures at 25-35°C., All substrates except leaves give better yield at 85% RH rather than 65%. Leaves give the better yield on 65% RH as at 249g. Substrate A, a combination of wheat straw and sawdust, yielded the most favorable outcomes, exhibiting an average DPI of 7, NFB of 50, and a yield of 375g, with a remarkable BE of 75%. Moreover, the hypothesis is proved that a combination of substrates can provide more yield and results demonstrate that a combination can give more 17.9 % yield. The best substrates in terms of all quality indicators are cardboard and substrate A (wheat straw plus sawdust). This research recommends the Cardboard while focusing on the best Diameter of the Stem as the greatest diameter of the stem in cardboard is 53 but substrate A is the best overall for all factors. It is also recommended to use the combination of the substrates just like substrate A for fast growth because substrate A has less TDM in all conditions. So, this study provides valuable insights into optimizing mushroom cultivation practices by strategically selecting suitable substrates and environmental settings to enhance both yield and quality.

Keywords: Mushroom Farming, Biological Efficiency (BE), First Primordial Initiation (DPI), Yield Optimization.

INTRODUCTION

Mushrooms are enigmatic creatures that have epigeous or hypogenous fruiting bodies with plenty of nutritional benefits (Bao *et al*., 2004) and are macro-filamentous fungi (out of a total of 97330 other filamentous fungi (Patel *et al*., 2012). Mushrooms are abundant in biodiversity, and there are numerous varieties, each with special qualities and traits with 2000 edible out of 14-22000 mushrooms all over the world (Hawksworth, 2001). Multiple kinds of mushrooms, particularly oyster mushrooms (*Pleurotus* spp.), are raised for profit motives in the mushroom business. So mushrooms require various favorable environments like temperature conditions, humidity, and substrate conditions (Haimid *et al*., 2013).

It is generally understood that mushrooms in their

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natural environments have to face the simultaneous effects of a wide range of environmental variables such as temperature, light and humidity; which are the most critical environmental elements (Bret, 1987), these can have an impact on the rate of all biological activities such as reproduction, development, growth, and survival, among others (Wada, 1991). In the context of the influences of temperature and substrate on Oyster Mushrooms, some studies have been explored by Japanese, such as (Uemoto, 1968; Numaguchi and Tanaka, 1986 a; Numaguchi, 1994 a, b), however, the role of the environmental characteristics and substrate of interest was only evaluated separately in there researches, not in interactions, when only one element was altered and the others were kept as constant as a checker.

The oyster mushroom scientifically known as *Pleurotus ostreatus,* was originally officially described in 1975 by N. J. Freiherr one of the Dutch scientists. Later, a German scientist incorporated these oyster mushroom species into the genus *Pleurotus*. Oyster mushrooms, also known as *Pleurotus* species, are an edible mushroom that can be found all over the world but is notably prevalent in southeast, Europe, and China. Compared to other types of mushrooms, oyster mushrooms are the most straight forward, inexpensive, and quickest to cultivate. They also require a small amount of preparation and manufacturing technology (Mandeel *et al*., 2005). *Agaricaceae* is a family that has Oyster mushrooms that have no chlorophyll and are sporophytic in mode of nutrition (Randive, 2012). Spawn is a substrate in which mycelium is impregnated and grows, and it acts as a seed in the development of mushrooms (Senatore and Soriente, 2004), a variety of grains is used to produce spawn like sorghum, barley, and paddy grains also (Khonga *et al*., 2013) on comparison, sorghum is a mycelium carrier than wheat and rice grains because it has short spawn running time (Jongman *et al*., 2018). It is critical to keep the compost and substrate from drying out (BISWAS *et al*., 2011).

