Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Assessment of agronomic crops-based residues for growth and nutritional profile of *Pleurotus eryngii*

Avaliação de resíduos de cultivos agronômicos para crescimento e perfil nutricional de *Pleurotus eryngii*

M. A. Khatana[a]*, M. M. Jahangir[a], M. Amjad[a] and M. Shahid[b]

[a]University of Agriculture, Institute of Horticultural Sciences, Faisalabad, Pakistan
[b]University of Agriculture, Department of Chemistry and Biochemistry, Faisalabad, Pakistan

Abstract

Among edible mushrooms, *Pleurotus eryngii* is unique due to its flavor, admirable medicinal and nutritional profiling. Pakistan is an agricultural country diverse in various crops. However, the residues of the horticultural and agronomic crops are wasted without utilization in the food chain. Hence, a study was performed to assess the performance of relatively low-cost, easily available crops residues i.e. cotton, rice, wheat, mustard and water chestnut for yield and nutrition enhancement of *Pleurotus eryngii* strains P9 (China) and P10 (PSU-USA). The results revealed that morphological attributes i.e. mycelium run, fruit development, yield and biological efficiency were significantly higher by using cotton waste as compared to other substrates. Regarding biochemical attributes i.e. total soluble solids (12.67 °Brix), phenolics (259.6 mg/100g), moisture (92.3%) and ascorbic acid contents (2.9 mg/100ml) were also significantly higher by using cotton waste. Whereas, acidity (0.30%), reducing sugar (7.67%), non-reducing (4.33%) and total sugars contents (12%) were found highest by using mustard straw. Nutrient analysis of substrates showed that nutrient levels were increased after harvesting of crop as compared to before harvesting levels. Overall results revealed that cotton waste and mustard straw are promising substrates for *Pleurotus eryngii* better growth and have potential in yield and nutrition enhancement. Moreover, P10 strain performed better as compared to P9.

Keywords: agronomic, cotton waste, mycelium, mustard straw, residues.

Resumo

Entre os cogumelos comestíveis, *Pleurotus eryngii* é único por causa de seu sabor e seu admirável perfil medicinal e nutricional. O Paquistão é um país agrícola com diversas culturas. No entanto, os resíduos das culturas hortícolas e agronômicas são desperdiçados sem aproveitamento na cadeia alimentar. Assim, um estudo foi realizado para avaliar o desempenho de resíduos de culturas com custos relativamente baixos e facilmente disponíveis, ou seja, algodão, arroz, trigo, mostarda e castanha-de-água, para o aumento da produtividade e nutrição de cepas de *P. eryngii* P9 (China) e P10 (PSU-EUA). Os resultados revelaram que os atributos morfológicos, ou seja, função do micélio, desenvolvimento de frutos, rendimento e eficiência biológica, foram significativamente maiores usando resíduos de algodão em comparação com outros substratos. Em relação aos atributos bioquímicos, ou seja, sólidos solúveis totais (12,67 °Brix), fenólicos (259,6 mg / 100 g), umidade (92,3%) e teores de ácido ascórbico (2,9 mg / 100 ml), também foram significativamente maiores usando resíduos de algodão. Já os teores de acidez (0,30%), açúcares redutores (7,67%), não redutores (4,33%) e açúcares totais (12%) foram os mais elevados na palha de mostarda. A análise de nutrientes dos substratos mostrou que os níveis de nutrientes aumentaram após a colheita da cultura em comparação com os níveis antes da colheita. Os resultados gerais revelaram que os resíduos de algodão e a palha de mostarda são substratos promissores para o melhor crescimento de *P. eryngii* e têm potencial na melhoria da produtividade e nutrição. Além disso, a cepa P10 apresentou melhor desempenho em comparação com a P9.

Palavras-chave: agronômico, resíduos de algodão, micélio, palha de mostarda, resíduos.

1. Introduction

Mushrooms (genus *Pleurotus*) are gaining the world's attention due to their nutritional and medicinal importance in human life. It is being consumed on large scale due to its good flavor, taste and essential bioactive components. Unsaturated acidic fats, proteins and 9 vital amino acids are an integral part of mushrooms that have major role in our metabolism (Ergonul et al. 2013). The mushroom *Pleurotus eryngii* is a member of *Agaricaceae* family having gray to grayish brown pileus (3-8 cm), thick whitish stipe (0.9-4cm) and umbrella like fleshly body (3.6-15 cm). Its elevated

*e-mail: ahsansiddique2319@gmail.com
Received: March 5, 2022 – Accepted: April 5, 2022

biological efficiency and ecological importance make it a high-ranked mushroom. Around the globe, it is consumed as a food and for medicinal usage due to enrichment of metabolites, dietary fiber and minerals like sodium, iron, calcium, copper and potassium which makes it a great health supplement (Rodriguez-Estrada et al., 2009).

Pleurotus eryngii is a commercial cultivated mushroom in Asia and Europe with its wide adaptation under different climatic conditions of China, Japan and Taiwan etc. (Korea, 2012). Globally it is found in natural forests and hardwoods while cottonwood, poplar, birch, conifers, elm trees, maple wood and willow are major substrates of *Pleurotus eryngii*, it has facultative behavior as it grows wild on trees, which are about to die or on deciduous woods (Shah et al., 2004; Bing et al., 2010). In 2011, Korea exported 3900 tons of *Pleurotus eryngii* to regions of Europe and North America out of total produce i.e. 54820 tons and its share was 33% in Koreas' market (Korea, 2012).

Mushrooms rely on substrates for their nutritional requirements as substrates contain lignin, the cellulose that lysis gave food and nutrition to mushroom and promotes its mycelium run and fruit development (Chang and Miles, 2004). Different species gave a dissimilar response on variant substrates regarding mycelium run, fruit development and yield (Biley et al., 2000). Lignin and cellulose-containing crops residuals were utilized for cultivation of *Pleurotus eryngii* like rice, soybean, millets and wheat hay, cotton waste, shells of peanut, wheat bran, grass clippings, sugarcane bagasse, corn cobs and seed hulls of cotton etc. (Jonathan et al., 2008). Humans are not being able to consume crops-based residues which are produced in great amount. Therefore, they may be utilized for mushroom production as these residues provide nutrition to mushrooms for its life cycle which has high market demand. Suitable and cheap substrate selection for *Pleurotus eryngii* production is important for its better development and yield. Mushrooms production on numerous substrates gives different protein contents (Gothwal et al., 2012).

Numerous crops are cultivated on a larger area of Pakistan including cotton, wheat and rice etc. Cultivated area under cotton, wheat and rice was 2373 thousand hectares, 8740 thousand hectares and 2810 thousand hectares, respectively (Pakistan, 2019). Major crops in Punjab, Pakistan includes wheat, rice, cotton, vegetables etc. whose waste material can be used as substrates while, abundant fruit peel is produced in KPK, Kashmir, Gilgit Baltistan and Balochistan and numerous agronomic and horticultural crops are cultivated on large scale in Sindh. Due to this vast range and easy availability of agricultural waste materials in Pakistan these can be utilized as substrates for *Pleurotus eryngii* cultivation. Most people used these waste materials as fodder for animals, to burn for cooking purpose, used in bricks kilns and many crop residues are burnt in field that pollute the environment and disturb the climate.

By considering the nutritional and medicinal importance of *Pleurotus eryngii*, a study was designed to evaluate vast range of available agricultural residues considering their low cost, ease of availability and environment friendly approaches. Moreover, the performance of these substrates was assessed based on growth, nutrition and yield improvement of *Pleurotus eryngii* strains (P9 and P10). This study will also be helpful for production technology development for mushroom growers.

2. Materials and Methods

The study was conducted at Medicinal and Mushroom Lab, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. An experiment was performed to check the morphological and biochemical attributes of king oyster mushroom (*Pleurotus eryngii)* strains i.e. P9 (China) and P10 (PSU-USA) by utilizing substrates alone and their combinations (1:1) with each other i.e. (as shown in Table 1).

2.1. Strains and substrates obtention

Spawn of two strains P9 (China) and P10 (PSU-USA) of *Pleurotus eryngii* were purchased from Medicinal and Mushroom Laboratory, University of Agriculture, Faisalabad. While, agricultural waste materials were collected from agronomy farm of University of Agriculture, Faisalabad and Ayyub Agriculture Research Institute, Faisalabad, Pakistan.

