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ENHANCING OYSTER MUSHROOM (*PLEUROTUS* SPP.) YIELD PERFORMANCE ACROSS VARIOUS SUBSTRATES THROUGH CHEMICAL AND BIOLOGICAL SUPPLEMENTS

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ABSTRACT

Oyster mushroom *Pleurotus ostreatus* is economically important, edible mushroom and known for its medicinal properties. Hence, integration of non-conventional crops in prevailing agricultural system can improve the economic status of the farmer. The aim of the study was to evaluated the suitability of various substrates with addition of chemicals and biological supplements to improve yield production. The spawn was prepared in Laboratory of Plant Pathology Agriculture Peshawar from the pure culture of *P. ostreatus*. In this study, diverse substrate materials were collected, chopped, and treated with a combination of chemical and biological supplements (10g/L) dissolved in water, employing the lamp spawning method. Each plastic bag was incubated with 200g of prepared spawn until entire bag become white, indicating full colonization. Subsequently, the treated substrates underwent incubation at 25°C. The appreciable yields were obtained on chemical fortified substrates as compared to non-supplemented treatments which have a significant effect on the mushroom production. Overall, the highest mean yield (388 g) was recorded on wheat straw substrate supplemented with diammonium. In addition, biological supplemented substrate was significantly enhanced the yield performance of *P. ostreatus* expect of chicken manure substrate. The high yield (372 g) was observed on cotton waste substrate augmented with biological supplement rice husk. It is concluded that the addition of external nutrients including chemical and biological supplements can increase the yield of oyster mushroom and will be useful tool for the farmers to add supplements in substrates.

Keywords: Oyster mushroom, substrate, biological nutrients, diammonium.

INTRODUCTION

Mushrooms are fungus which have been extensively utilize in numerous medicinal product in Japan, Korea, and China as considered as major protein source for food. It is reported that about 56 edible mushrooms have been reported from all the provinces of Pakistan (Kishwar *et al.,* 2007). Recently, mushrooms are becoming increasingly popular among Pakistani people due to its nutritional value and its medicinal value. Some of the species such as *Agaricus bisporus*, *Auricularisa* spp., and others are threatened to extinction (Tariq *et al.,* 2020). Furthermore, Oyster mushrooms are among the most popular mushrooms that are easy to cultivate and adoptable to local environmental conditions (Hussain *et al.,* 2023). While in other countries, a special attention has been made to using them as dietary supplement to

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improve health condition. The impact that mushroom products have on the gut microbiota and the inflammatory process, owing to the presence of β-glucans, enzymes, and secondary metabolites, supports their nutritional significance. They can be used as direct prebiotics, indirect probiotics, or both (Bell *et al.,* 2022; Fokunang *et al.,* 2022). Mushrooms are a rich source of vitamins, minerals, and protein. Fresh mushrooms typically contain between 85% to 95% water, 4% carbohydrates, 3% protein, 1% minerals/vitamins, and 0.1% fat. In addition, mushrooms are rich in phosphorus, copper, potassium, iron, and contain small amounts of calcium. Most mushroom cultivars belong to the phylum Basidiomycota. Among them, oyster mushroom *Pleurotus ostreatus* has been successfully cultivated and commercialized, although some others belong to the phylum Ascomycota (Rubini *et al.,* 2014; Vital *et al.,* 2015; Liu *et al.,* 2018). The production and consumption of edible mushrooms have ongoing for many years in developed countries. However, due to relatively simple cultivation process of oyster mushrooms, interest of the cultivation has surged in the past decade (Mendoza-Mendoza *et al.,* 2015). Oyster mushrooms are also called "meat of the forest, which means high nutrition and can be compared with meat, eggs and milk (Mata and Estrada, 2005). Several findings proved that mushrooms have anti-cancer, anti-cholesterol and anti-ulcer properties and also found useful in lung diseases and diabetes. Nowadays, due to their natural ability to thrive under various conditions and temperatures, the cultivation of oyster mushrooms has become an important aspect of agribusiness. However, mushroom cultivation is limited in developing countries, therefore their industries are still in its infancy (Kausar and Banjwa, 2005). The primary issue concerning to mushroom cultivation technology is the lack of technical knowledge require for its growth. The *Pleurotus* species stands out as one of the most efficient lignocellulose solid-decomposing white-rot fungi, offering an effective method for harvesting and recycling agricultural waste products. Possessing distinctive properties, it thrives in various plant parts that are low in nutrients (Raman *et al.,* 2021).

