Cultivation of *Agaricus bisporus* X25 on Reed Plant (*Phragmites australis*) Straw Decomposed by Using Actinomycetes

Agaricus bisporus X25'in Actinomycetes Kullanarak Saz Bitkisi (*Phragmites australis*) Üzerinde Yetiştirilmesi

Research Article

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ABSTRACT

The reed plant is available as economic alternative instead of wheat straw in state unavailable or the high prices to use it in the preparing compost for cultivation of *Agaricus bisporus* X25. In significant (*P*<0.05), use of *Streptomyces* inoculum led to increase the total yield of *A. bisporus* X25 during 21 days to 430 g/box (g/5 kg compost based on wet matter) compared without treatment (376.94 g/box). The treated reed straw showed higher yield 496.33 g/box. Using bacterial inoculum given biological efficiency 26.3% compared without treatment 23.8%. The best biological efficiency was 29.3% with treated reed straw compost using bacterium *Streptomyces*, while, the lower value was 20.8% for the mixture compost in same previous case. Quality and size of fruiting bodies were varied from compost to another one depended on diameter of cap (pileus), length of stipe. The bigger diameter had been recorded 55.83 mm for fruits of reed straw compost while the lower diameter was 42.33 mm on fruits of control compost.

Key Words

Bio-recycling, compost, food, white button mushroom.

ÖZ

Saz bitkisi, *Agaricus bisporus* X25'in yetiştirilmesi için buğday samanının, hazırlama kompostunda kullanılmak üzere bulunmadığı veya yüksek maliyetli olduğu durumlarda ekonomik bir alternatif olarak kullanılır. Belirgin olarak (P<0.05) *Streptomyces inoclum*'unun (aşı) kullanılması iyileştirme yapılmayan durumla (376.94 g/kutu) karşılaştırıldığında 21 gün süresince 430 g/kutu (ıslak maddeye bağlı olarak g/5 kg kompost) miktarına kadar toplam *A. bisporus* X25 veriminde artışa neden olmuştur. İyileştirilmiş saz samanı 496.33 g/kutu olmak üzere yüksek verim göstermiştir. Bakteriyel inoklum kullanılması iyileştirme yapılmayan durumla karşılaştırıldığında (%23.8) biyolojik etkinlik sağlamıştır (%26.3). *Streptomyces* bakterisi kullanılarak iyileştirilmiş saz samanı ile en iyi biyolojik etkinlik değeri %29.3 olup, önceki durumdaki kompost karışımı için değer %20.8'dir. Sporokarpların kalitesi ve büyüklüğü komposttan diğer başlığın çapına ve sapın uzunluğuna bağlı olarak değişmektedir. Kontrol kompostu meyveleri üzerindeki saz samanı meyveleri için kaydedilmiş en büyük çap 55.83 mm iken en düşük çap 42.33 mm'dir.

Anahtar Kelimeler

Bio-geri dönüşüm, kompost, gıda, beyaz düğme mantar.

Article History: Received: Jul 7, 2016; Revised: Sep 15, 2016; Accepted: Sep 20, 2016; Available Online: Apr 1, 2017.

DOI: 10.15671/HJBC.2017.153

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INTRODUCTION

The edible mushroom *Agaricus bisporus*, one of fleshy fungi, belongs to Basidiomycota. It has high nutritive value due to its high protein content [1]. *A. bisporus* is a mushroom that grows upon decomposed organic matters. Generally, edible mushrooms don't have ability for using energy of sun to produce the carbohydrates because lacking chlorophylls [2]. The potential of bio-conversion of lignocellulosic wastes into value-added products was tested in recent studies [3]. *A. bisporus* can be grown on wheat straw [4], sawdust mixed with wheat straw [5], waste tea leaves [6,7], waste paper [8,9], common reed [10-12] and sunflower head composts [13].

Medicinally, Hippocrates first referred to medicinal mushrooms in treatment in 400 B.C., while the first mention of cultivation of mushroom was in 1652 when appeared in the field naturally [14]. Extracts of *A. bisporus* has antimicrobial [15] and anticancer activities [16], also it used to treat atherosclerosis and diabetes; and to treat obesity because of its low calories [17]. *A bisporus* has some useful mineral elements such as Na, K, Pb, Co, Fe, Cr, Cu, Ni, Zn, Mn and Cd. These microelements encourage the citizens for consuming healthy food [18]. Also, it is contains elemental P, S, Ca and Li, without starch and rich of essential vitamins A, B, C, and D [19].

