Nutrient Requirements And Environmental Conditions For The Cultivation Of The Medicinal Mushroom (Lentinula Edodes) (Berk.) In Ghana

E. H. Quaicoe, C. Amoah, M. Obodai, G. T. Odamtten

ABSTRACT: The optimum nutrient and environmental growth conditions of three strains of Lentinula edodes (Le 75, Le P and Quagu) were investigated for their ability to grow under different temperatures, on some selected natural media, at different light durations as well as different acidities. Temperatures investigated ranged from 16°C to 30 °C. Optimal temperature for the development of all the three strains of L. edodes was at 25°C. The five selected natural media were; maize, rice, millet, sorghum meal agar and potatoes dextrose agar. The best mycelial growth on natural media was recorded on sorghum meal agar. The mycelia growth rates were also investigated under light durations. The best growth of L. edodes was in constant 24 hrs darkness for all the three strains and the differences between them were significant (P≤0.05). Mycelial filaments were relatively dense when it was incubated in alternating shifts of dark/light conditions; this showed that light is an important factor in the mycelial characteristics of this fungus. This baseline data obtained on these parameters is necessary for the cultivation of Lentinula edodes in Ghana as it is being introduced into the country for the first time.

Keywords: Lentinula edodes, Shiitake; mycelial, mushroom, Lentinan, lectin

INTRODUCTION

Mushrooms are popular and widely consumed in the diet of most communities in Ghana [1]. Various species found include Termitomyces (termite mushroom) Pleurotus (oyster mushroom), Volvariella (oil palm mushroom), etc. Wild mushrooms are always abundant during the rainy season and are harvested for home use and for sale [2]. Depending on the variety, they contain high quality proteins with levels ranging from 21-40% dry weight. Mushrooms have also been found to have a good balance of amino acids compared to most plant foods and found to contain all nine essential amino acids.They also contain vitamins B1, B2, B6, B12 as well as C and D [3]. More than 14,000 different varieties of mushrooms exist in nature [4], however, less than 200 of these are widely accepted as food and only about 80 are cultivated commercially. One of the important commercially cultivated mushroom varieties is Lentinula edodes. Lentinula mushroom has long been favored by Asian people as a gourmet and medicinal mushroom. Commonly known as shiitake and found throughout North America, Europe, and Asia, it is traded in the international market as well as other parts of the world. It is the world's 2nd most cultivated mushroom.

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Its popularity is ever increasing partly because of its exotic flavor and also because of its nutritional and various medicinal properties [5]. In different parts of the world, shiitake is known by different names. The name Shiitake (shii-take) is derived from the Japanese words: "shii" meaning the hardwood of Pasania spp and "take" meaning the mushroom. The name shiitake is now the most popular specialty mushroom worldwide [6]. Shiitake mushrooms from the orient are not only delicious and nutritious food with great flavor and an enticing aroma, but they also contain a material well known for its medicinal benefits- lentinan. This is a water-soluble polysaccharide produced and extracted from shiitake. It is an approved anti-cancer drug used in the world for cancer preventive, in the treatment of colds and flu, virus infections, hepatitis, environmental allergies, diabetes, high blood pressure, immune weakness, high cholesterol, chronic fatigue syndrome, poor blood circulation, upper respiratory diseases, exhaustion and weakness following antitumor treatments, and in alleviating the side effects of chemotherapy [7]. Nutritionally, it also provides various amino acids such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine, as well as vitamins B1, B2, B12 and also mineral salts. Regular intake may prevent rickets, especially in infants, which could be due to a disorder of phosphorus and calcium metabolism caused by vitamin D deficiency. This is because Lentinula edodes contains the provitamin ergosterol, which is not present in much quantity in vegetables, but is converted to vitamin D in the presence of sunlight. Vitamin D increases resistance against illnesses and has preventive and curative effects on colds [8]. Two other constituents have been isolated from Lentinula edodes which tend to reduce serum cholesterol are: $C_6H_{11}O_4N_5$ and $C_9H_{11}O_3N_5$, namely [2(R), 3(R)–dihydroxy-4-(9-adenyl)-butyric acid and 2(R)-hydroxy-4-(9-adenyl)-butyric acid] [9]. Many of the world's undernourished population are in subtropical and tropical regions. Studies on shiitake production under such climates, such as in Ghana, will be helpful in encouraging mushroom growing in other parts of the sub regions to alleviate poverty. Currently the local Termitomyces lestestui has not been domesticated for commercial cultivation under laboratory conditions because of the intricate life cycle of this fungus to the termites in termite mound. Volvariella volvacea (locally called domo) has limited commercial cultivation by mushroom growers in Ghana because of the difficulty in spawn production and the attendant competition of contaminants especially Coprinus species which limit flushing of this mushroom. The cultivation of oyster mushrooms (Pleurotus species) on composted sawdust is a thriving farming industry so far in Ghana. The present production capacity is over 300 tons annually (MUGREAG, unpublished data). With this development, the Ministry of Food and Agriculture in 2011 pledge to spearhead the promotion of mushroom cultivation and consumption in Ghana. The ministry also promised to strengthen the farmers and stakeholders in mushroom cultivation [10]. With oyster mushroom already under cultivation in the country, studies of other exotic ones like shiitake under the climatic conditions in Ghana can be undertaken to diversify the existing mushroom farming crops, alleviate poverty and enrich the protein and health benefit associated with growing shiitake. There is always an advantage in the multiplicity of mushroom cultivation so far as poverty alleviation and nutrient supplementation of diets are concerned. In view of this, the present study was designed to obtain a baseline data on the physiological parameters necessary for the cultivation of Lentinula edodes in Ghana as it is being introduced into the country for the first time.

