



Mycelia growth performance of *Agaricus bisporus* in culture media of composts supplemented with *Sesbania sesban* straw and phosphate rock

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Abstract

Alternate composts are used to improve button mushroom growth. Egyptian pea (*Sesbania sesban*) straw is used for the first time in growing mushrooms. WHS2 medium (30% wheat straw, 45% horse manure, 15% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock) is considered best significant ($p < 0.05$) compost extract for mycelial growth rates of *Agaricus bisporus* which reached 86.33 mm compared with the control (WH) 80.83 mm after 14 days. However, the lower growth rates have been recorded 46.50 mm and 53.83 mm on WCS2 and WCS1 media, respectively. As in the solid media, WHS2 broth is exhibited higher biomass weight of *A. bisporus* 4.24 g/L significantly ($p < 0.05$), whereas, lower biomass weights were 1.60 and 1.73 g/L in WCS2 and WCS1 broths, respectively. Generally, *Agaricus bisporus* C9 (brown strain) has been growing best than *A. bisporus* F599 (white strain) as observed in the current study. In conclusion, using Egyptian pea (*S. sesban*) extract plus phosphate rock enhances the importance of the compost with horse manure compared with chicken manure for growing *A. bisporus*, *in vitro*.

Key words – Biomass – button mushroom – C:N ratio – decomposing – Egyptian pea

Introduction

Agaricus bisporus, is a famous edible fleshy mushroom, has the first production in the world. It belongs to genus *Agaricus* of Basidiomycota. Its common name is button mushroom (Chang & Miles 2004). It is rich in macro and microelements (Owaid 2015), proteins, polyphenols (Owaid et al. 2017b), fatty acids and vitamins, thus its fruiting bodies have an important role in nutrition as healthy fresh foods or useful herbs (Atila et al. 2017) in treating bacterial, candidal (Waithaka et al. 2017), fungal (Kumar & Yadav 2014) and cancer cases (Salih & Al-Mosawy 2010) and it is a potent antioxidant agent (Al-Jubori et al. 2016, Cardoso et al. 2017) and enhancing immunostimulatory, antitumor (Zhang et al. 2014) and anti-diabetic activities (Atila et al. 2017). Recently, *A. bisporus* fruiting bodies have been used to biosynthesize silver nanoparticles which enter into medical and industrial applications (Owaid et al. 2017c).

This mushroom has biodegradability of lignocelluloses by excretion of enzymes like laccase for utilization of substrates as carbon and an energy source (Kabel et al. 2017). The genome of *Agaricus bisporus* encodes a limited repertoire of lignin-modifying enzymes compared to wood-decaying white rot species (Morin et al. 2012). During composting phases, 66% of polysaccharides of walls were consumed by microbes, and only 17% of the polysaccharides were used during mushroom production (Iiyama et al. 1994). Thus, composting process is an essential and critical point for the cultivation of *A. bisporus* (Vieira & Pecchia 2018).

In Iraq, *A. bisporus* was cultivated on various composts consisted from *Triticum* wheat straw, *Oryza* rice straw, *Zea mays* corn stalk (HaoLin et al. 2017), *Phoenix dactylifera* date-palm trunk (Hamoodi & Hameed 2013), *Helianthus* sunflower residues (Muslat et al. 2014) and straw of *Phragmites australis* reed plant (Muslat et al. 2011, Owaid et al. 2018) mixed with some supplements like chicken manure, urea, and gypsum. *Sesbania sesban* is a species of plant belongs to the legume family (Fabaceae). Its common name is seseban in Arabic or sesbania or Egyptian pea or the Egyptian Riverhemp in English. The exact origin of *Sesbania sesban* is unclear, but it is widely distributed and cultivated throughout tropical Africa and Asia (Orwa et al. 2009). It has crude protein 209 g/kg, and tannin contents 31 g/kg (dry weight) (Kiatho 1997). Different parts of *Sesbania sesban* are considered for different purposes like weed control, anti-inflammation, antioxidant and antimicrobial activities (Nigussie & Alemayehu 2013).

The value of nitrogen in Egyptian pea (seasaban) residues and is reputed an unused cellulosic source for feed of livestock, so it is introduced in the compost formula instead of others carbon and nitrogen sources as supplements to enhance mycelial growth of button mushroom and reduce cost of production when it is applied in farms thus this investigation is considered the first attempt to use Egyptian pea straw with phosphate rock in compost composition of *A. bisporus* and applied toward the mycelia growth *in vitro*.

