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# **Article**

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# Mycodegradation of reed straw, Phragmites australis

# Owaid MN<sup>1,\*</sup>, Muslat MM<sup>2</sup>, Abed IA<sup>3</sup>

- <sup>1</sup> Department of Heet Education, General Directorate of Education in Anhar, Ministry of Education, Hit, Anhar 31007, Iraq
- <sup>2</sup> Department of Horticulture, College of Agriculture, University of Anbar, Ramadi, Anbar 31001, Iraq

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#### **Abstract**

The reed plant (*Phragmites australis*) is available as an economical alternative to wheat straw, when the last substrate is unavailable or has a high price, to apply it in the preparing compost for the cultivation of white button mushroom *Agaricus bisporus* X25. Reed straw compost recorded the best total yield (441 g/tray) compared with 371 g/tray by the wheat straw compost (control). Also, use of Actinomycetes *Streptomyces* sp. in the biodegradation processes of compost, lead to increase the total yield of *A. bisporus* X25 to 430 g/tray compared with the microbiologically nontreated composts (376 g/tray), after 21 days from primordia appearance significantly (p<0.05). Quality and size of fruiting bodies were varied from compost to another one depended on diameter of cap (pileus) and length of stipe. The more significant diameter had been recorded 56 mm for fruits on reed straw compost while the lower diameter was 42 mm on fruits of the control compost (wheat straw compost).

**Key words** – *Agaricus bisporus* – agricultural wastes – compost – mushroom – *Streptomyces* sp.

### Introduction

The white button mushroom *Agaricus bisporus*, one of the fleshy fungi, belongs to Basidiomycota. *A. bisporus* has a high nutritive value due to its high protein content compared with *Pleurotus* sp. (Chang & Miles 2004). *A. bisporus* is an edible mushroom that grows upon decomposed organic matters. Generally, mushrooms don't have the ability for using the energy of the sun to produce the carbohydrates in photosynthesis because lacking chlorophylls (Stephens 2003). The first mention of cultivation of mushroom was in 1652 when appeared in the field naturally (Beyer 2003). The potential of bioconversion of lignocellulosic wastes into value-added products was tested in last studies such as production of fresh fruits, feed of ruminants and production of enzymes (Zervakisn & Philippoussis 2000). *A. bisporus* can be grown on wheat straw (Baysal et al. 2007), sawdust mixed with wheat straw (Schmidt 2006), waste tea leaves (Simsek et al. 2008), waste paper (Sassine et al. 2007), common reed (Alheeti et al. 2010, Muslat et al. 2011a,b) and sunflower head composts (Muslat et al. 2014).

Medicinally, Hippocrates first referred to the medicinal mushrooms in the treatment in 400 B.C. (Beyer 2003). Extracts of *A. bisporus* has antibacterial (Tamberkar et al. 2006), antifungal (Atila et al. 2017) antioxidant, and anticancer activities (Roberts et al. 2008). Also it used to treat

<sup>&</sup>lt;sup>3</sup> Desert Studies Center, the University Campus, University of Anbar, Ramadi, Anbar 31001, Iraq

atherosclerosis and diabetes; and to treat obesity because of it has low calories (Halpern 2006). Recently, *A. bisporus* was used to biosynthesize silver nanoparticles as nano-drugs in so-called Green Chemistry (Atila et al. 2017). *A bisporus* has some useful mineral elements such as Na, K, Pb, Co, Fe, Cr, Cu, Ni, Zn, Mn and Cd which encourage the citizens for consuming it as a healthy food (Owaid 2015). Also, it contains elemental P, S, Ca and Li, without starch, rich of essential vitamins A, B, C, and D (USDA 2011) and essential amino acids (Muslat et al. 2014).

The genus *Streptomyces* is one of the most genera of Actinomycetes in soil; it belongs to the family Streptomycetaceae and order Actinomycetales. It is filament bacteria, aerobic and Grampositive bacteria (Locci 1989). The bacteria *Streptomyces* spp. has a wide distribution in the various environments (soil, water, and air). But the soil is a major source of this bacterium (David & Sheare 1988). Also, it finds in other ecological media such as organic fertilizers, manure, and plant residues. The warm places are adequate to grow huge biomass of this bacterium; also the organic matters are a useful and a necessary factor in developing this microorganism (Motr 2009). This bacterium is capable to degrade the simple forms of cellulose and produce its biomsss which is considered as nitrogen source for mycelial of mushroom later (Muslat et al. 2014). Thus this bacterium was used to decompose composts in this work to raise the total yield of *A. bisporus* X25 after treatment of those composts by Actinomycetes in a low cost and an eco-friendly method.

