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COMPARATIVE STUDY ON CULTIVATION AND YIELD PERFORMANCE OF OYSTER MUSHROOM (*PLEUROTUS FLORIDANUS*) BY USING DIFFERENT SUPPLEMENTS TO SORGHUM SPAWN AND RICE STRAW.

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ABSTRACT

Popularity of Oyster mushroom is increasing for its ease of cultivation, high yield potential as well as high nutritional and medicinal values. This investigation was carried out to evaluate the effect of some supplements to spawn (sorghum grains) and also spawn level on *Pleurotus floridanus* yield and biological efficiency.The highest average yield (721g/500g straw) and biological efficiency (144%) were achieved with sorghum supplemented with milled cotton seeds. The range of 5-7% spawn level was the best. In addition *Pleurotus floridanus* was cultivated on rice straw alone or in combinations with some selected agro wastes viz., cotton stalk, broad bean grain husks, rice bran. The Highest yield and intracellular proteins were achieved in rice straw amended with broad bean grain husks followed by cotton stalks.

This investigation was undertaken during the research mushroom project at mushroom house at Faculty of Science.

INTRODUCTION

Environmental pollution is considered as one of the most dangerous problems that threaten the community. It is headed by getting rid of agricultural wastes which increased annually in Egypt (about 23 million tones of agricultural wastes including rice straw are accumulated annually) [**Hamdan (1990)**].

Burning of agricultural wastes is responsible for the black cloud which causes air pollution with toxic gases that affect the people health. On the other hand growth in human population necessitates the need to increase food production especially new sources of proteins.

Mushrooms represent one of the world's greatest untapped resources of nutritious food. Cultivated mushrooms are a good source of protein, essential and non-essential amino acids, minerals and vitamins [**FAO (2004) and Manzi *et al.*, (1999)**].

Oyster mushrooms have a medicinal effect [**El-Komy & El-Fallal (2006) and Toson *et al.*, (2003 a & b)**].Cultivation of edible mushrooms may be the only currently economical biotechnology for lignocellulose organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution [**Obodai** ***et al.*****, (2003 a & b)**].

Oyster mushroom cultivation (*Pleurotus* spp.) has increased tremendously worldwide during the last few decades. However, its cultivation in Egypt needs urgently additional researches to increase its yield.

The first stage of mushroom cultivation is production of strong spawns with increasing mycelial growth. It is equal to seed of higher plants. Spawn quality is considered the most important part in mushroom production [**Mohammadi & Purjam (2003)**].Spawn grains such as millet, wheat, sorghum and corn have been reported to affect carpophores production.Sorghum grains are used in Egypt.There are various additives that are known to stimulate fruiting [**Nwanze** ***et al.*****, (2005 a & b)**].

Although oyster mushrooms are commonly grown on pasteurized straw of wheat and rice, they can be cultivated on a variety of substrates that contain cellulose and lignin.

The aim of this investigation is to study the effect of some additives to sorghum grains on the growth and yield of mushrooms.

Based on our earlier studies and local availabilities of the agricultural wastes, we will also study the utilization of rice straw (as the main mushroom bed) supplemented with cotton stalks, rice husks and broad bean grain husks for the cultivation and production of *P. floridanus*.

MATERIAL AND METHODS

Spawn Preparation:

P. floridanus was obtained from American Type Culture Collection (ATCC). It was maintained on potato dextrose agar (PDA) slopes at 4 °C. Sorghum grains were mixed thoroughly with 1:4 calcium carbonate and calcium sulphate respectively in a ratio of 3 parts/100 g of grain weight. The mixture was filled in a wide mouthed bottle, plugged tightly and sterilized at 15 psi, 121 °C for 30 min.

Fully grown culture of *P. floridanus* was used for inoculation the sterilized grains. Jars were incubated at 28 °C for 15 days in dark to give an optimum time for spawn production plate (1A). Six different substrates were selected to be added as 10% to sorghum grains. These are milled cotton seed, broad bean grains husk, vine waste, okra receptacles, corn cob brackets and used tea leaves.

