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RECYCLING OF DATE-PALM FIBER TO PRODUCE *Pleurotus cornucopiae* var. *citrinopileatus* MUSHROOM

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Abstract

In this study, some local available organic matters, which are including wheat straw (*Triticum aestivum*), sawdust, and fiber of date palm (*Phoenix dactylifera* L.), were used for growing and cultivating of bright yellow oyster mushroom *Pleurotus cornucopiae* var. *citrinopileatus*. The possibility of using date palm fiber (in mixtures with other organic residues) as a substrate for the cultivation and production of fruiting bodies of *P. cornucopiae* var. *citrinopileatus* was investigated. This mushroom is capable of biorecycling and utilization of some mixtures of lignocellulosic substrates successfully, especially the mixture S3 (50% wheat straw, 30% sawdust, and 20% date palm fiber). The lower mycelia completion time was 17 days, that shown in bags of the S3 substrate. Date-palm fiber substrate exhibited best growth intensity level (moderate) significantly (p<0.05). The total yield and biological efficiency percent recorded approx. 90 g and 23% on the S3 substrate respectively, as a higher percent significantly (p<0.05), while sawdust substrate alone was an unsuitable medium for cultivation and production of this mushroom. Finally, the use of date-palm fibers in mixtures is usefulness in producing a fresh edible and medicinal mushroom.

Key words: Yellow oyster mushroom, date palm fiber substrate, morphological properties, yield.

Introduction

Pleurotus cornucopiae var. *citrinopileatus*, bright yellow oyster mushroom, is a white rot fungal strain, one of seventy species of genus *Pleurotus* which belongs to the higher Basidiomycota (Chang and Miles, 2004). This species produces light-yellow fruit bodies with an edible stem centrally located in relation to the pileus. The fruit bodies are distinguished by their high taste values. The growth of its mycelium is slower than that of other species; therefore, the substrate is more susceptible to the danger of infection (Ziombra, 2000). *P. cornucopiae* could be suggested as a new potential source of natural therapeutic use in many remedies. The presence of mevinolin compound in *P. cornucopiae* tissue leads to use its basidiocarp as a pharmacological agent to decrease the level of cholesterol (Pieckenstain et al., 1999). The antihypertensive effect of D-mannitol of *P. cornucopiae* was confirmed in spontaneously hypertensive rates by oral administration (Hagiwara et al., 2005). Further, *P. cornucopiae* possesses nephroprotective, antioxidant and anticancer properties *in vivo* (Kim,et al., 2009; Suman et al., 2014). Recently, it is consider to be useful for pharmaceutical and food industry because of its content from 6-Octadecenoic acid, cis-13-Octadecenoic acid and cis-Vaccenic acid and some macro and micro minerals such as K, Na, Ca, P, S, Al, Mg, Fe, Cu, Mn, Zn and Se (Parmar and Kumar, 2015). From another hand, industrially, *P. cornucopiae* var. *citrinopileatus* capable of decolorization for different textile dyestuffs *in vitro* (Kalms et al., 2007).

Pleurotus cornucopiae is useful to degrade hemicellulose, cellulose and lignin in some botanical organic wastes (Chaudhary et al., 1994). Furthermore, *P. cornucopiae* was successfully grown and cultivated at 18 and 23 °C with a 12 h photoperiod and light intensity of about 500 lux on barley straw, rice straw (Delmas and Mamoun, 1982; Baukhandi, 1989), sugarcane bagasse (Chaudhary et al., 1994), rye straw (Ziombra, 2000), wheat straw, cottonseed hulls, switch grass (*Panicum virgatum*) (Royse, 2002; Royse et al., 2004), rubber tree sawdust with rice bran (Owaid et al., 2015) and some of their mixtures. Recently, only few studies used date-palm residues and wastes in evaluation of growing and production of some edible mushrooms such as *Agaricus bisporus* (Hamodi and A.M. Hameed, 2013), *Pleurotus ostreatus* (Daneshvar and Heidari, 2008; Hassan, 2011; Kabirifard et al., 2012; Al-Qarawi et al., 2013; Alananbeh et al., 2014; Owaid et al., 2016b), *Pleurotus florida* (Alkoaik et al., 2015), *Pleurotus eryngii* (Owaid et al., 2016a), *Pleurotus salmoneostramineus* (Owaid et al., 2016b), *Pleurotus sajor caju* and *Calocybe indica* (Al Shamsi, 2016).

