1165
Arab Univ.
J. Agric. Sci.,
Ain Shams Univ., Cairo
Special Issue, 26(2A), 1165-1173, 2018

INFLUENCE OF NUTRITIONAL AND CLIMATIC CONDITIONS ON MYCELIAL GROWTH OF THREE OYSTER MUSHROOM STRAINS

[87]

Norhan H. Abdel Aziz[1]; Nahed, S. Yousef[2]; M.E. El-Haddad[3] and T.S. El-Tayeb[3]

1- Central Lab. for Agric. Climate, Agric. Res. Center (ARC), Giza, Egypt
2- Food Sci. and Tech. Dept., Fac. of Home Economic, Al-Azhar Univ., Tanta, Egypt
3- Agric. Microbiology Dept., Fac. of Agric., Ain Shams Univ., Cairo, Egypt

Keywords: Oyster mushroom, optimal conditions, nutrient media, mycelial growth rate.

ABSTRACT

The mycelial growth rate of three oyster mushroom strains namely *Pleurotus erengii, P. ostreatus* and *P. florida* was examined on six different agar media (malt extract, Potato dextrose, rose bengal, corn meal, czapek's dox and waksman's glucose agar media) to select the most suitable one. Effect of pH was also examined at different values (5.0, 5.5, 6.0, 6.5 and 7.0) on the selected medium. Results indicated that Malt extract agar medium was the most suitable one for mycelium growth of all the tested mushroom strains, being the highest (9 cm) for *P. erengii* and *P. ostreatus* at pH 7 and 9 cm at pH 6 -6.5 for *P. florida* after 6 days of incubation. The mycelia growth of the tested oyster mushroom strains was also examined at different temperatures (15, 20, 25, 30 and 35ºC) and different levels of relative humidity (50, 65, 75, 85, 95 and 100 %) on malt extract agar medium. The highest mycelial growth rate was obtained at 25°C and relative humidity of 65% after 6 days of incubation for the three tested oyster mushrooms.

INTRODUCTION

Mushroom is one of the man's earliest food which have come to be recognized as highly nutritive food, low in calories, rich in protein and certain vitamins. A distinctive feature of mushroom protein is that it comprises of all the essential amino acids and has highly digestible value. Its nutritional value can be compared to those of eggs, milk, and meat **(Oei, 2003).** The vitamins of mushrooms are not destroyed by cooking, drying and freezing. Mushroom has been used as a food and in medicine by different civilizations since immemorial time, due to its delicious taste and dietetic qualities. Mushroom has also beneficial qualities, like lowering the blood cholesterol level, warding against cancer and invigorating hair growth. **Morais et al (2000) and Sánchez (2004)** reported that the fresh mushroom contains about 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and vitamins. The total energetic value of mushroom caps is between 250 and 350 cal/kg of fresh mushrooms weight **(Oliver and Delmas, 1987; Laborde, 1995).** The production of mushrooms is regarded as the second most important Commercial microbial technology next to yeast **(Pathak et al 2009).** Oyster mushroom (*Pleurotus* spp.) cultivation has increased tremendously throughout the world during the last few decades **(Royse, 2002).** These mushrooms accounted for 14.2% of the total world production of edible mushrooms in 1997 **(Chang, 1999).** Although commonly grown on pasteurized straw of wheat or rice, they could be also cultivated on a wide variety of substrates that contain lignin and cellulose. Oyster mushrooms cultivation can play an important role in managing organic wastes which have become problematic for disposal. Oyster mushrooms can grow at moderate temperatures ranging from 20 to 30°C and humidity ranging from 55 to 80% for a period of 6 to 8 months in a year. It could also be cultivated in summer season, by providing the extra humidity for its growth. Hilly areas (above 900 m) are also suit-

(Received 22 October, 2017)
(Revised 29 October, 2017)
(Accepted 31 October, 2017)

able for its growth. Three primary factors can affect the yield of oyster mushrooms, i.e. temperature, compost component and humidity. The process of cultivating oyster mushrooms has 3 main steps: isolating mushroom from fruiting bodies, preparing primary and secondary spawn and cultivating mushrooms from these spawns to harvest fruiting bodies **(Dung, 2003).**

The objectives of this study are to determine the best nutritive medium, the optimal pH value, relative humidity and temperature for the *in vitro* cultivation of each of the three tested mushroom strains.

