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RESEARCH ARTICLE

Mycelial Growth and Primordial Initiation of *Agaricus bitorquis* and Related Species on Agar Media

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ABSTRACT

The mycelial growth and primordial initiation of the strains of *Agaricus bitorquis* and other species isolated from different areas of Pakistan, and their comparison was investigated with cultivated strains including AS60, AS61, AS65, AS51, W20, W2F, K26, and K32. In contrast to the most important and widely cultivated mushroom, *Agaricus bisporus*, which grows best at 25°C and produces fruiting bodies below 20°C, the tropical *Agaricus* species show optimum mycelial growth around 30°C. Comparison of the growth rates of different isolates showed that most of the strains grew within the range of 20-30°C with the optimum at 30°C. Most of the strains formed primordia at both 25° and 30°C whereas; W2-F and K32 produced areas of dense hyphal aggregates at 30°C. *A. bisporus* (AS51) failed to form pin heads at either temperature. Strain AS60 and W20 initiated most of the pin heads at 30°C. Strain AS60 formed pin heads at both the temperatures with the maximum number at 30°C after full colonization of the agar surface.

INTRODUCTION

Mushroom has been extensively used as food since ancient times, due to its medicinal and nutritive values (Manzi et al., 2001).There is large diversity of cultivated mushrooms, being a good source of protein, vitamins, comparable with meat, eggs and milk (Khan and Kausar, 1981). Some of them even contain significant amount of vitamin C, as well as the minerals potassium, phosphorus, calcium and magnesium. Mushrooms contain biological active compounds of medicinal value, used as complementary medicine as well as dietary supplements for anticancer, antiviral, immuno-potentiating, hypocholesterolemic, and hepatoprotective agents. These new compounds, termed as mushrooms nutriceuticals are extractable from either the fungal mycelium or fruiting body (Chang and Buswell, 2003). Both liquid and most commonly, semi-solid media are widely used in studies on mycelial growth (Stamets, 1993; Friel and McLoughlin, 2000). Linear growth rates can be compared under different environmental conditions and such measurements provide a basis for determining the suitable conditions for growth. The growth and reproduction of fungi depends on genetic factors as well as on nutritional and environmental conditions. The optimum condition for one species may be different from other fungi (Arita et al., 1980). Investigation of both mycelial growth and fruiting bodies are essential for the selection of thermo-tolerant mushroom strains (Pahil, 1992). Growth medium is among the most important factors because it supplies necessary nutrients for the growth of mycelium and for the development of fruiting body as the mycelial growth requires short time in comparison with fruiting body development (Rohul et al., 2008, Kalmis and Kalyoncu, 2006).

Recording linear extension on agar media is often considered the simplest practical method of measuring fungal growth. This is mostly studied in Petri-dishes and the increase in colony diameter is recorded at suitable intervals. Such linear growth measurements are useful in screening procedures for comparing mushroom strains. As the nutrient medium can influence growth, the same medium must be used throughout all screening trials (Chang and Miles, 1989).

The mushroom (*Agaricus bisporus*) has requirement for "casing layer" that has the specific physical, chemical and microbiological properties which stimulate and promote the initiation of primordia (Noble et al., 2003). Generally, strains of *A. bisporus* do not form primordia on artificial agar media. However, Noble et al. (2003) succeeded in obtaining primordia formation in *A. bisporus,* on malt extract agar. Pin heads were formed after complete colonization of the agar surface. Liaqat et al. (2013) determined the growth and formation of primordia in *A. bitorquis*. They further pointed out that this character is associated with mushroom production in compost cultures.

The strains of *Agaricus bitorquis*, obtained from HRI, Littlehampton, were compared with wild isolates of *A. bitorquis* and related species., collected from Pakistan, to evaluate their growth, temperature tolerance and fruiting on semi-solid media. The aim of these studies was to determine the growth characteristics of these strains which are of importance for the preparation of spawn and in adjusting the temperature for spawn running of mushroom to obtain maximum productivity.

MATERIALS AND METHODS

Selection of strains

The strains used in this study include both wild and cultivated strains of *Agaricus bitorquis* and related species of temperate and tropical origin. Four strains W20, W-2F, K26 and K32 of *A. bitorquis* were obtained from Horticulture Research Institute, Wellsbournes, U.K. The strain AS51 of *A. bisporus* was isolated from the fruiting body obtained from the market. The remainder strains AS60 (*Agaricus* sp), AS 61 identified as *A. xanthodermatie* (Smith et al., 2006) and AS65 of *A. bitorquis* were isolated from the fruiting bodies growing wild in different areas of Punjab province in Pakistan.

