Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

EFFECTS OF DIFFERENT CULTURE MEDIA, TEMPERATURE AND pH LEVELS ON THE GROWTH OF WILD AND EXOTIC *PLEUROTUS* SPECIES

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A B S T R A C T

A series of experiments were carried out to investigate the effects of various growth conditions on growth and development of *Pleurotus* species. In first experiment six different *Pleurotus strains Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm*, Pleurotus sajor-caju* (Fr.) Singer*, Pleurotus eryngii* (DC.) Quél*, Pleurotus columbinus* (DC.) Quél (exotic) and *Pleurotus sapidus* (Schulzer) Sacc. (FW-133) were cultured on different agar media viz. PDA (Potato dextrose agar), MEA (Malt extract agar) and WEA (Wheat extract agar). Among these media Potato dextrose agar medium (PDA) was found to be the best medium than malt extract agar (MEA) and wheat extract agar (WEA) for the growth of mycelium of all *Pleurotus* species. In second experiment the six *Pleurotus* species were cultured aseptically on PDA at different temperature ranges and found mostly *Pleurotus species* grows best at 25°C, while the *P. sapidus* (FW-133) shows mycelial growth at 35°C. In third experiment these six *Pleurotus* strains were aseptically inoculated on PDA at 25 °C. It was noted that the species exhibited maximum mycelial growth at pH 6, whereas the minimum growth was recorded at pH 4.

Keywords: Temperature, pH, media, wild *Pleurotus* species, mycelial growth.

INTRODUCTION

Oyster mushroom locally known as "Dhingri" grows wild on logs and stumps of trees in forests of Azad Kashmir, Khyber Pakhtunkhwa Province and other plantations in plains of Punjab and Sindh, during monsoon. It belongs to the class Basidiomycetes subclass Hollobasidiomycetidae, order Agricales and family Pleurotaceae (Alexopolous and Mims, 1996) and comprises about 40 species (Jose and Janardhanan, 2000). They are widely used as delicacies in different parts of the world because of their excellent flavor and taste (Jonathan and Esho, 2010).

During the rainy season, edible and non-edible species of mushrooms usually grow on different natural materials such as garden soil, decaying wood, termite nest, tree trunks, under the shade provided by trees; leaf litters, tea, coffee and rubber plantations. Wildly growing mushrooms are considered as highly nutritious food which contains protein, amino acids, vitamins, crude fiber, lipids, sugars, glycogen and important mineral contents, which are essential for normal functioning of the human body (Gbolagade *et al*., 2006).

Mushroom growth is highly influenced by several factors such as spawn, growing media, pH, temperature, moisture content and light intensity (Kadiri and Kehinde, 1999). The maintenance and production of a reliable pure culture spawn with required potentials is a key operation and the first critical stage for successful mushroom cultivation. Storage and maintenance of mushroom species in a pure, viable and stable condition is essential for their use as reference strain, both in research and industrial scales (Bhatt *et al*., 2010). Study of mycelial behavior is also important in studying the life cycle and other cultivation aspects of medicinally important mushrooms. Mushroom production has been limited throughout the world due to incompetence; incapability and lack of technical knowledge to culture edible mushrooms. Spawn (active mycelium) production is one of the major limiting factors to mushroom cultivation all over the world (Stanley, 2010). The high temperatures between 27ºC and 35ºC during the day has been a major problem for the farmers of tropical regions who wish to grow mushrooms (Gaitán and Salmones, 2008).

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Therefore, the selection for tolerance to high temperatures in the cultivable mushroom species is essential to obtain optimum yield and quality (Miles and Chang, 1997). The identification of suitable agar media, substrate and incubation temperature is essential to obtain high yield and quality of mushroom. Previous studies reported the effects of different agar media and substrates on growth and quality of mushroom mycelium but most of these involved the use of only few media. Therefore, the present study aimed at investigating the behavior of various oyster mushroom strains under different media, temperature, and pH requirements for mycelial growth.

MATERIALS AND METHODS

Experimental layout: Three experiments were conducted. The first experiment was performed to evaluate the effect of different media on the mycelial growth of wild and exotic *Pleurotus* spp.; the second and third experiments were performed to determine the effects of temperature and pH respectively on agar media. All the experiments were carried out in the growth room of Medicinal and Mushroom Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. The experiments were laid out in a completely randomized design with three replications in each treatment. Each experiment was repeated three times and the data presented are the mean of values obtained from these experiments.