They typically flourish in the trash and colonize decomposing organic matter in nature. They exude enzymes that break down the decaying organic matter into tiny fragments that are subsequently taken in as meals (Utami and Susilawati, 2017). The cultivation of oyster mushrooms, including *P. ostreatus*, is simpler, quicker, and more efficient than that of other mushroom species (Mandeel *et al*., 2005). The significant protein content is 30–40% on a dry weight basis. According to our most recent findings, key phases in the management of cholesterol homeostasis are impacted by the consumption of oyster mushrooms in the diet. The biological transformation and biological efficacy ranged from 16.7 to 38.8% and 37.2 to 78.7%, respectively (Ibrahim *et al*., 2020). Mushrooms are recognized for their high vitamin C and B complex content, which is crucial for human wellness, and additionally for their protein content level, which varies from 1.6 to 2.5 percent along with the mineral (Nongthombam *et al*., 2021).

This paper endeavors to provide significant insights by accomplishing the following objectives: (1) Assessing the synergistic impacts of temperature, humidity, and diverse substrates on quality factors, (2) Determining the Optimal Growth Conditions for each quality parameter, (3) Investigating the influence of substrate combinations on mushroom growth, and (4) Offering recommendations based on the findings.

MATERIAL AND METHODS

This study was conducted at the University of Sargodha's Tissue Culture Lab, College of Agriculture, and for the purpose of examining all aspects of oyster mushroom (*Pleurotus ostreatus*) production and comparing yields based on various substrates.

Strategies for Experiment: Substrate A had to be prepared by combining sawdust, wheat straw, in a 1:1 ratio. To compare their yield potential, four more substrates sawdust, wheat straw, cardboard, and leaves were needed to prepare. Raw material was taken and 25 g of Plant fibers was added to raw material each substrate. For cardboard, s laboratory shredder was used to shred cardboard to generate cardboard substrate. Plant residues was manually chopped into short pieces to create plant fibers and mixed with all substrates. Each substrate received a complete moistening as these were dipped in the water having benomyl (0.06g/L) for 12 hours to reduce the chances of pathogen such as *Trichoderma* spp. (Gumede, 2008)

The surplus water from the substrates should be drained from them in the laboratory using inclined steel macro-holed sieves because they were submerged in hot water at 60 °C for 18 hours to achieve a moisture content of 65–75 % (Jour, 2014). This makes it possible to remove any remaining water and keep the moisture level at ideal levels. Addition of 25 g limes to each substrate and let it rest for a week. When 25 g of chicken dung is added, 5 g of oyster mushrooms' nitrogen shortfall is addressed. Adding $CaCO^3$ and $CaSO_4$ to maintain pH balance is also crucial.

Each substrate was placed as 500g inside a bag made of polythene of 7*10 inches then gaping openings of the bags were sealed by cotton and closed by rubber rings. Three copies of each of the six treatments were performed. The hen was placed in an Autoclave machine for being autoclaved at 121 °C and 15-20 pounds of pressure, the bags were allowed to cool. The spawns were created using the PDA-spawn production technique on wheat grains and PDA plates that had been sterilized in LFC and kept at 5 °C. The multilayered spawning method was utilized since it produces outcomes more effectively. The bags were opened, 10% dry weight of spawn was added, along with 25 ml of sterilized distilled water, and then holes were formed, producing 15 holes that were all the same diameter 5mm in comparisons.

Spawn Run: Every sample contains 2 bags to obtain average data. Every bag that was placed in the isolation room at the tissue culture lab, which is 8 meters long and 6 meters wide and contains an air conditioner for proper aeration and cooling as well as to assist in controlling temperature, had all of its quality criteria checked. The lab also had windows and fluorescent lights (20–50 lux) that could offer light during the day and at night, respectively. To determine the effects of temperature on the biological effectiveness of oyster mushrooms, this experiment will endure for approximately 7 months.

