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Department of Botany,
University of Calcutta,
Kolkata 700 019, India

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Cultivation of Pink Oyster mushroom *Pleurotus djamor* var. *roseus* on various agro-residues by low cost technique

RAMAN JEGADEESH*[1,2], HARIPRASATH LAKSHMANAN[2,4], JANG KAB-YEUL[1], VIKINESWARY SABARATNAM[3], AND NANJIAN RAAMAN[2]

[1]Mushroom Research Division, National Institute of Horticultural and Herbal Science, Rural Development Administration, Eumsung, Republic of Korea
[2]Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India
[3]Mushroom Research Centre, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia
[4]Department of Biochemistry, Karpagam Academy of Higher Education, Coimbatore 641 021, Tamil Nadu, India

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Cultivation of the oyster mushroom, *Pleurotus* spp., has increased greatly throughout the world and commonly grown on pasteurized agro wastes. It can be cultivated on a wide variety of lignocellulosic substrates, enabling it to play an important role in managing organic wastes whose disposal is problematic. Mushroom cultivation is a simple, low cost and environmentally friendly technology for the utilization of rural and agro-industrial residues. The substrate used for the cultivation of one such species is pink oyster mushroom, *Pleurotus djamor* var. *roseus*, which is becoming important as this is an unfamiliar edible mushroom and can be cultivated easily throughout the year. In the present study different substrates viz. paddy straw, sugarcane bagasse, coir pith, sorghum straw, ragi straw and mixed bed were used for the cultivation of pink oyster mushroom. The selected substrates were chopped into 5 cm long pieces and soaked in clean tap water for 12 h. The pre-soaked straw was sterilized for 30 min at 15 lb/sq inch pressure. After cooling the substrate, a handful of spawn of *P. djamor* var. *roseus* was inoculated in perforated polypropylene bags (15 cm x 25 cm) row by row until it covers the whole size of the bag. The inoculated bags were incubated under dark for 12-14 d with the humidity range at 80-90% for mycelial formation. Primordium initiation was observed on 17-22nd day after spawning. Maximum yield of *P. djamor* var. *roseus* was obtained using paddy straw. The biological efficacy, energy value, energy recovery, proximate composition and elemental analyses of the fruiting bodies obtained on these substrates were reported.

Key words: *Pleurotus djamor* var. *roseus*, edible mushroom, cultivation, primordial, biological efficacy

INTRODUCTION

Mushroom industry is a global and expanding industry, with world production greater than two million tons annually (Dhar, 2014). The foremost mushroom varieties cultivated are *Agaricus bisporus,* oyster mushroom *Pleurotus ostreatus* and *Lentinus edodes*. The industrial cultivation of edible mushrooms has been optimized, the ever blooming small-scale industry and local farms are increasing attempts to domesticate local wild mushrooms cultivation (Mwai and Muchane, 2016). Mushroom farming has become a very important cottage industrial activity in the integrated rural development programme. Individual mushroom productions respond differently to varying cultivation substrate and optimal condition in terms of yield (Lechner and Albertó, 2011). The cultivation of mushrooms through solid state fermentation contributes to the recycling of agro wastes, which are the main substrates for the cultivation of mushroom (Thongklang and Luangharn, 2016). Mushrooms have long been valued as delicious and

*Corresponding author : jegadeesh_ooty@rediffmail.com

nutritional food in many countries and they are appreciated for their chemical and nutritional characteristics Mushrooms are a rich source of digestible proteins, have high protein content compared to vegetables and lower protein content than meat and milk

