Effect of Different Stress Condition on Production of Oyster Mushroom (*Pleurotus florida*)

Paramjeet Prabhakar[1], Deepak Kumar[2]

Renaissance Biotech Pvt. Ltd, Patna, Bihar

Abstract - Mushrooms have been used as food supplement for thousands of years and in recent times. They can utilize almost all agricultural wastes as their substrates for their growth and metabolism. In fact, mushrooms also contain low fat, low calories and good vitamins. Mushrooms are valuable health foods since they are low in calories, fats, and minerals. Mushroom cultivation technology is friendly to the environment. Oyster mushrooms as additional, highly nutritious vegetable crops. We should start with these because they are easiest to grow; yet they are also high in protein.

Oyster mushrooms cultivation offers benefits to market gardens when it is integrated into the existing production system. This study recommends for large scale spawn production and mass cultivation due to having the characteristics of high growth rate, high biological efficiency. Most of the Oyster mushrooms species grow and perform well at pH near to neutral. Suitable Temperature, Moisture and pH are most an impotent factor for good production of Pleurotus florida.

My work has shown that optimal environmental conditions for different place in Bihar for large scale oyster mushrooms mainly Pleurotus florida production. We determined by establishing variability in growth response over a range of growth parameters: moisture (80-85 %), temperature (24-26 °C) and pH (6.5 - 7). The optimum oyster mushroom production could be attained through submerge cultivation of the fungus at temperature 25°C, pH 6.5 and 85% of moisture. Optimization of the growth conditions for oyster mushroom has been achieved by that result for high yields production.

Key Words: Oyster mushroom, Pleurotus florida, stress condition, moisture, temperature.

I. INTRODUCTION

The word “mushroom” was used for the edible members of macrofungi. Edible mushrooms once called the “food of the gods” and still treated as a garnish or delicacy. There are at least 12, 000 species of fungi that can be considered as mushrooms, with at least 2000 species are edible. Mushroom cultivation has great scope in India and in some of other developing countries because of the cheap and easily available raw materials needed for this activity. Mushrooms and other fleshy fungi are the premier recyclers of nutrients on the planet**.**

Etter (1929) produced sporocarps of P. ostreatus in culture. Zadrazil (1978) established the industrial production of Pleurotus in U.S.A and Europe. In India Bano and Srivastava (1962) were the first to cultivate Pleurotus, while Jandaik (1974) laid foundation for substrate preparation and fruit bodies production on a commercial scale.

The nutritional composition of Pleurotus species have been well-studied. It is important to understand how differing water, pH and temperature conditions affect the various strains regarding primordium development and basidiome production, and whether tolerance to water stress can be selected for. The main substrate for Pleurotus spawn is wheat (Triticum aestivum) grain (Stamets, 2000).

Pleurotus is an important genus of edible basidiomycetes which are commonly called oyster mushroom: all known species are edible, with several being commercially cultivated. The species are also rich in minerals, are good sources of protein, and have short life cycle. Different substrates have been used by several workers for the cultivation of *Pleurotus* spp. viz. cotton waste (Chang *et al*. 1981). Thomas *et al*. (1998) have reported rice straw, as the most widely used substrate in Asia for the cultivation of *Pleurotus* spp. Though different spawn substrates have been reported by various workers, grain spawn is most widely used for *Pleurotus* cultivation. It has been reported that jowar and bajra grains are superior over wheat grains. Layer spawning has been found to be superior in comparison to other methods. The rate of spawn varies from 3-15 per cent of dry weight of the substrate but the optimum is 10 per cent (Easwaramoorthy, S. Ramaraj, B. and Shanmugam, N. (1983).

Pleurotus species have been used by human cultures all over the world for their nutritional value, medicinal properties and other beneficial effects. Oyster mushrooms are a good source of dietary fibre and other valuable nutrients.They also contain a number of biologically active compounds with therapeutic activities. Oyster mushrooms modulate the immune system, inhibit tumour growth and inflammation, have hypoglycaemic and antithrombotic activities, lower blood lipid concentrations, prevent high blood pressure and atherosclerosis, and have antimicrobial and other activities (N. Gunde-Cimerman, 1999).