Quality Parameters Assessment: The number of effective fruiting bodies (NFB), the days needed for first primordial initiation (DPI), and biological efficiency were all consistently tracked. On all bags, biological effectiveness (BE) was seen. Researchers utilize NFB to analyze consistency, quality, yield estimation, research, and development. After harvesting, it was simple to count them manually. Because DMS is the distinguishing characteristic of oyster mushrooms, the diameter is measured using a screw gauge in millimeters for more accuracy and precision. Following harvest, the yield was quantified in grams using a weight balance. All of the information listed in the results was gathered. The weight of fruiting bodies per dry weight of substrate at the same weight unit, multiplied by 100, is the definition of biological efficiency. Yield was assessed using molecular weight balance (Mandeel *et al*., 2005)

$$\text{Biological efficiency} = \frac{\text{Weight of fresh mushroom fruiting bodies}}{\text{Weight of dry substrate}} \times 100$$

RESULTS

This study delves into the multifaceted aspects of quality in oyster mushroom cultivation, looking at parameters like the number of fruiting bodies, Days Required for First Primordial Initiation (DPI), yield in grams per 500g of substrate, diameter of the oyster mushroom stem, total days for maturation, and the impact of temperature variations and different substrate types on biological efficiency. The sections below, give in-depth evaluations and analyses based on two main assessments—Table 1 and Table 2.

For testing the impacts of three temperature and relative humidity ranges, two bags of Substrate A with a dry weight of 500g were used, as shown in Table 1. Table 1 presents an array of quality indicators, including NFB (number of fruiting bodies), DPI (days for first primordial initiation), mushroom yield (measured in grams per 500g of substrate), stem diameter, and total maturation days. These parameters were assessed across multiple temperature ranges and encompassed two sample measurements.

Temperature	Relative Humidity	NFB	DPI	Yield in g / packet	Diameter (mm) of Stem	Total days for Maturation
10-15°C (winter)	85	49	22	249	44	28
	65	43	25	228	46	28
	Average	46	23	239	45	28
15-25°C (march)	85	52	19	280	48	31
	65	48	17	279	53	31
	Average	50	18	280	50	31
25-35°C(early summer)	85	19	26	94	14	45
	65	19	30	102	16	48
	Average	19	28	98	15	46

Five major substrates were tested for their impacts on yield and biological efficiency using two bags of each substrate, with a dry weight of 500g, while maintaining temperature at 15 to 25°C and relative humidity of sample 1 is deal with 85% and sample 2 with 65 %, respectively, as indicated in Table 2.

Substrates	Samples	DPI	NFB	Yield in g /packet	DIAMETER of STEM	Total days of maturation
Cardboard	1	8	44	380	54	30
	2	10	46	351	52	30
	Average	9	45	366	53	30
Sawdust	1	7	50	260	45	41
	2	5	42	241	36	42
	Average	6	46	251	41	42
Wheat straw	1	11	46	340	34	35
	2	11	40	300	34	35
	Average	11	43	320	34	35
Leaves	1	8	28	232	28	48
	2	9	18	249	30	48
	Average	8	23	241	29	48
Wheat-straw and sawdust combination	1	7	53	397	53	31
	2	6	46	353	43	31
	Average	7	50	375	48	31

Table 2 provides a comprehensive overview of quality metrics, including NFB (number of fruiting bodies), DPI (days for first primordial initiation), mushroom yield (measured in grams per 500g of substrate), stem diameter, and total maturation days. These parameters were evaluated with two samples for each substrate, offering a robust analysis of mushroom growth.

Figures A–F depicts the results of various experiments.