Materials & Methods

Mushroom strains

Two strains of mushrooms are *Agaricus bisporus* F599 (white button mushroom) and *Agaricus bisporus* C9 (brown button mushroom), obtained from ITALSPAWN, Italy by Hameediyah Mushroom Farm (HMF), Ramadi, Kilo 18, Anbar, Iraq. They are subcultured on PDA (Oxoid, England) and used in this investigation.

Chemicals and Organic matters

There are six matters viz.; wheat straw, Egyptian pea (*Sesbania sesban*, Fig. 1) straw, horse manure and chicken manure obtained from agricultural fields in Ramadi, Iraq. The powder of phosphate rock obtained from State Company for Phosphate in Anbar in raw form. This fertilizer contains macro and microelements viz.; Co, Cr, Ni, Pb, Fe, Zn, Cu, Mn and Cd (Owaid & Abed 2015). CaSO₄ obtained from the local market. As in Table 1, six formulas were prepared after composting processes achievement as mentioned by Owaid (2009).

Preparing compost extract media

Six compost extracts were used in this work. About 250 g of each the ready compost after three weeks of composting was extracted in 400 ml D.W, stand for 24 h, then crushed using a blender for 10 min and filtered twice by the gauze. The produced aqueous extracts were completed to the volume 500 ml by D.W individually. For broth culture, they are used directly while in case the solid media, 15 g agar per liter was added to the aqueous extracts to prepare the solid media. These media were sterilized within Autoclave at temperature 121 °C and pressure 15 psi for 15 min. The solid media were poured in 9 cm Petri dishes whereas the liquid media (50 ml broth) were poured into 100 ml-volumic flasks separately.

One 7-days mycelial disk put in the center of Petri dish and incubated in the incubator at 25 C while the one disk put in 50 ml broth and incubated at the same temperature. PDA and PDB were

used as a control. The measurements are mycelial growth rate after 14 days and the biomass weight after 30 days was recorded and statistical analysis applied by two-way analysis of variance using CRD (Completely Randomized Design) by GenStat software at a probability less than 5%.



Fig. 1 – Straw of Egyptian pea (*Sesbania sesban*)

Table 1 Compositions of composts and its carbon:nitrogen (C:N) ratio (On the basis of dry weight)

Treatments	Wheat straw	Horse manure	Chicken manure	Egyptian pea straw	CaSO ₄	Phosphate rock	C:N ratio
WH	45%	45%	-	-	5%	5%	42.5:1
WHS1	40%	20%	-	30%	5%	5%	38.8:1
WHS2	30%	45%	-	15%	5%	5%	35.8:1
WC	45%	-	45%	-	5%	5%	37.5:1
WCS1	40%	-	20%	30%	5%	5%	36.6:1
WCS2	30%	-	45%	15%	5%	5%	30.8:1

Legend: C:N ratio of Wheat straw: 60:1, horse manure: 25:1, chicken manure: 15:1, Egyptian pea (*Sesbania sesban*) straw: 20:1.

Characteristics of extracts

Take 1 g of the dried powder of each formula (compost) individually as in Table 1, mix with 5 ml D.W and put in a shaker for 1 hr. Use these aqueous extracts to determine pH and EC (Owaid et al. 2017a). Also, C:N ratios were calculated after assessing carbon content as in method of Page (1982) and nitrogen content by Kjeldahl method using Gallenkamp Kjeldahl Apparatus (Sawhney & Singh 2000)

Results and Discussion

Properties of compost extracts

Properties of compost extracts are including pH, EC, nitrogen content, and carbon:nitrogen (C:N) ratio as shown in Table 2. The pH of extracts was alkaline close to neutral which ranged from 7.24 to 7.75. C:N ratio of extracts ranged from 12.6 to 24.1. WCS1 extract exhibited lower C:N ratio (12.6) and higher nitrogen content (2.7%) compared with the control, WH medium which showed higher C:N ratio (24.1) and lower nitrogen content (1.63%) respectively. This phenomenon appears because of the compositions of WCS1 compost which have a high percentage of Egyptian pea (*Sesbania sesban*) straw which is rich in nitrogen content compared with the wheat straw (Table 1). This straw leads to the best microbial growth that reflects on biomass of compost and finally on the nitrogen content in WCS1 and WHS1 composts compared with the control during composting processes (Vos et al. 2017).

