

# Journal of Agricultural Chemistry and Biotechnology

Journal homepage & Available online at: [www.jacb.journals.ekb.eg](http://www.jacb.journals.ekb.eg)

## Effect of Mixed Substrates with Rice Straw on Cultivation of *Pleurotus ostreatus*

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### ABSTRACT

This study was carried out to investigate the effect of several agro-industrial wastes and mixed substrates with rice straw (w/w) on production of (*Pleurotus ostreatus*). Mixed substrates with rice straw at rate 1:1 by weight showed higher effect on the first stage, second and third stages. Data indicated that the time for production ranged from 12 - 25.5, from 22 - 52.25 and from 25 - 55 days respectively. When mixed substrates with rice straw the results were found within the period ranged from 11.75 - 18.5, from 22.5 - 28.5 and from 26.5 - 32 days respectively. Mixed substrates with rice straw reduce the first stage time of oyster compare with the control substrates. Therefore, reduce pinheads and fruiting bodies formation period. The mixed substrates rice straw with waste paper (RS+WP) gave the maximum of oyster mushroom produced per 1 kg dry ~ 578.50 g and gave the maximum biological efficiency 57.85 %. Finally, mixed substrates with rice straw increase in biological efficiency of *P. ostreatus* over control substrates.

**Keywords:** Agro-industrial wastes, Mixed substrates, *Pleurotus ostreatus*.

### INTRODUCTION

The principal cultivated mushroom worldwide is *Agaricus bisporus* followed by *Pleurotus* spp., and other mushrooms that have already an important grade. (Sánchez, 2004, Rühl *et al.*, 2008, Barny, 2009 & Afify *et al.*, 2022).

Since cultivated mushrooms can grow on different wastes they constitute a source for obtaining food protein from such wastes and thus they can be marshaled to aid in solving many problems of global importance including protein shortages, resources recovery and environmental management (Zadrazil, 1980).

In this respect, oyster mushroom is a important source of valuable food protein, and an organism with the ability to utilize various substrats. The utilization of waste paper for the production of cultured mushroom, this will provide an economical gain and protect the environment from pollution. (Brenneman and Guttman 1994, Soto *et al.*, 1999, Wang *et al.*, 2001).

The optimal substrate to cultivate *P. ostreatus* appeared with mixed substrates (Thongklang, and Luangham 2016). Also, Abd El-Razk *et al.*, (2020) reported that *P.ostreatus* cultivation is an effective bioconversion process that is transfer agro-wastes into potentially valuable source of natural use in functional food products.

The aim of this study was to investigate experimentally the effect of mixed substrates with rice straw (w/w) on cultivation of *P. ostreatus* on various wastes in order to give food protein with bid rid of environmental pollution.

### MATERIALS AND METHODS

#### *Pleurotus ostreatus* :

In this study, from Unit of mushroom, NRC, Douki, Cairo, Egypt, cultivar of mushroom (*P. ostreatus*) as sorghum (*Sorghum bicolor* L.) spawns were obtained.

#### Culture and maintenance medium :

The *Pleurotus ostreatus* was grown on malt agar medium (25% malt extract and 1.5% agar (Oxoid) at 28 °C). The fungal culture on malt agar medium were maintained at 5 °C and subcultured monthly (Atlas, 1995), until used.

#### Substrate preparation and cultivation condition:

All substrates were obtained from Mansoura city, Dakhliya, Egypt. Four supplements were prepared for this experiment as rice straw (*Oryza sativa*), sawdust, waste paper and industrial cardboard were used as a substrate. Small pieces of 2 – 5 cm these substrates were chopped and prepared before use according to Espindola *et al.*, (2007). Rice straw (RS), Sawdust (SD), Waste paper (WP), Cardborad industries (CI) are mixed with rice straw at rate 1:1 by weight {(RS + SD), (RS + WP), and (RS + CI)}.