Strains collection: Mushroom species were collected from different localities of Punjab province such as University of Agriculture, Faisalabad, Guttwala Forest area Faisalabad, Changa Manga Forest area Lahore. From these collected species *Pleurotus* spp. were identified on morphological characteristics in Mushroom Lab (Institute of Horticultural Sciences) and Mycology Lab (Department of Plant Pathology), University of Agriculture, Faisalabad. Species of *Pleurotus* genus used for the study are shown in Table 1.

Table 1. Source of different wild and exotic *Pleurotus* species.

Mushroom Species	Source
Pleurotus sapidus (FW-133)	Wild Pakistani strain
Pleurotus ostreatus	USA
Pleurotus sajor-caju	Canada
Pleurotus columbinus	UK
Pleurotus eryngii	USA

FW=Faisalabad wild

Experiment No. 1. Effect of different culture media on growth of *Pleurotus* species.

Culture Preparation:

Following culture media were used for the mycelial growth of *Pleurotus* species.

- Potato dextrose agar (PDA): PDA medium was prepared by adding 20 g each of potato starch, dextrose, agar and 1 L distilled water and autoclaved it at 121°C for 15 minute.
- Malt extract Agar (MEA): MEA medium was prepare by adding 20 g each of malt extract, dextrose, agar and 1L distilled water and autoclaved as described earlier.
- Wheat extract agar (WEA): WEA medium was prepared by adding extract of 200g wheat grains, 20 g dextrose, 20 g agar and 1 L distilled water and autoclaved as described earlier.

The medium were then poured in clean 90 mm Petri dishes. Streptomycin was added in the sterilized medium at the rate of 1 g/L. Medium were cooled at 40°C; the strains were inoculated in above mentioned media and incubated for ten days to prepare solidified plates of PDA, MEA and WEA under sterile conditions at 25. The radial growth was measured following the method reported by Zharare *et al*. (2010).

Isolations from the fresh specimens, collected from the wild collection of *Pleurotus* spp. were made by following the standard tissue culture technique. Fresh samples of *Pleurotus* spp. were cut across the pileal region with the help of a sterilized sharp blade to get 2-3 mm bits. These bits were dipped in 0.1% mercuric chloride solution, with the help of sterilized forceps, for 5-10 seconds and were washed five times using sterilized distilled water and later placed on sterilized filter paper to remove excess moisture. The four measurements were than averaged to obtain mean radial growth per petri dish.

Pleurotus strains were inoculated in petri plates containing sterilized media i.e. PDA, MEA, WEA in a laminar flow hood. A small block (ca. $5mm^2$) of mycelium from an actively growing pure culture was placed at the center of the media at the point where the radial lines intercepted.

Experiment No. 2. Effect of different temperature levels on the growth of *Pleurotus* species.

After standardization of agar media in experiment 1 for the mycelial growth of *Pleurotus* species temperature was optimized. *Pleurotus* strains (wild and exotic) were

inoculated separately in the Petri dishes of PDA media and incubated at 15°C, 20°C, 25°C, 30°C and 35°C in 3 replicates and mycelial growth was recorded in cm/day.

Experiment No. 3. Effect of different pH levels on the growth of *Pleurotus* species.

After optimization of temperature, these species were optimized for their pH requirement. Therefore, different levels of pH (4, 5, 6, 7 and 8) of the PDA media were maintained by adding 1 M solutions of HCl and NaOH. Inoculations were made by the same way as in the first experiment in three replicates and the plates were incubated at 25°C. Mycelial growth was also measured the same way as in temperature experiment.

Statistical analyses: The data collected from three experiments for each study, was analyzed statistically using the MSTAT-C (Russel and Eisensmith, 1983). Fisher's analysis of variance technique was used to test the overall significance of the data, while the Least Significant Difference (LSD) test ($P \leq 0.05$) was used to compare the differences among treatment means (Steel *et al*., 1997).

