**“Growth hormones for enhanced development and nutritional profiling of *Pleurotus florida* (Mont.) Singer”**

**Abstract**

Oyster mushrooms (*Pleurotus florida*) are a sustainable solution for food security, offering high nutrition and ease of cultivation on agro-waste substrates like wheat straw. Unlike traditional crops, they require minimal space and no fertile soil. This study evaluated the effects of growth regulators (CK, NAA and GA₃) on mushroom growth, yield, and nutrition. The T9 treatment [wheat straw + GA₃ (10ppm)] showed the best results, with early pinhead initiation (18 days), superior morphological traits (84 fruiting bodies/bag, stalk length 9.81 cm, cap diameter 12.15 cm), and highest total yield (1451.31 g) across three flushes. It also exhibited the highest biological efficiency (107.5%). Nutritional analysis revealed varying mineral contents across treatments: T6 [wheat straw + NAA (10ppm)] had the highest calcium (11.31 mg) and nitrogen (5.47 mg), while T9 recorded the highest zinc (6.64 mg). These findings highlight the potential of growth regulators in enhancing Oyster mushroom productivity and nutritional value.

**Key words: -** Oyster mushroom, growth regulators (CK, NAA and GA₃), spawn running, pin head formation, biological efficiency, nutritional analysis.

1. **Introduction**

Mushrooms have achieved global recognition due to their significant nutritional composition and bioactive compounds with demonstrated therapeutic effects. Fungiculture represents an ecologically efficient bioconversion strategy, valorizing lignocellulosic waste substrates into high-value mycoprotein products. This biotechnology demonstrates substantial potential for enhancing sustainability in agroforestry systems, with applications spanning both developing economies like India and industrialized nations worldwide.

Fungi play a vital role in nutrient cycling and the decomposition of organic matter, thereby sustaining ecosystem health. Furthermore, they are utilized in various biotechnological applications, such as the synthesis of antibiotics, enzymes, and biofuels . Recent progress in mycological research has emphasized elucidating the genetic and molecular mechanisms of fungi, driving innovations in sustainable agriculture and the discovery of new therapeutic compounds ( ICAR, 2023).

The majority of research efforts have been directed toward determining the optimal cultivation methodology for *Pleurotus* spp. (Oyster mushrooms) utilizing agricultural byproducts, with an emphasis on identifying the most economically viable substrate. Although the cultivation protocol for Oyster mushrooms is relatively simplistic, it demonstrates high productivity while preserving biological efficiency (Zenebe Girmay *et al.,* 2016 ).

Various researchers have conducted studies to find out suitable substrates for optimal growth of mushroom to obtain maximum yield and reducing the time duration for appearance of pin head, spawn run days and days to fruit. Researchers like Sharma *et al* ., (2023) and Sharma and Dal (2024) conducted elaborate research in this area using substrate, composed primarily of sawdust and straw, which served as the growth medium for mushroom cultivation. Thus they concluded that by optimizing substrate composition, growers can enhance yields, potentially increasing profitability and sustainability in the mushroom industry.

Hormonal studies in mushroom production are crucial in India due to their potential to enhance yield, optimize cultivation practices and improve economic viability. Mushrooms, being highly sensitive to phytohormones and growth regulators, exhibit significant variations in mycelial growth, fruiting body formation and overall productivity under hormonal influence.Godse *et al*., (2021) empirically demonstrated that exogenous application of gibberellic acid (GA₃) at concentrations of 15 and 20 ppm respectively elicited statistically significant enhancements in mycelial expansion, manifesting as increased colony diameters (89.98 and 90.00 mm) under *in vitro* conditions.

Nirdesh *et al*., (2019) demonstrated that *Pleurotus sajor-caju* exhibited a quintuple-flush harvesting pattern, with maximal productivity occurring during the initial flush, followed by progressively diminished yields in subsequent second and third flushes across all experimental treatments. The investigation further revealed that an optimized substrate composition comprising 75% wheat straw (*Triticum aestivum*), 25% mustard straw (Brassica spp.), supplemented with 100g wheat bran (*Triticum aestivum*) generated peak yields of 1483g per cultivation bag.

