

## Production of Oyster Mushroom (*Pleurotus ostreatus*) on Tomato Tuff Agro waste

Kholoud M. Ananbeh and Ahmad M. Al-Momany \*

### ABSTRACT

An experiment was conducted to examine the ability of Oyster mushroom (*Pleurotus ostreatus*) to grow on tomato tuff agro waste mixed with wheat straw. Six treatments containing different concentrations from tomato tuff from 10-50% in the growing medium were examined including the control, which contained only 90% wheat straw with the fixed additives (wheat bran 5% and gypsum 5 %). Substrates were placed in transparent plastic bags after inoculation with spawn at a rate of 5% of the dry weight and incubation at 20-25°C for 10 days. Three days were needed for pinheads to start appearing, and then between 3-7 days were needed for the maturity of the basidiomete. Several growth parameters were studied including incubation period, primordial induction and fructification period, earliness, average weight of individual basidiomata, average yield for each treatment, diameter of the pileus, Biological Efficiency (BE%). Proximate analysis for protein, crude fat, crude fiber, ash, carbohydrates, minerals and moisture content were performed. It was found that adding up to 30% tomato tuff to the growing substrate gave satisfactory results (the yield 417-478g/bag, average cap weight 21-29g/cap, average cap diameter 9.2-10cm/cap, and BE% 84-96%). Carbohydrate and protein contents were the highest in *Pleurotus* basidiomete in tomato tuff 3 and tomato tuff 2 treatments respectively. The lowest fiber content was in tomato tuff 2. Ash content was similar in both tomato tuff 2 and 3, while fat content was positively correlated with tomato tuff percentage in the growing substrate.

For mineral contents in mushrooms the trend was not the same in all treatments. Both macro elements K and P and Na were negatively correlated with tomato tuff percentage except for tomato tuff 2 in P content. Mg and Fe contents were positively correlated with tomato tuff percentage except for tomato tuff 4 in Fe content. The highest Cu and Mn contents were found in tomato tuff 4, while the same treatment contained the least Ca and Fe contents. Zn content was the highest in tomato tuff 3 and the lowest (0.91ppm) was in tomato tuff 2 treatment.

**KEYWORDS:** Mushroom, *Pleurotus ostreatus*, Basidiomata, Biological Efficiency, Tomato Tuff, Cultivation, Agro Waste.

### 1. INTRODUCTION

The Oyster mushroom (*Pleurotus* sp.) is known by this name because of typical shape of pileus resembling to Oyster shell (Wani and Sawani, 1998), it is a wood destroying saprophytic fungus (Croan, 2000; Chang and Hayes, 1978), and sometimes appearing as a parasite is wide spread in the temperate zone. It is a cosmopolitan genus (Chang and Hayes, 1978) grown commercially in

the Far East countries and United States for several years ago (Royse and Schisler, 1987). German scientists were the first who studied this fungus (Guler and Muh, 1988). For both mycelial growth and fruit body development on lignin, and cellulose; materials such as corn cobs, all grain straws, paper wood shavings, sawdust, nutshells and vegetable wastes are sufficient. They commonly found in forests and wood land, where they grow on fallen branches, dead tree stumps and on folen logs (Croan, 2000). *Pleurotus spp.* is characterized by their rapid growth and high saprophytic colonization ability of the mycelium (Hoglov, 1999; Alian *et al.*, 1989; Al-shimi *et al.*, 1990; Madbouly, 1987). Sporophores may develop

---

\* Department of Plant Protection, Faculty of Agriculture, University of Jordan, Amman, Jordan. Received on 17/2/2008 and Accepted for Publication on 27/8/2008.

