

**STUDIES ON THE PRODUCTION  
OF MUSHROOM ON  
AGRICULTURAL WASTES**

**By**

**MOHSSEN HASSAN MOHAMED EL-BAGORY**

*B.Sc. (Agric.), Tanta Univ., A.R.E., 1988*

**Thesis**

*Submitted in Partial Fulfillment of the  
Requirements for the degree*

*of*

**Master of Science**

*in*

**AGRIC. MICROBIOLOGY**

**Agricultural Botany Department**

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**Kafr El-Sheikh**

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**APPROVED BY:**

*M. E. K. Wahim*

*El-Sayed El-Kady*

*M. A. El-Shorkawey*

*F. I. El-Hawany*

Date / / 1997

Committee in Charge

## **SUPERVISION COMMITTEE**

**Prof. Dr. M. E. K. Ibrahim**

Professor of Agricultural Microbiology,  
Faculty of Agriculture, Kafr El-Sheikh,  
Tanta University.

**Dr. M. A. A. Hassan**

Associate Professor of Plant Pathology,  
Faculty of Agriculture, Kafr El-Sheikh,  
Tanta University.

**Dr. Sh. M. A. El-Gremi**

Lecturer of Agricultural Microbiology  
Faculty of Agriculture, Kafr El-Sheikh,  
Tanta University.

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## I. INTRODUCTION

Edible mushrooms have received a remarkable amount of interest in recent decades with the realization that they are a good source of delicious food with high nutritional attributes. They are rich in protein (20-40% of the dry weight) and contain nearly all vitamins, particularly the B group. Their mineral content is also quite high ; phosphorus, potassium, calcium and iron are the most notable minerals in mushrooms. They are low in cholesterol and contain fibers, which helps in preventing several intestinal disorders.

Due to their nutritional value and spicy flavour mushrooms are widely cultivated in USA, Europe and Japan. The total mushroom production world-wide has increased more than ten-fold in the past 25 years from about 350.000 tons in 1965 to about 4.3 million tons in 1991. Based on recent and historical trends, it is likely that diversification of the mushroom industry will continue in the USA and other western countries. In 1993-1994 the USA produced 346188 tons of mushroom, while Japan produced 351027 tons in the same period. In the USA, *Agaricus* accounted for over 90% of the total mushroom production value, while *Lentinula*, *Flammulina*, *Pleurotus*, *Hypsizgus*, *Pholiotas* and *Grifola* were the main genera cultivated in Japan. As consumers became more aware of the additional culinary characteristics offered by a variety of mushrooms, demands for "speciality mushrooms" should increase substantially in the future (Daniel, 1995).

In recent years attention has been paid to mushrooms as a weapon against starvation. It is well known that about 60% of the world's population is insufficiently fed. Protein deficiency is almost restricted to the under developed and developing countries. Lelley (1988) argued that the increased cultivation of mushrooms in the third world countries could make a significant contribution to the feeding of their population. This could be achieved even without diverting land and other agricultural resources, but only by converting agricultural wastes into tasty and nutritionally valuable mushrooms.

The third world countries have agriculture or agro-based industries as the main activity of their population. The most important agriculture products of these countries include rice, wheat, corn, millet, sugar cane, vegetables and fruits, cotton and fiber plants. Production and processing of these crops usually result in accumulation of plant residues which are mostly regarded as wastes. These plant wastes contain cellulose, hemicellulose and lignin. Edible mushrooms are able to flourish on materials containing these substances. Among these mushrooms the species belonging to *Pleurotus* are the most promising for cultivation in developing countries. *Pleurotus sp.* are primary decomposers. So, they can thrive on a wide range of agricultural waste materials without the need for costly and complicated treatment of substrates. Methods of cultivation are generally simple and can be modified to suit the local socio-economic conditions. *Pleurotus* are high yielding ; in a few weeks 1 kg of dry plant material can be used to produce 0.5-0.75 kg of fresh, tasty and nutritious fruit bodies. The spent substrate left after production of fruit bodies consists of degraded cellulose and lignin and is protein-rich,

compared to the original substrate. It can be used for animal feeding or as organic fertilizer.

In Egypt, growing and consumption of mushrooms are relatively new. The first farm producing *Agaricus bisporus* started in 1985. Also, a few studies on the cultivation of *Pleurotus sp.* have been made (Abd El-Kawi 1989, Abd El-Rehem 1991 and Hassan 1992).

The main objectives of the present study were :

1. Evaluating the performance of four species of *Pleurotus* mushrooms on different agricultural wastes.
2. Studying the effect of spawn ratio and supplements on the mushroom yield.
3. Testing simpler methods for substrate preparation.
4. Evaluating the nutritive value of the produced mushrooms.
5. Preliminary evaluation of the spent substrate as animal feed.

## **2. REVIEW OF LITERATURE**

### **2.1. The nutritional attributes of edible mushrooms:**

#### **2.1.1. Protein:**

Published values for the protein content of four popular edible mushrooms, *Agaricus bisporus*, *Lentinus edodes*, *Pleurotus spp.* and *Volvariella volvacea*, which are commercially cultivated in various countries ; range from 1.75 to 3.63% of their fresh weight (Chang, 1980).

The protein content of edible mushrooms, in general, is about twice that of asparagus and cabbage and 4 times and 12 times those of oranges and apples, respectively. On a dry weight basis, mushrooms normally contain 19 to 35% protein as compared to 7.3% in rice, 13.2% in wheat, 39.1% in soybean and 25.2% in milk. Therefore, in amount of crude protein, mushroom rank below most animal meats but well above most other foods, including milk, which is an animal product (Bano and Rajarathnam, 1982a and Naire, 1982).

#### **2.1.2. Essential amino acids:**

Altamura *et al.*, (1967) and Huges *et al.*, (1958), reported that various species of mushrooms contain, in addition to the common amino acids and amides, the less common amino acids and related nitrogenous compounds such as methionine sulfoxide, B-alanine, cystic acid, hydroxy-prolines, amino adipic acid, phosphoserine, cystathione, canavarine ; creatinine, citrulline, omithine, glucosamine and ethanolamine.

### 2.1.3. Fat:

The fat content in different species of mushrooms ranges from 1.1 to 8.3% on a dry weight basis, with an average content of 4.0%. Huang *et al.* (1985), reported that six commonly cultivated mushrooms namely, *Volvariella volvacea*, *Lentinus edodes*, *Agaricus bisporus*, *Pleurotus sajor-caju*, *Auricularia auricula* and *Termella fuciformis* have a higher percentage of saponifiable lipids than non saponifiable lipids.

### 2.1.4. Vitamins:

It has been reported that edible mushrooms are a good source for several vitamins including thiamine (vitamin B<sub>1</sub>), riboflavin (vitamin B<sub>2</sub>), niacin, biotin and ascorbic acid (vitamin c) (Crisan and Sands, 1978). The thiamine content per 100 g dry weight ranges from 0.35 mg in *V. volvacea*, to 1.14 mg in *A. bisporus*, from 1.16 to 4.8 mg in *Pleurotus spp.*, to 7.8 mg in *L. edodes*. The niacin content varied from 54.9 mg in *L. edodes* to 55.7 mg in *A. bisporus*, to 64.88 mg in *V. volvacea*, to 46.0 to 108.7 mg in *Pleurotus spp.*

### 2.1.5. Carbohydrates and fibers:

According to Crisan and Sands (1978), pentoses, methylpentoses, hexoses, as well as disaccharides, amino sugar alcohols and sugar acids are constituents of mushroom carbohydrates. *Pleurotus* species contain carbohydrates ranging from 46.6 to 81.8% (as compared to 60% in *A. bisporus*) on a dry weight basis (Bano and Rajarathnam, 1982a). The fiber content ranges from 7.4 to 27.6% in *Pleurotus* species (as compared to 10.4% in *A. bisporus* and to 4.0 to 20.0% in *V. volvacea*) (Yoshioka *et al.*, 1975). Fiber is considered to be an important ingredient in a balanced and

healthy diet. Anderson and Ward, (1979) reported that feeding diabetic patients with high fiber diets reduces their daily insulin requirement and stabilizes their blood glucose profile, possibly by decreasing the rate of glucose absorption and or delaying gastric emptying.

#### **2.1.6. Minerals:**

Mushrooms are a good source of minerals. The minerals present in the substrate are taken up by the growing mycelium and translocated to the sporophores, as in higher plants. The mineral of highest content is potassium, followed by phosphorus, sodium, calcium and magnesium. These are considered to be the major mineral constituents while copper, zinc, manganese, molybdenum and cadmium make up the minor elements (Bano and Rajarathnam, 1982a ; Bano *et al.*, 1981 and Li and Chang, 1982).

#### **2.1.7. Nuclie Acids:**

Li and Chang (1982) reported that *Pleurotus sajor-caju* contains the highest amount of nucleic acids among the four edible mushrooms studied (4.06% on a dry weight basis). This is equivalent to 0.51% on a wet basis. Accordingly, it is safe to consume as much as 392.5 g of fresh *P. sajor-caju* daily. The limit could be even higher in the case of the other mushrooms with a lower nucleic acid contents or around 20% more after being cooked. Therefore, the content of nucleic acid in edible mushrooms should not be a limit to their use as daily vegetable.

## 2.2. Production of *Pleurotus* mushrooms:

### 2.2.1. Suitability of substrates and efficiency of species:

Several substrates including lignocellulosic residues have been used for cultivation of *Pleurotus* isolates.

Cultivation techniques have been set up by Ferri, (1972). The author indicated the cultivation techniques on organic substrate composted of corn cobs.

Zadrazil (1973) studied the growth of *Pleurotus florida* in plastic bags each holding 25 kg substrate inoculated with 3% wheat inoculum and held at about 20°C. The yield (cap weight as a percentage of the wet weight of the substrate) was 20.6 for the first flush and 4.2 for the second.

Bano and Nagaraja (1974) used paddy straw as substrate for cultivating *Pleurotus flabellatus*.

Balazs (1978) reported that corn cobs and straw mixture (50-50%) produced the highest yield of *Pleurotus* mushrooms.

Bano *et al.* (1979) grew *Pleurotus flabellatus* and *P. sajor-caju* in a locally constructed village shed in Mysor, India during the rainy (temperature 20-26°C, R.H. 70-80%) and winter (T. 15-25°C, R.H. 80%) seasons. Two types of bed were used viz, a polyethylene bag, 35 times 55 cm with 0.9 kg straw (PB) and cylindrical hanging bed containing 3.6 kg straw (HB). Both were inoculated with 3% spawn and 1% horsegram powder. Vegetative growth required 10-20 days for *P. flabellatus*.

Average rainy season yields for both (PB) and (HB) were 50.05-51.00% for *P. flabellatus* and 83.3-83.9% for *P. sajor-caju*. Maximum yield of both *P. flabellatus* and *P. sajor-caju* were obtained at 20-26°C and R.H. 70%. In winter, vegetative growth of *P. flabellatus* was delayed and the yield reduced to 35.5-41.6% but the second yield of *P. sajor-caju* remained satisfactory at 69-70%.

Poo-Chow (1980) reported that perforated polyethylene bags were used as containers to hold 2 kg of unsterilized raw cotton waste as the basic component of the substrate. A fast growing strain of *P. florida* was used. The spawn was placed in layers and the bags were held at 20°C±3°C for 9-21 days and then at 26°C to 30°C for fruiting. Five flushes were harvested at 7-10 days intervals.

Losovoi (1980) reported that various strains of *P. ostreatus* and one of *P. cornucopiae* were grown successfully on sterile blocks of fresh beach sawdust with added gypsum (1-2%), chalk (1-2%), Urea (0.3-0.5%) and NPK (0.3-0.5%). The yield obtained was 25-30% on weight of mixture. Best yields were obtained with *P. ostreatus*.

Flick, (1981) stated that sterilized spent mushroom compost inoculated with 11 species of edible fungi *P. ostreatus*, *P. ostreatus* Var. *florida* and *Volvariella volvacea* grew well.

Puri *et al.* (1981) reported that baddy straw was found to be a suitable substrate, in which average yields amount to 300 g/kg of dry substrate.



Sivaprakasam and Kandswamy (1981) studied the suitability of some waste materials for the cultivation of *P. sajor-caju*. The best results were obtained on substrates of waste paper, sugar cane bagasse, hulled and powdered maize cobs and rice straw. These gave yields of 183, 176, 176, 173 and 163 g/m<sup>2</sup>, respectively.

Nout and Keya (1983) found that cotton hull waste was a superior substrate for cultivation of *Pleurotus* in Kenya.

Bisht and Harsh (1984) showed the possibility of using some weeds like *Lantana camara* and other wastes, ie., waste paper as a substrate for *P. ostreatus* cultivation.

Chakravarty and Sarkar (1984) found that rice straw was a suitable substrate to growing *P. sajor-caju*.

Punkow (1984) used wheat straw as a substrate for growing oyster mushrooms.

Henicus and Voros (1985) reported that oyster mushrooms *P. ostreatus* grew well on a substrate of maize stalks.

Das *et al.* (1987) used agricultural wastes as substrates for cultivation of *P. flabellatus* and *P. sajor-caju* such as wheat straw, rice straw, maize stems, banana pseudostems and water hyacinth which were

used successfully. The best average yields of the two studied species were 1650 and 1970 g, respectively produced from 3 kg dry wheat straw.

Guzman-Davalos *et al.* (1987) studied the sugar cane bagasse as a substrate for *Pleurotus* production in Jalisco state, Mexico. *P. ostreatus* reached a biological efficiency of 49.08 and *P. floridanus* 51.05%.

Morales (1987) studied the cultivation of *P. ostreatus* on cardamon pulp which proved to be a good substrate giving a good yield of mushrooms and a biological efficiency of 113.64% (a production of 1091 g fresh fruit bodies/8 kg fresh substrate was obtained).

Nicolini *et al.* (1987) compared the cultivation of *P. ostreatus* and *Agrocybe aegerita* on wheat straw, orange peel and distillery grape stalks, separately or mixed. Good levels of substrate colonization on orange peel and grape stalks were achieved by *P. ostreatus* and *A. aegerita* which degraded 50-60% and 20-30% of lignin content, respectively.

In Egypt, Abd El-Kawi (1989), studied the growth of three *Pleurotus* species namely, *P. ostreatus*, *P. sajor-caju* and *P. florida* on rice straw. He found that *P. florida* gave a good yield followed by *P. sajor-caju* and *P. ostreatus*.

Abd El-Rehem, Nahed, (1991), cultivated two *Pleurotus* species viz., *P. sajor-caju* and *P. columbinus* on three lignocellulosic materials namely ; sawdust "wood shaving", rice straw and water hyacinth. She found that the two species of *Pleurotus* gave the highest yield on rice

straw substrate followed by saw dust “wood shaving” and water hyacinth under Egyptian conditions.

Hassan (1992) studied the growth of *P. cornucopiae*, *P. flabellatus* on different substrates viz., rice straw, wheat straw, broad bean straw, wastes of tomato and wastes of cowpea separately or mixed. He found that the highest yield was obtained when the *Pleurotus* species were grown on a broad bean straw separately or mixed with tomato or cowpea wastes (50 and 75% from each).

### **2.2.2. Preparation of substrates:**

Substrates for cultivation of *Pleurotus* mushroom can be prepared by several methods. The success of any method depends on its ability to fulfill the following requirements : to eliminate or kill the weed fungi in the substrate or at least to inhibit their growth, to kill nematods, insects and their dormant stages in the substrate, to precondition the substrate for the attack of *Pleurotus* and to compete economically .

Methods used for the preparation of substrate for *Pleurotus* can be summarized into 5 distinctive methods (Schies, 1991):

1. Sterilization of wet substrate under pressure in autoclave.
2. Partial sterilization (Pasteurization) of wet substrate.
3. Partial sterilization (Pasteurization) of dry substrate (xerotherm method).
4. Aerobic fermentation of wet substrate.
5. Semianaerobic fermentation.

The semianaerobic fermentation is still relatively unknown for most growers of mushroom. Schies (1991) and Lelley, (1986) studied this method extensively. They concluded that this method for substrate preparation requires less energy than the other methods. In addition, semianaerobic fermentation allows the production of small amount of substrate as well as large quantities and does not requires special technical devices or measures. Therefore they suggested this method to be used in countries of the third world, and recommended further research on the coarse of the semi-anaerobic fermentation at higher temperatures as may be found in tropical and subtropical regions.

### **2.2.3. Supplementation of substrates:**

Various organic and inorganic supplements including cotton seed meal, horse gram powder, soybean meal, alfalfa meal, coconut cake, palm kernel cake, rice bran, wheat bran, tobacco dust and spawn mat have been tried in the cultivation of various *Pleurotus spp.* with increased yields (Bano *et al.*, 1979 ; Bano and Rajarethnam 1983 ; Gunasegaran and Graham 1987, Royse and Schlsler 1987 ; Zadrazil and Kurtzman 1982).

Losovoi (1980) reported that various strains of *P. ostreatus* and one of *P. cornucopiae* were grown successfully on sterile blocks of fresh beach sawdust with added gypsum (1-2%), chalk (1-2%), Urea (0.3-0.5%) and NPK (0.3-0.5%). The yield obtained was 25-30% on weight of mixture. Best yields were obtained with *P. ostreatus*.

The addition of nutrients like oat meal to the substrate gave better yield with most agricultural wastes (Quimio, 1980).

A number of studies using rice and other straws have shown increased yields with nitrogen rich supplements (Zadrazil 1980 ; Muller and Gawley 1983 ; Rajarathnam *et al.*, 1986 ; Gunasegaran and Graham 1987 ; Royse and Bahler 1988).

