International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol. 3, No.1, pp 497-504, Jan-Mar 2011

Optimization of mycelia growth and antimicrobial activity of new edible mushroom, *Lentinus tuberregium* (Fr.). Tamil Nadu.,India

J. Manjunathan* ,V. Kaviyarasan

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai-600 025,India

Corres.author: jmanjunathan@gmail.com

Abstract: Growth requirements of *Lentinus tuberregium* (Fr.), an Indian edible mushroom was optimized using different carbon, nitrogen, vitamins and amino acids followed by different ratio of C:N showed significant increment on biomass of mycelium with the amendment of dextrose, yeast extract, thiamine and glycine. Different rational supplement of dextrose and yeast extract confirmed the effective mycelia formation with 1:3 and 1:5 ratio and *in vitro* antimicrobial properties of *L. tuberregium* culture filterate extracted using four different solvent systems (Hexane, Dichloromethane, Chloroform and Ethyl acetate) were discussed. The activity was evaluated by well diffusion tests using bacteria and yeasts. Vancomycine and fluconazole were used as positive controls for bacteria and yeasts, respectively. The crude extracts of *L. tuberregium* have relatively high antimicrobial activity. Among the four organic extracts ethyl acetate extract showed more effective and inhibited the growth of human pathogenic bacteria, and yeast.
Key words: Otimization, *Lentinus tuberregium,* edible mushroom, antimicrobial activity.

INTRODUCTION

Lentinus tuberregium (Fr), is highly nutritious and compare favourably with other foreign edible species. The cost effectiveness of meat and fish people are turning to mushrooms as an alternative source of protein. Local people collect the mushroom in the wild and consumed as food or used as condiments to add to their food. Mushrooms are used extensively, especially by the local people as food item and for medicinal purposes[1, 2, and 3,4,5,6]. Inspite of the importance of this mushroom in India it has never been cultivated else where in the world. The mushroom is usually collected in the wild and consumed when a large collection is made, they are either sun-dried or smoked and then stored for longer.

It has been known that macro fungi are used as a valuable food source and traditional medicines since Greek and Roman antiquity[7]. Discords, first century Greek physician, knew that *Laricifomes* (*Fomitopsis*) *officinalis* (Vill.) Kotl. & Pouzar (*Fomitopsidaceae*) can be used for treatment of "consumption", a disease now known as tuberculosis[8] . It is believed that mushrooms need antibacterial compounds to survive in their natural environment. Antimicrobial compounds could be isolated from many mushroom species and some proved to be of benefit for humans[9]. In early studies performed by Anchel, Hervey and Wilkins in 1941, diverse antibiotic activity was detected in basidiocarp or mycelia culture extracts of more than 2000 fungal species[10] . In recent *in vitro* studies, screening for the antimicrobial activity of basidiomycete strains, some studies were done both of in basidiocarp and in submerged culture. Antimicrobial activities of basidiomycete strains from different countries were screened in submerged culture[10,11,12] . Similarly[10], detected 14 mushroom isolates with significant activity against one or more of the target microorganisms[10,13] (Rosa *et al*., 2003). Zjawiony 2004 observed that 75% of polypore fungi that have been tested show strong antimicrobial activity[13]. Antimicrobial activities of mushroom exopolysaccharides such as lentinan (from *Lentinus edodes*), schizophyllan (from *Schizophyllum*

commune) and PSK ("Polysaccharide Kureha" from *Trametes versicolor*) have also been reported [8,14,12]. In this context, the antimicrobial activity of submerged mycelia of newly isolated mushroom strain are reported here. In the present work, optimization of mycelia growth and antimicrobial activity of *L. tuberregium* were carried out. This will serve as a base or provide information on nutrients that can help in the mycelial growth for *L. tuberregium*.