Preparation of initial cultures

Cultures of isolates were prepared by the tissue culture method. The fruiting bodies were surface sterilized with 75% alcohol, cut in halves using a sterile scalpel. Small pieces of internal tissues from the upper part of the stipe of the fruiting bodies or pieces from the gills were removed aseptically and transferred to 2% malt agar slants.

Maintenance of cultures

All strains were maintained on slants in 9 cm petri dishes containing malt extract and peptone agar (MEM). The composition of the media was, Malt extract 20g., Mycological peptone 5g, Agar 15g; Pencillin 0.2g Streptomycin 0.2g; Distilled water 1 Lit. After autoclaving at 15 psi for 15 minutes, the pH of the medium was 6.5.

Growth studies on agar media

The temperature requirements of different strains of *Agaricus bitorquis* and related species viz., AS60, AS61, AS65, W20, W2-F, K26 and K32 and one strain (AS51) of *A. bisporus* were studied on malt extract agar. The sterilized agar selected for individual experiments was dispensed aseptically into sterile, disposable 9 cm petri dishes. Except where otherwise indicated, 5 replicate plates were poured for each strain and treatment tested. Agar plates were inoculated centrally with standard inoculum discs and incubated in the appropriate conditions. Means of measurements of radial extension of colonies along two diameters at right angles were recorded daily.

Statistical analysis

The results obtained were subjected to statistical analysis using the techniques elaborated by Steel et al. (1997). The values presented in tables are means ± standard deviation. The graphs along with regression equation and R^2 were drawn using EXCEL Add-ins.

RESULTS

Temperature requirements of *Agaricus* strains

Comparison of growth rates of different isolates of *A. bitorquis* and *A. bisporus*, as indicated by daily measurements of colony diameter on malt agar. Strain AS60, AS61, AS65, W20, W2-F, K26 and K32 produced maximum growth at 30°C (Fig. 1). The regression equation shown in the graphs indicated the positive impact of temperature on the mycelial growth with the exception of AS-51. The fastest growth was recorded in strain AS60 which covered the agar surface in 9 cm Petri-dishes within two weeks of incubation. The same strain showed marked tendency with the increasing temperature as it revealed with the maximum average diameter. The regression equation ($y = 0.85x + 2.22$ with $R^2 = 0.6757$) also supported the claims. Other strains took about 3-4 weeks. Maximum growth of strain AS51 of *Agaricus bisporus* was recorded at 25°C. Growth of strains K26 and K32 did not extend beyond the inoculum at 35°C, whereas AS61, AS65, W20 and W20 produced very slow growth at this temperature. It was also observed that the growth of strain AS60 was faster, even at sub-optimal temperatures of 20°C and 25°C than the growth of other strains at their optimum temperature levels.

Mycelial growth and primordium initiation by *A. bitorquis* and *A. bisporus* on agar medium:

In commercial mushroom production, fruiting body formation is achieved by covering the compost with casing soil. In the growth studies at different temperatures, some strains of *A. bitorquis* showed a tendency to form the primordia on agar medium. The data regarding colony diameter at different temperatures and days is presented in Table 1. (Most of the strains formed primordia at both 25° and 30°C, whereas, W2-F and K32 produced areas of dense hyphal aggregates at 30°C (Table 2). *A. bisporus* (AS51)