MATERIALS AND METHODS

Tested strains

The pure cultures of *P. erengii , P. ostreatus* and *P. florida* are a part of Mushroom Laboratory Culture Collection (MLCC), allocated at Mushroom Production and Research Unit,Central Laboratory for Agricultural Climate, Agricultural Research Center (ARC). Their numbers in this collection are 555, 21, and 14 in respective order. These cultures were kindly provided from Germany, Somycel-France and STCPL–Hong Kong respectively.

Media used

The following media were tested to find out the best one, giving the highest linear growth rate of the examined mushroom strains. Their components are expressed as (g/l):

Waksman,s glucose agar (WGA) medium (Waksman, 1922)

Glucose 10.0, peptone5.0, beef extract 5.0, distilled water up to 1000 ml, agar12.5 and. pH 7.0.

Malt extract agar(MEA) medium (Gutz and Doe, 1973)

Malt extract 30.0, peptone5.0, distilled water up to 1000 ml, agar 15.0 and pH 5.4.

Corn meal agar (CMA) medium, quarter – strength (ATCC-2221) (Hazen and Reed, 1955)

Corn meal infusion 250.0 ml, distilled deionized water up to 1000 ml, agar15.0 and pH 5.6

Potato dextrose agar(PDA) medium (Ahmed, 2001)

Potato extract 200.0 ml, dextrose 20.0, distilled water up to 1000 ml, agar 15.0 and pH 5.6

Rose bengal agar (RBA) medium (Smith and Dawson, 1944)

Peptone 5.0, dextrose 10.0, KH_2 PO_4 1.0, $MgSO_4.7H_2O$ 0.50, Rose bengal 0.05, Chloramphenicol. 0.10, distilled water up to 1000 ml, agar 15.0 and pH 7.2.

Czapek's dox agar(CDA) medium (Prauser and Folta, 1968)

Sucrose 20.0, $NaNo_3$ 2.0, KH_2 PO_4 1.0, $MgSO_4.7H_2O$ 0.50, KCL 0.50, Fe_2SO_4 0.01, distilled water up to 1000 ml, agar 15.0 and pH 7.0.

Effect of different nutrient media on the growth of the tested mushroom strains

The aforementioned media were tested to select the most suitable one, giving the highest mycelial growth rate of the tested mushroom strains. The media were adjusted to their sutible pH before autoclaving. Media were sterilized by autoclaving at 121^{o}C, for 20 minutes, 15~20 ml of each medium was aseptically poured into plates. A 0.5 cm diameter plug of an inoculum was picked up with sterilized cork borer from 7days old culture, grown on potato dextrose agar medium and placed in the center of each plate of the tested six media and incubated for 6 days at 25 °C. The mycelia radial growth was measured after 2,4 and 6 days of incubation.

Effect of different pH levels on the growth of the tested mushroom strains

A 0.5 cm diameter plug of an inoculum was picked up with sterilized cork borer from 7 days old culture, grown on the selected medium and placed in the center of each plate The medium was adjusted to different levels of pH (5, 5.5, 6, 6.5 and 7) with the addition of 1NNaOH or 1NHCl before autoclaving at 121^{o}C for 20 minutes. Three replicates of each treatment were prepared. Plates were incubated for 6 days at 25 °C. The mycelial radial growth was measured after 2,4 and 6 days of incubation.

Effect of different temperatures on the growth of the tested mushroom strains

The effect of different temperatures, *i.e.* 15, 20, 25, 30 and 35^{o}C on the mycelium linear growth (cm) of the tested strains were determined on the selected medium. For each treatment, three replicates were inoculated with 0.5 cm agar discs taken from actively growing cultures with sterilized cork borer .The mycelial radial growth was measured after 6 days of incubation.