The neutral values of pH in those composts may be returned to using CaSO₄ with phosphate rock, and this is suitable for button mushroom growth and close to the optimal pH (Royse 2008). At the same time, this fertilizer, phosphate rock, is considered the main source for phosphorus element which is regarded an important source for a microbial community in the compost through the composting processes (Abed & Rasheed 2013). Thus, the phosphate rock was used to increase production of some mushrooms like *A. bisporus* (Abed & Rasheed 2013) and *Pleurotus* spp. (Owaid et al. 2015). The role of phosphate-rock-solubilizing bacteria and fungi in compost is important for increasing phosphate rock solubility after mixing with cellulosic wastes during composting processes (Al-Tae'e 2008, Faraj 2014).

Also, the value of C:N (carbon:nitrogen) ratio is a benefit for production this type of mushroom which needs this feature a ratio of 20:1 as an optimal value which comes from the degradation of bacterial biomass through the composting processes outdoor (Buscot & Varma 2005). The higher electrical conduction (EC) showed 17.67 ds/m in WCS1 compost followed 15.08 and 14.74 ds/m WCS2 and WC composts respectively compared with 9.85, 11.22 and 11.86 ds/m for WH, WHS2 and WHS1 composts respectively that may be return to increase EC and TDS in chicken manure compared as horse manure.

Table 2 Physical and chemical characteristics of aqueous extracts of composts

Features	WH	WHS1	WHS2	WC	WCS1	WCS2	LSD (<i>p</i> <0.05)
pH	7.67	7.73	7.75	7.64	7.58	7.24	0.02
EC (ds/m)	9.58	11.86	11.22	14.74	17.67	15.08	0.09
Nitrogen content %	1.63	2.5	2	2.3	2.7	2.6	0.15
C:N ratio	24.1	14.8	16.9	15.9	12.6	12.7	1.29

Legend: EC: electrical conductivity, WH compost (control): 45% wheat straw, 45% horse manure, 5% CaSO₄ and 5% phosphate rock. WHS1 compost: 40% wheat straw, 20% horse manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock. WHS2 compost: 30% wheat straw, 45% horse manure, 15% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock, WC compost: 45% wheat straw, 45% chicken manure, 5% CaSO₄ and 5% phosphate rock. WCS1 compost: 40% wheat straw, 20% chicken manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock. WCS2 compost: 30% wheat straw, 45% chicken manure, 15% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock.

Mycelial growth of *A. bisporus* in the solid media of compost extracts

Generally, *Agaricus bisporus* C9 (brown strain) is considered the best mushroom than *A. bisporus* F599 (white strain), Table 3, Fig. 2. This is relating to genetic characteristics of *A. bisporus* that have been limited so far (Hildén et al. 2013). Also, those results show that type of compost extract affected significantly (*p*<0.05) on mycelial growth of *A. bisporus* F599 and *A. bisporus* C9. The medium WHS2 shows best growth reaches 88.00 mm and 84.66 mm, followed by 82.33 mm and 79.33 mm in WH medium for *A. bisporus* C9 and *A. bisporus* F599 respectively after 14 days. *A. bisporus* C9 and *A. bisporus* F599 exhibit mycelial growth 68.33 mm and 64.33 mm in WC medium and 66.33 mm and 63.33 mm in WHS1 medium respectively. The lower growth rate was recorded 44.00 mm and 49.00 mm in WCS2 medium, followed 52.00 mm and 55.66 mm in WCS1 medium for *A. bisporus* F599 and *A. bisporus* C9 respectively. The differences among composts due to different specifications of pH, EC, and carbon:nitrogen ratio (Table 2).

The medium WHS2 showed best mycelial growth because it has optimal nitrogen content 2% compared with the higher nitrogen content of WCS1 and WCS2 media reached to 2.7% and 2.6% respectively (Table 2) which negatively reflected on mycelial growth rate in WCS1 and WCS2 media because that lead to dispersed colonies in the medium (Calvo 2010) as in Fig. 2.

Also, higher values of EC in the media of WC, WCS1, and WCS2 (containing horse manure) may be slightly affected on the growth *in vitro* (Table 2) compared with WH, WHS1 and WHS2 media (containing chicken manure). Generally, the finding horse manure was suitable more than chicken manure in this test that agrees with many studies in button mushroom cultivation. But in the mushroom farm, usually, electrical conductivity (EC) did not influence on the growth of many

types of mushroom in the mushroom farms thus values of EC cannot influence on this investigation (Royse 2008).