Bano and Rajarathnam (1982b) used chopped paddy straw as a substrate and studied the effect of protein supplementation of the substrate on the yield and protein content of the fruiting bodies. Protein supplementation greatly enhanced yield. The best results being obtained with horsegram powder or yeast mud at 4.4 and 2.2% (dry weight basis), respectively. The highest protein contents of fruiting bodies were obtained with ground nut cake (3.3 or 4.4%) and were associated with relatively low yields.

Visscher (1984) studied the effect of amending the dry straw substrate with rice bran at 7.5 or 15.0% on yield of *P. pulmonarius* and *P. columbinus*. The highest concentration gave the best results .

Sharma and Jandaik (1985) reported that *Pleurotus* waste which constituted 40% of the contaminated straw during spawn running and cropping and 60% of the left-over substrate after the cultivation of *P. sajor-caju* did not support better yield than the fresh wheat straw, but an average yield of 853 g/kg of air dried substrate could be obtained when the waste was supplemented with 5% wheat bran .

El-Kattan and Bahran (1986) reported that rice straw as a medium for cultivation of an oyster mushroom could be supplemented with organic additives containing nitrogenous compounds at a reasonably high level. Wheat bran, soyabean meal, or dried berseem were added at rates of 1-5%.

Bisaria *et al.* (1987) cultivated oyster mushroom, *P. sajor-caju* on a number of agro-residues and their mixtures. Biological efficiency, defined as the percentage conversion of substrate into fruit bodies on a dry weight basis, was maximum on paddy straw supplemented with cotton seed (12-82 g/100 g substrate).

Ertan (1987) amended wheat straw medium with crushed barley, wheat bran, cotton seed residues or cotton lint at 10, 125, 250 or 375 g/3kg. Mycelial development and fructification of *P. ostreatus* took 26.7 and 36.7 days, respectively, with the unamended control medium. These developmental periods were shortest with media supplemented with 250 g cotton lint (14.7 and 26.8 days, respectively) and 250 g crushed barley (15.3 and 20.9 days, respectively).

Gunasegaran and Graham (1987) noticed that yield of the phoenix mushroom increased significantly by adding some organic additives i.e. rice bran, palm kernel cake, corn meal, coconut cake and tobacco dust. On the other hand it was reduced to zero by soya bean meal which stimulated the competitive growth of *Coprinus spp.*

Pettipher (1988) reported that cultivation of the oyster mushroom (*Pleurotus ostreatus*) on saw dust and coco-bean shell waste with organic supplements gave better yields.

Vischer (1989) studied the effect of adding various amendments to straw substrate on yield of *P. ostreatus* and *P. Pulmonarius*. The amendments comprised rice bran, lucerne meal, feather meal, millichamp 3000 (soya bean), poultry manure and gypsum. They were mixed with the straw at filling when the moisture content of the straw had been raised to between 72 and 78%. The reactions to the amendments varied between strains and even between species. Lucerne meal gave better results than rice bran, which was usually better than straw alone. The *P. ostreatus* strain Somycel 3200 grew best without amendments. It did not respond to lucerne meal whereas poultry manure delayed harvest and resulted in bare patches. Lucerne meal, feather meal, millichamp 3000 and gypsum depressed yields.

Upadhyay and Vijay, (1991) studied the effect of addition of various nitrogen supplements to wheat straw. Wheat bran, rice bran, and cotton seed meal at 5 and 10% (of dryweight of the substrate) and brewers grain at 10 and 20% (pretreated with 2% formaldehyde solution) were added to wheat straw before spawning. All the species i.e. *P. ostreatus*, *P. sp. cfr. Florida*, *P. cornucopiae*, *P. fossulatus* and *P. eryngii* behaved differently with addition of the above supplements. Dried brewers grain 20% gave the highest yield with *P. eryngii* and *P. fossulatus* (73 and 43% B.E.) followed by cotton seed meal. However, rice bran 5% was found superior to other supplements with *P. ostreatus*.

#### **2.2.4. Spawn ratio:**

El-Kattan and Bahrán (1986), studied the influence of spawn inoculum on yield of fruit bodies and found that 4% spawn ratio attained high percentage of yield/dry substrate weight.

Abd El-Kawi (1989) studied the effect of spawn ratio on biological efficiency of *Pleurotus spp.* on rice straw. Although 6% increased the (B.E.) the best spawn ratio was 4% because he did not find significant differences between the yield at 4 or 6% spawn ratio.

Hassan (1992) studied the effect of the spawn ratio on production of *Pleurotus* species. He found that the yield produced at 5% spawn ratio was higher than at 3%. However, the latter ratio (3%) was more economic and was recommended therefore, by the author.

#### **2.2.5. Spent substrate as an animal feed:**

The composition of the substrate before spawning and after mushroom harvesting show variable changes in their constituents. The residue after harvesting is referred to as a spent or compost substrate. Generally, the spent could be used for feeding of animals or as a manure for plants.

Rangaswami *et al.* (1975) reported that growing *P. sajor-caju* on N-free medium, increased N-content of the substrate up to 30 days after spawning, then N-content declined. After harvesting of the sporophores the substrate contained 15-18% protein.



Zadrazil (1975b) reported that *Pleurotus spp.* during their growth on rice straw, caused variable changes in its constituents i.e., crude protein, fiber, carbohydrates, lignin, amino acids and ash content. He found that straw fermented with *P. cornucopiae* for 120 days at 22°C and 25°C attained a digestibility of 60-70% high quality. In addition, the C/N ratio of straw was reduced due to the increase in crude protein. He also reported that when *P. florida* was grown on wheat straw, the nutrient extraction by the mycelium and fruiting bodies altered the proportion of minerals in the substrate ; Ca and Mg showed a relative increase and N, P and K a slight decrease.

Zadrazil (1977) reported an increase of 12 percentage units in the in-vitro digestibility of wheat straw incubated with *P. florida*.

Bano *et al.* (1986) revealed the suitability of spent straw after cultivation of the mushroom, *P. sajor-caju* and found that the extract of spent straw did not contain any mycotoxins. Moreover, they stated that in spite of the fact that body weights of albino rats, albino mice and weights of vital organs of the treated animals were decreased when higher concentrations of the “spent straw” were utilized, no abnormalities were recorded in histopathological examinations of the vital organs or the haematological observations. They concluded that feeding “spent straw” to these animals did not cause any toxic hazards.

Muller and Trosch (1986) when tested 22 basidiomycetes for lignin-cellulose and hemicellulose degradation reported that *P. florida* was the fastest in delignification.

Tsang *et al.* (1987) found that cellulose digestibility of the residues after *Pleurotus* species harvesting, was generally higher than that of the original straw. They concluded that it is not feasible to simultaneously produce *Pleurotus* and a highly delignified residue from wheat straw.

Gupta and Langer (1988) mentioned that *P. cornucopiae*, *P. florida* and *P. ostreatus* exhibited promising properties for the decomposition of lignin cellulose materials, mainly the cereal straw, to increase their value as animal feeds.

Silanikove *et al.* (1988) reported that organic matter losses after *Pleurotus sp.* growth were less for the cotton straw substrate than for the wheat straw substrate as shown by the increase in ash content of the substrate. During the growth cycle of the fungus the ligno-cellulose content of the substrate decreased and the detergent soluble content increase indicating that the quality of the substrates as feed stuffs for ruminants had been improved.

Li and Wang (1989) determined the nutritive value of the substrate after cultivation of *P. spidus* with a view to its use as a poultry feed, using cotton seed hulls as a substrate for cultivation. It was observed that, waste from *P. spidus* cultivation contained more water, crude ash and crude protein but less crude fat and crude fiber.

### **3. MATERIALS AND METHODS**

#### **3.1. Species of *Pleurotus*:**

Four species of *Pleurotus* mushroom were used throughout the present study, namely : *Pleurotus sajor-caju* (Fr.) Sing ; *P. pulmonarius* (Fr.) Que'l ; *P. cornucopiae* (paulex Fr.) and *P. ostreatus* (Jacques Fr.) Kummer. Cultures of the first three species were kindly offered by Research Institute for Mushroom Cultivation of the Rheinland Chamber of Agriculture in Krefeld, Germany. The fourth culture was kindly offered by the National Research Center, Cairo, Egypt.

Cultures of *Pleurotus spp.* were maintained on Potato Dextrose Agar (PDA) kept at 4°C and subcultured every 2-3 months. Cultures were renewed every season by isolation from the inner parts of selected fruit bodies under aseptic conditions on (PDA) or malt agar medium.

#### **3.2. Preparation of spawn :**

##### **3.2.1. Spawn of *Pleurotus* :**

Spawn of *Pleurotus spp.* was prepared according to Mueller, 1983 and Anonymous, 1986. Wheat grains were soaked in boiling water for 30 min., then separated from water and left to cool for 4 h. Grains were mixed thoroughly with calcium carbonate and calcium sulphate added at the ratios of 6% and 3% w/w, respectively. Amended wheat grains were filled in glass bottles 600 ml capacity at the rate of 200 g/bottle and sterilized in autoclave for 1 h. at 125°C.

After sterilization bottles were left to cool and each bottle was then inoculated with 3-5 disks of (PDA) fungus culture. Bottles were incubated at 26-28°C for about 20-30 days (until full growth).

### **3.3. Substrates:**

Agricultural residues which were used as substrate for the cultivation of *Pleurotus spp.* in the present study were : rice straw, wheat straw, maize straw and sugar cane bagasse. Dry and sound (not rotten or decayed) batches of these agricultural wastes were chopped to small particles of about 3-6 cm (Lelley, 1988).

#### **3.3.1. Substrate treatment:**

Two methods were applied for the preparation of the substrates: Pasteurization and semianaerobic fermentation.

##### **3.3.1.1. Pasteurization:**

In this method, substrates were soaked in tap water for 24 h., then the excess water was drained off. The wet substrates were pasteurized with live steam for 4 h., at 70-80°C. The pasteurized substrates were allowed to cool overnight, then spawned and bagged.

##### **3.3.1.2. Semianaerobic fermentation:**

Semianaerobic fermentation was carried out as described by Lelley (1988). The dry chopped substrate was placed in a water tight concrete basin (1x0.8x1 m), pressed with a wooden cover and weighted down with heavy stones. Water was poured into the basin until the water level was about 10-20 cm above the cover. The fermentation was carried out either

in the presence or without the addition of the fungicide benomyl. In the former case, benomyl was added to the water at the beginning of the fermentation at the concentration of 100 ppm (50 ppm. active ingredient). The required quantity of benomyl was calculated and suspended in 3-4 liter of water and then added to fermentation water.

Fermentation continued for 10 days, with the substrate remaining always under water the whole time. The temperature of the fermentation mixture ranged between 15-18°C throughout the fermentation process. Changes in pH of the fermentation mixture as well as microbial counts were determined at 0, 2, 5 and 10 days of the fermentation process. After ten days the water was drained away. The substrate was spread on clean plastic sheets for 24 h. and then spawned and bagged.

### **3.3.2. Supplementation:**

The effect of some supplements to improve the nutritional properties of substrate and maximize the yield of fruit bodies was tested using pasteurized wheat straw as substrate. Supplements tested were rice bran, wheat bran, peat moss, gypsum and a balanced mixture of these supplements. They were sterilized by autoclaving at 120°C for 1 h, left to cool and then added to the substrate just prior to spawning in the rate of 5% w/w. Each treatment was represented by 5 replicats. Yield was expressed as g fruit bodies/bag (4 kg wet substrate) and as biological efficiency (B.E.).

### **3.4. Cultivation procedure:**

#### **3.4.1. Spawning of substrates:**

Spawn was added to the damp substrate at the rate of 4% (if it is not specifically stated) and evenly mixed into the substrate. Spawned substrate was filled into 50 x 35 cm polyethylene bags, which were perforated before to permit enough aeration needed for fungal vegetative growth. The holes were 1 cm wide and spaced 20 cm apart from each other. The upper third of bag was not perforated. Each bag was filled with 4 kg spawned substrate and compressed. Bags were then tied firmly .

#### **3.4.2. Colonization phase:**

Bags were placed in dark at 22-25°C until the substrate was fully colonized by the fungal mycelium (2-3 weeks) contaminated bags were discarded. The relative humidity of the air around the bags ranged from 80-95% (Fig. 1).

#### **3.4.3. Fructification and harvesting:**

After 3 weeks, sound bags were transferred to a cultivation room (a glass house covered with shading net) with temperature of 20-28°C and 80-95% relative humidity. Bags were water atomized whenever needed to facilitate primordia induction.

Fruiting initials (primordia) appeared from the holes of the plastic bags were allowed to grow until maturity (Fig. 2). They were harvested from the substrate when the edges of most of the fruiting bodies in the cluster no longer pointed downwards but were more or less horizontal (Lelley *et al.*, 1988) mature fruiting bodies were gently broken daily and



Fig. (1): Bags of spawned substrate kept in dark at 22-25°C and 80-95% RH.



Fig. (2): Bags in cultivation room at harvesting phase.

weighted. Yield experiments were terminated 80 days after spawning. The total yield of each experimental treatment was calculated and expressed as g fruit bodies/bag (4 kg wet substrate) and as biological efficiency B.E. as the following :

$$\text{B.E.} = \frac{\text{Fresh weight of harvested fruit bodies}}{\text{Dry weight of substrate}}$$

### **3.5. Yield experiments:**

#### **3.5.1. Effect of spawn ratio:**

The effect of spawn ratio on the yield of fruit bodies was tested using three ratios of spawn (2%, 4% and 6% w/w of damp substrate) of either *P. pulmonarius*, *P. ostreatus*, *P. sajor-caju* and *P. cornucopiae*. Substrate used was pasteurized rice straw. Each treatment was represented by 5 replicates. Yield was expressed as g fruit bodies/bag (4 kg wet substrate) and as B.E.

#### **3.5.2. Effect of *Pleurotus spp.*:**

#### **3.5.3. Effect of substrate:**

The effect of *Pleurotus spp.* and that of the substrate on the yield of fruit bodies were tested in a combined experiment. Four *Pleurotus* species were used namely *P. pulmonarius*, *P. ostreatus*, *P. sajor-caju* and *P. cornucopiae*. Substrates tested were pasteurized rice straw, wheat straw, maize straw and sugar cane bagasse. Spawn ratio applied was 4% w/w. Each treatment was represented by 5 replicates. Yield was expressed as g fruit bodies/bag (4 kg wet substrate) and as B.E.



#### **3.5.4. Effect of method of substrate preparation :**

Rice straw, wheat straw, maize straw and sugar cane bagasse were prepared by either pasteurization or semianaerobic fermentation. The latter was carried out either in the presence of the fungicide benomyl (100 ppm) or without benomyl. Treated substrates were spawned (4%) with either *P. pulmonarius*, *P. ostreatus*, *P. sajor-caju* or *P. cornucopiae*. Each treatment was represented by 5 replicates. Yield was expressed as g fruit bodies/bag (4 kg wet substrate) or as B.E. In addition, the ratio of contaminated bags to the total bags in each method was also determined.

#### **3.6. Feeding experiments:**

Feeding experiments were carried out to evaluate the suitability of spent substrates for animal feeding. Experiments were carried out in the experimental Farm of the Department of Animal Production, Faculty of Agriculture, Kafr El-Sheikh, Tanta University .

##### **3.6.1. Feed stuff:**

Spent substrate left after the production of *Pleurotus* mushroom on rice straw was collected, dried and stored. Sample was taken for chemical analysis. Dry matter, crude protein, ether extract, crude fiber and ash were determined.

##### **3.6.2. Experimental animals :**

Three Baladi goats aged two years old and weighted about 30 kg were used to proceed the digestion trials for the evaluation of feed stuff.

### **3.6.3. Digestibility trials:**

To determine the nutritive value of spent substrate, one digestibility trial was carried out. The ration was offered to goats to cover maintenance requirements (1% of body weight of each head spent substrate and the remaining of concentrate ration) according to NRC (1976). Digestibility trial consisted of a 15 days preliminary period followed by a 6 days collection period. Feces samples (10%) were collected, dried at 65°C for 16 h. The daily dried feces samples, from each animal were thoroughly mixed and ground for chemical analysis.

## **3.7. Chemical analysis:**

### **3.7.1. Moisture content:**

Moisture content was determined by drying the sample in an oven at 105°C until constant weight according to (A.O.A.C. 1980).

### **3.7.2. Crude protein :**

Crude protein of stip and cap together usually calculated from the nitrogen content, as determined by the micro-kjeldahl method (Horwitz, 1980), using the conversion factor, ( N x 6.25 ).

Crude protein of substrate and spent was determined by the macro-kjeldahl technique using the conversion factor, ( N x 6.25 ) according to A.O.A.C. (1980).

**3.7.3. Fats:**

Crude fat was determined by Soxhlet procedure similar to basic method described by A.O.A.C. (1980) modified by Chang and Quimio (1982).

**3.7.4. Crude fiber:**

A known weight of the ground sample (2 g) was mixed with sulphuric acid (200 ml 1.25% w/v). The mixture was boiled under reflux condenser for 30 minutes, filtered through a Gooch crucible provided with asbestos mat then thoroughly washed with hot distilled water according to Chang and Quimio (1982).

**3.7.5. Ash:**

The ash content was determined according to the official method (A.O.A.C. 1980) by heating the sample in a muffle at 550°C to constant weight.

**3.7.6. Determination of benomyl residues:**

Carbamates (including benomyl) are anticholinesterases (Casida, 1963). So, determination of benomyl residues based on its effect on acetylcholinesterase activity.

The method of Ellman et al. (1961) was used for assaying the activity of acetylcholinesterase (AChE) in bovine blood. The method is based on the hydrolysis of Acetylthiocholine (ASch) as a substrate by the enzyme (AChE) to produce thiocholine and acetic acid. Thiocholine reacts with dithiobisnitrobenzoate (DTNB) to produce the yellow colour

anion of 5-thio-2-nitrobenzoic acid. The rate of colour formation as a function of enzyme activity is measured spectrophotometrically at 412 nm.