MATERIALS AND METHODS

The fruitbody of *L. tuber-regium* were collected in keeriparai forest Kanyakumari district, Tamil Nadu, India. The culture was maintained on Potato Dextrose Agar for further investigation. The mycelial growth was determined by a mycelial dry weight method. The basal medium used in this study was that described[15, 16]. The basal medium and supplementary compounds were dissolved in 1 litre of distilled water. 100 ml was dispensed into 250 ml flat bottom flasks and the pH was adjusted to 6 . The mouth of each flask was sealed with cotton wool and covered with aluminium foil paper before sterilization. The media after cooling were then inoculated with 5 mm diameter agar block of 7-9 days old mycelium of the mushroom. The flask was incubated at room temperature for 28 days. Each experiment was replicated three times. The mycelium in each flask was filtered through a pre-weighed 9 cm diameter filter paper, and dried at 85^{o}C for 10 h and recorded the fresh weight

Carbon source

Each five g of six different carbon sources of dextrose, lactose, sucrose, maltose, manitol and starch were amended in the basal medium having the PH of 6. The mycelia disc of 5mm was cut from 9-10 days old mycelia mat, and this blocks were aseptically inoculated in the flasks including the control flask ie basal medium without any carbon source. All the flasks were kept undisturbed for 28 days of incubation. After the incubation period the mycelia mat collected and recorded the results.

Nitrogen source

Two grams of each nitrogen sources such as, sodium nitrate calcium nitrate, ammonium nitrate, peptone, yeast extract , beef extract, and urea were added to the basal medium containing fructose (10g) KH_2PO_4 (0.5 g), $MgSO_4.7H_2O$ (0.5 g), thiamine hydrochloride (500 µg) and made up to 1 litre with distilled water[15]. Sterilization, inoculation and assessment of dry weight were carried out as described for the carbon sources above. Basal medium alone was used as control.

Vitamin source

Vitamins were selected to this study such as, biotin, ascorbic acid, thiamine, and tocoferrol. The basal medium was the same for determination of nitrogen sources. Each vitamin was added 500 µg of each to the basal medium and made up to 1 litre. The set up was treated the same way as for carbon, nitrogen and amino acids. Basal medium alone was used as control.

Amino acid source

The amino acids of were selected aspartic acid, cysteine, phenyl alanine, tyrosine, methionine, L-glutamic acid, DL-leucine, histidine, L-leucine, tryptophan, DL-dopa, proline, DL-2-amino butric acid, DL-threonine, Isoleucine, hydroxy proline, glycine, DL-valine, L-cysteine, L-lysine mono hydrochloride, DL-serine, L-ornithine mono hydro chloride, L-arginine monohydro chloride, and DL-alanine. The basal medium was used the same as that of nitrogen source. For each amino acid (500 µg) was added to the basal medium and made up to 1 litre and dispensed into the flasks which were treated as described above. Basal medium alone without amino acid was used as control.

Carbon to nitrogen ratio (C/N)

The basal medium was similar to that used for nitrogen compounds but glucose was varied composition with yeast extract as sources of C/N. Concentration of 0.15 g/litre of dextrose and yeast extract in the basal medium serve as 1:1 ratio (Fasidi and Olorunmaiye, 1994). Other ratio was prepared proportionately i.e., 1:1, 1:3, 1:5 and 5:1, 3:1.

Determination of antimicrobial activity

In vitro antimicrobial susceptibility studies were performed using the following strains; *Bacillus subtilis* (M-441), *Staphylococcus aureus* (M-96), *Micrococcus luteus* (M-1541), *Escherichia coli* (ATTC 25992), *Candida albicans* (M-227), *Salmonella flerineri* (M-1457) *Salmonella typhi* (M-733) (isolate obtained from MTCC and ATCC Chandighar). Antimicrobial activities of all extracts and fractions were screened by the well diffusion method.

Agar well method

Test microorganisms were activated in Mueller Hinton Broth (37°C, 150 rpm, 24 h). The Mc Farland (No: 0.5) standard is used to adjust the turbidity to prepare inoculum from overnight grown bacteria and yeast cultures. 100 µl of crude extract (100 mg/ml) were added to each well (6 mm diameter holes cut in the agar gel). The plates were incubated at 37°C for 24 h for bacteria, and at 30°C for 48 h for

yeasts. Antimicrobial activity was determined by measuring the radius of the clear inhibition zone around each well [12]. Standard antibacterial agents, vancomycine and fluconazole (30 µg/disk) were used as positive controls for bacteria. Disks injected with 20 µL of 20% DMSO were observed as negative control. Each experiment was replicated three times and the results were expressed as average values.

Fig. 1 Growth of *Lentinus tuberregium* on different carbon source.

