KAVAKA 49: 72-76 (2017)

Evaluation of physical parameters for vegetative growth of *Pleurotus cystidiosus*

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(Submitted in November, 2017; Accepted on December 18, 2017)

ABSTRACT

Present investigation pertains to study the impact of different culture media, temperature range, pH range, incubation period and effect of light and darkness on the vegetative growth of *Pleurotus cystidiosus* O.K.Mill. Out of the media selected for evaluation, best mycelial growth (6.3cm) was recorded in Malt Extract Agar (solid medium) and Yeast Glucose Medium (liquid medium), which is 7.9 mg/ml. Out of the six different temperatures (15, 20, 25, 30, 35, 40˚C), best mycelial growth was obtained at 30 ± 1˚C in both solid (6.3cm) as well as liquid medium (8.2 mg/ml). Acidic pH (6.5) supported the best mycelial growth in solid (6.3 cm) as well as liquid medium (12.9 mg/ml), when the mycelium was grown in media with pH ranging from 3.0-10.0. For incubation period, the experiment was conducted for sixteen days in Yeast Glucose Medium and maximum mycelial growth (6.93 mg/ml) was recorded on 13th day of incubation. Under light condition mycelial growth was quite less in both solid (5.06 cm) as well as liquid media (6.46 mg/ml) in comparison to dark condition under which growth was 6.3 cm in solid medium and 7.63 mg/ml in the liquid medium, which is comparatively on the higher side.

Keywords:- Coremia, culture media, incubation period, oyster mushroom, pH, temperature.

INTRODUCTION

Edible and medicinal properties of mushrooms are documented in the records of many ancient civilizations (Rout *et al.,* 2015). Edible *Pleurotus* (Fr.) P. Kumm. species refers to Oyster mushroom or Dhingri which are very good dietary foods having positive effect on metabolism, (Ginter and Bobeck, 1987; Trinci 1992; Jong and Birmingham, 1993). The mushroom cultivation is a valuable agribusiness and particularly Oyster mushroom with excellent flavor and taste (Shah *et al.*, 2004) is one of the excellent option for the entrepreneurs. It is reported to be the second widely cultivated mushroom worldwide after *Agaricus bisporus* (Kues and Liu, 2000). Species of *Pleurotus* are widely cultivated in Asia, America and Europe due to their low cost production technology and higher biological efficiency (Mane *et al.*, 2007). Several factors such as spawn, growing media, temperature, pH, moisture content and light intensity are reported to alter the mushroom growth (Kadiri and Kehinde, 1999). Considering obvious prospective of *Pleurotus cystidiosus,* various experiments were designed to ascertain the effect of solid and liquid media, temperature and pH range, incubation period and dark and light condition on the vegetative growth as well as mycelial biomass of the test fungus under laboratory conditions for standardization of production techniques for increasing yield.

MATERIALS AND METHODS

Experimental design: The experiments were conducted in Mycology and Plant Pathology laboratory, Department of Botany, Punjabi University, Patiala. The experiments were arranged in a randomized complete design with three replicates per treatment.

The material: Viable mycelial culture was raised from tissue culture of *Pleurotus cystidiosus* picked from the bark of living stem of *Lagerstromia speciosa* at Punjabi University Campus, Patiala. Pure culture of the mushroom was raised aseptically from the pileus portion where the lamellae join the stipe. The mushroom culture was again sub-cultured aseptically on sterile Potato Dextrose Agar (PDA) medium slants. These inoculated slants were kept at 28±2˚C for further analysis. This whole procedure of culturing was done aseptically under laminar air flow.

