



Optimization of Critical Factors for *In Vitro* Vegetative Growth of *Pleurotus cornucopiae* to Improve Branched Oyster Mushroom Production

Arunesh Kumar¹, R. S. Jarial², Kumud Jarial², Savita Jandaik¹, Meenu Gupta³, Chidembra Bhardwaj⁴, Satish Kumar Sharma¹, Ankita⁵ and Surender Kumar^{6*}

¹Dept. of Plant Pathology, ³Dept. of Vegetable Science, ⁶Dept. of Biotechnology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh (173 230), India

²Dept. of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh (177 001), India

⁴Dept. of Agriculture, Khalsa College Amritsar, Punjab (143 002), India

⁵Dept. of Forestry, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar (848 125), India

Corresponding Author

Surender Kumar
e-mail: kr.surender.001@gmail.com

Article History

Received on 18th April, 2025
Received in revised form on 05th July, 2025
Accepted in final form on 19th July, 2025
Published on 31st July, 2025

Abstract

The present investigation was carried out during (January–April) 2025 at Mushroom Research Laboratory, Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur (HP) in which effect of different cultural parameters on vegetative growth of *P. cornucopiae* was evaluated. Mushroom, an edible fungus, grows on moist surfaces such as wood or other organic matter including natural or semi-synthetic compost and consume globally due to their high nutritious vegetarian delicacy. The first crucial step in the successful generation of spawn was the preservation and restoration of a pure mycelium culture of exceptional quality. The fungus *P. cornucopiae*, branched oyster mushroom was widely used due to its nutritional and medicinal properties. Its cultivation was rather new in Indian conditions and much work has not yet been done. The solid and liquid media including potato dextrose, malt extract, oat meal, Asthana and Hawker's and Czapek's dox were evaluated in the present study for *P. cornucopiae* growth in which maximum diametric growth on potato dextrose agar and minimum on Asthana and Hawker's agar were recorded. Similarly, maximum biomass was observed in potato dextrose broth and minimum in Asthana and Hawker's broth. The growth was maximum under neutral and minimum under acidic condition, which ranged from sparse, fluffy to thick strand, and optimum at 25°C and pH 7. It will require to validate these findings through comparative studies on different mushroom varieties.

Keywords: Mushroom, *Pleurotus cornucopiae*, liquid media, temperature, pH

1. Introduction

Global climate change has resulted drastic and negative impacts on soil and water being essential for optimal crop productivity, which has become even more challenging with increasing global population and reduced cultivation area. Mushroom is a better option to ensure global food security (Lee et al., 2018; Raman et al., 2020; Iwalokun et al., 2007), which is highly nutritious and vegetarian exotic substitute to meat and eggs. Mushrooms are rich of protein, vitamins (B, C and D, fiber, essential minerals (K, P, Se and Zn)

and amino acids (Kim et al., 2015; Jeet et al., 2022; Uzomba et al., 2024). They are thrived to growth in both tropical and temperate regions with minimum economic and operational requirements for commercial growth (Aditya et al., 2024) such as natural or synthetic media including lignocellulose substrates such as crop residue, processed waste, horticultural waste, saw dust, wood chips and natural or semi synthetic compost were used as substrates and required 20–30°C temperature and 55–75% relative humidity (Upamanya et al., 2021; Lee et al., 2018).



Asia is leading in the oyster mushroom cultivation due to low cost and production technology, easy availability of substrates, temperature tolerance and high yield capacity. The global mushroom production is reaching to \$86.6 billion by 2025 with an estimated annual growth rate of 9.5% from 2021 to 2028 (Sganzerla et al., 2022) in which oyster mushroom, the second most cultivated mushroom worldwide, has a significant contribution. China with 74% yield is dominated leader in oyster mushroom globally, followed by European countries, South Korea, and India (Aditya et al., 2024).

In India, commercially important edible mushrooms are white button mushroom (*Agaricus bisporus*) with 85% cultivation and oyster mushroom (*Pleurotus* spp.) with 24.1% cultivation (Sharma et al., 2019; Upamanya et al., 2021). *Pleurotus cornucopiae* is the branched oyster mushroom species, which is easy to cultivate with little investment and environment friendly way of using waste materials to produce highly nutritious food. was not practiced very commonly in India. It is characterized by a cap in younger stage, which is cream colored and then becomes yellowish-ochraceous and turns darker ochraceous-dark brown at maturity. The cap is funnel shaped while the inner flesh is white, thin with a pleasant odour and mild taste (Krishnapriya et al., 2017; Aditya et al., 2024). The optimum temperature range for its cultivation is between 25 to 35°C in both temperate and tropical regions (Jang et al., 2005). Substrates rich of lignocellulosic materials including wheat straw, sawdust, palm cones, corn cobs, sugarcane pulp, coconut fiber, sugarcane pulp and cotton waste commonly used for mushroom production by promoting the mycelial growth and fruit formation (Liaqat et al., 2014; Das et al., 2014; Kataoka et al., 2025).