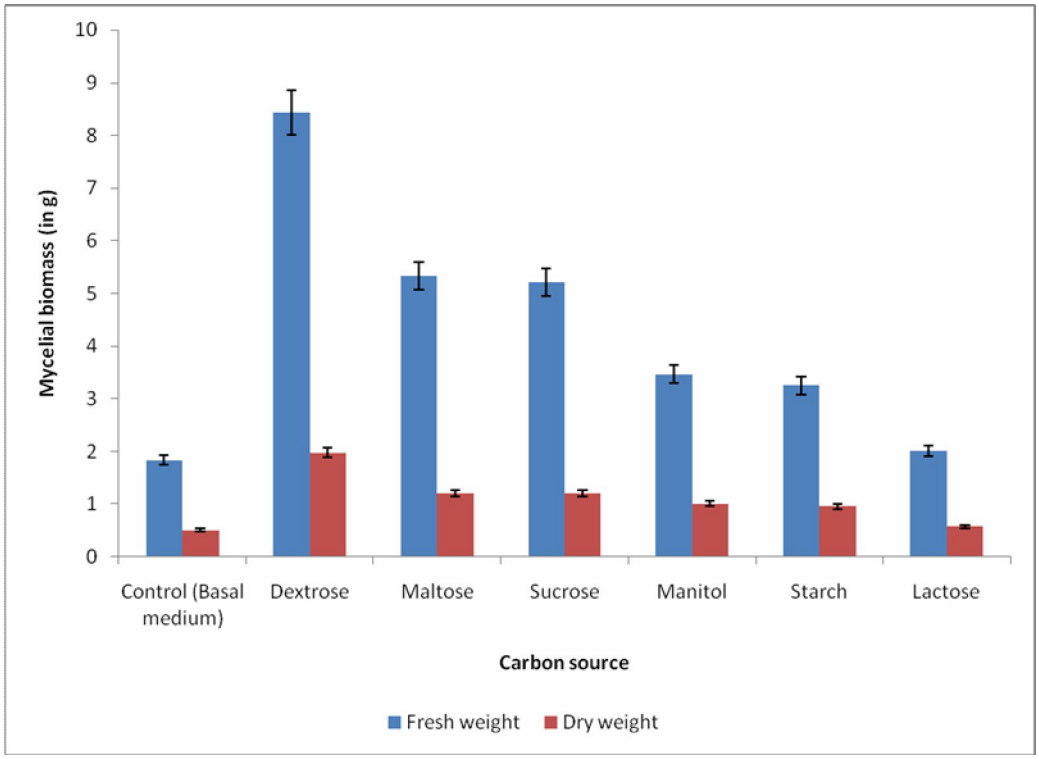


Fig. 2 Growth of *Lentinus tuberregium* on different amino Nitrogen source.