# **Materials & Methods**

#### Microbial strain

The spawn of white button mushroom *Agaricus bisporus* X25 was obtained from Le Lion Company, France by the Hameediyah Mushroom Farm (HMF), Ramadi, Kilo 18, Iraq. The bacterium *Streptomyces* sp. (strain O3) obtained from Department of Biology, College of Science, University of Anbar, Iraq which isolated from Ramadi soils, Iraq. This bacterium was used as bioagent to biodegrade of media at concentration 10<sup>6</sup> cfu (cells)/kg of dry matter during the composting process.

# Preparing inoculum of Streptomyces sp.

The bacterium *Streptomyces* sp. is maintained using Gauze's Agar medium (GAM) which constituents of 20 g soluble starch, 1 g KNO<sub>3</sub>, 0.5 g NaCl, 0.5 g MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.5 g K<sub>2</sub>HPO<sub>4</sub> and 0.001 g FeSO<sub>4</sub>.5H<sub>2</sub>O which dissolved in 1 L of the tap water. The pH of GAM was adjusted to 7.2-7.4, agar 15 g added, dissolved and autoclaved (Motr 2009). The Nutrient Broth is used to prepare the *Streptomyces* inoculum at the concentration  $10^6$  CFU/ml and applied ml/kg of dry matter.

# Preparing compost, composting, pasteurization and the Cultivation of A. bisporus X25

Below, details of each formula which obtained from local gardens in Heet district, Iraq. The principal lignocellulosic matters are formed from wheat (*Triticum sativum*) straw, reed (*Phragmites australis*) straw. Six treatments were used in this test including A: wheat straw compost, B: reed straw compost, C: the mixture compost (1:1), AS: the decomposed wheat straw compost using *Streptomyces* sp., BS: the decomposed reed straw compost using *Streptomyces* sp., CS: the decomposed mixture compost using *Streptomyces* sp., see Table 1.

Solid state fermentation was achieved outdoor and indoor during phase 1 and phase 2 respectively as mentioned by Owaid (2009). After pasteurization of compost and cooling, mushroom spawn was applied within bed at percent 2% based on dry weight using Ruffling Method until mycelial growth completion. Pasteurizing the casing layer (consisted of peat moss and soil) was applied using formalin at concentration 500 ppm. Other processes were used as mentioned by Owaid (2009). Casing layer was used in-depth 2.5 cm as mentioned by Royse (2008). At 18-16 °C temperature and 90% relative humidity, some determinations of this mushroom were calculated using six replications. The total yield of *A. bisporus* X25 for the period 21 days after primordia appearance, biological efficiency, number of fruiting bodies, diameter of cap, length of

stipe and the ratio of diameter of cap to length of stipe were determined in six composts with or without decomposition using bacterium *Streptomyces* sp.

**Table 1** Properties and compositions of composts of *Agaricus bisporus* X25 in this work

Materials (kg)	A	В	С	AS	BS	CS
Wheat straw	50	-	25	50	-	25
Reed straw	-	50	25	-	50	25
Chicken manure	41.2	41.2	41.2	41.2	41.2	41.2
Urea	2.47	2.47	2.47	2.47	2.47	2.47
Gypsum	6.33	6.33	6.33	6.33	6.33	6.33
Streptomyces sp. applying	-	-	-	Yes	Yes	Yes

# **Statistical Analysis**

Statistical significance was determined using CRD (two ways) analysis of variance (ANOVA) by applying GenStat program (VSN International Ltd., UK). Significant differences at p<0.05 were considered. All the experiments were done in six replicates.

#### **Results**

Influence of the treatments (type of compost and using *Streptomyces* or no) on the crop of *Agaricus bisporus* X25 was reported and discussed in this study. The treated reed straw compost using *Streptomyces* sp. (BS) showed higher total yield (496 g/tray) significantly (p<0.05), followed 400, 397 and 396 g/tray for C, AS and CS composts respectively, compared with the lower total yield which showed on wheat straw compost (control, A) (344 g/tray) in this work (Table 2). The best biological efficiency was 29.3% on the decomposed reed straw compost using *Streptomyces* bacterium (BS), followed by 28.8% and 25.1% on AS and C composts respectively. But, the lower biological efficiency was 20.8% on CS compost compared with 22.7% by the control (A). The higher fruits number was observed 18 fruits on the treated wheat straw compost using *Streptomyces* (AS) compared with 17 fruits by the control (wheat straw compost, A) significantly (p<0.05). The lower number was 7 fruits on the reed straw compost (B) which increased to 8 fruits when using bacteria in the decomposing process (BS compost).