Growth Substrates:

Rice straw was used as a main substrate for mushroom cultivation. In another experiment, 5% of four different substrates, rough cotton stalk, fine cotton stalk, rice grain husks and broad bean seeds husks were added to rice straw.

Preparation of the Substrates for Cultivation:

Chopped Rice straw were soaked in water overnight and drained, resulting in a moisture content of approximately 70% plate (1B). The rice straw and the four additive substrates were pasteurized by steaming for 5 hours plate (1C) . After cooling the rice straw were filled in plastic bags. The four additive substrates were thoroughly mixed by hand with rice straw.

Spawning and Spawn Running:

Spawn was broadcast at the rate of 5 %. The distribution of the spawn throughout the substrate was done by ranking with hands. The bags were arranged randomly on clean and surface sterilized shelves. The temperature in the cultivation unit was maintained at approximately 30 °C and relative humidity at 85 % by using foggers and watering the floor twice a day.

Harvesting:

After fully covering of the substrate with the white mycelial growth, two dozen nail size holes equally spaced were made around the bags to provide aeration. The relative humidity was maintained at about 85% by watering the floor and using foggers plate (1D). The fruit bodies were daily harvested plate (1E).

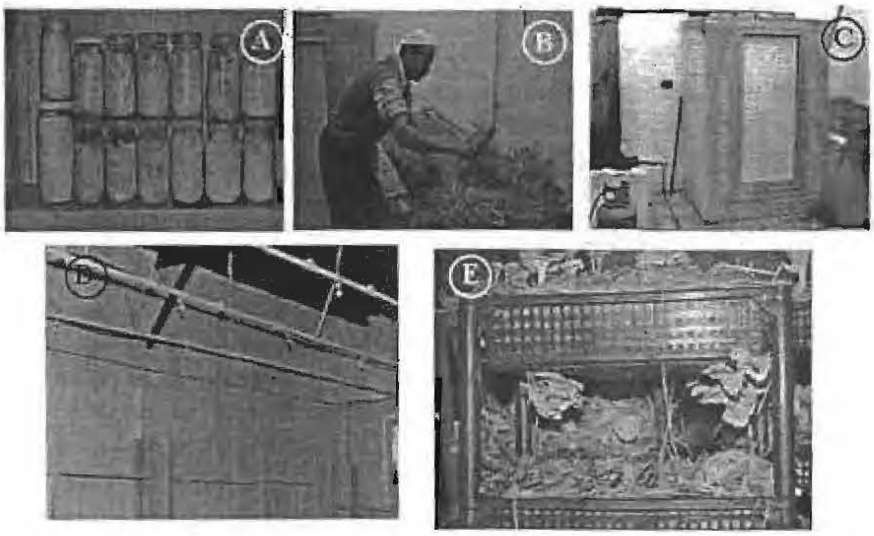


Plate (1): Stages of mushroom cultivation. A, spawn prepared in jars; B, preparation of substrate; C pasteurization by steam; D, mushroom room with foggers; E, fruit bodies appearance.

Determination of Intracellular Protein:

Harvested fruit bodies were washed three times with distilled water, extracted in 20 mL of NaOH (1M), boiled for 20 minutes, allowed to cool and then centrifuged. The clarified solution was used to determine the intracellular protein concentration of the organisms using Bradford method (1976). 1.0 mL of the extract was mixed with 5.0 mL protein reagent and incubated at room temperature. After 5 minutes, the optical density at 595 nm was recorded. Protein concentration was determined from a standard curve with bovine serum albumin (BSA) ranging from 0 to 100 $\mu g\ mL^{-1}$.

Analysis of variance (ANOVA) was carried out using SYSTAT-Bogram [**Wilkinson (1997)**].

RESULTS

It is clear from **table (1)** that sorghum grains supplemented with milled cotton seeds and broad bean grains husks enhanced the earlier appearance of fruit bodies than the other treatments including the control. In contrary, addition of corn cob brackets leaves or used tea leaves to sorghum grains delayed the formation of fruit bodies.