Oyster mushrooms were commercially cultivated worldwide, due to the unique ability to produce extra cellular lignocellulolytic enzymes and allowing then to grow in a wide range of agro-wastes Sánchez, 2010; Royse, 2013; Elisashvili *et al*. 2008; The oyster mushrooms production is the second most important mushrooms cultivated in the world, accounting for 25% of total global production The genus *Pleurotus* consists of nearly 50 species and widely distributed in both tropical and temperate regions of the world. In India about 26 species have been reported including *Pleurotus eryngii, P. citrinopileatus, P. flabellatus, P. ostreatus, P.djamor* var. *roseus* and *P. florida*. Unlike other mushroom species, growing oyster mushroom is becoming more popular throughout the world because of their ability to grow in various lignocelluloses. Further, the yield is high, without any additional growth requirements such as compost, manure, limestone, casing or temperature shocks. They can be easily grown on very wide temperature, relatively humidity and CO_2 tolerance (Thulasi *et al*. 2010; Ahmad Khan *et al*. 2012). In India, prevalence of varied, agro-climatic conditions and availability of vast quantities of lignocellulosic raw materials have stimulated the cultivation of *Pleurotus* spp. However, it is not necessary to process substrates for cultivation of *Pleurotus* species . Recently the local farmers have been looking for alternative methods and the substrates that are easily available with higher yields and better quality.

Pleurotus djamor var. *roseus* is an edible mushroom, belonging to the family *Pleurotaceae* in the order *Agaricales*. It is commonly called as roseus mushroom, pink oyster or salman pink oyster because of its pink sporophore, large sized fruit bodies and delicious flavour. It can be grown at 26°C and 35°C and with relative humidity above 80%. The main aim of the present study is to cultivate *P. djamor* var. *roseus* mushroom on low-cost techniques and to determine its growth, yield and biological efficacy on different agro wastes. To the best of our knowledge, this is the first data on the cultivation the *P*. *djamor* var. *roseus* on different agro wastes.

MATERIALS AND METHODS

Sample collection and isolation of pure culture

Pleurotus djamor var. *roseus* fresh basidiomata were collected from decomposed wood materials (*Ficus*-tree) in the forests of Indian Institute of Technology (IIT) campus, Chennai, India and their macroscopic and microscopic characters were recorded. The molecular and classical taxonomy results supported the identification of the isolated strain as *P. djamor* var. *roseus*. The sequence data obtained in the present study was deposited in the GenBank database (Maryland, USA) with accession No. GU350628. A small piece of inner tissue of the fresh pileus was aseptically removed using a sterile forceps. Then, it was immediately placed on the surface of potato dextrose agar (PDA) plates and the plates were incubated at ambient temperature, from 20-24°C for 5-6 days. The pure culture obtained was maintained on PDA slants at 4±1°C and subcultured at regular intervals for further studies.

Mass cultivation of Pleurotus djamor var. roseus

In the present study, paddy straw, sugarcane bagasse, coir pith, sorghum straw, ragi straw and mixed bed were used as substrates for cultivation of pink oyster mushroom. Mushroom cultivation involved in two major steps ie, 1. Spawn preparation and 2.Substrate preparation

Preparation of spawn

One kg of healthy grains of sorghum (*Sorghum vulgare*), finger millet (*Eleusine coracana*), wheat (*Triticum vulgare*), paddy (*Oryza sativa*), foxtail (*Setaria italica*) and bajar (*Pennisetum glaucum*) were washed thoroughly in tap water and boiled in 1.5 L of water for 30 min till the grains turned soft. The grains were than spread on used newspapers to drain out the excess water. To the drained grains, 15 g of calcium carbonate was mixed to prevent the grains from clumping. Further, 200 g each of these grains were placed separately in polypropylene bags (18 cm X 12 cm), plugged with cotton and sterilized at 15 psi of pressure for 20 min. After cooling at room

temperature, the grains were inoculated with 8 mm disc of pure culture of *P. djamor* var. *roseus* mycelium grown on PDA medium under aseptic condition. The inoculated polypropylene bags were then incubated at 25±1ºC in the dark. After 12-15 days, all the grains were colonised with mycelia and was used as inoculum for the mushroom cultivation.