RESULTS

Experiment 1: *Pleurotus spp*. showed significant ($P \leq 0.05$) differences regarding mycelial growth on different growing agar media i.e. PDA, MEA and WEA. Among these growing agar media, on an average PDA showed fastest (0.52 cm/day) mycelial growth of *Pleurotus* spp. (Figure 1) followed by MEA (0.47 cm/day) while, slowest mycelial growth of all *Pleurotus* spp. (0.41 cm/day) were observed on WEA. As far as different *Pleurotus* spp. are concerned, *P. sapidus* (FW-133) showed the highest mycelial growth which was about 2.2-fold higher as compared to *P. eryngii* (Figure 2). Similarly, the interactive effect of species and agar media also showed that the *P. sapidus* (FW-133) exhibited the highest mycelium growth on PDA media (Figure 3).

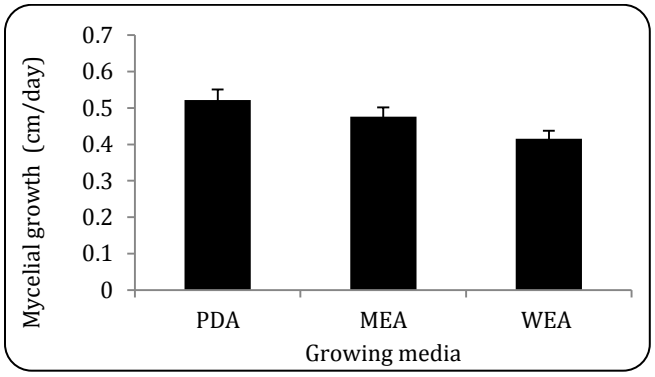

Figure 1. The comparison among agar media for the mycelial growth of *Pleurotus* spp. Vertical bars represent ± S.E of means (n=15).

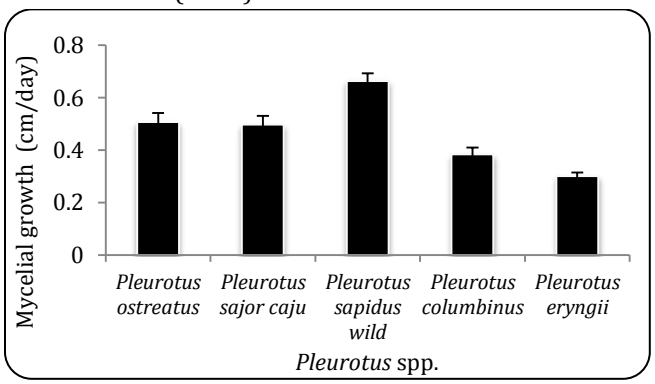

Figure 2. The mycelial growth recorded in various *Pleurotus* spp. on different agar media. Vertical bars represent ± S.E of means (n=9).