The genus *Streptomyces* is one of most genera of Actinomycetes in economical state; it belongs to the family Streptomycetaceae and order Actinomycetals [20]. It is filament bacteria, aerobic and gram positive charge [21]. The bacterium *Streptomyces* has wide distribution in the various environments (soil, water and air). But the soil is a major source for this bacterium [22]. Also, it finds in other ecological media such as organic fertilizers, manure, plant matters and upon surface of plant [23]. The warm places are more adequate to grow huge numbers of this bacterium; also the organic matters are useful and necessary factor to grow this microorganism [24]. Actinomycetes diminished rapidly with the latter disappearing after 6 weeks [25], thus this bacterium was used to decompose composts in this work to raise total yield of *Agaricus bisporus* X25 on those local composts.

MATERIALS and METHODS

Mushroom Strain

The mushroom spawn of white button mushroom *Agaricus bisporus* X25 was obtained from Le Lion Company, France by the Hameediyah Mushroom Farm (HMF), Ramadi, Kilo 18.

Bactria Strain

Bactrium *Streptomyces* obtained from Department of Biology, College of Science, University of Anbar which isolated from Ramadi soils, Iraq. This bacterium was used as inoculum at concentration 10^6 cfu (cells)/g of solid substrate/compost during composting process.

Preparing Inoculum of *Streptomyces*

This bacterium *Streptomyces* maintenances using Gauze Agar medium which composing from 20 g soluble starch, 1 g KNO_3, 0.5 g NaCl, 0.5 g $MgSO_4.7H_2O$, 0.5 g K_2HPO_4 and 0.001 g $FeSO_4.5H_2O$ which dissolved in 1 L of tap water. The pH of medium was adjusted to 7.2-7.4 then agar added and autoclaved. Then nutrient broth used to prepare the *Streptomyces* inoculum at concentration 10^6 cfu/g of solid compost.

Proportions of Mushroom Composts

Below, details of each formula and its C:N ratio which obtained from local gardens in Hit, Iraq. The main lignocellulosic matters are formed from

Table 1. Properties and compositions of composts of harvested *Agaricus bisporus* X25 in this study.

Materials (kg)	Wheat straw compost	Reed straw compost	Mixture compost 1:1
Wheat straw	50	-	25
Reed straw	-	50	25
Chicken manure	41.2	41.2	41.2
Urea	2.47	2.47	2.47
Gypsum	6.33	6.33	6.33
C:N Ratio	10:1	22:1	20:1

Figure 1. Mycelial growth completion of *A. bisporus* X25 on reed straw compost in the box.

wheat (*Triticum sativum*) straw, reed (*Phragmites australis*) straw and their mixture 1:1 [26].

Composting, Pasteurization and Cultivation

Solid state fermentation was achieved outdoor and indoor during phase 1 and phase 2 respectively as mentioned by Alheeti [26]. 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 (Figure 1). Pasteurizing casing layer was applied which consisted from peat moss and soil. Other processes were used as mentioned by Alheeti [26]. Casing layer was used in depth 2.5 cm as mentioned by Royse [27].

Determinations

Total yield for 21 days, 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 three composts with or without decomposition using bacterium *Streptomyces*.

Statistical Analysis

Statistical significance was determined by using CRD (two ways) analysis of variance (ANOVA) by implementing GenStat Discovery Edition computer program version 7 DE3 (VSN International Ltd., UK). Significant differences at $P < 0.05$ were considered. All the experiments were done in six replicates.

RESULTS and DISCUSSION

In significant ($P < 0.05$), the treatment using bacterium *Streptomyces* inoculum led to increase the total yield of fresh *Agaricus bisporus* X25 during 21 days to 430 g/box (g/box which equal 5 kg compost based on wet matter) compared without treatment (376.94 g/box). The treated reed straw compost showed higher yield 496.33

Table 2. Productivity and biological efficiency of *Agaricus bisporus* X25 after 21 days.

Treatments	Yield (g/box) (box= 5 kg based on wet matters)		Mean of inoculum
	Wheat straw compost	Mixture compost (1:1)	
Without treatment	344.83	400.67	376.94
With bacterial treatment	397.50	396.17	430.00
LSD (P <0.05)	5.878		3.394
Without treatment	Biological efficiency (%)		
	22.7	25.1	23.8
With bacterial treatment	28.8	20.8	26.3
LSD (*P* <0.05)	10.82		6.24

Figure 2. Fruiting bodies of *Agaricus bisporus* X25 in mushroom room. **Legend**: A2: wheat straw compost, B2: reed straw compost, C2: the mixture compost (1:1), AS3: wheat straw compost which decomposed using bacterial inoculum *Streptomyces*, BS3: reed straw compost which decomposed using *Streptomyces*. CS3: mixture compost which decomposed using *Streptomyces*.

g/box significantly ($P < 0.05$), followed 400.67, 397.50 and 396.17 g/box for the mixture compost with and without treatment and wheat straw compost with treatment respectively. While the lower productivity was 344.83 g/box for wheat straw compost (control) in this trial (Table 2 and Figure 2).

