

Short Communication

Addition of *Sesbania sesban* in compost for reducing fraction of horse manure and enhancing the productivity of *Agaricus bisporus*

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Abstract

This study aimed to use straw of *Sesbania sesban* (Egyptian pea) in the formulation of a compost for producing *Agaricus bisporus* F599 and C9 by using three casing layers namely Topterra, Kekkila, and Local. The highest total yield was 1031 g/bag on B compost with Topterra casing layer for *A. bisporus* F599, while *A. bisporus* C9 cultivated on compost-A with Local casing layer exhibited the lowest yield of 684 g/bag. The best biological efficiency was 45.80% for *A. bisporus* F599 on compost-B with Topterra. Furthermore, *A. bisporus* C9 cultivated on compost-A with Local showed the lowest biological efficiency of 30.39%. *A. bisporus* F599 recorded higher average fruit weights of 35.31 and 34.00 g on compost-B in casings with Local and Kekkila, respectively. *A. bisporus* C9 on compost-B with Topterra recorded the largest number of fruit at 35.0 fruit/bag. Finally, a compost of *S. sesban* (compost-B) is suitable for cultivating this mushroom, especially with Topterra.

Keywords: agricultural wastes, button mushroom, composting, peat moss, mushroom

1. Introduction

Button mushroom (*Agaricus bisporus*) is the first ranked mushroom product in the world. *A. bisporus* is among the most famous macrofungi and belongs to Basidiomycota division (Chang & Miles, 2004). Button mushroom is rich in nutrients and various minerals, such as proteins, essential aminoacids, polyphenols, and vitamins, so this fungal species has medicinal and nutritional uses (Owaid *et al.*, 2017). Also, the medical uses are associated with various benefits like antitumor activity (Salih and Al-Mosawy, 2010), along with

antibacterial, antifungal (Kumar *et al.*, 2016), larvicidal (Arul *et al.*, 2017), and antioxidant activities (Kimatu *et al.*, 2017), or improving the human immune system (Kavyani *et al.*, 2012). Recently, this mushroom has been used to mycosynthesize green nanoparticles like AgNPs as green nano-drugs (Atil *et al.*, 2017; Owaid *et al.*, 2017).

Generally, edible mushrooms can mycodegrade various organic substrates like sawdust, wheat straw, etc. (Owaid, Al-Saeedi, & Abed, 2018). The genome of button mushroom encodes a special repertoire of lignin-modifying enzymes comparable to white-rot fungi that decay wood (Morin *et al.*, 2012). Therefore, *Agaricus bisporus* can release lignocellulolytic enzymes such as cellulases and ligninases and degrade the compost as a carbon and energy source (Rana and Rana, 2011). The compost is composed of lignocellulosic wastes mixed with several supplements such as urea, chicken

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or horse manure, and calcium sulfate, and the composting process utilizes and recycles these agricultural wastes to good fresh mushrooms (Owaid *et al.*, 2017). At mushroom farms, *A. bisporus* varieties are successfully grown and cultivated on various types of composts including straws of wheat and rice, corn stalks (HaoLin *et al.*, 2017), residues of sunflower (Muslat *et al.*, 2014), straws of reed plant (*Phragmites australis*) (Owaid *et al.*, 2018), or date-palm trunk matter (Hamoodi and Hameed, 2013).

Recently, Egyptian pea (*Sesbania sesban*) was used to grow and cultivate white and brown button mushrooms in the laboratory (Rashid, Abed, & Owaid, 2018). The precise origin of *S. sesban* was not apparent, but it is planted throughout Africa and tropical Asia (Orwa *et al.*, 2009) and used as antimicrobial, antioxidant, and weed control agent (Nigussie & Alemayehu, 2013). Egyptian pea is a plant species related to the legume family (Fabaceae), and it is called seseban in Arabic as a common name or Egyptian Riverhemp and Egyptian pea; and it is sesbania in English. Based on dry weight, *S. sesban* contains 209 g/kg total protein (Kiatho, 1997).