MATERIALS AND METHODS

Mushroom strains used and maintenance

Three strains of Lentinula edodes (Le75, Le P and Quagu) were used for these studies. These were obtained from the Mushroom Unit of the CSIR-Food Research Institute, Accra, Ghana. Stock cultures of the three different strains of L. edodes (Le75, Le P and Quagu) were maintained on Potato Dextrose Agar. These were sub- cultured fortnightly for the studies.

Cereal grains and media used

Cereal grains used were bought from the Madina market, Accra namely, Rice, Wheat, Millet and Sorghum which were used in the preparation of the media:

i) Rice Meal Agar,
ii) Wheat Meal Agar,
iii) Millet Meal Agar,
iv) Sorghum Meal Agar. Potato Dextrose Agar (PDA) was also used.

Media preparation

About 200g of all the cereal grains were milled and heated at 60^{o}C for 30 min in 500 ml distilled water, strained and made up to 1 litre, 20 g of glucose and 12 g of agar were then added. It was sterilized by autoclaving at 15 psi for 45min.

Method of inoculation

About 30 ml of each media was poured into 90 mm Petri dishes and allowed to solidify. A 3 mm disc of the mycelium of each strain was taken from the advancing end of the culture which was used as inoculum in all investigations for both liquid and solid media studies. In the solid media, the disc bearing the culture was placed at the centre of the culture where two lines along two diameters at the bottom of the plate intersect.

Assessment of radial growth

Radial growth of the mycelium was assessed by measuring the growth of the fungus along two diameters drawn at right angles at the bottom of the Petri plates prior to inoculation. Measurements were made daily and the period of incubation varied from one experiment to the other. Influence of media and temperature on growth of three strains of Lentinula edodes. The three strains were cultured in the different media and incubated at different temperatures (16^{o}C, 20^{o}C, 25^{o}C and 30^{o}C).The growth rate was assessed as described above.

Measurement of pH of media

The pH of the media was measured using a Beckman pH meter (model 40). An extra medium in excess of the number of replicates required for a treatment was used for measuring the pH after autoclaving. Adjustment of the pH of the media when required was done by adding the appropriate McIlvaine Buffer solution. The buffer solutions were prepared according to the McIlvaine Buffer standard . The adjusted media were checked with a pH meter to ascertain the exact pH obtained.

Effect of light and its duration on radial vegetative growth of L. edodes

This was assessed by inoculating the best media (sorghum meal agar) with 3 mm discs of the fungus taken from the advancing edge, of the culture from all the three strains of L. edodes.

