Journal of Applied Botany and Food Quality 95, 167 - 173 (2022), DOI:10.5073/JABFQ.2022.095.021

[1]Institute of Botany, University of the Punjab, Lahore, Pakistan

Domestication and element analysis of the giant edible *Macrocybe gigantea* from Pakistan

Aneeqa Ghafoor[1], Abdul Rehman Niazi[1]*, Najam-ul-Sehar Afshan[1]

(Submitted: January 11, 2022; Accepted: June 22, 2022)

Summary

During a survey of mushrooms of Pakistan, *Macrocybe gigantea* was collected from University of the Punjab, Lahore, Pakistan under the *Morus* species. For the domestication of this wild species, its culturability and cultivation potential was assessed by using different synthetic culture media and substrates. Among these different media used, maximum cultural growth was observed on Potato Dextrose Agar (PDA) medium at 30 °C followed by Malt Extract Agar (MEA), Compost Extract Agar (CEA), Glucose Peptone Agar (GPA), and Saboraud Dextrose Agar (SDA). Strains on PDA medium were used for production of spawning material on wheat, sorghum and barley grains. Sorghum grains at 30 °C were the best combination for spawn production. A mixed substrate of wheat straw and Tea waste at 30 °C produced the highest yield. Mineral analysis of the wild and cultivated strain revealed that both forms enrich Potassium and Calcium. These findings show that this giant edible mushroom species could be domesticated on the number of media and substrates. Its domestication can provide nutritional, economical, medicinal and tasty food to the growing population that would otherwise be restricted to natural production at a specific time of the year.

Keywords: Biological efficiency, Culturability, Cultivation potential, *Macrocybe gigantea,* Spawn.

Introduction

The genus *Macrocybe* Pegler & Lodge emerged out in 1998 as separate entity from the genus *Tricholoma* on the basis of morphological and molecular evidences (Pegler et al., 1998). Species of *Tricholoma* are obligatory ectomycorrhizal with clampless hyphae while the species of *Macrocybe* are large, saprophytic with clamped hyphae (Pegler et al., 1998; Razzaq et al., 2017). Recent comparative genomic approaches also support that *Macrocybe* is a stand-alone genus from *Tricholoma* (Kui et al., 2021). It has nine well-recognized species, distributed in the tropical regions of the world, which include *M. gigantea* (Massee) Pegler & Lodge, *M. pachymeres* (Berk. & Broome) Pegler & Lodge, *M. praegrandis* (Berk.) *M. spectabilis* (Peerally & Sutra) Pegler & Lodge, *M. crassa* (Sacc.) Pegler & Lodge, *M. praegrandis* (Berk.) Pegler & Lodge, *M. titans* (H.E. Bigelow & Kimbr.) Pegler, Lodge & Nakasone, *M. lobayensis* (R. Heim) Pegler & Lodge and *Macrocybe sardoa* Vizzini, Consiglio & M. Marchetti and most of these species are edible (Pegler et al., 1998; Duong et al., 2017; Vizzini et al., 2020). Initially, the *Macrocybe* species were only studied for pharmaceutical purposes (Chatterjee et al., 2011), while their farming is relatively new as compared to other commonly cultivable mushrooms. *M. gigantea* (Massee) Pegler & Lodge belongs to family Tricholomataceae under the order Agaricales and was first reported as *Tricholoma giganteum* (Massee, 1912).

This giant mushroom is mainly confined to the pantropical regions (Asia and Africa) and commonly found in the West Bengal region. However, it is also reported from the Japan, USA, and Korea (Khatua and Acharya, 2016; Ghosh and Acharya, 2022). It grows gregariously in shady, grassy areas or with angiospermic trees in high temperature and humidity and occurs in hylaea and subtropical rain forests in Africa and Asia (Huang, 2001). It is mostly grown in cluster form of about 20 to 30kg (Kui et al., 2021). It has a smooth cap, whitish to greyish white in color, the gills are straw yellow in color and are crowded. The stipe is 15-18 cm in length and 6 cm in diameter and its color is same as that of the cap. The spore print is white (Pegler et al., 1998; Kui et al., 2021).

It contains water-soluble polysaccharides and certain bioactive compounds that have a pharmaceutical value (Kui et al., 2021) as antitumor, anticancer, antioxidant and antimicrobial agent (Chatterjee et al., 2011; Gaur and Rao, 2016). Macrocybin, a natural triglyceride present in *Macrocybe* species reduces tumor cells both in vitro and in vivo by interacting with the actin cytoskeleton (Vilarino et al., 2020). Ethanolic extracts of *M. gigantea* revealed the presence of antioxidants like Vitamin E, colchicines, and 5-methyldioadenosine. (Acharya et al., 2012). It is also rich in mineral elements such as Ca (38-470 mg/kg), Mg (84-550 mg/kg) and Zn (16-160 mg/kg) (Liu et al., 2012). *M. gigantea* is also valuable because it sustains high temperature range 30~38 °C in addition to nutrient and taste (Amin et al., 2010).