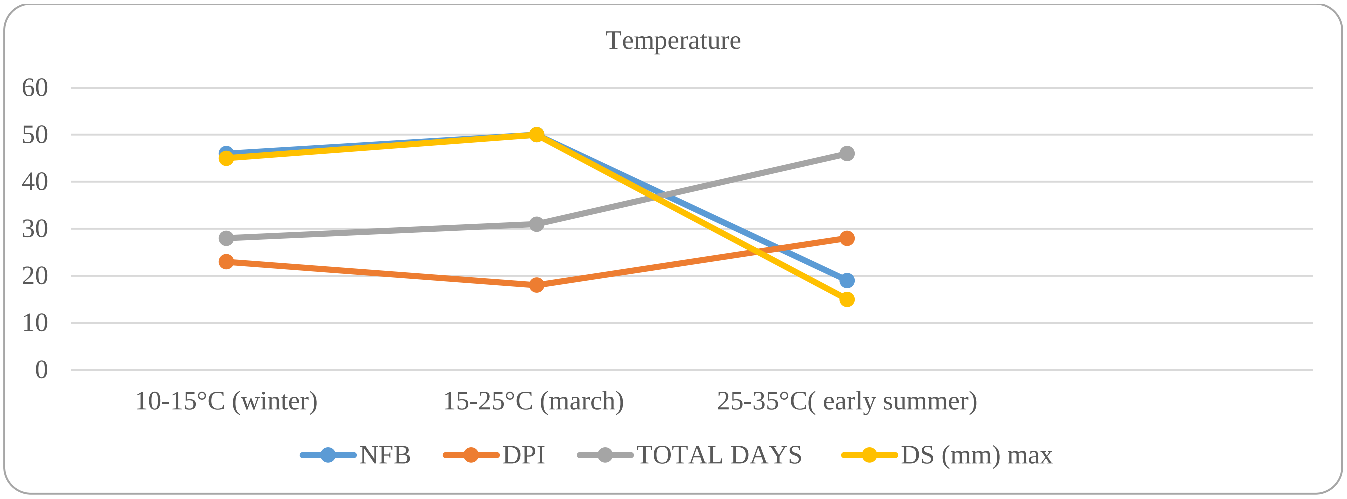

Chart 1 displays the changes in all quality indicators, including NFB (number of fruiting bodies), DPI (diameter of oyster mushroom stem), and total days needed for maturation owing to temperature variations.

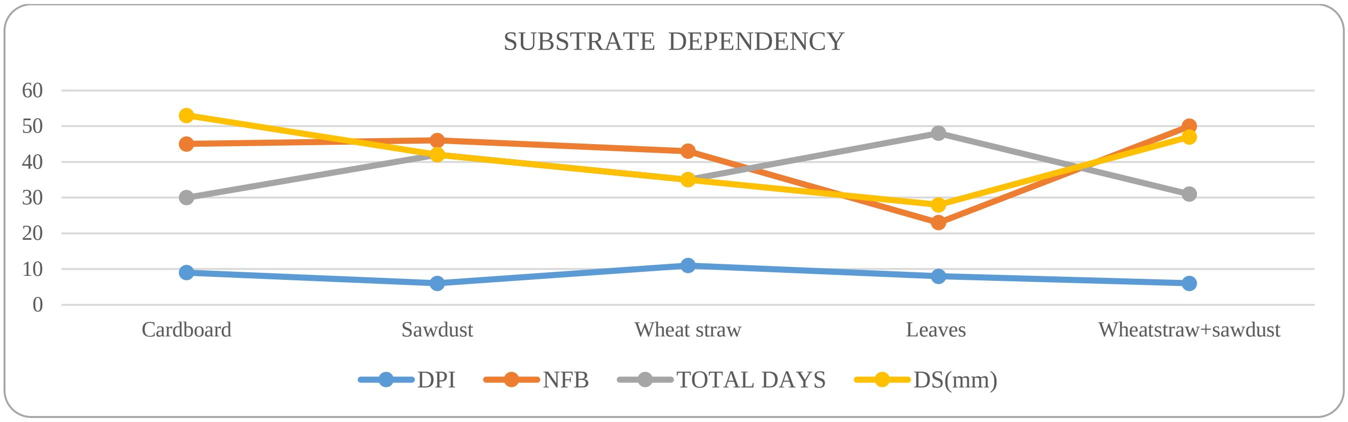

Chart 2 displays the changes in all quality indicators, including NFB (number of fruiting bodies), The number of days needed for initial primordial initiation (DPI, size of the oyster mushroom's stem, Days required overall for maturation due to different substrates

DISCUSSION

Effect of the Environmental Factors: Role of Humidity-Temperature-Substrate Correlations: In terms of yield, the production is higher at 85% humidity compared to 65%, maintaining the same temperature conditions overall. However, at significantly higher temperatures, lower humidity proves to be more effective than higher humidity. For instance, as indicated in Table 1, treatments at 25-35°C with 65% relative humidity result in an average yield of 102g of oysters, whereas treatments at the same temperature range with 85% relative humidity yield slightly less at 98g.