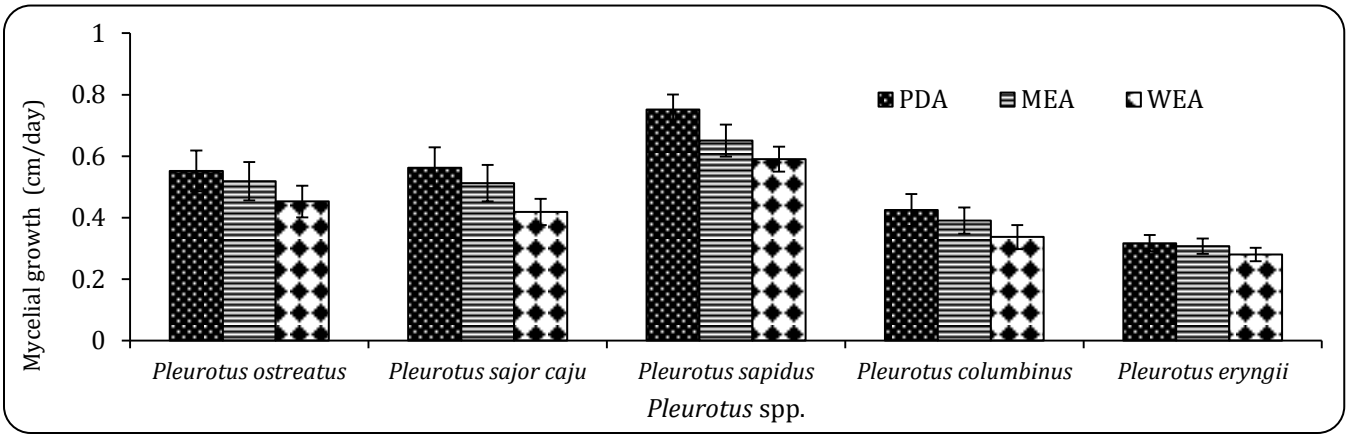

Figure 3. The interactive effect between different growing agar media and *Pleurotus* spp. Vertical bars represents ± S.E of means (n=3).

Experiment 2: Among different temperature levels *Pleurotus* spp. showed significant variation of mycelial growth (Figure 4). All the *Pleurotus* spp. showed their highest mycelium growth at 25°C (Figure 5). The fastest

mycelial radial growth (0.90 cm/day) was exhibited by *P. sapidus* (FW-133) followed by *P. sajor-caju* (0.80 cm/day), *P. ostreatus* (0.79 cm), *P. columbinus* (0.64 cm/day) while the slowest mycelial growth was observed in *P. eryngii* which was about 0.43 cm on day^{-1} at 25°C in dark (Figure 6). However, at 35°C mycelium growth was stopped in all the *Pleurotus* spp. except *P. sapidus* (FW-133) with 0.63 cm/day mycelial growth.

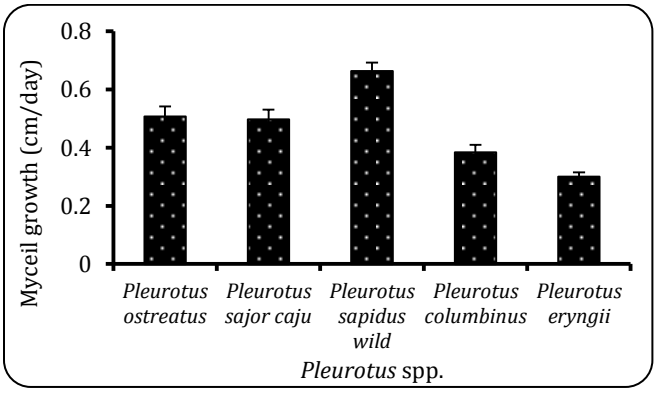

Figure 4. The mycelial growth of *Pleurotus* species on different temperatures levels. Vertical bars represents ± S.E of means (n=15).

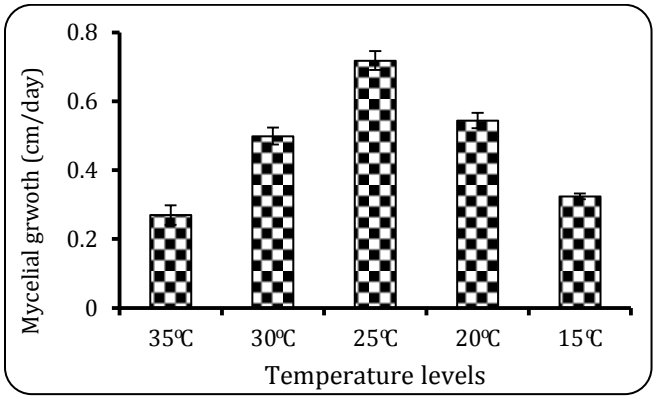

Figure 5. The effect of different temperature levels on mycelial growth of *Pleurotus* species. vertical bars represent ± S.E of means (n=15).