The first crucial step in ensuring the success of spawn production is the preservation and resuscitation of pure mycelium of exceptional quality (Sharma et al., 2019b). Solid culture medium is considered appropriate for the growth of *Pleurotus* spp. (Vieira et al., 2013). Which found optimum on oat meal agar, potato dextrose agar, malt extract agar (Suharban and Nair, 1994; Nasim et al., 2001), corn extract agar (Khandakar et al., 2008) and cassava pillings extract agar (Stanley and Nyenke, 2011). Hence, the experiment was conducted to evaluate the effects of different cultural parameters including media composition, pH and temperature on vegetative growth of *P. cornucopiae*.

2. Materials and Methods

The present investigation was carried out during (January–April) 2025 at Mushroom Research Laboratory, Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur (HP), which is located within 30° 51'N and 77° 11'E longitude 1250 m above the sea level.

2.1. Evaluation of basal medium

Cultural studies were undertaken with the standard procedure laid down by Lilly and Barnett (1951) and Tuite (1969) with

some modifications. Five different nutrient media viz., potato dextrose, malt extract, oat meal, Asthana and Hawker's and Czapek's dox were evaluated for mycelial growth of *P. cornucopiae* in both solid and liquid media. A 5 mm bit of test fungus from pure culture was inoculated on solid and liquid media using a sterilized cork borer and incubated in a BOD incubator at 25±1°C. In the case of solid media, observations on average diametric growth of mycelia, pigmentation was recorded after 7 days of inoculation. The average fungal biomass after 7, 14, and 21 days of inoculation was estimated in the liquid media by filtering through Whatman No.1 filter paper and repeatedly washed with distilled water and dried at 70°C overnight. The dry weight of the fungus was calculated by using the following formula:

Dry weight=(Weight of filter paper+mycelium)-(Weight of filter paper)

2.2. Effect of pH on *P. cornucopiae* growth

The growth of *P. cornucopiae* was evaluated in potato dextrose agar at different pH regimes viz., 4.0, 5.0, 6.0, 7.0, and 8.0, adjusted with the help of pH meter by inoculated with test fungus (5 mm bit) and incubated in BOD incubator at 25 ± 1°C. The data were recorded in terms of average diametric growth and type of growth after 7 days of inoculation.

2.3. Effect of temperature on *P. cornucopiae* growth

To study the effect of temperature, Petri plates containing PDA as a basal medium was inoculated with 5 mm bits of tested fungus and incubated at different temperatures viz., 10, 15, 20, 25, 30, and 35°C for 7 days. The average diametric growth (mm) and type of growth was recorded for each treatment with three replicates.

3. Results and Discussion

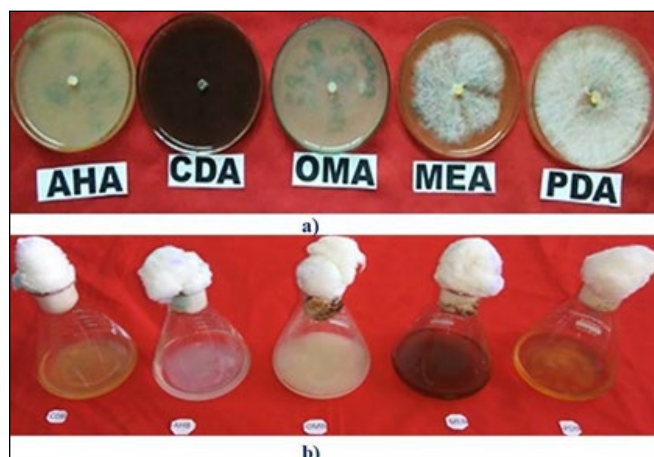
Potato dextrose agar medium supported maximum (88.55 mm) mycelial growth, which was statistically at par with malt extract agar medium (85.55 mm) and significant followed by oatmeal agar (80.88 mm) (Table 1). However, a significant minimum (43.22 mm) mycelial growth was recorded in Asthana and Hawker's agar medium. Mycelial character of the test fungus was varied from white to translucent in different media with thick strands, thin strands, and sparse growth (Figure 1a). These results were agreed with Rawte and Diwan (2011) and Sardar et al. (2015), who tested different media for culturing *Pleurotus* species and concluded that potato dextrose agar medium was best for the growth of *Pleurotus* species. Similar results were noticed by Kumla et al. (2013) who observed the best mycelial growth of *Pleurotus giganteus* on potato dextrose agar medium.