2.2. Media preparation

For media preparation firstly material was soaked in water thoroughly and a heap was formed on floor. During heap formation lime was added at the rate of four percent of weight of substrate. Subsequently that when excessive water leached out from heap, it was covered with polythene sheet and remained closed for ten days for composting process accomplishment. After process of composting polypropylene bags (20-30 cm) were filled with media and tied up loosely with rubber bands. After that pasteurization of bags was accomplished at 50-60°C for 2 to 3 hours and bags were kept for one night to cool down. Next day, spawning was achieved at the rate of 1.5% of wet weight of media and bags were placed in growing room for mycelium run and cropping.

2.3. Environmental conditions maintenance

Temperature and humidity requirements for *Pleurotus eryngii* growth varies at different growing stages therefore,

Table 1. Crops-residues and their combinations with each other used as substrates.

Treatments	Treatments
T_0: Wheat straw (W) (100%)	T_8: W + WCP (50%: 50%)
T_1: Cotton waste (CW)	T_9: CW + RS (50%: 50%)
T_2: Rice straw (RS) (100%)	T_{10}: CW + MS (50%: 50%)
T_3: Mustard straw (MS) (100%)	T_{11}: CW + WCP (50%: 50%)
T_4: Water chestnut peel (WCP) (100%)	T_{12}: RS + MS (50%: 50%)
T_5: W + CW (50%: 50%)	T_{13}: RS + WCP (50%: 50%)
T_6: W + RS (50%: 50%)	T_{14}: MS+ WCP (50%: 50%)
T_7: W + MS (50%: 50%)	

temperature of growth room was sustained at 24-25 °C, 10-15 °C and 15-21 °C while humidity was maintained at 90-95%, 95-100% and 85-90% for spawn run initiation, pinheads development and fructification respectively. During pinhead's development and fructification light requirement was accomplished at 500-1000 Lux/day and CO_2 level was maintained 5000-20000 ppm, 500-1000 ppm and ≤ 2000 ppm, however ventilation was performed 1 time, 4-8 times and 4-5 times for spawn run initiation, pinhead's development and fructification respectively (Stamets, 2000).

2.4. Morphological attributes

Numerous morphological attributes were studied which include spawn run initiation duration (days), mycelium run completion duration (days), pinhead initiation duration (days), number of pinheads, 1st and 2nd flush completion duration (days), number of flushes, mushroom stipe length and pileus diameter (cm), weight of 1st and 2nd flush (g), yield (g) and biological efficiency (%) BE = (weight of harvest / weight of dry substrate) x 100%.

2.5. Biochemical attributes (TSS, acidity, sugars, phenolics, moisture and ascorbic acid)

Total soluble solids from mushroom were measured by using hand-held refractometer (RX 5000-Atago Japan) at 25 ± 2 °C (room temperature) and value expressed in °Brix. Acidity (%), reducing, non-reducing and total sugars (%) were calculated by adopted titration method (Hortwitz, 1960) while ascorbic acid by employing titration method (Ruck, 1963). Moreover, moisture content (%) of mushroom was measured by adopting standard method (AOAC, 1990). While total phenolics contents were measured by employing Folin-Ciocalteu reagent and TPC were recorded by using spectrophotometer (UV-1800, Shimadzu, Japanese and European Pharmacopoeia) at 750 nm and were expressed as mg of standard gallic acid (GAE) equivalents per 100g of extract (Sanchez-Rangel et al., 2013).

2.6. Substrates pH and nutrients quantification

Substrates mineral contents i.e. Nitrogen, phosphorus and potassium before and after attaining crop were estimated by using Kjeldahl, spectrophotometer (UV-1800, Shimadzu, Japanese and European Pharmacopoeia) and flame photometer (FP-902, PG Instruments Limited, United kingdom) respectively (Chapman and Parker, 1961; Yoshida et al. 1976). Moreover, digital pH meter (HI 98107, Hanna instruments, Mauritius) was used to determine pH of substrates.

2.7. Statistical analysis

Experiment was performed by adopting two factors factorial under a completely randomized design and treatments were replicated five times. Moreover, data analysis was performed by using Tukey HSD test (Steel et al., 1997).

3. Results

3.1. Spawn run commencement, mycelium completion, pinhead initiation duration (days) and number of pinheads/bag:

Minimum duration (2.3 days) for spawn run initiation was observed for T1 while maximum duration (11.7 days) was observed for T13. In strains comparison minimum and maximum duration (2.3 days and 11.3 days, respectively) for mycelium run commencement was observed for P10 while minimum and maximum duration (2.7 days and 11.7 days, respectively) was observed for P9. Regarding mycelium run accomplishment, minimum duration (37.3 days) was observed for T1 while maximum (57.7 days) was observed for T13. However, minimum duration (37.3 days and 38.3 days) for mycelium run accomplishment was observed in P10 and P9 strain respectively.

Moreover, among various treatments minimum pinheads initiation duration (43 days) was observed for T1 while maximum (65.3 days) was observed for T13. Results regarding strains comparison revealed that minimum and maximum duration (43 days and 64.3 days, respectively) for pinheads initiation was noted for P10 while minimum and maximum duration (44 days and 65.3 days, respectively) was noted for P9. Whereas, among various treatments maximum pinheads (46.7) were observed for T1 while minimum pinheads (15) were observed for T13. Regarding strains comparison, minimum and maximum pinheads (16.7 and 46.7, respectively) were noted for P10 while minimum and maximum pinheads (15 and 45.3, respectively) were noted for P9 (see Figure 1).

3.2. Duration (days) for first and second flush accomplishment and no. of flushes/bag:

Between several treatments, the minimum duration (48.7 days) for 1st flush accomplishment was observed for T1 while highest (71 days) was observed for T13. Results regarding strains contrast revealed that minimum and maximum duration (48.7 days and 70 days, respectively) was noted for P10 while minimum and maximum duration (49.3 days and 71 days, respectively) was noted for P9. While, minimum duration (56.7 days) for second flush completion was observed for T1 while highest (89 days) was observed for T13. Result regarding strains comparison revealed that minimum and maximum duration (56.7 days and 78.7 days, respectively) was noted for P10 while minimum and maximum duration (57.3 days and 79 days, respectively) was noted for P9. Moreover, minimum number of flushes (1.3) was observed for T13 and maximum (6) was observed for T1. Result regarding strains effect on number of flushes revealed that minimum and the maximum number of pinheads observed for P10 (1.7 and 6, respectively) while for P9 (1.3 and 5.7, respectively) was observed. Overall results revealed that P10 strain performed better than P9 (see Figure 2).

3.3. Pileus diameter and stipe length (cm)

Maximum diameter (7.5 cm) was noted for T1 while the least diameter (3.2 cm) was noted for T13. Results regarding strains comparison revealed that P10 strain gave

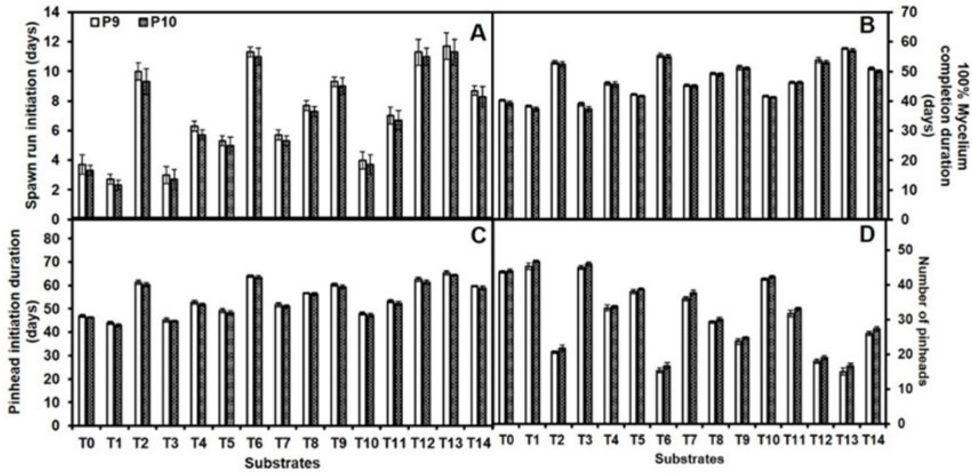

Figure 1. Effect of numerous substrates on (A) spawn run initiation (days), (B) mycelium completion duration (days), (C) pinheads initiation (days) and (D) number of pinheads/bag. Vertical bars represents ± S.E. of means and bars are invisible where values are smaller than the symbol. Tukey HSD ($P \leq 0.05$) for A: Treatment (T):1.982, Variety (V): 0.409 and T × V: NS (Non significant); B: Treatment (T):2.051, Variety (V): 0.424 and T × V: NS (Non significant); C: Treatment (T):2.580, Variety (V): 0.533 and T × V: NS (Non significant); D: Treatment (T):2.214, Variety (V): 0.456 and T × V: NS (Non significant).