Keeping the importance of role of phytohormones in increasing mushroom production, the research study titled “Studies on the precision application of growth hormones for the cultivation of *Pleurotus florida* (Mont.) Singer” was undertaken.

1. **Materials and Methods**
	1. **Experimental site**

The present investigation was conducted at the Mushroom Research and Development Centre, Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur from 2023 to 2025.

* 1. **Substrate preparation and chemical sterilization**

The wheat straw used in this study was first thoroughly washed in fresh water before undergoing chemical treatment by soaking for 18–20 hours in a formalin solution (2-4%). On the next day, all the wet wheat straw was separated from the water and the additional water was carefully drained. The substrates were kept on a concrete floor that had been sterilized with a 2- 4% formalin sterilization. Thus, the substrates were prepared for use in the mushroom culture procedure.

**2.3 Spawning**

Fresh spawn (15-20 days old) was used in a pre-fumigated room (disinfected with 2% formaldehyde for 36 hours). Polypropylene bags (75×45 cm) were filled by layering substrate and 50g spawn alternately, topped with a final substrate layer. Bags were sealed, perforated with 8-10 holes, and placed on racks for optimal mycelial growth.

**2.4 Method of Spawning**

The polypropylene bag method was selected for *Pleurotus florida* cultivation over the tray-based method due to its superior effectiveness and efficiency. After inoculation, the spawn-impregnated substrates were transferred to a cultivation chamber under sterile conditions. The chamber was sterilized using 2% formaldehyde vapor for 48 hours, followed by thorough aeration to remove residual vapors before cultivation began.

* 1. **Experimental Details**

The present investigation was conducted using a completely randomized design (CRD) with nine treatments and three replications per treatment. The substrate consisted of pure wheat straw supplemented with different growth hormones (CK, NAA and GA3), with 5 kg (dry weight) per bag, and spawning was carried out at 10% of the dry substrate weight to ensure optimal mycelial colonization.

* 1. **Details of various treatments**

|  |  |  |
| --- | --- | --- |
| S.No.  | Treatment | Details |
| 1. | T1 | Wheat straw 5kg + Cytokinin(CK) @ 2ppm |
| 2. | T2 | Wheat straw 5kg + Cytokinin(CK) @ 6ppm |
| 3. | T3 | Wheat straw 5kg + Cytokinin(CK) @ 10ppm |
| 4. | T4 | Wheat straw 5kg + Naphthalene acetic acid (NAA) @ 2ppm |
| 5. | T5 | Wheat straw 5kg + Naphthalene acetic acid (NAA) @ 6ppm |
| 6. | T6 | Wheat straw 5kg + Naphthalene acetic acid (NAA) @ 10ppm |
| 7. | T7 | Wheat straw 5kg + Gibberellic acid (GA3) @ 2ppm |
| 8. | T8 | Wheat straw 5kg + Gibberellic acid (GA3) @ 6ppm |
| 9. | T9 | Wheat straw 5kg + Gibberellic acid (GA3) @ 10ppm |
| 10. | T10 (Control) | Wheat straw 5kg |

The recorded observations focused on various parameters, including the number of primordia and fruiting bodies, as well as the fresh and dry weight of the samples. Additionally, the study measured the total content of both macro and micro elements. The macro elements included nitrogen (N), phosphorus (P), calcium (Ca) and magnesium (Mg), while the micro elements comprised iron (Fe), zinc (Zn) and copper (Cu). These detailed observations provided a comprehensive analysis of the different aspects of the study.