as white mycelium or with cream-colored pilei, or brownish brown, dark brown, black brown, gray, dark gray, blue gray or black gray ones. The individual specimens often grow in layers on top of each other (Wood and Smith, 1988; Hoglov, 1999). *Pleurotus* can be divided into 4 sections with a total of 39 species (Hoglov, 1999; Chang and Hayes, 1978). The higher price for fresh Oyster mushroom reflects, in part, the high cost of wheat straw and less reliable technology available to growers for cultivating these important species (Sharadqah, 2000). In Jordan, mushroom growers imports wheat straw from Saudi Arabia and this stuff is expensive and very important for feeding animals. Tomato tuff is available in Jordan in large quantity after making tomato juice and paste and this material spoils our environment. The objectives of this study were to decrease the cost of mushroom production by finding other cheap and suitable substrates for Oyster mushroom cultivation mainly by recycling agricultural wastes available as tomato tuff, and to develop a simple technique for Oyster mushroom production. The productivity as well as mushroom quality produced on tomato tuff was also investigated.

## 2. MATERIALS AND METHODS

### Fungal Culture

The basidiomycetous fungus *Pleurotus ostreatus* (Jacquin: Fries) P. Kummer was obtained from the White Button Establishment in Al-salt, Jordan. The strain used was P015 and obtained as ready spawns grown on wheat seeds. For our experiment, the fungal culture was prepared from a pure culture of the strain which was isolated on Malt Extract Agar (MEA) media. Our inoculum was produced from strain P015 by growing the fungus on boiled wheat seeds and incubated at 25°C for 10 days. Spawn was used at the rate of 5 gm mycelial clump for 100 gm of the growing medium (dry weight basis).

### Substrate Preparation

Each treatment was mixed with the additives as mentioned before and placed in a cloth bag and then completely submerged in a water bath inside a large drum at 100°C for 1-2 hours. This was done to eliminate insects and pathogens that may be found in the substrate. The working area was disinfected using house hold bleach diluted to 5% to avoid contamination. The bags containing the substrate were removed from the water

bath, allowed to drain, cooled at about 30-40 C° and prepared for cultivation. Then it was placed in large plastic bags in order to allow the manipulation of mixing the spawn with the substrate by shaking manually, and then it was inoculated with *Pleurotus* inoculum at a rate of 5% on the dry weight basis. Each bag contained 0.5 kg dry substrate and considered as one plot. Bags were then tied at the top by a nylon thread and punctured by a clean nail or fork in a form of (+) shape for ventilation (Ananbeh and Almomany, 2005).

The experiment was carried out in a glass house. Six treatments containing different concentrations from tomato tuff as shown down were used including the control treatment. Each treatment contained 0.5 kg dry matter. Completely Randomized Design (CRD) was used with four replicates. Data were statistically analyzed and treatments were compared using Waller Duncan multiple range test (Steel and Torrie, 1980). The additives used were 5% wheat bran and 5% gypsum. The control treatment was composed of 90% straw, 5% gypsum and 5% wheat bran.

### Treatments:

The amount of additives used for each media was similar, only the percentage of tomato tuff was subtracted from the percentage of wheat straw, the conducted treatments were prepared on a dry weight basis, and those were in addition to the control:

Tomato tuff 1: 10% tomato tuff: 80% straw: 5% wheat bran: 5% gypsum.

Tomato tuff 2: 20% tomato tuff: 70% straw: 5% wheat bran: 5% gypsum.

Tomato tuff 3: 30% tomato tuff: 60% straw: 5% wheat bran: 5% gypsum.

Tomato tuff 4: 40% tomato tuff: 50% straw: 5% wheat bran: 5% gypsum.

Tomato tuff 5: 90% tomato tuff: 5% wheat bran: 5% gypsum.

### Substrate Inoculation, Incubation and Culture Conditions

After pasteurization, substrates were placed inside plastic bags and inoculated with spawn at a rate of 5% of their dry weight, and then were placed for 10 days inside incubators at 20-25°C and under humid conditions between 80-95% with complete darkness during the first days of incubation until the compost was completely colonized by the mycelium. After that, the colonized

substrates were exposed to a cold shock around 4-5°C for 1-2 days to improve induction of the first fruiting structures. During fruiting period, ventilation was very important, so the bag's upper parts were opened and air was allowed to enter. Temperature was recorded by thermograph, (it was around 18-25°C on an average), and relative humidity was between 80-90%, it was obtained by watering the bags twice daily, and spraying water on the floor. Relative humidity was measured by a hygograph.