The oyster mushroom has the saprophytic ability to colonize and grow on agriculture wastage. In mushroom cultivation, both cellulose and lignin materials such as cotton waste, sorghum waste, sugarcane leaves, sawdust, wheat straw, and many others agriculture/industries waste are required for the spawning process and fruit growth (Yang *et al.,* 2013). Therefore, many agriculture waste materials are used as a substrate in production of *Pleurotus* (Mandeel *et al.,* 2005; Sánchez, 2010). The diverse composition of the substrates has potential to improve production when supplemented with adequate quantity of additives (Miles and Chang, 2004; Naraian *et al.,* 2009; Hu *et al.,* 2011). Various supplemented substrate has been recommended for cultivation of oyster mushroom. These supplements are not limited to chemicals such as diammonium phosphate, ammonium nitrate, and potassium, but also include biological supplements (Belewu and Belewu, 2005; Ratuchne *et al.,* 2016). The main macronutrients that fungi require for their structural and energy needs are nitrogen, phosphorus, and potassium (Miles and Chang, 2004). Biological supplements such as chicken manure, rice husk, wheat bran, soybean, and mustard cake have been found to effectively boost mushroom production (Rose *et al.,* 2022). Incorporating the correct quantity of these supplements into the substrate can increase the yield of the mushrooms (Salami *et al.,* 2017). The aim of the study is to explore the use of eco-friendly supplements that have potential to reduce waste material in environment while simultaneously promote the mushroom production. The utilization of these natural substrates could potentially supply high amounts of essential micronutrients such as carbon and nitrogen which are crucial for achieving optimal growth and to increase the mushroom yield production (Rodias *et al.,* 2020). The utilization of low-cost agricultural waste materials for economically important end products will lead to the development of low-input farming techniques for rural communities. This will be a significant step towards decreasing the poverty rate (Bhatti *et al.,* 2007), and also reduce burden on climate change factor. This research aims to evaluate the yield performance of oyster mushrooms on various agriculture crop waste material substrates with the addition of some nutrients under controlled condition.

MATERIALS AND METHODS

Mycelial culture establishment and spawning: The mushroom spawn production and its inoculation of substrate is illustrated in Figure 1. The mushroom mycelial culture was cultured on the potato dextrose agar (PDA) medium in test tube. For production of spawn, the pure mycelium from slants containing PDA medium was used. The barley and sorghum grains were soaked and washed with water, and boiled for 15 minutes. Next, the boiled grains were put on a sieve to drain completely, and were spread them on a clean sheet to dry. The ingredients were mixed thoroughly and moisture content was adjusted to 55–60%. These grains were mixed with calcium carbonate and the mixture was distributed equally into 250 mL sterile jar, at the rate of 200g seeds per plastic autoclave bag and autoclaved at 121°C for 30 minutes, and then cooled the jar at room temperature. The cooled *P. ostreatus* spawns were evenly distributed into the seeds within the jar. The jars were incubated in a ventilated incubator at 26°C for 12 days and periodically shake the jar thoroughly by hand to distribute the mycelium within the grain. After 15days of incubation, the spawn is now ready for inoculation of the substrate.

Figure 1. Cultivation of oyster mushroom. [A] the oyster spawn produced from pure mycelia, [B] preparation of wheat substrate bags for mushroom production, [C] mycelia covered the whole substrate bag, and [D] the mature flushes of oyster mushroom.