Quality and size of fruiting bodies were varied from compost to another one depended on the diameter of cap (pileus) and the length of stipe. The biggest diameter had been recorded on fruits of reed straw compost (B) 56 mm, while the smaller diameter was 42 mm for the fruits on the control compost (A). The bigger length was 34 mm on fruits of the treated wheat straw compost using *Streptomyces* (AS) compared with the control (29 mm) as a lower value (Table 2). The higher ratio of diameter of cap to the length of stipe (DC/LS ratio) was 1.8 on the mixture compost (C) while the lower ratio was 1.2 on the treated wheat straw compost using *Streptomyces* (AS). DC/LS ratio is useful for the quality of the mushroom when equal or more than 1.

**Table 2** Properties of the harvested *Agaricus bisporus* X25 during 21 days after primordia appearance

Features	A	В	С	AS	BS	CS	LSD
							(p < 0.05)
Total Yield (g/tray)	344	385	400	397	496	396	5.8
Biological efficiency (%)	22.7	23.6	25.1	28.8	29.3	20.8	10.82
No. of Fruiting bodies	17	7	9	18	8	10	0.9
Diameter of cap (mm)	42	56	55	43	55	50	2.5
Length of stipe (mm)	29	32	29	34	30	28	1.7
DC/LS ratio	1.4	1.7	1.8	1.2	1.8	1.7	0.11

Legend: Tray: (5 kg based on wet matter), DC/LS ratio: diameter of cap to length of stipe ratio. A: wheat straw compost, B: reed straw compost, C: the mixture compost (1:1), AS: the decomposed wheat straw

compost using *Streptomyces*, BS: the decomposed reed straw compost using *Streptomyces*, CS: the decomposed mixture compost using *Streptomyces*.

Effect of the organic source and using *Streptomyces* sp. bacterium on the cultivation of *A. bisporus* X25 was tested and recorded. The compost type or the carbon source has a significant (p<0.05) influence on most properties of *Agaricus bisporus* X25 as shown in Table 3. The reed straw compost showed the best total yield 441 g/tray then it decreased to 398 g/tray on the mixture compost 1:1 in comparison with the control (wheat straw compost) which reached 371 g/tray. The biological efficiency of wheat straw and reed straw composts reached 26% without any effects. The more significant fruits number has recorded 18 fruiting bodies on the wheat straw compost, significantly (p<0.05), compared with other composts. The reed straw compost has only eight fruits and significant cap's diameter reached 56 mm. The length of stipe was approx. 31 mm as average but the ratio of the diameter of cap to the length of stipe (DC/LS ratio) was more than one on all composts.

**Table 3** Effect of using *Streptomyces* sp. on properties of the harvested *A. bisporus* 

Treatments	Total yield (g/tray)	Biological efficiency %	Fruit bodies No.	Diameter of cap (mm)	Length of stipe (mm)	DS/LS ratio
Wheat straw compost	371	26	18	43	32	1.30
Reed straw compost	441	26	8	56	31	1.75
The mixture compost 1:1	398	23	10	53	29	1.75
LSD ( $p < 0.05$ )	4.1	7.6	0.6	1.8	1.2	0.083

Legend: Tray: 5 kg based on wet matter, DC/LS ratio: diameter of cap to length of stipe ratio.

Significantly, (p<0.05), using Streptomyces sp. bacterium led to increase the total yield of fresh Agaricus bisporus X25 after 21 days from primordia appearance that reached 430 g/tray (5 kg compost based on wet matter) compared with the microbiologically non-treated composts (376 g/tray), see Table 4. Also, the previous table showed the biological efficiency of A. bisporus X25 cultivated on the microbiologically treated composts. The treated composts using Streptomyces gave a biological efficiency of 26% compared with the non-treated compost of 24%. Using bacteria led to increase the number of fruiting bodies to 12 fruits significantly (p<0.05), compared with 11 fruiting bodies on the microbiologically non-treated composts. Quality and size of fruiting bodies were varied from compost to another one depending on the diameter of cap (pileus), the length of stipe and the ratio of the diameter of cap to the length of stipe of fruiting bodies (Table 4). The diameter of cap recorded approx. 50 mm in this test, generally. Using bacteria in the treatments gives diameter of cap 49 mm compared with 51 mm without the biological treatment. Also, using Streptomyces as a decomposer for composts leads to increase the length of stipe to 31 mm compared with 30 mm without microbiologically treating. If the ratio of the diameter of cap to the length of stipe of fruiting bodies was more than 1, that meaning it is useful for quality of mushroom.