Due to these nutritional and therapeutic attributes, (Chatterjee et al., 2011; Niazi and Ghafoor, 2021) it could be advantageous to grow this fungus at industrial scale for maximum benefits. *M. gigantea* can meet the demand of food of a growing population due to both nutritional and therapeutic peculiarities. However, in wild form, there is a chance of radioactive contamination, which can be overcome by the cultivation under controlled conditions (Falandysz et al., 2015). Cultivation practices of *M. gigantea*, *M. crassa*, and *M. lobayense* are described in literature but none of the species farming status reached the industrial scale in any country. Initially, *M. gigantea* was commonly cultivated as *Tricholoma gigentium* (Yoshikazu and Takashi 1997; Dadwal, 1984; Lu, 1992; Kim et al., 1998; Kinjo and Miyagi, 2006; Chen et al., 2012; Inyod et al., 2016) as one of the largest edibles tricholomatoid agarics of southeast Asia. It is being cultivated on a small scale in India and China but requires more research and refinement. Generally, it requires a temperature between 25-35 °C, 70-80% relative humidity and light of 8-10 h for its growth (Razaq et al., 2016; Inyod et al., 2016; Verma et al., 2017; Akhtar et al., 2019; Devi and Sumbali, 2021). From Pakistan, only one species of *M. gigantea* is reported (Razaq et al., 2016). Pakistan's climatic conditions are favourable for its natural growth but its domestication was never tried before.

The aim of this study was to optimize the standard cultivation requirements of the *M. gigantea* and to compare the element composition of the wild and artificially cultivated fruiting bodies to get maximum benefit from this edible mushroom. Its domestication can compete with the nutritional peculiarities of the widely growing edible fungal species like button and oyster strains.

* Corresponding author

A step towards Sustainable Development Goal 2 "Zero Hunger"
The findings will help open new possibilities for food production, namely large scale production of *Macrocybe gigantea*, and contribute to sustainability by optimizing the use of waste materials as substrates for food production.

Material and methods

Sampling and experimental design

Basidiomata of the *M. gigantea* were collected in gregarious form under the *Morus* species from University of the Punjab, Lahore Pakistan. The collected specimen were photographed using an Android camera and identified by macro-microscopically and phylogenetically according to the literature available (Razzaq et al., 2016). The experiments were carried out in Fungal Biology and Systematics Research lab, Institute of Botany, University of the Punjab, Lahore. Specimen *M. gigantea* (LAH03821), was deposited in the Herbarium, Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH) for ready reference.

Evaluation of culturability

Culturability of *M. gigantea* was assessed according to the method described by Siddiq et al., 2018. Small tissues from inner unexposed part of the fruiting body were placed onto five different nutrient agar media i.e, Malt extract agar (2% MEA: agar 20 g, malt extract 20 g dissolved into 1000 mL dH2O), Potato dextrose agar (2% PDA: thin potato slices 200 g, glucose 20 g, agar 20 g per liter of dH2O), Glucose peptone agar medium (2% GPA: 20 g peptone, 20 g dextrose, 5 g Nacl, 15 g agar dissolved into 1000 ml dH2O), Saboraud dextrose agar (2% SDA: 15 g agar, 40 g dextrose, 10 g peptone dissolved into 1000 ml dH2O) and Compost extract agar (2% CEA: 20 g agar,10 g glucose dissolved into 1000 mL wheat straw water based filtrate). Inoculated petri plates were sealed with paraffin film and then incubated at different temperatures i.e., 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C. Mycelial growth characteristics were observed on regular basis. Each effect was determined in triplicates. These mushroom cultures were also deposited in the Herbarium Culture Collection of University of the Punjab, Lahore (as LAH#072021C(ABCDE).

Spawn production

Spawn was prepared by following Pal and Thupa (1979) described methodology. Three types of cereal grains viz., sorghum, wheat, and barley grains were used as the substrate to determine the spawn production efficiency. For spawn preparation, grains were washed and soaked for overnight, boiled for half an hour and excess water from grains was removed by spreading them on blotting paper. Three quarters of the each 1 L filter jars was filled with boiled grains supplemented with gypsum (2 g) and lime (1 g) and then autoclaved. Spawns were prepared by inoculating mycelial discs from pure PDA culture on sterilized grains in a laminar air flow cabinet. Inoculated grains were incubated at 30 °C. Effect of grains on production of spawning material was determined in triplicates.

Substrate production

Wheat straw, sawdust and tea waste were used as the raw material for substrate production. Dried wheat straw collected from the field area, University of the Punjab, Lahore, Sawdust collected from the furniture shop, while tea-waste collected from the Hostel Canteens, University of the Punjab, Lahore. Tea waste is just the left-over residues of tea (water containing tea) after usage. It is enriched with cellulose, hemicellulose, proteins, lipids, polyphenols as well as many minerals (Zhu et al., 2013). Six types of substrates were prepared. Three of pure types i.e., wheat straw, sawdust and tea-waste while three were of mixed type i.e., sawdust and wheat straw, tea waste and saw dust and tea waste and wheat straw. For substrate production (pure and mixed types), raw materials were sprinkled with water and piled up, 65% moisture maintained during the substrate production process of ten days. Piles were turned every second day. Chicken manure and urea (one fourth of the substrates (25 g/1 kg) were added as supplements for nitrogen (N) and carbon (C) source on the second and last turning while gypsum (15 g/kg) was added and thoroughly mixed before the pasteurization process. When substrates were prepared, 700 g/bag were filled in polypropylene bags (20 × 15cm) and autoclaved for 3 to 4 hours for sterilization purpose. Experiment was performed in triplicates.