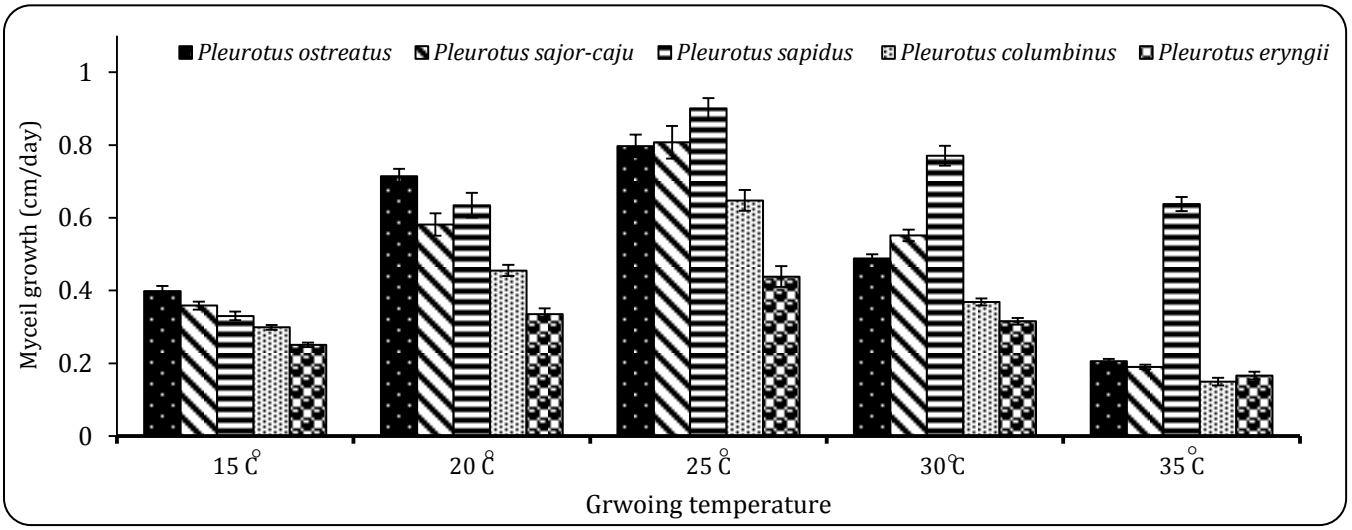

Figure 6. The interactive effect of *Pleurotus* species and different temperature levels for the mycelial growth. vertical bars represent ± S.E of means (n=3).

Experiment 3: Influence of pH media on the growth of mushroom was studied in the absence of light at 25°C. The pH levels of PDA were significantly affected the mycelium growth of oyster mushroom. Maximum growth of *Pleurotus* spp. was observed at pH 6 while minimum mycelial growth was observed at pH 4 (Figure 7). Among the *Pleurotus* spp., *P. sapidus* (FW-133) exhibited highest growth (0.923 cm/day) followed by *P. sajor-caju* (0.85cm/day), *P. ostreatus* (0.81cm/day), *P. columbinus* (0.68 cm/day) and *P. eryngii* (King oyster mushroom) (0.433 cm/day) at different pH levels (Figure 8).

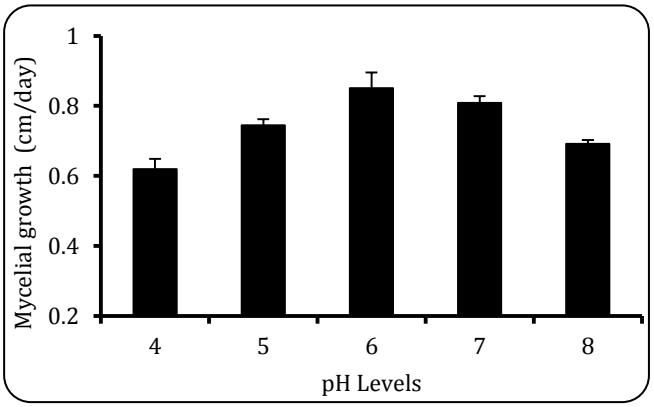

Figure 7. The mycelial growth of *Pleurotus* species at different pH levels. vertical bars represent ± S.E of means (n=15).