Generally, some reports were demonstrated that date-palm wastes might be burned in the rural areas for cooking and/or heating processes. In Iraq, Iran, Saudi Arabia and United Arab Emirates (UAE), date-palm wastes and residues were introduced from some local universities as agro-substrate to grow and cultivate *A. bisporus*, *Pleurotus* spp. and *Calocybe indica*. The increasing availability of date-palm wastes has mushroom growers to consider the use of these huge lignocellulosic wastes to produce an edible mushroom in farms in these countries successfully. Iraq is one of the developing countries around the world, the pollution has increased because of the high amount of date-palm wastes that are buried or burned. Thus, this work leads to the need of serious solutions for myco-recycling these wastes. According to this issue, this experiment was achieved in Iraq for evaluation of growth, total productivity and biological efficiency of mushroom *Pleurotus cornucopiae* var. *citrinopileatus* on date-palm fibrillum, sawdust, wheat straw and their mixtures amended with a raw fertilizer phosphate rock by using an easy method and in a low cost.

Materials and methods

Strains

Mycelia of the bright yellow oyster mushroom *Pleurotus cornucopiae* var. *citrinopileatus* was obtained from the MushroomBox Co., UK, and subcultured on potato dextrose agar medium at 25 C° for this experiment. Spawn was proliferated on seeds of millet (*Pennisetum americanum*) as mentioned by (Chang and Miles, 2004).

Agro-substrates and supplements

In this experiment, using locally surplus organic wastes (Fig. 1), available in Heet city, Iraq, were the wheat straw (*Triticum aestivum*), sawdust, and fibrillum (fibers of date palm, *Phoenix dactylifera* L.), which first chopped into small pieces (5×5) cm and mixed together in five combinations to use in this experiment. There are S1 substrate (wheat straw alone as a control), S2 substrate (70% wheat straw, 20% sawdust and 10% date palm fiber), S3 substrate (50% wheat straw, 30% sawdust and 20% date palm fiber), S4 substrate (sawdust alone) and S5 substrate (date palm fibers alone). All substrates were amended with 5% fertilizer phosphate rock (based on dry weight), obtained from State Company for Phosphate in Anbar province, Iraq.

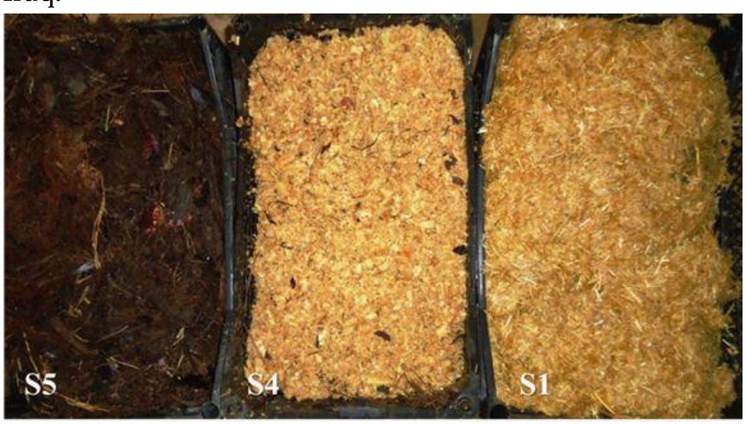

Fig 1: Organic materials; S1: wheat straw (control); S4: sawdust of factories of wood; S5: date palm tree surface fiber from bark surface or Mesh (Fiber) as a source of fibrous particles.

Preparation of agro-substrates and cultivation of *P. cornucopiae* var. *citrinopileatus*

After soaking of agro-substrates, all mixtures were disinfected by boiling in a hot water for 2 h, cooled, put on a clean place to drain out excess water and mixed with 5% rock phosphate powder. The inoculation with 4% mushroom's spawn based on wet weight (Delmas and Mamoun, 1983) was applied in the Layers Method within polyethylene bags (which capacity 30×50 cm) contain 1.5 kg of the substrate. The inoculated bags were transferred into an incubation room, darkly incubated at 22-25 °C for the spawn running. After colonization of each substrate, the polyethylene was removed; then the temperature and relative humidity (HR) maintained at 16-18 °C and 90% respectively, lighted 12 h/d by using fluorescent light and fresh aeration with spray watering twice a day for the fruiting stage (Baukhandi, 1989).