Sesbania sesban straw (Egyptian pea) is cellulosic matter that is not used to feed the livestock, therefore it is suited for composting processes as an alternative to straws of wheat to prepare the compost and to enhance cultivating and producing *Agaricus bisporus*. The novelty of this study is in using of this straw to decrease the cost of production in farms of *Agaricus bisporus* and to improve the properties of fruiting bodies, and this experiment is considered the first attempt to use *S. sesban* straw with various casing layers in the production of *A. bisporus* in the mushroom farm.

2. Methods and Materials

2.1. Mushroom strains

In this study, 2 strains of button mushroom namely *Agaricus bisporus* C9 (brown strain) and *A. bisporus* F599 (white strain) were provided from ITALSPAWN (Italy) by HMF (Hameediyah Mushroom Farm), Ramadi, Iraq. These strains were subcultured on potato dextrose agar (PDA) plates (Oxoid, England) and used in the current work.

2.2. Organic matters, composting and spawning

There were three types of organic matter, viz. straws of wheat, straws of Egyptian pea (*S. sesban*), and horse manure, which were obtained from agrofields around Ramadi city. The phosphate rock powder was obtained from State Company For Phosphate in Anbar in a raw form. Calcium sulfate (CaSO₄) was purchased from the market. In this experiment, two compost formulations were the control treatment named A compost: 45% horse manure, 0% straw of *S. sesban*, 45% wheat straw, and 5% CaSO₄ supplemented with 5% phosphate rock; and B compost: 20% horse manure, 30% straw of *S. sesban*, 40% wheat straw, and 5% CaSO₄ supplemented with 5% phosphate rock, as shown in Table 1. The composts were cooled and inoculated with 2% spawn based on wet weight after composting processes and pasteurization, as described by (Owaid, 2009).

2.3. Mushroom casing and harvesting

Three mushroom casing layers were applied. They were TOPTERRA (mushroom casing ready mix with pH 7.11 - 7.16, Holland) as control, KEKKILA peat substrate (NPK 14:16:18) plus 10% CaCO₃ to raise pH from 5.4 to 7.5-7.2 (professional substrate, Finland), and LOCAL casing layer composed of sand, coal and peat moss plus CaCO₃ (pH 7.5-7.2). All the casing layers were pasteurized at 70 °C, cooled and applied after completing mycelia growth, covered by 2.5 cm, and held at 16 °C for fruiting body appearance as mentioned by (Owaid, 2009). The fruitbodies of *Agaricus bisporus* C9 and F599 were harvested over 21 days and the total yield, biological efficiency (BE), number of fruitbodies, and average fruit weight (AFW) were recorded.

2.4. Statistical analysis

Completely Randomized Design (CRD) in 2-ways was implemented using GenStat software (VSN Inter. Ltd., UK). Mean differences with $p < 0.05$ were considered significant. Each experimental treatment was done in triplicate.

3. Results

Table 2 exhibits the effects of the two compost types (A and B) and the three casing layers (Topterra, Kekkila, and the Local type) on the total yield of *Agaricus bisporus* C9 (brown button strain) and on *Agaricus bisporus* F599 (white button strain) at three weeks after the harvesting. The casing layer influenced significantly ($p < 0.05$) the mushroom yield. The best results of strain F599 and strain C9 yields were 1031 and 928 g/bag (bag had 6 kg wet compost) on B compost with casing layer Topterra, followed by 911 and 905 g/bag for *A. bisporus* F599 on A compost with Topterra and B compost with Kekkila, respectively. Furthermore, *A. bisporus* C9 and *A. bisporus* F599 cultivated on A compost with Local casing layer showed lower yields at 684 and 702 g/bag respectively, followed by 706 g/bag for *A. bisporus* C9 on A compost plus Kekkila. Generally, Topterra casing layer showed the best yields of 979 and 877 g/bag on B and A composts at average 928.2 g/bag differed significantly ($p < 0.05$), and decreased on using Kekkila and local casing layer to 772.6 and 747.1 g/bag respectively.