#### **Preparation of samples:**

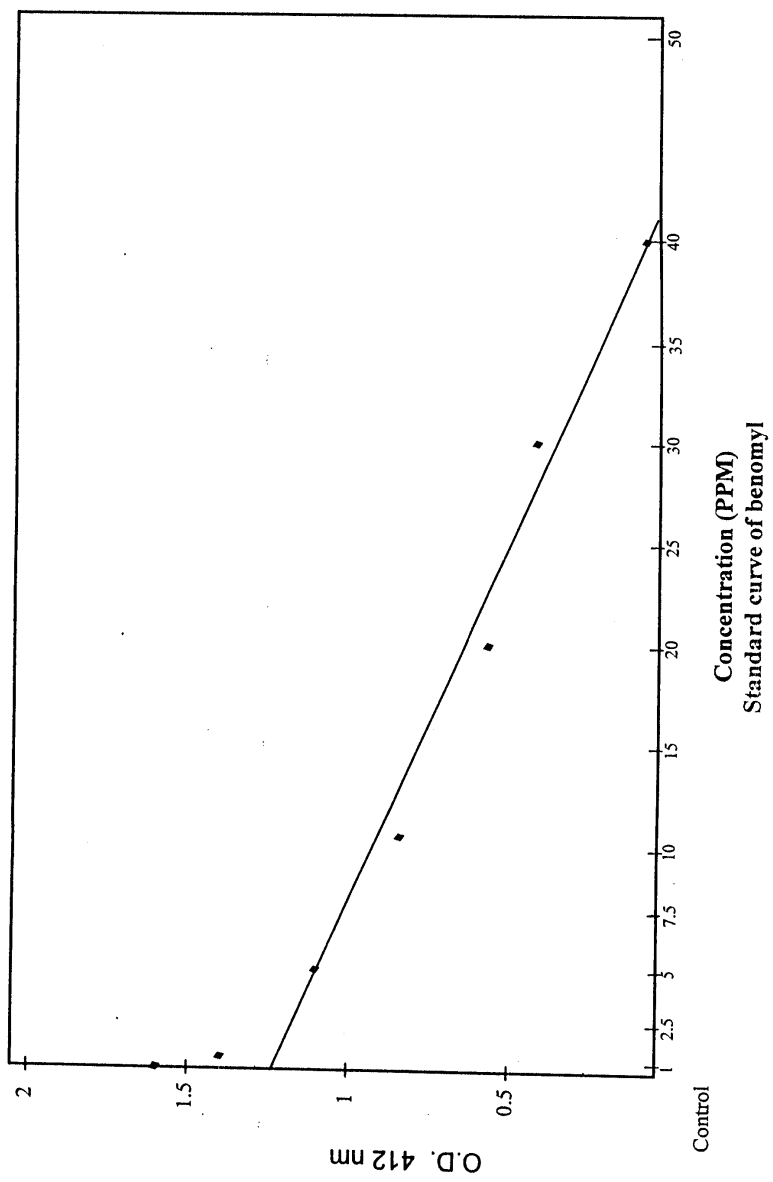
Chloroform ( $\text{CHCl}_3$ ) as a good organic solvent for benomyl (Bleidner et al. (1978) was used to extract benomyl residues from either fermented substrates, spent substrates or fruit bodies. Chloroform was added to samples at the rate of 3:1 (v/w). Samples with chloroform were thoroughly blended at high speed twice for 5 min., and then were filtered on sodium sulphate anhydrous. Obtained chloroform phase was dried under vacuum using rotary evaporator and dried residues were resolved in 1 ml acetone for biochemical determination.

#### **Solutions:**

- i) Buffer phosphate, 0.1 M, pH 8.0.
- ii) Substrate, acetylthiocholine iodide, 0.075 M (21.67 mg/ml).
- iii) Reagent, Dithiobisnitrobenzoic acid (DTNB) 0.01 M of the 5:5-dithiobis-2-nitrobenzoic acid (39.6 mg were dissolved in 10 ml, pH 7.0 phosphate buffer 0.1 M, and 15 mg of sodium bicarbonate were added).

#### **Procedure:**

1. Of a prepared sample, 5  $\mu\text{l}$  were transferred to a test tube and kept until dryness before adding 6 ml of buffer phosphate (pH 8.0, 0.1 M) containing 10  $\mu\text{l}$  from fairly stable suspension of bovine blood. Tubes were incubated at 37°C for 30 min.
2. Exactly 3 ml of the incubated mixture were pipetted into a cuvette.



Control

Concentration (PPM)  
Standard curve of benomyl

3. Of the DTNB reagent, 25  $\mu$ l were added and the cuvette was placed in the photometer. The photometer was adjusted so that the absorbance (at 412 nm) of the suspension in the cuvette was zero.
4. Of the substrate, 20  $\mu$ l were added to this cuvette, and changes in absorbance at 412 nm were recorded for at least 6 min.

The blank consists of buffer, substrate, sample and DTNB solutions. The standard curve was prepared using different concentrations of benomyl. Two tests and one blank were run for each sample. Means of the changes in absorbance/min. (O.D.) were plotted on the standard curve and residues of benomyl were assessed.

### **3.8. Microbiological analysis:**

Counts of bacteria as well as molds and yeasts in the fermentation water during the semianaerobic fermentation were determined. Samples (10 ml) were taken under aseptic conditions from the fermentation water on the 1st, 2nd, 5th and 10th day. Samples were diluted with sterilized tap water up to  $10^{-7}$ .

Counts of bacteria was determined using the poured agar plate technique. Portions of 1 ml of each dilution stage were pipetted into sterile petri dishes and 15-20 ml portions of molten and cooled to about  $45^{\circ}\text{C}$  tryptone glucose yeast agar medium were poured in plates, mixed evenly in and left to solidify. After solidification the medium, plates were incubated in an inverted position for 2-3 days at  $30^{\circ}\text{C}$ , count of bacteria was determined from the number of colonies on each of 5 plates per

dilution. Only plates exhibiting between 30-300 colonies were used. the mean value was calculated and then multiplied by the dilution factor.

Count of molds and yeasts was determined by spreading of 0.1 ml portions of each dilution stage into petri dishes containing sterilized malt agar medium. Five plates were used per dilution. Plates were incubated at 25°C for 4-5 days. Colonies of molds and yeasts were counted, the mean value was calculated and multiplied by the dilution factor.

### 3.9. Media:

#### 3.9.1. Malt agar medium:

	g per liter
Malt extract	30.0 g
Peptone	3.0 g
Agar agar	14.0 g
pH	4.0±0.1

#### 3.9.2. Potato dextrose agar (PDA):

	g per liter
Potato	200.0 g
Dextrose	20.0 g
Agar agar	14.0 g
pH	7.0±0.1

#### 3.9.3. Tryptone glucose yeast agar medium:

	g per liter
Peptone from casein	5.0 g
Yeast extract	2.5 g
D (+) glucose	1.0 g
Agar agar	14.0 g
pH	7.0±0.1

**3.10. Statistical analysis:**

Experiments were designed as complete randomized and obtained data were statistically analyzed according to the methods of Gomez & Gomez (1984) using the least significant differences (at  $P < 0.05$ ), means were compared.



## 4. Experimental Results

### 4.1. Yield of fruit bodies of *Pleurotus* spp.:

#### 4.1.1. Effect of spawn ratio on the yield of fruit bodies:

Data in Table (1) and illustrated in Fig. (1) represent the influence of spawn ratio on the yield of fruit bodies of *P. pulmonarius*, *P. ostreatus*, *P. sajor-caju* and *P. cornucopiae* grown on rice straw. Results are expressed as biological efficiency and as g fruit bodies/bag containing 4 kg substrate.

Results indicate that increasing the spawn ratio from 2 to 4 and 6% of the substrate significantly increased the yield of fruit bodies (B.E. and g/bag) of the tested *Pleurotus* spp. However, *P. cornucopiae* was an exception, as it did not respond similarly to the higher spawn ratio. It is worthy to note that the first flushes of fruit bodies appeared earlier at higher rather than at lower spawn ratios.

The highest yield of fruit bodies recorded at 2% spawn ratio was that of *P. cornucopiae* (B.E. 0.83 ; 625 g/bag) whereas the lowest was that of *P. sajor-caju* (B.E. 0.49 ; 365 g/bag); *P. pulmonarius* recorded the highest yield at both 4% and 6% spawn ratios (B.E. 1.08 ; 813 g/bag and B.E. 1.24 ; 930 g/bag), whereas *P. sajor-caju* recorded the lowest yield at the same spawning ratios (B.E. 0.66 ; 498 g/bag and B.E. 0.74 ; 563 g/bag).

To evaluate the yield increase affected by increasing the spawn ratio, means of yield attained at 2, 4 and 6% were compared. Increasing

Table (1). Effect of spawn ratio on the yield of fruit bodies of *Pleurotus* spp. grown on rice straw.

Spawn ratio	2%		4%		6%		Mean	
	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag
<i>P. pulmonarius</i>	0.68	513	1.08	813	1.24	930	1.00	752
<i>P. ostreatus</i>	0.56	420	0.76	571	0.88	662	0.73	551
<i>P. sajor-caju</i>	0.49	365	0.66	498	0.74	563	0.63	475
<i>P. cornucopiae</i>	0.83	625	0.78	582	0.90	677	0.83	628
<b>Mean</b>	0.64	481	0.82	616	0.94	708	0.80	602

(B.E.) LSD 0.05  
 Species = 0.13  
 Ratio = 0.11  
 Species x Ratio = 0.22

(Yield) LSD 0.05  
 Species = 95.66  
 Ratio = 82.84  
 Species x Ratio = 165.69

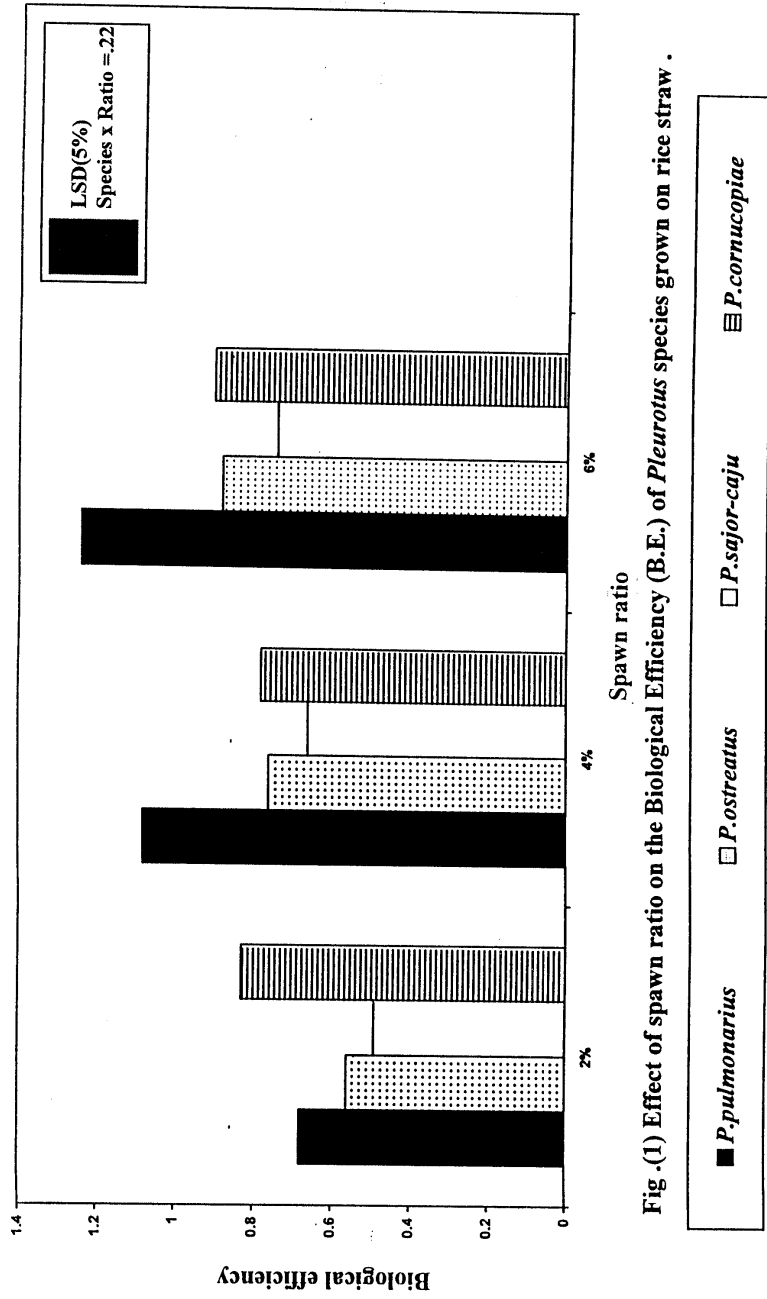


Fig. (1) Effect of spawn ratio on the Biological Efficiency (B.E.) of *Pleurotus* species grown on rice straw .

spawn ratio from 2 to 4% resulted in about 28% yield increase. However, increasing spawn ratio from 4 to 6% was less effective, as it increased the yield by about 14%.

Therefore, 4% spawn ratio seemed to be more suitable in the light of cost-benefit ratio, and was used in the next experiments.

#### **4.1.2. Performance of different *Pleurotus spp.* grown on different substrates:**

Data presented in Table (2) and illustrated in Fig. (2) represent the yield of fruit bodies (B.E. and g/bag) of *P. pulmonarius*, *P. ostreatus*, *P. sajor-caju* and *P. cornucopiae* grown on rice straw, wheat straw, maize straw and sugar cane bagasse. Substrate were prepared by pasteurization and spawned at 4%.

Results indicate that all the tested agricultural wastes can be used as substrates for the production of fruit bodies of *Pleurotus spp.* However, rice straw, wheat straw and maize straw were more suitable than sugar cane bagasse for growing and production of fruit bodies of *Pleurotus* mushroom. The mean biological efficiencies achieved using rice straw, wheat straw and maize straw were not significantly different (0.93, 0.91 and 1.10), whereas the mean biological efficiency achieved using sugar cane bagasse was significantly lower (0.53). The same trend was noticed in relation to achievable fruit bodies per bag containing 4 kg substrate. It reached 718, 727 and 1028 g fruit bodies/bag containing 4 kg substrate of rice straw, wheat straw and maize straw, respectively, while it reached 335 g/bag containing 4 kg of sugar cane bagasse .

Table (2). Yield of fruit bodies of different *Pleurotus* species grown on different agricultural wastes.

Substrates	Rice straw		Wheat straw		Maize straw		Sugar cane bagasse		Mean	
	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag
<i>P. pulmonarius</i>	1.15	863	0.90	717	1.11	1035	0.56	355	0.93	743
<i>P. ostreatus</i>	0.96	744	0.77	613	1.04	973	0.33	210	0.78	635
<i>P. sajor-caju</i>	0.65	488	1.03	820	0.49	457	0.48	305	0.66	518
<i>P. cornucopiae</i>	0.98	775	0.96	757	1.77	1645	0.75	470	1.12	912
<b>Mean</b>	0.93	718	0.91	727	1.10	1028	0.53	335	0.87	702

(B.E.) LSD 0.05  
Species = 0.20  
Substrates = 0.20  
Species x Substrate = 0.41

(Yield) LSD 0.05  
Species = 157.6  
Substrates = 157.6  
Species x Substrates = 315.24

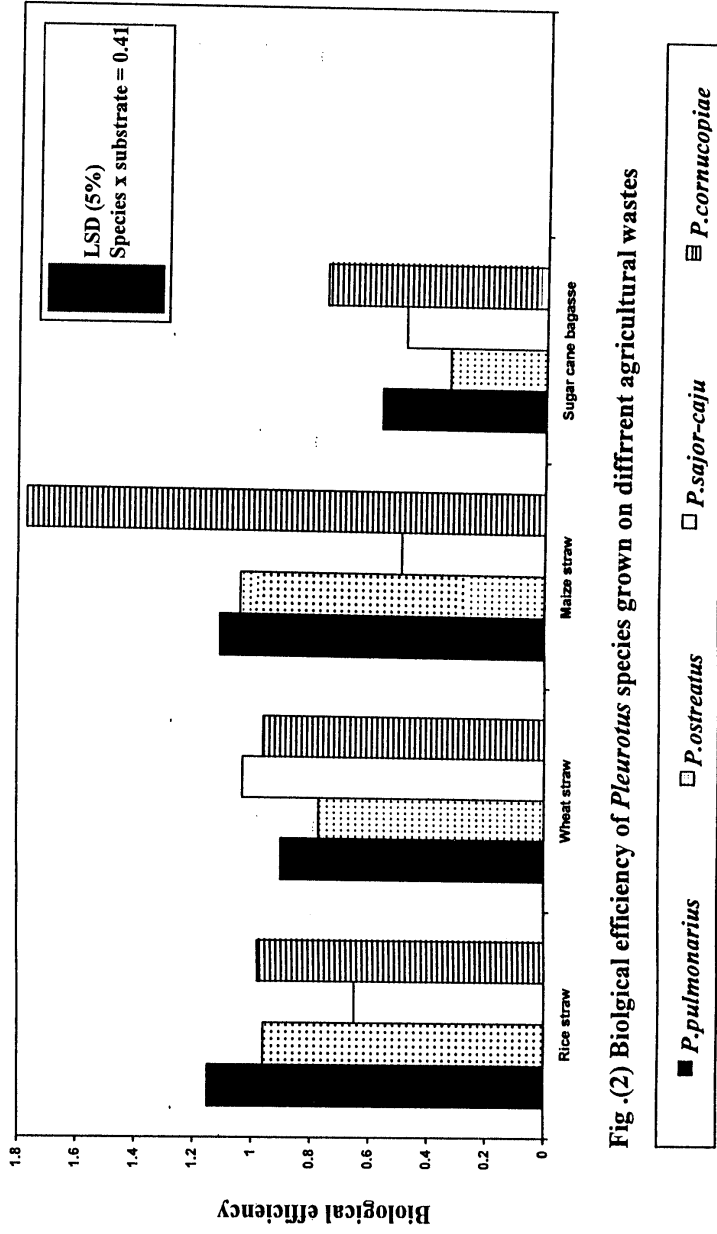


Fig.(2) Biological efficiency of *Pleurotus* species grown on different agricultural wastes

Also the performance of the tested *Pleurotus species* varied significantly on the different substrates. Significant species x substrate interactions were observed. The highest yield (B.E. 1.77 ; 1645 g/bag containing 4 kg substrate) attained was that of *P. cornucopiae* grown on maize straw, whereas the lowest was that of *P. ostreatus* grown on sugar cane bagasse (B.E. 0.33 ; 210 g/bag containing 4 kg substrate).