Determinations are including mycelium completion time (MCT), growth intensity level (GIL), primordial formation time (PFT), times of the cropping, the total yield, biological efficiency, clusters' number, fruiting bodies' number, the diameter of pileus, the thickness of pileus, the length of stipe and the diameter of stipe (Chang and Miles, 2004).

Statistical Analysis

The data has been expressed using their means and standard deviation (SD). The data were collected in triplicates and analyzed in one-way analysis of variance (ANOVA) by version 9.0 of SAS statistical program for windows 7 (SAS Institute Inc., USA). Significance of difference was determined by DMRT (Duncan's Multiple Range Test) accordingly. Probability values least than 0.05 were considered to be statistically significant.

Results and discussion

Growth properties of *P. cornucopiae* :

Using various agro-substrates in oyster mushroom cultivation has an effect on its growth properties. In significant (p<0.05), S4 (sawdust), S2 (70% wheat straw, 20% sawdust and 10% date palm fiber) and S5 (date palm fiber) substrates exhibited long mycelium completion time (MCT) of yellow oyster mushroom *Pleurotus cornucopiae* var. *citrinopileatus* 23, 22.3 and 22 days respectively, while S3 substrate (50% wheat straw, 30% sawdust and 20% date palm fiber) showed lower MCT (17 days) compared with the control medium (S1, wheat straw) (18 days) as showed in Table 1. S5 substrate exhibited higher primordial formation time (PFT) 36 days significantly (p<0.05), followed by S4, S3 and S2 substrates 27, 25 and 23 days respectively, whereas the control recorded the lowest time about 18 days. S1 and S5 substrates had 2nd growth intensity level (GIL) significantly (p<0.05), while the others had the 1st level (table 1). In general, fungi have certain requirements in order to digest and decay date-palm parts which containing complex carbohydrates (Al-Juruf, 2002). These results are in line with the finding of Kabirifard et al. (2012) who reported, this genus grows on date palm leaves faster than the wheat stubble.

Table 1: Growth properties of *P. cornucopiae* var. *citrinopileatus* on different substrates types.

Substrate type	Mycelium Completion Time MCT (days)	Growth Intensity Level (GIL)	Primordial Formation Time PFT (days)
S1	18.3[b]	2[a]	18.3[d]
S2	22.3[a]	1[b]	23.0[c]
S3	17.3[b]	1[b]	25.0[bc]
S4	23.0[a]	1[b]	27.0[b]
S5	22.0[a]	2[a]	36.0[a]
Mean ± MSD	20.6 ± 1.89	1.4 ± 0.0	25.9 ± 1.27

Legend: Growth Intensity Level (GIL): 1: Light, 2: Moderate, 3: Vigorous. S1: a wheat straw substrate, S2 (70% wheat straw, 20% sawdust and 10% date palm fiber), S3: (50% wheat straw, 30% sawdust and 20% date palm fiber), S4: sawdust, S5: date palm fiber. Means of the same superscript letter(s) along each column are not significantly different (p<0.05) using Duncan's multiple range test (DMRT). MSD: Mean of standard deviation.

Yield properties of *P. cornucopiae*:

Yellow oyster mushroom *P. cornucopiae* var. *citrinopileatus* had three flushes of total yield during 30 days on S2 substrate and the control, significantly (p<0.05) as a higher value, while S3 and S5 exhibited 2 flushes, while S4 had only one flush (table 2). Hardwood sawdust substrate (S4) has been affected on the yield of *P. cornucopiae* var. *citrinopileatus* negatively at average 0.7 g/bag, while S5 substrate (date palm fibers) exhibited 14.1 g/bag. All that reflected on increasing total yield of mixtures substrates S2 and S3 media of 68.9 g/bag and 90.1 g/bag respectively (Fig. 2), compared with control (102 g/bag). Using these cellulosic materials affected on composition of mixture substrates; in another mean, date-palm fiber has a positive correlation with yield while sawdust has a negative correlation (Owaid et al., 2016b).