The conditions used were as follows:

Continuous light: Fifteen plates (five replicate for each strain) earmarked for continuous light treatment were exposed to continuous light (100) lux light intensity for up to 11 days. Continuous darkness: Fifteen plates (five replicate for each strain) were covered with black polythene bags and then incubated in darkness inside an incubator at 25 ^{o}C for 11 days. Alternating light/ darkness: The Petri plates were exposed for 11 days under these conditions.

Alternating 4 hrs light/4 hrs darkness

Alternating 8 hrs light/8 hrs darkness

Alternating 12 hrs light/12 hrs darkness

In each instance, the radial growth of the fungus was measured daily along two diameters. There were five replicates for each treatment.

RESULTS

Influence of media and temperature on growth of three strains of Lentinula edodes.

Variations of mycelia growth were recorded on the media tested and on the temperatures used. In the case of maize meal agar, the best strains recorded were Quagu, Le P and Le 75 at 16^{o}C, 20^{o}C, and 25^{o}C respectively (Fig.1) whiles Le P obtained the best growth at both 16^{o}C and 25^{o}C followed by Quagu at 20^{o}C on rice meal agar (Fig. 2). Strain Le P again had the best growth at both 20^{o}C and 25^{o}C on millet meal agar whilst Quagu had its best growth at 16^{o}C (Fig.3). Sorghum meal agar and a temperature of 25^{o}C was the best growth medium and best temperature respectively for all the strains tested. At 30^{o}C only strain Le 75 recorded a slow mycelia growth rate on all the media. Strain Quagu recorded the best growth at both 16^{o}C and 25^{o}C whilst Le75 recorded the best growth at 20°C on same medium (Fig. 4). Fig. 5 shows an interesting growth pattern where all strains attained a maximum at 25°C and a minimum growth at 20 °C. Analysis of variance of data obtained showed that the type of media used under a particular temperature significantly (P≤0.05) influenced the radial growth per day.

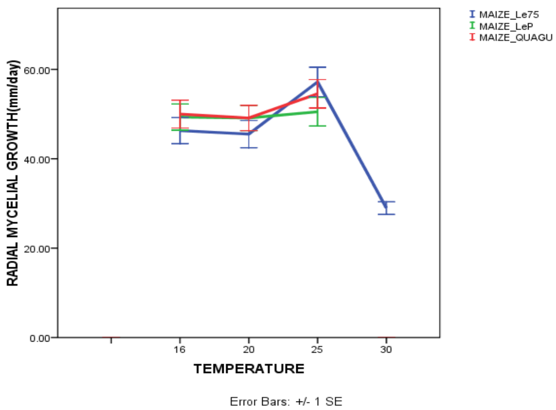


Figure 1. *Radial growth of L. edodes (Le 75, Le P and Quagu) on maize meal agar*

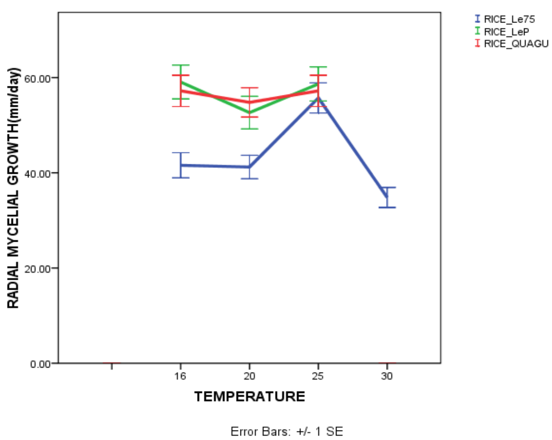


Figure 2. *Radial growth of L. edodes strains (Le 75, Le P and Quagu) on rice meal agar*

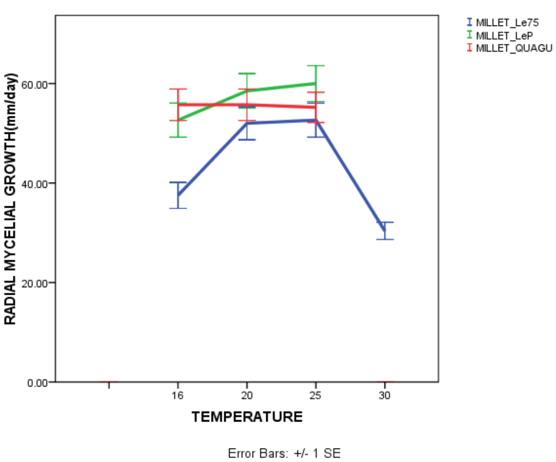


Figure 3. *Radial growth of L. edodes strains (Le 75, Le P and Quagu) on millet meal agar*