Evaluation of different relative humidity(RH) levels on radial growth of tested *Pleurotus* strains

After optimization of temperature, *Pleurotus* species were optimized for their RH. Therefore, different levels of RH (50, 65, 75, 85, 95 and 100 %) of the selected medium were adjusted according to **Solomon (1951)** by taking accurate weight of either NaCl or KOH, and then dissolved in 100 ml deionized distilled water to obtain the required levels of RH . Petri-dishes (9 cm –diameter) containing 15~20 ml of the selected medium were inoculated with agar discs (0.5 cm), taken from actively growing cultures with sterilized cork borer, then the plates were converted. Ten ml of each prepared solution were placed in the cover of each Petri dish, then sealed with para film and incubated at 25°C for 6 days. The diameter of the mycelium extension was measured every 2 days with the help of a ruler until completion of growth in any plate. Diameter of mycelial growth was measured as described by **Imtiaj et al (2009).**

Average mycelial growth was calculated as follows:

1st Petri dish (AB1+CD1+EF1) /3 = R1
2nd Petri dish (AB2+CD2+EF2) /3 = R2
3rd Petri dish (AB3+CD3+EF3) /3 = R3
Note: AB, CD and EF referrers to different three measures (cm) for each plate (three replicates, R1, R2 & R3 for each treatment).
Average mycelial growth of each strain is (R1+ R2+ R3) /3

Statistical analysis

The obtained results were statistically analyzed by using Statistical Analysis System **(SAS, 2006**). Least Significant Difference (LSD) test was used to test significance between means according to **Snedecor and Cochran (1991).**

RESULTS AND DISCUSSION

Oyster mushroom requires different nutrients and certain environmental conditions in order to grow and reproduce **(Ravimannan et al 2014).** Effect of different media on mycelial growth of the tested mushroom strains (*P.erengii, P. ostreatus* and *P. florida**)*** are presented in **Table (1)**. There are significant differences between radial growth in all tested media during the incubation period. The maximum linear growth rates for *P. ostreatus, P.erengii* and *P. florida* were recorded on malt extract agar medium being 8.1,7.39and7.39cmin respective order after 6 days of incubation followed in general by PDA and RBA (7.5 cm). The CDA medium had the lowest radial growth rate as compared to the other tested media after 6 days of incubation. Radial growth of *P.erengii* and *P. florida* recorded 5 cm in CDA medium. In general, CDA medium gave lower values of radial growth for tested mushroom strains after 6 days of incubation (**Shim et al 2003).** Such differences in mycelial growth detected on the tested agar media may be due to the availability of different carbon sources and other required nutrients. Mycelium growth was marginally better on a medium containing glucose and sucrose than other carbon sources **(Santiago, 1983).** These results are in agreement with the findings of **Hoa and Wang (2015),** who reported that *Pleurotus* spp. showed faster growth of mycelium on malt extract and potato dextrose agar media, compared to the other tested media.

Results presented in **Table (2).** showed the effect of different pH levels on radial growth of *P. erengii, P. ostreatus* and *P. florida* growing on malt extract agar medium. The maximum linear growth rate (9 cm) was found at pH 7 for *P. erengii* and *P. ostreatus* after 6 days of incubation, and pH 6-6.5 (9cm) for *P. florida.* Low radial growth was observed at pH 6 (6.83 and 6.90 cm) for *P. erengii* and *P. ostreatus* respectively and the lowest radial growth of *P. florida* (7.5cm) was recorded at pH 5.

pH is considered one of the prime factor that affects the growth of fungi. **Karacanci (1997) and Sardar et al (2015)** noticed that maximum mycelial growth of *P. ostreatus* was recorded at pH 6.5. These results are in accordance with the findings of **Suharban and Nair (1994),** who reported that *Pleurotus* spp. grow faster on slightly acidic medium than basic one.