The irrespective of the days of observation, maximum (676.00 mg) average biomass was recorded in potato dextrose broth (Figure 1b) followed by malt extract broth (587.66 mg) and oat meal broth (531.33 mg) (Table 2). However, significantly minimum (268.66 mg) fungal biomass was recorded in



Table 1: Effect of different solid media on mycelial growth of the *Pleurotus cornucopiae*

Media	Average diametric growth (mm)	Colour of mycelium	Type of growth
Potato dextrose agar	88.55	White	Thin strandy
Malt extract agar	85.55	White	Thick strand
Oat meal agar	80.88	Translucent to white	Sparse
Czapek's dox agar	52.55	Translucent	Sparse
Asthana and Hawker's agar	43.22	Translucent	Sparse
Mean	70.15		
SEm±	2.35		
CD ($p=0.05$)	5.23		

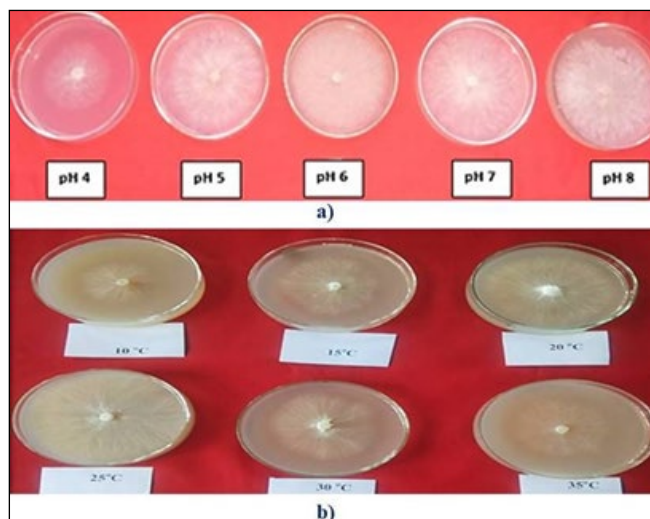
Figure 1: Effects of different solid (a) and liquid media (b) on the growth of *P. cornucopiae*

Asthana and Hawker's broth followed by Czapek's dox broth (299.00 mg). Irrespective of the liquid media tested, maximum (690.00 mg) fungal biomass was observed after 21 days of inoculation followed significantly by 14 (471.00 mg) and 7 days (256.60 mg) after inoculation. The maximum (987.00 mg) fungal biomass was supported by potato dextrose broth after 21 days of inoculation, which was minimum (146.00 mg) for Asthana and Hawker's broth after 7 days of inoculation. Similar findings were observed by Amita and Atri (2017) in the vegetative growth of *P. cystidiosus* in the yeast glucose medium (7.9 mg ml^{-1}) followed by potato dextrose broth (6.9 mg ml^{-1}) while, minimum growth recorded in Asthana and Hawker broth (0.08 mg ml^{-1}).

The average diametric growth of *P. cornucopiae* was maximum

Table 2: Effect of different liquid media on *Pleurotus cornucopiae* biomass production

Media	Average biomass after days of inoculation (mg)			
	7 days	14 days	21 days	Mean
Potato dextrose broth	365.00	676.00	987.00	676.00
Malt extract broth	317.00	581.00	865.00	587.66
Oat meal broth	284.00	503.00	807.00	531.33
Czapek's dox broth	171.00	309.00	417.00	299.00
Asthana and Hawker's broth	146.00	286.00	374.00	268.66
Mean	256.60	471.00	690.00	
	SEm±	CD ($p=0.05$)		
Days	1.14	3.33		
Treatment	1.48	4.30		
Interaction	2.56	7.45		

Figure 2: Effects of pH (a) and temperature (b) on the growth of *P. cornucopiae*

(83.25 mm) at pH 7.0 which was statistically at par with growth (81.83 mm) at pH 6.0 (Table 3), while significantly slower diametric growth (53.91 mm) was recorded at pH 4.0 (53.91 mm) after 7 days of inoculation. The mycelial growth was thick strandy at pH 7.0 and 8.0, thin strandy and fluffy at pH 5.0 and 6.0 and sparse growth at pH 4.0 (Figure 2a). The optimum pH was in accordance with the observations of Yadav and Chandra (2014), who reported best fungal growth for all the strains of *Pleurotus* spp. at pH 7.0 and Fellal et al. (2003) who obtained an optimum growth of *P. columbinus* and *P. pulmonarius* at pH 7.0.