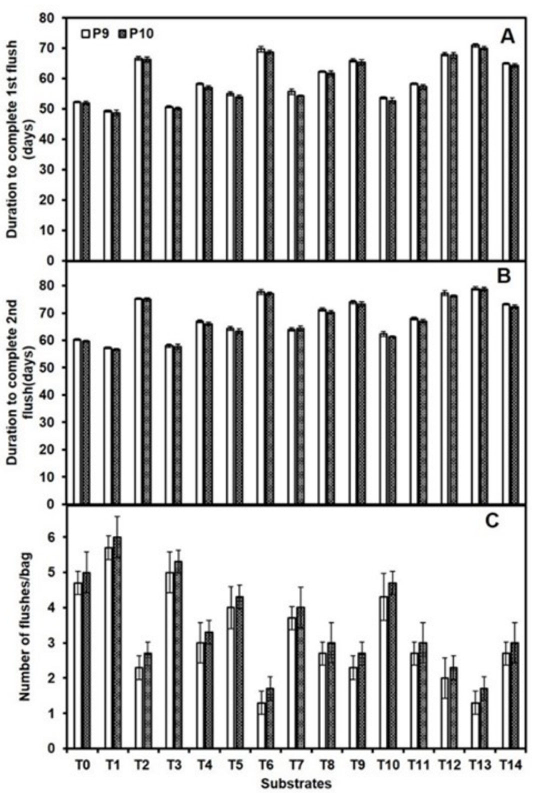

Figure 2. Effect of numerous substrates on (A) duration to complete 1st flush (days), (B) duration to complete 2nd flush (days) and (C) number of flushes/bag. Vertical bars represents ± S.E. of means and bars are invisible where values are smaller than the symbol. Tukey HSD ($P \leq 0.05$) for A: Treatment (T):2.235, Variety (V): 0.462 and T × V: NS (Non significant); B: Treatment (T):2.224, Variety (V): 0.4596 and T × V: NS (Non significant); C: Treatment (T):1.565, Variety (V): 0.323 and T × V: NS (Non significant).

better results regarding pileus diameter than P9 strain as the minimum and maximum diameter (3.4 cm and 7.5 cm respectively) was noted for P10, compared to P9 having minimum (3.2 cm) and maximum (7.3 cm) diameter. Moreover, maximum stipe length (8.4 cm) was noted for T1 while least length (3.8 cm) was noted for T13. Results regarding strains comparison revealed that minimum and maximum length (4.2 cm and 8.4 cm respectively) was observed for P10 while minimum and maximum length (3.8 cm and 8.3 cm respectively) was observed for P9 (see Figure 3).

3.4. First and second flush weight (g), Yield (g) and Biological efficiency (%)

Maximum first flush weight (141.7 g) was observed for T1 while minimum (70.3 g) was observed for T13. Minimum and maximum weight (72 g and 141.7 g correspondingly) was noted in P10 strain while minimum and maximum weight (70.3 g and 140.3 g respectively) was observed for P9 strain. While, maximum 2nd flush weight (98 g) was observed for T1 while minimum (48 g) was observed for T13. Minimum and maximum weight (48.7 g and 98 g respectively) was observed in P10 strain while minimum and maximum weight (48 g and 97.3 g respectively) was observed for P9 strain. Whereas, maximum yield (239.7 g) was observed for T1 while minimum yield (118.3 g) was observed for T13. Results regarding strains comparison revealed that minimum and maximum yield (120.7 g and 239.7 g correspondingly) was for P10 while minimum and maximum yield (118.3 g and 237.7 g correspondingly) was for P9. Additionally, maximum biological efficiency (68.5%) was observed for T1 while minimum (33.8%) was observed for T13. Results regarding strains comparison revealed that P10 strain gave better results regarding biological efficiency than P9 strain (see Figure 4).

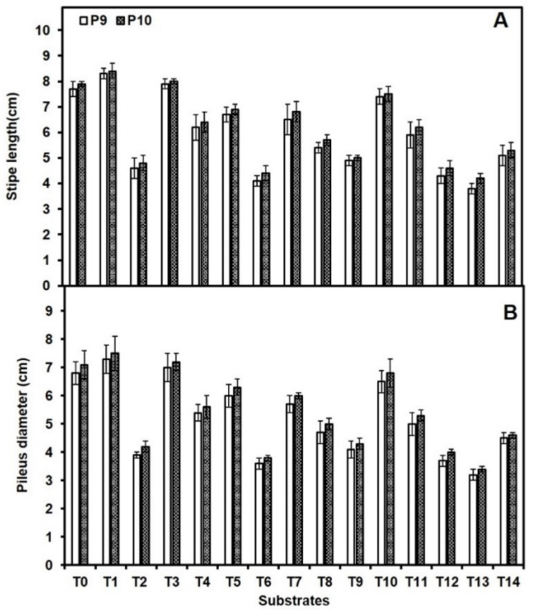

Figure 3. Effect of numerous substrates on (A) mushroom stipe length (cm) and (B) diameter of pileus (cm). Vertical bars represents ± S.E. of means and bars are invisible where values are smaller than the symbol. Tukey HSD (P ≤ 0.05) for A: Treatment (T):1.104, Variety (V): NS (Non significant) and T × V: NS (Non significant); B: Treatment (T):1.131, Variety (V): 0.233 and T × V: NS (Non significant).

3.5. Total phenolics (mg/100g), acidity (%), ascorbic acid (mg/100ml) and moisture (%) contents of mushroom:

The highest phenolics contents (259.6 mg/100g) were observed for T1 and minimum (191.9 mg/100g) was observed for T13. Results related to strains showed that the highest phenolics contents (259.6 mg/100g and 259.4 mg/100g) were observed for P10 and P9, respectively. Moreover, maximum acidity (0.30%) was observed for T3 and minimum (0.10%) was observed for T13. Maximum and minimum acidity observed in P10 strain was (0.30% and 0.10% respectively) while maximum and minimum acidity observed in P9 strain was (0.29% and 0.10% respectively). While, highest ascorbic acid contents (2.9 mg/100ml) was observed for T1 and minimum (0.7 mg/100ml) was observed for T13. Maximum ascorbic acid contents observed in P10 strain was (2.9 mg/100ml) while in P9 strain was (2.8 mg/100ml). Minimum value of ascorbic acid contents observed in P10 strain was (0.8 mg/100ml) and in P9 strain was (0.7 mg/100ml). Furthermore, maximum (92.3%) moisture level was observed for T1 and the least (60%) was observed for T13. Result related to strains comparison showed that maximum moisture percentage observed in P10 strain was (92.3%) while minimum (61%) was observed while maximum moisture percentage observed in P9 strain was (91.3%) whereas minimum (60%) was observed (see Figure 5).