In plastic bag, substrates were spawned at a ratio 4% of sorghum spawn (w/w) and closed with a plastic neck. In semi dark room, the bags were then incubated at 25 – 30 °C and 60 – 65% relative humidity for 2 – 3 weeks until spawn run was completed (El-Sawah, 2000).

#### Cropping:

After 2 weeks in case with WS + CI, 3 weeks for RS and 4 weeks for SD when a complete first stage, the bags were opened.

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DOI: 10.21608/jacb.2022.136557.1023

The second day of opening the bags the compact mass of the substrate and mycelium was watered daily with distilled sterilized water. Second stage (4 – 5 cm in diameter) after 3 – 8 days of opening, Young mushrooms attained the normal size in about 3 – 5 days when the first crop was collected from each bag. Mature fruiting bodies were harvested, the fresh weight and spawn running (first stage) recorded after the harvest. Biological efficiency (BE) was calculated as percentage yield of fruiting bodies in relation to dry weight of the substrate.

**Statistical analysis:**

The obtained data was subjected to the analysis of LSD test to compare between mean values of all treatments (SPSS, 1999).

**RESULTS AND DISCUSSION**

**The growth of *Pleurotus ostreatus* on substrates:**

Table (1) show, the growth of mushroom on tested various wastes as abbreviations : RS; SD; WP and CI.

**Spawn running (first stage) :**

The mycelium totally colonized the tested wastes within 12.00 to 25.50 days. The shortest first stage period was determined as average being 12.00 days on WP, CI and the longest was 25.50 days on SD (Afify et al., 2012) as shown in Table 1.

The first, second and final are three important stages in the production of oyster. Temperature 25 °C for first stage and 25-30 °C for fructification showed highest results (Afify et al., 2012).

**Pinheads formation (second stage):**

These is second stage, small pinheads like structures were observed, (Shah et al., 2004). As seen in Table 1 the second stage appearing 3.50 – 39.75 days, RS expended the shortest time to pinned in 22.00 days, sawdust and waste paper pinned in 31.00 and 48.25 days respectively. While a longer time to pinned 52.25 days with cardboard.

**Fruiting bodies formation (third or final stage):**

The final stage, of *P. ostreatus* appeared 2.75 – 4.50 days and took 25.00 – 55.00 days later after spawning. RS and CI took a lower time to fruiting bodies formatted in 2.75 days after second stage. SD took a longer time to fruiting bodies formatted in 4.50 days after second stage, CI took a longer time to fruiting bodies formatted in 55.00 days later after spawning (Table 1). Mandeel et al., (2005) and Kulshreshtha et al., (2010) reported that in their cultivation of oyster mushroom on various wastes and bioremediation of industrial waste.

**Table 1. The three phases in cultivation of oyster at 25 – 30 °C with a various treatments (days).**

Treatments	First stage	Second stage	Final stage
RS	18.50	22.00	25.00
SD	25.50	31.00	35.50
WP	12.00	48.25	52.00
CI	12.50	52.25	55.00

**The growth of *P. ostreatus* on mixed substrates with rice straw (w/w):**

Mixed substrates with rice straw at rate 1:1 by weight had valuable effect on the three stages the growth of oyster. The obtained results are presented in Table 2.

In the first stage, *P. ostreatus* was determined as average 11.75 – 18.50 days. The lowest period was determined as average 11.75 days on RS + WP (Afify et al., 2012) and the highest was 18.50 days on RS + SD.

The second stage started appearing 4.00 – 16.75 days. While, the lowest period was determined as 22.50 days on RS + SD and the highest period was 28.50 days on RS + WP. The third stage appeared 2.50 – 4.00 days and took 26.50 – 32.00 days later after spawning. But the lowest period 26.50 days on RS + SD and the highest period was 32.00 days on RS + WP as shown in Table 2