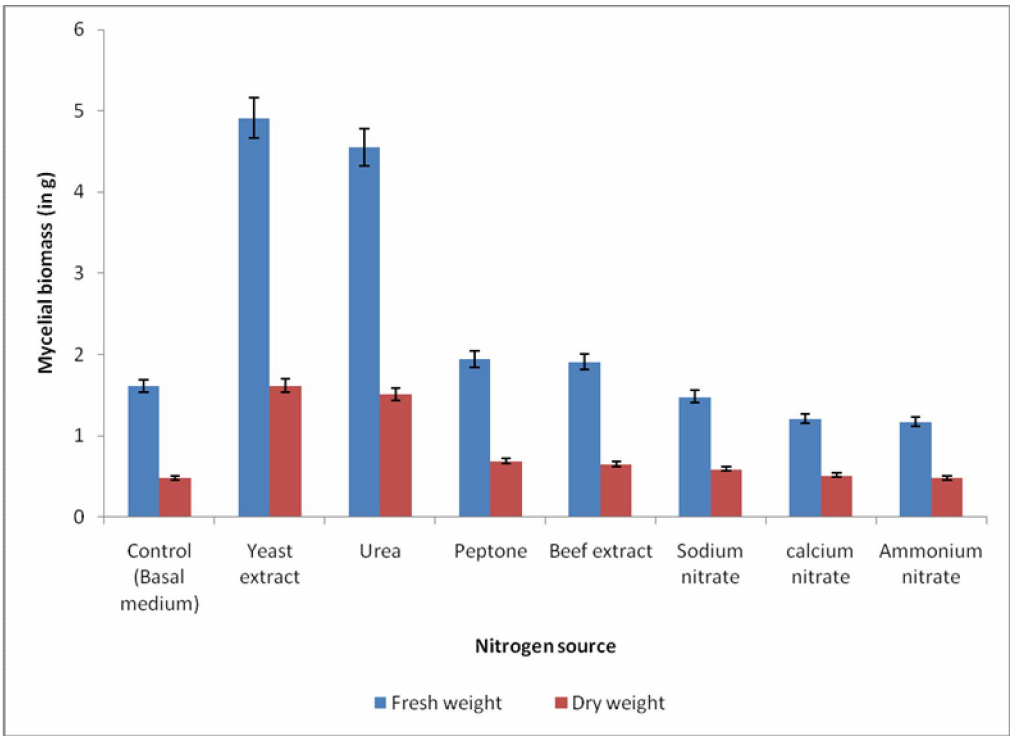


Fig.3 Growth of *Lentinus tuberregium* on different vitamin source.

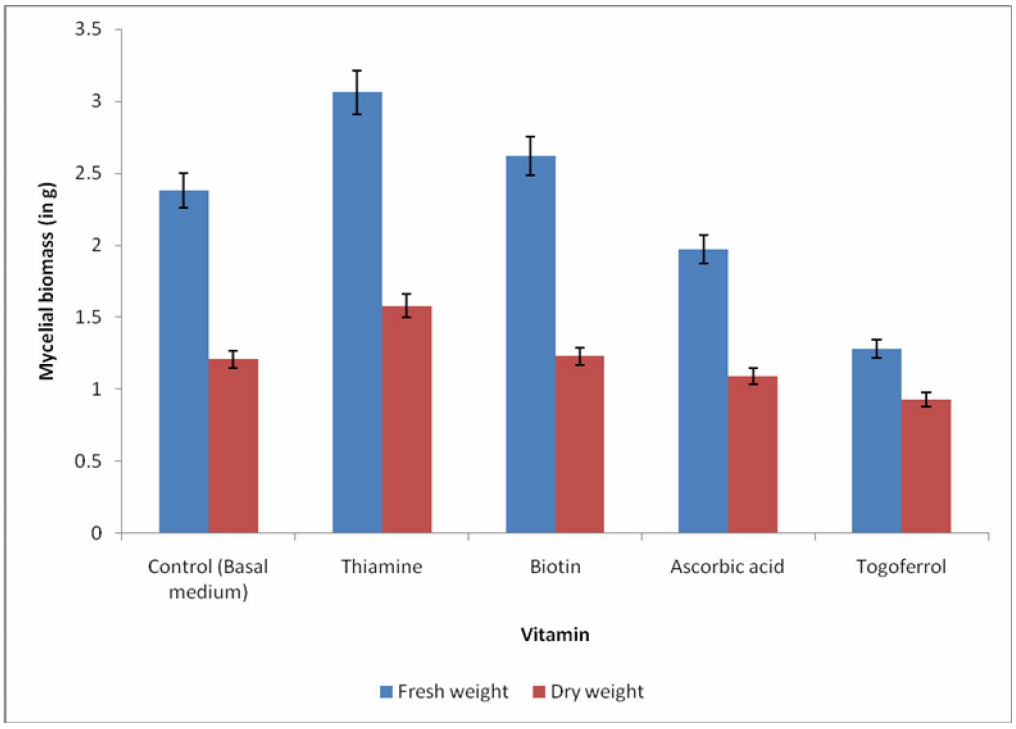


Fig. 4 Growth of *Lentinus tuberregium* on different amino acids source.