3.6. TSS (°Brix) and sugars contents (%)

Maximum (12.67 °Brix) TSS was observed for T1 and minimum (4.33 °Brix) was observed for T13. Highest TSS

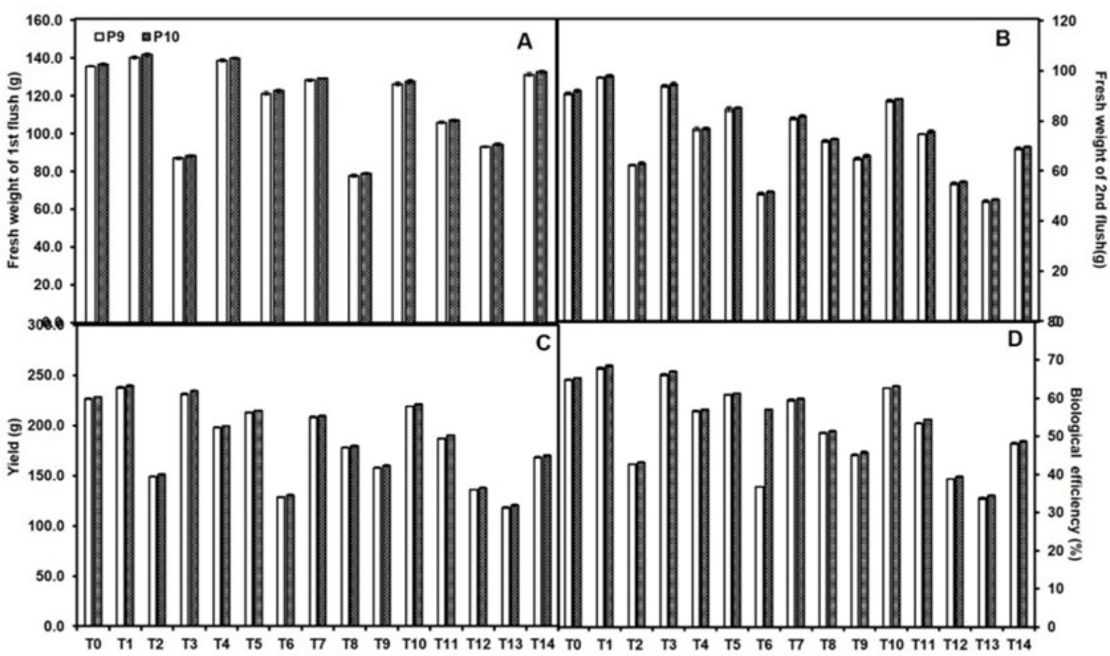

Figure 4. Effect of numerous substrates on (A) 1st flush weight (g) and (B) 2nd flush weight (g), (C) Yield (g) and (D) Biological efficiency (%). Vertical bars represents ± S.E. of means and bars are invisible where values are smaller than the symbol. Tukey HSD (P ≤ 0.05) for A: Treatment (T):2.694, Variety (V): 0.557 and T × V: NS (Non significant); B: Treatment (T):1.923, Variety (V): 0.397 and T × V: NS (Non significant); C: Treatment (T):2.286, Variety (V): 0.472 and T × V: NS (Non significant); D: Treatment (T):0.656, Variety (V): 0.135 and T × V: NS (Non significant).

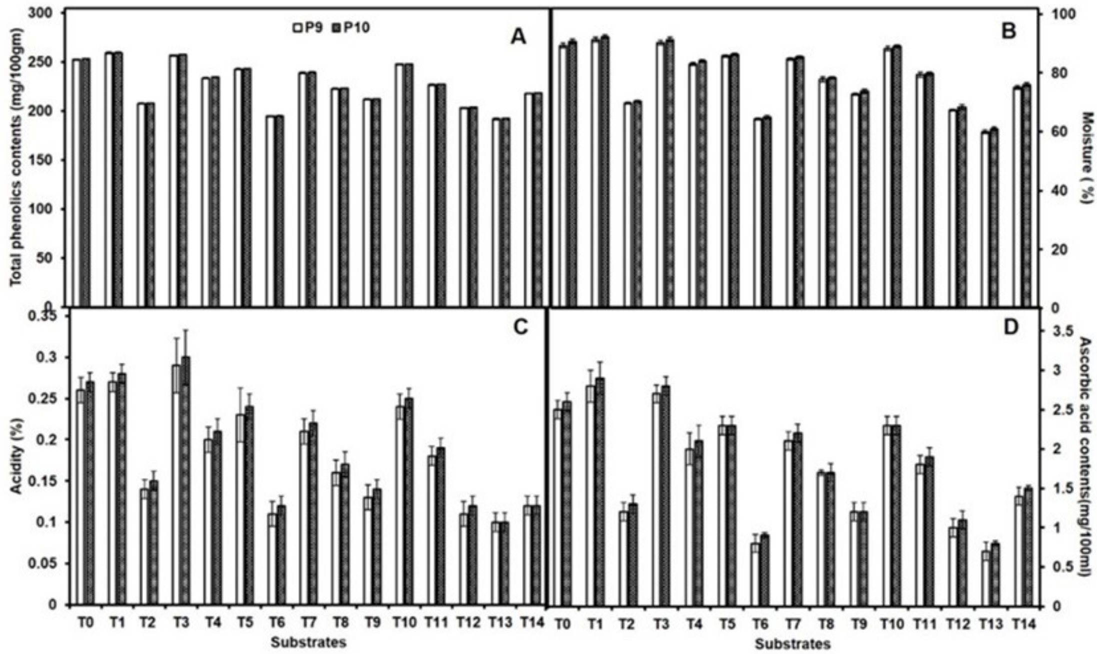

Figure 5. Effect of numerous substrates on (A) total phenolics (mg/100g), (B) acidity (%), (C) moisture (%) and (D) ascorbic acid (mg/100ml) contents. Vertical bars represents ± S.E. of means and bars are invisible where values are smaller than the symbol. Tukey HSD ($P \leq 0.05$) for A: Treatment (T):1.930, Variety (V): 0.398 and T × V: NS (Non significant); B: Treatment (T):0.058, Variety (V): NS (Non significant) and T × V: NS (Non significant); C: Treatment (T):2.245, Variety (V): 0.464 and T × V: NS (Non significant); D: Treatment (T):0.340, Variety (V): NS (Non significant) and T × V: NS (Non significant).

observed for P10 strain was (12.67 °Brix) while lowest was (5 °Brix) whereas, highest TSS was observed for P9 strain was (12 °Brix) whereas lowest was (4.33 °Brix). Furthermore, among various treatments maximum (7.67%) reducing sugars contents were found for T1 while minimum (2%) were found for T13. Result related to strains' affect revealed that least and highest reducing sugars contents for P10 strain were (7.67% and 2.33% respectively) while minimum and maximum reducing sugars contents were for P9 strain were (7.33% and 2%). Whereas, among different treatments highest (4.33%) non-reducing sugars contents were found for T1 while minimum (2.77%) were found for T13. Result related to strains comparison unveiled that minimum and maximum non-reducing sugars contents for P10 strain were (2.83% and 4.33% respectively) while for P9 strain were (2.77% and 4.23%). Whereas, among different treatments maximum (12%) total sugars contents was found for T1 while minimum (4.77%) was found for T13. Result related to strains' affect unveiled that lowest and highest sugar contents for P10 strain were (5.17% and 12% respectively) while minimum and maximum sugar contents for P9 strain were (4.77% and 11.57%) (see Figure 6).

3.7. pH, NPK (%) contents of substrates formerly to cropping

Among these substrates maximum (8.7) pH was observed for T12 while minimum (7.6) pH was observed for T11. Maximum nitrogen contents (9.8%) were observed for T8 while, a minimum (4.2%) were observed for T0. Whereas, maximum (0.17%) phosphorus contents were observed for T10 while minimum (0.02%) was observed for T0. Moreover, maximum (0.41%) potassium % was observed for T2 and minimum (0.18%) was observed for T0 and T4 (see Figure 7).

3.8. NPK(%) contents of substrates after cropping

Maximum (10.3%) nitrogen contents were observed for T8 while minimum (4.7%) was observed for T0. While, maximum (0.18%) phosphorus contents were observed for T10 while minimum (0.03%) was observed for T8 while, maximum (0.43%) potassium % was observed for T2 whereas least (0.18%) was detected for T8 (see Figure 8).