Table (1): Average growth time for the appearance of the first and second flushes of *Pleurotus floridanus*

Spawn composition	First flush (days)	Second flush (days)
Sorghum	47	67
Sorghum + milled cotton seed	39	47
Sorghum + broad bean grains husk	43	58
Sorghum + vine peduncles	44	67
Sorghum + okra receptacles	44	65
Sorghum + corn cob brackets	67	76
Sorghum + boiled tea leaves	54	80

Results represented in **fig. (1) & (2)** show that sorghum grains supplemented with milled cotton seeds, vine peduncles and broad bean husks supported significantly higher mushroom production (721, 637 ad 510g/500g straw respectively) and biological efficiency(144, 127 and 102% respectively) compared with the control (sorghum grains without any addition) (490g/500g straw and 98%). However, sorghum grains supplemented with corn cob brackets leaves or used tea leaves suppressed the production (429 and 353g/500 g straw respectively).

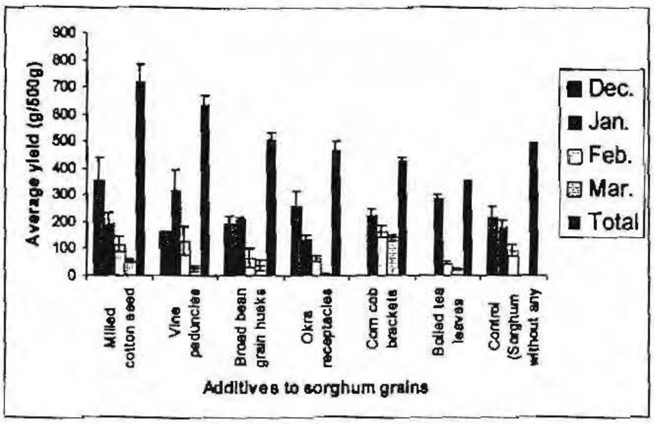


Fig (1): Effect of some additives to sorghum grains on monthly average yield of *Pleurotus floridanus*.

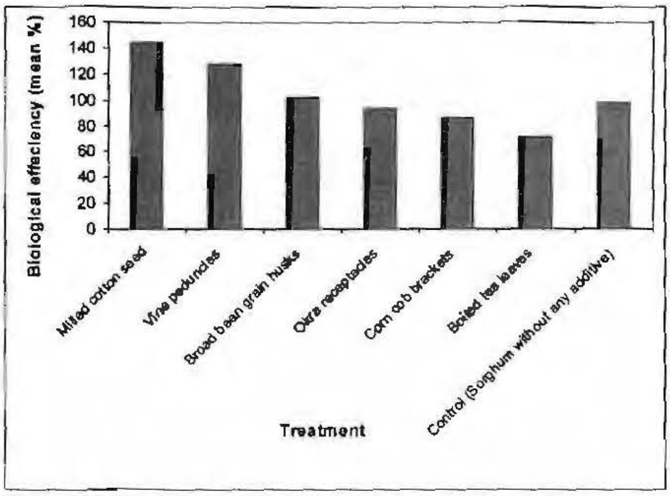


Fig (2): Influence of spawn composition on biological efficiency (mean %) of *Pleurotus floridanus*.

It can be observed from **table (2)** that the fruit body production is related to the loss in the substrate weight, since the highest loss in weight was caused by sorghum grains supplemented with milled cotton seeds, vine peduncles and broad bean husks (60, 53.3 and 52%).