Spawning

Sterilized substrate bags were kept until their temperature reached 28 °C (for spawning), then they were inoculated with the spawn prepared on sorghum grains (25 g/700 g). The bag mouths were loosely tied with the rubber bands and incubated at different temperatures.

Spawn running

Spawn running period was observed on different substrates at different temperatures. During the spawn running, relative humidity (70%) was maintained by humidifier and ventilation fan at different incubation temperatures. When the spawn running was almost completed, casing with autoclaved tea-waste (tea containing water) was done to maintain the moisture of the substrates. After pinhead emergence, bags were transferred to the cropping room with relative humidity of 85%, maintained by continuous ventilation.

Biological efficiency

Biological efficiency (dry weight basis) of different types of substrates up to three flushes was observed as per 700 g of the substrate bags.

$$\text{Biological efficiency} = \frac{\text{weight of fresh mushroom}}{\text{dry weight of substrate}} \times 100\ \%$$

Element analysis

Element analysis of the wild and cultivated fruiting bodies on the mixed substrate of wheat straw and Tea waste was pursued by using standard procedures described by Horwitz et al. (1970). Powder samples were subjected to digestion by wet digestion method to check the mineral contents present in the samples. Minerals i.e., Potassium (K), Zinc (Zn), Calcium (Ca), Nickle (Ni), Copper (Cu) and Cobalt (Co) were analyzed through wet digestion method. Standard solutions of 5, 10, 15 20 and 25 ppm were prepared from the stock solution of the five required metals except potassium. For potassium, standard solution of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 ppm were prepared from the stock solution (1000 mL) of potassium and were analyzed in Atomic Absorption (AA) Spectrophotometer (PerkinElmer AAnalyst100) and in Flame Photometer (JANWAY PFP 7).

Statistical analysis of the data

Completely randomized design was used to determine the different parameters i.e., culturability, spawn production and cultivation potential. All the treatments were evaluated in triplicates and two-way Analysis of variance was applied to determine the significant differences between different treatments. SPSS software package was used for the statistical analysis. Data is also expressed as mean value ± S.E.

Results and discussion

Screening the most effective culture medium and optimization of temperature

Mycelium extension rate and density always rely on the suitable culture medium utilized for culturing (Reyes et al., 2009). Mycelial features of *M. gigantea* were evaluated on various nutritive solid media at different temperatures. White colored mycelium appeared in cultures without any exudates, with regular pattern and moderate to abundant density on different solid media. (Fig. 1). Cottony mycelium with fibrillar growth was observed on all the media used for the evaluation of mycelial growth potential. Findings of this study were similar with Dulay et al., 2021; in which they observed the same mycelial characteristics for *L. tigrinus* on the majority of carbon sources used for its culturing.

Amongst the different media utilized for the culturing of *M. gigantea,* PDA proved the most efficient in terms of mycelium extension rate (13.96±0.033 mm/day) and density (abundant). SDA medium (6.43±0.035 mm/day) at 15 °C was found to be the least appropriate for the mycelial growth of this species (Fig. 1). The current results coincide with Sardar et al., 2015 as they found that PDA is the best medium for the growth of mycelium of various *Pleurotus* species. Our findings were also in concurrent with the Roy and Krishnappa, 2018; they evaluated the medium growth specificity of the *M. gigantea* on different nutritive solid media such as PDA, MEA, SDA and Czapek Dextrose Agar (CDA). They determined the maximum mycelial growth on the PDA medium followed by MEA. Jo et al., 2002 evaluated the cultural potential of *Ganoderma lucidum* and screened the PDA as the appropriate medium for the mycelial growth. However, our findings were contrasted with the Shim et al., 2005 as they found the minimum mycelial density of *Macrolepiota procera* on the PDA medium.

Vegetative growth of mushrooms needs a specific range of temperature for proliferation due to its effect on metabolic reactions. Different basidiomycetes species grow in a wide range of temperatures, while the best temperature is between 20-30 °C (Vahidi et al., 2004; Lai et al., 2014). For the vegetative growth of *M. gigantea,* temperature suitability was evaluated and 30 °C was determined as the optimum temperature on all the solid nutritive media used for culturability testing. At 35 °C, mycelium growth slowed down. Our findings were also in line with the temperature response of various tropical mushrooms like *Lentinus squarrosulus* (Leon et al., 2017), *Collybia reinakeana* (Reyes et al., 1997) and *Volvariella volvacea* (Reyes et al., 1998). All media used for the evaluation of culturability of *M. gigantea* were proven to be supportive for its growth, with PDA being the best option at 30 °C. The mycelium extension rate (mm/day) on different media at different temperatures differed significantly at $p<0.001$ as shown in Tab. 1.