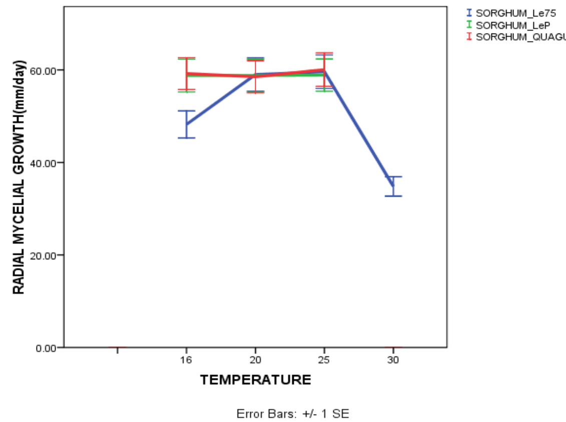


Figure 4. *Radial growth of L. edodes strains (Le 75, Le P and Quagu) on sorghum*

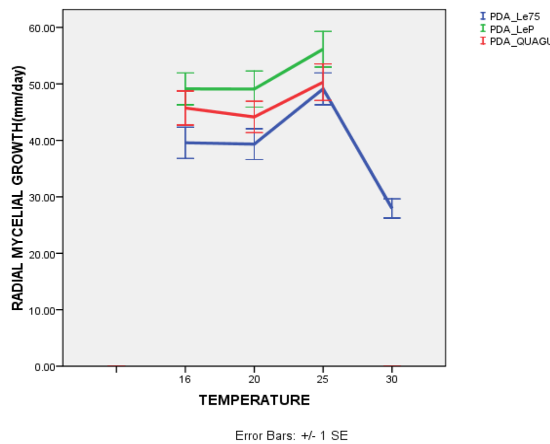


Figure 5. *Radial growth of L. edodes strains (Le 75, Le P and Quagu) on potato dextrose agar*

Light quality and light duration effect on mycelia growth

The best medium, sorghum meal agar was used in this study. The best growth was obtained in continuous darkness where the entire plate was covered in 10 days followed by alternating 12 hr darkness /12 hr light (Fig 6). Continuous 24 hrs light declined radial growth of all the three strains of L. edodes. The differences observed were statistically significant (P≤0.05).

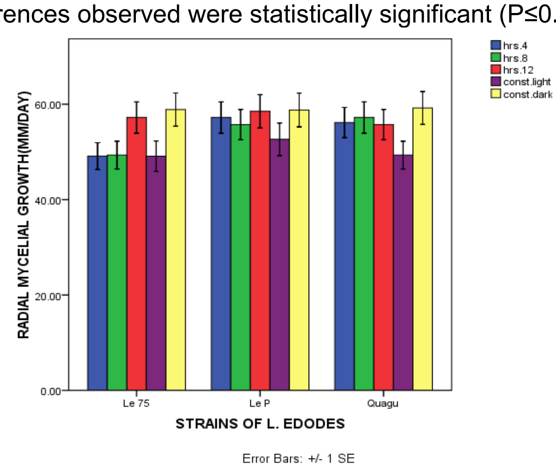


Figure 6. *Effect of light duration on radial growth of three Lentinula edodes strains at 25 °C on sorghum meal agar.*

Morphological characteristics of mushroom mycelium
In this present study various forms of mycelia growth were observed. Plate 1 illustrates the growth of strain Le P on sorghum meal agar. In constant dark and alternating 12hrs light and dark, both rhizomorphic and cottony mycelia were observed; on the other three light regimes more of the rhizomorphic mycelia types were seen. The same pattern was found on Plate 2 for the Quagu strain and strain Le 75 which had only the rhizomorphic type of mycelium growth (Plate 3).