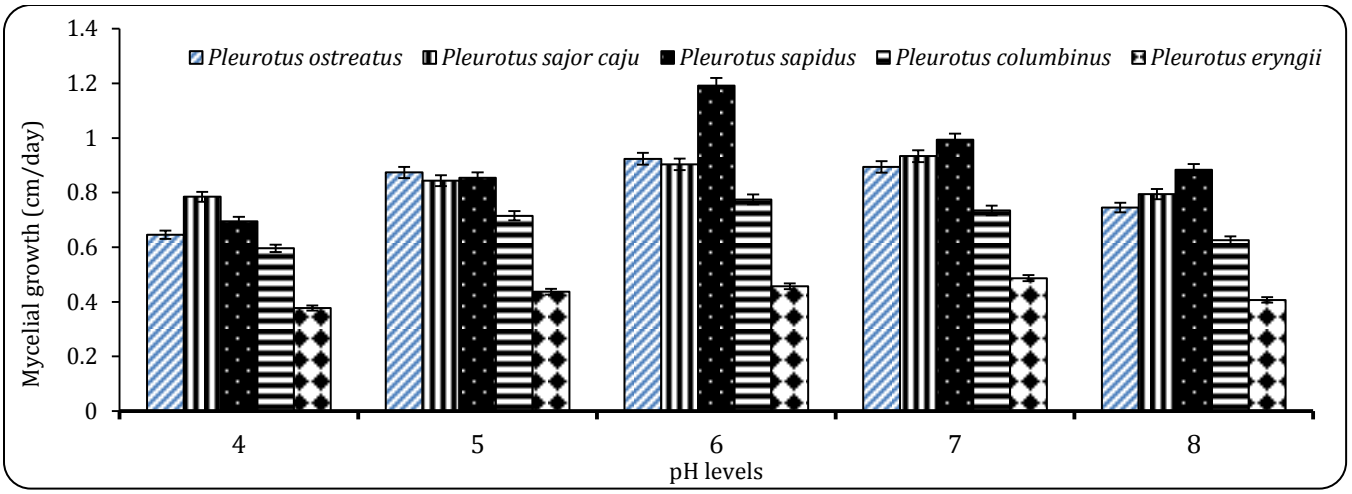

Figure 8. The interactive effect of *Pleurotus* species and pH levels for mycelial growth. Vertical bars represent ± S.E of means (n=3)

DISCUSSION

Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce (Ravimannan *et al*., 2014). Among different growing agar media used in this investigation i.e. PDA, MEA, and WEA, Potato dextrose agar proved to be the best media for the mycelial growth of *Pleurotus* species. This difference of mycelial growth on different agar media may be due to availability of different carbon sources and other required nutrients. Mycelium growth was marginally better on a medium containing glucose and sucrose than other sources (Santiago, 1983). Moreover, PDA might exhibit higher carbon sources and nutrients for mushroom mycelia in petri plate. Our results are in agreement with the findings of Gibriell *et al*. (1996); Hussain and Hussain, (2004) who reported that *Pleurotus* spp. showed fastest growth of mycelium on potato dextrose agar among different media used.

In general, temperature and pH are the most important environmental factors that control the growth of microorganisms. pH and temperature effects the growth of *Pleurotus* spp. through affecting the enzyme activity in the cell (Sopit, 2006). For mycelial growth, pH is another important factor that affects the growth potential of fungus. In this study, it was observed that most of *Pleurotus* spp. showed superior growth at pH 6. Among these test species, *P. sapidus* (FW-133) showed maximum growth adaptability scale at pH (6-7). It might be attributed to genetic differences found in different species of genus *Pleurotus*. As, Karacanci (1997) noticed maximum mycelial growth in *P. ostreatus* at pH 6.5 These results are in accordance to the findings of (Suharban and Nair, 1994), who reported that mycelia of the *Pleurotus* spp. grow faster on slightly acidic medium than basic media. Oyster mushroom (*Pleurotus* spp.) studied in this study decreased its mycelial growth at pH 4 and 5 levels. Overall, decrease in growth may be due to reduced hyphal growth that subsequently had affected its enzymatic activity of respective mycelia. Likewise observation has also been reported previously by Zadrazil (1978) that *P. ostreatus* and *P. eryngii* showed significant decrease in mycelial growth at pH 4.0 which is too acidic. However, Some *Pleurotus* spp. are characterized for wider growth adaptability scale for pH i.e. 5-8 as reported by Yadav (2001). The mycelial growth of *P. ostreatus* was recorded best at pH 7.0 by Bugarski *et al*. (2000). It is evident from the results that *P. sapidus* (FW-133) can perform best in term of its mycelial growth around pH (6-7) in sub-tropical region (Punjab) Pakistan.