Table 1. Effect of different nutrient media on liner growth rate of mushroom strains *P. erengii, P. ostreatus and P. florida* at 25°C

Tested agar media	Interval (days)	Linear growth rate (cm)		
		Tested mushroom strains		
		P. erengii	*P. ostreatus*	*P. florida*
PDA	2	1.81	3.20	1.71
	4	3.10	5.40	2.90
	6	7.50	7.30	5.50
MEA	2	2.53	2.75	2.90
	4	6.66	6.50	6.40
	6	7.93	8.10	7.93
CDA	2	1.13	2.00	1.10
	4	3.30	2.80	3.30
	6	5.00	5.60	5.00
CMA	2	1.60	2.30	1.70
	4	4.75	5.60	4.75
	6	6.30	6.80	6.30
WGA	2	1.58	2.30	1.60
	4	4.81	5.40	4.81
	6	6.30	6.80	6.30
RBA	2	2.68	3.00	2.50
	4	5.80	5.90	5.80
	6	6.90	7.20	7.50
LSD		0.53	0.75	0.50

PDA = Potato dextrose agar medium, MEA = Malt extract agar medium, CDA = Czapek' sdox agar medium, CMA = Corn meal agar medium, WGA = Waks man glucose agar medium, RBA = Rose bengal agar medium

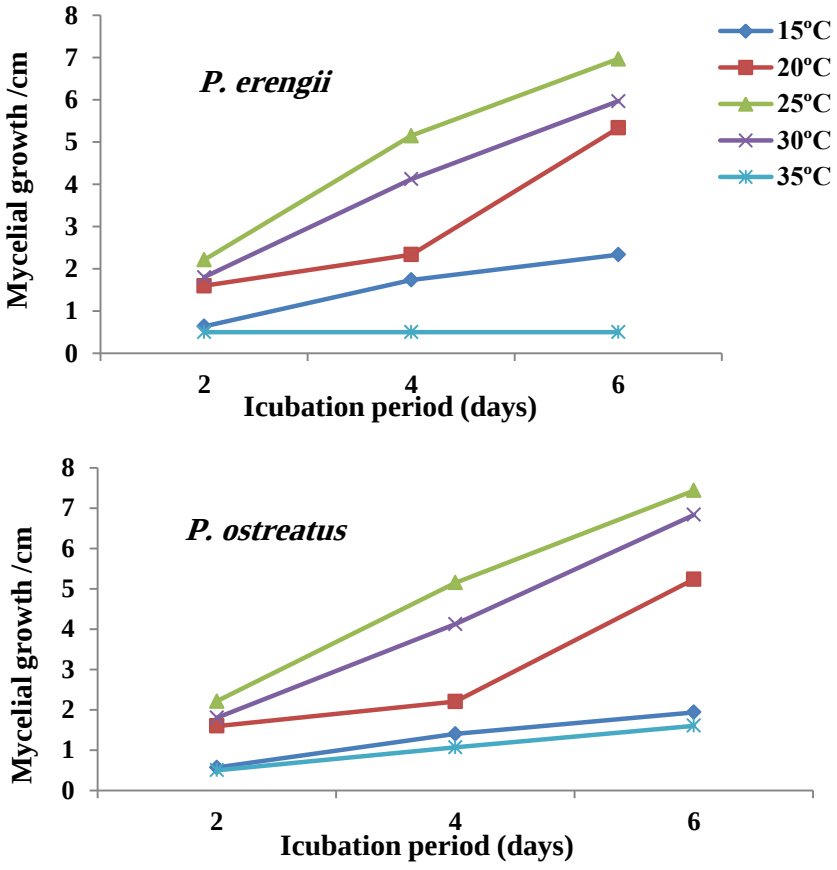
Table 2. Effect of different pH levels on liner growth rate of mushroom strains on MEA medium at 25°C