Plate1. Photographs showing various mycelia morphologies in the different light regimes for strain Le P. From left to right: constant 24hrs dark, 12hrs light/12hrs dark, 8hrs light/8hrs dark, 4 hrs light/4hrs dark and constant 24hrs light (x1/3)

Plate 2. Photographs showing various mycelia morphologies in the different light regimes for strain Quagu. From left to right: constant 24hrs dark, 12hrs light/12hrs dark, 8hrs light/8hrs dark, 4 hrs light/4hrs dark and constant 24hrs light (x 1/3)

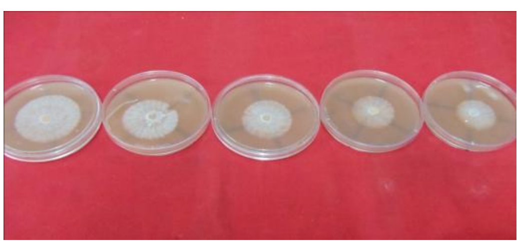
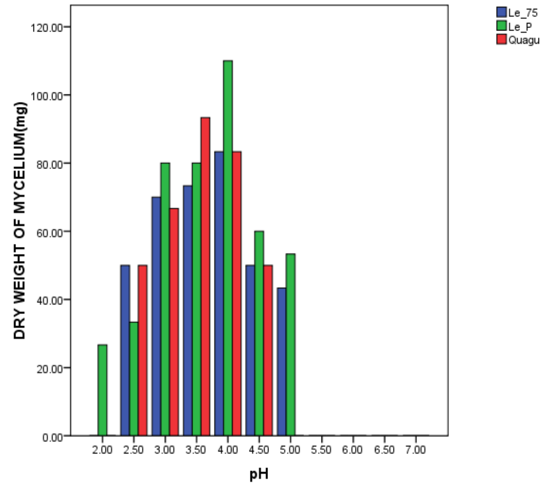


Plate 3. Photographs showing various morphologies in the different light regimes for strain Le75. From left to right: constant 24hrs dark, 12hrs light/12hrs dark, 8hrs light/8hrs dark, 4 hrs light/4hrs dark and constant 24hrs light (x 1/3)

Effect of pH on the vegetative growth of three strains of Lentinula edodes at 25°C
Vegetative growth of mycelium of the three L. edodes strains at different pH's is summarized in Fig 7. Over the range of pH tested, the three strains of L. edodes showed unimodal optimal growth curve. Strains Le 75 and Quagu did not grow at pH 2.0 whilst there was limited growth of strain Le 75 attaining a meagre vegetative growth of 80 mg in 11days at pH 3.0 and 3.5. The strain Quagu grew best at pH 3.5 (95mg) while strain Le P and Le 75 grew best at pH 4.0.with a dry weight of 110mg and 85mg respectively. Vegetative growth of Le P was however significantly ($P<0.05$) greater than Le 75 at the same pH levels (Fig 7).



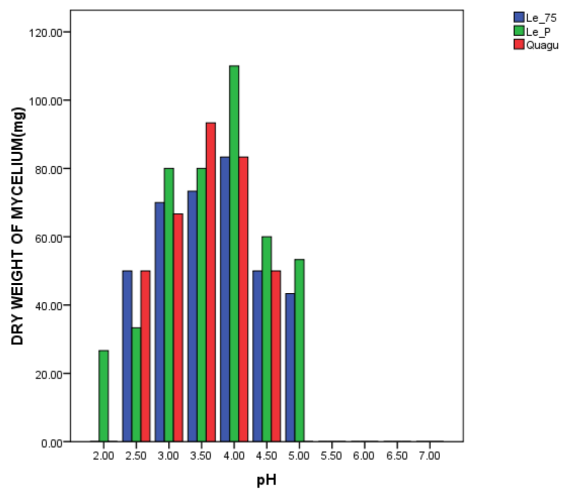


Figure 7. *Vegetative growth of three strains of L. edodes provided by McIlvaines buffer system at 25 °C for 11 days.*