### **Harvesting and Measurement of Parameters**

Mushrooms were harvested when the pilei were fully mature and before they started to curl up. Remnants of the substrate attached to stipes were removed and the mushroom clusters were weighed. After the mushrooms were harvested, several parameters were evaluated to test the suitability of the used substrates for the cultivation of the Oyster mushroom. These parameters included: length of production cycle (incubation, primordial induction and fructification), earliness, (defined as the time elapsed between the day of inoculation and the day of the first harvest), the average weight of individual basidiomata (determined as quotient of the total weight of fresh mushrooms harvested by their total number), the average yield for each treatment, diameter of the pilei, color, Biological Efficiency (BE %), (calculated as the percentage yield of fresh mushroom over the dry weight of the substrate).

### **Proximate and Mineral Analysis**

Proximate analysis was determined according to the guidelines of the Association of Official Analytical Chemists (AOAC, 1995) for protein, crude fiber, ash, carbohydrate, minerals and moisture. Mineral analysis was performed by the wet ashing procedure; iron, zinc, calcium, magnesium, copper and manganese were determined by atomic absorption spectrophotometer, while sodium and potassium were determined by flame photometry (AOAC, 1995), and phosphorous was determined by using Olsen's method (Olsen and Dean, 1995).

## **3. RESULTS AND DISCUSSION**

As shown in Table (1), the incubation period was the longest in tomato tuff 4 where 40% of tomato tuff was used, it reached 2 weeks. Control treatment ranked

second, and it differs by 1 day from the longest and the shortest incubation period. Tomato tuff 1, tomato tuff 2, and tomato tuff 3 came in the third level, with no significant differences among them; they were the lowest in their values and were considered better for early cropping. By longer incubation period a marked delay in the fruiting structure formation will be observed (Wood and Smith, 1988). *Pleurotus* mycelium didn't spread in 90% tomato tuff media (tomato tuff 5), and so no parameters were taken on this treatment and it was excluded from the experiment. Tomato tuff in high percentage was not a suitable growing medium for *Pleurotus* due to low degradable and less soluble lignin and cellulose content. Organic matter in soluble form is needed for growing fungi and some are slow lignin decomposers. Adding more olive cake to the substrate may be detrimental to the growth of Oyster mushroom because seed fraction of olive cake is rich in lignin (Sharadqah, 2000) and this affects biodegradation rates of the substrate; lignin acts as a barrier for the breakdown of cellulose and delays the appearance of basidiomete, this may explains the results *et al* on growth parameters including incubation period, earliness and yield (Royse and Schisler, 1987).

Primordial induction time was increased when tomato tuff percentage was increased in the substrate. It was the lowest in tomato tuff 1, and the highest in tomato tuff 4 but with no significant difference from control, tomato tuff 2, and tomato tuff 3. No significant differences for earliness were noted among the different substrates used. *Pleurotus* mycelium ramifies the substrate slowly and pin heads formed after good colonization (Ananbeh, and Almomany, 2005).

Table (1) showed that there were no significant differences among the whole treatments in yield obtained except for that of tomato tuff 4 where it was 314g/bag. The yield of other treatments ranged between 413-478 g/bag. Average cap weight ranged between 20.6-28.6 g per cap, but with no significant differences among different substrates.