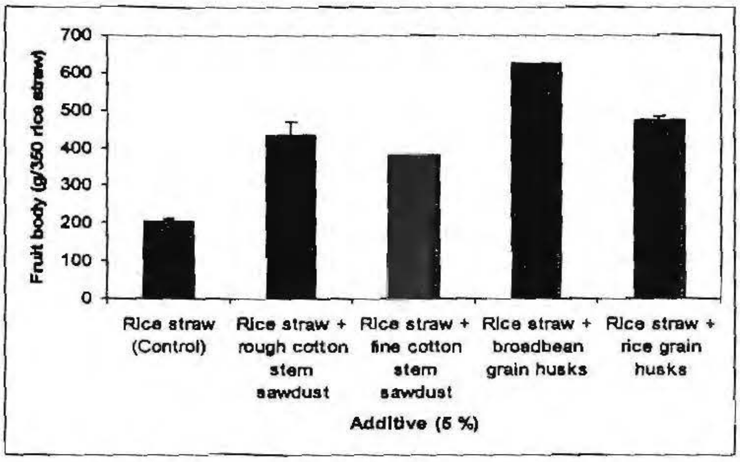
Table (2): Effect of spawn composition on straw utilization by *Pleurotus floridanus*

Spawn composition	% Loss in weight
Sorghum	48.6
Sorghum + milled cotton seed	60.0
Sorghum + broad bean grains husk	52.0
Sorghum + vine waste	53.3
Sorghum + okra receptacles	41.3
Sorghum + corn cob brackets	30.6
Sorghum + used tea leaves	26.6

All the supplementations to rice straw significantly increased fruit body relative to the control (rice straw without any supplementation). It is headed by rice straw supplemented with broad bean husk which produce 626g/350g straw and achieved biological efficiency at 178.9% **Fig (3) & table (3).** In addition the highest protein content was found in supplemental nutrition of broad bean husk to rice straw **Fig (4).** On the contrary, lower protein content was recorded in rice straw supplemented with rice husks and rough cotton stems powder compared with the control (rice straw).

Table (3): Effect of some additives to rice straw on the biological efficiency of *Pleurotus floridanus.*

Additives	Biological efficiency (%)
Rice straw + rough cotton stem sawdust	124,5
Rice straw + rice grain husks	132,28
Rice straw + fine cotton stem sawdust	108,85
Rice straw + broad bean grain husks	178,85
Rice straw (Control(	58,3



Anova test : F=123.8, p <0.001

Fig. (3): Effect of some additives to rice straw on the total yield of *Pleurotus floridanus* (Fruit body (g)/ 350 g rice straw).

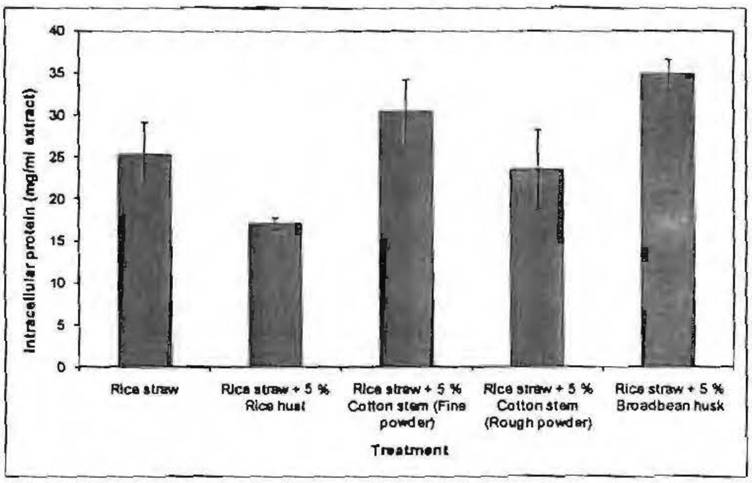


Fig. (4): Effect of different treatments on intracellular protein content (mg/ml extract) of *Pleurotus floridanus*.

Fig. (5) & (6) show that there is increase in yield and biological efficiency with increasing spawn rate, and 7% is the best spawn rate. whereas 3% spawn level resulted in significantly lower mushroom yield than the other three levels.