Also, Table 2 showed the biological efficiency of *A. bisporus* X25 cultivated on three composts which treated with or without bacterial inoculum. The treated compost given biological efficiency 26.3% compared without treatment 23.8%. The best biological efficiency was 29.3% with decomposed reed straw compost using bacteria *Streptomyces*, while, the lower biological efficiency was 20.8% for the mixture compost in same previous case.

Table 3 showed influence of treated compost using bacteria toward fruiting bodies number which reached to 12 fruits compared with approx. 11 fruits in case without treatment. The higher fruits number was observed on the treated wheat straw compost approx. 18 compared with the control (wheat straw compost) approx. 17 significantly (P <0.05). The lower number was 7 fruits on reed straw compost. The treated mixture compost had 10 fruits.

Quality and size of fruiting bodies were varied from compost to another depended on diameter of cap (pileus), length of stipe (Table 4) and ratio of diameter of cap to length of stipe of fruiting bodies (Table 5). Diameter of cap recorded approx. 50 mm in this trail generally. Using bacteria in treatment gives diameter of cap 49.89 mm compared without treatment (51.22 mm). The bigger diameter had been noted on fruits of reed straw compost 55.83 mm while the smaller diameter was 42.33 mm on fruits of control compost. Also, using *Streptomyces* as a decomposer for composts leads to increase length of stipe to 31.28 mm compared without

Table 3. Number of *Agaricus bisporus* X25 fruiting bodies.

Treatments	Wheat straw compost	Reed straw compost	Mixture compost (1:1)	Mean of inoculum
Without treatment	16.50	7.17	8.83	10.83
With bacterial treatment	18.33	7.67	10.00	12.00
LSD (P <0.05)	0.932			0.538

Table 4. Size of fruiting bodes of *Agaricus bisporus* X25.

Treatments	Average of diameter of cap (mm)		Mean of inoculum
	Wheat straw compost	Mixture compost (1:1)	
Without treatment	42.33	55.50	51.22
With bacterial treatment	43.83	50.67	49.89
LSD (P <0.05)	2.566		1.482
	Average of length of stipe (mm)		
Without treatment	29.00	29.83	30.56
With bacterial treatment	34.17	28.83	31.28
LSD (P <0.05)	1.708		0.986

Table 5. Ratio of diameter of cap to length of stipe of fruiting bodies.

Treatments	Wheat straw compost	Reed straw compost	Mixture compost (1:1)	Mean of inoculum
Without treatment	1.467	1.712	1.858	1.679
With bacterial treatment	1.283	1.800	1.775	1.619
LSD (*P* <0.05)	0.1185			0.0684

treatment (30.56 mm) the bigger length was 34.17 mm on fruits of the treated wheat straw compost compared with the control one (29 mm) as a lower value (Table 4).

If ratio of diameter of cap to length of stipe of fruiting bodies was more than 1, that meaning useful in quality of mushroom. These results had been given significant (*P* <0.05) value at level less than 0.05 (Table 5). The higher ratio was 1.85 with the mixture compost while the lower ratio was 1.28 with the treated wheat straw compost using *Streptomyces*.

Compost type or carbon source have significant (*P* <0.05) influence on most properties of *Agaricus bisporus* X25 which seen in Figure 3. Reed straw compost recorded best yield (440.83 g/box) and biological efficiency (22.4%) for wheat straw and the mixture composts, respectively.

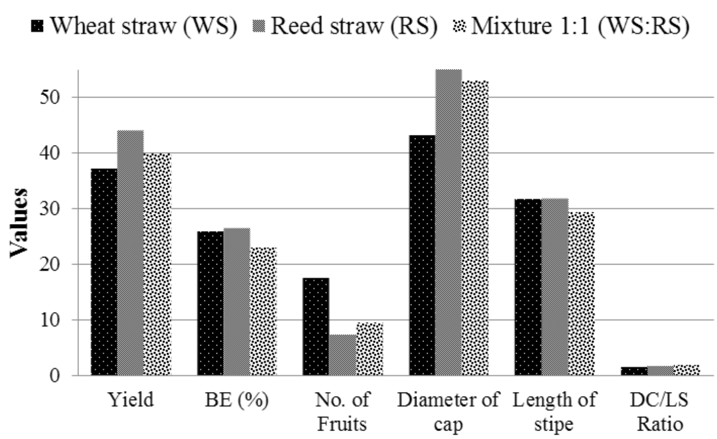

Figure 3. Type on some characteristics of mushroom *A. bisporus*. **Legend:** LSD ($p<0.05$) for Yield (g/bog) =4.15, BE (%) =7.65, No. of Fruits =0.659, Diameter of cap (mm) =1.81, Length of stipe (mm) =1.20, DC/LS Ratio=0.083.