The influences of two compost types and three casing layers (Topterra, Kekkila and Local) on the biological efficiency of two strains of *A. bisporus* are summarized in Table 3 at 21 days after harvesting. The casing layer influenced significantly ($p < 0.05$) the biological efficiency of the mushroom. The best biological efficiencies of strain F599 and strain C9 were 45.80% and 41.25% on B compost with casing layer Topterra, followed by 40.50% and 40.22% for *A. bisporus* F599 on A compost with Topterra and B compost with Kekkila, respectively. Furthermore, *A. bisporus* C9 and *A. bisporus* F599 cultivated on A compost with Local casing layer showed lower biological efficiencies at 30.39% and 31.19%, respectively, followed by 31.35% for *A. bisporus* C9 on A compost plus Kekkila. Generally, Topterra casing layer showed the best biological efficiencies of 43.52% and 38.98%

Table 1. Compositions of composts and their carbon:nitrogen (C:N) ratios (On dry weight basis)

Treatment	Wheat straw	Horse manure	<i>Sesbania sesban</i> straw	CaSO ₄	Phosphate rock	C:N ratio
A	45%	45%	-	5%	5%	42.5:1
B	40%	20%	30%	5%	5%	38.8:1

Legend: C:N ratio of wheat straw: 60:1, horse manure: 25:1, *S. sesban* straw: 20:1

Table 2. Total yield of *A. bisporus* after three weeks (g/bag)

Casing layer Compost	Topterra		Kekkila		Local		LSD ($p < 0.05$)
	A	B	A	B	A	B	
<i>A. bisporus</i> F599	911	1031	735	905	702	882	7.96
<i>A. bisporus</i> C9	843	928	706	745	684	721	
Mean of composts	877	979	720	825	693	801	n.s
Mean by casing layer	928.2		772.6		747.1		3.37

Legend: A compost (control): 45% wheat straw, 45% horse manure, 5% CaSO₄ and 5% phosphate rock. B compost: 40% wheat straw, 20% horse manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock

Table 3. Percentage of the biological efficiency of *A. bisporus* after three weeks

Casing layer Compost	Topterra		Kekkila		Local		LSD ($p < 0.05$)
	A	B	A	B	A	B	
<i>A. bisporus</i> F599	40.50	45.80	32.67	40.22	31.19	39.19	0.352
<i>A. bisporus</i> C9	37.46	41.25	31.35	33.09	30.39	32.05	
Mean of composts	38.98	43.52	32.01	36.65	30.79	35.62	n.s
Mean by casing layer	41.25		34.33		33.20		0.149

Legend: A compost (control): 45% wheat straw, 45% horse manure, 5% CaSO₄ and 5% phosphate rock. B compost: 40% wheat straw, 20% horse manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock

on B and A compost at average 41.25% being significantly different ($p < 0.05$), which then decreased using Kekkila and Local casing layers to 34.33% and 33.20% respectively.

The number of fruiting bodies of the mushroom is considered an indicator of the quantity of mushroom production. The type of compost and casing layer have influenced significantly ($p < 0.05$) the number of fruiting bodies, as shown in Table 4. *A. bisporus* C9 on B and A composts with Topterra recorded the most fruits at 35.0 and 32.3 fruit/bag, followed by *A. bisporus* F599 and *A. bisporus* C9 at 32.0 and 31.3 fruit/bag on B compost with Topterra and Kekkila, respectively. The lowest numbers of fruits were 22.0 and 23.0 fruit/bag for *A. bisporus* F599 on A compost with Local and Kekkila respectively. Generally, Topterra with B and A composts showed the best numbers of fruits reaching 33.5 and 30.1 fruit/bag respectively, while the lowest numbers were 24.3 and 25.8 fruit/bag in case A compost with Local and Kekkila respectively. Finally, Topterra recorded significant differences ($p < 0.05$), which reached a rate of 31.8 fruit/bag, followed by 27.3 and 25.8 fruit/bag in the case of Kekkila and Local casing layers, respectively.