Preparation of substrate

Well dried (free from mould fungi) paddy straw, sugarcane bagasse, coir pith, sorghum straw, ragi straw and mixed substrates were evaluated for the cultivation of *P. djamor* var. *roseus*. The selected substrates were chopped into 5 cm long pieces and transferred to gunny bags. The bags were soaked in clean tap water for 12 h. The excess water was drained out and the pre-soaked substrates were sterilized for 30 min at 15 psi pressure. After cooling the substrates, the spawn of *P. djamor* var. *roseus* was inoculated layer by layer until it covered the bags. The inoculated bags were incubated in dark for 12-14 days at a humidity range 80-90% for mycelial growth. When mycelial growth had covered the whole substrate in the bags, the bags were placed hanging and exposed to light of 2000-3000 lux units intensity for development of fruiting bodies.

Spawn running

Spawn running room was mainly used to keep the beds for running spawn. The temperature of the spawn running room was maintained between 25 to 30ºC. Light is not required in the spawn running room. Thatched shed was preferred for mushroom growing. Sheds were built in east west direction to avoid direct effect of sun and to reduce the inside temperature. The roof top is covered with chicken mesh to prevent the entry of rats, squirrels and snakes etc. The sides of the shed were covered with coconut plant leaves. The floor of the shed was filled with sand to a uniform height about 15 cm. Racks were built to accommodate the mushroom beds and inner side of the shed was covered with jute gunny bags. Water was sprinkled twice in a day on the floor and gunny bags to maintain the required temperature (28 - 30°C) and relative humidity (75 - 85%).

Cropping and Harvesting

Pin heads were formed after 2-4 d and the fruiting bodies were harvested at the right stage and the beds were again maintained for second and third cropping. Mushroom pin heads appeared on 3rd or 4th d of mushroom beds. Matured mushrooms were seen 3-4 d after pin head formation. The matured mushrooms were harvested before spraying water. Second and third harvests were obtained after scraping the surface of beds to 1 to 2 cm deep after the first harvest. The entire cropping was completed in 50 - 55 d. The fruiting bodies were collected prior to over maturation (detoriation). The biological efficiency (BE) was determined by the following formula.

$$BE\ (\%) = \frac{\text{Total fresh weight of the basidiomata}}{\text{Dry weight of the substrate}} \times 100$$

Determination of proximate composition and energy values

Proximate analysis, the total ash, acid value, reducing and non-reducing sugar, crude fibre (Maynard 1970), Saponification value, total protein , total lipid, iodine value and carbohydrate were determined according to the respective methods. The nutraceutical analysis of basidiocarp was compared with primordia and the energy values were calculated by using the following formula:

Energy value (Kcal/100g) = Protein × 2.62 + Fat × 8.37 + Carbohydrate × 4.2

Estimation of elements

Elemental analysis was performed by ICP-OES (model Optima 5300 DVICP-OES, Perkin Elmer). The ash content was determined using one gram of dried and ground mushroom powder in a porcelain crucible. It was ashed for 2 h at 500ºC and allowed to cool. The ash was wetted with 10 drop of H_2O and 3-4 mL HNO_3 (1+1) was added carefully. Excess HNO_3 was evaporated on a hot plate set at 100-120ºC. The crucible was returned to the furnace and a shed in 1 mL HCL (1+1) and it was transferred quantitatively to 50 mL volumetric flask. Then, the digested solution was filtered through Whatmann filter paper. The filtered suspension was used for mineral determination.

RESULTS AND DISCUSSION

Fungal materials source and maintenance of culture

The pure *P. djamor* var. *roseus* culture obtained was maintained on PDA slants at 4±1°C and

subcultured at regular intervals for further studies (Homolka, 2013). Maintenance of pure mycelial cultures is a necessity for ultimate spawn preparation and production. The pure culture was preserved at Centre for Advanced Studies in Botany, University of Madras, Chennai, India.