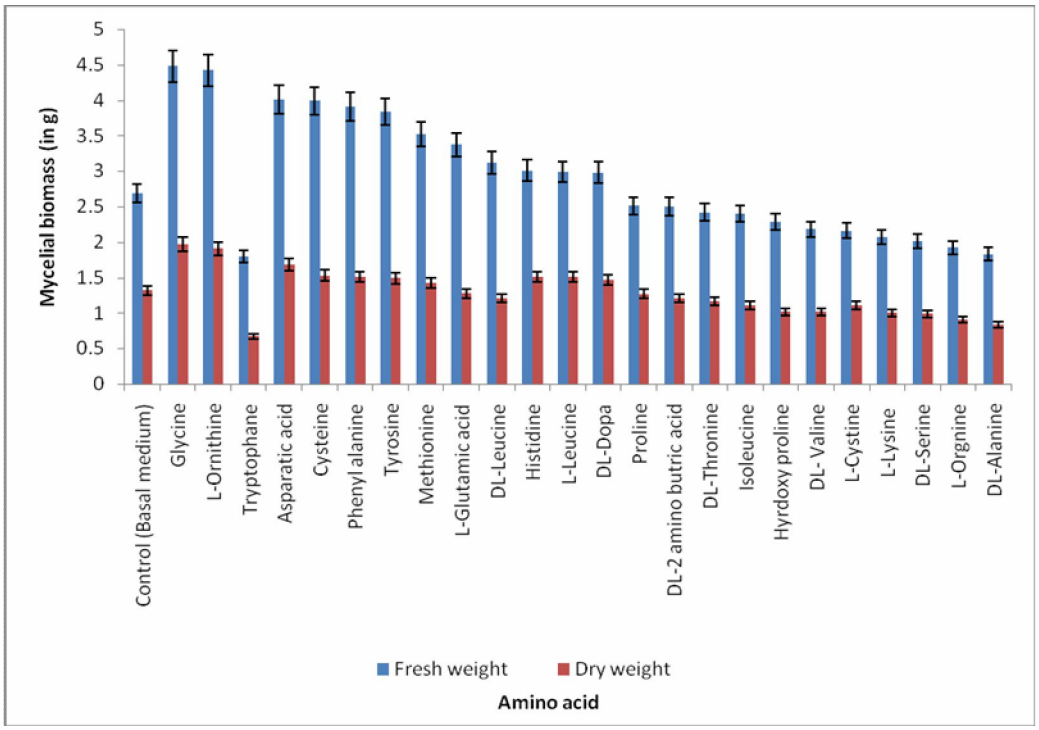


Fig.5 Growth of *Lentinus tuberregium* on different carbon to nitrogen ratio.

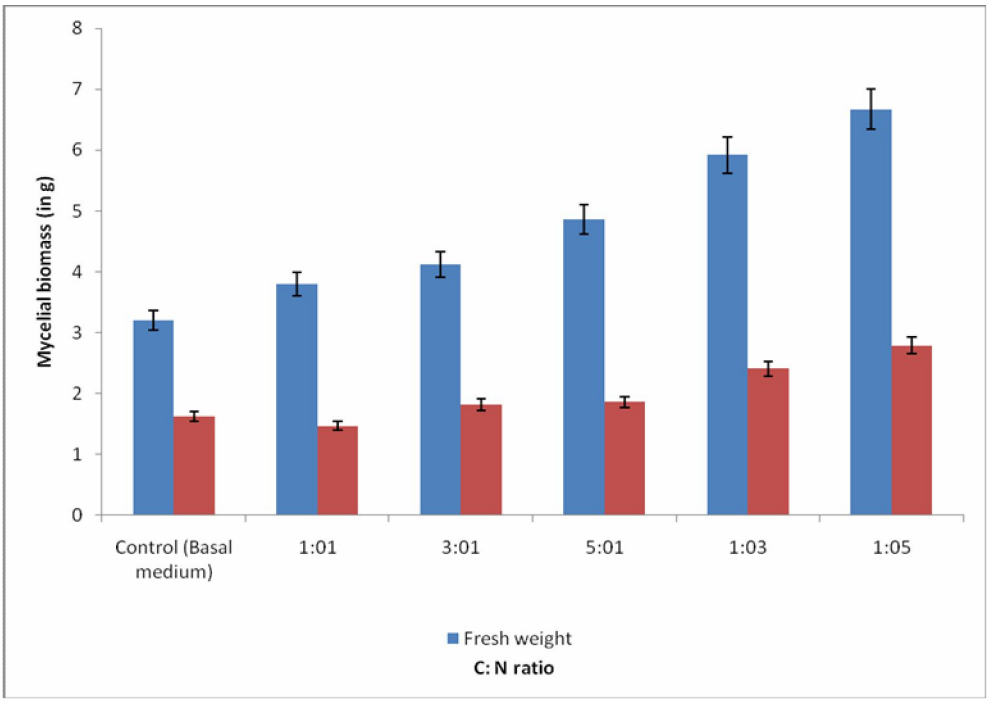
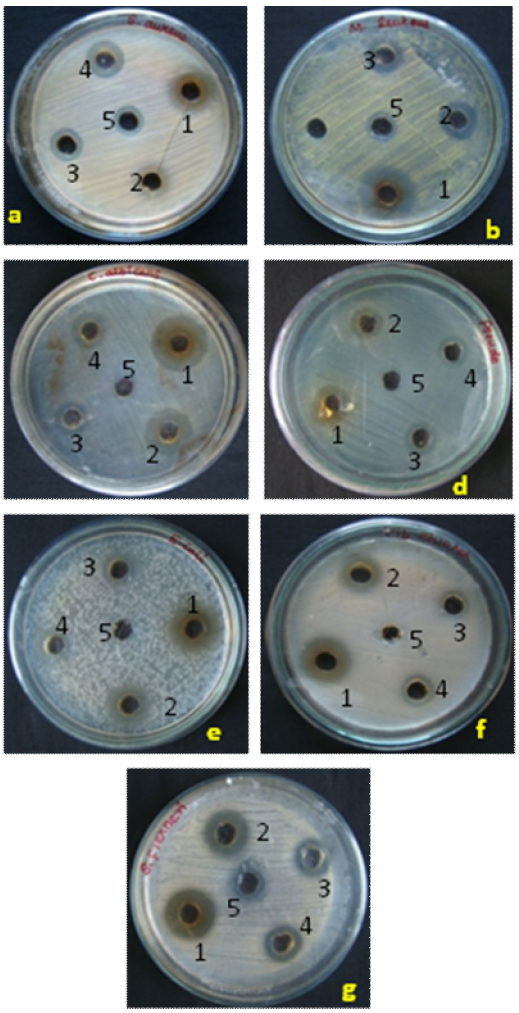