Temperature is an important aspect in the selection of mushroom for the tropics where high temperature remains for most of the time. Mushroom test (*Pleurotus*) species are usually attributed for higher growth rate at 25ºC. Previously, optimum temperature within this range was reported by Zharare *et al*. (2010) by observing 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 (Kausar, 1988). However, *P. sapidus* (FW-133), collected from the sub-tropical region has tendency to survive and continue its enzymatic activity

even at 35ºC. Temperature sensitivity index of *Pleurotus* spp. revealed greater genetic difference among the genus *Pleurotus*. Similar results were found 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 35ºC temperature.

CONCLUSION

Pleurotus spp. performed best when grown at temperature of 25°C for the fastest mycelial growth. However, *P. sapidus* (FW-133) can tolerate temperature of 35°C, which can be grown on sub-tropical high temperature areas. As for as different growing media are concerned PDA media proved to be the best media for the growth of *Pleurotus* spp. pH level must be maintained at 6 for best growth of *Pleurotus* spp.

ACKNOWLEDGEMENT

The authors are grateful to Higher Education Commission (HEC) of Pakistan for financial support to this study (Av-7-206).

REFERENCE

Alexopoulos, C. J., C. W. Mims and M. Blackwell. 1996. Introductory Mycology. John Willey and Sons. Inc., New York, 868.

Bhatt, P., R.P. Singh and S.C. Sati. 2010. Evaluation of different *Pleurotus* hybrids for their growth requirements in-vitro. Indian. Phytopath. 63: 424-426.

Bugarski, D., D. J. Gvozdenovic, J. Cervenski and A. Takac. 2000, October. 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 (pp. 319-323).

Gaitán, H.R. and D. Salmones. 2008. Obtaining and characterizing *Pleurotus ostreatus* strains for commercial cultivation under warm environmental conditions. Scientia. Hortic. 118:106-110.

Gbolagade, J.S., I.O. Fasidi, E.J. Ajayi and A.A. Sobowale. 2006. Effect of physico- chemical factors and semi-synthetic media on vegetative growth of *Lentinus subnudus* (Berk.), an edible mushroom from Nigeria. Food. Chem. 99: 742-747.

Gibriel, A.Y., M. Ahmed, N. Rasmy, I. Rizk and N.S. Abdel-Rehem. 1996. Cultivation of oyster mushrooms (*Pleurotus spp*.): Evaluations of different media and organic substrates. In: Mushroom biology and Mushroom Products Proceedings of the 2nd International Conference, Pennsylvania, USA (pp. 415-421).

Hussain, A. and N. Hussain. 2004. Evaluation of different media, spawn and substrates for the cultivation of *Pleurotus ostreatus* in Muzaffarabad (Pakistan). Mycopath. 2:67-69.

Jonathan, S. G. and E.O. Esho. 2010. Fungi and aflatoxin detection in two oyster mushrooms *Pleurotus ostreatus* and *Pleurotus Pulmonarius* from Nigeria. Electronic J. Env. Agric. Food. Chem. 9: 1722-1730.

Jose, N. and K.K. Janardhanan. 2000. Antioxidant and antitumor activity of *Pleurotus florida*. Curr. Sci. 79: 941-943.

Kadiri, M. and I.A. Kehinde. 1999. Production of grain mother and planting spawns of *Lentinus subnudus*. Niger. J. Bot. 12: 37-44.

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 10-13th September, 7.

Kausar, T. 1988. Cultivation of mushrooms using crop residues as substrate, Ph.D. Thesis. Dept. Bot. Univ. Pun. Lahore, Pak.

Miles, P.G. and S.T. Chang. 1997. Mushroom Biology: Concise Basics and Current Developments. World Sci. Press, Hong Kong. 105-106.

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

Russell, D. F. 1986. MSTAT-C package programme. Crop and Soil Science Department, Michigan State University, USA.

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

Stanley, O. H and G. Awi-Waadi. 2010. Effect of substrates of spawn production on Mycelia of Oyster Mushroom species. Res. J. Appl. Sci. 5: 161-164.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. McGraw Hill Book Co., New York.

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

Wei, S.H., Y.Y. Jie, H.L. Rong, H.L. Li and M.G. Yan. 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.

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