Cultivation of P. djamor var. roseus

In mushroom cultivation, spawn preparation is one of the basic techniques. In the present study, six different grains were screened for the spawn production, which are frequently available throughout the year. Among the six different grains, the maximum spawn run period (16 days) was recorded in paddy (*Oryza sativa*) and wheat (*Triticum vulgare*), followed by foxtail (*Setaria italica*) and bajar (*Pennisetum glaucum*) (14 day). The short spawn run period (12 days) was recorded in the finger millet (*Eleusine coracana*) and sorghum (*Sorghum vulgare*) (Fig. 1). In most laboratories, cereal grains such as wheat (Elhami *et al*., 2008; Chang, 2009; Stanley, 2010), rye (Chang, 2009), sorghum (Chang, 2009; Stanley, 2010), rice, millet (Elhami *et al*., 2008; Stanley, 2010) and white maize (Stanley, 2010) are used as the mother spawn. Failure to achieve a satisfactory harvest may often be traced to unsatisfactory spawn used (Chang, 2009).

Five different lignocellulosic residues were used as substrates for cultivation of *P. djamor* var. *roseus*. Compact mass of whitish and cottony growth was formed within a period of 2-3 weeks due to complete impregnation of mycelium into the substrate at 20-24ºC. A quick spawn run time was recorded in paddy straw (10 d) followed by ragi straw, corn straw, mixed substrate, sugarcane bagasse and coir pith as 11, 13, 12, 14 and 18 days respectively (Table 1). In *Pleurotus* spp. the spawn run takes about 12-25d at 24±1ºC. The maximum spawn run period was observed in *P. pulmonarius* was on 25th day (Akinmusire *et al*. 2011). In the present study, we observed a shorter spawn run period of 21 days for *P. djamor* var. *roseus* on paddy straw and corn straw substrate compared to reported literature. However, other substrates such as sugarcane bagasse and coir pith recorded a longer spawn run period of 24 days (Table 1). After spawn run the colonized substrates were transferred to cultivation thatched shed and clear plastic bags containing substrates were hanged with nylon rope with distance 20-30cm (Mamiro *et al*. 2014).

Yield and Biological efficiency

The yield of mushrooms (*P. ostreatus*, *P. sajor-caju*, *P. platypus*, *P. citrinopileatus*) was reported to be in the range of 0.08-4.79 kg fresh weight of dry substrate (Girmay *et al*. 2016), with a biological efficiency of 7.66-74.17%. In the present study, maximum yield (120.07%) of *P. djamor* var. *roseus* was recorded in paddy straw substrate followed by Ragi straw (119.74%), mixed substrate (111.1%), corn straw (96.19%), sugarcane bagasse (85.13%) and in coir pith (57.26%). The data revealed that the maximum yield was in paddy straw and ragi straw substrates, in addition mixed substrates also showed significant yield (Table 1).

Table 1: Spawn run period and biological efficiency of *Pleurotus djamor* var. *roseus*

Substrate	Spawn run time (d)	Primordia initiation (d)	First harvest (d)	Yield (g)	Biological efficiency (%)
Paddy straw	11.33 ± 0.89^{a}	16.67 ± 0.58^{a}	21 ± 0.89^{a}	600.37 ± 27.02^{d}	120.07±5.40^{d}
Ragi straw	12 ± 1.55^{ab}	18 ± 1.0^{ab}	21.67 ± 1.53^{a}	596.87 ± 28.70^{d}	119.37±5.74^{d}
Corn straw	13.33 ± 1.79^{bc}	18 ± 1.0^{ab}	21.33 ± 1.53^{a}	475.58 ± 26.96^{b}	095.12±5.39^{b}
Coir pith	17.67 ± 1.55^{d}	22 ± 2.0^{c}	26 ± 1.0^{b}	282.77 ± 21.0^{a}	056.55±4.20^{a}
Sugarcane bagasse	12.67 ± 0.59^{ab}	20.33 ± 0.58^{c}	24 ± 1.0^{b}	490.60 ± 22.99^{b}	098.12±4.54^{b}
Mixed substrate	15 ± 0.89^{c}	20 ± 1.0^{bc}	24.33 ± 0.58^{b}	548.88 ± 25.62^{c}	109.78±5.13^{c}

Values are mean ± S.D.
Values with the same alphabet are considered not significant at p value > 0.05.