Fig-6 Screening of Antimicrobial activity



RESULTS AND DISCUSSION

L. tuberregium shows different preferences for carbon sources for its metabolism (Fig. 1). According[17], the ability of an organism to utilize the carbohydrate depends on type of enzyme produced by the organism. In this study, dextrose was best source of carbon for this mushroom. This shows that *L.tuber-regium* produces enzymes that utilize dextrose better than any other carbon source. [18]also reported that *Volvoriella volvacea* utilizes glucose and starch better than other carbon sources. Chakavarty and Mllick (1979) obtained more growth of *V. volvacea* with glucose than starch. Luo (1993) [19]also reported that fructose, glucose and maltose were the most suitable carbon sources for *Auricularia auricular*. [20]reported that the best utilizable carbon sources for *Lentinus subnudus* were fructose, maltose, dextrin and glucose. This study showed that *L.tuber-regium* utilizes dextrose better than maltose, manitol, lactose and starch. The least carbon sources were lactose [21]reported that glucose has been good respiratory substrate.

L.tuberregium utilises organic nitrogen better than inorganic nitrogen (Fig.2). This observation are agreement with the report[16] of who observed that yeast extract which is a complex nitrogen source sustained the greatest growth of *P. tuberregium*. [20]reported peptone as the best nitrogen source for *L. subnudus* [22]also reported that *V. volvacea* frequently responds better to organic nitrogen than inorganic nitrogen. [18]reported that the best yield of *Volvoriella* were obtained on media containing peptone or potassium

Table-1-Antimicrobial activity

Pathogens	Zone of inhibition (mm in diameter)				
	Hexane(1)	Dichloromethane(2)	Ethyl acetate(3)	Chloroform(4)	Control 4%DMSO(5)
S.aureus	9	9	11	10	-
M.luteus	-	10	12	9	-
P.aureginosa	8	10	8	8	-
E.coli	-	11	6	7	-
S.typhi	8	10	12	9	-
S.flexneri	10	9	13	8	-
C.albicans	8	9	10	9	-

nitrate. In the same vein [19]reported that organic nitrogen sources such as yeast extract and peptone are the preferred nitrogen sources for *A. auricular*. In this study, *L.tuber-regium* showed preference for organic nitrogen than inorganic nitrogen.

Thiamine proved best among the vitamins followed by biotin and tocoferrol. According to[23] who found that thiamine stimulates mycelial growth of *Cercospora arachidicola* in liquid culture. [24]also reported that thiamine is required for good growth in mushrooms. Also, [19]reported that different vitamins produce different effects on myelial growth within a certain concentration range. [25]reported that combined amino acids stimulate greater growth than single amino acids. The least effective vitamin in this study was ascorbic acid (Fig. 3)[16]reported that ascorbic acid, folic acid and riboflavin did not support good growth of *P. tuberregium*.