Tested mushroom Strains	Interval (days)	Linear growth rate / cm on MEA medium				
		pH levels				
		5	5.5	6	6.5	7
P. erengii	2	2.00	2.53	2.70	3.20	3.00
	4	6.00	6.66	5.78	7.00	6.66
	6	7.20	7.93	6.83	8.16	9.00
P. ostreatus	2	2.50	2.75	3.51	3.28	4.35
	4	6.80	6.50	7.00	5.90	6.80
	6	8.00	8.10	6.90	8.30	9.00
P. florida	2	2.80	2.90	3.11	2.50	2.90
	4	6.00	6.40	7.80	7.00	6.50
	6	7.50	7.90	9.00	9.00	8.10
LSD		0.56	0.64	0.90	0.55	0.40

Accordingly, in the light of the aforementioned results it could be concluded that most of *Pleurotus* spp. showed superior growth at pH 6 to 7. This might be attributed to genetic differences found in different species of genus *Pleurotus* **(Sardar et al 2015).**

The growth of oyster mushroom (*Pleurotus* spp.) was decreased at pH 5. Such decrease in growth may be due to reduction of its enzymatic activities. Likewise observation has also been previously reported by **Zadrazil (1978),** who mentioned that *P. ostreatus* and *P. erengii* showed significant decreases in their mycelial growth at pH 4.0, which is too acidic. However, some *Pleurotus* spp. are characterized by wider growth adaptability scale for pH i.e. 5-8 as reported by **Yadav (2001).** The mycelial growth of *P. ostreatus* was better at pH 7.0 as reported by **Bugarski et al (2000).**

Temperature is an important aspect in the selection of mushroom. The tropics, areas where high temperature remains most of the time, are not recommended for mushroom production. Tested oyster mushroom species exerted their highest growth rate at 25°C, as shown in **Fig. (1).** Optimum temperature was reported within this range by **Zharare et al (2010),** who observed maximum growth of *Pleurotus* strains. Thus, it appears that 20-25ºC was universal temperature range for the mycelial growth of mushrooms. In addition, fungus also exhibited maximum enzymatic activity within this range and growth inhibition at elevated temperature was reported **(Sardar et al 2015).** Favorable temperature for growth was recorded at 25°C and the lowest growth has shown at 15 and 35°C **Fig. (1). Shim et al (2003)** reported that the mycelial growth of *Paecilomyces fumosoroseus* had been expedited gradually in proportion to the rise of temperature and the most suitable temperature was 25°C. Similar results were obtained by **Wei et al (2002),** who reported a temperature range of 20-31ºC for the hyphal growth of *P. flabellatus* and concluded that a temperature of 25ºC is the optimum. Similarly, **Zharare et al (2010)** found that *P. sajor-caju* can tolerate high temperature up to 35ºC. Temperature and pH affected the growth of *Pleurotus* spp. through suppressing their enzymatic activities in the cell **(Sopit, 2006).**