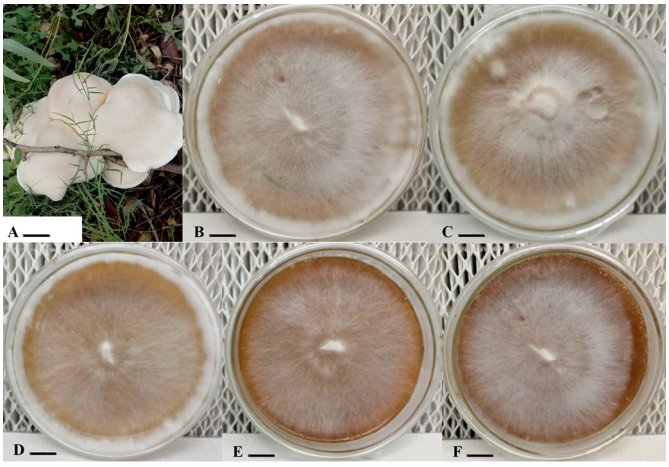

Fig. 1 (A-F): A: Basidiomata of *M. gigantea*; B-F: Cultures on different nutrient agar media at 30 °C after 12 days of inoculation; B: on PDA; C: on MEA; D: on CEA; E: on GPA; F: on SDA.
Scale bar: A: 2 cm, B-F: 1 cm

Spawn production

Spawn is the medium for the transformation of the mushroom mycelium to the growing substrate (Wozniak, 2009). It promotes quick colonization of the mushroom substrate and initiates successful fruiting (Chang, 2009). The colonization rate of the active mycelium (cultured on the PDA medium) was checked on cereal grains (sorghum, wheat and barley) at 30 °C. Mycelium colonized more quickly on sorghum (*Sorghum bicolor*) followed by wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) (Fig. 2). Full spawning material (whole grains covered with the mycelium) was ready on sorghum grains after 13 days of inoculation.

The ample growth of mycelium on sorghum grains was due to its moisture and nutrient composition (Leder, 2004). This is in agreement with Devi and Sumbali (2021) for the spawn production of

Tab. 1: Mycelial growth rate (mm/day) of *M. gigantea* on different media at different temperatures. CEA, Compost Extract Agar; PDA, Potato dextrose agar; MEA, Malt Extract Agar; SDA, Saboraud Dextrose Agar; GPA Glucose Peptone Agar

Types of media	Mycelium extension rate (mm/day)					
	Temperatures					
	15 °C	20 °C	25 °C	30 °C	35 °C	P-value
PDA	7.33±0.033	10.27±0.057	12.33±0.035	13.96±0.033	12.85±0.033	<0.001
MEA	7.25±0.036	9.83±0.035	11.28±0.033	12.87±0.033	11.95±0.033	<0.001
CEA	6.95±0.043	9.3±0.033	10.4±0.057	12.26±0.631	11.36±0.036	<0.001
GPA	6.76±0.033	8.96±0.033	9.9±0.057	11.24±0.033	10.96±0.036	<0.001
SDA	6.43±0.035	6.96±0.033	7.86±0.033	8.56±0.036	7.93±0.035	<0.001
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	

Values given are mean ± Standard error. Media type and temperature have significant impact over Mycelium growth rate ($p<0.001$). Moreover, the joint effect of media and temperature has also a significant impact over Mycelium extension rate ($p<0.001$). In addition, LSD test was applied and found the significant differences between all possible combinations of media types ($p<0.001$) and between all possible combinations of temperatures ($p<0.001$).

(Green-Yellow-Red) Scheme was applied on the tables for quick readability of the most and least effective treatment on all the parameters. Green color indicated the best treatment while red color showed the least effective.

Fig. 2 (A, B, C): Spawn production of *M. gigantea* on A: sorghum grains; B: wheat grains; C: barley grains at 30 ºC after 17 days of inoculation. Scale bar: A-C: 1 cm.

M. gigantea. Leon et al. (2017) observed the shortest incubation period for spawn production of wild strain of *L. squarrosulus* on sorghum grains from the Philippines. These grains were also found to be the good spawning material for *Agaricus blazei and Agrocybe aegerita* (Galamgam, 2009; Marcelo, 2011). Bharti (2019) found wheat grains while Pamitha (2014) and Duong et al. (2017) found paddy grains as the suitable substrate for spawn preparation of *M. gigantea*. Tab. 2 shows that the days required to complete spawn production of *M. gigantea* on sorghum, wheat and barley grains at 30 °C significantly differed at ($p<0.001$).

Tab. 2: Efficiency of wheat, sorghum and barley grains for spawn production of *M. gigantea*

Types of grains	Days required to complete spawn production at 30 °C
Sorghum	13 d 15 h ±1.15h
Wheat	16 d 22.3 h ± 1.20 h
Barley	20 d 22 h ± 1.15 h

The results reported were run in triplicates and stated as Mean± Standard error.

Determination of efficient lignocellulosic substrate for fruiting and yield of *M. gigantea*

The success of a newly cultivated strain depends on both economical and biological factors (Thawthong et al., 2014). Temperature is one of the key biological factors for successful fruiting of any mushroom or the conversion of the dikaryotic mycelium into the fruiting body. Mata et al. (2005) reported that different factors like, temperature, light and humidity of the incubation room influence the spawn running of mushrooms. Spawn prepared on sorghum grains was used to determine the spawn running time of *M. gigantea* on six different substrates (three were of pure type and three mixed substrate) at various temperatures, 20 °C, 25 °C, 30 °C, 35 °C, and 40 °C. At 30 °C, minimum spawn running period was observed on the tea waste + wheat straw (19.86±0.033d) followed by pure wheat straw (21.34±0.035d) with 70% humidity level of the incubation rooms (Tab. 3). This is similar to the work of Devi and Sumbali (2021), who found the lowest spawn running period (16 days) on wheat straw substrate.