Fig 2: Formation of *P. cornucopiae* var. *citrinopileatus* Clusters on S2 and S3 substrates

The mixtures S2 and S3 exhibited best biological efficiency 19.19% and 22.83% compared with the control (29.62%). The sawdust substrate has been affected negatively on the biological efficiency of *P. cornucopiae* var. *citrinopileatus* mushroom least than 1%, while date palm fibers substrate exhibited approx. 3%. The results of mixtures agrees with the biological efficiency of rice straw and barley straw mixture (20%) (Delmas and Mamoun, 1982), also, compatible with results of the biological efficiency of *P. ostreatus* (20%-30%) on date-palm residues composed from its fibers (Al-Qarawi et al., 2013).

The mixtures S2 and S3 showed best fruiting bodies' number 39 and 31 fruits compared with the control (42 fruits), followed by S5 substrate which recorded 8 fruits, while S4 substrate was unsuitable medium to cultivate *P. cornucopiae* var. *citrinopileatus* (two fruits only). Fruiting bodies weight rate was 3.15 g/boy for fruits on S3 substrate compared with fruits of the control substrate 2.43 g/body, while S4 showed poor fruits weight with rate 0.55 g/body. Clusters' number reached to 9 clusters on S3 substrate compared with 8 ones on the control, whereas S4 had only one cluster.

That agrees with some studies which referred to that hardwood sawdust is unsuitable for cultivating *Pleurotus ostreatus* (Owaid et al., 2016b), they demonstrated that the sawdust extract exhibits poor mycelial growth of oyster mushroom. Furthermore, Onuoha (2007) mentioned that the sawdust may be reduced production of oyster mushroom relatively. The reason may be related with pretreating the wood in factories with fungicides (Kalpana et al., 2011). Thus, the sawdust must be not used alone but mixed with other agro-substrates (Davis and Aegerter, 2000).

Generally, oyster mushroom species are able to cleavage of cellulosic matters of date palm fibers (Owaid et al., 2016b). The mixture substrate S3, which contains around 20% of date-palm fibers, is a best substrate recorded best results in most of the parameters measured compared with the substrate S2 (10% date-palm fibers) and other substrates which composed from one type. These results agrees with results of *Pleurotus ostreatus* that achieved by Alananbeh et al. (2014). The yield of *Pleurotus* spp. depended on the type of straw used as substrate (Ziombra, 2000). *Pleurotus* spp. enables to degrade and convert lignocellulosic compounds into protein rich food (Chang and Miles, 2004). Finally, using palm fibers mixed with others as a substrate to growing plants in soilless successfully (Latigui et al., 2013). Thus, date-palm fiber substrate as a substitution to wheat straw was succeeded.

Table 2: Quantity properties of cultivated *Pleurotus cornucopiae* var. *citrinopileatus* on various cellulosic matters

Substrate type	No. of Flushes	Flushes weight (yield) (g/bag)	Biological efficiency (%)	Fruits number	Fruit weight (g)	Clusters' No.
S1	3[a]	102.0[a]	29.62[a]	42[a]	2.43[a]	8.3[a]
S2	3[a]	68.9[a]	19.19[b]	39[a]	1.70[ab]	6.5[b]

S3	2^{b}	90.1^{a}	22.83^{ab}	31^{a}	3.15^{a}	8.5^{a}
S4	1^{c}	0.7^{b}	0.16^{c}	2^{b}	0.55^{b}	1.0^{c}
S5	2^{b}	14.1^{b}	2.84^{c}	8^{b}	1.75^{ab}	2.0^{c}
Mean±MSD	2.2±0.00	55.18±14.58	16.26±3.96	24.4 ±7.25	1.96 ±0.61	5.5 ± 0. 52

Legend: Growth Intensity Level (GIL): 1: Light, 2: Moderate, 3: Vigorous. S1: a wheat straw substrate, S2 (70% wheat straw, 20% sawdust and 10% date palm fiber), S3: (50% wheat straw, 30% sawdust and 20% date palm fiber), S4: sawdust, S5: date palm fiber. Means of **the** same superscript letter(s) along each column are not significantly different (p<0.05) using Duncan's multiple range test (DMRT). MSD: Mean of standard deviation.