DISCUSSION
The basic conditions that enable a fungus to successfully colonize the substrate and perform maximum fructification are the temperature, the substrate pH, the status of the nutrients within the substrate, the level of inoculum, virulence and the competition on the part of other microorganisms [9]. Generally, strain Le P grew faster as compared to Le 75 and Quagu at almost all temperatures tested, and the differences between them were significant ($P \leq 0.05$). This observation could be attributed to the fact that this strain has the ability to adapt easily at a given temperature [11]. The optimum temperature at which all strains of L. edodes utilized the nutrients best was at 25°C, and full colonization of culture plates were obtained between 11 and 14 days. This temperature was within the temperature range for the development of most mesophilic fungi. According to some researchers, the optimal temperatures for maximum mycelial growth of the L. edodes was recorded between 15°C to 25°C although each strain has its own optimum temperature [12]. [13] also recorded best

mycelial growth of L. edodes at 23 ºC and a weaker growth at 4ºC. In this present study 30ºC was extremely unfavorable. Only strain Le 75 was able to grow feebly at a very slow rate, whereas no growth was recorded for strains Le P and Quagu. This temperature was lethal and mycelial started turning yellowish. It is known that shiitake is vulnerable to high temperatures and mycelial stops growing above 30°C and turns yellow with serious mycelial damage occurring at 36 °C. [4] showed that mycelia of L. edodes turns reddish and die above 40°C. These results indicate an interesting characteristic for cultivation of these strains at different temperatures. It also agrees with [11] who reported that L. edodes radial growth was highest when incubation temperature was between 20ºC - 25ºC and decreased at temperatures above 30ºC. Vegetative growth of L. edodes has also been reported to decline at temperature above 30 ºC [14], whilst [13] observed that temperature 31ºC was lethal for the strains used. The above results confirms the fact that for the fungus to carry out successful colonization and to counteract competitive species and perform maximum fructification, certain basic environmental conditions must be met depending on the type and strain of the fungus, and all the three different strains used for this research performed differently at different temperatures. Studies on the growth of the fungus on different media for all the three strains showed that sorghum meal agar was the best. This could be due to the fact that the grain is higher in protein and lower in fat content than the other cereals, or that the fungus is able to utilize the nutrients on this medium more than the others. [15] reported that sorghum emerged as an ideal material under Indian conditions for the production of shiitake spawn. [16] also showed that out of many cereal grains such as rice and millet used for making spawn, sorghum grains was the most suitable. Shiitake cultivation requires the use of light energy for the formation of basidiospores and dispersal whilst mycelial growth can occur in darkness without light. Under weak diffused light, mycelial grow better than under direct strong light. In darkness, mycelial grows 3-4 times faster than under light [17]. Light was found not essential for mycelial growth of the three strains of Lentinula edodes, and the differences between growth of the three strains were significant ($P \leq 0.05$). The best growth was in continuous darkness which was essential for fast mycelial growth [18]; [19]. [20] using the medicinal mushroom Lyophyllum decastes also reported that photoperiod showed significant effect on the growth of fungal mycelium. The highest mycelial growth was observed in total darkness, followed by 12-hour alternating shifts of dark and light and then complete light. As mycelium grows on a solid medium, it shows a remarkable diversity of forms. In this study these forms of mycelium were all present. Mycelial colony was relatively dense when it was incubated in alternating shifts of dark/light conditions; this showed that light is an important factor in the growth and yield of this fungus. Another important requirement for optimum growth of a fungus on a substrate is its pH preference. It was observed that, L. edodes was able to grow over a wide range of pH and greater biomass production was detected in media with a pH of 3.5 and 4.0 , resulting in 90 mg and 110 mg of dried biomass for strains Quagu and Le P respectively. Results of other studies indicated that the optimum pH for L. edodes mycelial growth was pH 3.0-4.0 [21]. This low final pH measured after mycelial growth indicates acid production by L. edodes. Results are in agreement with those of other investigators who found varied pH ranges in liquid substrates used for L. edodes growth. The results obtained in this study will be useful for evaluating liquid media to enhance L. edodes mycelial biomass and to evaluate metabolites of interest produced by this fungus, such as antibacterial compounds, enzymes and immunotherapeutic agents. This phenomenon has particular importance in production of the fruiting bodies of the fungi for commercial purposes as is the case with L. edodes. Formation of fruit bodies for this species is best on substrates with acidic reaction. This should certainly be taken into consideration for the pH value of the substrate is the key factor for the infection process, substrate colonization or rot progress and dynamics [13]. This study has established both the nutrient and environmental conditions required for three strains of L. edodes for subsequent cultivation studies.

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