Furthermore, cultivation potential, in the form of fruiting bodies and yield, was evaluated on six types of substrates, three were of pure substrates, (wheat straw, tea waste and saw dust) and other three were the combination of two substrates (wheat straw+ tea waste, tea

Tab. 3: Days required to complete Spawn Running Period on different substrates at variable temperatures

Types of substrates	Days required to complete Spawn running period					
	Temperatures					
	15 °C	20 °C	25 °C	30 °C	35 °C	P-value
Tea waste & wheat straw	29.96±0.033	25.93±0.035	22.96±0.033	19.86±0.033	21.86±0.006	<0.001
Pure wheat straw	27.93±0.035	26.93±0.035	24.96±0.036	21.34±0.035	24.93±0.035	<0.001
Saw dust & wheat straw	28.92±0.039	27.96±0.033	26.85±0.033	24.96±0.033	26.96±0.033	<0.001
Tea waste & saw dust	31.93±0.035	28.96±0.033	28.33±0.035	27.85±0.036	29.95±0.033	<0.001
Pure saw dust	Not Initiated	30.93±0.035	29.96±0.033	27.96±0.033	30.93±0.035	<0.001
Pure tea waste	Not Initiated	32.91±0.044	30.56±0.036	29.93±0.035	31.3±0.033	<0.001
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	

Values given are mean ± Standard error. Substrate types and temperature have significant impact over spawn running time ($p<0.001$). Moreover, the joint effect of substrates and temperature has also a significant impact over spawn running time ($p<0.001$). In addition, LSD test was applied and found the significant differences between all possible combinations of substrate types ($p<0.001$) except between Tea waste & wheat straw and pure tea waste; and between all possible combinations of temperatures ($p<0.005$) except 15 ºC & 20 ºC ($p=0.086$) and 20 ºC and 25 ºC ($p=0.737$).

(Green-Yellow-Red) Scheme was applied on the tables for quick readability of the most and least effective treatment on all the parameters. Green color indicated the best treatment while red color showed the least effective.

waste+ saw dust and saw dust+ wheat straw) at 30 °C with humidity level 85%. The mixture of tea waste and wheat straw was found to be the best substrate for the cultivation of *M. gigantea* at 30 °C in the term of fruiting and yield (86.77±0.035g) followed by the wheat straw (79.85±0.035). Tea-waste was used for the first time as the growing medium for the cultivation of *M. gigantea* that was proved effective. YANG et al. (2016) revealed the tea-waste as the economic and suitable substrate for the fruiting and yield of *P. ostreatus*. Lignin cellulosic waste supplemented with tea-waste could be an efficient source of growing substrate for the cultivation of various saprophytic mushrooms like *Ganoderma lucidum* (PEKSEN and YAKUPOGLU, 2009).

As far as the efficiency of substrates were concerned, INYOD et al. (2016) obtained the maximum yield of *M. crassa* on saw-dust. ATRI and LATA (2013) obtained the maximum yield of *L. cladopus* from the substrate of the mixture of wheat straw and paddy straw. Our research was also related to the findings of DULAY et al., 2021. They observed the combination of rice straw and saw dust at different temperatures as the suitable substrate for the cultivation of *Lentinus* species. Tab. 4 revealing the yield obtained from varieties of substrates and Fig. 3 representing the different stages of fruiting body production of *M. gigantea*.

Element analysis

The Element analysis was conducted to determine macro and trace elements in the nutritionally and medicinally significant *Macrocybe gigantea*. This species was enriched with macronutrients like potassium and calcium while trace elements (nickel and cobalt) were not detected in both wild and cultivated basidiomata (Tab. 5). Enrichment of essential elements and absence of toxic metals showed their suitability to enrich a diet. These results were in agreement with the findings of LIU et al., 2012 and MALLIKARJUNA et al., 2013.

Tab. 5: Minerals concentration present in the *Macrocybe gigantea*

Mushrooms	Essential and Trace minerals (mg/g)					
	Ca	Co	Cu	K	Zn	Ni
M. gigantea wild	1.54	<LOD	0.524	69	0.20	<LOD
M. gigantea cultivated	1.46	<LOD	0.422	71	0.11	<LOD

<LOD indicates limit of detection; Calcium (Ca), Cobalt (Co), Copper (Cu), Potassium (K), Zinc (Zn), Nickel (Ni)

Conclusion

It can be concluded that *M. gigantea* could easily be grown on different media but PDA medium at 30 °C was proved the best combination for the growth of this mushroom. Tea waste medium was used for the first time as the growth medium for *M. gigantea* and found very effective. However, different combinations of the substrates and media like paddy straw with tea waste, cotton waste with tea waste, tea waste with banana peels etc at 30 °C should be investigated in more detail to enhance the yield and biological efficiency of this nutritious mushroom.

Conflict of interest

No potential conflict of interest was reported by the authors.