Comparing the mean values of yield achieved by the tested *Pleurotus species* reveals that *P. cornucopiae* achieved the highest mean yield of fruit bodies (B.E. 1.12 and 912 g/bag containing 4 kg substrate). It was significantly higher than the means of all tested *Pleurotus species*. The lowest mean yield was that of *P. sajor-caju* (B.E. 0.66 ; 518 g/bag containing 4 kg substrate), which was not significantly different from that of *P. ostreatus* (B.E. 0.78 ; 635 g/bag containing 4 kg substrate).

#### **4.1. 3. Effect of different supplements on the yield of fruit bodies:**

Data in Table (3) and illustrated in Fig. (3) represent the effect of some supplements on the yield of fruit bodies of *P. pulmonarius*, *P. ostreatus*, *P. sajor-caju* and *P. cornucopiae* grown on wheat straw. Supplements were autoclaved and added to pasteurized substrate at 5% ratio just prior to spawning.

Results indicate that supplements tested varied in their effect on yield of fruit bodies. The addition of either rice bran or wheat bran significantly increased the yield of fruit bodies of all tested *Pleurotus*

Table (3). Effect of different supplements on yield of fruit bodies of *Pleurotus* species grown on wheat straw.

Species	<i>P. pluronarius</i>		<i>P. ostreatus</i>		<i>P. sajor-caju</i>		<i>P. cornucopiae</i>		Mean	
	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag
Rice bran 5%	0.72	750	1.07	855	0.94	750	1.53	1225	1.07	895
Wheat bran	0.68	540	0.75	597	0.81	650	0.96	765	0.80	638
Peat moss	0.71	570	0.52	415	0.59	475	0.83	660	0.66	530
Gypsum	0.41	331	0.55	437	0.31	250	0.58	462	0.46	370
Mixture	0.52	413	0.46	365	0.64	513	0.79	630	0.60	480
Control	0.72	578	0.64	511	0.67	534	0.65	517	0.67	535
Mean	0.63	530	0.67	530	0.66	529	0.89	710	0.71	575

(B.E.) LSD 0.05  
 Species = 0.08  
 Supplements = 0.09  
 Supplements x species= 0.19

(Fruit bodies) LSD 0.05  
 Species = 61.12  
 Supplements = 74.85  
 Supplements x species= 149.70



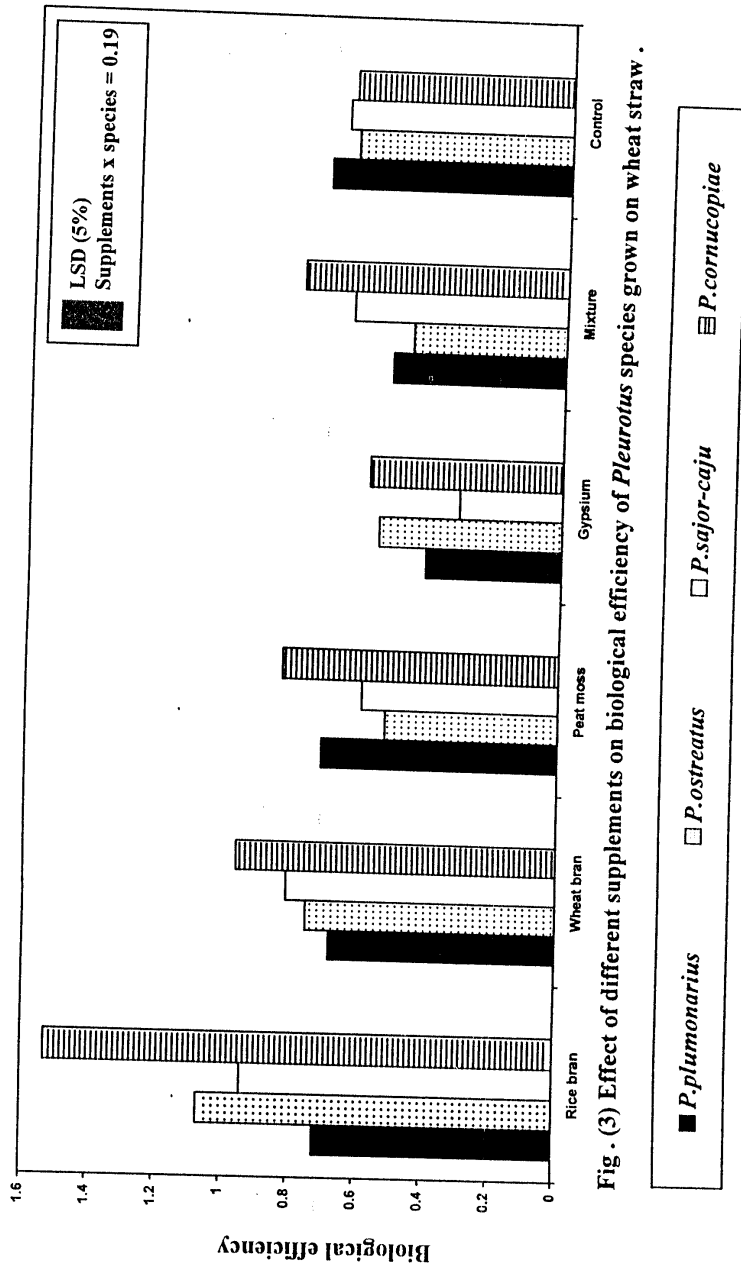


Fig. (3) Effect of different supplements on biological efficiency of *Pleurotus* species grown on wheat straw .

species compared with the other supplements and with the non supplemented substrate (control). Addition of 5% rice bran to wheat straw substrate increased the biological efficiency from 0.67 (non supplemented control) to 1.07, which represent an increase in yield of about 67.0%. The increase of yield affected by the addition of 5% wheat bran was less pronounced, though still significant ; the biological efficiency increased from 0.67 to 0.80, which represent an increase in yield of about 19%.

Addition of 5% of either peat moss or a balanced mixture of all supplements tested did not significantly affect the yield of fruit bodies. However, amendment of wheat straw with 5% gypsum significantly decreased the yield of fruit bodies of all *Pleurotus species* as compared to non supplemented wheat straw (control) and other supplements. The biological efficiency decreased from 0.67 to 0.46 which corresponds to a decrease in yield of about 31%.

The highest yield recorded in the present experiment was that of *P. cornucopiae* grown on wheat straw supplemented with rice bran (1.53 and 1225 g/bag containing 4 kg substrate). The lowest yield was that of *P. sajor-caju* on wheat straw supplemented with gypsum (B.E. 0.31 and 250 g/bag containing 4 kg substrate). It is worthy to note that comparing the means of yield achieved by the tested *Pleurotus species* in this experiment reveals again that *P. cornucopiae* was the most efficient species. The means of yield achieved by *P. cornucopiae* were significantly higher and surpassed by far those of the other *Pleurotus species*.

#### 4.1.4. Effect of method used for substrate preparation on yield of fruit bodies:

##### 4.1.4.1. Substrates prepared by semianaerobic fermentation in the presence of benomyl:

Data in Table (4) and illustrated in Fig. (4) represent the yield of fruit bodies of *P. pulmonarius*, *P. ostreatus*, *P. sajor-caju* and *P. cornucopiae* grown on rice straw, wheat straw, maize straw and sugar cane bagasse, which had been prepared by semianaerobic fermentation (10 days) in the presence of 100 ppm benomyl (50% active ingredient). Spawning ratio used was 4%.

Results indicate that rice straw was more suitable for the production of fruit bodies than the other tested substrates. The mean values of biological efficiency (0.95) and fruit bodies /bag containing 4 kg substrate (711 g) achieved on rice straw were significantly higher than those achieved on other substrates. Sugar cane bagasse gave the lowest mean values of biological efficiency (0.51) and of fruit bodies /bag (322 g), though not significantly different from those achieved on wheat straw or maize straw.

Results also indicate that *P. cornucopiae* was more efficient than the other tested *Pleurotus species*. The mean values of biological efficiency (0.82) and of fruit bodies /bag containing 4 kg substrate (605 g) achieved by *P. cornucopiae* were significantly higher than those of *P. ostreatus* and *P. sajor-caju*, but was not significantly different from that of *P. pulmonarius*.

Table (4). Yield of fruit bodies of *Pleurotus* species grown on different substrates prepared by semianaerobic fermentation in the presence of 100 ppm benomyl.

Substrates	Rice straw		Wheat straw		Maize straw		Sugar cane bagasse		Mean	
	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag
<i>P. pulmonarius</i>	1.06	795	0.72	578	0.59	555	0.49	307	0.72	559
<i>P. ostreatus</i>	0.81	607	0.64	511	0.58	538	0.52	327	0.64	496
<i>P. sajor-caju</i>	0.94	705	0.67	534	0.52	498	0.51	322	0.66	515
<i>P. cornucopiae</i>	0.98	736	0.65	417	0.78	730	0.85	535	0.82	605
<b>Mean</b>	0.95	711	0.67	510	0.62	580	0.59	373	0.71	543

(B.E.) LSD 0.05

Species = 0.10

Substrates = 0.10

Species x Substrate = 0.20

(Yield) LSD 0.05

Species = 76.05

Substrates = 76.05

Species x Substrates = 152.1

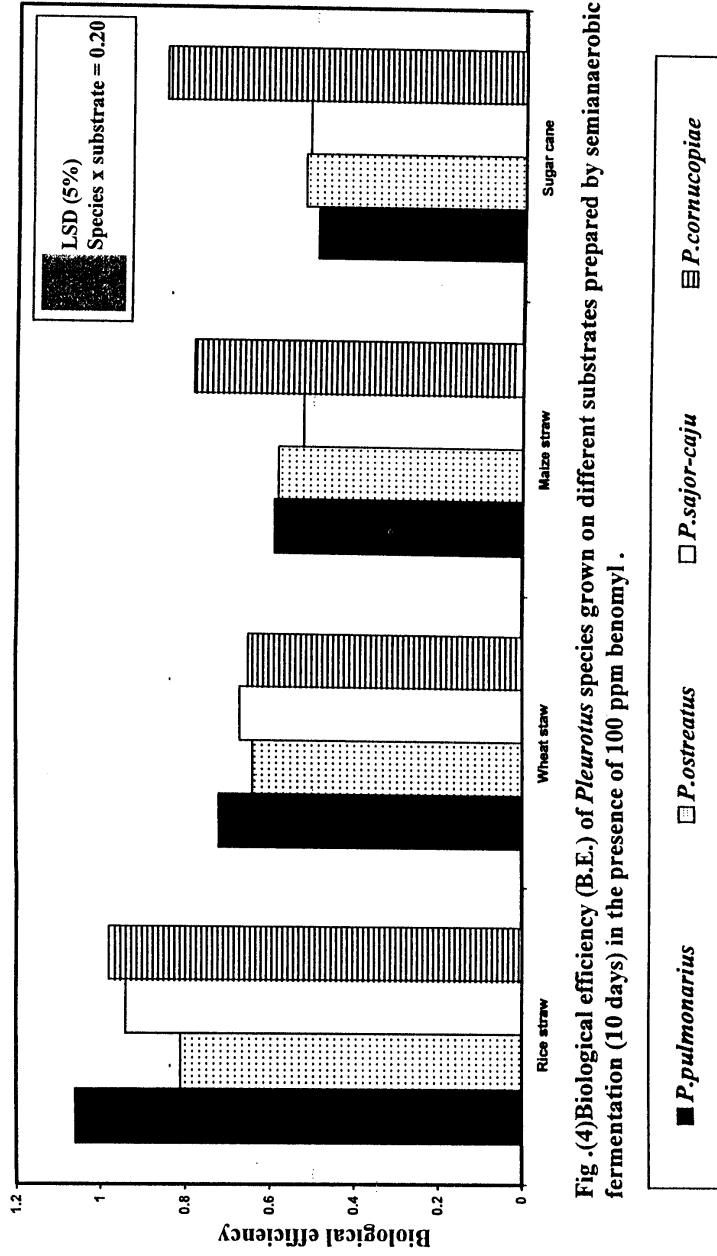


Fig.(4) Biological efficiency (B.E.) of *Pleurotus* species grown on different substrates prepared by semianaerobic fermentation (10 days) in the presence of 100 ppm benomyl.

The highest yield of fruit bodies recorded on substrates prepared by semianaerobic fermentation in the presence of benomyl was that of *P. pulmonarius* grown on rice straw (B.E. 1.06 ; 795 g fruit bodies /bag containing 4 kg substrate). The lowest, however, was that of *P. sajor-caju* grown on sugar cane bagasse (B.E. 0.51 ; 322 g fruit bodies /bag containing 4 kg substrate).

#### **4.1.4.2. Substrates prepared by semianaerobic fermentation without benomyl:**

Data in Table (5) and illustrated in Fig. (5) represent the yield of fruit bodies of *P. pulmonarius*, *P. ostreatus*, *P. sajor-caju* and *P. cornucopiae* grown on rice straw, wheat straw, maize straw and sugar cane bagasse, which had been prepared by semianaerobic fermentation (10 days) without the addition of the fungicide benomyl. Spawning ratio used was 4%.

Results indicate again that rice straw was more suitable for the production of fruit bodies than the other tested substrates. The mean values of biological efficiency (0.82) and fruit bodies /bag containing 4 kg substrate (615 g) achieved on rice straw were significantly higher than those achieved on other substrates. Also sugar cane bagasse gave the lowest mean values of biological efficiency (0.48 and of fruiting bodies /bag (305 g).

No significant difference could be detected between the performance of the tested *Pleurotus species* grown on substrates prepared by semianaerobic fermentation without the fungicide benomyl.

Table (5). Yield of fruit bodies of *Pleurotus* species grown on different substrates prepared by semianaerobic fermentation without benomyl.

Substrates	Rice straw		Wheat straw		Maize straw		Sugar cane bagasse		Mean	
	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag	B.E.	Fruit bodies g/bag
<i>P. pulmonarius</i>	1.08	813	0.59	474	0.58	540	0.37	230	0.65	514
<i>P. ostreatus</i>	0.76	568	0.63	506	0.56	526	0.36	227	0.58	457
<i>P. sajor-caju</i>	0.66	498	0.72	573	0.46	424	0.44	280	0.57	444
<i>P. cornucopiae</i>	0.78	582	0.48	387	0.62	580	0.77	483	0.66	508
Mean	0.82	615	0.61	485	0.56	518	0.48	305	0.62	481

(B.E.) LSD 0.05 (Yield) LSD 0.05  
 Species = 0.086 Species = 67.45  
 Substrates = 0.086 Substrates = 67.45  
 Species x Substrate = 0.17 Species x Substrates = 134.91

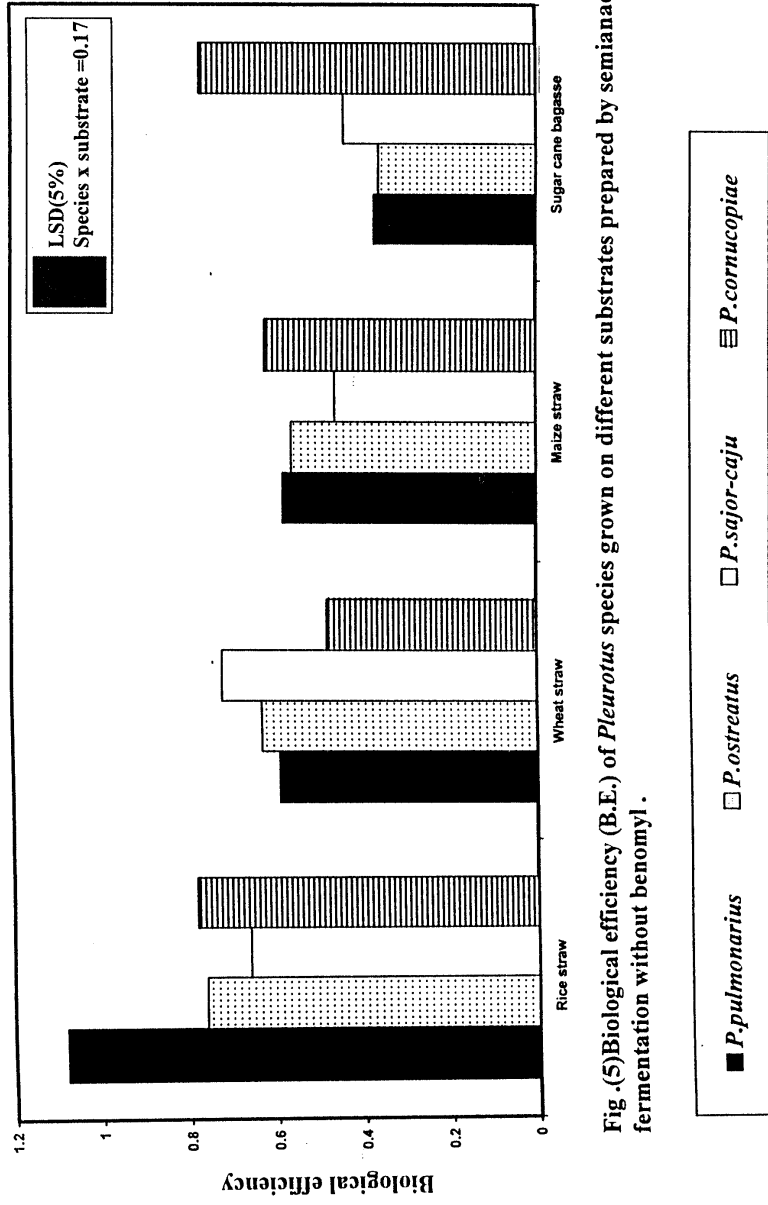
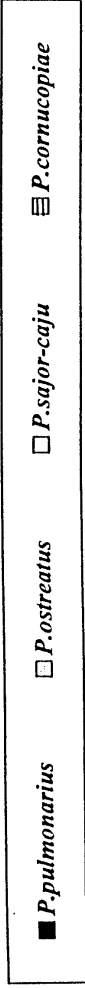


Fig. (5) Biological efficiency (B.E.) of *Pleurotus* species grown on different substrates prepared by semianaerobic fermentation without benomyl.