Effect of compost type on the mycelial growth rate

According to Table 3, generally, WHS2 medium is considered best significant ($p < 0.05$) compost extract for growth rates of *A. bisporus* which recorded 86.33 mm after 14 days, compared with the control (WH) 80.83 mm. However, the lower growth rate was 46.50 mm and 53.83 mm on WCS2 and WCS1 media respectively.

WH and WC media are superior due to the difference of carbon sources as through large percentages of various lignocellulosic residues. The availability or scarcity of carbon and type of its sources determine the rate of mycelial growth of mushroom, development, and appearance of *A. bisporus* (Calvo 2010). The high content of nitrogen in the compost extracts influences on mycelial growth negatively (Royse 2008) as shown in other media (WHS1, WHS2, WCS1, and WCS2).

WHS2 medium is regarded the best formula/compost in case of mycelial growth rate that might return to its adequate properties of pH, EC, and C:N ratio. Subsequently, it improves conditions of the microbial growth in the compost during decomposing stages (Owaid 2009).

Table 3 Mycelial growth rate of *A. bisporus* on the solid media of different compost extracts after 14 days

Mushroom Strains	WH	WHS1	WHS2	WC	WCS1	WCS2	Mean
<i>A. bisporus</i> F599	79.33	63.33	84.66	64.33	52.00	44.00	64.60
<i>A. bisporus</i> C9	82.33	66.33	88.00	68.33	55.66	49.00	68.27
Mean of media	80.83	64.83	86.33	66.33	53.83	46.5	66.44
LSD($p < 0.05$)				15.4			0.51

Legend: WH compost (control): 45% wheat straw, 45% horse manure, 5% CaSO₄ and 5% phosphate rock. WHS1 compost: 40% wheat straw, 20% horse manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock. WHS2 compost: 30% wheat straw, 45% horse manure, 15% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock, WC compost: 45% wheat straw, 45% chicken manure, 5% CaSO₄ and 5% phosphate rock. WCS1 compost: 40% wheat straw, 20% chicken manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock. WCS2 compost: 30% wheat straw, 45% chicken manure, 15% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock.

Biomass weight of *Agaricus bisporus* in broth of compost extracts

It is difficult to determine the suitability of the solid medium for the biomass weight because it is not possible to measure the growth density, so the biomass measurement is used in the liquid medium (broth). In general, *A. bisporus* C9 recorded best significant ($p < 0.05$) biomass weight (2.75 g/L) compared with *A. bisporus* F599 (2.63 g/L) in liquid media of compost extracts after 30 days. Table 3 shows that WHS2 broth significantly ($p < 0.05$) recorded the best result of formation *Agaricus bisporus* biomass reached to 4.36 g/L and 4.13 g/L for *A. bisporus* C9 and *A. bisporus* F599 respectively, that return to the adequate C:N ratio in those composts which determine the rate of mycelial growth of *A. bisporus* (Calvo 2010). Next, WHS1 exhibits biomass weight of 3.50 g/L and 3.42 g/L, followed by 2.69 g/L and 2.57 g/L in WC broth compared with the control (WH broth) 2.50 g/L and 2.45 g/L for *A. bisporus* C9 and *A. bisporus* F599, respectively. While WCS1 and WCS2 recorded lower biomass reached to 1.56 g/L and 1.66 g/L for *A. bisporus* F599 and then 1.65 g/L and 1.81 g/L for *A. bisporus* C9 respectively. WHS2 compost is suitable for growth this mushroom in solid and liquid media of compost due to its characteristics which let for performance best growths (Tables 3 & 4).

Effect of compost type on the biomass of mushroom in broths

In the broths as in the solid media, WHS2 broth is exhibited best biomass weight of *A. bisporus* 4.24 g/L significantly ($p < 0.05$), followed rates 3.46 and 2.63 g/L by WHS1 and WC broths respectively. Furthermore, the lower biomass weight was 1.60 and 1.73 g/L on WCS2 and WCS1 broths respectively as shown in Table 4. WHS2 broth is regarded the best medium to

synthesis the fungal biomass that might return to the suitable properties of this compost (Table 2). Indeed, using Egyptian pea (*Sesbania sesban*) straw reflects on improving nitrogen content of WHS2 compost which consists of 30% wheat straw, 45% horse manure, 15% Egyptian pea straw, 5% CaSO₄ and 5% phosphate rock (dry matter basis).