* 1. **Observations recorded**

Various observations including radial mycelial growth, growth behaviour (spawn run period, pinhead formation, and three harvesting phases), growth parameters (number, weight, stalk length, cap diameter, and stalk diameter of fruiting bodies), yield potential (per harvest and total), biological efficiency, and fresh/dry weights. Conducted in a Completely Randomized Design (CRD) with 10 treatments and 3 replications, the study used *Pleurotus florida* (Mont.) Singer. Harvesting flushes were monitored at different intervals to assess productivity.

* 1. **Statistical Analysis**

Each treatment was replicated thrice and values were means ± SE. The data were computed using SPSS software version 21.

The experiments were designed and conducted using a Completely Randomized Design (CRD). Where applicable, the data underwent appropriate transformations and were analyzed using an analysis of variance (ANOVA) table. The critical difference was calculated at a 5% level of significance and the F-value was determined to compare the means of different treatments. This statistical approach ensured the reliability and accuracy of the experimental results. C.D. was calculated by following formula:

$$C.D=\frac{\sqrt{2VE}}{2r}X t at 5$$

Where,

C.D. = Critical difference

VE = Error variance

r = No. of replication is the value at 5% level of significance for error degree of freedom.

**3. Results and discussion**

**3.1 Effect of** **exogenous application of growth hormones at different concentrations on spawn running, pin head initiation and harvesting period of *Pleurotus florida.***

The results as observed in Table 1 indicates that T9 (wheat straw + GA3 10ppm) showed the fastest spawn run (12 days vs. 19 in control), aligning with past studies (Mukhopadhyay *et al*., 2005). It also had the earliest pinhead formation (18 days vs. 24 days in control). Harvesting was quickest in T9 (24, 32, 43 days for 1st to 3rd flushes), matching findings by Godse *et al*. (2021). GA3-treated substrates enhance mycelial growth, reducing production time. Further , the research conducted by various researchers such as Sharma and Lal (2024) showed that use of wheat straw as substrate helped reducing the spawn run time (8 days), pinhead initiation (9 days), fruiting formation (11 days), maximum number of mature fruiting bodies (32), length of stipe (2.70cm), yield (200.3g), biological efficiency (164%) and benefit-cost ratio (1:2.8).

**Table 1: Effect of** **growth hormones on spawn running, pin head initiation and harvesting period of *Pleurotus florida***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  **Treatment**  **details** | **Spawn run periods (days)** | **Pinhead initiation periods (days)** | **First harvesting****(days)** | **Second harvesting****(days)** | **Third harvesting****(days)** | **Total harvesting periods****(days)** |
| T1 | 18.00 | 22.00 | 31.00 | 41.00 | 51.00 | 51.00 |
| T2 | 15.00 | 21.00 | 28.00 | 38.00 | 48.00 | 48.00 |
| T3 | 14.00 | 20.00 | 26.00 | 34.00 | 45.00 | 45.00 |
| T4 | 17.00 | 22.00 | 30.00 | 40.00 | 51.00 | 51.00 |
| T5 | 15.00 | 20.00 | 28.00 | 37.00 | 47.00 | 47.00 |
| T6 | 13.00 | 19.00 | 25.00 | 33.00 | 44.00 | 44.00 |
| T7 | 17.00 | 22.00 | 30.00 | 39.00 | 49.00 | 49.00 |
| T8 | 15.00 | 20.00 | 27.00 | 36. 00 | 46.00 | 46.00 |
| T9 | 12.00 | 18.00 | 24.00 | 32.00 | 43.00 | 43.00 |
| T10 (Control) | 19.00 | 24.00 | 32.00 | 42.00 | 52.00 | 52.00 |
| **C.D at 5%** | 1.450 | 1.949 | 2.638 | 3.491 | 4.497 | 2.860 |
| **SE(m)** | 0.488 | 0.656 | 0.887 | 1.175 | 1.513 | 0.969 |
| **SE(d)** | 0.690 | 0.928 | 1.255 | 1.661 | 2.140 | 1.371 |

# **3.2 Effect of distinct growth hormones at different concentrations on the phenological attributes (Fruiting bodies, their weight, stalk length and cap diameter) of *Pleurotus florida***