Glycine proved to be the best amino acid; this is followed by L-ornithine mono hydrochloride (Fig. 4). [15]Reported that asparagine and aspartic acid have been employed in increasing the mycelial growth and fruit body production in *Agaricus bisporus*. Hayes (1981) [26]reported that higher and lower concentrations of these amino acids are found to be either ineffective or inhibitory for the mycelial growth of mushrooms.

[27]Reported that the ratio of carbon to nitrogen (C: N) balance in mushroom substrate is very important. A well balanced carbon to nitrogen ratio enhances the growth and development of mushrooms while an imbalance of C: N impedes their growth[28, 29] In this study the C: N ratio of 1:3 and 1:5 supported best growth of the mushroom (Fig. 5), growth was reduced above or below this levels. [16] also reported C: N ratio of 1:3 and 1:5 for *P. tuberregium*. According to[28, 27] over-supplementation of mushroom substrates with nitrogen and carbohydrates impedes mycelial growth of mushrooms. As the ratio of C: N increased, the mycelial growth of *L. tuberregium* also increased up to a point after which further increase in carbon decreased the mycelial growth. The same was applicable to nitrogen. In this study, the vegetative growth of *L. tuberregium* was greatly improved by carbon, nitrogen, vitamins and amino acids. Carbon to nitrogen of 1:3 and 1:5 was best and however, *L.tuber-regium* can be cultivated on substrates containing C: N ratio of 1:3 or 1:5, Where as antibacterial activity of submerged strain against test microorganisms was compared with the results of positive control antibiotics of vancomycine and fluconazole. The antibacterial activity of mushroom sample varied according to the solvents. Different solvent extracts of this strain were active against bacteria. These extracts were the most active to inhibit the growth of *Salmonella flexineri*, *Micrococcus luteus* and *Salmonella typhi*. In the present study, *Lentinus tuberregium* showed activity against some of the studied test microorganisms (Table 1). The activities of *Lentinus tuberregium* were higher than positive control (vancomycine or fluconazole) against *Salmonella typhi, Micrococcus luteus*, and *Salmonella flexineri*. But, in the present study, our *L.tuberregium* strain did not show any activity against *E.coli*. (Fig-6)

These combine activity of antimicrobial increase the chance of the mushroom for medicinal purposes. The fact that the Basidiomycetes have been insufficiently investigated coupled with the broad range of structural types of antibiotics. However, basidiomycetes may be a source of new and useful bioactive compounds. To our knowledge, no investigation has been performed for comparing antimicrobial activity potential of basidiomycetes strains in different life forms. Further studies on isolation and identification of the active compounds may provide a better source for developing new therapeutic agents.