Preparation of the substrates: Various substrates material including sorghum waste, wheat straw, sawdust, sugarcane and maize leaves were gathered from diverse field in local areas and cotton waste as a by-product from cotton mill was collected. Initially, the substrate was dried before of any degradation in whole process. The large agro-waste straws were chopped into small pieces in range of 6 to 8 cm. The biological materials were purchased from local market. To absorb moisture and soften, the substrate materials were submerged in water for a full day, after which they were placed on a wire sieve to drain off the excess water. The chemical nutrients from nitrogen sources include urea, di-ammonium phosphate (DAP), ammonium nitrate (AN) and biological supplements such as chicken manure (CK), rice husk (RH), wheat bran (WB), soybean meal (SM) and mustard cake (MC) at rate of 10g/L in a water (H_2O), were added into the substrates. Polyethylene bags were filled with 1kg of each substrate medium. These bags were closed with ribbon band and sterilized at 121°C for 15 minutes and were then cooled down to room temperature.

The spawn inoculated into these substrates by adding nutrients using lamp spawning method. Simultaneously, the spawn was amended in to each substrate at 2% (w/w) and inoculated at 25°C under control condition. The plastic bag surface was puncher with help of sterilized blade for aeration within the bag. After mycelia colonized the substrate, polyethylene bags were cut with the help of sterilized scissor to grow out the mushroom in the cropping room. These bags were placed on shelves for further growth under control condition. The moisture content and humidity were checked twice a day at 9.00 am and

3.00 pm by using digital thermometer. The mycelia of the substrate were watered twice a day (during morning at 9.00 am and afternoon at 3.00 pm) using water pot to maintain water activity and humidity around 70-90%.

Yield calculation and comparison of supplements on various substrates: The mature fruiting bodies were harvested by clean hands without damaging after two to three days of emergence. In order to harvest the mushrooms from the second and third flushes, the substrates were kept in the same conditions for an additional seven days. For fresh weight measurements, mushrooms from various substrates and treatments were stored apart. Efficacy of chemical and biological nutrients supplement were compared for mushroom yield after three flushes were calculated by adding their means in gram. The yield (means) of mushrooms in three flushes was calculated using the following formula to compare the chemical and biological efficiencies (Iqbal *et al.,* 2005).

$$BE = \frac{\text{Weight of fresh mushroom harvested per bag}}{\text{Weight of dry substrate per bag before inoculation}} X100$$

STATISTICAL ANALYSIS

Data obtained during the study was analyzed by Analysis of Variance (ANOVA). The experiment was designed using randomized complete block design (RCBD) in three replications in each treatment. Using the IBM SPPS version 20.0 software package, the treatment means were separated by Duncan's multiple range test to compare the mean of significance differences ($p < 0.05$) among different treatments.

RESULTS

Efficacy of chemicals and biological supplements: The effects of chemicals and biological supplements on agro wastes substrate on the growth of *P. ostreatus* yield was evaluated and are shown in (Table 1 & 2). After three flushes, the oyster mushroom yield on different chemical and biological substrates were compared (Table 1 & 2). Of the seven substrates used, the highest mushroom mean weights (388 g) were obtained in three flushes on wheat straw substrate fortified with chemical diammonium phosphate (DAP), respectively (Table 1). Among five biological supplements; rice husk (RH) and soybean meal (SM) fortification in cotton waste substrate resulted the highest yield (Table 2).

Overall obtained yield (in means) of *P. ostreatus* was recorded after thee flushes with addition of chemical supplements i.e. urea, diammonium phosphate (DAP), ammonium nitrate (AN) on wheat straw substrate were 351, 388, 361, 344 g respectively as compare to control (344 g) treatment. On cotton substrate with addition of urea, DAP and AN resulted mean yield were 381, 377, 381, 357 g as compared to control (357 g) treatment. The mushroom yield on sorghum waste substrate with addition of chemical, the obtained yield was 343g, 345g, 356g as compared to control (338 g) as followed by yield on paddy straw with addition of chemical supplements were 350, 347, 358 g (Table 1). Saw dust substrate with addition of nutrients, the obtained yield was 353, 340, 344 g as compared to control that was lower as 335 g (Table 1). On sugar cane with addition of nutrients, obtained yield was 357, 341,346 g while in control it was 333 g (Table 1). Oyster mushroom yield after three flushes on maize substrate with supplementation of urea, DAP and AN were 349, 352, 354 g respectively while in control, it was 341 g (Table 1).

Table 1. The obtained yield in three flushes of Oyster mushroom (*Ploeurotus sp.*) on different substrates with addition of chemical substrates.