Determination of the best medium for vegetative growth of *Pleurotus cystidiosus*: Fourteen solid media ie., Malt Extract Agar (MEA), Yeast Extract Agar (YEA), Potato Malt Agar (PMA), Potato Dextrose Agar (PDA), Yeast Glucose Agar (YGA), Yeast Potato Dextrose Agar (YPDA), Glucose Peptone Yeast Agar (GPYA), Glucose Asperigine Medium (GAM), Milk Powder Agar (MPA), Elliot Agar (EA), Wood Extract Agar (WEA), Dimmick Agar (DA), Wheat Grain Extract Agar (WGEA), Czepak Dox Agar (CDA) and fourteen liquid media ie., Yeast Glucose Medium (YGM), Potato Dextrose Broth (PDB), Malt Broth (MB), Glucose Peptone Medium (GPM), Peptone Water (PW), Glucose Asperigine Medium (GAM), Malt Peptone Broth (MPB), Richard Solution (RS), Koser Citrate Medium (KCM), Dimmick Medium (DM), Czepak Dox Medium (CDM), Bilai Medium (BM), Asthana and Hawker Medium (AHM) and Will Mineral Salt Medium (WMSM) were used for evaluation in the present study. Composition of different media employed is based on Tuite (1969). All the prepared media were sterilized in an autoclave at 15 lbs pressure for 30 minutes. Excessive heating of the culture media was avoided. The inoculation in solid media was done in the conical flasks each containing 20 ml of different solid media. For this purpose a mycelial disc cut with the help of a cork borer bearing 0.006 gm of mycelial load was placed in the centre of conical flasks. The growth in the solid medium was evaluated by measuring the diameter of the mycelium on an average daily basis for 12 days, (Stanley and Nyenke, 2011).

The inoculum of the liquid medium was prepared by taking small amount of fungus in 100 ml conical flasks carrying 20 ml of liquid medium. The inoculated flasks were incubated at 28±2˚C for 12 days, after which a mycelium mat appeared in the medium, which was homogenized with the help of homogenizer. An amount of 5 ml of this homogenate was added to each flask, containing 20 ml of the liquid medium followed by incubation at 28±2˚C. The mycelium mat was

taken out after 15 days of growth and dried at 65˚C for 48 hrs in a pre-weighed watch glass so as to determine the dry weight of mycelium. Before drying, the mycelium mat was thoroughly washed with double distilled water, so as to remove remaining traces of medium from the mycelium.

The medium (solid as well as liquid) with the best vegetative growth was used as the basal medium for further studies and subsequent experiments.

Effect of Temperature: To establish the optimum temperature requirement for mycelial growth, the experiments were conducted in both solid as well as liquid media. To study the effect of temperature on vegetative growth of *P. cystidiosus*, Malt Extact Agar (solid medium) and Yeast Glucose Medium (liquid medium) selected as the basal media were used. Each of the inoculated flasks were incubated at varied temperatures ranging from 15, 20, 25, 30, 35 and 40˚C.

Effect of Hydrogen Ion Concentration: The experiment for determination of best suited Hydrogen Ion Concentration (pH) for the growth of mushroom mycelium was performed on the solid (MEA) as well as liquid media (YGM). For conducting the experiment, pH of the basal medium was adjusted ranging from pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 by using 1N NaOH and 1N HCL. The pH of the medium was stabilized by using the citrate and phosphate buffers.

Incubation Period: This experiment was conducted by calculating the no. of days required in incubation for achieving maximum vegetative growth of *P. cystidiosus* in liquid medium (YGM). For this purpose inoculated flasks of Yeast Glucose Medium were incubated at 30±1˚C and observations were recorded for 16 days at regular intervals of 24 hrs.

Effect of Light and Darkness: The experiment on light intensity was conducted to ascertain the effect of different light intensities such as proper darkness and light intensity of 100 lux, on the vegetative growth of fungus in both solid (MEA) as well as liquid medium (YGM).