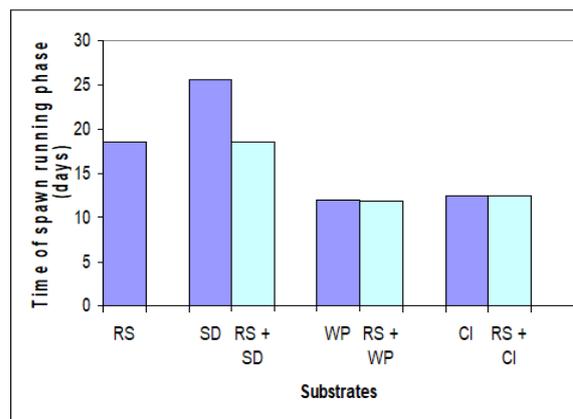
Mixed substrates with rice straw reduce the first stage of *P. ostreatus* over control substrates (Fig. 1). Therefore, reduce pinheads and fruiting bodies formation period.

These results are in assurance with those obtained by Akyuz and Yildiz (2008) and Kulshreshtha et al., (2010) in their evaluation of wastes for the production of *Pleurotus eryngii* (DC. ex Fr.) Quel and bioremediation of industrial waste through mushroom cultivation.

At the same time Thongklang and Luangharn (2016) the first fruiting bodies of *P. ostreatus* appeared on day 30 after inoculation.

**Table 2. Effect of mixed substrates with rice straw on growth days of *P. ostreatus* at 25 – 30 °C.**

Treatments (w/w)	First stage	Second stage	Third stage
RS+SD	18.50	22.50	26.50
RS+WP	11.75	28.50	32.00
RS+CI	12.50	27.50	30.00



**Fig. 1. Effect of mixed substrates with rice straw on growth of oyster mushroom**

RS ; Rice straw. SD; Sawdust. WP; Waste paper. CI; Cardboard industrial. RS + SD ; Rice straw + Sawdust. RS + WP ; Rice straw + Waste paper. RS + CI ; Rice straw + Cardboard industrial .

**Biological efficiency(BE) and yield :**

These BE was calculated because some substrates were denser than others (Akyuz and Yildiz, 2008 & Afify et al.,2012).

The analysis of variance different substrate effects on yield of mushroom revealed significant differences (P<0.05) between used substrates.

The data are presented in Table (3) showed that the highest total weight of mushroom produced per 1 kg dry substrate of rice straw was 652.38 g , followed by sawdust ~ 251.20 g , but the lowest yield was reported on WP and CI.

The maximum BE was recorded with RS (65.24 %), followed by SD (25.12%).

**Table 3. Effect of various substrates on Yields and Biological efficiency of oyster.**

Treatments	Total fresh weight of fungus g/kg substrate dry weight	(BE%)
RS	652.38	65.24
SD	251.20	25.12
WP	112.14	11.21
CI	117.93	11.83
LSD at 5 %	6.558	0.655

**Effect of mixed substrates with rice straw (w/w) on yield and biological efficiency:**

Rice straw is the principal substrate for oyster mushroom growing (Baysal *et al.*, 2003).

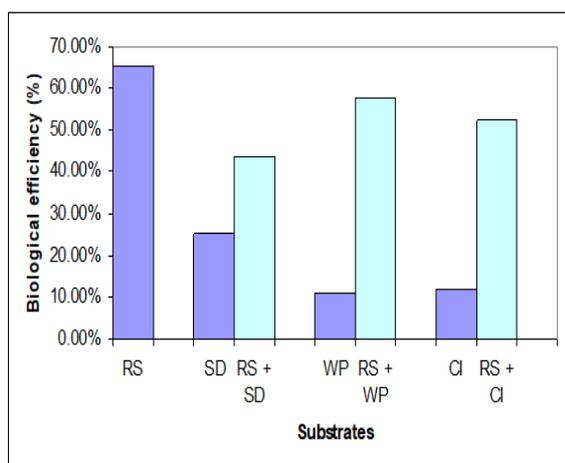
The effect of mixed substrates on yield performance of *P. ostreatus* was shown (Table 4). The highest total weight of mushroom produced per 1 kg dry mixed substrate was recorded on RS + WP ~ 578.50 g , followed by RS + CI ~ 524.37 g , and the lowest yield was recorded on RS + SD)~ 435,92 g. The biological efficiency of *P. ostreatus* production when mixed substrates with RS (Table 4). The maximum BE of 57.85% was reported on RS + WP, followed by RS + CI 52.44%.