The results as observed from Table 2 indicates that significant variations were observed in stipe length and pileus width across different treatments, with T9 (wheat straw 5 kg + GA3 10ppm) yielding the highest measurements of 9.81 cm and 12.15 cm, respectively, while the control (T10) showed the lowest values of 5.69 cm and 8.75 cm. These results align with previous research by Singh and Prasad (2012), who noted that GA3-treated bags produced longer stipes . Additionally, T9 also produced the highest number of fruiting bodies (84), compared to the control (55). Similar results were reported by Sarkar and Chowdhury (2013), who observed peak fruiting body counts at 10 ppm and 20 ppm GA3, further validating the study's conclusions.

# **Table 2:- Effect of distinct growth hormones on the phenological attributes (Fruiting bodies, their weight, stalk length and cap diameter) of *Pleurotus florida***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment details** | **Maximum****individual weight of fruiting body (g)** | **Stalk****length (cm)** | **Cap****diameter (cm)** | **Number****of fruiting bodies** | **Per cent increased on fruiting bodies over control**  |
| T1 | 24.72 | 7.86 | 9.20 | 61.00 | 10.9 |
| T2 | 26.93 | 8.59 | 10.52 | 69.00 | 25.4 |
| T3 | 29.09 | 9.56 | 11.35 | 79.00 |  43.6 |
| T4 | 25.28 | 8.28 | 9.58 | 63.00 | 14.5 |
| T5 | 28.11 | 8.69 | 10.72 | 73.00 | 32.7 |
| T6 | 29.84 | 9.79 | 11.65 | 80.00 | 45.4 |
| T7 | 25.71 | 8.49 | 10.35 | 65.00 | 18.1 |
| T8 | 28.57 | 9.46 | 10.98 | 78.00 | 41.8 |
| T9 | 32.05 | 9.81 | 12.15 | 84.00 | 52.7 |
|  T10 (Control) | 23.47 | 5.69 | 8.75 | 55.00 | -- |
| **C.D at 5%** | 1.771 | 0.570 | 0.674 | 4.335 |  |
| **SE(m)** | 0.600 | 0.193 | 0.228 | 1.574 |  |
| **SE(d)** | 0.849 | 0.273 | 0.323 | 2.226 |  |

**3.3 Effect of distinct growth hormones** **at different concentrations on the yield potential and biological efficiency of Oyster mushroom (*Pleurotus florida).***

 The results as obtained from Table 3 indicates that the highest yield was observed in T9 (wheat straw 5 kg + GA3 10ppm) with 1451.31 g, followed by T6 (NAA 10ppm) and T3 (CK 10ppm) with 1425.30 g and 1351.97 g, respectively. Additionally, T9 showed the highest fresh (1451.31 g) and dry weight (177.21 g), while the control group had the lowest. Studies by Tolera *et al*. (2017) and Rashid *et al*. (2016) supported these results, noting high moisture content in fresh mushrooms. Biological efficiency was also highest in T9 (107.50%), followed by T6 (105.57%), with the control at 74.09%. Researchers like Chourasia *et al*. (2020) highlighted that growth regulators improve biological efficiency due to mushrooms' rich nutrient content, emphasizing their importance in nutritious diets. Nutritional composition varied significantly across treatments, further proving the impact of growth regulators.