RESULTS

Evaluation of solid media (MEA) for the vegetative growth of *P. cystidiosus*:- From amongst the solid media used for evaluation maximum colony diameter of 6.3 cm was recorded in Malt Extract Agar after 12 days of incubation. The mycelium was found growing at the rate of 0.60 cm in diameter on an average daily basis. After 3 days of inoculation coremia appeared in all the flasks inoculated. The mycelium formed a thick mat with concentric rings, and in due course of time all the coremial droplets were formed on the mycelium mat, **(Fig. 1)**. The next comparable vegetative growth was observed in Yeast Extract Agar (5.4 cm), Potato Malt Agar (3.5 cm), Potato Dextrose Agar (3.4 cm), Yeast Glucose Agar (3.3 cm) and Yeast Potato Dextrose Agar (3.1) media. Least mycelial growth was recorded in Czepak Dox Agar (2.1 cm) in which mycelium was found growing at the rate of 0.14 cm in diameter on an average daily basis. The histogram showing mean colony diameter of the fungus with standard deviation is presented in **Fig. 2**.

Fig. 1 Coremia formation on the mycelial mat

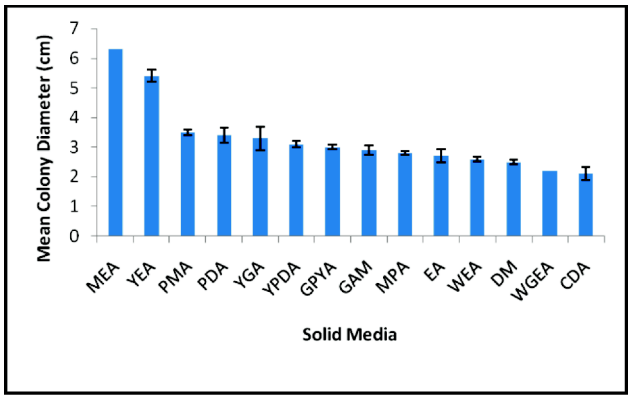

Fig. 2 Histogram showing the effect of solid media on the mycelial growth of *Pleurotus cystidiosus*

Evaluation of the liquid media(YGM) for the vegetative growth of *P. cystidiosus*:- Yeast Glucose Medium supported maximum yield (7.9 mg/ml) followed by Potato Dextrose Broth (6.9 mg/ml) while minimum growth is recorded in Asthana and Hawker medium (0.08 mg/ml). There was practically no growth in Will Mineral Salt medium. The mean dry weight of the mycelium obtained with standard deviation in different liquid media used for evaluation is presented in **Fig. 3**.

Evaluation of temperature requirements for mycelial growth of *P. cystidiosus* in solid (MEA) and liquid media (YGM):- The maximum mycelial growth of 6.3 cm was recorded in solid medium and 8.3 mg/ml in liquid medium giving quite dense and thick mycelium mat at incubation temperature of 30˚C. The mycelium was growing @ 0.61 cm in diameter per day on an average on daily basis in solid medium. The second best growth was recorded at 25˚C i.e.,

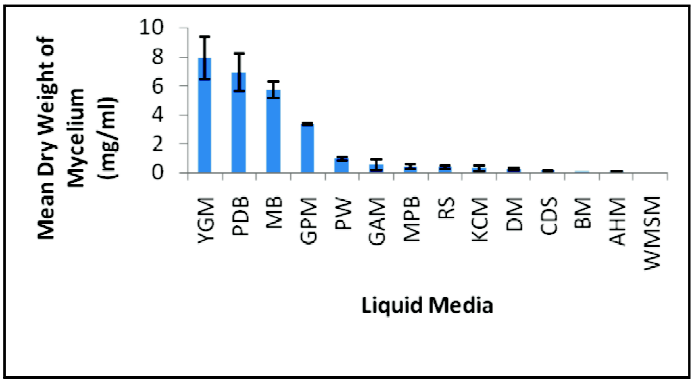

Fig. 3 Histogram showing the effect of liquid media on the mycelial growth of *Pleurotus cystidiosus*

(4.3 cm) with growth rate of 0.38 cm per day and 7.4 mg/ml in solid and liquid media. In comparison at 35˚C the mycelial growth was less prominent. The minimum mycelial growth was recorded at 20˚C (2.1 cm and 1.60 mg/ml). There was practically no mycelial growth at 15˚C and 40˚C in both the media. The mean colony diameter and dry weight of the fungus in both solid and liquid medium is presented in **Figs. 4 and 5**, respectively.