4. Discussion

Numerous factors viz. growing conditions, humidity level, temperature, availability of nutrients, oxygen, carbon dioxide and growing media (substrates) have a great impact on mycelium run of various strains of *Pleurotus eryngii* mushroom (Hassan et al. 2010). Moisture plays a vital role in the growth of *Pleurotus eryngii*, if the moisture percentage of substrates ranges from 70-75% it grows well (Akyuz and Yildiz, 2008). Likewise, various other factors like growing room environmental conditions, temperature, humidity, substrate physiology, micro and macro elements of substrates and quality of spawn have an impact on pinheads' formation. Due to

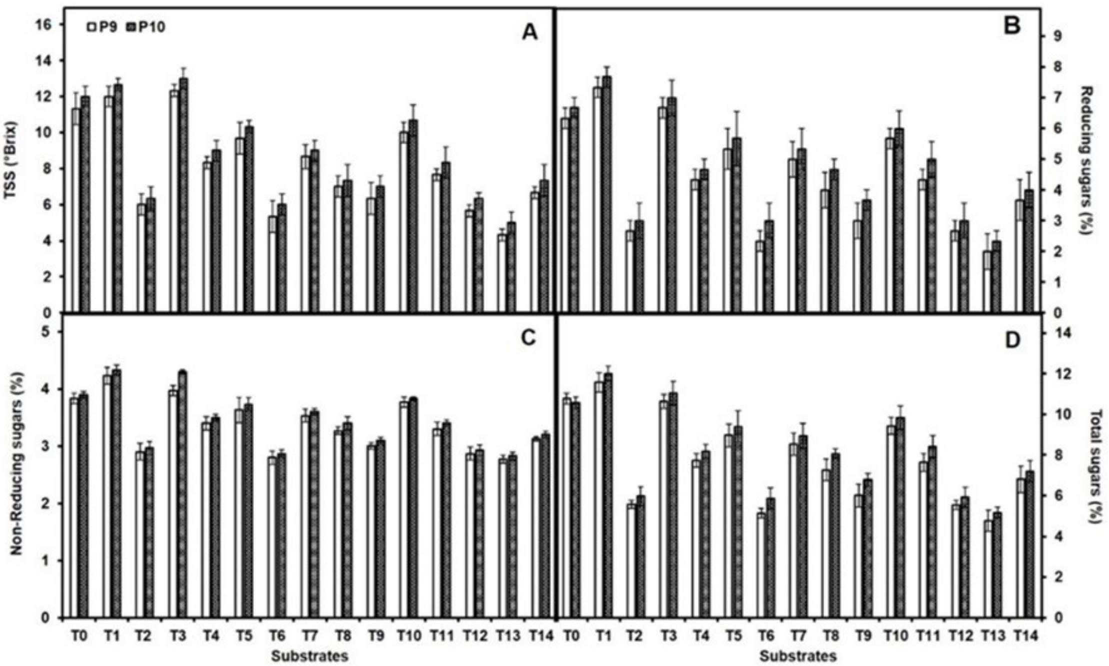

Figure 6. Effect of numerous substrates on (A) TSS (°Brix), Reducing sugars (%), (C) Non-Reducing sugars (%) and (D) Total sugars (%). Vertical bars represents ± S.E. of means and bars are invisible where values are smaller than the symbol. Tukey HSD ($P \leq 0.05$) for A: Treatment (T):2.214, Variety (V): 0.458 and T × V: NS (Non significant); B: Treatment (T):1.759, Variety (V): 0.364 and T × V: NS (Non significant); C: Treatment (T):0.345, Variety (V): 0.071 and T × V: NS (Non significant); D: Treatment (T):1.639, Variety (V): 0.339 and T × V: NS (Non significant).

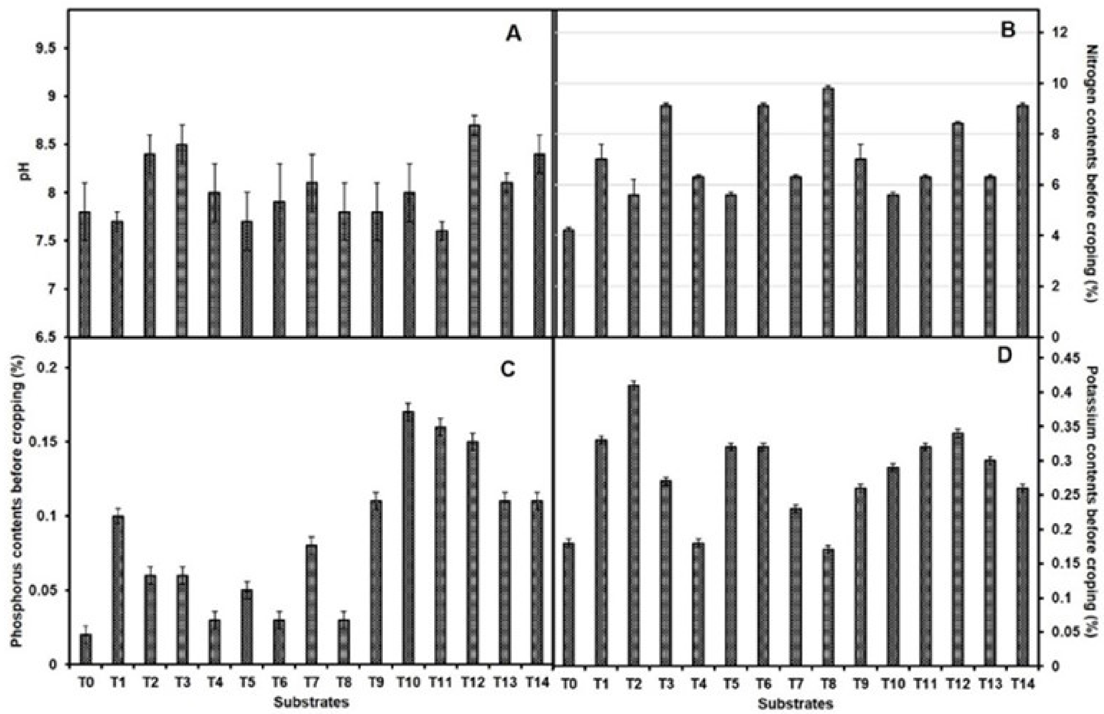

Figure 7. (A) pH, (B) Nitrogen, (C) Phosphorus, (D) Potassium (%) contents of numerous substrates formerly to cropping. Vertical bars represents ± S.E. of means and bars are invisible where values are smaller than the symbol. Tukey HSD ($P \leq 0.05$) for A: Treatment (T): NS (Non-significant); B: Treatment (T):1.372; C: Treatment (T): 0.063; D: Treatment (T):0.301.

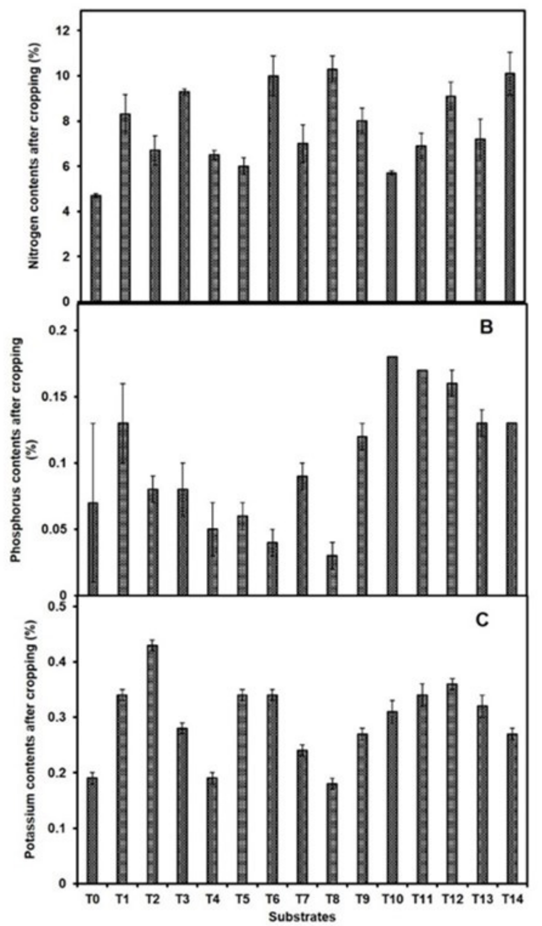

Figure 8. (A) Nitrogen, (B) Phosphorus, (C) Potassium (%) contents of numerous substrates after cropping. Vertical bars represents ± S.E. of means and bars are invisible where values are smaller than the symbol. Tukey HSD ($P \leq 0.05$) for A: Treatment (T): 3.272; B: Treatment (T):0.097; C: Treatment (T):0.058.

the temperature and humidity fluctuations number of pinheads was suppressed. The optimum temperature for pinhead formation is 16-22 °C and direct application of water during pinhead formation has negative impact on pinheads development (Singh et al., 2011).