Average cap diameter was ranged from 7.2-10.1 cm per cap. It was the highest in tomato tuff 3 (10.1cm), with no significant differences in it from control, tomato tuff 1, and tomato tuff 2. Tomato tuff 4, which had the lowest average diameter, didn't differ significantly from the control treatment. It was successful to grow *Pleurotus* on tomato tuff by adding up to 30% from tomato tuff to growth medium. Table (1) showed that BE% was not

significantly different between control, tomato tuff 1, tomato tuff 2, and tomato tuff 3. But all of them were significantly different from tomato tuff 4, which had the lowest BE% that reached about 63%. Since biological

efficiency correlates with yield; these results were expected, and so the same trend of yield parameter has been noted in this study.

**Table 1. Effect of different rates of tomato tuff substrate amendments on incubation period, primordial induction, earliness, yield, average weight, average diameter, and biological efficiency (BE %) of *P. ostreatus*.**

Treatments	Inc. per* (Days)	Prim. Ind. (Days)	Earl. (Days)	Yield g/0.5kg	A.wt (g/cap)	A.D (cm/cap)	BE%
Control	13b**	30.3a	37.5a	413.1a	20.7a	8.4ab	82.6a
Tomato tuff 1	12c	25.0b	34.3a	478.2a	20.9a	9.2a	95.6a
Tomato tuff 2	12c	28.8a	36.5a	416.8a	25.8a	9.3a	83.4a
Tomato tuff 3	12c	28.0a	37.5a	456.3a	28.6a	10.1a	91.3a
Tomato tuff 4	14a	29.5a	37.8a	313.9b	20.6a	7.2b	62.8b

(\*): Inc.per: Incubation period, Prim.Ind: Primordial induction, Earl. Earliness, A. wt: Average weight, A.D: Average diameter, BE%: Biological efficiency.

(\*\*): Means within each column followed by the same letter were not significantly different according to Duncan's Multiple Range Test (P= 0.05).

**Table 2. Effect of different rates of tomato tuff substrate amendments on proximate composition of *P. ostreatus* basidiomete.**

Treatments	Protein %	Fat %	Ash %	Fiber %	CHO %	Energy Kcal**
Control	25.6c*	0.77ab	6.7a	20.2a	46.8a	296.2b
Tomato tuff 1	32.6ab	0.59b	5.9a	20.3a	40.7b	298.4b
Tomato tuff 2	35.2a	0.70ab	6.3a	17.0b	40.7b	310.1a
Tomato tuff 3	30.3b	0.91a	6.3a	20.3a	42.2b	298.1b
Tomato tuff 4	34.7a	0.70ab	5.7a	18.0b	40.9b	308.6a

(\*): Means within each column followed by the same letter were not significantly different according to Duncan's multiple Range Test (P= 0.05).

(\*\*): Energy: calculated by using proximate analysis values and using the values 4, 9, and 4 Kcal/100g soluble carbohydrates, fat and protein, respectively.

Table (2) showed that protein content of *Pleurotus ostreatus* was increased when tomato tuff content increased, due to the ability of the mushroom to utilize the protein content in tomato tuff itself. Protein content was the highest in tomato tuff 2, tomato tuff 4, and tomato tuff 1, respectively but they didn't differ significantly from each other. In tomato tuff 3, protein content was low but it was higher and significantly different from the control. The lowest protein value was found in the control treatment where it reached 25.6%.

Fat content was less than 1% and ranged between 0.5-0.9%. Tomato tuff 3 showed the highest fat content, but it was not significantly different from control, tomato tuff 2, and tomato tuff 4. Tomato tuff 1 showed the least fat content where it reached 0.59%.

No significant differences were noted for ash content in mushroom grown in different substrates (Table 2), and its value ranged between 5.9 in tomato tuff 1 to 6.7% in the control. It was shown in Table (2) that fiber content was the highest in control, tomato tuff 1, and tomato tuff 3 where it reached 20%. Both tomato tuff 2 and tomato tuff 4 came in the second level where there values were 17 and 18%, respectively.