Maintaining a constant relative humidity of 65%, the samples treated at 15 to 25°C exhibit the highest yield, followed by those treated at 10 to 15°C, with the least yield observed in samples treated at 25-35°C, as detailed in both Table 1 and Table 2.

Role of Humidity-Substrate Correlations: At temperature ranging from 15 to 25°C, the relationship with various relative humidity results in different yields on the same substrate. For instance, referring to Table 2, all substrates except leaves exhibit a better yield at 85% relative humidity compared to 65%. This implies that leaves yield better results at 65% relative humidity, specifically at 249g.

Role of Combination of Substrates: From the table 2 it is observed that there is great impact on the yield when we combine 2 different substrates (50% by 50% by weight). For example, the yield of wheat straw and sawdust is 320g and 251g respectively but when we combined these 2 substrates and this mixture lead to substrate A, which give best yield 375 g. Same is the case which is observed in the, table 1

Average of yield of both substrates=320+251/2 =285.5 g

Average of yield of substrate A =375 g

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SUBTRACT **=** 375-285.5=98.5
BE = (95.5/500)*100=17.9%

After Combination of Substrate give BE 17.9 % more than yield: Effects of Environmental Conditions: There is a huge impact of the environmental elements on the growth of the oyster mushrooms as described below:

Effect of Temperature on Yield: Temperature is an important growing factor to focus on for cultivating every living thing. Changing in temperature increase or decrease the living thing's grooming. Results indicate that the best yield occurs between 15 and 25°C, while at 10 to 15°C (winter), the yield weight stand at 280 g per 500 g of substrate A (wheat straw plus sawdust and plant fibers) on an average basis in just 28 days. 25–35 °C treatments reduce the production of substrate A (wheat straw, sawdust, and plant fibers) by up to 98g per 500g on average bases and the best relative humidity (RH), as shown in Table 1, is 85%.

Effect of Temperature On Nfb: A distinct linear trend can be seen in the line graph (Line Graph 1) that illustrates NFB variations. More there will be NFB count more will be yield. NFB count is higher at 15-25°C. The NFB declines proportionately to temperature changes from the ideal range of 15–25°C. The quantity of fruiting bodies drops at both lower and higher temperatures, as NFB was 46 and 19 at 10-15°C and 25-35°C respectively, demonstrating how sensitive this species of mushroom is to temperature changes during cultivation.

Effect of Temperature On Diameter Of Stem Of Mushrooms: The results show a linear correlation between moderate temperatures (15–25°C) and the diameter of the oyster mushroom stem. In this temperature range, the mushroom stem grows more strongly, and the stem diameter decreases at both lower and higher temperatures from 15–25°C.

Effect of Temperature on Biological Efficiency: The larger yields of 280g and better overall growth suggest that the biological efficiency is apparently at its highest at 15–25°C. The information points to a linear link between biological efficiency and temperature. Biological efficiency decreases proportionately to temperature deviations from the ideal range of 15–25°C as BE is 47.8% and 19.6% at 10-15°C and 25–35°C respectively.