**Table 4** Effect of using *Streptomyces* sp. on properties of the harvested A. bisporus

Treatments	Total yield (g/tray)	Biological efficiency %	Fruiting bodies No.	Diameter of cap (mm)	Length of stipe (mm)	DS/LS ratio
Without using the bacteria	376	24	11	51	30	1.63
Using the bacteria	430	26	12	49	31	1.56
LSD ( <i>p</i> <0.05)	3.3	6.2	0.5	1.4	0.9	0.068

Legend: Tray: 5 kg based on wet matter, DC/LS ratio: diameter of cap to length of stipe ratio.

# **Discussion**

In this study, biological efficiency depends on total yield after 21 days from primordia appearance as demonstrated by the positive correlation between the total yield and the biological efficiency (Table 5). According to compost weight, the mushroom yield on reed straw compost increased compared with the wheat straw and the mixture composts. The variances in the yield may be returned to difference these composts in their capability in ecological and nutritional requirements because the differences of compositions of composts. Also, the content of cellulose, hemicellulose, and lignin was varied in these composts, which took place in the numerous decomposition levels (Edit et al. 2006). When mushroom yield increased, fruits sizes increased too as seen in Table 5, which demonstrated finding a positive correlation among mushroom characteristics.

**Table 5** Correlation among properties of mushroom A. bisporus X25

Correlation	Total yield	BE	No. of	Diameter	Length of	DC/LS
			Fruits	of cap	stipe	ratio
BE	0.483	1.000				
No. of Fruits	0.084	0.267	1.000			
Diameter of cap	0.153	-0.155	-0.865	1.000		
Length of stipe	-0.012	0.151	0.176	-0.116	1.000	
DC/LS ratio	0.127	-0.202	-0.770	0.848	-0.618	1.000

Legend: BE: biological efficiency, DC/LS ratio: diameter of cap to length of stipe ratio.

From another side, type and ratio of constituents of the compost have a useful effect toward production of mushroom, which shows an increasing number of fruiting bodies in the control compost due to finding more of nutrients in wheat straw and the mixture composts compared with the reed compost (Muslat et al. 2011b). All that led to increasing surfaces of compost constituents to occur most biodegradation and formation of fungal biomass. These results made to produce high fruits, sometimes appear in cluster form on casing layer (Aswad 2005). The number of fruiting bodies decreased on the reed straw compost in comparison with wheat straw, and the mixture composts that due to different proportions and constituents of each medium (Owaid 2009).

The reason for the high yield when using *Streptomyces* sp. in the decomposition process may be returned to the ability of Actinomycetes biomass to give high levels of amino acids that lead to improving agro-media for *A. bisporus* cultivation (Aswad 2005). Extracts of bacteria also could inhibit the growth of undesirable microorganisms such as green molds *Trichoderma* sp. and other pathogenic fungi (Fermor & Grant 1985) because of recording alkaline levels in compost when finding *Streptomyces* sp. (Muslat et al. 2011a). *Streptomyces* sp. adjusts pH of media to alkaline by producing ammonia which helps to precipitate CaCO<sub>3</sub>. It produces bioactive matters like antibiotics (Adekunle 1985). Likewise, Inbar et al. (2005) referred to produce metabolism byproducts such as antibiotics and extracellular enzymes in compost which has force toward some fungal pathogens.

Streptomyces sp. has critical ecological roles in the decomposition of organic matters such as plant wastes, manure, and organic fertilizers by using keratinase and chitinase as nitrogen and carbon sources (Chigaleichik et al. 1978) and cellulose (Ishaque & Kluepfel 1980). Motr (2009) used isolated Streptomyces sp. isolated from the soil in the decomposing biotechnology because it is capable of handling and utilizing lignin and cellulosic matters as a carbon source with inhibitory activity against pathogens and undesirable fungi. Streptomyces sp. is useful in increasing yield and essential physiochemical properties of compost by its byproducts and its biomass (Aswad 2005). That agrees with the positive correlation between the nitrogen content in compost and mushroom from the side (Royse 2008), and with results of this experiment from another one. The growth of bacteria especially Streptomyces sp. is essential to growing A. bisporus because of its effects on C:N ratio which leads to induce the primordial formation (Wood 1976, Chen et al. 2013). This bacterium plays a useful role to prevent stay asexual phase of A. bisporus and induce fruiting

bodies formation (Wood 1976). These results agree with findings of Straatsma et al. (1989), Sanchez & Royse (2008), Zarenejad et al. (2012), they referred for producing a suitable compost for *A. bisporus* cultivation by using *Scytalidium thermophilum* and some thermophilic fungi.

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