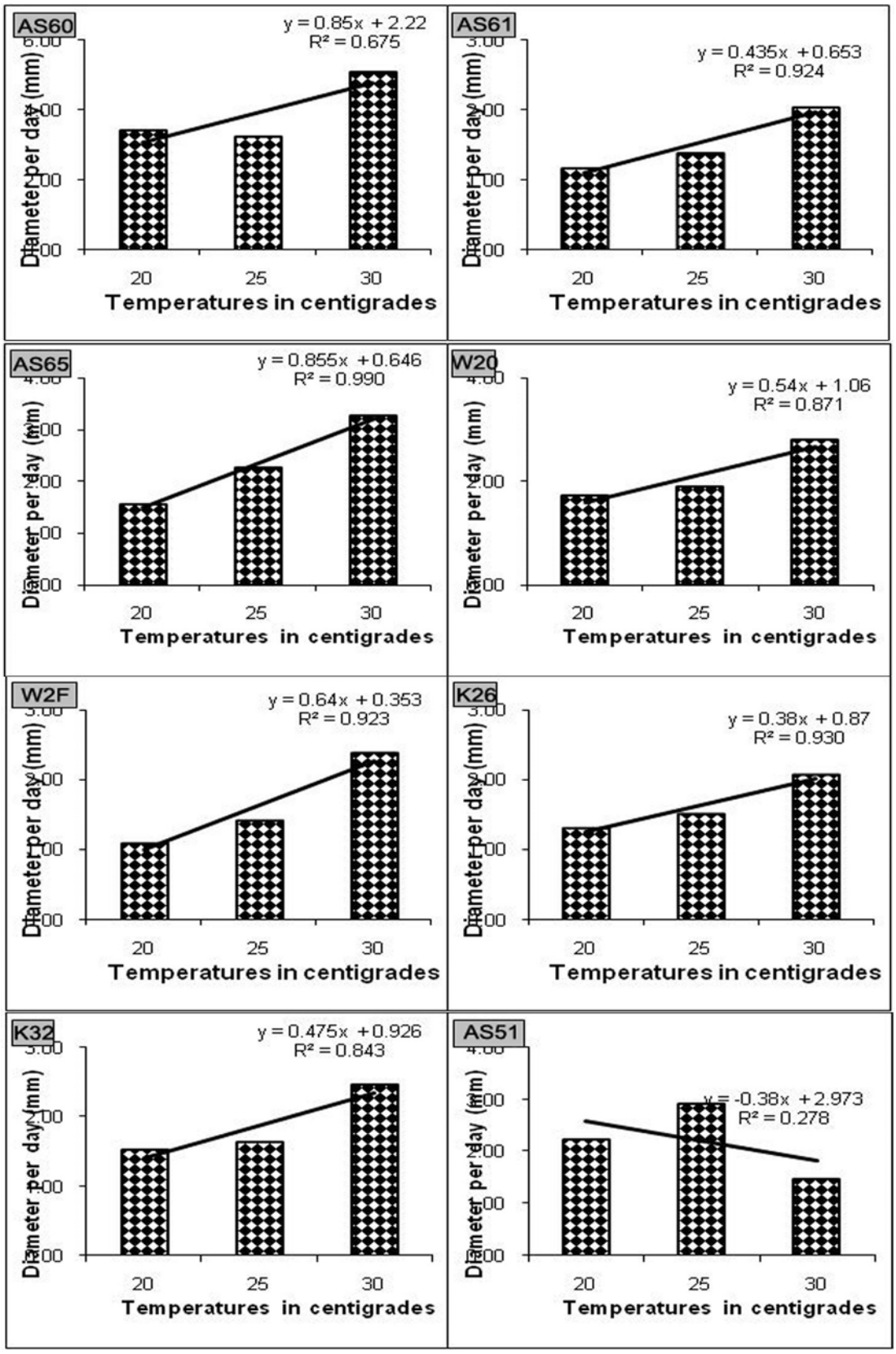


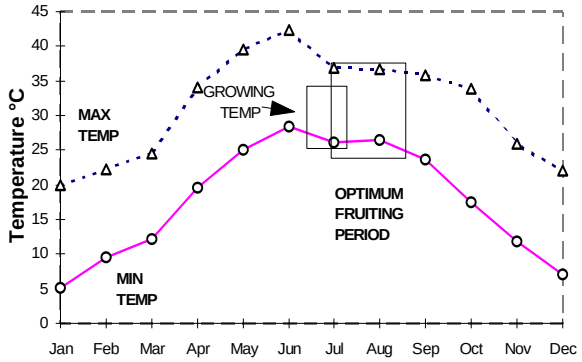
Fig. 1: Rate of mycelial growth (mm per day) for different strains of *Agaricus* species at various temperatures (means ± SE from 5 replicate cultures). LSD at 0.05 = 0.549.