Chemical supplements	Wheat straw substrate	Cotton waste substrate	Sorghum waste substrate	Paddy straw	Saw dust	Sugarcane leaves	Maize leaves
Urea	351g ± 0.145 c	381g ± 0.298 a	343g ± 0.431 c	350g ± 0.321 b	353g ± 0.577 a	357g ± 0.471 a	349g ± 0.632 c
Diammonium phosphate	388g ± 1.766 a	377g ± 1.007 b	345g ± 1.883 b	347g ± 1.442 c	340g ± 2.211 c	341g ± 1.973 c	352g ± 1.247 b
Ammonium nitrate	361g ± 1.136 b	381g ± 1.009 a	356g ± 1.981 a	358g ± 1.638 a	344g ± 0.995 b	346g ± 0.027 b	354g ± 0.913 a
Control	344g ± 0.957 d	357g ± 0.892 c	338g ± 0.835 d	340g ± 1.002 d	335g ± 0.757 d	333g ± 1.110 d	341g ± 0.997 d

The ± sign indicate standard deviation of 3 replication and letters showed significantly differ (P <0.05 LSD test).

Biological supplementation to substrate: With the exception of chicken manure to substrates made of wheat straw, cotton wastes, sugarcane, and maize leaves, adding biological supplements to substrate greatly increased yield. Low yields of 342, 347, 330, and 339 g were obtained on these four substrates fortified with chicken manure, while high yields of 344, 347, 333, and 341 g were obtained in the control group (Table 2). *P. ostreatus* produced yields of 350, 342, and 367 g on wheat straw substrate when rice husk, chicken manure, and wheat bran were added. The highest yields were obtained on mustard cake (350 g) and soybean (367 g) in the control group (Table 2).

Table 2. The obtained yield (means) in three flushes of Oyster mushroom (*Ploeurotus* spp) on different substrates with addition of biological substrates.

Biological supplements	Wheat straw substrate	Cotton waste substrate	Sorghum waste substrate	Paddy straw	Saw dust	Sugar cane leaves	Maize leaves
Rice husk	350g ± 1.117 b	372g ± 1.271 a	350g ± 1.851 c	361g ± 1.083 b	344g ± 1.992 b	330g± 2.445 e	338g ± 1.027 f
Chicken manure	342g ± 0.757 d	347g ± 1.002 f	344g ± 0.757 d	348g ± 1.110 c	336g ± 1.110 e	330g ± 0.997 e	339g ± 1.077 e
Wheat bran	367g ± 1.614 a	370g ± 2.020 c	361g ± 1.100 a	368g ± 2.311 a	356g ± 1.055 a	358g ± 1.087 a	350g ± 1.350 a
Mustard cake	350g ± 0.913 b	360g ± 0.359 d	340g ± 1.110 e	337g ± 0.884 e	341g ± 1.114 c	343g ± 1.773 b	347g ± 1.037 c
Soybean meal	367g ± 1.724 a	371g ± 2.203 b	358g ± 1.220 b	335g ± 1.971 f	338g ± 1.011 d	342g ± 1.429 c	349g ± 1.477 b
Control	344g ± 1.551 c	357g ± 1.654 e	338g ± 2.171 f	340g ± 1.055 d	335g ± 1.267 f	333g ± 0.882 d	341g ± 0.449 d

The ± sign indicate standard deviation of 3 replication and letters showed significantly differ (P <0.05 LSD test).