The average diametric growth of *P. cornucopiae* was significantly maximum (85.00 mm) at 25°C followed by 20°C

Table 3: Effect of pH on mycelial growth of *Pleurotus cornucopiae*

Treatments (pH)	Average diametric growth (mm)	Growth pattern
4.0	53.91	Sparse
5.0	72.08	Thin strand
6.0	81.83	Fluffy
7.0	83.25	Thick strand
8.0	75.25	Thick strand
Mean	73.26	
SEm±	1.81	
CD ($p=0.05$)	4.04	

(67.41 mm) and 30°C (60.66 mm), while slower and minimum growth was recorded at 10°C (39.16 mm) (Figure 2b; Table 4). The mycelium was thick strandy, thin strandy and strandy at different temperatures. More or less similar results were reported by Rangad and Jandaik (1977) who obtained highest mycelial growth of *P. ostreatus* at 25°C and Zhao and Cui (2002) who found 25°C as the optimum temperature for mycelial growth of *P. ferulae*.

Khan et al. (2013) reported that pH is an important factor

Table 4: Effect of temperature on mycelial growth of *Pleurotus cornucopiae*

Temperature (°C)	Average diametric growth (mm)	Growth pattern
10	39.16	Strandy
15	57.91	Strandy
20	67.41	Thin strand
25	85.00	Thick strand
30	60.66	Thin strand
35	55.00	Strandy
Mean	60.85	
SEm±	0.83	
CD ($p=0.05$)	1.81	

for the production of oyster mushroom (*Pleurotus* sp.) which grew well with high yield at slightly basic pH. The pH 5–6 and 6.2 and 6.4 were found best for the growth of *Pleurotus* spp., *P. florida* and *P. citrinopileatus*, and *P. ostreatus* (Singh et al., 2000; Mukherjee and Nandi, 2000). The *P. ostreatus* growth was decreased in pH between 6.5 and 7.5 (Ibekwe et al., 2008). Peksen et al. (1999) found good mycelial growth when spores were kept at 35°C for three days before incubation at 25°C on malt extract agar. Ayman et al. (2009) studied effect of temperature on mycelia growth of *Pleurotus* spp., and found 25°C as optimum temperature for its growth.

In the case of liquid media, Rawte and Diwan (2011) evaluated

Richard's Broth, Asthana Hawker's, Czapek's dox and potato dextrose broth for biomass production and found maximum growth of *P. florida* on Asthana Hawker's media. Growth was probably best in the dark but continuous light (below 250 lux) was best for fruiting. *P. cornucopiae* has been successfully grown at 20–28°C (Date, 1997). Cultivation of *P. cornucopiae*, the branched oyster mushroom was rather new in Indian conditions and much work has not yet been done on this species. With this background, the present study was undertaken to study the effect of different cultural parameters on the vegetative growth of *P. cornucopiae*.

4. Conclusion

The evaluation of *P. cornucopiae* revealed maximum diametric growth on potato dextrose agar and minimum on Asthana and Hawker's agar. Similarly, maximum biomass was observed in potato dextrose broth and minimum in Asthana and Hawker's broth. The growth was maximum under neutral and minimum under acidic condition. The *P. cornucopiae* growth was ranged from sparse, fluffy to thick strand, and optimum at 25°C and pH 7. It would require to validate these findings through comparative studies on different mushroom varieties.

5. Acknowledgment

The authors are thankful to Department of Plant Pathology, Dr. Y.S.P. University of Horticulture and Forestry, Nauni (H.P.), India, for providing research facilities and assistance during this study.