Carbohydrate content of mushroom grown on different tomato tuff substrates was significantly less than the control. Tomato tuff 1, tomato tuff 2, tomato tuff 3, and tomato tuff 4 concerning carbohydrate contents were not significantly different from each other and their values ranged from 40-42%.

Tomato tuff 2 and tomato tuff 4 had the highest

energy level; 310 and 308 Kcal, respectively. Control, tomato tuff 1, and tomato tuff 3 came next in their energy content where there were no significant differences among them as shown in table (2). Proximate analysis for

Oyster mushroom obtained in this study was applicable with those recorded by Sanjust, *et al.*, 1991; Guler and Axoxlu, 1999; Chang and Hayes, 1978; Oei, 1991; and Ananbeh and Almomany, 2005.

**Table 3. Effect of different rates of tomato tuff substrate amendments on mineral contents in (ppm) of *P.ostreatus* basidiomete.**

Treatments	K	P	Na	Ca	Mg	Zn	Fe	Cu	Mn
Control	2973a*	1837bc	92.3a	1.7bc	29.6a	1.0ab	1.78a	0.18a	0.18a
Tomato tuff 1	2211b	2066a	88.3a	1.9ab	17.5a	1.3ab	1.77a	0.16a	0.21a
Tomato tuff 2	2095b	1732c	79b	2.1a	19.5a	0.9b	1.85a	0.17a	0.18a
Tomato tuff 3	2061b	1999ab	78b	1.4c	21.4a	1.3a	1.94a	0.16a	0.16a
Tomato tuff 4	1804b	1912abc	77b	0.8d	23.9a	1.0ab	1.63a	0.25a	0.24a

(\*): Means within each column followed by the same letter were not significantly different according to Duncan's multiple Range Test (P= 0.05).

Table (3) shows that K concentration was the highest in the control treatment which was significantly different from the other treatments. Phosphorus was high in its concentration in tomato tuff 1 with no significant differences from tomato tuff 3 and tomato tuff 4. The control treatment didn't differ significantly from tomato tuff 2, tomato tuff 3 and tomato tuff 4 in its P concentration. Sodium was the highest in its concentration in both the control and tomato tuff 1. The rest treatments didn't differ significantly from each other. Potassium, P and Na, concentrations were negatively correlated with tomato tuff percentage in the substrate. More growth induced dilution of some elements in tissues (Croan, 2000). For calcium, tomato tuff 2 had the highest amount but it didn't differ significantly from tomato tuff 1 which in its term didn't differ significantly from the control. Tomato tuff 3 didn't differ significantly from the control treatment. Tomato tuff 4 had the lowest Ca concentration. Calcium concentration was not correlated in any way with tomato tuff content in the substrate. There were no significant differences among the whole

treatments in their magnesium contents but it was higher in the control treatment when compared to the other treatments. Zinc was the highest in its concentration in tomato tuff 3 with no significant differences from the control, tomato tuff 1, and tomato tuff 4. Tomato tuff 2 had the lowest Zn concentration. Iron, copper, and manganese didn't differ significantly in their concentration among the whole treatments. The highest Cu and Mn contents were found in tomato tuff 4, while the same treatment contained the least Ca and Fe content. Same results were obtained in *Pleurotus ostreatus* grown on olive cake agro waste (Ananbeh and Almomany, 2005; Madbouly, 1987). Recycling of agro wastes is helpful to increase organic content of any growing substrate. Tomato tuff is an example of a substitute for wheat straw which can be used for growing *Pleurotus ostreatus* by adding up to 30% to the growing medium without causing any negative results on mushroom growth. Further treatments of tomato tuff are required to increase the availability of organic matter and other mineral contents