The highest yield of fruit bodies recorded on substrates prepared by semianaerobic fermentation without benomyl was that of *P. pulmonarius* grown on rice straw (B.E. 1.08 ; 813 g fruit bodies /bag containing 4 kg substrate). The lowest yield was that of *P. ostreatus* grown on sugar cane bagasse (B.E. 0.36 ; 227 g fruit bodies /bag containing 4 kg substrate).

#### **4.1.4.3. Changes of pH and microbial counts in fermentation liquid during the semianaerobic fermentation:**

##### **4.1.4.3.1. Changes of pH:**

The pH of the fermentation liquid was measured daily during the semianaerobic fermentation of rice straw, wheat straw, maize straw and sugar cane bagasse either in the presence or absence of benomyl. Results presented in Table (6) and illustrated in Fig. (6) show that a slight acidification of the substrates took place during the semianaerobic fermentation. The decrease of pH value took a wave-like pattern ; and seemed not being affected by the presence of benomyl. In case of rice straw fermented in the presence of 100 ppm benomyl the pH decreased from 7.3 to 5.91 in the second day. It increased again to 6.7 on the third day to decrease again on the fifth day and so on. The same wave like decrease of pH was also observed with or without benomyl during the semianaerobic fermentation of wheat straw and maize straw.

Noteworthy is the characteristic low pH of sugar cane bagasse. After an initial decrease of pH from 7.1 to 4.3 - 4.7 in the first day, no further decrease could be noticed.

**Table (6). Values of pH measured during semianaerobic fermentation of some substrates.**

Treatments	Rice straw		Wheat straw		Maize straw		Sugar cane bagasse	
	Benomyl	Without Benomyl	Benomyl	Without Benomyl	Benomyl	Without Benomyl	Benomyl	Without Benomyl
0	7.30	7.30	7.50	7.50	7.30	7.40	6.10	6.20
1	6.84	6.80	6.74	6.73	6.16	6.02	4.31	4.70
2	5.88	5.91	5.31	5.29	5.39	5.43	4.20	4.64
3	6.69	6.70	6.35	6.31	5.84	5.86	4.47	4.60
4	6.01	6.07	5.39	5.64	5.91	5.89	4.47	4.55
5	5.81	5.78	5.51	5.71	6.02	5.95	4.46	4.50
6	5.94	5.95	5.75	6.06	6.01	5.93	4.37	4.49
7	5.91	6.01	5.72	5.92	5.76	5.63	4.54	4.53
8	5.97	5.93	5.61	5.77	5.47	5.41	4.33	4.43
9	6.02	5.71	5.61	6.20	5.33	5.23	4.21	4.30
10	6.33	6.52	6.00	5.81	5.27	5.37	4.50	4.75

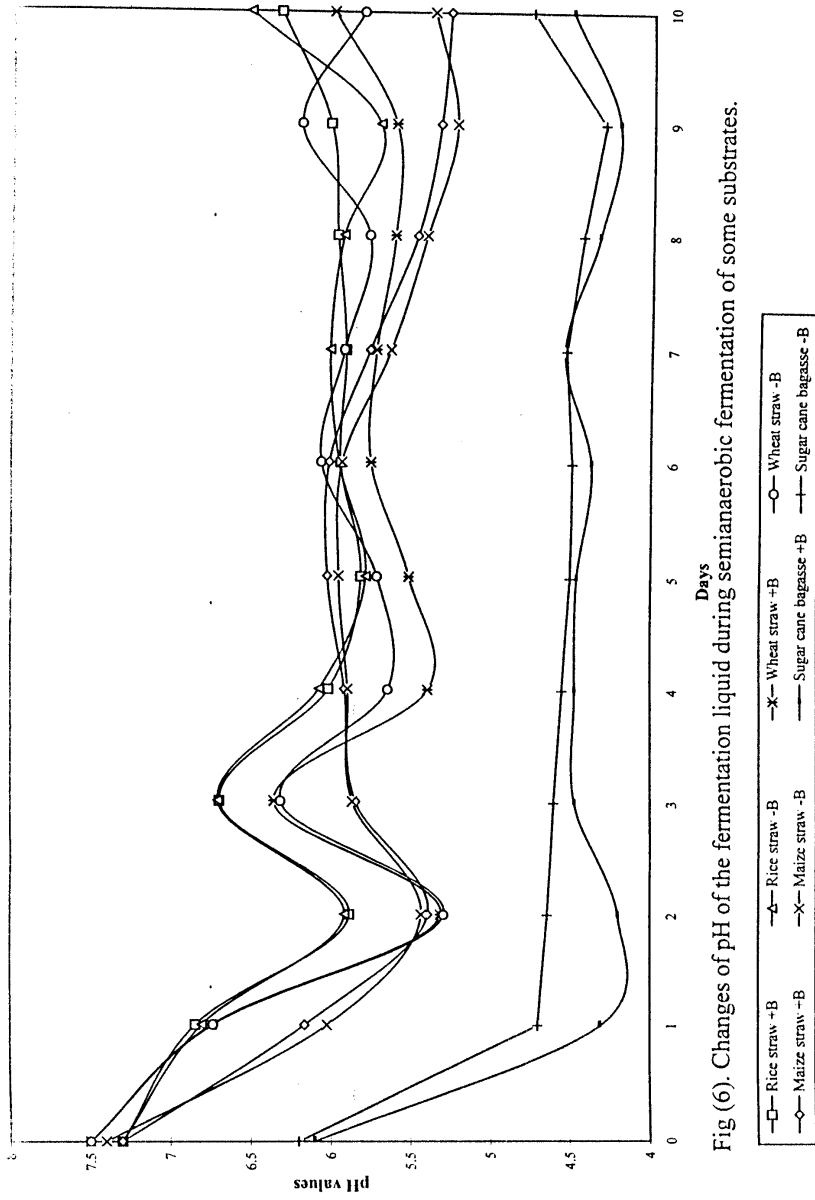


Fig (6). Changes of pH of the fermentation liquid during semianaerobic fermentation of some substrates.

#### 4.1.4.3.2. Changes of microbial counts:

Data presented in Tables (7-10) and illustrated in Fig. (7) represent bacterial counts as well as counts of fungi and yeasts attained in the fermentation liquid during semianaerobic fermentation of rice straw, wheat straw, maize straw and sugar cane bagasse either in the presence or absence of benomyl.

Results indicate that counts of bacteria as well as those of fungi and yeasts increased in the first 2 days of the fermentation process and then began to decrease up to the 10 days. This phenomenon was observed during the semianaerobic fermentation of all substrates tested. Although the same pattern was also observed in the presence of the fungicide benomyl, the counts of bacteria, fungi and yeasts attained in the presence of benomyl were markedly less than those attained without benomyl.

During the semianaerobic fermentation of rice straw, for example counts of bacteria increased in the first two days from  $4 \times 10^6$  CFU/ml to  $4 \times 10^7$  CFU/ml in the absence of benomyl. Counts of bacteria decreased gradually thereafter and reached  $2.3 \times 10^6$  CFU/ml at the end of the fermentation process. In the presence of benomyl, counts of bacteria increased in the first 2 days from  $3.1 \times 10^4$  CFU/ml to  $1.7 \times 10^7$  CFU/ml, then decreased gradually and reached  $2.7 \times 10^6$  CFU/ml at the end of the fermentation process.

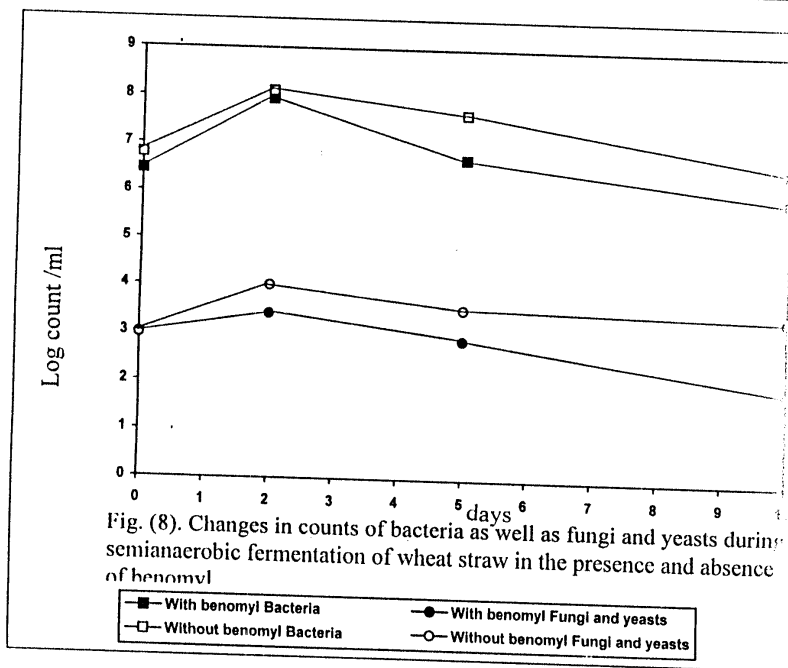
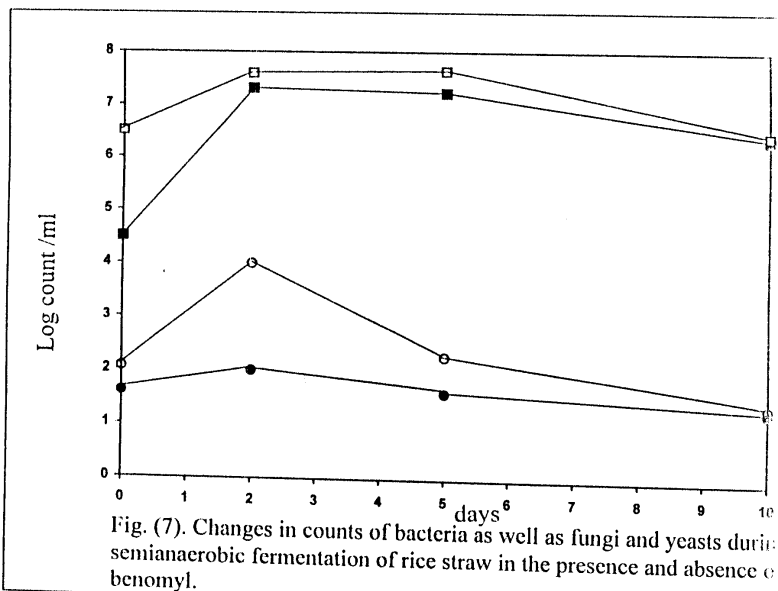
Counts of fungi and yeasts attained during semianaerobic fermentation were less, by far, than bacterial counts, except in case of sugar cane bagasse, where counts of fungi and yeasts were comparable

**Table (7). Microbial counts of bacteria, fungi and yeasts attained during semianaerobic fermentation of rice straw.**

Treatments	Days	0	2	5	10
	Microorganisms				
With benomyl	Bacteria	$3.10 \times 10^4$	$2.00 \times 10^7$	$1.70 \times 10^7$	$2.34 \times 10^6$
	Fungi and yeasts	$0.40 \times 10^2$	$1.00 \times 10^2$	$0.40 \times 10^2$	$0.20 \times 10^2$
Without benomyl	Bacteria	$4.00 \times 10^6$	$4.00 \times 10^7$	$1.10 \times 10^7$	$2.70 \times 10^6$
	Fungi and yeasts	$1.15 \times 10^2$	$1.01 \times 10^4$	$1.90 \times 10^2$	$0.25 \times 10^2$

**Table (8). Microbial counts of bacteria, fungi and yeasts attained during semianaerobic fermentation of wheat straw.**

Treatments	Days	0	2	5	10
	Microorganisms				
With benomyl	Bacteria	$2.85 \times 10^6$	$8.95 \times 10^7$	$5.20 \times 10^6$	$8.60 \times 10^5$
	Fungi and yeasts	$9.05 \times 10^2$	$2.55 \times 10^3$	$0.77 \times 10^3$	$0.75 \times 10^2$
Without benomyl	Bacteria	$6.00 \times 10^6$	$1.29 \times 10^8$	$4.60 \times 10^7$	$3.42 \times 10^6$
	Fungi and yeasts	$9.65 \times 10^2$	$1.01 \times 10^4$	$3.40 \times 10^3$	$0.26 \times 10^4$



**Table (9). Microbial counts of bacteria, fungi and yeasts attained during semianaerobic fermentation of maize straw.**

Treatments	Days	0	2	5	10
	Microorganisms				
With benomyl	Bacteria	$8.75 \times 10^5$	$1.59 \times 10^7$	$2.80 \times 10^6$	$1.33 \times 10^6$
	Fungi and yeasts	$0.10 \times 10^4$	$2.62 \times 10^4$	$2.45 \times 10^4$	$0.40 \times 10^2$
Without benomyl	Bacteria	$4.47 \times 10^6$	$2.25 \times 10^7$	$1.23 \times 10^7$	$6.05 \times 10^6$
	Fungi and yeasts	$4.60 \times 10^4$	$2.32 \times 10^5$	$1.20 \times 10^5$	$0.35 \times 10^4$

**Table (10). Microbial counts of bacteria, fungi and yeasts attained during semianaerobic fermentation of sugar cane bagasse.**

Treatments	Days	0	2	5	10
	Microorganisms				
With benomyl	Bacteria	$2.88 \times 10^6$	$4.82 \times 10^7$	$2.70 \times 10^7$	$2.06 \times 10^7$
	Fungi and yeasts	$1.35 \times 10^5$	$3.04 \times 10^6$	$3.35 \times 10^6$	$2.25 \times 10^5$
Without benomyl	Bacteria	$1.23 \times 10^7$	$1.06 \times 10^8$	$3.90 \times 10^7$	$2.27 \times 10^7$
	Fungi and yeasts	$1.36 \times 10^6$	$8.22 \times 10^6$	$4.40 \times 10^6$	$1.59 \times 10^6$

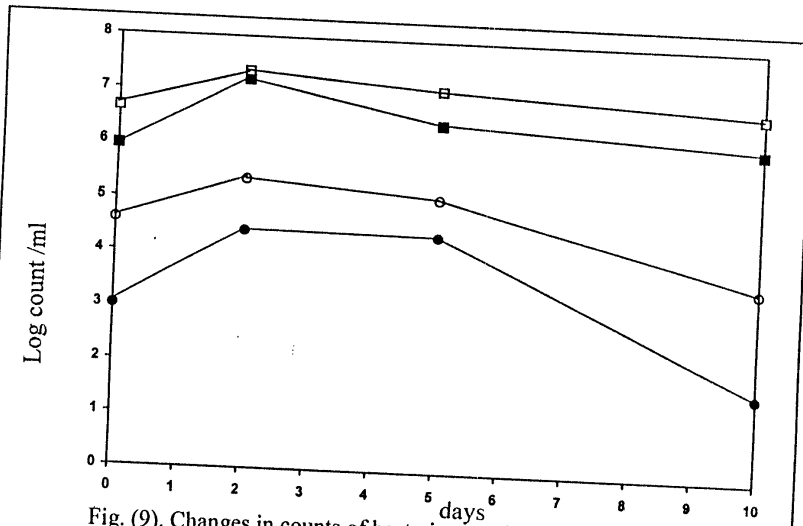


Fig. (9). Changes in counts of bacteria as well as fungi and yeasts during semianaerobic fermentation of maize straw in the presence and absence of benomyl.

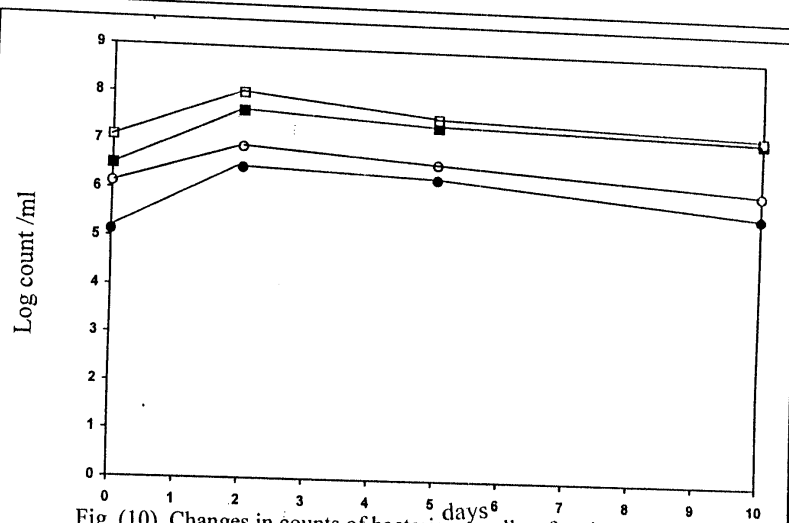


Fig. (10). Changes in counts of bacteria as well as fungi and yeasts during semianaerobic fermentation of sugar cane bagasse in the presence and absence of benomyl.