Oyster mushrooms are good source of protein. Pleurotus spp. (oyster mushrooms) also contain some unsaturated

fatty acids, provide several of the vitamins B, and vitamin D. Some even contain significant vitamin C, as well as the minerals potassium, phosphorus, calcium, and magnesium**.** Oyster mushrooms are most popular and two different species of this mushroom like *P. ostreatus*, and *P. florida*, are commercially cultivated all over the year by using sawdust and/or rice straw as main substrate. But the productions of these mushrooms are not economically beneficial in every season. The environmental variation is supposed to be the main cause behind this problem. But the performances of these species of oyster mushroom have not yet properly been investigated in the climatic conditions of different condition.

Therefore the present study was undertaken to identify the effect of different stress condition such as pH, temperature, and moisture on production of oyster mushrooms. Because, Environment of Bihar varies place to place

II. MATERIAL & METHODS

Firstly we have Mushroom spawn is prepared on cereal gains i.e. wheat.

The cereal grains thoroughly washed in sufficient water 3-4 times to remove soil debris, straw particles and undesirable seeds and grasses. Washed grains soaked in sufficient water for 20-30 minutes (20 kg wheat grains kept in 35 liters water). After boiling, grain should absorb 55-60 % moisture. Spreading on sieve made of fine wire mesh or muslin cloth for excess water removed from boiled grain. The grains were left for 2-5 hours for water evaporation from surface. The grain mixed with gypsum (calcium sulfate, $CaSO_4$) and chalk powder (calcium carbonate, $CaCO_3$) and the pH was 7-7.5 and did not from lumps. The 200g gypsum and 50g calcium carbonate mixed separately the mixture of both thoroughly mixed with 10 kg grains.

a. *Master/Mother spawn preparation***:** 300g prepared substrate (boiled cereal grains coated with gypsum and calcium carbonate) was filled in glucose/milk bottles up to 2/3 volume. Plug cotton and autoclave at 22 lb p.s.i. pressure at 126^{o}C for 2 hours. These autoclaved bottles left in the room for 24 hours for evaporated excess moisture accumulated inside the bottle. Autoclaved bottles containing sterilized grains exposed to UV light inside the inoculation chamber for 30 minutes. Sterilized grains ware inoculated in BOD at 25^{o}C for 20-25 days and gently shake on 5th and 10th day after inoculation. Fully colonized mother spawn bottles can be used for inoculating commercial spawn bags after 2-3 weeks.

b. *Commerical Spawn production:* Polypropylene bags Used instead of bottles for grain filling (for ½ and 1 kg spawn, the bags used 35X17.5 cm and 40X20 cm size). Polypropylene bags should be double sealing at the bottom. Filled gains plugged with the help of a Polypropylene neck and non-absorbent cotton. Polypropylene bags of 150 gauges should be used. The bags sterilized at 22 lb p.s.i. pressure for 1.5 – 2 hours in an autoclave. Autoclaved bags shake well before inoculation for water droplets accumulated inside the bags reabsorbed by the grains. The sterilized bags kept in the laminar flow under UV light for about 30 minutes.

c. *Harvesting:* Formation of a complete mushroom occurred one week after the colonized bag has been transferred into the cropping room. The appearance of those mushrooms occurs in flush and about 3 flushes occur in a week continuously for a period of four weeks. A clean scalpel was used to detach the fruit bodies at the base of the stipe from the bags. Weight and numbers of the fruit bodies were recorded per replicate bag before the fruit bodies were consumed.

d. *Cultivation condition:* Common cultivation is used for mushroom production under different condition. The control was taken at Temperature @ 22 ^{O}C, Moisture 80%, and pH 6.5 shown in Table 1.