### REFERENCES

- Alian, A., Madbouly, F. and Gadallah, A. 1989. Cultivation of *Pleurotus columbinus* on cannery wastes. *Second Conference of Food Science and Technology for Mediterranean Countries*. Cairo.
- Al-shimi, M.M., Aliann, A.M., Madbouly, F.H. and Raouf, M.S. 1990. Cultivation and processing of mushrooms. 2- Drying and freeze-drying of *Pleurotus ostreatus*. Cairo University, Egypt.
- Ananbeh, K. M. and Almomany, A. R. 2005. Production of Oyster mushroom *Pleurotus ostreatus* on olive cake agro waste. *Dirasat*, 32 (1):64-70.
- Association of Official Analytical Chemists (A.O.A.C). 1995. Official methods of analysis of the association of official analytical chemists, sixteenth edition. Publishing Association of Official Analytical Chemists, Virginia, USA .
- Chang, S.T. and Hayes, W.A. 1978. *The Biology and Cultivation of Edible Mushrooms*. 1st edition. Academic Press, INC. London.
- Croan, S.C. 2000. Conversion of wood waste into value

- added products by edible and medicinal *Pleurotus* (Fr.) P.Karst. Species (Agaricales s.I, Basidiomycetes). *International Journal of Medicinal Mushrooms*. 2:73-80.
- Guler, M. and Axoxlu, Y.S. 1999. Comparison of composition elements on different substrates of Oyster mushrooms (*Pleurotus* spp.) growing under plastic sheet. *XI World Forestry Congress*, Vol(3), Topic 15.(Abstract) Available at <http://www.fao.org/forestry/foda/wforcong/PUBLI/V315E/3-27.htm>
- Guler, M. and Muh, Y.Z.1988. *Kayin Mantari Yetistirme Teknigi*. Yayin No.669, seri No: 16. Arastirma planlama ve koordinasyon dairesi baskanligi. Ankara.
- Hoglov, J. 1999. *Pleurotus flabellatus culturing on water hyacinth, Eichhornia crassipes*. M.Sc. Thesis, University of Daresalaam, Tanzania, Stockholm.
- Madbouly, F.H.1987. *Some Studies on Cultivation and Preservation of Mushroom*. Ph.D. thesis, Cairo University, Egypt.
- Oei, P. 1991. *Manual on Mushroom Cultivation. Techniques species and opportunities for commercial application in developing countries*. Transfer of Technology for Development (Amsterdam) and Technical Center for Agricultural and Rural co-operation (Wageningen).
- Olsen, S.R. and Dean, L.A. 1995. Phosphorus. In: Black, C.A.(editor), *Methods of Soil Analysis*. Part two. American Society of Agronomy, Inc., Madison.
- Royse, D.J. and Schisler, L.C. 1987. Yield and size of *Pleurotus ostreatus* and *P.sajor-caju* as affected by delayed-release nutrients. *Applied Microbiology and Biotechnology*, 26: 191-194.
- Sanjust, E., Pompel, R., Rescigano, A., Rinaldi, A. and Ballero, M. 1991. Olive milling wastewater as a medium for growth of four *Pleurotus* species. *Applied Biochemistry and Biotechnology*, 31: 223-235.
- Sharadqah, W.S.N. 2000. *Lignin Biodegradation During the Process of Biological Conversion of Olive Mill By – Product (Jift)*. M.Sc. Thesis, Jordan University of Science and Technology, Jordan.
- Steel, R.G.D. and Torrie, J.H. 1980. *Principles and Procedures of Statistics, 2<sup>nd</sup> edition*. McGraw-Hill Book Company, NewYork.
- Wani, P.V. and Sawani, D.M. 1998. Oyster-A mushroom of broad adaptability: an overview. *Journal of Maharashtra Agriculture University*, 23 (3): 230-237.
- Wood, D. A. and Smith, J.F. 1988. The cultivation of mushroom (Part II). *Mushroom Journal*, 188: 665-674.

### (*Pleurotus ostreatus*)

\*

	%	%	%
	7-3	%5	3
/ 29-21)	( / 417-478)	%30	%30-10
	.(%96-84)	( / 10-9.2)	(

.2008/8/27

2008/2/17

\*