REFERENCES

1. Oso, B, A., Mushrooms in yoruba mythology and medicinal practices. Economic Botany, 1977, 31, 367-371.
2. Chu, K, K., Ho, S, S., Chow, A, H., Coriolus versicolor*:* a medicinal mushroom with promising immunotherapeutic values. J. Clin. Pharmacol, 2002, 42, 976 – 984.
3. Akpaja, E, O., Isikhuemhen, O, S., Okhuoya, J, A., Ethnomycology and usage of edible and medicinal mushrooms among the Igbo people of Nigeria. Int. J. Med. Mushrooms, 2003, 5, 131 – 319.
4. Kuforiji, O, O., Fasidi, I, O., Odunfa, S, A., Nutritive value of *Pleurotus tuberregium* cultivated on different agro-industrial wastes. Niger. J. Microbiol, 2003, 17, 68 – 70
5. Chiroro, C, K., Poverty alleviation by mushroom growing in Zimbabwe. Mushroom growing for a living worldwide. Heineart Inc. Seoul, Korea, 2004, P. 298.
6. Okhuoya, J, A., Akpaja, E ,O., Abbot, O., Cultivation of *Lentinus squarrosulus* (Mont) Singer on sawdust of selected tropical tree species. Int. Journal of Mushroom Scienc, 2005, 2, 41 – 46.
7. Anke T., Basidiomycetes: A source for new bioactive secondary metabolites. Progress in Industrial Microbiology, 1989, 27, 51 – 66.
8. Stamets, P., . Novel antimicrobials from mushrooms. HerbalGram, 2002, 54, 29-33.
9. Lindequist, U., Niedermeyer, T, H, J., Jülich, W, D., The pharmacological potential of mushrooms. Ecam, 2005, 2, 285–299.
10. Rosa, L, E., Machado, K, M, G., Jacob, C, C., Capelari, M., Rosa, C, A., Zani, C, L., Screening of Brazilian Basidiomycetes for antimicrobial activity. Memórias do Instituto Oswald Cruz, 2003, 98, 967-974.
11. Wasser, S, P., Weis A., Medicinal properties of substances occurring in higher basidimycetes mushrooms: current perspectives. International Journal of Medicinal Mushrooms, 1999, 1, 31-62.
12. Ezeronye, O, U., Daba, A, S,, Okwujiako, I, A., Onumajuru, I, C., Antibacterial effects of crude polysaccharide extracts from sclerotium and fruitbody (sporophore) of *Pleurotus tuberregium* (Fried) Singer on some clinical isolates. International Journal of Molecular Medicine and Advance Sciences, 2005, 1, 202-205
13. Zjawiony, J, K., Biologically active compounds from Aphyllophorales (polypore) fungi. Journal of Natural Products, 2004, 67, 300-310.
14. Chihara G., Immunopharmacology of lentinan, a polysaccharide isolated from *Lentinus edodes*: Its application as a host defense potentiator. International Journal of Oriental Medicine, 1992, 17, 55-77.
15. Chandra, A., Purkayastha, R, P., Physiological studies on Indian mushrooms. Transactions of British Mycological Society, 1977, 69, 63 – 70.
16. Fasidi, I O., Olorunmaiye, K, S., Studies on the requirements for vegetative growth of Pleurotus tuberregium (Fr) Sing., a Nigerian mushroom. Food Chemistry, 1994, 50, 397 – 401.
17. Cochrane, V, W., Physiology of Fungi. John Wiley and Sons, New York, 1958, 296p.
18. Ofosu-Asiedu, A., Schmid, O., Liese, W., Growth studies of *Volvoriella volvacea* for cultivation on wood waste Materials and organis, 1984, 19, 241 – 251.
19. Luo, X, C., Biology of Artificial log cultivation of Auricularia mushroom. In: Chang, S. T., Buswell J, A., Siu-wai Chiu (eds) mushroom biology and mushroom cultivation. Chinese University Press, Hong Kon, 1993, p. 370.
20. Kadiri, M., Fasidi, I, O., Growth requirements of *Lentinus subnudus* Berk. A Nigerian edible mushroom. Chemical Microbiology Technology, 1994, 16, 80 – 84.
21. Hammond, W, B, J., Changes in composition of harvested mushrooms (*Agaricus bisporus*). Phytochemistry, 1978, 18, 415 – 418.
22. Chang, S, T., Biology and cultivation of *Volvoriella volvocea.* In: Chang, S. T., Buswell, J, A., and Chiu, S, W., (eds). Mushroom Biology and Mushroom Products. Chinese University Press. Hong Kong. Chang ST, Quimio TH 1982, 370p.
23. Lander, K, E., Growth of *Cercospora arachidicola* in a glucose phosphate, asparagine-thiamine agar medium. Phytopathology, 1964, 54, 1236 – 1240
24. Madunagu, B, E., Collection and studies on the cultivation of *Pleurotus squarrosulus* (Mont) Sing. Niger. J. Sci, 1988, 22, 51 – 55.
25. Nolan, R, A., The Phycomycetes catenaries anguillulae growth requirements. J. Gen. Microbiol, 1970, 60, 167 – 18.
26. Hayes, W, A., Interrelated studies of physical, chemical and biological factors in casing soils and relationships with productivity in commercial culture of *Agaricus bisporus* Lange. Mushroom Science, 1981, 11, 103 – 129.
27. Stamets, P., Growing Gourmet and Medicinal Mushroom. Ten Speed Press , California, 2000, 574.
28. Stamets P., Chilton, J,S., The Mushroom Cultivator. A Practical Guide to Growing Mushrooms at Home. Agarikon Press Olympia Washington, 1983, p. 415.

29. Okhuoya, J, A., Isikhuemhen, O, S., Tomo, H ,A., Effect of soil factors on growth and yield during sporophore induction from sclerotia of *Pleurotus tuberregium* (Fr.) Sing. The Int. J. Mushroom Sci, 2000, *3*, 3 – 7.

*****