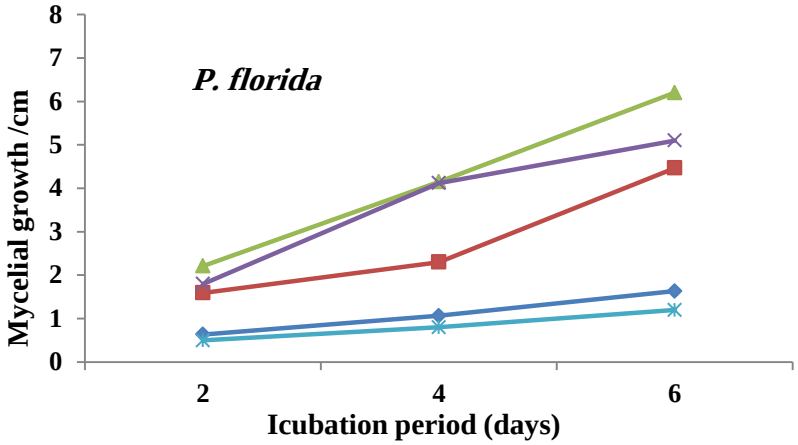
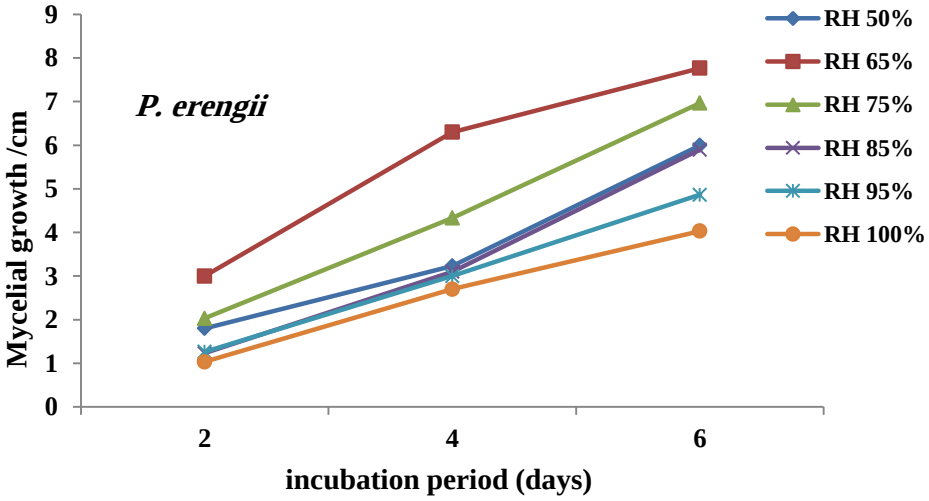


Fig. 1. Effect of different temperature on mycelial growth of *P. erengii, P. ostreatus and P. florida.*

The effect of relative humidity on radial growth of tested *Pleurotus* strains was studied under *in vitro* condition .Results are presented in Figure(2).There were significant differences in radial growth of *Pleurotus* strains at different levels of relative humidity. Maximum radial growth (8 cm) of the three tested *Pleurotus* strains was noticed at 65 % relative humidity followed by 75 % (6.9 cm) while, it was significantly low at 95 and 100 % . **Shukla (2003)** observed maximum mycelial growth (90 mm) of *Calocybe indica* at 75 and 100 % relative humidity while it was significantly low at 25 % (80 mm) followed by 50 % relative humidity (83.25 mm).

Accordingly, it could be concluded that, *P. erengii, P. ostreatus* and *P. florida* showed best growth when grown at temperature of 25°C and 65% relative humidity. As for among the different growing media, MEA medium proved to be the best one for the growth of *Pleurotus* spp. pH level must be maintained at 6-7 for best growth of oyster mushroom strains.