Table 1: Ouster mushroom was put in different condition

Parameters	Stages						
Temperature	10^{0}C -15^{0}C	15^{0}C -20^{0}C	20^{0}C -25^{0}C	25^{0}C -30^{0}C	30^{0}C -35^{0}C	35 ^{0}C -40 ^{0}C	40 ^{0}C-45 ^{0}C
pH	4.5	5.5	6.5	7.5	8.5	9.5	10
Moisture	55%-60%	60%-65%	65%-70%	70%-75%	75%-80%	80%-85%	85%-90%

III. RESULTS AND DISCUSSION

The water content of the wheat grain generally increased with each successive boiling, for example in the experiment using narrow-mouthed bottles and increased number of shorter boiling periods, water content increased from 39.90 % (boiled twice) to 66.29 % (boiled 6 times). Burst grain occurred for the following treatments 5 min (boiled for 5 and 6 times) with a lot of burst grain for the boiled 6 times treatment for 5 min boiling treatments.

The average number of days taken by each respective treatment to show the presence of visible contamination ranged from 5-9 days. The number of days taken before contamination for all the treatments varied significantly. The 30 minute boiling treatment resulted in the highest number of days before contamination appeared. Contamination generally occurred within the period of a week on the inoculated grain whilst it took one to two weeks on the grain not inoculated. The autoclaved grain took 30 days to show contamination.

Good quality spawn characterized as sterilized wheat grain covered with either rhizomorphic mycelia or sparse cottony mycelia was obtained for the 30 min for the assessment of sterilization of wheat grain by use of narrow78 mouthed bottles. Figure 1.A & 1.B shown the result of master commercial spawn.

Figure 1.A. Master Spawn

Figure 1.B. Commercial spawn production

Figure 1.A&B: Production of *Pleurotus* (oyster mushrooms) master spawn by using narrow-mouthed bottles and commercial spawn by using poly bag.

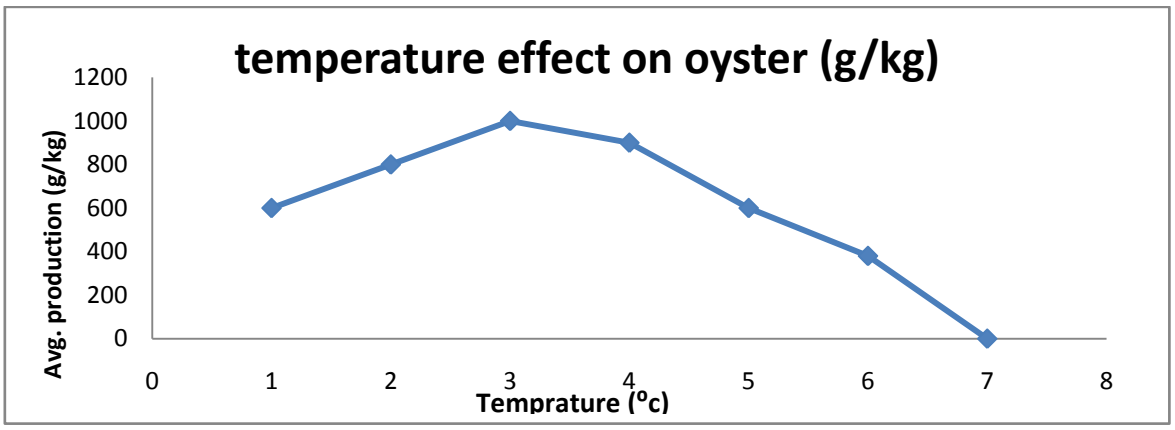
A. *Studies on the effect of Temperature.*

After cultivation of Oyster mushrooms with per kg spawn in the field. The maximum growth was observed at temperatures of 20^0C -25^0C whereas minimum growth was observed till temperatures 35^0C -40^0C and there was no growth above temperatures of 40^{0C}. This reveals revels that the optimum temperature was 20^0C -25^0C. Result shown in Table 2 and Graph 1.