6. References

- Aditya, Neeraj, Jarial, R.S., Jarial, K., Bhatia, J.N., 2024. Comprehensive review on oyster mushroom species (Agaricomycetes): Morphology, nutrition, cultivation and future aspects. *Heliyon* 10(5), e26539.
- Amita, Atri, N.S., 2017. Evaluation of physical parameters for vegetative growth of *Pleurotus cystidiosus*. *Mycological Society of India* 49, 72–76.
- Ayman, S.D., Sanaa, S.K., Fatma, E.N., William, A.B., 2008. Production of mushroom (*Pleurotus ostreatus*) in Egypt a source of nutritional and medicinal food (Specialty mushrooms). *World Journal of Agricultural Sciences* 4(5), 630–634. ISSN 1817–3047.
- Das, D., Kadiruzzaman, M., Adhikary, S., Kabir, M., Akhtaruzzaman, M., 2014. Yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates. *Bangladesh Journal of Agricultural Research* 38, 613–623.
- Date, K., 1997. Cultivation of Tamogitake (*Pleurotus cornucopiae*). *Food reviews international* 13, 401–405.
- Fellal, E.I., Dein, E.I., Khalil, 2003. Studies on some requirements for vegetative growth of two *Pleurotus* spp. *Egyptian Journal of Microbiology* 38(1), 27–38.
- Golak-Siwulska, I., Kałużewicz, A., Spiżewski, T., Siwulski, M., Sobieralski, K., 2018. Bioactive compounds and