Table 1: Colony diameter (mm) after 15d and 30d on 2% malt extract agar in Agaricus strains 25°C and 30°C (Means ± SE of 5 replicates)

Strains	25°C		30°C	
	15 days	30 days	15 days	30 days
AS60	50.5±1.43	78.0±0.51	78.0±0.65	99.0±3.61*
AS61	25.2±0.74	56.5±1.52	49.3±1.09	81.4±0.61
AS65	32.5±1.16	61.2±1.89	51.2±1.49	85.7±1.02
W20	23.2±1.11	50.5±2.03	43.2±1.26	75.7±0.91
W2-F	17.4±0.45	44.0±1.59	31.3±0.97	78.0±0.92
K26	32.0±0.68	60.0±1.09	45.5±0.96	70.3±1.86
K32	15.3±0.97	50.1±1.00	29.5±1.11	77.5±0.29
AS51	46.5±1.50	75.0±0.4	10.1±0.59	21.9±0.68

Table 2: Number of primordia in *Agaricus* strains at 25°C and 30°C (Means ± SE of 5 replicates)

Strains	Primordia	
	25°C	30°C
AS60	25.1±2.4	9.5±1.9
AS61	15.4±1.2	Large numbers of hyphal aggregates
AS65	12.7±1.4	10.0±1.2
W20	6.0±0.9	11.0±1.6
W2-F	25.5±1.6	Large numbers of hyphal aggregates
K26	10.8±1.4	2.0±0.4
K32	11.3±1.3	Large number of hyphal aggregates
AS51	Nil	Nil



Correlation Coefficients: Temperature x Average Mycellial Growth = 0.42

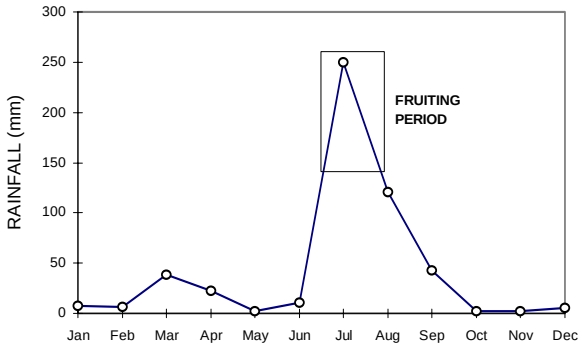


Fig. 2 & 3: Meteorological data at Faisalabad, compared with maximum growth and fruiting. Note that fruiting occurred during monsoons in July-August.

failed to form pin heads at either temperature. Strain AS60 and W20 initiated most of the pin heads at 30°C. Strain K26 initiated primordia in the centre of the culture all around the inoculum at 25°C and only few primordia appeared at 30°C. Fully developed primordia appeared in the centre at 25°C forming a ring around the inoculum. In strain W20, primordia were scattered within a radius of 2 cm from the inoculum at 30°C but only a few pin heads were visible close to the inoculum at 25°C. Large numbers of pin heads were produced by strain W2-F at 25°C, forming a circle at a distance of 2cm from the inoculum, but only hyphal aggregates formed the appearance of typical cultures with fruiting primordia is shown in plates 1, 2 & 3.

Strain AS60 formed pin heads at both the temperatures with the maximum number at 30°C after full colonization of the agar surface. These primordia developed above and below the agar, lifting the medium upward but few primordia appeared on the agar surface during the development of actively growing mycelium.

DISCUSSION

The isolates AS60, AS61 and AS65 were collected from the fruiting bodies in the field. The period of maximum fruiting is associated with high humidity in the monsoon season and occurs after the temperature and rainfall maximum. At Faisalabad, the warm and moist season in July and August received maximum rainfall. Because of rain and cloudy weather during the monsoon, the temperature declines, whereas the natural soil moisture and humidity increase. Thus with comparatively lower temperature, decreasing rainfall and high natural moisture and humidity are favourable for mycelial growth and fruiting of these mushrooms.

The laboratory data on temperature relationships were then compared with climatological data for the areas where these strains grow (Fig 2 and 3). The larger box, in each figure delimits the regular fruiting season, while the smaller box delimits the range over which *Agaricus bitorquis* strains were found to grow on agar in these experiments. Fruiting occurred only in months when the average maximum temperature was greater than the minimum growth temperature required for mycelium growth. The period of fruiting is clearly mediated by the availability of moisture, while in the Punjab province in Pakistan which has monsoons in July and August, maximum fruiting is associated with periods when there is adequate natural moisture. These strains were collected from shady places under the trees, where the temperature is further lowered which can be related to the growing temperature in the laboratory (Fig. 3).