Table 2: Temperature effect on *Pleurotus florida* cultivation

Temperature	Avg. Production (g/kg)
10^0C - 15^0C	600
15^0C - 20^0C	800
20^0C - 25^0C	1000
25^0C - 30^0C	900
30^0C - 35^0C	600
35^0C - 40^0C	380
40^0C - 45^0C	0

Graph 1: Temperature effect.



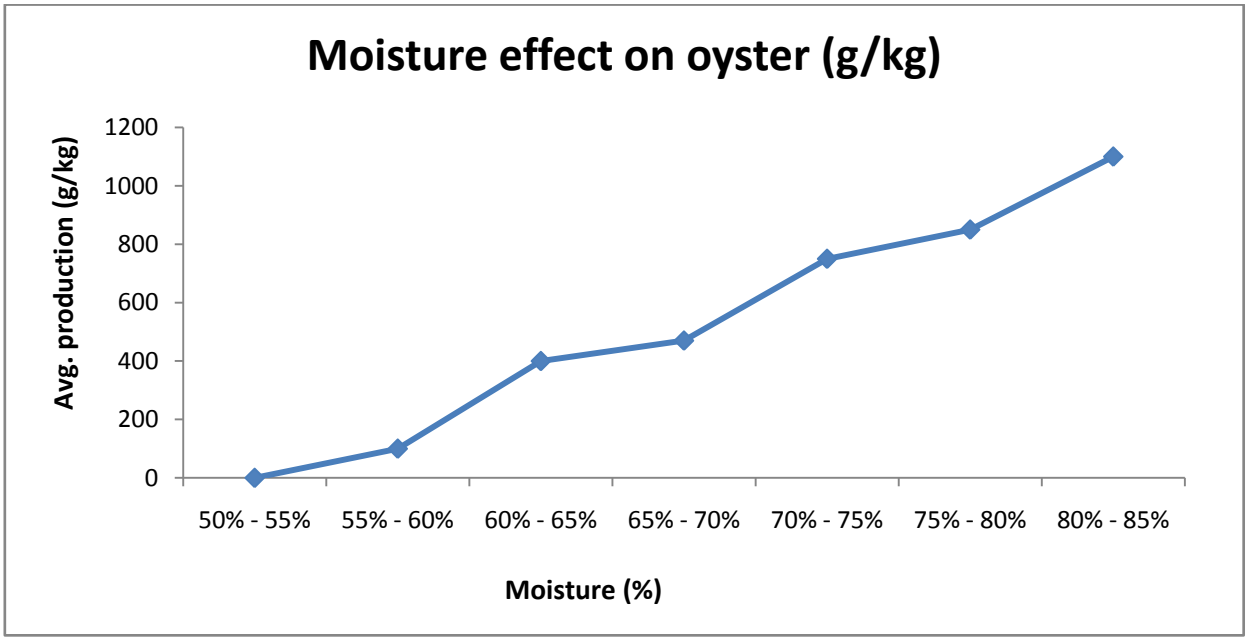
B. Studies on the effect of Moisture.

After cultivation of Oyster mushrooms with per kg spawn in the field. The maximum growth was observed at moisture of 80% - 85% but this moisture is not favor for industry due to less stability so industry prefer 75% - 80%. Whereas minimal growth was observed till moisture 55% -60% and there was no growth below moisture of 55%. This reveals revels that the optimum moisture was 75% - 80%. Result shown in Table 3.

Table 3: moisture effect on *Pleurotus florida* cultivation

Moisture (%)	Avg. Production (g/kg)
50% - 55%	0
55% - 60%	100
60% - 65%	400
65% - 70%	470
70% - 75%	750
75% - 80%	850
80% - 85%	1100

Graph 2: Moisture effect on *Pleurotus florida* cultivation



C. Studies on the effect of pH.

After cultivation of Oyster mushrooms with per kg spawn in the field. The maximum growth was observed

at pH of 6.5. Whereas minimal growth was observed at pH 4.5 and there was no growth below pH 4 and above pH 9. This reveals revels that the optimum temperature was 6.5. Result shown in Table4.