Also, WHS1 and WHS2 broths exhibited best mushroom biomass because of their suitable nitrogen content or C:N ratio compared to other broths and the control. The suitability these composts extracts may be returned to nitrogen content which obtained from microbial community biomass during composting phases at optimal condition because of constituents of these formulas (Vieira & Pecchia 2018). This investigative work agrees with other studies and agriculture reports in the button mushroom farms globally.

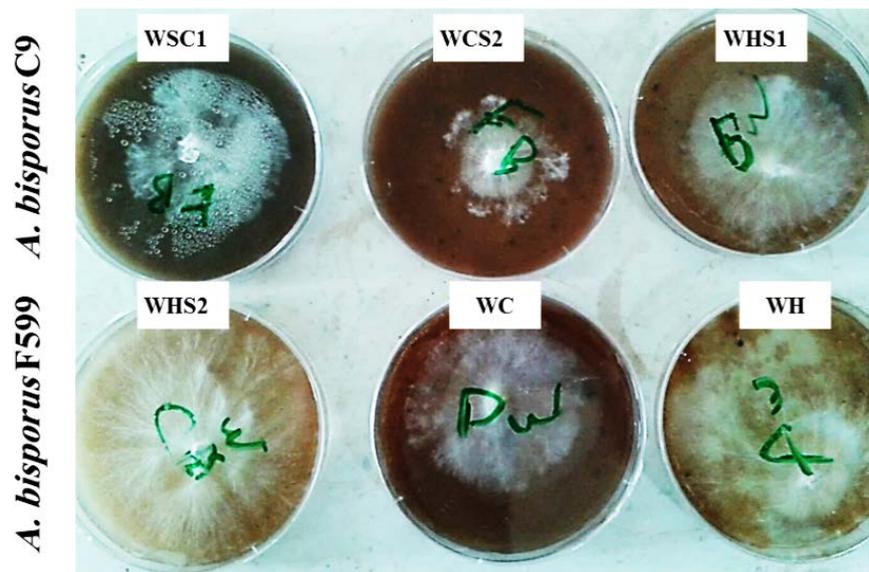


Fig 2 – Pattern of mycelial growth of *A. bisporus* after 14 days

Legend: WH compost (control): 45% wheat straw, 45% horse manure, 5% CaSO₄ and 5% phosphate rock. WHS1 compost: 40% wheat straw, 20% horse manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock. WHS2 compost: 30% wheat straw, 45% horse manure, 15% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock, WC compost: 45% wheat straw, 45% chicken manure, 5% CaSO₄ and 5% Phosphate rock. WCS1 compost: 40% wheat straw, 20% chicken manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock. WCS2 compost: 30% wheat straw, 45% chicken manure, 15% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock.

Table 4 Biomass weights of *A. bisporus* in the broth of various compost extracts after 30 days

Mushroom Strains	WH	WHS1	WHS2	WC	WCS1	WCS2	Mean
<i>A. bisporus</i> F599	2.45	3.42	4.13	2.57	1.56	1.66	2.63
<i>A. bisporus</i> C9	2.50	3.50	4.36	2.69	1.65	1.81	2.75
Mean of media	2.48	3.46	4.24	2.63	1.60	1.73	2.69
LSD ($p < 0.05$)				0.04			0.01

Legend: WH compost (control): 45% wheat straw, 45% horse manure, 5% CaSO₄ and 5% phosphate rock. WHS1 compost: 40% wheat straw, 20% horse manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock. WHS2 compost: 30% wheat straw, 45% horse manure, 15% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock, WC compost: 45% wheat straw, 45% chicken manure, 5% CaSO₄ and 5% phosphate rock. WCS1 compost: 40% wheat straw, 20% chicken manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock. WCS2 compost: 30% wheat straw, 45% chicken manure, 15% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock.

Conclusion

WHS2 extract showed significant ($p < 0.05$) growth in the solid and liquid media compared with the control. In solid media, *Agaricus bisporus* recorded best growth rate on WHS2 medium

(30% wheat straw, 45% horse manure, 15% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock) is 86.33 mm after 14 days, compared with 80.83 mm by the control (WH, 45% wheat straw, 45% horse manure, 5% CaSO₄ and 5% phosphate rock). Also, WHS2 broth is exhibited best biomass weight of *A. bisporus* 4.24 g/L significantly ($p < 0.05$). Finally, *Agaricus bisporus* C9 (brown strain) is considered the best mushroom than *A. bisporus* F599 (white strain), generally. In conclusion, using Egyptian pea (*Sesbania sesban*) extract with phosphate rock enhances the compost property.

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