The literature suggests that the optimum-fruiting temperature for other basidiomycetes is generally lower than the optimum for mycelial growth. As has been

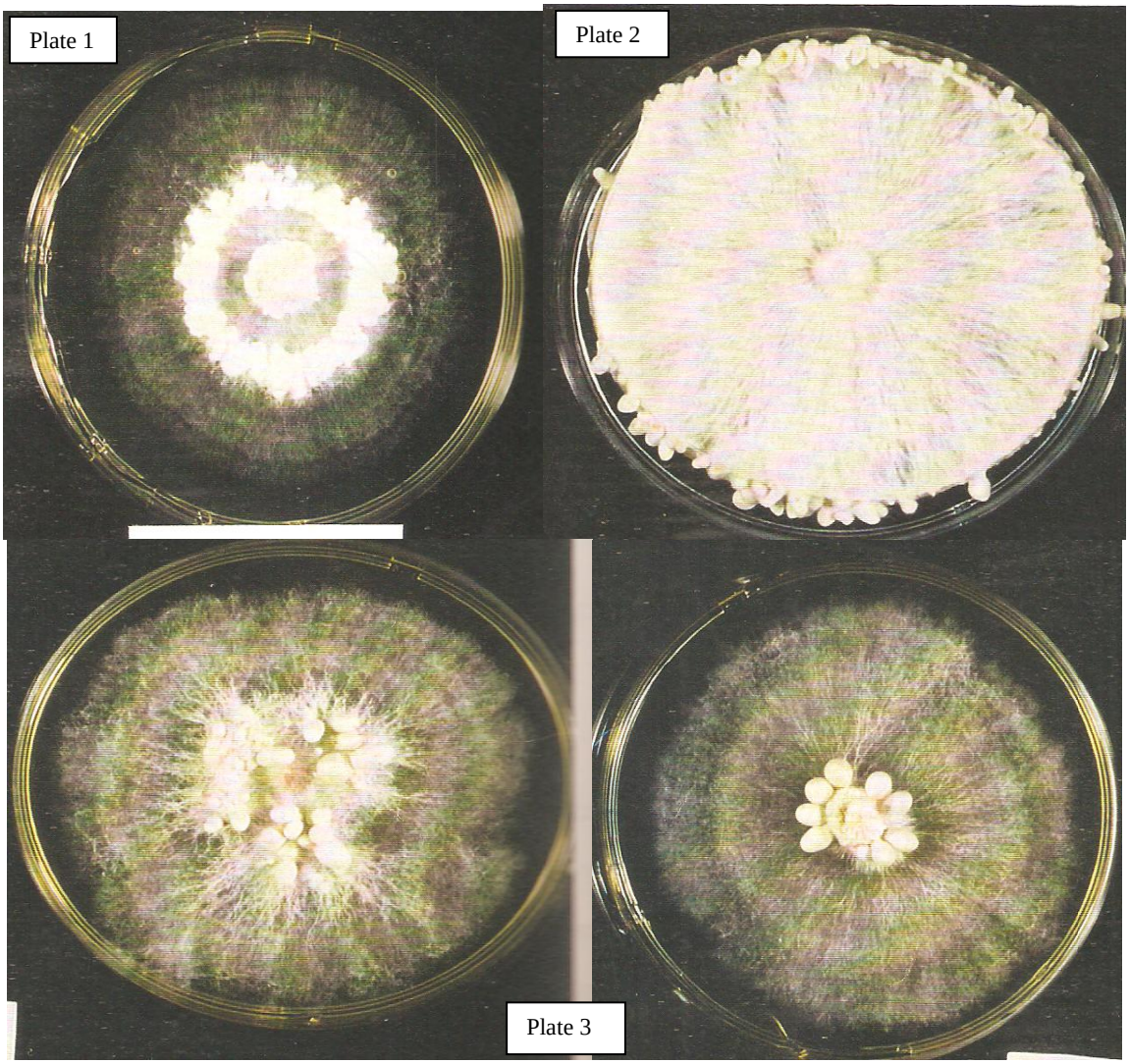


Plate 1: Hyphal aggregates of strain W2-F
Plate 2: Primordia formation of strain AS60 on the edges of agar plate
Plate 3: Promordia formation of strain W20 on agar surface