Nutritive values of P. djamor var. roseus

Several studies have been previously carried out on the chemical composition and nutritional properties of edible mushrooms by many researchers (Toledo *et al.* 2016; Teklit, 2015; Upadhyaya *et al.* 2017). In general, mushroom fruiting bodies on a dry weight basis contain about 39.9% carbohydrate, 17.5% protein, 2.9% fats and

rest of them are minerals and vitamins (Demirbas, 2001). In the present study, the carbohydrate level of pink oyster mushroom (*P. djamor* var. *roseus*) was recorded to be 54.75% and 56.64% in matured basidiomata and primordia, respectively. *Pleurotus* spp. having considerable proportions of the carbohydrates and also consists of dietary fibres, which cannot easily be digested by humans and function essentially as dietary fibre Edible fungi are highly valued as a good source of protein and their protein contents usually range between 15% and 35% of dry weight. The protein content of Indian varieties of *Pleurotus* species was found to be around 21.6%, whereas the FAO (1973) report showed that the protein in *Pleurotus* spp is about 30.4%. In the present study, the maximum amount of protein was observed in matured basidiomata of *P. djamor* var. *roseus* (35.50%) followed by primordia (31.49%) (Table 2). A high content of protein and nitrogen source has been reported to be effective in shortening the growth period and increasing both the yield and biological efficiency (Adebayo *et al*., 2009; Fanadzo *et al*., 2010; Jafarpour *et al*., 2010) and the protein content may vary depends on the species and agro climatic factors. Mushrooms possess a wide range of crude fat content from minimum 2 % to maximum 8.0% on dry weight basis. Lipids from mushrooms are highly suitable for human to the least risk of atherosclerosis . In the present study, the fat content of *P. djamor* var. *roseus* is comparatively less in both matured basidiomata (1.72%) and primordia (1.63%). The fibre and ash content of *P. djamor* var. *roseus* in matured basidiomata is 14.6% and 5.90%, respectively and in primordial it is recorded as 8% and 8.40%, respectively primordial (Table 2). The energy value of *P. djamor* var. *roseus* was calculated in three different constituents based on the protein, carbohydrate and fat contents (Table 3). The matured basidiomata showed maximum amount of energy value (295.36 Kcal) than the primordia (291.76 Kcal).

Several studies have been carried out on the chemical composition and nutritional properties of edible mushrooms by many researchers. In general, mushroom fruiting bodies on a dry weight basis contain about 39.9% carbohydrate, 17.5% protein, 2.9% fats and rest of them are minerals and vitamins. Mushrooms are considered as protein-rich crops. The protein content of *Agaricus bisporus* has been recorded as 28.0% . Whereas,

Table 2: Proximate analysis of *Pleurotus djamor* var. *roseus*.

Name of the principle	Content (g / 100 g dry powder)	
	Primordia	Matured Basidiomata
Protein	31.48 ± 1.97^{de}	35.50 ± 1.78^{ef}
Carbohydrate	46.58 ± 2.57^{g}	44.75 ± 2.02^{g}
Fat	1.63 ± 0.03^{a}	1.72 ± 0.03^{a}
Reducing sugar	28.29 ± 1.23^{d}	37.10 ± 2.87^{f}
Non-reducing sugar	33.60 ± 1.28^{ef}	19.36 ± 1.48^{c}
Total ash	8.40 ± 0.17^{b}	5.90 ± 0.05^{b}
Crude fiber	8.00 ± 1.0^{b}	14.60 ± 0.43^{c}
Acid value	1.99 ± 0.01^{a}	0.63 ± 0.01^{a}
Iodine value	9.03 ± 0.06^{b}	9.63 ± 0.03^{b}
Saponification value	106.64 ± 9.61^{i}	92.68 ± 6.90^{h}

Values are mean ± S.D.
Values with the same alphabet are considered not significant at p value > 0.05.