DISCUSSION

The *Pleurotus spp.,* are naturally cultivate on decomposed plant parts, which are usually deprived of vitamins and nutrients. According to reports, lingo-cellulosic substances are essential for the development of fruiting bodies and mycelial growth because they provide good sources of carbon and nitrogen in different substrates at a ratio of 50:1 (Du Plooy, 2007; Thakur, 2020). Most of these agro-waste substrates have nitrogen content ranging from 0.5 and 0.8%, therefore by adding nitrogen to the various substrate higher mushroom yields (Naraian *et al.,* 2009). Thus, it is necessary to supplement other additives with substrate materials containing organic or inorganic nitrogen. The addition of chemical and biological supplements to different agro-waste substrates has enhanced appreciable yield of the mushroom. It was reported that various agro substrates including cotton waste, wheat straw, sorghum waste, sawdust, sugarcane leaves were suitable natural substrates on which *Pleurotus* spp grew very well (Ashraf *et al.,* 2013; Chanakya *et al.,* 2015; Iqbal *et al.,* 2023). The addition of supplements is beneficial for the production of *P. ostreatus* because as these supplements serve as a major source of external nutrients and vitamins necessary for stimulating high mushroom yields (Hoa *et al.,* 2015). The supplements were found best for *P. ostreatus* growth (Khan *et al.,* 2012; Hoa *et al.,* 2015). Our results are in line with observation of Tesfay *et al.* (2020) for mushroom cultivation. In this study, high yield of fruiting body was observed on substrates amended with supplements, while the lower growth was recorded in control treatment without amendments of supplements (Ashraf *et al.,* 2013). The various chemical and biological supplements have stimulatory effect on mushroom cultivation with source of nitrogen (Curvetto *et al.,* 2002). Comparing the results in this experiment, the addition of various chemicals, such as potassium, diammonium phosphate, and urea, we found that the fruit body yield was higher than that of the control. Suggesting that these chemicals are good source of nitrogen which has essential macronutrients for better growth of mushroom. However, adequate number of supplements is necessary for better growth of mycelium and fruiting body (Naraian *et al.,* 2009). it is reported that substrate amended with supplements; can potentially enhance the mushroom yield (Pant *et al.,* 2006). In the current study, the highest yield efficiency has been achieved with biological supplements such as rice husk, wheat bran, mustard cake amendments on agro waste substrate as previously reported (Buendía *et al.,* 2016). The fruit body yield was significantly improved than the control when mushroom was supplemented by various chemicals and biological compounds were compared. Our results fully concur with the previously mentioned observations (Sharma *et al.,* 2013). The two biological supplements viz., wheat bran and soybean cake (SM) showed the best results and are considered as first-generation supplements (Sobhan, 2006). In previous studies, SM was found to be a better supplement, as it serves is a good source of fats and protein. Additionally, it increased mushroom yields by prompting mycelium growth and fruiting bodies due to presence of amino acids (Obodai *et al.,* 2003). In view of the above results, it was also found that agro waste products; cotton waste, wheat straw, sorghum waste, sawdust, sugarcane leaves can be used successfully in mushroom cultivation. Various researchers confirmed that supplementation of various compounds at adequate concentrations exhibited a better result by enhancing growth rate and fruit body yield. Our results are in line with Onyango *et al.* (2011) that Mustard cake (MC) is also the best supplement to promote mushroom growth and its fruit. Therefore, *Pleurotus sp.,* cultivation on agricultural waste substrate i.e. cotton, wheat, maize, sorghum, paddy straw and saw dust amended with adequate concentrations of 2 % for biological supplements and chemical supplements (0.5 %), respectively is suggested to enhance mushroom yield. The addition of rice husk greatly enhanced mycelium growth and fruit body formation on agro-waste substrates, resulting in a high mushroom yield (Obodai *et al.,* 2003). The supplementation of chicken manure to wheat straw, cotton wastes, sugarcane and maize leaves substrate resulted in yield losses and this may be due to presence of high nitrogen content of the substrate (Sreekrishnan *et al.,* 2004; Bellettini *et al.,* 2019).

CONCLUSION

P. ostreatus is known for its medicinal properties and also contribute to agribusiness. The yield performance of oyster mushrooms grown on various substrates with the addition of various biological nutrients improved its production as compared to non-supplemented treatments. So, it is suggested to use these substrates with the addition of various biological nutrients to facilitate the farmer in profitable agribusiness.

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Shazia Akram	:	Designed the study, methodology and writing original draft of manuscript
Rizwan Khan	:	Analyzed the data, edit the manuscript and prepared figures and help in performing experiment
Owais Iqbal	:	Help in data analysis, review the manuscript and prepared figures
Muhammad Nabeel	:	Review manuscript
Sumbal Zaman	:	Proofread the manuscript
Nimra Khanzada	:	Edit the manuscript
Muhammad A. Khanzada	:	Helped in editing the manuscript
Muhammad A. Rajput	:	Help in data analysis