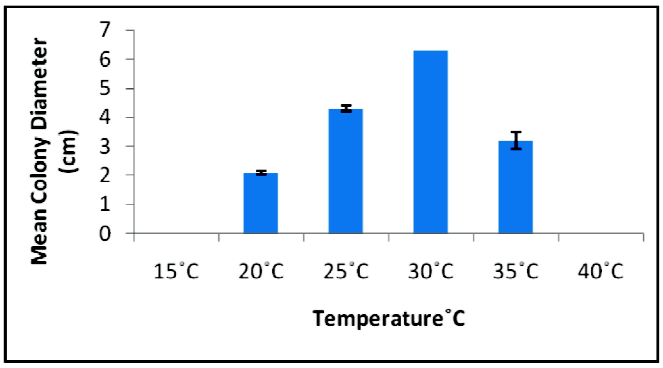

Fig. 4 Histogram showing the effect of different temperatures on the mycelial growth of *Pleurotus cystidiosus* on Malt Extract Agar (solid medium)

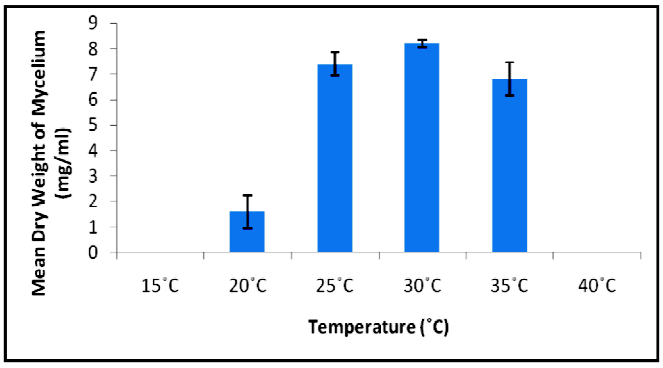

Fig. 5 Histogram showing the effect of different temperatures on the mycelial growth of *Pleurotus cystidiosus* on Yeast Glucose Medium (liquid medium)

Evaluation of different Hydrogen-Ion Concentrations for the vegetative growth of *P. cystidiosus*:- To study the effect of pH, on vegetative growth of *P. cystidiosus*, Malt Extract Agar (solid medium) and Yeast Glucose Medium (liquid medium) were prepared with different pH levels ranging from pH 3.0-10.0. The maximum growth was recorded in the medium with pH 6.5, (6.3 cm) with growth rate of 0.47 cm/day and 12.9 mg/ml while minimum growth was there in pH 4.0 (2.1 cm) with growth rate of 0.18 cm and 3.1 mg/ml in both solid and liquid media. There was practically no growth at pH 3.0 and 3.5. The results with standard deviation are presented in **Figs. 6 and 7**.

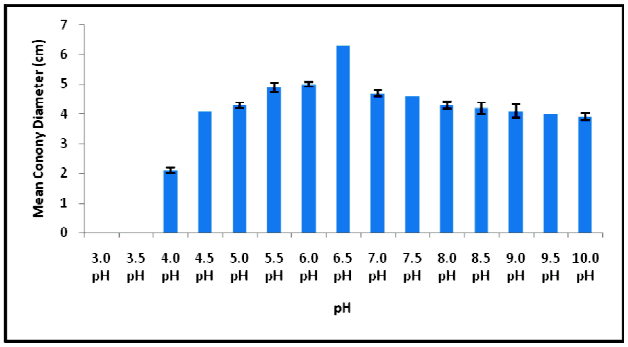

Fig. 6 Histogram showing the effect of different pH levels in solid medium on the mycelial growth of *Pleurotus cystidiosus*