**Table 3:- Effect of growth hormones on the yield potential and biological efficiency of Oyster mushroom (*Pleurotus florida)***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Treatment details** | **Total weight of first flush (g)** | **Total weight of second flush(g)** |  **Total weight of third flush(g)** | **Total****yield (g)** | **Per cent increase of yield over control** | **Biological****efficiency (%)** |
| T1 | 373.21 | 337.21 | 335.55 | 1045.97 | 4.59 | 77.40 |
| T2 | 388.88 | 383.88 | 360.55 | 1133.31 | 13.33 | 83.94 |
| T3 | 467.55 | 457.21 | 427.21 | 1351.97 | 35.19 | 100.14 |
| T4 | 376.55 | 347.21 | 340.55 | 1064.31 | 6.431 | 78.83 |
| T5 | 395.55 | 393.88 | 373.88 | 1163.31 | 16.33 | 86.17 |
| T6 | 482.21 | 472.21 | 470.88 | 1425.30 | 42.53 | 105.57 |
| T7 | 377.21 | 360.88 | 350.55 | 1088.64 | 8.86 | 80.64 |
| T8 | 402.21 | 413.88 | 377.21 | 1193.30 | 19.33 | 88.39 |
| T9 | 497.21 | 480.55 | 473.55 | 1451.31 | 45.13 | 107.50 |
|  T10 ( Control ) | 365.88 | 330.88 | 320.55 | 1000.31 | ----------- | 74.09 |
| **C.D at 5%** | 5.613 | 5.462 |  3.261 | 324.56 |  |  |
| **SE(m)** | 1.965 | 1.534 | 1.213 | 110.31 |  |  |
| **SE(d)** | 2.663 | 0.781 | 1.654 | 156.22 |  |  |

**3.4** **Effect of distinct growth hormones at different concentrations on concentrations on nutritional composition of *Pleurotus florida***

 The results as observed from Table 4 revealed significant variations in mineral content across different treatments due to growth regulator application. The treatment T6 (wheat straw 5 kg + NAA 10ppm) showed the highest calcium (11.31 mg) and nitrogen (5.32 mg), while T9 (wheat straw 5 kg + GA3 10ppm) had the highest zinc (6.64 mg) content. The control (T10) consistently recorded the lowest levels, such as calcium (9.98 mg) and zinc (6.29 mg). Phosphorus content peaked in T3 (wheat straw 5 kg + CK 10ppm) at 1.45 mg, whereas T5 (wheat straw 5 kg + NAA 6ppm) had the highest copper (39.85 mg) content. Additionally, T6 and T3 exhibited the highest iron (59.20 mg) and magnesium (50.68 mg), respectively, compared to the control’s lowest values (iron: 51.25 mg; magnesium: 47.70 mg).

**Table 4:- Effect of growth hormones on nutritional composition of *Pleurotus florida***

|  |  |
| --- | --- |
| **Treatment details** | **Nutrient contents(mg\100g)** |
| **N** | **P** | **Ca** | **Mg** | **Fe** | **Zn** | **Cu** |
| T1 | 4.99 | 1.20 | 10.99 | 48.70 | 53.27 | 6.44 | 38.17 |
| T2 | 5.24 | 1.13 | 10.70 | 49.56 | 53.16 | 6.46 | 39.22 |
| T3 | 5.13 | 1.45 | 11.20 | 50.68 | 54.34 | 6.53 | 39.60 |
| T4 | 5.16 | 1.13 | 10.98 | 49.09 | 51.36 | 6.35 | 38.15 |
| T5 | 5.15 | 1.14 | 11.00 | 48.38 | 51.41 | 6.36 | 39.85 |
| T6 | 5.47 | 1.23 | 11.31 | 49.80 | 69.20 | 6.54 | 39.60 |
| T7 | 5.18 | 1.22 | 11.06 | 49.01 | 53.78 | 6.47 | 39.39 |
| T8 | 4.99 | 1.14 | 11.07 | 48.09 | 53.17 | 6.44 | 38.15 |
| T9 | 5.11 | 1.03 | 11.20 | 49.02 | 54.56 | 6.64 | 39.41 |
| T10 (Control) | 4.88 | 0.94 | 9.98 | 47.70 | 51.25 | 6.29 | 37.85 |
| **C.D at 5%** | 0.142 | 0.122 | 0.939 |  0.877 | 0.619 | 0.171 | 0.854 |
| **SE(m)** | 0.056 | 0.041 | 0.341 | 0.365 | 0.275 | 0.042 | 0.493 |
| **SE(d)** | 0.073 | 0.058 | 0.462 | 0.516 | 0.436 | 0.060 | 0.597 |