Morphological properties of *P. cornucopiae*:

Morphological properties of oyster mushroom had been related with the fruiting bodies size such as diameter and thickness of pileus and stipe (Owaid et al., 2016b). The size of fruiting bodies is useful to determine the morphology of yellow oyster mushroom *P. cornucopiae* var. *citrinopileatus* (table 3). The best diameter of pileus 54.5 mm on S3 substrate significantly (p<0.05), while S4 substrate produced a smallest diameter 28.5 mm compared with the control (S1) at average of 43.6 mm. S2 and S5 substrates led to producing fruits with a diameter approx. 40 mm.

Fruits of the yellow mushroom *P. cornucopiae* var. *citrinopileatus* recorded thickness of pileus at mean 2.7±0.44 mm in this experiment. Significantly (p<0.05) S3 substrate showed the highest thickness around 3.3 mm, while S4 substrate showed only 2.0 mm compared with the control 2.6 mm. Fruiting bodies of mushroom on S2 and S5 had a thickness approx. 3 mm.

About properties of fruit's stipe, S4 was the best substrate about 15.7 mm compared with the control 19.0 mm, while the substrate S3 gave 32.2 mm significantly (p<0.05). The diameter of stipe, significantly (p<0.05) S3 had the best diameter (50 mm) compared with the fruits of the control substrate (40 mm), whereas S4 substrate had the lowest (27 mm). S2 and S5 showed fruits with diameter 48 mm. D/L ratios reached average 1.88±0.08, which means all treatments had D/L ratio less than two. Average of D/L ratio similar findings of Owaid et al. (2016b), while sawdust substrate showed very poor morphology properties of this mushroom when was used alone. That is agrees with the result of Davis and Aegerter (2000), they referred to must be use the sawdust with other agro-substrates.

Table 3: Morphological properties of cultivated *Pleurotus cornucopiae* var. *citrinopileatus* fruit bodies on various cellulosic matters

Substrate type	Diameter of pileus mm	Thickness of pileus mm	Length of stipe mm	Diameter of stipe mm	D/L ratio
S1	43.6^{b}	2.6^{ab}	19.0^{c}	40^{b}	2.3^{a}
S2	39.7^{b}	2.7^{ab}	24.7^{b}	48^{ab}	1.6^{b}

S3	54.5[a]	3.3[a]	32.2[a]	50[a]	1.7[b]
S4	28.5[c]	2.0[b]	15.7[d]	27 [c]	1.8[b]
S5	40.0[b]	3.2[ab]	23.0[b]	48[ab]	1.7[b]
Mean ± MSD	41.65 ± 1.59	2.7 ± 0.44	22.5 ± 1.11	42 ± 0.36	1.88 ± 0.08

Legend: Growth Intensity Level (GIL): 1: Light, 2: Moderate, 3: Vigorous. S1: a wheat straw substrate, S2 (70% wheat straw, 20% sawdust and 10% date palm fiber), S3: (50% wheat straw, 30% sawdust and 20% date palm fiber), S4: sawdust, S5: date palm fiber. Means of the same superscript letter(s) along each column are not significantly different (p<0.05) using Duncan's multiple range test (DMRT). MSD: Mean of standard deviation.

Conclusion

Five locally surplus organic mixtures consist of wheat straw, sawdust and date palm fiber, were used to cultivate yellow oyster mushroom *P. cornucopiae* var. *citrinopileatus*. In conclusion, the shorter period for completion of mycelia in polyethylene bags was 17 days, as the best time on S3 substrate. Date-palm fiber substrate exhibited best growth intensity level (moderate) significantly (p<0.05). The total yield, and biological efficiency percent recorded approx. 90 g and 23% on the S3 substrate, respectively, as a higher percent significantly (p<0.05), while sawdust substrate alone was an unsuitable medium for cultivation and production of this mushroom. Finally, the use of date-palm fibers in mixtures is usefulness in producing a fresh edible and medicinal mushroom.

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