Table 6. Correlation among properties of mushroom.

Correlation	yield	BE	Fruits No.	Diameter of cap	Length of stipe	DC/LS ratio
BE	0.483	1.000				
Fruits No.	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.

The bigger fruits number was approx. 17 fruiting bodies on wheat straw compost, significantly ($P < 0.05$). While bigger cap's diameter was 55.5 mm on reed straw compost and lower length of stipe was 29.33 mm on the mixture compost which given best DC/LS ratio (1.8).

In this study, biological efficiency is depend on total yield during 21 days as demonstrated by positive correlation between productivity (total yield) and biological efficiency (Table 6). According to compost weight, the mushroom yield on reed straw compost increased compared with the wheat straw and the mixture composts. The differences in yield may be return to difference those composts in capability of composts in ecological and nutritional requirements. Also, content of cellulose, hemicellulose and lignin was varied in these composts, which took place in numerous decomposition levels [28,29]. When mushroom yield increased, fruits sizes increased too as seen in Table 6 which demonstrated that in positive correlation between these characteristics.

From other side, type and size of compost constituents have important effect toward mushroom production, which demonstrates increasing number of fruiting bodies in the control compost because of its available nutrients in wheat straw and the mixture composts more than reed straw compost [12]. All that, led to increase surfaces of compost constituents which gave most decomposition and bigger fungal biomass. These results make to form greater fruits, sometimes appear in cluster form on casing layer [30]. Number of fruiting bodies decreased on reed straw compost in comparison with wheat straw and the mixture composts that due to difference proportions and constituents of each medium [26].

The reason of the high yield by using *Streptomyces* as an inoculum during composting phase may be return to ability this bacterium to give proteins, which lead to improve agro-media for *A. bisporus* cultivation. Extracts of bacteria also could inhibit growth of undesirable microorganisms such as green molds *Trichoderma* sp. and other pathogenic fungi [31] because of recording alkaline levels in compost when using *Streptomyces* [11]. *Streptomyces* adjust pH of media to alkaline by producing ammonia which helps to precipitate $CaCO_3$. It produces bioactive matters like antibiotics [23]. Also, Inbar et al. [32] referred to produce metabolism byproducts such as antibiotics and extracellular enzymes in compost which has force toward some fungal pathogens.

Streptomyces has important ecological roles in decomposition of organic matters such as plant wastes, manure and organic fertilizers by using keratinase and chitinase as nitrogen and carbon sources [33] and cellulose [34]. Hopkins et al. [35] and Motr [36] used isolated *Streptomyces* in the decomposing biotechnology because it is capable to use and utilize lignin and cellulosic matters as carbon source with inhibitory activity against pathogens and undesirable fungi. *Streptomyces* is useful in increasing yield and important physiochemical properties of compost by its byproducts and its biomass [30]. That agrees with positive correlation between nitrogen content in compost and in mushroom from side [27], and with results of this experiment form other one.

Growth of bacteria especially *Streptomyces* essential to grow *A. bisporus* because of its effective on C:N ratio which lead to induce the primordial formation [37,38]. This bacterium play useful role to prevent stay vegetable phase of *A. bisporus* and induce fruiting bodies formation [39]. These results agree with findings of Sanchez and Royse [40] and Straatsma et al. [41], they refereed for producing chosen compost for *A. bispoorus* cultivation by using *Scytalidium thermophilum* and some thermophilic fungi to decomposed lignocellulosic wastes.

CONCLUSIONS

The treatment of compost using *Streptomyces* inoculum leads to increase the total yield of *A. bisporus* X25 during 21 days to 430 g/box compared without treatment (376.94 g/box). The treated reed straw compost showed higher yield 496.33 g/box. Using bacterial inoculum given biological efficiency 26.3% compared without treatment 23.8%. The best biological efficiency was 29.3% with the treated reed straw compost, while the lower biological efficiency was 20.8% for the treated mixture compost. Quality and size of fruiting bodies were varied from compost to another depended on diameter of cap (pileus), length of stipe. The bigger diameter had been showed on fruits of reed straw compost (55.83 mm) while the smaller diameter was 42.33 mm on fruits of control.

ACKNOWLEDGEMENTS

Authors thank Mr. Fahad Al- Khirbit and the Hameediyah Mushroom Farm (HMF), Kilo 18, Ramadi, Iraq, to supply spawn for this trial.

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