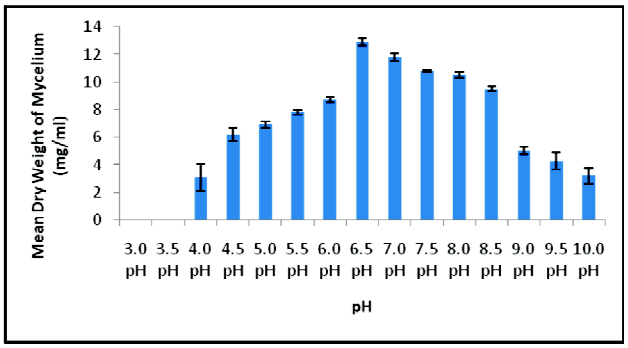

Fig. 7 Histogram showing the effect of different pH levels in liquid medium on the mycelial growth of *Pleurotus cystidiosus*

Determination of the incubation period for maximum vegetative growth of *P. cystidiosus*:- For determining the growth, dry weight method was used. The fungal mycelium was harvested on daily basis so as to measure the increase or decrease in growth with time. Maximum mycelial growth of 6.93 mg/ml was recorded after thirteen days of incubation. There after decrease in growth was observed. The vegetative growth of mycelium obtained at different incubation periods is presented in **Figs. 8 and 9**.

Effect of Light and Darkness on mycelial growth of *P. cystidiosus* in solid medium (MEA) and liquid Medium

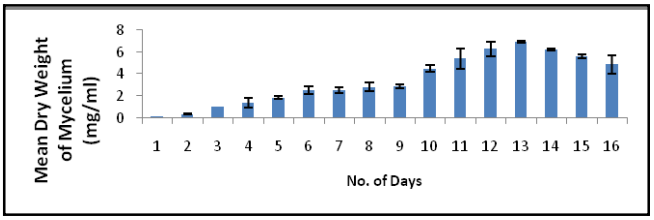

Fig. 8 Histogram showing the effect of different incubation periods on the mycelial growth of *Pleurotus cystidiosus*

(YGM):- The dark condition supported the best mycelial growth (6.3 cm and 7.63 mg/ml) as compared to light condition, where growth was comparatively less (5.06 cm and 6.46 mg/ml) in both solid (MEA) and liquid medium (YGM)

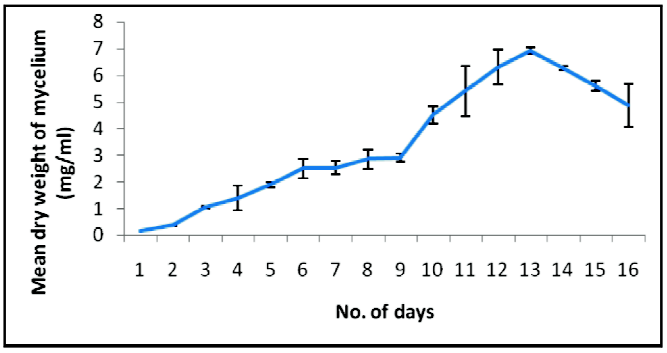

Fig. 9 Linear graph showing the mycelial growth of *Pleurotus cystidiosus* during incubation period

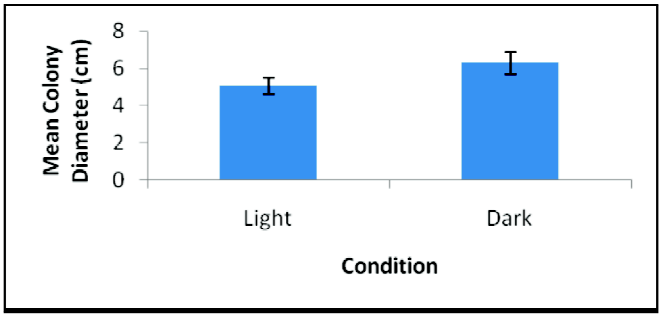

Fig. 10 Histogram showing the mycelial growth of *Pleurotus cystidiosus* in solid medium