Table 5 exhibits the average fruit weights (AFW) of *A. bisporus* three weeks after harvesting. *A. bisporus* F599 recorded higher average fruit weight at 35.31 and 34.00 g on B compost in case of Local casing layer and Kekkila, respectively, followed by 32.57 g on A compost with Topterra. The lowest AFW showed for fruits of *A. bisporus* C9 on B compost with Kekkila and Local reaching 23.77 and

24.06 g, respectively. Generally, B compost with Local recorded higher AFW (29.68 g), while A compost with Kekkila exhibited the lower AFW, which reached 287.29 g. Furthermore, the casing layer type influenced AFW generally, as mentioned in Table 5. Topterra recorded the highest AFW (29.34 g) while Local and Kekkila recorded 29.24 and 28.58 g, respectively.

The effect of the interaction between the casing layer type and mushroom strain is presented in Figure 1. The higher total yields of strain F599 and strain C9 were 971.0 and 885.5 g/bag on Topterra, respectively, followed by *A. bisporus* F599 on Kekkila, which recorded 820.0 g/bag. Furthermore, the lower total yields were 702.5 and 725.5 g/bag for *A. bisporus* C9 on Local and Kekkila, respectively. The biological efficiencies of strain F599 and strain C9 were 43.15% and 39.35% on Topterra, respectively, followed by 36.44% for *A. bisporus* F599 on Kekkila. The growth of *A. bisporus* C9 on the Local casing layer and Kekkila showed lower biological efficiency at 31.22% and 32.22%, respectively.

The largest numbers of fruiting bodies were 33.65, and 30.00 fruit/bag for strain C9 and strain F599 cultivated on Topterra, respectively, followed by 29.95 fruit/bag for *A. bisporus* C9 on Kekkila. Furthermore, the smallest numbers were 23.50 and 24.80 fruit/bag for *A. bisporus* F599 on Local and Kekkila, respectively. The highest average fruit weights were 33.62, 32.98, and 32.38 g for *A. bisporus* F599 on Local, Kekkila, and Topterra, respectively, while *A. bisporus* C9

Table 4. Fruiting body numbers of *A. bisporus* after three weeks (fruit/bag)

Casing layer	Topterra		Kekkila		Local		LSD ($p < 0.05$)
	A	B	A	B	A	B	
<i>A. bisporus</i> F599	28.0	32.0	23.0	26.6	22.0	25.0	n.s
<i>A. bisporus</i> C9	32.3	35.0	28.6	31.3	26.6	30.0	
Mean of composts	30.1	33.5	25.8	28.9	24.3	27.5	n.s
Mean by casing layer	31.8		27.3		25.8		0.684

Legend: A compost (control): 45% wheat straw, 45% horse manure, 5% CaSO₄ and 5% phosphate rock. B compost: 40% wheat straw, 20% horse manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock.

Table 5. Average fruit weight (AFW) of *A. bisporus* after three weeks (gram)

Casing layer	Topterra		Kekkila		Local		LSD ($p < 0.05$)
	A	B	A	B	A	B	
<i>A. bisporus</i> F599	32.57	32.20	31.96	34.00	31.94	35.31	1.735
<i>A. bisporus</i> C9	26.07	26.53	24.62	23.77	25.65	24.06	
Mean of composts	29.32	29.36	28.29	28.88	28.79	29.68	n.s
Mean by casing layer	29.34		28.58		29.24		n.s

Legend: A compost (control): 45% wheat straw, 45% horse manure, 5% CaSO₄ and 5% phosphate rock. B compost: 40% wheat straw, 20% horse manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock