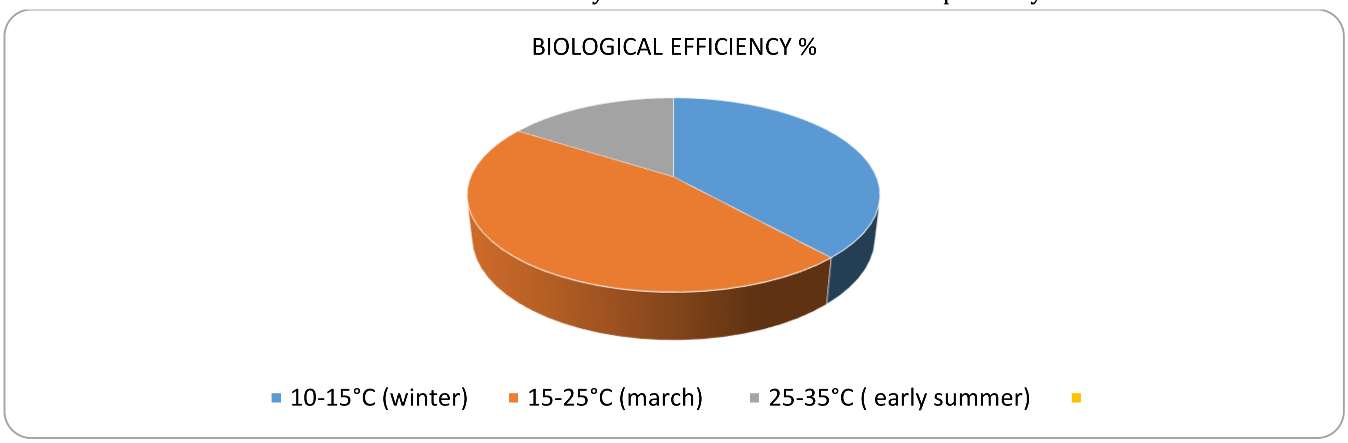

Chart 3 compares biological efficiency percentages for various temperatures.

Effect of Temperature On Total Days Of Maturation: According to the study, the total days for maturation (TDM) are shorter at temperatures ranging from 10 to 15°C. The more there will be shorter the TDM, the more will be cultivated and it is 31 and 46 for 15-25°C and 25-35°C. This information is critical for mushroom producers, emphasizing the significance of maintaining certain temperature conditions in order to accomplish efficient and timely Oyster mushroom development.

Effect of Temperature On DPI: The following phase that initiates a run of mycelial growth is the appearance of pinheads. DPI increased in direct proportion to temperature at 15-25 °C (Table 1). DPI will increase when to moves above and down from the ideal temperature of 15-25 °C as it is 23 and 28 at 10-15°C and 25-35°C respectively.

Effects of Substrates: The substrate is an important growing medium for Oyster mushroom grooming. For the purpose of verifying precise measurements, each substrate was made separately and placed in two plastic bags. These were set up in the laboratory's culture room, which was kept at a comfortable 15 to 25 °C in March and had an 85% humidity level. At the same time, an equal amount of spawn was added to the bags. Data is collected by averaging the yields of 2 treatments for each substrate,

Effects of Substrate On Yield: The variation in yield is evident among diverse substrates, with Substrate A giving the most at 375 grams, while the leaves substrate give relatively less yield, as seen in Table 2. Using a comprehensive approach, the average yields of two treatments for each substrate, as shown in Table 2, highlight the subtle variances, such as cardboard, sawdust, wheat straw, leaves, and wheat-straw and sawdust combination have yields in g of 366,251,220,241,375, respectively. This careful analysis offers useful insights for optimal growing techniques by providing a deep insight into the productivity dynamics linked with various substrates.

Effects of Substrates On NFB: NFB in table 2 was higher in substrate A, of 50 due to the presence of more nutrients that promote growth, conversely, NFB count is lowest in leaves. The rate of NFB in Cardboard and Sawdust are relatively the same. In Wheat straw, NFB is noted at 43. These all variations show that NFB is also dependent on the substrate type.

Effects of Substrate On Diameter of Stem of Mushrooms: Highest DMS is observed in Cardboard at 53 mm, followed by substrate A which shows DMS at 48mm. The rate in Wheat straw and Leaves exhibit relatively parity and low DMS of 34 mm and 29 mm respectively. Sawdust substrate shows a slightly smaller average diameter of 41 mm, suggesting a moderate stem. The data indicates that the type of substrate has a notable impact on the diameter of the Oyster mushroom stem. The choice of substrate influences the nutritional composition and growth conditions, directly affecting the development and thickness of the mushroom.

Effects of Substrate on Biological Efficiency: The results underscore the influence of substrate variations on biological efficiency. Analysis, as depicted in Pie Chart 3, the cardboard shows the BE rate at 73.2%, sawdust shows at 50%, wheat straw stands at 67.2%, leaves at 48.2%, and the combination of wheat straw and sawdust shows the BE rate of 75%. These findings shed light on the substrate-specific nuances that influence biological efficiency, giving useful information for optimizing cultivation tactics.