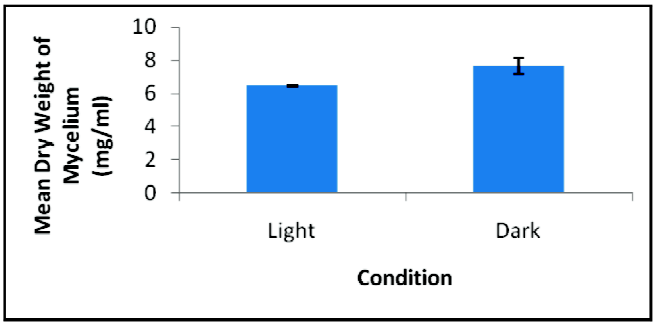

Fig. 11 Histogram showing the mycelial growth of *Pleurotus cystidiosus* in liquid medium

when incubated at 30 ± 1˚C. The mean colony diameter of the fungus and dry weight of the mycelium is depicted in **Figs. 10 and 11**.

DISCUSSION

Food is required by every living organism for its growth and reproduction and oyster mushrooms are not an exception to it. It is necessary to furnish those compounds in the media which are required for its growth and other life processes in order to culture the oyster mushroom in laboratory (Thi and Chun, 2015).

Based upon the present studies conducted with different solid media the best mycelial growth was observed in Malt Extract Agar with respect to radial growth, thickness of mycelial mat and uniformity of colony characters while Platt *et al*. (1975) reported homogenized sorghum grain medium as the best medium for mycelial growth of *Pleurotus cystidiosus.* Many other earlier workers have recommended Malt Extract Agar (Pandey and Tewari, 1991; Nasim *et al*., 2001; Singh, 2005) and Potato Dextrose Agar (Hasan *et al*., 2015 and Bankole and Salami, 2017) as the best medium for mycelial growth of *Pleurotus* species. Yeast Glucose Medium amongst the liquid media supported maximum mycelial growth on dry weight basis. There is hardly any report available regarding recommended best liquid medium for the vegetative growth of *Pleurotus cystidiosus*. On the other hand for the *Pleurotus* species, Potato Dextrose Broth has been reported to be supporting maximum mycelial growth by earlier workers (Suharban and Nair, 1991 and Pardeep *et al.,* 2002). Temperature is a very important factor for mycelial growth of fungi. Maximum vegetative growth was documented when temperature was maintained at 30±1˚C in both solid and liquid media while Thi and Chun (2015) reported best mycelial growth of *Pleurotus cystidiosus* at 28˚C. pH is generally considered to be one the most important environmental factor that alters the growth and extension of fungal mycelia (Kang *et al.,* 2002; Akinyele and Adetuyi, 2005; Okwulehie *et al*., 2006). Present observations are in conformity with the observations of Srivastava and Bano, (1970), Jandaik and Kapoor, (1975) and Hasan *et al.,* (2015) that most of the *Pleurotus* species require an optimum pH of 5.6 and 6, respectively for best mycelial growth. The maximum mycelial growth of 6.93 mg/ml was recorded on 13th day of incubation. Earlier Singh (2010) evaluated incubation period of 13 days for the maximum vegetative growth of *Lentinus squarrousulus* (Mont.) Singer. Light, exerts a significant impact on the growth and development process of carpophores of *Pleurotus*, along with other external features. It is reported to act as a signal for triggering the various biophysical and biochemical processes which are reported to lead to morphological and phototropic reactions (Trukhonovets, 1991). During the present investigation the mycelial growth of *P. cystidiosus* was found best under dark conditions, both in solid as well as liquid media which is in conformity with similar such observations by Rout *et al.* (2015) that low light intensity near darkness or darkness is suitable for good vegetative growth in all *Pleurotus* species.

CONCLUSION

From the above discussion it can be concluded that for vegetative growth of *P. cystidiosus* Malt Extract Agar (MEA) is the best solid medium and Yeast Glucose Medium (YGM) is the best liquid medium. The optimum temperature for the vegetative growth of fungus in solid as well as in liquid medium has been evaluated at temperature 30±1˚C. Acidic pH and incubation period of 13 days supported maximum mycelial yield. Dark condition gave better yield in solid as well as liquid media as compared to light condition.

ACKNOWLEDGMENTS

Authors are thankful to Head, Department of Botany, Punjabi University Patiala (Punjab), India for providing necessary laboratory facilities.

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