Authors contribution

Aneeqa Ghafoor: Collection, analysis, methodology and writing
Abdul Rehman Niazi: Identification, supervision, analysis and methodology
Najam-ul-Sehar Afshan: Formal analysis, visualization

Tab. 4: Yield (g/700 g) obtained from different types of substrates at 30 °C

Types of substrates	Biological Efficiency (yield g/700 g)			
	1st flush yield	2nd flush yield	3rd flush yield	Total yield
Tea waste & wheat straw	39.96±0.033	25.96±0.033	20.85±0.033	86.77±0.035
Pure wheat straw	37.96±0.036	22.96±0.033	18.93±0.033	79.85±0.035
Sawdust & wheat straw	30.96±0.036	21.93±0.035	17.95±0.033	70.84±0.033
Tea waste & saw dust	26.96±0.033	19.94±0.047	Not appeared	46.9±0.035
Pure saw dust	21.40±0.057	18.93±0.065	Not appeared	40.33±0.057
Pure tea waste	20.93±0.033	17.93±0.033	Not appeared	38.86±0.044
P-value	<0.001	<0.001	<0.001	<0.001

Values given are mean ± Standard error. Substrate types have significant impact over the total yield (p<0.001).

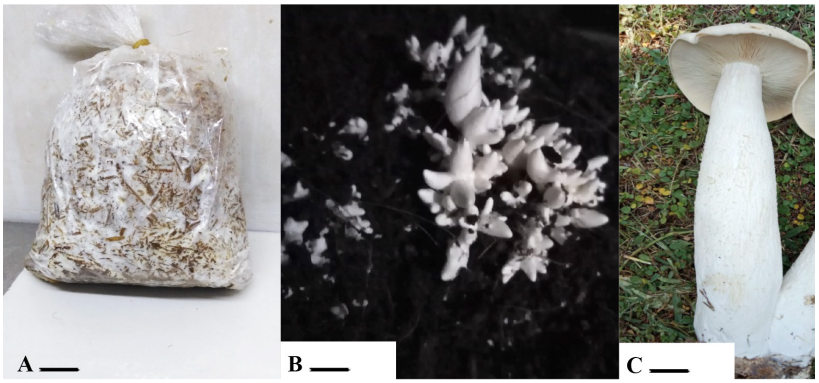

Fig. 3 (A, B, C): A: Spawned compost; B: pinheads and C: Harvested fruiting body of *M. gigantea*. Scale bar (A-C): 2 cm

Acknowledgements
Authors are very grateful to University of the Punjab, Lahore, Pakistan, for providing funds to conduct this project during fiscal year 2021/2022. We are highly thankful to Prof. Dr. Rehan Ahmad (College of Statistics and Actuarial Sciences, University of the Punjab, Lahore, Pakistan) for his help in the statistical analyses of the manuscript and giving useful suggestions.

References

Acharya, K., Chatterjee, S., Biswas, G., Chatterjee, A., Saha, G.K., 2012: Hepatoprotective effect of a wild edible mushroom on carbon tetrachloride-induced hepatotoxicity in mice. Int. J. Pharm. 4(3), 285-288.

Akhtar, N.S., Kumar, C., Dayaram, Kumar, P.M., 2019: Cultivation technology of *Tricholoma giganteum* on agricultural waste to promote sustainable agriculture and doubling farmers income. J. Pharmacogn. Phytochem. 8(6), 2356-2361.

Amin, R., Khair, A., AIam, N., Lee, T.S., 2010: Effect of different substrates and casing materials on the growth and yield of *Calocybe indica*. Mycobiology. 38(2), 97-1. DOI: 10.4489/MYCO.2010.38.2.097

Atri, N.S., Lata, 2013: Studies for culturing and cultivation of *Lentinus cladopus* Lev. Mycosphere. 4(4), 675-82. DOI: 10.5943/mycosphere/4/4/3

Bharti, V., 2019: Standardization of cultivation technology of new tropical mushroom *Macrocybe gigantea* (Massee) Pegler & Lodge. Ph.D Thesis, Department of Plant Pathology, Sher-E-Kashmir University of Agricultural Sciences and Technology (SKUAST), Jammu.

Chang, S.T., 2009: Training Manual on Mushroom Cultivation Technology. United Nations, Beijing, China.

Chatterjee, S., Saha, G.K., Acharya, K., 2011: Antioxidant activities of extracts obtained by different fractionation from *Tricholoma giganteum* basidiocarps. Pharma. online. 3, 88-97.

Chen, L., Wei, S., Huang, Z., Chen, Z., Qin, X., Wang, Q., Wang, C., 2012: Effect of temperature, pH and moisture content of culture medium on mycelium growth of *Tricholoma giganteum*. Southwest China J. Agric. Sci. 25(2), 597-600.

Dadwal, V.S., 1984: A note on the fruit body production of *Tricholoma giganteum* Massee. Curr. Sci. 53(17), 931-933.

de Leon, A.M., Guinto, L.J., De Ramos, P.D., Kalaw, S.P., 2017: Enriched cultivation of *Lentinus squarrosulus* (Mont.) singer: a newly domesticated wild edible mushroom in the Philippines. Mycosphere. 8(3), 615-29. DOI: 10.5943/mycosphere/8/3/9

Devi, S., Sumbali, G., 2021: Suitability of three different cereal grains for spawn development and their impact on the growth and yield of *Macrocybe gigantea* (Massee) Pegler and Lod. J. Appl. Nat. Sci. 13(1), 204-209. DOI: 10.31018/jans.v13i1.2547

Dulay, R.M.R., Cabrera, E.C., Kalaw, S.P., Reyes, R.G., 2021: Optimization of culture conditions for mycelial growth and fruiting body production of naturally-occurring Philippine mushroom *Lentinus swartzii* Berk. J. Appl. Biol. Biotechnol. 9(3), 17-25. DOI: 10.7324/JABB.2021.9303

Duong, P.N., Duy, V.D., Anh, N.T., Xuan, B.T.T., Tham, L.X., 2017: Studying identifcation and cultivation of *Macrocybe titans*, a new record species for Vietnam collected in Cat Tien National Park, south of Vietnam. Tap. Chi. Sinh. Hoc. 39, 172-181.