■ With benomyl Bacteria	● With benomyl Fungi and yeasts
□ Without benomyl Bacteria	○ Without benomyl Fungi and yeasts



with those of bacteria. However, change in counts of fungi and yeasts during the fermentation process followed in general the same pattern as in bacteria ; i.e. an increase in the first two days followed by a gradual decrease up to the end of the fermentation process.

#### **4.1.4.4. Residues of benomyl in substrates, fruit bodies and spent:**

Rice straw, wheat straw, maize straw and sugar cane bagasse were fermented semianaerobically for 10 days in the presence of 100 ppm benomyl (50% active ingredient). At the end of the fermentation, residues of benomyl in substrates were determined, and substrates were spawned with either *P. pulmonarius*, *P. ostreatus*, *P. sajor-caju* or *P. cornucopiae*. Residues of benomyl were determined also in the early flushes of fruit bodies, and in spent substrates left at the end (80 days after spawning).

Results in Table (11) show that the fermented substrates contained 5.37-13.09 ppm benomyl at the end of the fermentation process which lasted for 10 days. However, no residues of benomyl could be detected in fruit bodies produced on these substrates. Since the sensitivity of the determination method lays at 0.1 ppm of benomyl, it seems therefore justified to assume that fruit bodies of *Pleurotus species* grown on such substrates, would contain less than 0.1 ppm benomyl. Spent substrates left at the end of experiment (80 days after spawning) showed a benomyl content ranging from 0.67-0.97 ppm.

**Table (11). Residues of benomyl in substrates fermented semi - anaerobically in fruit bodies grown on the fermented substrates and in spent left after harvesting.**

Sample	Benomyl ppm (mg/kg)
<b>1. Substrates at the end of 10 days fermentation in the presence of 100 ppm benomyl:</b>	
Rice straw	13.09
Wheat straw	10.29
Maize straw	10.15
Sugar cane bagasse	5.37
<b>2. Fruit bodies grown on the fermented substrates:</b>	
<i>P. pulmonarius</i>	< 0.1
<i>P. ostreatus</i>	<0.1
<i>P. sajor-caju</i>	<0.1
<i>P. cornucopiae</i>	<0.1
<b>3. Spent substrates left after harvesting:</b>	
Rice straw	0.97
Wheat straw	0.76
Maize straw	0.91
Sugar cane bagasse	0.67

#### **4.1.4.5. Comparison between yields of fruit bodies of *Pleurotus* grown on substrates prepared by different methods:**

Data in Table (12) and illustrated in Fig. (12) represent the yield of fruit bodies of *P. pulmonarius*, *P. ostreatus*, *P. sajor-caju* and *P. cornucopiae* grown on rice straw, wheat straw, maize straw and sugar cane bagasse, which had been prepared by pasteurization, or by semianaerobic fermentation either in the presence or absence of benomyl.

Results indicate that preparation the substrate with pasteurization resulted in a significant increase in yield of fruit bodies compared with semianaerobic fermentation. The mean values of biological efficiencies and fruit bodies /bag achieved on pasteurized substrate (B.E. 0.87 ; 702 g fruit bodies /bag containing 4 kg substrate) were significantly higher than those achieved on substrates prepared by either semianaerobic fermentation in the presence of benomyl (B.E. 0.71 ; 549 g fruit bodies /bag containing 4 kg substrate) or without benomyl (B. E. 0.62 ; 468 g fruit bodies /bag containing 4 kg substrate). The highest yield obtained by using pasteurization was that of *P. cornucopiae* on maize straw (B. E. 1.77 ; 1645 g /bag containing 4 kg substrate).

On the other hand, fermentation of substrates in the presence of benomyl resulted in significant increase in yield of fruit bodies compared with substrates fermented without benomyl. The biological efficiency increased from 0.62 to 0.71 and the yield per bag from 468 g to 549 g.

It is worthy to state that yield results considered were those of sound bags in each treatment. Contaminated bags were discarded. A

Table (12). Comparison between yield of fruit bodies of *Pleurotus* species as affected by methods of substrates preparation.

Species	Substrates	Pasteurization		Semianacrobic fermentation				Mean	
		B.E.	Fruit bodies g /bag	Treated with benomyl		untreated with benomyl		B.E.	Fruit bodies g /bag
				B.E.	Fruit bodies g /bag	B.E.	Fruit bodies g /bag		
<i>P. pulmonarius</i>	rice straw	1.15	863	1.06	795	1.08	813		
	wheat straw	0.90	717	0.72	578	0.58	474		
	maize straw	1.11	1035	0.60	555	0.58	540		
	sugar cane bagasse	0.56	355	0.49	307	0.36	230		
	Mean	0.93	743	0.72	559	0.65	514		
<i>P. ostreatus</i>	rice straw	0.96	744	0.81	607	0.76	568		
	wheat straw	0.77	613	0.64	511	0.63	506		
	maize straw	1.04	973	0.58	538	0.56	526		
	sugar cane bagasse	0.33	210	0.52	327	0.36	227		
	Mean	0.78	635	0.64	496	0.85	457		
<i>P. sajor-caju</i>	rice straw	0.65	488	0.94	705	0.66	498		
	wheat straw	1.03	820	0.67	534	0.72	573		
	maize straw	0.49	457	0.53	488	0.45	424		
	sugar cane bagasse	0.48	305	0.51	322	0.44	280		
	Mean	0.66	517	0.66	512	0.57	394		
<i>P. cornucopiae</i>	rice straw	0.98	775	0.98	736	0.78	582		
	wheat straw	0.96	757	0.65	517	0.48	387		
	maize straw	1.77	1645	0.78	730	0.62	580		
	sugar cane bagasse	0.75	470	0.84	533	0.77	483		
	Mean	1.12	912	0.81	629	0.66	508		
Total mean		0.87	702	0.71	549	0.62	468		

(B.E.) LSD 0.05

Species = 0.13

Substrates = 0.08

Methods = 0.07

Species x Substrates = 0.16

Substrates x methods = 0.13

Species x methods = 0.13

Species x substrates x methods = 0.27

(Yield) LSD 0.05

Species = 122.1

Substrates = 122.1

Methods = 105.7

Species x Substrates = 211.4

Substrates x methods = 211.4

Species x methods = 211.4

Species x substrates x methods = 211.4

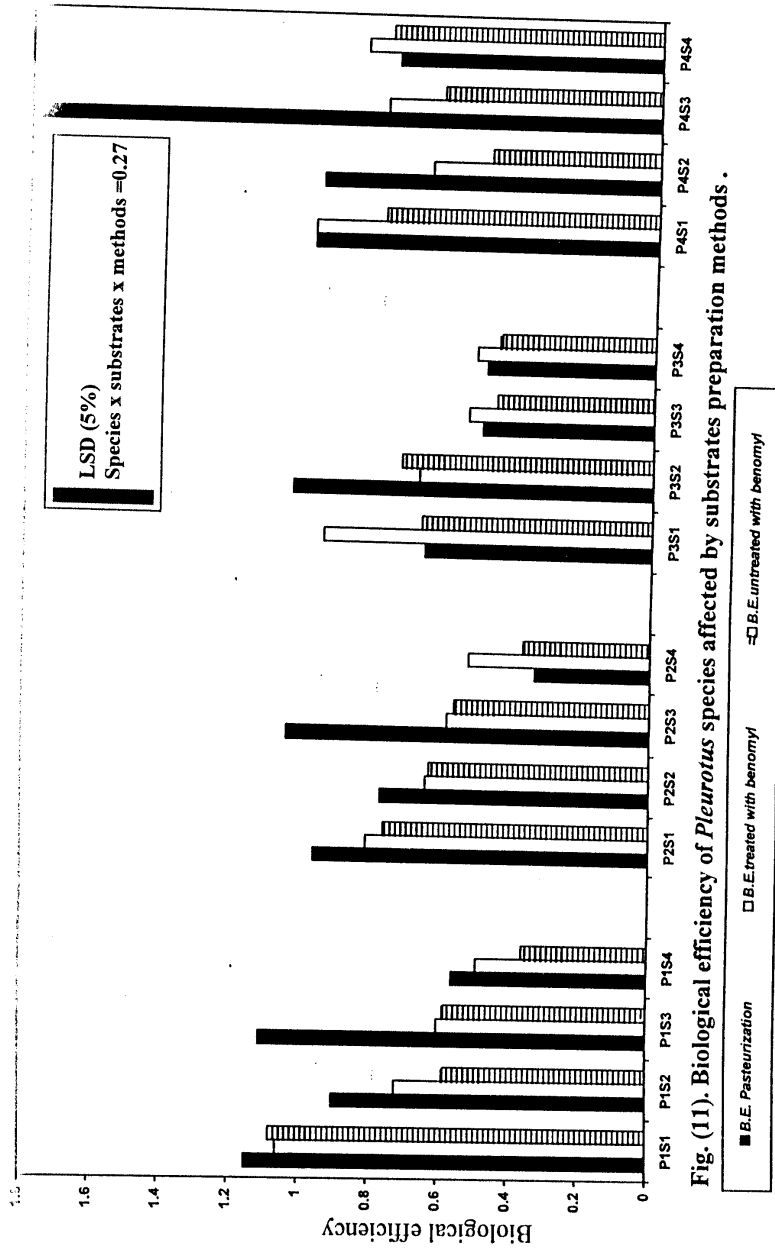


Fig. (11). Biological efficiency of *Pleurotus* species affected by substrates preparation methods .

■ B.E. Pasteurization      □ B.E. untreated with benomyl  
 P1: *P. pulmonarius*    P2: *P. ostreatus*    P3: *P. sajor-caju*    P4: *P. cornucopiae*  
 S1: rice straw    S2: wheat straw    S3: maize straw    S4: sugar cane bagasse

preliminary evaluation of the overall contamination ratio in relation to method of substrate preparation indicate that semianaerobic fermentation in the presence of benomyl exhibited the least contamination ratio (13%), followed by semianaerobic fermentation without benomyl (24%). The highest contamination ratio (41%) was found in substrates prepared by pasteurization.

## **4.2. Chemical composition of fruit bodies of *Pleurotus* mushroom:**

### **4.2.1. Moisture:**

Table (13) show the moisture % of fruit bodies of different *Pleurotus* species grown on different substrates. Results indicate that fruit bodies grown on rice straw or wheat straw contained significantly higher moisture (91.51%, 91.64%) compared with those grown on maize straw or sugar cane bagasse (88.47% and 88.93%). It is clear that the moisture content of fruit bodies depends to some extent on the water holding capacity of the substrate and on conditions prevailing during harvesting. Results also indicate that *P. ostreatus* and *P. sajor-caju* had significantly less moisture in their fruit bodies (87.46%, 87.88%) than *P. pulmonarius* and *P. cornucopiae* (92.35%, 92.85%). The mean value of 90.14% could be considered a representative value of moisture content of *Pleurotus* fruit bodies regardless of species or substrates.

### **4.2.2. Protein :**

Results of experiments carried out to determine the protein content in fruit bodies of *Pleurotus* species are presented in Table (14). Results indicate significant differences between species of *Pleurotus*. *P. ostreatus*

**Table (13). Moisture percentage of fruit bodies of *Pleurotus* species grown on different substrates.**

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
<i>Pleurotus pulmonarius</i>	96.94	94.52	90.75	87.18	92.35
<i>Pleurotus ostreatus</i>	86.66	86.88	86.65	89.67	87.46
<i>Pleurotus sajor-caju</i>	88.35	90.30	86.06	86.83	87.88
<i>Pleurotus cornucopiae</i>	94.10	94.84	90.40	92.04	92.85
<b>Mean</b>	<b>91.51</b>	<b>91.64</b>	<b>88.47</b>	<b>88.93</b>	<b>90.14</b>

LSD (5%)

Species = 1.10

Substrates = 1.10

Species x substrates = 2.20

**Table (14). Protein percentage in harvested fruit bodies *Pleurotus* species grown on different substrates.**

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
<i>Pleurotus pulmonarius</i>	19.37	17.24	16.53	12.60	16.43
<i>Pleurotus ostreatus</i>	13.45	22.95	21.57	22.90	20.27
<i>Pleurotus sajor-caju</i>	21.92	20.63	16.18	18.01	19.19
<i>Pleurotus cornucopiae</i>	18.58	17.69	16.81	17.96	17.76
<b>Mean</b>	<b>18.38</b>	<b>19.63</b>	<b>17.77</b>	<b>17.87</b>	<b>18.41</b>

LSD (5%)

Species = 2.04

Substrates = 2.04

Species x substrates = 4.07

gave the highest mean value of protein content in fruit bodies (20.27%) whereas the lowest was that of *P. pulmonarius* (16.43%). Results also indicate that substrates on which fruit bodies were produced had no influence on their protein content. So, no significant differences could be noticed between protein content of fruit bodies produced on either rice straw, wheat straw, maize straw or sugar cane bagasse (18.38, 19.63, 17.77 and 17.87, respectively).

However, a significant species x substrate interaction was observed for protein content of *Pleurotus* fruit bodies. The highest protein content of fruit bodies achieved was that of *P. ostreatus* grown on wheat straw (22.95%), while the lowest was that of *P. pulmonarius* grown on sugar cane bagasse (12.60%).

#### **4.2.3. Fibers:**

Table (15) show percentages of fibers in fruit bodies of *Pleurotus* species grown on different substrates. Results indicate that no significant differences in fiber % could be detected between fruit bodies of the tested *Pleurotus* species. Substrates on which fruit bodies were produced had clearly no influence on the percentage of fibers in fruit bodies. In addition, no significant species x substrate interaction could be observed for fiber % of the fruit bodies. The mean value of 5.67% could be considered a representative value of fiber content in *Pleurotus* mushroom regardless of species or substrates.



**Table (15) Fiber content (%) of dry weight of fruit bodies of *Pleurotus* species grown on different substrates.**

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
<i>Pleurotus pulmonarius</i>	5.63	5.52	4.97	5.68	5.45
<i>Pleurotus ostreatus</i>	6.06	6.76	4.75	4.78	5.59
<i>Pleurotus sajor-caju</i>	4.51	5.55	6.85	5.97	5.72
<i>Pleurotus cornucopiae</i>	4.92	5.86	7.64	5.27	5.92
Mean	5.28	5.92	6.05	5.27	5.67

LSD (5%)

Species = 1.24

Substrates = 1.24

Species x substrates = 2.497

**Table (16). Fat content (%) of dry weight of fruit bodies of *Pleurotus* species grown on different substrates.**

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
<i>Pleurotus pulmonarius</i>	8.11	4.29	5.77	6.06	6.06
<i>Pleurotus ostreatus</i>	9.96	4.88	10.68	8.50	8.51
<i>Pleurotus sajor-caju</i>	9.62	7.43	4.73	4.54	6.58
<i>Pleurotus cornucopiae</i>	7.42	5.53	8.78	6.37	7.02
Mean	8.78	5.53	7.49	6.37	7.04

LSD (5%)

Species = 0.82

Substrates = 0.82

Species x substrates = 1.63

#### 4.2.4. Fats :

Table (16) show the fat content in fruit bodies of *Pleurotus* species grown on different substrates. Results indicate significant differences between species and between substrates. *P. ostreatus* contained the highest mean value of fat (8.51%), whereas *P. pulmonarius* showed the least mean value of 6.06%. Fruit bodies produced on rice straw contained significantly higher fat content (8.78%) compared with other substrates ; whereas those produced on wheat straw, contained significantly lower fat content (5.53%).

A significant species x substrates interaction was observed for fat percentage of fruit bodies. The highest fat percent was achieved in fruit bodies of *P. ostreatus* grown on maize straw, while the lowest was that achieved in fruit bodies of *P. pulmonarius* grown on wheat straw.

#### 4.2.5. Carbohydrates:

Data in Table (17) show the calculated carbohydrates % in fruit bodies of *Pleurotus* species grown on different substrates. results indicate significant differences between species and between substrates.

The highest mean value of carbohydrate percentage in the harvested fruit bodies was noticed with *P. pulmonarius* (57.47%) while the lowest was that of *P. ostreatus* (51.72%). Also the highest mean value of carbohydrate percentage in the harvested fruit bodies was recorded with sugar cane bagasse substrate, (56.74%), while the lowest was that of rice straw substrate (53.17%).

**Table (17). Calculated carbohydrates content (%) of dry weight of fruit bodies of *Pleurotus* species grown on different substrates.**

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
<i>Pleurotus pulmonarius</i>	51.48	58.36	57.74	62.28	57.47
<i>Pleurotus ostreatus</i>	57.70	48.12	50.70	50.36	51.72
<i>Pleurotus sajor-caju</i>	49.62	49.93	57.56	56.67	53.45
<i>Pleurotus cornucopiae</i>	53.88	57.40	51.27	57.63	55.05
Mean	53.17	53.45	54.32	56.74	54.42

LSD (5%)  
 Species = 2.32  
 Substrates = 2.32  
 Species x substrates = 4.63

**Table (18). Ash percentage in harvested fruit bodies of *Pleurotus* species grown on different substrates.**

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
<i>Pleurotus pulmonarius</i>	15.41	14.59	14.99	13.38	14.59
<i>Pleurotus ostreatus</i>	12.83	17.29	12.30	13.46	13.97
<i>Pleurotus sajor-caju</i>	14.33	16.46	14.68	14.81	15.07
<i>Pleurotus cornucopiae</i>	15.20	13.52	15.50	12.77	14.25
Mean	14.44	15.46	14.37	13.60	14.47

LSD (5%)  
 Species = 0.96  
 Substrates = 0.96  
 Species x substrates = 1.9

A significant species x substrates interaction was observed for carbohydrate percentage of the harvested fruit bodies. The highest carbohydrate percentage in the harvested fruit bodies was achieved by *P. pulmonarius* grown on sugar cane bagasse (62.28%), while the lowest was that achieved by *P. ostreatus* grown on wheat straw substrate (48.12%).