it has been reported that *A. bisporus* contains crude protein 34.84%, crude fat 2.28% and crude ash 9.23%. The protein content of Indian varieties of *Pleurotus* spp. has been recorded as 21.6 %. It was reported that the protein content of *P. eous* was 27.4%, whereas, it is also reported that it was 26.6 – 34.1% in Pleurotus spp. The Ferula (*Pleurotus ferulae*) and white-ling mushrooms (*P. nebrodensis*) contain less carbohydrate (47.8% and 46.2%, respectively) than red oyster mushroom and purple spore oyster mushroom

Table 3: Energy values at different stages of *P. djamor* var. *roseus*

Stages	Nutrient content g/100g			Energy value (Kcal/100g)
	Protein	Fat	Carbohydrate	
Primordia	31.48	1.63	46.58	291.75
Matured basidiomata	35.50	1.72	44.75	295.36

Energy value (Kcal/100g) = Protein x 2.62 + Fat x 8.37 + Carbohydrate x 4.2

(59.9% and 57.1%, respectively). However, the carbohydrate contents were all in the range between 44.0–74.3% (Crisan and Sands 1978) and 46.6– 81.8%. In the present study, the carbohydrate level of pink oyster mushroom (*P. djamor* var. *roseus*) was recorded 54.75, 56.64 and 58.90% in matured basidiomata, primordia and mycelial mat, respectively (Table 1).

Edible fungi are highly valued as a good source of protein and their protein contents usually in the range between 15% and 35% of dry weight . The maximum amount of protein was observed in matured basidiomata of *P. djamor var. roseus* (35.50%) followed by mycelial mat (33.31%) and primordia (31.49%). The protein content may vary depending on the species and agro-climatic factors (Table 1).

The fibre content in red oyster mushroom (17.2%) was much higher than those in the other oyster mushroom species like white and yellow winter mushrooms (*Flammulina velutipes*). The fibre contents in ferula mushroom, purple spore oyster mushroom and white-ling mushroom ranged from 11.2% to 15.0% and were quite high in comparison with those in king oyster mushrooms (*P. eryngii*) which showed 5.97–9.15% and many other edible mushrooms (3.70–11.1%) . In the present study, the fibre content of *P. djamor var. roseus* ranged from 11% to 14.6%, with a minimum amount of 5.6% recorded in primordia and maximum level in the mycelial mat and matured basidiomata, which was reported similar to the purple spore oyster mushroom and white-ling mushrooms. The more amount of fibre content leads to the reduction in the accumulation of fat in our body system and blood stream. (Table 1). The crude fat content of mushrooms ranged from 1.1–9.23% and ash contents ranged from 3.84% to 5.83% in species of *Pleurotus* spp. such as P*. djamor*, *P. ferulae*, *P. nebrodensis* and *P. sapidus*, whereas in *P. djamor var. roseus* the fat content was very less in matured basidiomata (1.72%) and primordia (1.63%).

Table 4: Elemental analysis of *Pleurotus djamor* var.*roseus*

	Element present (mg/kg)	
Name of the elements	Primordia	Matured Basidiomata
Iron	19.1 ± 0.01^{d}	17.32 ± 0.03^{c}
Sodium	47.5 ± 0.1^{f}	67.12 ± 0.02^{h}
Potassium	2300.17 ± 0.29^{k}	2218.33 ± 0.58^{n}
Magnesium	120.8 ± 0.1^{i}	167.37 ± 0.55^{j}
Phosphorous	672.7 ± 0.1^{m}	503.1 ± 0.1^{i}
Calcium	20.47 ± 0.03^{e}	49.67 ± 0.04^{g}
Zinc	1.07 ± 0.03^{b}	1.21 ± 0.00^{a}

Values are mean ± S.D.
Values with the same alphabet are considered not significant at p value > 0.5.