Mixed substrates with rice straw increase in biological efficiency of *P. ostreatus* over control substrates ( Fig. 2 ).

Akyuz and Yildiz (2008) and Kulshreshtha *et al.*, (2010) reported that in their evaluation of cellulosic wastes for the cultivation of *Pleurotus eryngii* (DC. ex Fr. ) Quel and bioremediation of industrial waste through mushroom cultivation.

**Table 4. Effect of mixed different substrates with rice straw (w/w) on Yields and Biological efficiency of *P. ostreatus*.**

Treatments (w/w)	Total fresh weight of fungus g/kg substrate dry weight	(BE %)
RS+SD	435.91	43.59
RS+WP	578.50	57.85
RS+CI	524.37	52.44
LSD at 5 %	4.228	0.423



**Fig. 2. Effect of mixed substrates with rice straw (w/w) on Biological efficiency of *P. ostreatus*.**

RS ; Rice straw. SD; Sawdust. WP; Waste paper. CI; Cardboard industrial. RS + SD ; Rice straw + Sawdust. RS + WP ; Rice straw + Waste paper . RS + CI ; Rice straw + Cardboard industrial .

Thongklang and Luangharn (2016) found that the best substrate to cultivate *P.ostreatus* is mixed substrates.

Also, Abd El-Razek, *et al.*, (2020) conculed that the *P.ostreatus* cultivation is an effective bioconversion process that is capable to transferring agro-wastes such as rice and wheat straws, into potentially valuable for food production as mushrooms.

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**تأثير خلط المواد المستخدمة في الزراعة مع قش الأرز على زراعة فطر عيش غراب النوع *Pleurotus ostreatus***  
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يتم في هذه الدراسة بحث تأثير المخلفات الزراعية والصناعية المختلفة، وكذا خلط هذه المواد مع قش الأرز لاستخدامها في زراعة فطر عيش غراب الأوبستر نوع بليروتس اويستراتس، فعند خلط المواد المستخدمة في الزراعة مع قش الأرز بنسبة ( ١ : ١ ) وجد لها تأثير ملحوظ على مراحل نمو وانتاج الفطر . ووجد ان بالزراعة على المخلفات الزراعية والصناعية المختلفة ان الوقت اللازم لنمو الاسبون وتكوين رؤوس الدبابيس وتكوين الاجسام الثمرية تم خلال الفترة من ١٢ إلى ٢٥،٥ ومن ٢٢ إلى ٥٢،٢٥ و من ٢٥ إلى ٥٥ يوم على التوالي . وعند خلط هذه المخلفات مع قش الأرز كانت النتائج المتحصل عليها للوقت اللازم لنمو الاسبون وتكوين رؤوس الدبابيس وتكوين الاجسام الثمرية في خلال الفترة من ١١،٧٥ إلى ١٨،٥ و من ٢٢،٥ إلى ٢٨،٥ و من ٢٦،٥ إلى ٣٢ يوم على التوالي ويتضح من ذلك ان خلط المواد المستخدمة في الزراعة مع قش الأرز أدى إلى تقليل فترة نمو الاسبون وبالتالي تقل فترات تكوين رؤوس الدبابيس وتكوين الاجسام الثمرية للفطر . كما تم الحصول على أعلى محصول للفطر عند خلط قش الأرز مع مخلفات الورق حيث تم الحصول على ٥٧٨،٥ جم / كجم مواد مختلطة . كما سجل خليط قش الأرز مع مخلفات الورق أعلى كفاءة بيولوجية للفطر وكانت ٥٧،٨٥ % أى ان : خلط المواد المستخدمة في الزراعة مع قش الأرز أدى إلى زيادة الكفاءة البيولوجية للفطر .