#### **4.2.6. Ash :**

Data in Table (18) show percentages of ash in fruit bodies of *Pleurotus species* grown on different substrates. Results indicate significant differences between species and between substrates. The highest mean value of ash percentage in the harvested fruit bodies was noticed in *P. sajor-caju* (15.07%), whereas the lowest was that of *P. ostreatus* (13.97%). Also the highest mean value of ash percentage in fruit bodies was recorded in those produced on wheat straw (15.46%), whereas the lowest was in fruit bodies grown sugar cane bagasse (13.60%).

A significant species x substrates interaction was observed for ash percentage in the fruit bodies. The highest ash percent was achieved in *P. ostreatus* grown on wheat straw (17.29%), while the lowest was that of same specie grown on maize straw (12.30%).

### **4.3. Substrate as animal feed:**

#### **4.3.1. Chemical composition of spent:**

##### **4.3.1.1. Moisture:**

Table (19) show the moisture % of spent of different substrates left after growth and harvesting of fruit bodies of different *Pleurotus species*. Results indicate that spent of rice straw exhibited the least mean value of

**Table (19). Moisture content (%) in spent substrate of *Pleurotus* species.**

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
Control (Untreated)	87.02	83.21	87.55	88.79	86.64
<i>Pleurotus pulmonarius</i>	54.26	66.88	81.30	84.80	71.81
<i>Pleurotus ostreatus</i>	73.29	73.30	80.89	88.99	79.12
<i>Pleurotus sajor-caju</i>	52.15	70.65	80.39	85.39	72.14
<i>Pleurotus cornucopiae</i>	80.21	60.74	71.98	83.99	74.23
Mean	64.98	67.89	78.64	85.79	74.33

LSD (5%)  
 Species = 0.47  
 Substrates = 0.42  
 Species x substrates = 0.93

**Table (20). Protein percentage in spent substrate of *Pleurotus* species.**

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
Control (Untreated)	2.94	2.60	1.71	2.00	2.31
<i>Pleurotus pulmonarius</i>	11.11	11.90	7.59	12.56	10.78
<i>Pleurotus ostreatus</i>	9.47	11.49	10.48	10.10	10.38
<i>Pleurotus sajor-caju</i>	7.77	18.73	5.85	7.85	10.05
<i>Pleurotus cornucopiae</i>	11.87	10.10	9.24	11.52	10.68
Mean	10.05	13.06	8.29	10.51	10.48

LSD (5%)  
 Species = 2.68  
 Substrates = 2.40  
 Species x substrates = 5.37

moisture % (64.89%) compared with other spent substrates, whereas spent of sugar cane bagasse contained the highest value (85.79). Moisture content of spent substrate varies obviously between the different substrates depending on the water holding capacity of each substrate and on conditions prevailing during harvesting. Results also indicate that *P. pulmonarius* and *P. sajor-caju* had less moisture in their spent substrates (71.81 and 72.14%) than *P. cornucopiae* and *P. ostreatus* (74.23 and 79.12). The mean value of 74.33% could be considered a representative value of moisture content of spent substrates regardless of species or substrates.

#### **4.3.1.2. Protein:**

Results of experiment carried out to determine the protein content in spent substrates left after growth and harvesting of different *Pleurotus* species are presented in Table (20). Results reveal significant increases in protein content of all spent substrates compared with the untreated substrates. So, the protein content of rice straw increased from 2.94% to 10.05% in spent, in wheat straw from 2.60% to 13.06% in maize straw from 1.71% to 8.29% and in sugar cane bagasse from 2.00% to 10.51%. It is obvious from these results that using agricultural wastes for the production of *Pleurotus* fruit bodies leads also to a significant increase in the protein content of the spent substrate left after harvesting the fruit bodies.

#### **4.3.1.3. Crude fiber:**

Data in Table (21) represent percentages of fiber in spent substrates after harvesting of fruit bodies of *Pleurotus* mushroom compared with the

**Table ( 21). Fiber percentage spent substrate of *Pleurotus* species.**

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
Control (Untreated)	35.61	35.60	36.39	38.37	36.49
<i>Pleurotus pulmonarius</i>	17.40	25.87	19.31	33.51	24.02
<i>Pleurotus ostreatus</i>	18.62	31.70	19.61	28.35	24.57
<i>Pleurotus sajor-caju</i>	19.06	24.79	24.25	35.41	25.89
<i>Pleurotus cornucopiae</i>	18.87	30.57	20.23	29.17	24.71
Mean	18.49	28.23	20.85	31.61	24.80

LSD (5%)  
 Species = 2.49  
 Substrates = 2.22  
 Species x substrates = 4.97

**Table ( 22). Fat percentage in spent substrate of *Pleurotus* species.**

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
Control (Untreated)	1.59	1.59	1.09	1.40	1.42
<i>Pleurotus pulmonarius</i>	1.75	1.61	1.07	0.49	1.23
<i>Pleurotus ostreatus</i>	0.70	1.86	1.75	0.54	1.22
<i>Pleurotus sajor-caju</i>	1.57	2.13	1.85	1.10	1.66
<i>Pleurotus cornucopiae</i>	0.59	0.73	0.43	0.61	0.59
Mean	1.16	1.58	1.28	0.69	1.18

LSD (5%)  
 Species = 0.10  
 Substrates = 0.10  
 Species x substrates = 0.23

untreated substrates. Results indicate significant reduction in fiber content of all spent substrates compared with the original untreated substrates. The fiber content of rice straw decreased from 35.61% to 18.49% is spent in wheat straw from 35.60% to 28.23%, in maize straw from 36.39% to 20.85% and in sugar cane bagasse from 38.37% to 31.61%. Comparing the mean value of fiber content of the untreated substrates (36.49%) with that of the spent substrate (24.80%) an overall decrease of 32% can be calculated.

#### **4.3.1.4. Fat:**

Data in Table (22) represent percentages of fat in spent substrates after harvesting of fruit bodies of *Pleurotus* mushroom compared with the untreated substrates. No significant differences could be detected between the fat content of spent of wheat straw or maize straw and their corresponding original untreated substrates. However, a slight decrease in the fat content of the spent of rice straw and sugar cane bagasse was noticed compared to the fat content of the original substrates.

#### **3.4.1.5. Carbohydrates:**

Data in Table (23) represent percentages of carbohydrates in spent substrate after harvesting of fruit bodies of *Pleurotus* mushroom compared with the untreated substrates. No significant differences could be detected between the carbohydrates content of each spent substrate and its corresponding original untreated substrate.



Table ( 23). Carbohydrate percentage in spent substrate of *Pleurotus* species.

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
Control (Untreated)	43.80	45.63	53.42	51.61	50.49
<i>Pleurotus pulmonarius</i>	43.14	50.80	60.19	47.18	51.10
<i>Pleurotus ostreatus</i>	47.55	44.79	56.21	54.19	51.53
<i>Pleurotus sajor-caju</i>	46.94	32.88	57.53	49.06	47.44
<i>Pleurotus cornucopiae</i>	43.99	41.50	57.69	52.47	49.74
Mean	45.40	42.49	57.92	50.72	49.94

LSD (5%)  
 Species = 3.24  
 Substrates = 3.01  
 Species x substrates = 6.11

Table ( 24). Ash percentage in spent substrate of *Pleurotus* species.

Species \ Substrates	Rice straw	Wheat straw	Maize straw	Sugar cane bagasse	Mean
Control (Untreated)	16.06	13.33	7.39	6.62	10.85
<i>Pleurotus pulmonarius</i>	26.60	9.82	11.84	6.26	13.63
<i>Pleurotus ostreatus</i>	23.66	10.16	11.85	6.82	13.12
<i>Pleurotus sajor-caju</i>	24.66	21.47	10.52	6.58	15.81
<i>Pleurotus cornucopiae</i>	24.68	17.10	12.41	6.23	15.11
Mean	24.90	14.64	11.66	6.47	14.42

LSD (5%)  
 Species = 1.25  
 Substrates = 1.12  
 Species x substrates = 2.50

#### **3.4.1.6. Ash:**

Data in Table (24) represent percentages of ash in spent substrates after harvesting of fruit bodies of *Pleurotus* mushroom compared with the untreated substrates. Results show a significant increase in ash content of the rice straw and maize straw compared with their original untreated substrates. However, the increase of ash content was minimal in case of wheat straw spent, and not significantly different from that of the original untreated substrates. The same was noticed also regarding the ash content of sugar cane bagasse spent.

#### **4.3.2. Nutritive value of spent:**

Data in Table (25) represent the proximate chemical composition of rice straw spent and of the original untreated rice straw, which were used in the digestibility trial. It is clear that the spent had higher contents of protein and ash, but a lower content of fiber compared to the untreated substrate.

Results of digestibility trial shown in Table (26) indicate that digestibility coefficient of the crude protein increased from 63.35 to 70.78% by using spent instead of the untreated substrate (control) in the diet. Also the digestibility coefficient of fat increased from 80.87 to 82.53 and that of fiber from 30.10 to 32.78% whereas that of nitrogen free extract (NFE) from 71.58 to 80.18%. The higher content of crude protein in spent as well as higher digestibility coefficient of protein resulted in an increase of DCP from 7.90 to 8.98 in diet containing spent. The improvement of the digestibility coefficient of protein, fat, fiber and NFE has led to an increase of the TDN from 61.31 to 63.36%. Results of DCP

**Table ( 25). Proximate chemical composition of rice straw spent compared with untreated rice straw .**

<b>Treatment</b>	<b>Protein</b>	<b>Fat</b>	<b>Ash</b>	<b>Fiber</b>	<b>NFE</b>
<b>Rice straw spent</b>	<b>10.36</b>	<b>10.31</b>	<b>26.13</b>	<b>22.79</b>	<b>39.41</b>
<b>Raw rice straw untreated (control)</b>	<b>2.50</b>	<b>1.59</b>	<b>15.45</b>	<b>35.61</b>	<b>44.85</b>

**Table ( 26). Mean values of digestibility coefficients digested crude protein (DCP) and total digested nutrients (TDN) of diet containing spent rice straw as feed for goats.**

<b>Treatment</b>	<b>Protein</b>	<b>Fat</b>	<b>Fiber</b>	<b>NFE</b>	<b>DCP</b>	<b>TDN</b>
<b>Rice straw spent</b>	<b>70.78</b>	<b>82.53</b>	<b>32.78</b>	<b>80.18</b>	<b>8.98</b>	<b>63.36</b>
<b>Raw rice straw untreated (control)</b>	<b>63.35</b>	<b>80.87</b>	<b>30.10</b>	<b>71.58</b>	<b>7.90</b>	<b>61.31</b>

and TDN show that inclusion of rice straw spent in ration instead of the untreated substrate increases the feeding value (nutritive quality) of the ration.

## 5. Discussion

Yields of *Pleurotus* mushroom are affected by many factors, some of which have been dealt with in the present study. Bearing in mind the abundance of agricultural wastes in our region (Kafr El-Sheikh governorate) and the suitable climatic conditions prevailing for more than 9 months yearly, we tried to test some yield factors in order to simplify the production process and raise the yield of fruit bodies.

Results of experiments carried out to explore the effect of spawn ratio on the yield of fruit bodies indicated that increasing the spawning ratio of substrate from 2% to 4% significantly increased the yield by about 28%. A further increase of spawn ratio from 4% to 6% resulted in a significant but less pronounced increase (15%) of fruit bodies. Increasing the spawn ratio obviously decreases the time of mycelial colonization and reduces the competition of the other microbial contaminants. A preliminary cost/ benefit analysis revealed that 4% spawn ratio is more economic than 2% and 6%. This may lead us to recommend 4% spawn ratio to obtain economic yields. El-Kattan (1986) and Abd El-Kawi (1989) found also that 4% was the best spawn ratio for *Pleurotus* mushroom. Hassan (1992), however recommended the use of 3% spawn ratio.

Results of experiments carried out to evaluate the performance of different *Pleurotus* species on different agricultural wastes revealed significant differences in the productivity of the tested *Pleurotus* species regardless of substrate. *P. cornucopiae* was the most efficient species and

gave the highest yield followed by *P. pulmonarius*, *P. ostreatus* and *P. sajor-caju*. Similar findings about the distinguished performance of *P. cornucopiae* have been recently reported. Upadhyay and Vijay (1991) found that *P. cornucopiae* had yielded double compared with *P. ostreatus* when both species were cultivated on wheat straw. Zirvakies and Balis (1991) also reported during a broad scale survey of *Pleurotus* species in Greece that *P. cornucopiae* appeared to be capable to exploit the cellulose substrate with greater efficiency compared with *P. sajor-caju*, *P. ostreatus* or *P. pulmonarius* depending on the fact that *P. cornucopiae* was physiologically distinguished with higher cellulose degradation efficacy.

Results also indicated the suitability of all tested agricultural wastes (rice straw, wheat straw, maize straw and sugar cane bagasse) as substrates for cultivation of *Pleurotus* mushroom. Actually, *Pleurotus* species can grow on almost all kinds of lignocellulosic materials as repeatedly reported (Losovoi, 1980 ; Puri *et al.*, 1981 ; Sivaprakasam and Kandswamy, 1981 ; Bisht and Harsh, 1984 ; Punkow, 1984 ; Henicus and Voros, 1985 ; Das *et al.*, 1987 ; Guzman-Davalos *et al.*, 1987 ; Morales, 1987 ; Nicolini *et al.*, 1987 ; Abd El-kawi, 1989 ; Abd El-Rehem, 1991 and Hassan, 1992.

In the present study maize straw proved to be more suitable than the other tested substrates followed by rice straw, wheat straw and then sugar cane bagasse. The mean value of biological efficiency attained on these substrates were 1.10, 0.93, 0.91 and 0.53, respectively. However, the biological efficiency attained on each substrate varies significantly according to the cultivated species. The biological efficiency achieved by

*P. cornucopiae* on rice straw, for example, was about 3 times of that achieved by *P. sajor-caju* on the same substrate. Sugar cane bagasse was found in our study to be the least suitable substrate for *Pleurotus* cultivation. The biological efficiency attained on sugar cane bagasse was relatively low (0.53), but in agreement with the results reported by Guzman-Davalos et al., 1987 for the same substrate (0.49 and 0.51). The relative low biological efficiency noticed on sugar cane bagasse could be partially attributed to its weak water holding capacity and poor compact structure. In addition, abundance of soluble carbohydrates, may have stimulated numerous saprophytic contaminants, which competed the cultivated *Pleurotus*.

The effect of different supplements on the yield of fruit bodies was also studied. Results indicated that *Pleurotus* species did not respond similarly to the different tested supplements. However, the addition of 5% rice bran to wheat straw substrate at spawning improved the biological efficiency by 67% compared with control treatment (non supplemented substrate). Wheat bran added at the same rate achieved only 19% improvement. The remaining supplements (peat moss, gypsum or balanced mixture of all supplements) applied at spawning at the rate of 5% have either no effect or even adverse effect on the yield of fruit bodies.

The beneficial effect of rice bran on the yield of fruit bodies in our study is in agreement with that reported by Visscher (1984) using *P. pulmonarius* and *P. columbinus*. Also Upadhyay and Vijay (1991) reported improvement in the biological efficiency of *P. ostreatus* on

wheat straw supplemented with rice bran. However, they observed adverse effects on *P. fossulatus* using chicken manure, brewer's grain or even rice bran. So, supplementation does not always positively enhance the productivity of mushrooms, but occasionally, its incompatibility reduces the yield. Royse and Schlsler (1987), using wheat straw supplemented with 168 g/kg dry substrate of the two commercial supplements Spawn Mate® and Fast Break®, came to the results that the second supplement dramatically decreased the yield of an isolate of *P. sajor-caju*, while the first supplement or a balanced mixture of both (at the same ratio) increased the yield.

The relevancy of a relatively new, simple and less energy consuming method for substrate preparation (semianaerobic fermentation) was evaluated in comparison with the well known pasteurization. So, the substrates were chopped and immersed into water, containing either no or 100 ppm of the fungicide benomyl (50% active ingredient) for ten days. The water was drained off and after 6 h substrates were spawned. Results indicated that during the semianaerobic fermentation the pH value of the fermentation water dropped from about 7.40 within ten days down to about 6 in the presence of benomyl. The drop in pH value was less pronounced in the absence of the fungicide. Results also indicated that the microbial counts (counts of bacteria, fungi and yeasts) increased during the first two days of fermentation and then slowly decreased. The same pattern was observed either in the presence or absence of the fungicide benomyl. However, the microbial counts were significantly lower in the presence of benomyl. Changes in pH and microbial counts determined in



the present study are in general agreement with those reported by Schies (1991).

Results of experiments carried out to trace the fungicide benomyl in the products indicated that after ten days fermentation, the substrates contained a residual quantity of 5.37-13.09 ppm. The early flushes of fruit bodies harvested 3-4 weeks after spawning had less than 0.1 ppm. The spent substrate left after harvesting (80 days after spawning) contained (0.67-0.97 ppm). These results are in general agreement with those reported by Schies, (1991), who could detect 0.08 ppm of benomyl (as carbendazim) in fruit bodies of *Pleurotus* which had been produced on substrates prepared by semianaerobic fermentation with 100 ppm benomyl.

A comparison between yields of fruit bodies of *Pleurotus spp.* grown on substrates prepared by pasteurization and semianaerobic fermentation indicated that pasteurization achieved significantly higher yield (B.E. 0.87) than those achieved by methods of semianaerobic fermentation. However, if the high energy requirement of pasteurization as well as the high contamination rate of pasteurized substrate (about 41%) are considered, the semianaerobic fermentation methods seem to be better qualified for the application in developing countries.