Element analysis

The mushrooms are relatively good source of nutritional and having appreciated therapeutic properties as well as their mineral composition (Kuldo *et al.* 2014). Mineral constituents are reflected with growth condition of the mushroom. High concentration of potassium and phosphorous presented in the mushrooms as well as sodium is relatively less in mushroom species; thus, mushrooms are said to be good for patients with hypertension. In the present study, low concentration of zinc was recorded from matured basidiomata 12.1 mg and primordia 11.0 mg/kg dry weight. Zinc concentrations of mushroom samples, in the literature, have been reported in the ranges of 33.5– 89.5 µg/g and 45–188 µg/g. The Iron content was recorded in primordia and basidiomata ranged from 19.1 to 17.3 mg/kg. In adequate iron content in a daily diet is very important in order to decrease the incidence of anemia (Gençcelep *et al.* 2009). Phosphorus content was recorded maximum in primordia 672.7 mg/kg and minimum in matured basidiomata 503.1 mg/kg. These exotic mushrooms can contribute to human nutrition for phosphorus intake since recommended daily intake of phosphorus is 0.7g. Calcium level of *P. djamor var. roseus* was recorded maximum in matured basidiomata 49.7 mg/kg and minimum level in primordia 20.5 mg/ kg. Magnesium and potassium content in basidiomata and primordia were varied from 167.37 & 2218.33 mg to 120.8 & 2300.17 mg/kg. There is a good balance between the high content of Potassium and low content of sodium curing high blood pressure. Mostly the mushrooms contained considerably amounts of minerals and the levels were higher than those of toxic elements. The edible mushroom *P. djamor* var. *roseus* was a good source of minerals including iron, sodium, potassium, magnesium, manganese, phosphorus, calcium and zinc (Table 4). The results obtained for proximate composition and trace elements in analyzed *P. djamor* var *roseus* seem suitable for daily intake at nutritional levels.

Different agro wastes were successfully used as a basic substrate material for the cultivation of *P. djamor* var. *roseus*. Paddy straw, ragi straw, corn straw and mixed substrate showed excellent biological efficiency. The other advantage of using paddy and ragi straw substrates were low or no cost throughout the year. Therefore, mushroom

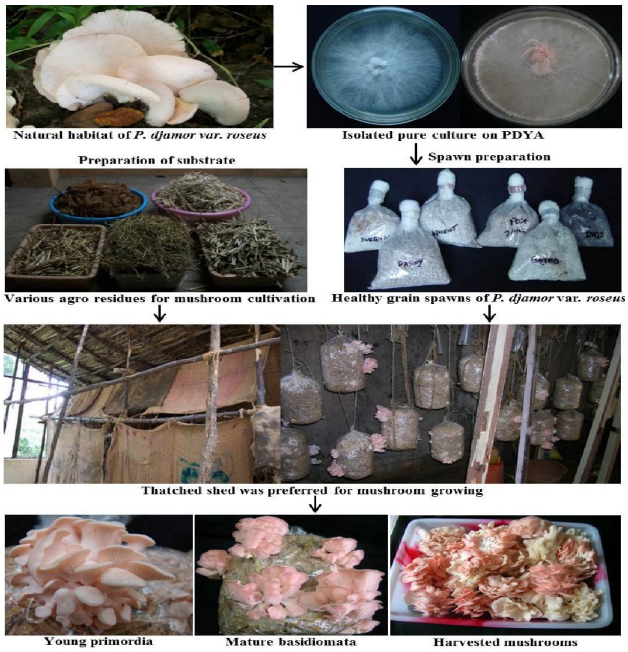

Fig. 1 : Cultivation *P. djamor* var. *roseus* by low cost technique

cultivation proves to be a highly efficient method for disposing of agriculture residues, such as rice and ragi straw, as well as producing nutritious human food. The alternative uses for the spend straw after mushroom production include animal feed, soil conditioner and the feed stock for energy generation via combustion or anaerobic digestion. The study attempts to contribute to the knowledge of the easy and low cost cultivation and nutritional properties of the mushroom. *P. djmor* var *roseus* might be beneficial nutritive content and have therapeutic values in reducing the risk factor of atherosclerosis due to high fiber contents.

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