Table 4: pH effect on *Pleurotus florida* cultivation

pH	Avg. Production (g/kg)
3.5	0
4.5	100
5.5	550
6.5	900
7.5	600
8.5	550
9.5	0

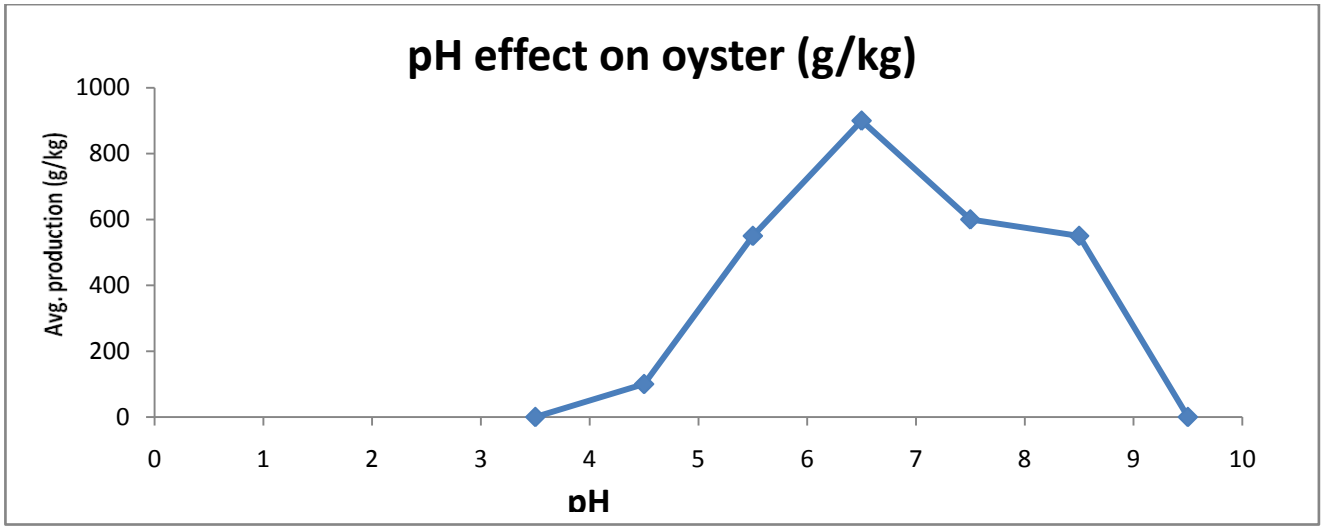
Commercial cultivation of mushrooms is not for everyone. It requires someone who is familiar with fungi life cycles and willing to commit time and money to research, designing a system, and developing a business. The mushroom cultivator must be able to carry out operations on time, be attentive to details, and be vigilant about pest invasions.

The moisture contents of oyster mushroom were found 80-85%. In the moisture contain 85-90% the highest mushroom yield got but this moisture % water content is highly found. For this regain 85-90% moisture contain mushroom less stable than 80-85%. So all oyster mushroom production industry preferred 80-85% moisture condition for large scale production.

Temperature had a remarkable effect on mushroom yield production by oyster mushroom. The minimum and maximum temperatures for growth were 20°C and 25°C. Optimum temperature for growth production by oyster mushroom contrasts the report of Mahmond et al. (2004). The optimum temperature for cultivation of oyster mushroom was 25°C. This observation agree favorably well with the findings of Chi et al. (1996) and that of Jonathan and Fasidi (2001).

Ability of oyster mushroom to grow optimally at pH 6.5 is that several kinds of mushrooms have more acidic pH optima for mushroom yield during their submerge cultivation (Kim et al., 2003). Oyster mushroom appears to be able to grow over a wide range of pH value from 5.5 - 7.5.