Falandysz, J., Zhang, J., Zalewska, T., Apanel, A., Wang, Y., Wiejak, A. 2015: Distribution and possible dietary intake of radioactive 137Cs, 40K and 226Ra with the pantropical mushroom *Macrocybe gigantea* in SW China. J. Environ. Sci. Health A 50(9), 941-945.

Galamgam, H.D., Reyes, R.G., Kalaw, S.P., 2009: Biophysiology of hemimatsuke (*Agaricus blazei*) strain 2. J. Trop. Biol.7, 42.

Gaur, T., Rao, P.B., 2016: Antioxidant potential of the giant mushroom, *Macrocybe gigantea* (Agaricomycetes), from India in different drying methods. Int. J. Med. Mushrooms 18(2). DOI: 10.1615/IntJMedMushrooms.v18.i2.40

Horwitz, W., Chichilo, P., Reynolds, H., 1970: Official methods of analysis of the association of official analytical chemists. Official methods of analysis of the Association of Official Analytical Chemists.

Inyod, T., Sassanarakit, S., Payapanon, A., Keawsompong, S., 2016: Selection of *Macrocybe crassa* mushroom for commercial production. Agric. Nat. Resour. 50(3), 186-191. DOI: 10.1016/j.anres.2016.06.006

Jo, W.S., Cho, Y.J., Cho, D.H., Park, S.D., Yoo, Y.B., Seok, S.J., 2009: Culture conditions for the mycelial growth of *Ganoderma applanatum*. Mycobiology. 37(2), 94-102. DOI: 10.4489/MYCO.2009.37.2.094

Khatua, S., Acharya, K., 2016: Influence of extraction parameters on physico-chemical characters and antioxidant activity of water-soluble polysaccharides from *Macrocybe gigantea* (Massee) Pegler & Lodge. J. Food Sci. Technol. 53(4), 1878-1888. DOI: 10.1007/s13197-015-2145-0

Kim, H.K., Kim, Y.S., Seok, S.J., Kim, G.P., Cha, D.Y., 1998: Artificial cultivation of *Tricholoma giganteum* collected in Korea (I)-Morphological charateristics of fruitbody and environmental condition in habitat of *T. giganteum*. Kor. J. Mycol. 26(2), 182-186.

Kinjo, K., Miyagi, T., 2006: Nutritional requirements for mycelial growth and artificial cultivation of *Tricholoma giganteum*. J. Wood. Res. 52(5), 320-326.

Kui, L., Zhang, Z., Wang, Y., Zhang, Y., Li, S., Dong, X., Dong, Y., 2021: Genome assembly and analyses of the macrofungus *Macrocybe gigantea*. Biomed Res. Int. 2021, 1-10. DOI: 10.1155/2021/6656365

Lai, W.H., Siti Murni, M.J., Fauzi, D., Abas Mazni, O., Saleh, N.M., 2011: Optimal culture conditions for mycelial growth of *Lignosus rhinocerus*. Mycobiology. 39(2), 92-5. DOI: 10.4489/MYCO.2011.39.2.092

Léder, I., 2004: Sorghum and millets. Cultivated plants, primarily as food sources. 1, 66-84.

Liu, H., Zhang, J., Li, T., Shi, Y., Wang, Y., 2012: Mineral element levels in wild edible mushrooms from Yunnan, China. Biol. Trace Elem. Res. 147(1), 341-345. DOI: 10.1007/s12011-012-9321-0

Lu, C.Y., Zhong, Y.J., Zhang, M., 1992: Studies on the *Tricholoma giganteum* from Jishou. Microbiology.

Mallikarjuna, S.E., Ranjini, A., Haware, D.J., Vijayalakshmi, M.R., Shashirekha, M.N., Rajarathnam, S., 2013: Mineral composition of four edible mushrooms. J. Chem. 2013, 1-5. DOI: 10.1155/2013/805284

Marcelo, V.A., 2011: Biophysiology of *Agrocybe aegerita*. Undergraduate thesis. Central Luzon State University, Science City of Munoz, Nueva Ecija.

Massee, G., 1912: Fungi exotici, XIV. Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew). 1912(6), 253-5.

Mata, G., Hernandez, D.M., Andreu, L.G., 2005: Changes in lignocellulolytic enzyme activities in six *Pleurotus* spp. Strains cultivated on coffee pulp in confrontation with *Trichodema* spp. World. J. Microbiol. Biotechnol. 21, 143-150. DOI: 10.1007/s11274-004-3041-3

Niazi, A.R., Ghafoor, A., 2021: Different ways to exploit mushrooms: A review. All Life. 14(1), 450-60. DOI: 10.1080/26895293.2021.1919570

Pal, J., Thupa, C.D., 1979: Cultivation of oyster (mushrooms) made easy. Ind. J. Mushroom. 5, 17-20.