Substrate structure is an important factor that influenced the mycelium run on growing substrate and fruiting of mushroom (Tripathy, 2010). Moreover, *Pleurotus eryngii* yield and pinheads formation was influenced by lignocellulosic material containing substrates and supplements which cause enhancement in yield by decomposing numerous components of substrates (Hassan et al., 2010; Jafarpour and Eghbalsaeed, 2012; Kazemi et al., 2016; Tripathy, 2010; Zadrazil et al., 1996). The findings of our experiment depicted that king oyster mushroom growth pattern varied for different substrates i.e. mycelium run, and pinhead formation were better on cotton waste, mustard straw, wheat straw and their combinations with each other as compared to water chestnut, rice straw and their combinations with other substrates (see Figure 1). The density of mycelium has impact on fruit body development duration (Ohga, 2000). Moisture retaining capacity of wheat straw is low that cause a reduction in fruit body development (Wang, 2010; Yang et al., 2013), these findings are similar to our results which showed that cotton waste and mustard straw give better pinheads formation as compared to wheat straw (see Figure 1).

Flushes development and their numbers are dependent on growing conditions like humidity, temperature, substrates properties and quality spawn, when these conditions are optimum more number of flushes can be obtained. Flushes yield can also be enhanced by removing dead pinheads from surface of growing bags (Stamets and Chilton, 1983). Minimum duration for flushes development and maximum number of flushes was observed for cotton waste as compared to other substrates which showed that substrates structure, moisture holding capacity and other optimum conditions etc. has a prominent impact (see Figure 2). Different substrates affect the stipe length of different *Pleurotus* species. Mushrooms like oyster and button mushroom if it bears long stipe it is consider as a poor quality mushroom. Moreover, in case of *Pleurotus eryngii* lengthy stipe is a desirable character. Substrates nutritional imbalance causes irregularity in stipe length (Samuel and Eugene, 2012; Zadrazil, 1978; Oei, 1996). Pileus diameter of variant species of *Pleurotus* lies between ranges 2.89-5.59 cm, due to substrates characteristics. Substrates firmness, structure and physiological properties have impact on pileus diameter. Moreover, substrates which have good moisture retaining capability produced mushroom with big pileus (Mondal et al., 2010; Chukwurah et al., 2013).

Physio-chemical properties of substrates affect the yield of mushroom. King oyster yield can be enhanced by chemical augmentation of substrates or combination of various substrates. Furthermore, numerous lignin and cellulose containing substrates cause fluctuation in biological efficiency and yield (Rodriguez-Estrada and Royse, 2007; Liang et al., 2009). Moreover, yield and biological efficiency are positively co-related with nutrients composition of substrates and 50-80% biological efficacy range of *Pleurotus eryngii* was observed (Peksen and Yakupoglu, 2009; Rodriguez-Estrada, 2008). Biological efficiency is affected by spawn quality, strain and substrate and supplement interaction (Mandeel et al., 2005). Our results also showed similar response i.e. yield and biological efficiency of king oyster mushroom was good for cotton waste as compared to other substrates (see Figure 4).

Biological efficiency 85.2% and 25.6/100g (wet media) yield was calculated when wheat hay and millet hay combination was used with supplementation of 10% rice bran whereas when wheat straw was used alone it gave 48% biological efficiency and 14.4 g/100g (wet media) yield. Furthermore, when soybean is supplemented with extra 6% ground soybean it causes up to 35% increment in the yield. *Pleurotus eryngii* yield was observed more in sawdust as compared to rice straw (Kirbag and Akyuz, 2008; Rodriguez-Estrada and Royse, 2007; Moonmoon et al., 2010). Our findings also depicted that maximum biological efficiency was observed for cotton waste and minimum was for rice and water chest nut combination (see Figure 4).

Moisture is a quality parameter of fruits, vegetables and mushrooms and loss of moisture in these commodities causes a reduction in turgidity, weight and firmness (Hatfield and Knee, 1988; Van den Berg, 1981). Moisture contents of mushrooms are high and vary among different strains and are affected by growing conditions, harvesting and storage circumstances. Among *Pleurotus* species *Pleurotus eryngii* have more export potential due to low moisture contents and good shelf life (Kang et al., 2000).

Ascorbic acid through signals of hormones plays part in plant development regulation and antioxidation (Pastori et al., 2003). Moreover, it also plays role in inhibition of reactive oxygen species during process of respiration, worked as cofactor in numerous enzymes and has a role in cell growth (Lee and Kader, 2000). Total soluble solids (TSS) are a vital quality attribute of texture and structure of numerous fruits and include solid substances viz. sugars, minerals and vitamins etc. (Peck et al., 2006).

Glucose, sucrose and sorbitol collectively called as total sugars have an important role in fruit quality, sweetness and flavor. Sucrose has a vital role in regulation of osmotic pressure, metabolism pathway and antioxidation system activation for elimination of free radicals (Nishizawa et al., 2008). Moreover, reducing sugars comprise of glucose polymers, oligosaccharides, starch, mono-saccharides and starches which have reducing ends (Campbell and Farrell, 2012).

Among different substrates growing period is extended in some substrates which is due to the composition of substrates. Some substrates have such compounds which are harmful for metabolic processes however; some varieties have resistance against such compounds. Due to these compounds some chemical changes occur in substrates which alter the pH and nutrient composition leading to toxicity (McGary et al., 2013).

Nitrogen-containing basal media enhanced yield and biological efficiency (Fanadzo et al., 2010). Moreover, during the early stage of mycelium growth of *Pleurotus* species nitrogen requirement is less as compared to carbon requirement. Furthermore, ammonium and nitrates have toxic effect on pinhead formation which causes reduction in yield (Mandeel et al., 2005). Nitrogen is an integral part of purines, vitamins, polysaccharides and pyrimidines which utilized for the formation of chitin to provide support to cell wall of mushroom (Miles and Chang, 2004). Nitrogen has known effect on yield, biological efficacy, mycelium run, pinheads' formation and fruit body development (Shashirekha et al., 2002).

Phosphorus plays a noteworthy part in yield and development improvement of mushroom (David and Muthersbaugh, 1996). During numerous enzymatic activities and biological processes phosphorus act as co-factor in mushroom cells while in plants it acts as a mobile element (Khan et al., 2006). Potassium acts a key character in mobility of enzymes, synthesis and stability of ions, carbohydrates maintenance (Griffin, 1994). Plants and mushrooms have shown similar behavior of potassium uptake (Urban and Bystrzejewska-Piotrowska, 2003). Because of production of acetic acid, oxalic acid and pyruvic acid pH value of substrate is fluctuates throughout the growing period (Leu, 1992).

5. Conclusion

The current study revealed that numerous crops-based residues have positive impact on growth and nutritional enhancement of *Pleurotus eryngii* mushroom strains. Results related to growth and yield revealed that Cotton waste performed best while least yield and growth was observed for combination of Rice Straw and Water Chestnut Peal. Biochemical results stated that cotton waste performed better while rice straw and water chestnut peel combination performed poor. Other substrates which gave better results were mustard straw, wheat straw and combination of Cotton waste with mustard, cotton with wheat and wheat with mustard. Moreover, results regarding substrates unveiled that N, P and K percentage was increased in spent mushroom compost as compared to substrates prior to cropping. pH has variant values among all substrates. Overall results showed that cotton waste and mustard straw are promising substrates for *Pleurotus eryngii* cultivation and P10 strain is more nutritious and gave better growth as compared to P9 strain. Abundant amount of wastes of fruit, vegetables and agronomic crops are produced which yet have not been studied for mushroom production. Among these we have examined few substrates for our study. That's why in future other waste materials should be examined for production of mushroom so that farmers have a vast range of suitable substrates.