Graph 3: pH effect on Pleurotus florida cultivation



This work has shown that optimum oyster mushroom (*Pleurotus florida)* production could be attained through submerge cultivation of the fungus at temperature 25°C, pH 6.5 and 85% of moisture. This result may provide a sustainable means of adding value to submerge cultivation of which will result in production. The optimal conditions identified in this study may enhance worldwide production of oyster mushrooms, especially in the developing

countries such as India, Pakistan, Nepal, etc. Polyethylene glycol 6000, potassium chloride and sodium chloride at minute concentrations are recommended as growth enhancers in Pleurotus spawn and cultivation procedures.

*Acknowledgement*s: The authors are thankful to Biotech Consortium India Limited who give me financial support through Biotech Industrial Training Program and Mr. Deepak Kumar, Director, Renaissance Biotech Pvt. Ltd, for providing laboratory and library facilities.

REFERENCES

[1]. Bano, Z. and Nagarajan, N. (1979). The cultivation of mushrooms (*P. flabellatus*) on paddy straw packed in polythene bags with vents. Indian Food Packer 30:52–57.
[2]. Bano, Z. and Rajarathnam, S. (1982). *Pleurotus* mushrooms as a nutritious food. *In*: S.T. Chang and T. H. Quimio (ed.) Tropical Mushrooms: their Biological Nature and Cultivation Methods. The Chinese University Press, Hongkong, p. 363–380.
[3]. Bano, Z. and Srivastava, H. C. (1962). Studies on the cultivation of *Pleurotus* spp. on paddy-straw. Food Sci. 12:363–365.
[4]. Chang ST, Quimio TH (1981). Tropical mushroom: biological nature and cultivation method. The Chinese University Press, Shatin New Town. Chang ., *Int. J. Med. Mush. 1* 1999, 291–300.
[5]. Chi JeongHyun, Ha TaeMoon, Kim YangHo, Ju YoungCheul, J.H. Chi, T.M. Ha, Y.H. Kim and Y.C. Ju. (1996). Effect of storage temperature and packing method for keeping freshness of fresh mushrooms. *RDA Journal of Agricultural Science, Farm Management, Agricultural Engineering, Sericulture and Farm Products Utilization*. 38(1): 915-921.
[6]. Easwaramoorthy, S. Ramaraj, B. and Shanmugam, N. (1983). Studies on some factors influencing yield of *Pleurotus* mushroom. Paper presented at Symposium on Science and Technology of Edible Fungi. CSIR, RRL Srinagar, India.
[7]. Etter, B. E. (1929). New media for developing sporophores of wood-rot fungi. Mycologia 21:197–203.
[8]. Jandaik, C. L. (1974). Artificial cultivation of *Pleurotus sajor-caju.* Mushroom J. 22:405. Jandaik, C. L. and Kapoor, J. N. (1976). Studies on cultivation of *Pleurotus sajor-caju* (Fr.) Singer. Mushroom Sci. 9(1):667– 672.
[9]. Jonathan SG, Fasidi IO., *Food Chem*. (2001), 75: 303-307
[10]. Kim JH, Kim YS., (2003). A fibrinolytic metalloprotease from the fruiting bodies of an edible mushroom Armillariella mellea. Biosci Biotechnol Biochem; 63, 2130–6.
[11]. Nayana, J. & Janardhanan, K.K. (2000). Antioxidant and antitumour activity of *Pleurotus florida*. *Current Science*. 79: 941-943.
[12]. Stamets, P. (2000). *Growing Gourmet and Medicinal Mushrooms.* Ten Speed Press, PO Box 7123, Berkeley, CA 94707.
[13]. Zadrazil, F. (1978). Cultivation of *Pleurotus*, p. 521-557. *In*: S. T. Chang and Hayes, W. A. (eds). The Biology and cultivation of Edible Mushrooms. Academic Press New York.
[14]. N. Gunde-Cimerman, (1999), Medicinal value of the genus *Pleurotus* (Fr.) P. Karst. (Agaricales s.l., Basidiomycetes), *Int. J. Med. Mush. 1* 69–80.