- medicinal properties of oyster mushrooms (*Pleurotus* sp.). *Folia Horticulturae* 30(2), 191–201.
- Ibekwe, V.I., Azubuike, P.I., Ezeji, E.U., Chinakwe, E.C., 2008. Effect of Nutrient sources and Environmental Factors on the cultivation and yield of oyster mushroom (*Pleurotus ostreatus*). *Pakistan Journal of Nutrition* 7(2), 349–351.
- Iwalokun, B.A., Usen, U.A., Otunba, A.A., Olukoya, D.K., 2007. Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*. *African Journal of Biotechnology* 6(15), 1732–1739.
- Jang, I.J., Chung, K.C., Chang, H.Y., 2005. Excellent strain selection and optimal mycelial growth condition of *Pleurotus cornucopiae*. *Journal of Mushroom* 3(1), 40–44.
- Jeet, S., Mehta, S.K., Chugh, R.K., Raj, K., 2022. Eco-friendly and cost-effective methods of pasteurization of substrates for oyster mushroom cultivation. *International Journal of Bio-resource and Stress Management* 13(8), 868–876.
- Kataoka, R., Nigaki, A., Barua, B.S., Yamashita, K., 2025. Ergothioneine circulation in mushroom cultivation using food waste recycling. *Recycling* 10(3), 91.
- Khan, M.W., Ali, M.A., Khan, N.A., Khan, M.A., Rehman, A., Javed, N., 2013. Effect of different levels of lime and pH on mycelial growth and production efficiency of oyster mushroom (*Pleurotus* spp.). *Pakistan Journal of Botany* 45(1), 297–302.
- Khandakar, J., Yesmin, S., Moonmoom, M., 2008. Mycelial growth of *Pleurotus citrinopileatus* on different environmental condition, *Bangladesh Journal of Mushroom* 2, 55–62.
- Kim, S.H., Jakhar, R., Kang, S.C., 2015. Apoptotic properties of polysaccharide isolated from fruiting bodies of medicinal mushroom *Fomes fomentarius* in human lung carcinoma cell line. *Saudi Journal of Biological Sciences* 22, 484–490.
- Krishnapriya, P.J., Geetha, D., Priya, R.U., 2017. Morphological and molecular characterization of oyster mushroom of Kerala. *International Journal of Pure and Applied Bioscience* 5(6), 716–724.
- Kumla, J., Suwannarach, N., Jaiyasen, A., Bussaban, B., Lumyong, S., 2013. Development of an edible wild strain of Thai oyster mushroom for economic mushroom production. *Chiang Mai Journal of Science* 40(2), 161–172.
- Lee, S.J., Kim, H.H., Kim, S.H., Kim, I.S., Sung, N.J., 2018. Culture conditions of liquid spawn and the growth characteristics of *Pleurotus ostreatus*. *Journal of Mushroom* 16(3), 162–170.
- Liaqat, R., Shafiq, M., Naeem, M.S., Ali, M.A., Ali, S., Sardar, H., 2014. Growth and yield performance of oyster mushroom on different substrates. *Mycopath* 12(1), 9–15.
- Lilly, V.G., Barnett, H.L., 1951. *Physiology of the fungi*. McGraw-Hill, New York, 454p.
- Mukherjee, R., Nandi, B., 2000. Effect of pH on submerged mycelia production by *Pleurotus* species in a mycelium with lignocellulosic biomass. *Journal of Mycopathological Research* 38, 65–69.
- Nasim, G., Malik, S.H., Bajwa, R., Afzal, M., Mian, S.W., 2001. Effect of three different culture media on mycelial growth of oyster and chinese mushrooms. *Online Journal of Biological Sciences* 1(12), 1130–1133.
- Peksen, A.U., Hatat, G., Erper, I., 1999. Effect of different media and treatments on mycelial growth of some wild mushrooms and *Pleurotus sajor-caju*. *Ondokuzmayis Universitesi, Ziraat Fakultesi Dergisi* 14 (3), 44–53.
- Raman, J., Jang, K.Y., Oh, Y. L., Oh, M., 2020. Cultivation and nutritional value of prominent *Pleurotus* spp.: An overview. *Mycobiology* 49(1), 1–14.
- Rangad, C.O., Jandaik, C.L., 1977. Nitrogen fixation by *Pleurotus* species [edible fungi, India]. *Indian Journal of Mushrooms* 3(1), 13–17.
- Rawte, H., Diwan, R., 2011. Growth response of *Pleurotus* spp. on different basal media and different pH levels. *Journal of Ecobiotechnology* 3, 10–12.
- Sardar, H., Ali, M.A., Ayyub, C.M., Ahmed, R., 2015. Effects of different culture media, temperature and pH levels on the growth of wild and exotic *Pleurotus* species. *Pakistan Journal of Phytopathology* 27(2), 139–145.
- Sganzerla, W.G., Todorov, S.D., Silva, A.P.G., 2022. Research trends in the study of edible mushrooms: nutritional properties and health benefits. *International Journal of Medicinal Mushrooms* 24, 1–18.
- Sharma, A., Khanna, A.S., Raina, R., Kapoor, R., Thakur, K.S., 2019. Faunistic survey of insect-pests associated with *Agaricus bisporus*. *International Journal of Economic Plants* 6(3), 122–125.
- Sharma, S., Jarial, R.S., Jarial, K., 2019b. Studies on different cultural parameters on vegetative growth of straw mushroom (*Volvariella volvacea*). *International Journal of Bio-resource and Stress Management* 10(6), 628–635.
- Singh, S.K., Upadhyay, R.C., Verma, R.N., 2000. Physico-chemical preferences for efficient mycelial colonization in edible mushroom. *Mushroom Research* 9(2), 85–89.
- Stanley, H.O., Nyenke, C.U., 2011. Cultural studies on mycelia of *Pleurotus plumonarius* (oyster mushroom) in selected culture media. *International Journal of Science and Nature* 2(2), 183–185.
- Suharban, M., Nair, M.C., 1994. Physiological studies on *Pleurotus* spp. *Mushroom Resources* 3, 99–100.
- Tuite, J., 1969. *Plant pathological methods: fungi and bacteria*. Burgess Publishing Co. Minneapolis, U.S.A., 239p.
- Upamanya, G.K., Brahma, R., Sarma, R., Sharma, P., Das, K., 2021. Effect of different bag opening methods of oyster mushroom (*Pleurotus ostreatus*) on growth parameter, yield and benefit cost ratio. *International Journal of Economic Plants* 8(4), 207–210.
- Uzomba, C.G., Ezemagu, U.K., Ofoegbu, M.S., Lydia, N.,



- Goodness, E., Emelike, C., Mbajiorgu, E.F., 2024. Edible mushroom (*Pleurotus cornucopiae*) extract vs. glibenclamide on alloxan induced diabetes: sub-acute in vivo study of Nrf2 expression and renal toxicity. *Anatomy & Cell Biology* 57(3), 446–458.
- Vieira, F.R., Andrade, M.C., Minihoni, M.T., 2013. Growth of *Pleurotus ostreatus* in culture media based on formulated straw or grass. *African Journal of Agricultural Research* 8(20), 2345–2352. <http://hdl.handle.net/11449/137564>.
- Yadav, M.K., Chandra, R., 2014. Evaluation of culture media, pH and temperature for mycelial growth of different strains of *Pleurotus* spp. *Agricultural Science Digest* 34(4), 299–302.
- Zhao, G.Y., Cui, W., 2002. Determination of optimum pH and temperature for hyphal growth of *Pleurotus ferulae*. *Edible Fungi of China* 21, 18–19. ISSN 1003-8310. <https://www.cabidigitallibrary.org/doi/full/10.5555/20033081140>.