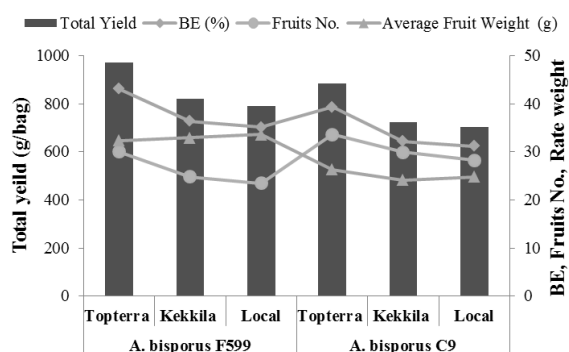


Figure 1. Effect of interactions between casing layer type and mushroom strain

showed lower average fruit weights at 24.19, 24.85, and 26.30 g on Kekkila, Local, and Topterra, respectively.

The type of compost and mushroom strain significantly ($p < 0.05$) influenced total yields, biological efficiencies, fruiting body number, and average fruit weight. The effect of the interaction between compost type and the mushroom strain is presented in Table 6. The highest total yields of strain F599 and strain C9 were 939.3 and 798.09 g/bag on B compost, respectively. At the same time, the lowest total yields were 744.3 and 782.6 g/bag for strain C9 and strain F599, respectively. The highest biological efficiencies of strain F599 and strain C9 were 41.73% and 35.46% on B compost, respectively. However, the lowest biological efficiencies were 33.06% and 34.78% for strain C9 and strain F599, respectively.

The largest fruiting body numbers were 32.10 and 29.16 fruit/bag for strain C9 on B and A compost, respectively, while the smallest numbers were 24.33 and 27.86 fruit/bag for strain F599 on A and B composts,

respectively. The highest average fruit weights were 33.83 and 32.15 g for strain F599 on B and A composts, while the lowest average fruit weights were 24.78 and 25.44 g for strain C9 on B and A composts, respectively. Finally, B compost was considered the best formulation for the quantity of the button mushroom in this investigation, superior to the control formula (A compost) as shown in Table 6.

4. Discussion

The high nitrogen content in Egyptian pea (*S. sesban*) residues and this being an unused cellulosic source in livestock feed (Nigussie and Alemayehu, 2013) led to testing it in compost formulation to replace other carbon and nitrogen source supplements, and to increase the productivity of *Agaricus bisporus* and reduce the cost in its production. *In vitro*, an extract of Egyptian pea straw has been used to enhance the mycelial growth of button mushroom (Rashid *et al.*, 2018). These considerations motivated using its compost to produce *A. bisporus* with various casing layers in the farm. Generally, the results of this experiment show that straw of *S. sesban* raises the nitrogen content in B compost because of the high range of nitrogen (C:N ratio 20:1) in waste of this plant (Kiatho, 1997; Nigussie and Alemayehu, 2013) compared with horse manure alone (C:N ratio 25:1). In compost parts, mycodegradable enzymes of *A. bisporus* are able to produce different organic acids that lead to lightly acidic pH (Jurak, 2015). After that, phosphorus becomes bio-available from the phosphate rock and manures to enhance the compost characteristics (Pagliari and Laboski, 2012) due to acidic and alkaline phosphomonoesterases in the compost containing horse and chicken manure, respectively. Phosphomonoesterase plays a useful role in the biochemistry of compost by mineralizing organic phosphates, which encourages work of mycodegradative enzymes in the compost (Vuorinen, 1999).

Table 6. Effect of interactions between compost type and mushroom strain

Mushroom	<i>A. bisporus</i> F599		<i>A. bisporus</i> C9		LSD ($p < 0.05$)	composts (mean)		LSD ($p < 0.05$)	
	Compost	A	B	A		B	A		B
Total Yield		782.6	939.3	744.3	798.0	6.825	763.4	868.5	5.217
BE		34.78	41.73	33.06	35.46	0.301	33.92	38.60	0.231
Fruits No.		24.33	27.86	29.16	32.1	n.s	26.75	29.98	0.345
AFW		32.15	33.83	25.44	24.78	2.021	28.8	29.31	0.351