**4. Conclusion**

Ensuring global food security with nutritious options is a challenge and here mushroom cultivation and production appears as a protein-rich, farm-free crop grown on agricultural waste as an ideal solution. Oyster mushrooms, in particular, offer higher nutrition, easier cultivation, and better yields than other mushroom species. The study conducted indicates that treatment T9 (wheat straw + GA3 10ppm) performed best with faster spawn running pinhead formation , and harvests . It yielded the most fruiting bodies, longest stipe, highest biological efficiency, and total yield . Thus , T9 treatment can be further advised for commercial cultivation of mushroom for getting profitable yields.

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. No AI or Chat gpt has been used for writing the content of this article

**References:**

1. Godse, D., Kumbhar, C., Jadhav, A., & Shitole, L. (2021). Effect of Growth Regulators and Micronutrients on Growth and Yield of *Pleurotus sajor-caju*. International Journal of Current Microbiology and Applied Sciences, 10(12), 240-250.
2. **ICAR (Indian Council of Agricultural Research).** (2023). Biotechnological applications of fungi in agriculture. ICAR Technical Bulletin.
3. **Mukhopadhyay, R., Chatterjee, S., Chatterjee, B. P., & Guha, A. K. (2005). Enhancement of biomass production of edible mushroom *Pleurotus sajor-caju* grown in whey by plant growth hormones. Process Biochemistry, 40(3-4), 1241-1244.**
4. **Kumar, N., Biswas, S. K., Lal, K. I. S. H. A. N., Baboo, D. I. P. A. K., & Hussain, A. R. S. H. A. D. (2019). Integrated effect of different substrates on growth parameters and yield of *Pleurotus sajor caju*. Journal of Mycopathology Research, 57(1), 35-40.**
5. **Rashid, M. H. O., Bhattacharjya, D. K., Paul, R. K., Rahaman, M. S., Rahaman, M. S., Miah, M. N., & Ahmed, K. U. (2016). Effect of different saw dust substrates on the growth and yield of Oyster Mushroom (*Pleurotus florida*). Bioresearch Communications-(BRC), 2(1), 193-199.**
6. **Sarker, R. R., & Chowdhury, A. K. M. S. H. (2013). Effect of different doses of GA3 application at primordia initiation stage on the growth and yield of Oyster mushroom. Journal of the Bangladesh Agricultural University, 11(1), 5-10.**
7. **Sharma, V., Kumar, D. M., Singh, S., Pandey, A., & Kumar, M. (2023). Application of substrate supplements to the yield oyster mushrooms (*Pleurotus ostreatus* var. *florida*). Int. J. Environ. Clim. Change, 13(11), 1878-1885.**
8. **Sharma, G. N., & Lal, A. A. (2024). Evaluation of Different Substrates for Growth and Yield Parameters of Pink Oyster Mushroom (*Pleurotus djamor*). Journal of Experimental Agriculture International, 46(12), 197-204.**
9. Singh, S. D., & Prasad, G. (2012). Effect of different Substrate supplements on the growth and yield of two species of Mushroom *Pleurotus florida* and *P. sajor-caju*. International Multidisciplinary Research Journal, **2**(3), 61-64.
10. Tolera, K. D., & Abera, S. (2017). Nutritional quality of Oyster Mushroom (*Pleurotus Ostreatus*) as affected by osmotic pretreatments and drying methods. *Food science & nutrition*, ***5***(5), 989-996.
11. Zenebe Girmay, Z. G., Weldesemayat Gorems, W. G., Getachew Birhanu, G. B., & Solomon Zewdie, S. Z. (2016). Growth and yield performance of *Pleurotus ostreatus* (Jacq. Fr.) Kumm (oyster mushroom) on different substrates.