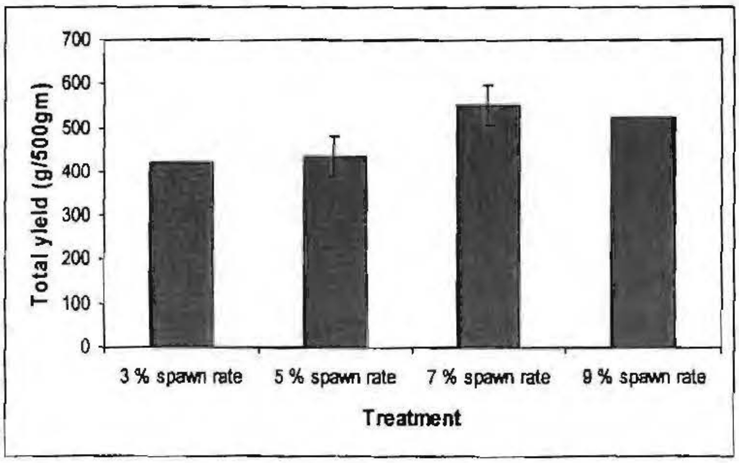


Fig. (5): Influence of spawn rate on total yield of *Pleurotus floridanus.*

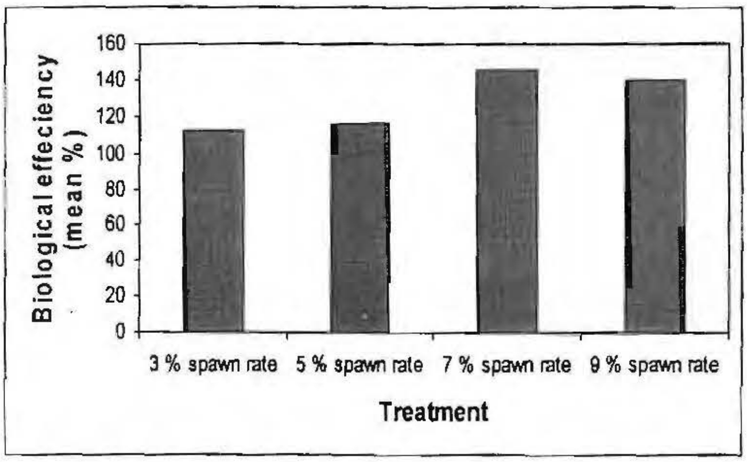


Fig. (6): Influence of spawn rate on biological efficiency (mean %) of *Pleurotus floridanus*

DISCUSSION

An adequate spawn is one possessing a mycelium that is capable of rapid growth when invading a particular substrate. Large grains have a greater food reserve and can sustain the mycelium for longer periods of time during stress [**Fritsche (1988)**]. He also added that different types of spawn may influence productivity. In this study, significant different yields were observed from different spawn composition.

From the industrial point of view, reduction of the time required for culture is one of the keys to reduce the running cost of the growing facilities. Consequently inexpensive methods for reduction of the time required for culture of mushrooms are needed. Providing nutritional supplements to the spawn has, as its principal objective, the production of a vigorous mycelium [**Matta *et al.*, (2002)**]. This investigation confirms the same conclusion, since addition of milled cotton seed, vine peduncle or broad bean husks to sorghum grains achieved the earlier colonization to the rice straw and earlier appearance of fruit bodies and recorded the highest productivity and highest biological efficiency. However, supplemental nutrition of cotton seed powder to the rice straw increased the yields, proteins and essential amino acids of *Pleurotus florida* [**Shashirekha *et al.*, (2005) and Nwanze *et al.*, (2005a)**] reported that various oils including cotton oil produced a highly significant effect on dry weights of *Lentinus squarrosulus* fruit bodies. It is thus evident that, sterol from various oils was responsible for the stimulation of fruiting response [**Nwanze *et al.*, (2005b)**]. Dry matter loss of the wheat or rice straw after oyster mushroom growth varied from 30 to 44 % [**Zhang *et al.*, (2002)**]. In this study 48% loss in rice straw dry weight was obtained after growth and harvest of *P. floridanus* fruit bodies. Our results also revealed that supplementing sorghum grains with cotton seeds powder enhancedthe loss in rice straw dry weight (60%).Similar results of [**Shashirekha *et al.*, (2002)**] who reported that due to cotton seed cake supplementation, there was an increase in the in vitro dry matter digestibility of rice straw by *P. sajor-caju.* However, the combinations that contain sorghum grains with corn cob brackets or boiled tea leaves resulted in a longer colonization period. Also yield performance and biological efficiency values decreased in the same variations. Similar observations, were obtained by [**Baysal (2009)**] who found that waste paper mixed with waste tea leaves gave the longer mycelia development period and the lower *P.ostreatus* mushroom yield than other substrate mixtures. In contrast, addition of waste tea leaves to wood wastes has been recorded by [**Sivrikaya *et al.*, (1999)**] to increase *P. florida* fruit bodies production.