Legend: A compost (control): 45% wheat straw, 45% horse manure, 5% CaSO₄ and 5% phosphate rock. B compost: 40% wheat straw, 20% horse manure, 30% Egyptian pea (*Sesbania sesban*) straw, 5% CaSO₄ and 5% phosphate rock. BE: biological efficiency, AFW: average fruit weight

In general, use of the straws of Egyptian pea leads to increased EC of compost due to minerals (Nigussie & Alemayehu, 2013). The properties of compost were improved by the presence of 30% *S. sesban* straw in the compost formulation, and this is reflected in the results on mushroom production, in agreement with Rashid *et al.* (Rashid *et al.*, 2018). The physicochemical properties and faster maturation of B compost than of A compost, as mentioned by (Kabbashi, 2011), were reflected in the yield, BE, and AFW of *A. bisporus* (Tables 2, 3 and 5).

The increased total yield of *A. bisporus* was shown with the compost having 30% *Sesbania sesban* straw (B Compost), and that agrees with results of (Rashid *et al.*, 2018). This issue is related to plant waste blends with horse manure, which improves properties of the compost and gives good characteristics toward growing *A. bisporus*. Also, Table 1 exhibits that C:N ratio of B compost was higher than that of A compost (control) at respectively 38.8:1 and 42.5:1, which may have positively influenced the yield, the biological efficiency, and the average fruit weight (Tables 2, 3 and 5).

Casing layer type significantly ($p < 0.05$) influenced the production of strains (*A. bisporus* F599 and *A. bisporus* C9), as seen in Figure 1. Kekkila and Local casing layers did not increase the total yield, BE, or AFW of the two mushroom strains compared with Topterra (control). But Kekkila was better than Local for all composts and mushroom strains, since the composition of Local from sand and coal decreased the nutritional value of casing layer (Zied, Minhoni, Kopytowski-Filho, & Andrade, 2010) compared to Kekkila (only peat moss with lime).

Table 6 exhibits the interactions between compost type and mushroom strain, indicating that strain F599 was absolutely better than strain C9 in all treatments. The reason for this is related to the genetic properties of these *A. bisporus* strains (Hildén, Mäkelä, Lankinen, & Lundell, 2013), which agrees with (Rashid *et al.*, 2018). AFW and fruit number have negative correlation and this agrees with prior studies (M.N. Owaid, Abed, & Nassar, 2015; M.N. Owaid, Abed, & Al-Saeedi, 2015). Tables 4 and 5 show this influence, which was clear in the interaction between casing layer type and the mushroom strain, as seen in Figure 1. Finally, *S. sesban* straws are suitable for inclusion in composts for mushroom, reducing the fraction of horse manure and enhancing the productivity of *A. bisporus*.

5. Conclusions

This study aimed to use Egyptian pea (*Sesbania sesban*) straws in the formulation of a compost for producing

two strains of *Agaricus bisporus* F599 (white) and C9 (brown) by using three alternative casing layers Topterra, Kekkila, and Local. The highest total yield was 1031 g/bag (bag: 6 kg wet compost) on B compost with Topterra casing layer for *A. bisporus* F599. The best biological efficiency was 45.80% for *A. bisporus* F599 on B compost with Topterra. Also, *A. bisporus* F599 recorded higher average fruit weights at 35.31 and 34.00 g on B compost with Local and Kekkila casings, respectively. *A. bisporus* C9 on B compost with Topterra recorded the largest number of fruits at 35.0 fruit/bag. Finally, compost of *S. sesban* (B compost) is superior to the control for the cultivation of *A. bisporus*, especially with Topterra. *Sesbania sesban* straws (Egyptian pea) were included in the compost as an alternative for wheat straws, to enhance cultivating and producing *Agaricus bisporus*. The novelty of this study was in using of this straw to decrease the cost of production of *A. bisporus* and to improve properties of fruiting bodies, and this experiment is considered the first attempt to use *S. sesban* straws with various casing layers in the production of *A. bisporus* in the mushroom farm.

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