Acknowledgements

The authors acknowledge the financial support of the Office of Research, Innovation and Commercialization (ORIC), University of Agriculture, Faisalabad, Pakistan for experimental studies.

References

AKYUZ, M. and YILDIZ, A., 2008. Evaluation of cellulosic wastes for the cultivation of *Pleurotus eryngii* (DC. ex Fr.) Quel. *African Journal of Biotechnology*, vol. 7, no. 1, pp. 1494-1499.

ASSOCIATION OF OFFICIAL CHEMISTS – AOAC, 1990. *Official methods of analysis of the Association of Official Analytical Chemists*. 15th ed. Arlington: AOAC.

BILEY, V.T., SOLOMKO, E.F. and BUCHALO, A.S., 2000. Growth of edible and medicinal mushrooms on commercial agar. In: L.J.L.D. VAN GRIENSVEN, ed. *Science and cultivation of edible fungi. Proceedings of the 15th International Congress on the Science and Cultivation of Edible Fungi*, 15-19 May 2000, Maastricht, Netherlands. Balkema Rotterdam, Netherland: A. A. Balkema, pp. 779-782.

BING, C.X., ZHONG, W.D., BIN, Z.M. and PING, G.X., 2010. Effects of different external environment on the mycelium growth of *Pleurotus eryngii. Anhui Nongye Daxue Xuebao*, vol. 37, no. 1, pp. 150-153.

CAMPBELL, M.K. and FARRELL, S.O., 2012. *Biochemistry*. USA: Marry Finch, pp. 459.

CHANG, S.T. and MILES, P.G. 2004. *Mushrooms cultivation nutritional value, medicinal effect and environmental impact*. Baikema, Netherland: CRC Press Rotterdam.

CHAPMAN, D.H. and PARKER, E.R., 1961. *Determination of NPK methods of analysis for soil, plant and waters*. USA: Pub Division Agriculture University California, pp. 150-179.

CHUKWURAH, N.F., EZE, S.C., CHIEJINA, N.V., ONYEONAGU, C.C., OKEZIE, C.E.A., UGWUOKE, K.I., UGWU, F.S.O., ARUAH, C.B., AKOBUEZE, E.U. and NKWONTA, C.G., 2013. Correlation of stipe length, pileus width and stipe girth of oyster mushroom (*Pleurotus ostreatus*) grown in different farm substrates. *Journal of Agricultural Biotechnology and Sustainable Development*, vol. 5, no. 3, pp. 54-60. http://dx.doi.org/10.5897/JABSD2013.0197.

DAVID, M.B. and MUTHERSBAUGH, H., 1996. Nutrient supplements that influence later break yield of *Agaricus bispourus. Canadian Journal of Plant Science*, vol. 76, no. 4, pp. 835-840. http://dx.doi.org/10.4141/cjps96-141.

ERGONUL, P.G., AKATA, I., KALYONCU, F. and ERGONUL, B., 2013. Fatty acid composition of six wild edible mushroom species. The Scientific World Journal, vol. 2013, pp. 1-4. http://dx.doi.org/10.1155/2013/163964.

FANADZO, M., ZIREVA, D.T., DUBE, E. and MASHINGAIDZE, A.B., 2010. Evaluation of various substrates and supplements for biological efficiency of *Pleurotus sajor-caju* and *Pleurotus ostreatus. African Journal of Biotechnology*, vol. 9, pp. 2756-2761.

GOTHWAL, R., GUPTA, A., KUMAR, A., SHARMA, S. and ALAPPAT, B.J., 2012. Feasibility of diary waste water and distillery spent wash effluents in increasing the yield potential of *Pleurotus flabellatus* and *Pleurotus sajor-caju* on baggase. *Biotech*, vol. 2, pp. 249-257.

GRIFFIN, D.H., 1994. *Fungal physiology*. New York, USA: Wiley.

HASSAN, F.R.H., MEDANY, M.G. and HUSSEIN, S.D.A., 2010. Cultivation of the King Oyster Mushroom (*Pleurotus eryngii*) in Egypt. *Australian Journal of Basic and Applied Sciences*, vol. 4, no. 1, pp. 99-105.

HATFIELD, S.G.S. and KNEE, M., 1988. Effects of water loss on apples in storage. *International Journal of Food Sciences*, vol. 23, pp. 575-583.

HORTWITZ, W., 1960. *Official and tentative methods of analysis*. Washington, DC: Association of the Official Agriculture Chemist, vol. 9, pp. 320-341.

JAFARPOUR, M. and EGHBALSAEED, S., 2012. High protein complementation with high fiber substrates for oyster mushroom cultures. *African Journal of Biotechnology*, vol. 11, pp. 3284-3289.

JONATHAN, S.G., FASIDI, I.O., AJAYI, A.O. and ADEGEYE, O., 2008. Biodegradation of Nigerian wood wastes by *Pleurotus tuber-regium* (Fries) Singer. *Bioresource Technology*, vol. 99, no. 4, pp. 807-811. http://dx.doi.org/10.1016/j.biortech.2007.01.005. PMid:17391957.

KANG, M.S., KANG, T.S., KANG, A.S., SHON, H.R. and SUNG, J.M., 2000. Studies on mycelia growth and artificial cultivation of Saesongi. *Korean Journal of Mycology*, vol. 28, pp. 73-80.

KAZEMI, E.J., JAFARPOUR, M. and EGHBALSAEED, S., 2016. King oyster mushroom production using various sources of agricultural wastes in Iran. *International Journal of Recycling of Organic Waste in Agriculture*, vol. 5, no. 1, pp. 17-24. http://dx.doi.org/10.1007/s40093-015-0113-3.

KHAN, M.S., ZAIDI, A. and WANI, P.A., 2006. Role of phosphate solubilizing microorganisms in sustainable agriculture. A review. *Agronomy for Sustainable Development*, vol. 26, pp. 1-15.

KIRBAG, S. and AKYUZ, M., 2008. Effect of various agro-residue on growing periods, yield and biological efficiency of *Pleurotus eryngii. Journal of Food Agriculture and Environment*, vol. 6, pp. 402-405.

KOREA. MINISTRY FOR FOOD, AGRICULTURE, FORESTRY AND FISHERIES – MFAFF, 2012. *An actual output for special crop*. Korea: MFAFF, pp. 54-57.

LEE, S.K. and KADER, A.A., 2000. Pre-harvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, vol. 20, no. 3, pp. 207-220. http://dx.doi.org/10.1016/S0925-5214(00)00133-2.

LEU, J.Y., 1992. *Studies on Agrocybe aegerita (Brig.) singer with special reference to ecological factors on mycelial growth and fructification*. Taichung, Taiwan, R.O.C.: National Chung Hsing University, M.Sc. Dissertation.

LIANG, Z.C., WU, C.Y., SHIEH, Z.L. and CHENG, S.L., 2009. Utilization of grass plants for cultivation of *Pleurotus citrinopileatus. International Biodeterioration & Biodegradation*, vol. 63, no. 4, pp. 509-514. http://dx.doi.org/10.1016/j.ibiod.2008.12.006.

MANDEEL, Q.A., AL-LAITH, A.A. and MOHAMED, S.A., 2005. Cultivation of oyster mushrooms (*Pleurotus* spp.) on various lignocellulosic wastes. *World Journal of Microbiology & Biotechnology*, vol. 21, no. 4, pp. 601-607. http://dx.doi.org/10.1007/s11274-004-3494-4.

MCGARY, K.L., SLOT, J.C. and ROKAS, A., 2013. Physical linkage of metabolic genes in fungi is an adaptation against the accumulation of toxic intermediate compounds. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 28, pp. 11481-11486. http://dx.doi.org/10.1073/pnas.1304461110. PMid:23798424.

MILES, P.G. and CHANG, S.T., 2004. *Mushrooms: cultivation, nutritional value, medicinal effect and environmental impact*. Boca Raton: CRC Press. http://dx.doi.org/10.1201/9780203492086.

MONDAL, S.R., REHANA, M.J., NOMAN, M.S. and ADHIKARY, S.K., 2010. Comparative study on growth and yield performance of oyster mushroom (*Pleurotus florida*) on different substrates. *Journal of Bangladesh Agricultural University*, vol. 8, no. 2, pp. 213-220. http://dx.doi.org/10.3329/jbau.v8i2.7928.