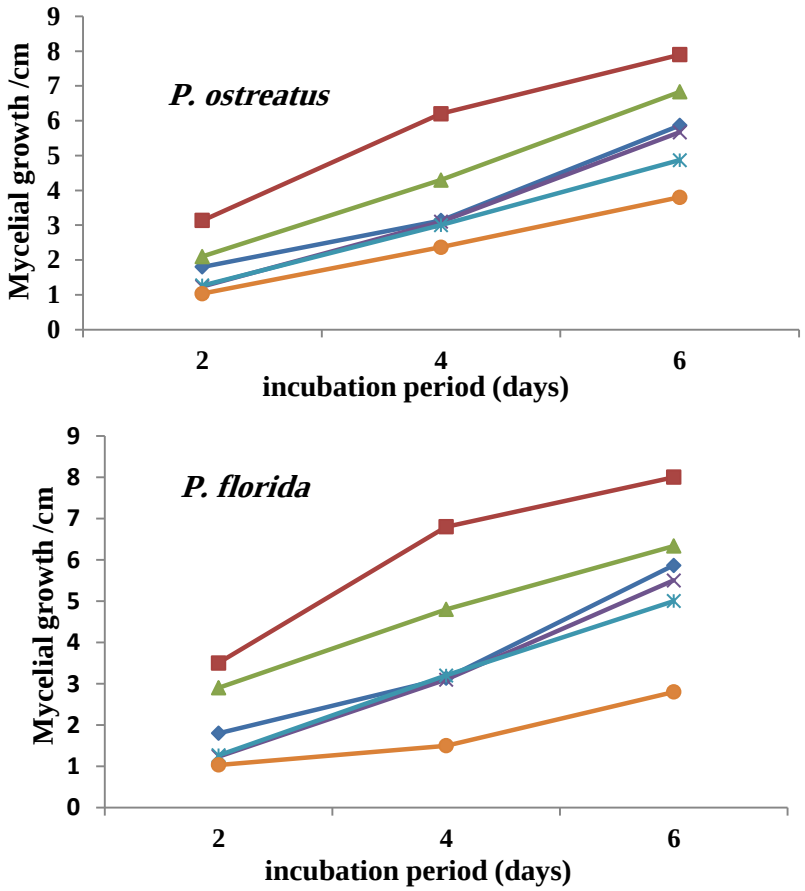


Fig. 2. Effect of different relative humidity (RH) levels on mycelial growth of *P. erengii* , *P. ostreatus* and *P. florida.*

REFERENCES

Ahmed, M.A. 2001. Dictionary of the Fungal Terminology. Academic Book Shop. Egypt. **pp. 230-231.**

Bugarski, D., Gvozdenovic, D.J., Cervenski, J. and Takac, A. 2000. Effect of major environmental conditions on the development of the mycelium and growth of the oyster mushroom (*Pleurotus ostreatus*). **In: II Balkan Symposium on Vegetables and Potatoes, 579, 319-323.**

Chang, S.T. 1999. World production of cultivated and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes*. **Int. J. Medici. Mush. 1, 291 - 300.**

Dung, L.B. 2003. Mushrooms in Tay Nguyen (in Vietnamese). Ha Noi: Science and Technique. Cited from **Sardar, H., Ali, M.A., Ayyub, C.M. and Ahmad, R. 2015.** Effects of different culture media, temperature and pH levels on the growth of wild and exotic *Pleurotus* species. **Pak. J. Phytopathol., 27(2), 139-145.**

Gutz, H. and Doe, F.J. 1973. Two different h¯mating types in *Schizosaccharomyces pombe*. **Genetics 74, 563–569.**

Hazen, E.L. and Reed, F.C. 1955. Monograph Laboratory Identification of Pathogenic Fungi Simplified**, American Lecture Series, Publ. No. 253, 100 p. Charles C. Thomas, Springfield, IL.**
https: //Catalog.hardydiagnostics.com.

Hoa, H.T. and Wang, C.L. 2015. The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). **Mycobiol. 43(1), 14-23.**

Imtiaj, A., Jayasinghe, C., Lee, G.W. and Lee, T.S. 2009. Comparative study of environmental and nutritional factors on the mycelial growth of

edible mushrooms. **J. Cult. Collect. 6(1), 97-105.**

Karacanci, S. 1997. An investigation on biomass production of *Pleurotus sajor-caju* and *Lentinus edodes* in submerged culture. **Paper presented in Indian Mushroom Conference – 97 at NCMRT Solan from 10-13thSeptember, 7. Cited from Sardar, H., Ali, M.A., Ayyub, C.M. and Ahmad, R. 2015.** Effects of different culture media, temperature and pH levels on the growth of wild and exotic *Pleurotus* species. **Pak. J. Phytopathol., 27(2), 139-145.**

Laborde, J. 1995. Dossier Pleurote. INRA, Centre de Recherches de Bordeaux, Station de Recherchessur les Champignons, Bordeaux, **pp. 17–18**

Morais, M.H., Ramos, A.C., Matos, N. and Santos-Oliveira, E.J. 2000. Production of shiitake mushroom (*Lentinus edodes*) on ligninocellulosic residues. **Food Sci. Techol. Int., 6, 123–128.**

Oei, P. 2003. Mushroom Cultivation 3rded. Appropriate technology for mushroom growers.Backhuys Publishers, Leiden. The Netherlands. **429 p.**

Oliver, J.M. and Delmas, J. 1987. Vers la maîtrise des champignons comestibles.**Biofutur 1, 23–41.**