observed in some strains of *A. bisporus* and *A. bitorquis*, the optimum temperature for fruiting for various mushrooms were generally lower than the optimum temperature for mycelial growth (spawn running). The optimum temperature for mycelial growth and spawn running of white button mushroom, *Agaricus bisporus* (Lange) Imbach is 25±2°C, while fruiting bodies are formed at 14-18°C (Gogoi et al., 2006). The mycelial growth and spawn running in Chinese mushroom, *Volvariella volvacea* (Fr.) Singer, takes place at 32-35°C, whereas basidiocarps are formed at 28 to 32°C (Shalawet and Tewari 2007). *P. ostreatus* produce good mycelial growth between 27-30°C and fruiting bodies are formed between 22°-25°C (Stamets and Pal, 1983). Spawn running in *Lentinus edodes* (Berkely) Pegler takes place at 21-27°C, where as primordial initiation starts at 10-16°C (Stamets, 1993). However, in *Podaxis pistillaris* (L. ex Pers)FR., mycelial growth takes place over a range of 20-45°C with optimum growth at 40°C, which corresponds to the optimum temperature for fruiting in nature.

Temperature is also considered a most critical factor for the preparation of spawn and maintaining cultures. The most suitable temperatures for thermo-tolerant strains of *A. bitorquis and related species* was found to be 30°C. The growth of strain AS65 was very slow on malt extract agar, but faster on wheat grains. However, spawn of this strain became compact and fluffy after prolonged incubation at 30°C. On the other hand, strain AS61 produced more vigorous growth at 30°C on both malt extract agar and wheat grains and spawn remained loose and suitable for mixing in the compost. The rest

of the strains showed steady growth which has been reported as desirable character for *A. bisporus* strains (Phutela and Garcha, 1986). At both 25° and 30°C the colony diameter observed after 15 or 30 days on agar plates was maximum for strains AS60 and AS61 and least for Strain K32., which is in close agreement with Fritsche (1981). This practice was adopted in the present study for various *A. bitorquis* strains particularly K26, K32 and W2-F. The spawn of these strains also deteriorated in quality if stored at higher temperatures. The results are helpful for the preparation of spawn and adjustment of temperature to provide suitable conditions for spawn running in mushroom cultivation to obtain maximum productivity. The indigenous mushrooms with higher temperature requirements for mycelial growth and fructification may be easier to grow under natural conditions in summer months in Pakistan and other countries with similar climate, than is *A. bisporus,* which has the optimum temperature for mycelial growth about 25°C and requires temperatures below 20°C for fruiting.

Different strains of *A. bitorquis* showed a remarkable ability to produce pin heads on agar medium especially on malt extract agar, while most of the strains of *A. bisporus* do not readily form primordia on the usual agar culture media. Different strains showed marked differences in their ability to form primordia. The formation of primordia may be dependent on the nature of the media used and Primordium formation by these strains occurred after the cessation of vegetative growth. Production of primordia was also related to the pH of the nutrient media, as the optimal pH range studied by Noble et al. (2003) for *A. bisporus* was 7.0-7.5. Humes and Hayes (1972) described methods and specified conditions required for primordium development on malt extract agar media for two white strains of *A. bisporus*. When important environmental factors, such as suitable temperature, relative humidity and prolonged incubation are favourable, some strains form primordia, if continuously subcultured from old malt extract cultures. The extent of the contribution of nutrition in primordia formation in *A. bitorquis* remains to be investigated.

The cultures grown in high ambient carbon dioxide levels failed to produce primordia in *A. bisporus* Noble et al. (2003). Most of the primordium formation in the majority of *A. bitorquis* strains were observed in the centre of colonies on agar medium, which may be directly related to age of mycelium and to high CO_2 requirements for primordia initiation, whereas in AS60, the majority of fruiting initials were formed on the edges of agar plates, where the lower CO_2 concentrations in unsealed plates may be present. Strains of *A. bisporus* have been isolated which produce large numbers of initials on malt agar medium (Elliot and Wood, 1978). Primordia formation in the commercial cultures varies between strains and within replicate cultures of the same strain and the number of primordia formed depends upon the quantity of mycelium in the colony and the type of nutrient medium used (Flegg et al., 1985).