MOONMOON, M., UDDIN, M.D.N., AHMED, S., SHELLY, N.J. and KHAN, M.D.A., 2010. Cultivation of different strains of king oyster mushroom (*Pleurotus eryngii*) on saw dust and rice straw in Bangladesh. *Saudi Journal of Biological Sciences*, vol. 17, no. 4, pp. 341-345. http://dx.doi.org/10.1016/j.sjbs.2010.05.004. PMid:23961095.

NISHIZAWA, A., YABUTA, Y. and SHIGEOKA, S., 2008. Galactional and raffinose constitute a novel function to protect plant from oxidative damage. *Plant Physiology*, vol. 147, no. 3, pp. 1251-1263. http://dx.doi.org/10.1104/pp.108.122465. PMid:18502973.

OEI, P., 1996. *Mushroom cultivation with special emphasis on Appropriate Techniques for developing countries*. Leiden (Netherlands): Tool Publications.

OHGA, S., 2000. Influence of wood species on the sawdust-based cultivation of *Pleurotus abalonus* and *Pleurotus eryngii. Journal of Wood Science*, vol. 46, no. 2, pp. 175-179. http://dx.doi.org/10.1007/BF00777368.

PAKISTAN. GOVERNMENT OF PAKISTAN, 2019. *Economic survey of Pakistan 2018-2019. Economic Advisors Wing*. Islamabad: Ministry of Finance.

PASTORI, G.M., KIDDLE, J., ANTONIW, J., BERNARD, S., VELJOVIC-JOVANOVIC, S., VERRIER, P.J., NOCTOR, G. and FOYER, C.H., 2003. Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. *The Plant Cell*, vol. 15, no. 4, pp. 939-951. http://dx.doi.org/10.1105/tpc.010538. PMid:12671089.

PECK, G.M., ANDREWS, P.K., REGANOLD, J.P. and FELLMAN, J.K., 2006. Apple orchard productivity and fruit quality under organic, conventional and integrated management. *HortScience*, vol. 41, no. 1, pp. 99-107. http://dx.doi.org/10.21273/HORTSCI.41.1.99.

PEKSEN, A. and YAKUPOGLU, G., 2009. Tea waste as a supplement for the cultivation of *Ganoderma lucidum. World Journal of Microbiology & Biotechnology*, vol. 25, no. 4, pp. 611-618. http://dx.doi.org/10.1007/s11274-008-9931-z.

RODRIGUEZ-ESTRADA, A.E. and ROYSE, D.J., 2007. Yield, size and bacterial blotch resistance of *Pleurotus eryngii* grown on cottonseed hulls/oak sawdust supplemented with manganese, copper and whole ground soybean. *Bioresource Technology*, vol. 98, no. 10, pp. 1898-1906. http://dx.doi.org/10.1016/j.biortech.2006.07.027. PMid:16973354.

RODRIGUEZ-ESTRADA, A.E., 2008. *Molecular phylogeny and increases of yield and the antioxidants selenium and ergothioneine in Basidiomata of Pleurotus eryngii*, 237 p. USA: Department of Plant Pathology, The Pennsylvania State University. Dissertation in Plant Pathology.

RODRIGUEZ-ESTRADA, A.E., JIMENEZ-GASCO, M.D.M. and ROYSE, D.J., 2009. Improvement of yield of *Pleurotus eryngii* var. eryngii by substrate supplementation and use of casing over lay. *Bioresource Technology*, vol. 100, no. 21, pp. 5270-5276. http://dx.doi.org/10.1016/j.biortech.2009.02.073. PMid:19560343.

RUCK, J.A., 1963. *Chemical methods for analysis of fruits and vegetables*. Ottawa: Canada Department of Agriculture. Research Station, Summerland, B.C., No. 1154.

SAMUEL, A.A. and EUGENE, T.L., 2012. Growth performance and yield of oyster mushroom (Pleurotus ostreatus) on different substrates composition in Buea south west Cameroon. *Science Journal of Biochemistry*, vol. 2012, pp. 1-6. http://dx.doi.org/10.7237/sjbch/139.

SANCHEZ-RANGEL, J.C., BENAVIDES, J.J., HEREDIA, B.J., CISNEROS-ZEVALLOS, L. and JACOBO-VELAZQUEZ, D.A., 2013. The Folin-Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Analytical Methods*, vol. 5, no. 21, pp. 5990-5999. http://dx.doi.org/10.1039/c3ay41125g.

SHAH, Z.A., ASHRAF, M. and ISHTIAQ, C.M., 2004. Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (wheat straw, leaves, saw dust). *Pakistan Journal of Nutrition*, vol. 3, no. 3, pp. 158-160. http://dx.doi.org/10.3923/pjn.2004.158.160.

SHASHIREKHA, M.N., RAJARATHNAM, S. and BANO, Z. RAJARATHNAM, S., SHASHIREKHA, M.N. and BANO, Z., 2002. Enhancement of bioconversion efficiency and chemistry of mushroom *Pleurotus sajor-caju* (Berk and Br) sacc, produced on spent rice straw substrates supplemented with oil seed cakes. *Food Chemistry*, vol. 76, no. 1, pp. 27-31. http://dx.doi.org/10.1016/S0308-8146(01)00244-8.

SINGH, M., VIJAY, B., KAMAL, S. and WAKCHAURE, G.C., 2011. *Mushroom cultivation, marketing and consumption. Directorate of mushroom research*. Índia: Indian Council of Agricultural Research.

STAMETS, P. and CHILTON, J.S., 1983. The mushroom cultivator a practical guide to growing mushrooms at home. Olympia, Washington: Agarikon Press, pp. 149-150.

STAMETS, P., 2000. *Growing gourmet and medicinal mushrooms*. 2nd ed. Berkeley, CA: Ten Speed Press, pp. 552.

STEEL, R.G.D., TORRIE, J.H. and DICKEY, D.A., 1997. *Principles and procedures of statistics: a biometrical approach*. 3rd ed. New York: McGraw Hill Book Co.

TRIPATHY, A., 2010. Yield evaluation of paddy straw mushrooms (*Volvariella* spp.) on various lignocellulosic waste. *International Journal of Applied Agricultural Research*, vol. 3, pp. 317-326.

URBAN, P.Ł. and BYSTRZEJEWSKA-PIOTROWSKA, G., 2003. Comparative analysis of cesium and potassium uptake in onion (*Allium cepa* L). *Czechoslovak Journal of Physics*, vol. 53, no. S1, pp. 91-96. http://dx.doi.org/10.1007/s10582-003-0014-y.

VAN DEN BERG, L., 1981. The role of humidity, temperature and atmospheric composition in maintaining vegetable quality during storage. *ACS Symposium Series*, vol. 170, pp. 95-107. http://dx.doi.org/10.1021/bk-1981-0170.ch008.

WANG, B.F., 2010. Use of wheat straw residue from a paper mill for *Pleurotus ostreatus* cultivation. *Acta Edulis Fungi*, vol. 17, pp. 30-31.

YANG, W.J., GUO, F.L. and WAN, Z.J., 2013. Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. *Saudi Journal of Biological Sciences*, vol. 20, no. 4, pp. 333-338. http://dx.doi.org/10.1016/j.sjbs.2013.02.006. PMid:24235869.

YOSHIDA, S., FORNO, D.A., COCK, J.H. and GEOMEZ, K.A., 1976. *Laboratory manual for physiological studies of rice*. 3rd ed. Manila: IRRI, pp. 17-22.

ZADRAZIL, F., 1978. Cultivation of *Pleurotus*. The biology and cultivation of edible mushrooms. Orlando, Florida: Academic press INC, vol. 1, pp. 62.

ZADRAZIL, F., KAMRA, D.N., ISIKHUEMHEN, O.S., SCHUCHARDT, F. and FLACHOWSKY, G., 1996. Bioconversion of lignocellulose into ruminant feed with white rot fungi. *Journal of Applied Animal Research*, vol. 10, no. 2, pp. 105-124. http://dx.doi.org/10.1080/09712119.1996.9706139.