Pathak, R., Joshi, N. and Dwivedi, R.R. 2009. Eco-friendly production of *Agaricus bisporus* (lange) imbach (white button mushroom). **Nat. Sci., 6, 57–60.**

Prauser, H. and Folta, R. 1968. Phagensensibilitat, Zellwandzusammensetzung and Taxonomie van actinomuceten. **Z. Allg. Mikrobiol, 8, 39-64.**

Ravimannan, N., Arulanantham, R., Pathmanathan, S. and Niranjan, K. 2014. Alternative culture media for fungal growth using different formulation of protein sources. **Annals. Biol. Res. 5, 36-39.**

Royse, D.J. 2002. Influence of spawn rate and commercial delayed release of nutrient levels on *Pleurotus cornocopiae* yield, size and time to production. **Appl. Microbiol. Biotechnol. 17, 191 – 200.**

Sánchez, C. 2004. Modern aspects of mushrooms culture technology. **Appl. Microbiol. Biotechnol. 64, 756–762.**

Santiago, C.M. Jr. 1983. Basidiocarp production in *Volvariella volvacea* in laboratory cultures. Kalikasan, **Philippines Journal of Biology, 12, 70-76.**

Sardar, H., Ali, M.A., Ayyub, C.M. and Ahmad, R. 2015. Effects of different culture media, temperature and pH levels on the growth of wild and exotic *Pleurotus* species. **Pak. J. Phytopathol., 27(2), 139-145.**

SAS 2006. Statistical Analysis System, SAS User's Guide Statistics. SAS Institute Inc., Editor, Cary, NC.

Shim, S.M., Lee, K.R., Kim, S.H., Im, K.H., Kim, J.W., Lee, U.Y., Shim, J.O., Lee, M.W. and Lee, T.S. 2003. The optimal culture conditions affecting the mycelial growth and fruiting body formation of *Paecilomyces fumosoroseus*. **Mycobiol., 31(4), 214–220.**

Shukla, C.S. 2003. Role of agronomical biochemical practices on growth and yield parameters of *Calocybe indica,* **Ph.D. Thesis Submitted to IGKV, Raipur, 148 p.**

Smith, N.R. and Dawson, V.T. 1944. The bacteriostatic action of rose bengal in media used for the plate counts of soil fungi. **Soil Sci., 58, 467-471.**

Snedecor, G.W. and Cochran, W.G. 1991. Statical Method 7th Ed. Iowa State Univ. Press, Iowa, USA.

Solomon, M.E. 1951. Control of humidity with potassium hydroxide, sulphuricacid, or other solutions. **Bul. Entomol. Res. 42, 543-554.**

Sopit, V. 2006. Oyster mushroom cultivation on different cellulosic substrates. **Res. J. Agri. Biol. Sci., 2(6), 548-551.**

Suharban, M. and Nair, M.C. 1994. Physiological studies on *Pleurotus* spp. **Mushroom Res. 3, 87-104.**

Waksman,S.A. 1922. A method for counting the number of fungi in the soil**. J Bacteriol 7(3), 339–341.**

Wei, S.H., Jie,Y.Y., Rong, H.L., Li, H.L. and Yan, M.G. 2002. Effects of different cultivation conditions on hyphal growth of *Pleurotus flabellatus*. **Edible Fungi of China. 23(2), 29-30.**

Yadav, B.R. 2001. Physical and nutritional factors in relation to growth of *Pleurotus sajor-caju*(Fr.) Singer. **J. Phytol. Res.,14, 95-98.**

Zadrazil, F. 1978. Cultivation of *Pleurotus*. The biology and cultivation of edible mushrooms by S.T. Chang and W.A. Hayes (eds). Academic press INC. **Orlando, Florida, 1(62), 521-558.**

Zharare, G.E., Kabanda, S.M. and Poku, J.Z. 2010. Effects of temperature and hydrogen peroxide on mycelial growth of eight *Pleurotus* strains. **Scientia Hortic., 125, 95-102.**