Pamitha, N.S., 2014: Medicinal and nutraceutical potential of giant mushroom (*Macrocybe gigantea* (Massee) Peglar & Lodge). M.Sc. thesis, Department of Plant Biotechnology, College of Agriculture, Vellayani

Pegler, D.N., Lodge, D.L., Nakasone, K.K., 1998: The pantropical genus *Macrocybe* Mycologia. 90(3), 494-504. DOI: 10.1080/00275514.1998.12026934

Peksen, A., Yakupoglu, G., 2009: Tea waste as a supplement for the cultivation of *Ganoderma lucidum*. World. J. Microbiol. Biotechnol. 25(4), 611-8. DOI: 10.1007/s11274-008-9931-z

Razaq, A., Nawaz, R., Khalid, A.N., 2016: An Asian edible mushroom, *Macrocybe gigantea*: its distribution and ITS-rDNA based phylogeny. Mycosphere. 7(4), 525-530. DOI: 10.5943/mycosphere/7/4/11

Reyes, R., Eguchi, F., Iijima, T., Higaki, M., 1997: *Collybia reinakeana*, a wild edible mushroom from the forest of Puncan, Nueva Ecija, Philippines. Mushroom. Sci. Biotechnol. 15, 99-102. DOI: 10.24465/apmsb.5.2_99

Reyes, R.G, Eguchi, F., Iijima, T., Higaki, M., 1998: Physiological con-

siderations for efficient colonization of fukorotake *Volvariella volvacea*. J. Wood. Sci. 44, 408-413.

Reyes, R.G., Lopez, L.L., Kumakura, K., Kalaw, S.P., Kikukawa, T., Eguchi, F., 2009: *Coprinus comatus*, a newly domesticated wild nutraceutical mushroom in the Philippines. J. Agric. Technol. 5(2), 299-316.

Roy, D.R., Krishnappa, M., 2018: Influence of solid media on growth of mycelia and antibacterial activity of wild macrofungi, *Macrocybe gigantea*. Int. J. Pharm. Sci. Res. 9(10), 4349-4354.

Sardar. H., Ali, M.A., Ayyub, C.M., Ahmed, R., 2015: Effects of different culture media, temperature and pH levels on the growth of wild and exotic *Pleurotus* species. Pak. J. Phytopathol. 27(2), 139-45.

Shim, S.M., Oh, Y.H., Lee, K.R., Kim, S.H., Im, K.H., Kim, J.W., Lee, U.Y., Shim, J.O., Shim, M.J., Lee, M.W., Ro, H.S., 2005: The characteristics of cultural conditions for the mycelial growth of *Macrolepiota procera*. Mycobiology 33(1), 15-18.

Siddiq, M., Ali, M.A., Maqsood, M., 2018: Mycelial growth performance of various wild and exotic strains of oyster mushroom (*Pleurotus* spp.) On different growing media. J. Agric. Res. 56(3), 187-91.

Thawthong, A., Karunarathna, S.C., Thongklang, N., Chukeatirote, E., Kakumyan, P., Chamyuang, S., Hyde, K.D., 2014: Discovering and domesticating wild tropical cultivatable mushrooms. Chiang Mai J. Sci. 41(4), 731-764.

Vahidi, H., Kobarfard, F., Namjoyan, F., 2004: Effect of cultivation conditions on growth and antifungal activity of *Mycena leptocephala*. Afr. J. Biotechnol. 3(11), 606-609.

Vilariño, M., García-Sanmartín, J., Ochoa-Callejero, L., López-Rodríguez, A., Blanco-Urgoiti, J., Martínez, A., 2020: Macrocybin, a mushroom natural triglyceride, reduces tumor growth *in vitro* and *in vivo* through caveolin-mediated interference with the actin cytoskeleton. Molecules. 25(24), 6010. DOI: 10.3390/molecules25246010

Vizzini, A., Consiglio, G., Marchetti, M., Alvarado, P., 2020: Insights into the Tricholomatineae (Agaricales, Agaricomycetes): a new arrangement of Biannulariaceae and Callistosporium, Callistosporiaceae fam. nov., Xerophorus stat. nov., and Pleurocollybia incorporated into Callistosporium. Fungal Divers. 101(1), 211-259. DOI: 10.1007/s13225-020-00441-x

Wozniak, W., 2009: Production and quality appraisal of mycelium of parasol mushroom *Macrolepiota procera* (Scop. ex Fr.) Sing, Herba. Pol. 55(3), 285-291.

Yang, D., Liang, J., Wang, Y., Sun, F., Tao, H., Xu, Q., Wan, X., 2016: Tea waste: an effective and economic substrate for oyster mushroom cultivation. J. Sci. Food Agric. 96(2), 680-684. DOI: 10.1002/jsfa.7140

Zhu, L.G., Cheng, X.X., Zhang, W.J., 2013: Research progress on the reuse of solid wastes in tea industry and its application in environmental improvement. Fujian J. Agric. Sci. 28, 1310-1315.

Address of the corresponding author:
Abdul Rehman Niazi, Institute of Botany, University of the Punjab, Lahore, 54590, Pakistan
E-mail: drarniazi.botany@pu.edu.pk