The fortification of rice straw with broad bean husks, rice husks and cotton stems induced significantly greater mushroom yield and higher biological efficiency compared with the control (rice straw). Broad bean husks supported the highest mushroom yield and protein, this referred to its nutritional value (Crude protein 20.8%, Lipid 0.767%, Crude fiber 9.59%,Ash 7.23% and Carbohydrates 56.11%) [**Khattab & Abdel Wahab (2005)**].

[**Hanai *et al.*, (2005)**] suggested the existence of both stimulatory and inhibitory substances in rice husks. They suggested the presence of phytoalexins including momilactone A which inhibits the mycelial growth. However, they recorded the stimulatory effect of rice husks on mycelial growth of some edible mushrooms and they recommended the development of new profitable cultivation methods for mushrooms using rice husks. In spite of being the second ranking in stimulating the mushroom yield, it causes a decrease in protein content in fruit bodies. This might be attributed to its low content of proteins (13% crude protein, 15% crude fiber, 13.5% crude fat and 10.6% ash) **[Ambreen *et al.*, (2006)]**.

Cotton stem was found to induce more growth of *Pleurotus* spp. **[Muhamad & Khan (1993)]**. It was recommended by **[El-Fallal (1995) and El-Fallal *et al.*, (2004)]** in oyster mushroom cultivation. The present findings came to the same results. The superiority of chopped (rough) cotton stem over ground (fine) one in yield production when added to rice straw was recorded in the present study.**[Zhang *et al.*, (2002)]** came to the same conclusion when they used ground and chopped straw.

This study revealed that the mushroom-yield increased with increasing spawn levels. The same results have been obtained with *P. sajor-caju* by **[Zhang *et al.*, (2002)]**.

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الملخص العربى

دراسة مقارنة علي زراعة وكفاءة إنتاج عيش الغراب المحاري (بلوروتس فلوريدانس) باستخدام إضافات مختلفة لحبوب الذرة العويجة وقش الأرز

أميرة علي الفلال – محمد إسماعيل أبو دبارة –هدى محمد الغرباوى

قسم النبات – كلية العلوم بدمياط– جامعة المنصورة– فرع دمياط

استهدف هذا البحث دراسة تأثير بعض الإضافات علي حبوب الذرة العويجة وكذلك معدل الاسبون علي الإنتاجية والكفاءة البيولوجية لعيش الغراب بلورتس فلوريدانس وكان أعلي إنتاج (٧٢١ جرام / ٥٠٠ جرام قش أرز) والكفاءة البيولوجية (١٤٤ %) عند إضافة بذور القطن المطحونة للذرة العويجة كما وجد أن معدل الاسبون ٥-٧ % يعطى أفضل إنتاجية. بالإضافة إلي ذلك تم زراعة بلوروتس فلوريدانس علي قش الأرز (بدون أي إضافات) أو بإضافة بعض المخلفات الزراعية الأخرى مثل سيقان القطن & قشربذور الفول & قشر(ردة) الأرز وكانت أعلي لنتاجية للفطر وأعلى محتوى بروتينى تم الحصول عليه في حالة استخدام قش الأرز مضاف اليه قشر حبوب الفول يليها استخدام سيقان القطن.