Our results indicated that semianaerobic fermentation in the presence of benomyl (100 ppm) achieved higher yield (B.E. 0.71) and exhibited less contamination ratio than in the absence of the fungicide. However, the residues of benomyl (B.E. 0.62) in fruit bodies (<0.1 ppm)

and in spent (<1 ppm), though minimal, may hinder the application of this method. Although there are no regulations concerning the maximum residue limits of benomyl on foodstuff and feed in Egypt, ecologists, public health authorities, and citizens are becoming increasingly aware of the increasing concentrations of toxic chemicals in nature's food chain. Therefore, it would be more appropriate to recommend the semianaerobic fermentation without benomyl to be the method of choice. The slight decrease in yield (14%) from 549 g/bag in the presence to 468 g/bag in the absence of benomyl would not justify the application of the fungicide with the environmental hazards, which may arise on the long run.

Results of chemical analysis of *Pleurotus* mushrooms supported the published reports about their nutritional attributes. The moisture content of fruit bodies ranges between 86.06 and 94.84% according to species and substrates. Similar values of moisture in *Pleurotus* fruit bodies were reported by Abd El-Rehem, Nahed, (1991) and Hassan (1992). Significant differences were found between *Pleurotus* species regarding protein content of fruit bodies. It ranges between 16.43-20.27% of the dry matter according to species. These values lay within the range of protein content reported for *Pleurotus* fruit bodies by Abd El-Kawi (1989) (13.2-14.3%), Abd El-Rehem, Nahed, (1991) (29.3%) and Hassan (1992) (20.9-38%).

The average fiber content of *Pleurotus* fruit bodies was found to be 4.51-7.64%, of the dry matter. These values agree well with those reported by Yoshioka et al. (1975) and Abd El-Rehem, Nahed, (1991), but is less than that reported by Hassan (1992). The fat content of fruit bodies varied significantly according to species and substrates. It ranged between

4.29-10.68% on dry weight basis with an average content of 7.04%, and is in agreement with fat contents reported by Abd El-Kawi (1989) (5.7-7.7%) and Abd El-Rehem, Nahed, (1991), (6.95%).

Fruit bodies of *Pleurotus species* contained carbohydrates ranging from 48.12 to 62.28% on dry weight basis with an average of 54.42%, which agree well with results reported by Bano and Rajarathnam (1982a) and Abd El-Kawi (1989). Our results indicated that ash percentage varied significantly according to species and substrates. It ranged between 12.30-17.29% with an average of 14.47%. This values is about 2 times greater than those reported by Abd El-Rehem, Nahed, (1991), and Hassan (1992).

Using agricultural wastes for the production of *Pleurotus* mushroom resulted in variable changes in their constituents. Chemical analysis of spent substrates left after harvesting of fruit bodies indicated significant increase (3-4 fold) in their protein contents compared to the corresponding raw substrates. This result is in agreement with that of Gupta and Langer (1988), who found that inoculation with *P. florida* increased crude protein content of wheat straw spent as compared with untreated wheat straw.

Meanwhile, growth of *Pleurotus* mushroom on the tested agricultural wastes was consistently accompanied with a marked and significant reduction in crude fiber of spent. The reduction in fiber content ranged between 18-48%. Similar results were reported by Bakshi et al. (1985), who used wheat straw with 42.92% crude fiber and the resultant spent contained 31.31% (27% reduction).

In addition, results indicated that ash content of spent substrates increased compared with the corresponding raw substrates. This result is in line with those reported by Zadrazil (1980), and Bakshi et al. (1985). However, the ash content of spent increased in different magnitude according to the agricultural wastes used. It increased significantly in maize straw spent (58%) and rice straw spent (55%), whereas the increase was minimal in case of wheat straw spent (10%) and sugar cane bagasse spent (2%). The variable increase in ash content of spent of different agricultural wastes was also noticed and reported by Badr (1993).

Results of digestibility trial carried out to explore the nutritive quality of rice straw spent as animal feed were promising. The digestibility of the crude protein, fat, fiber, and nitrogen free extract (NFE) increased by using rice straw spent instead of raw rice straw in diet. These results are in agreement with those of Zadrazil (1977), who reported an increase of 12 percentage units in the in vitro digestibility of wheat straw inoculated with *P. florida*. The higher content of crude protein in spent as well as the improvement of the digestibility of protein, fat, fiber and NFE have led to increases in the digested crude protein (DCP) and total digested nutrients (TDN). The increase in DCP and TDN show that inclusion of rice straw spent in ration instead of raw rice straw increases the feeding value of the ration. This result is supported by those of Gupta and Langer (1988) and Badr (1993), who reported that *Pleurotus* mushroom exhibited promising properties for the decomposition of lignin cellulose materials, mainly the cereal straw, to increase their value as animal feed.

Results of the present investigation show the importance of growing *Pleurotus* mushroom. They can grow on a wide range of plant wastes without the need for costly processing. Simple and cheap methods of cultivation are available. They are highly yielding and yield can be easily improved through simple measures (such as using the suitable species, raising the spawn ratio, supplementation). The fruit bodies are nutritious and the spent substrate left after harvesting of fruit bodies can be used for animal feeding.

Therefore, greater attention should be paid to *Pleurotus* cultivation in Egypt to make use of the large amount of agricultural wastes. The total yield of agricultural residues in Egypt is about 14 million tons yearly. Only one third of these residues is fed to livestock. The rest (about 10 million tons) consisting mainly of rice straw, maize straw and cobs, bagasse and cotton stems is used as bedding, processed as fertilizer or burnt for cooking. Assuming that all the 10 million tons of plant residues is available for mushroom growing, it could be used to produce about 180.000 tons of mushroom protein. This would cover the annual protein requirements of more than 6.5 million of our citizens and would be also a considerable contribution to their vitamin and mineral requirements. Spent substrates left after harvesting of fruit bodies are more suitable for animal feed than the raw substrates.

One of the main problems, which hinders the cultivation of mushroom so far in Egypt is that the consumption of mushroom is new and hence very limited. Therefore, more efforts should be directed also to

get the population accept the mushrooms. Working out of suitable nutritional education programmes may be helpful in the integration of mushrooms into the normal diet of the Egyptians.

## SUMMARY

The present study was conducted to make use of the large amounts of agricultural wastes available in our region (Kafir El-Sheikh Governorate) by using them for growing mushrooms. For this reason experiments were carried out to evaluate the performance of 4 *Pleurotus* species (*P. pulmonarius*, *P. ostreatus*, *P. sajor-caju*, *P. cornucopiae*) on different agricultural wastes (rice straw, wheat straw, maize straw, sugar cane bagasse). Experiments were also carried out to study the effect of spawn ratio and of some supplements on the yield of fruit bodies. In addition, the study aimed at evaluating the relevancy of new, simple and less energy consuming methods of substrate preparation. Furthermore, chemical composition of fruit bodies were determined to evaluate their nutritive value and spent left after harvesting of fruit bodies was subjected to digestibility trial to explore its nutritive quality as animal feed.

### Results can be summarized in the following :

1. Comparing the performance of the tested *Pleurotus* species revealed that *P. cornucopiae* was the most efficient species and gave the highest yield. Its yield averaged 912 g of fruit bodies/bag containing 4 kg of wet substrate, which corresponds to a biological efficiency (B.E.) of 1.12, followed by *P. pulmonarius* (B.E. 0.93) and *P. ostreatus* (B.E. 0.78), whereas the least efficient was *P. sajor-caju* (B.E. 0.66).
2. Results indicated the suitability of all tested agricultural wastes, as substrates for cultivation of *Pleurotus* mushroom. Maize straw proved to be the most suitable substrate followed by rice straw and wheat straw

then sugar cane bagasse. The average yield of fruit bodies/bag containing 4 kg of wet substrate was 1028, 718, 728 and 335 respectively, which corresponds to (B.E.) of 1.10, 0.93, 0.91 and 0.53 respectively.

3. Increasing the spawning ratio of rice straw from 2% to 4% increased the yield of fruit bodies by about 28%. A further increase of spawn ratio to 6% resulted in an additional increase of 15% of yield. A preliminary cost / benefit analysis revealed that 4% spawn ratio is more suitable than 2% or 6% for an economic production.
4. Supplementation of wheat straw substrate with 5% rice bran at spawning improved the yield of fruit bodies by about 67% compared with non supplemented substrate. Wheat bran added at the same rate achieved only 19% improvement. Some supplements such as gypsum had an adverse effect on yield ; addition of 5% gypsum reduced the yield by 31%.
5. Comparing the efficiency of some methods used for substrate preparation revealed that pasteurization achieved the highest yield (702 g fruit bodies /bag containing 4 kg wet substrate), followed by semianaerobic fermentation with benomyl (549 g /bag), whereas semianaerobic fermentation without benomyl gave the least yield (468 g/bag). However, the high energy requirement of pasteurization as well as the high contamination ratio of pasteurized substrates (41%) favors the adoption of semianaerobic fermentation methods in developing countries. Although the yield of semianaerobic fermentation with



benomyl is higher than without benomyl, and the residues of benomyl in fruit bodies (<0.1 ppm) and in spent (<1 ppm) are minimal. However, it would be better to carry out the fermentation without the fungicide to avoid the environmental hazards, which may arise on the long run.

6. Chemical analysis of *Pleurotus* fruit bodies indicated that they contain 16.43-20.27% crude protein, 4.51-7.64% fibers, 4.29-10.68% fat, 48.12-62.28% carbohydrates and 12.30-17.29% ash, on the dry weight basis, whereas the fresh fruit bodies contain 86.50-94.84 water.

7. Using agricultural wastes for the production of *Pleurotus* mushroom resulted in variable changes of their constituents. Spent substrates left after harvesting of fruit bodies showed 3-4 fold increase in their protein content, significant reduction (18-48%) in fiber content, and a variable increase ranging from 2-58% of ash content, compared with the corresponding raw substrates.

8. Results of digestibility trials indicated that the digestibility of the crude protein, fat, fiber and nitrogen free extract (NFE) as well as the digested crude protein (DCP) and the total digested nutrients (TDN) increased by using rice straw spent instead of raw rice straw in diet. These results show that application of rice straw for the production of *Pleurotus* fruit bodies increases its nutritive quality as animal feed.

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## الملخص العربي

أجريت هذه الدراسة بغرض الاستفادة من المخلفات الزراعية المتوافرة في البيئة المحلية وذلك بإستخدامها لإنتاج فطر عيش الغراب ولذلك فقد استهدفت الدراسة تقييم كفاءة أربعة أنواع من فطر عيش الغراب *P. cornucopiae*, *Pleurotus pulmonarius*, *P. sajor-caju*, *P. ostreatus*, عند نموها على أنواع مختلفة من المخلفات الزراعية هي قش الأرز وتبن القمح وقش الذرة ومصاصة القصب ، وكذا تحديد النسبة المثلى من اللقاح أو الإضافات المختلفة للحصول على أعلا محصول من الأجسام الثمرية ، كما استهدفت الدراسة أيضاً إختبار كفاءة بعض الطرق المستخدمة لإعداد المخلفات بغرض الوصول إلى طريقة سهلة ورخيصة يسهل إستخدامها بواسطة المنتج البسيط. كما إشمطت الدراسة على جزء خاص بتقييم القيمة الغذائية للأجسام الثمرية التي تستخدم كغذاء آدمي وكذلك للمخلفات المتبقية بعد إنتاج الأجسام الثمرية ومدى صلاحيتها للإستخدام كغذاء للحيوانات .

وتتلخص النتائج التي تم الحصول عليها في الآتي :

١. بمقارنة إنتاجية أنواع عيش الغراب المختبرة أتضح أن أعلا الأنواع إنتاجية تحت ظروف التجربة هو : *P. cornucopiae* حيث وصل متوسط إنتاجه على المخلفات المختبرة ٩١٢ جم من الأجسام الثمرية لكل كيس يحتوى على ٤ كجم وزن رطب من المخلفات بكفاءة بيولوجية ١,١٢ وتبعه في كفاءة الإنتاج *P. pulmonarius* بكفاءة بيولوجية ٠,٩٣ ثم *P. ostreatus* بكفاءة بيولوجية ٠,٧٨ أما أضعفهم إنتاجية فقد كان *P. sajor-caju* حيث بلغت كفاءته البيولوجية ٠,٦٦ .

٢. أتضح صلاحية المخلفات المختبرة لإنتاج عيش الغراب ، وكانت أفضل المخلفات المستخدمة لإنتاج أكبر محصول من الأجسام الثمرية هي قش الذرة وتبعها كل من قش الأرز وتبن القمح وكان أقلها مصاصة القصب ، وقد بلغ متوسط محصول الكيس الواحد (٤ كجم وزن رطب) ١,٠٢٨ ، ٧١٨ ، ٧٢٧ ، ٣٣٥ جم على الترتيب بكفاءة بيولوجية تعادل ١,١٠ ، ٠,٩٣ ، ٠,٩١ ، ٠,٥٣ على الترتيب.

٣. أدت زيادة نسبة اللقاح المضاف إلى قش الأرز من ٢ إلى ٤ ٪ إلى زيادة في محصول الأجسام الثمرية مقدارها ٢٨ ٪ ، وبزيادة نسبة اللقاح إلى ٦ ٪ تحققت زيادة إضافية مقدارها ١٥ ٪ ، ولكن نظراً لأن نسبة اللقاح تؤثر في التكلفة النهائية للمنتج لذلك يمكن اعتبار أن نسبة لقاح ٤ ٪ هي النسبة المثلى لإنتاج اقتصادي.

٤. تم إختبار تأثير بعض الإضافات على محصول الأجسام الثمرية الناتجة على تبين القمح وإتضح أن إضافة رجيع الكون بنسبة ٥ ٪ قد أدى إلى زيادة المحصول بمقدار ٦٧ ٪ في حين لم تؤد إضافة نفس النسبة من ردة القمح إلا إلى زيادة قدرها ١٩ ٪ . وقد كان لبعض الإضافات تأثيراً سلبياً مثل الجبس الزراعي حيث أدت إضافته بنسبة ٥ ٪ إلى خفض في المحصول مقدار ٣١ ٪.

٥. بمقارنة كفاءة بعض طرق إعداد المخلفات إتضح أن البسترة تعطي أعلا محصول حيث بلغ متوسط إنتاج الكيس (٤ كجم وزن رطب) ٧٠٢ جم من الاجسام الثمرية ، وتبعها طريقة التخمير شبه اللاهوائي في وجود المبيد الفطري بنليت حيث بلغ متوسط إنتاج الكيس ٥٤٩ جم وكان أقلها من حيث المحصول طريقة التخمير شبه اللاهوائي بدون بنليت حيث بلغ متوسط محصول الكيس ٤٦٨ جم.

ومع هذا فإن إرتفاع نسبة تلوث الأكياس بعد عملية البسترة (٤١ ٪) وإرتفاع تكلفة الطاقة المستخدمة تجعل إستخدام طرق التخمير شبه اللاهوائي أكثر ملائمة للبلاد النامية . ورغم ارتفاع محصول التخمير شبه اللاهوائي في وجود البنليت والإختفاض الملحوظ في بقايا المبيد على الاجسام الثمرية (> ٠,١ جزء في المليون) وفي المخلفات (> ١ جزء في المليون)، فإنه من الأفضل إجراء التخمير شبه لاهوائي بدون المبيد الفطري لتلافى الأخطار التي قد تنجم على المدى الطويل من إدخال مواد كيميائية سامة في سلسلة الغذاء.

٦. أظهرت نتائج التحليل الكيماوي للأجسام الثمرية للأصناف المختلفة لعيش الغراب التابعة لجنس *Pleurotus* إحتوائها على ١٦,٤٣-٢٠,٢٧ ٪ بروتين ، ٤,٥١-٧,٦٤ ٪ ألياف، ٤,٢٩-١٠,٦٨ ٪ دهون ، ٤٨,١٢-٦٢,٢٨ ٪ كربوهيدرات ، ١٢,٣٠-

١٧,٢٩٪ رماد على أساس الوزن الجاف . أما الأجسام الثمرية الطازجة فتحتوى على ٨٦,٥-٩٤,٨٤٪ رطوبة.

٧. إتضح من النتائج أن إستخدام المخلفات الزراعية المختبرة فى إنتاج الأجسام الثمرية لأنواع جنس *Pleurotus* يؤدي الى تغيرات فى التركيب الكيماوى لهذه المخلفات ، حيث أزداد محتواها من البروتين الخام بمقدار ٣-٤ أضعاف بمقارنته بالمخلفات التى لم ينمو عليها الفطر ، كما إنخفض محتواها من الألياف بمقدار ١٨-٤٨٪ وازداد محتواها من الرماد بنسب تتراوح ما بين ٢-٥٨٪.

٨. اتضح من تجربة هضم حقلية *in vivo* زيادة معامل هضم البروتين الخام والدهن والألياف والمستخلص الغير أروتى ، وكذا إرتفاع قيمة البروتين الخام المهضوم والمركبات الكلية المهضومة وذلك بإحلال قش الارز المتخلف بعد إنتاج وحصاد الأجسام الثمرية بدلاً من قش الأرز الخام فى تركيب العليقة . وتشير هذه النتائج إلى أن استخدام قش الأرز لإنتاج الاجسام الثمرية لعيش الغراب التابع لجنس *Pleurotus* يؤدي إلى تحسن خواصه كغذاء للحيوانات.

دراسات على إنتاج فطر عيش الغراب على  
المخلفات الزراعية

٤١٤



رسالة مقدمة من

محسن حسن محمد الباجوري

بكالوريوس العلوم الزراعية - شعبة أمراض نبات -  
كلية الزراعة بكفر الشيخ - جامعة طنطا ١٩٨٨

للحصول على درجة

الماجستير في العلوم الزراعية

ميكروبيولوجيا زراعية

قسم النباتات الزراعي

كلية الزراعة بكفر الشيخ

جامعة طنطا

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