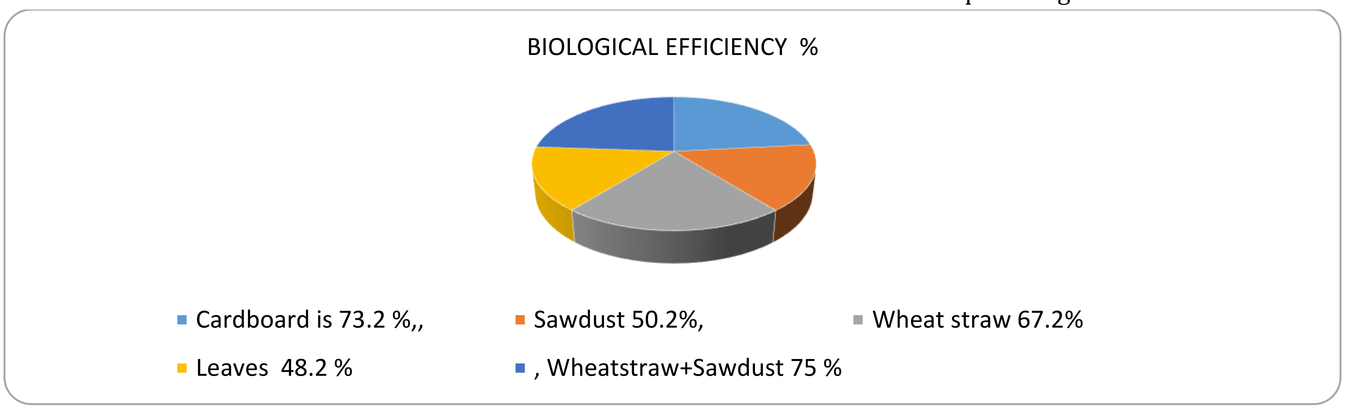

Chart 4 compares biological efficiency on various substrates.

Effects of Substrate on Total Days of Maturation: Substrate A (a mixture of wheat, straw, and sawdust) and cardboard both have lower TDMs of 31 and 30, respectively. Oyster mushrooms can take 48 days with leaves, as illustrated in tables 1 and 2. While Wheat straw give maturation in 35 days and Sawdust take 42 days,

Effect of Substrate On DPI: The Pinhead-like structures as shown in table 2 were developed earlier in the samples of Sawdust substrate in a period of six days, followed by Substrate A which took 7 days. Both these substrates are best to take DPI shorter, and the Cardboard took 9 days which also shows its good efficiency. Pinhead formation can be seen easily in leaves in 9 days and wheat straw substrate took 11 days, which was the maximum as stated in Table 2.

This extensive study delves into the complex interplay of environmental variables, substrate types, and critical quality indicators in oyster mushroom growing. The findings provide useful information for improving farming procedures and optimizing conditions to increase overall output and quality.

CONCLUSIONS

In conclusion, all objectives of this research paper are accomplished explained that there is a huge impact of the synergetic effects of temperature, humidity, and various substrates on the quality factors. Overall, oyster mushrooms yield the most at 10-15°C, 85% relative humidity, and 56% biological efficiency and that the best substrates in terms of all quality indicators are cardboard and substrate A (wheat straw plus sawdust). Secondly, hypothesis is proved that combination of substrates can provide more yield and results demonstrate that combination can give more 17.9 % yield. The number of fruiting bodies, or NFB, was highest for substrate A. The quantity of fruiting bodies drops at both lower and higher temperatures, as NFB was 46 and 19 at 10-15°C and 25-35°C, the recommendations are to get best yield, there should be mixture of substrates just like substrate A, should use 10-15°C and 85% relative humidity. This research recommends the Cardboard for taking best Diameter of Stem as the greatest diameter of the stem in cardboard is 53 but substrate A is best at all. It is also recommended to use the combination of the substrates just like substrate A also for fast growth because substrate A has less TDM in all conditions.

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