It can be concluded that AS51 failed to form pin heads at either temperature. Strain AS60 and W20 initiated most of the pin heads at 30°C. Strain AS60 formed pin heads at both the temperatures with the maximum number at 30°C after full colonization of the agar surface.

REFERENCES

Arita I, A Teratani and Y Shione, 1980. The optimal and critical temperatures for growth of *Pholiota adiposa*. Report Tottori Mycological Institute (Japan), 6: 49.

Chang ST and JA Buswell, 2003. Medicinal mushrooms. A prominent source of nutriceuticals for the 21st century. Current Topics in Nutraceutical Research, 1: 257-280.

Chang ST and PG Miles, 1989. Edible mushrooms and their cultivation, CRC Press, Boca Raton, Florida, USA.

Elliot TJ and DA Wood, 1978. A development variant of *Agaricus bisporus.* Transactions of the British Mycological Society, 70: 373-381.

Flegg PB, DM Spencer and DA Wood, 1985. The biology and technology of the cultivated mushroom. John Wiley & Sons, New York, USA.

Friel TM and AJ McLoughlin, 2000. Production of liquid inoclum/spawn of *Agaricus bisporus*. Biotechnolgy Letters, 22: 351-354.

Fritsche G, 1981. Some remarks on the breeding, maintenance of strains and spawn of *A. bisporus* and *A. bitorquis*. Mushroom Science, 1: 367-386.

Gogoi R, Y Rathalah and TR Borah, 2006. Mushroom Cultivation Technology. Scientific Publishers, Jodhpur, India.

Humes DP and WA Hayes, 1972. The production of fruit body primordia of *Agaricus bisporus* (lange) in agar. Mushroom Science, 8: 527-532.

Kalmis E and F Kalyoncu, 2006. Variations in the isolate obtained from Basidiospores of commercial mushroom *Lentinus edodes* (Shiitake). International Journal of Science and Technology, 1: 99-103.

Khan SM and AG Kausar, 1981. Prospects of mushroom culture in Pakistan. Mushroom Science, 11: 55-58.

Liaqat MR, MA Ali, NA Khan, MK and HU Khan, 2013. Influence of different casing materials on the growth and yield of thermo-tolerant strains of *Agaricus bitorquis.* Pakistan Journal of Phytopathololology, 25: 101-104.

Manzi P, A Aguzzi, L Pizzoferrato, 2001. Nutritional value of mushrooms widely consumed in Italy. Food Chemistry, 73: 321-325.

Noble R, TR Fermor, S Lincoln, A Dobrovin-Pennington, C Evered and A Mead, 2003. Primordia initiation of mushroom (Agaricus bisporus) strains on axenic casing materials. Mycologia, 95: 620-629.

Pahil VS, 1992. Cultivation and strain improvement of high temperature wild and cultivated *Agaricus* species. PhD, Thesis. Faculty of Science, University of London, UK.

Phutela RP and HS Garcha, 1986. Improving yield potential of the cultivated mushroom *Agaricus bisporus* (Lange) Sing. The Punjab Horticulture Journal, 2: 348-354.

Rohul A, N Alam, M Tania and KM Asaduzzaman, 2008. Study of mycelial growth of *Cordyceps senensis* in different media, at different pH level and temperature. Bangladesh Journal of Mushrooms, 2: 43-48.

Shalawet OP and R Tewari, 2007. Cultivation Technology of Paddy Straw mushroom (*Volvariella volvacea*). National Research Centre for Mushroom. Chambat, Solan, India.

Smith JE, MP Challan, PE White, RN Edmondson and D Chandler, 2006. Differential effect of Agaricus host species on the population development of *Megaselia halterata* (Diptera: Phpridae). Bulletin of Entomological Research, 96: 565-571.

Stamets P and JS Pal, 1983. The Mushroom Cultivator. A Practical Guide to Growing Mushrooms at Home. Agaricon Press. Olympia, Washington, USA.

Stamets P, 1993. Growing Gourmet and Medicinal Mushrooms. Ten Speed Press Berekely, CA, USA.

Steel RGD, JH Torrie and D Dickey, 1997. Principles and procedures of statistics: a biometrical approach, 3rd Ed. McGraw Hill Book Co Inc, New York, USA.