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UTILIZATION OF AGRICULTURAL AND ANIMAL WASTES IN GROWTH OF NOVEL IRAQI STRAINS OF EDIBLE MUSHROOMS *PLEUROTUS OSTREATU*S AND BROWN *AGARICUS BISPORUS*

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Abstract

This study was carried out to find a local natural media for growth of two strains of edible mushroom which were recorded the first time in Iraq-Missan governorate and belonging to the genus oyster mushroom *Pleurotus ostreatus* and brown *Agaricus bisporus.* Different natural media was used for this purpose involved two types of agricultural wastes of apple peel and banana peels s and potato peels, maize peels and date seeds in addition to the leaves of sugar plant *Stevia rebaudiana* the second type included animal wastes ,feather of chicken and wool of sheep and concentrations ranging from 10-40 g /L .Also studied The effect of some physical factors and conditions temperatures and pH on the growth rates of two species *Pleurotus ostreatus and Agaricus bisporus.* The results showed that the mean percentages of chicken feathers and sheep wool achieved the best growth rates for both species of edible mushrooms**,** the growth rate of two species of edible mushroom on feather and wool (1.20, 1.18) cm / day, (1.18, 1.17) cm / day then followed the leaves of *Stevia rebaudiana* (1.00, 0.94) cm/day respectively, and the rest of the other medium compared to the medium of Potato Dextrose Agar (PDA).The best concentration for the preparation of these media was 10 and 20 g / L compared with 30 and 40 g / L. The best pH value that could be used to growth of *Pleurotus ostreatus* and brown *Agaricus bisporus* were 6.5which achieved the highest growth rates in all natural culture media for both species of edible mushrooms, and the best temperature was 30 ºC regardless of the type of medium.

Keywords : Agro-wastes; animal wastes; *Pleurotus ostreatu*s ; brown *Agaricus bisporus* mushroom; Missan governorate; Iraqi strains

Introduction

The edible Mushrooms are characterized by their sweet taste and high nutritional value, encouraging consumers and for more than 2,000 years to consume (Silva *et al*., 2013). The two genera of *Agaricus* and *Pleurotus* are the most common in the world, while the rest of the fungi are concentrated in East Asia and some European countries (Jroyse, 2014). *Agaricus bisporus* is the first of the most wide spread food fungi in the world. *Agaricus* species or button mushroom is characterized by its high nutritional qualities as it contains all the essential amino acids as well as unsaturated fatty acids with flavor and taste. Many scientists have found evidence that there are effective biological compounds with anti-cancer and anti-cancer properties and anti-cholesterol. Against vulnerability and mobility (Ekhlas *et al*., 2018). *Pleurotus* species or oyster mushroom is the second largest producer in the world after *Agricus bisporus* (Adjapong *et al*., 2015). Its production accounts for about 14% of the world's production of all types of fungi. Its cultivation in low cost, and suitable for small farmers with limited potential, and the beginners with little experience (Owaid, 2014). The cultivated mushrooms is a biotechnology for production of bioprotein which is called mycoprotein, where plant and animal wastes are used as a growing medium, and prepared according to solid state fermentation technique to become a ready medium for growing mushrooms, the programs of cultivation and improvement for production of mother culture through the increasing the means of production of mushrooms to improve their quality (Shah *et al*., 2004). Agro-wastes that contain only lignocellulose are the basic material for the cultivation of oyster mushrooms, such as sawdust and field crops such as wheat, barley, rice and others, as well as leaves and stalks of millet, maize, banana leaves, yellow corn husks, rice husks (Buah *et al*., 2010). Also some industrial waste such as waste paper industry and cane sugar, which is a suitable material for the growth and cultivation of mushrooms on the oyster, and successful cultivation of mushrooms on the oysters agricultural communities prepared from the roots and stems of trees and Different natural material was used for this purpose involved powder cumin, onion, oatmeal, banana peel, date seeds, garlic, alfalfa, flowers of palm pollen in a concentration ranged from 10 to40 g/L. (Al-Badrani, 2010; Nayak *et al*., 2015; Rukaibaa *et al*., 2017).

Pleurotus has the ability to analyze different types of high-content cellulose and cellulose residues because it possesses an enzymatic system capable of analyzing such compounds in organic waste (Manso *et al*., 2011). It is one of the second most important fungi cultivated globally after the white mushroom (Kong, 2004). The date of culturing and the method influenced on the nutritional and chemical content of the mushroom, which varies according to light, aeration, irrigation and temperature (Gregori *et al*., 2007). The purpose from study to obtain the best natural media with the best temperature and pH value to growth of two strains of edible mushrooms ***Pleurotus ostreatu*s and *Agaricus bisporus*** **(brown mushroom)** isolated from Iraqi environment for the first time at Missan governorate-Iraq.

Material and Methods

The Site of Study and the References of strains

The current study was done in the Laboratory Biotechnology for Propagation and Production of edible mushrooms at college of Agriculture-Baghdad university-Baghdad- Iraq from 1-10-2018 to 30-5-2019 .Two strains of edible mushrooms were obtained from college of Agriculture which were isolated from Missan governorate and recorded for the first time in Iraq *Pleurotus ostreatu*s and *Agaricus bisporus* (brown mushroom) in National Center for Biotechnology Information- NCBI website:-

1. *Agaricus bisporus* strain RA999 - accession version (MK 208476.1)
2. *Pleurotus ostreatus* (oyster mushroom) - accession version (MF065714.1).

Effect the Type and Concentration of Medium on Growth Rate of Edible Mushroom

The natural Media were prepared according to the type and concentration which are considered agro-wastes (apple peels, potato peels, banana peels, maize peels, dates seeds, leaves of *Stevia rebaudiana* Bertoni) and animal wastes (feathers of chicken, wool of sheep). The natural media were prepared by cutting the peels and feathers of chicken, wool of sheep into small pieces and added to it the amount of distilled water and boiled for 30 minutes and cooled and put in a mixer blender for 5 minutes and filtered using medical gauze and complements the volume supernatant of distilled water to 1000 ml and adjusted the pH to 6.5 , four concentration from each natural medium were done (10, 20, 30, 40) g/L, each 500 ml from medium were prepared in Erlenmeyer flasks in 1000 ml sterilized in autoclave cooled and poured in sterilized plates then inoculated the plates by placing 7mm in diameter disk from the end of colony in age 7 days of each strains of mushrooms *Pleurotus ostreatus* and *Agaricus bisporus* then incubated at 35ºC, six replicates plates were used per treatment then estimated the rate of growth was calculated as follows:

$$\text{Growth rate (cm / day)} = x / y$$

X: represents the diameter of the petri-dish 8.5 cm

y: the arrival time of mycelium to the edge of perti-dish (day). (Rukaibaa *et al*., 2017)

Effect the Temperature on Growth Rate of Edible Mushrooms

The effect of different temperatures (25, 30, 35,40) with different natural media agro-wastes (apple peels, potato peels, banana peels, maize peels, dates seeds, leaves of *Stevia rebaudiana* Bertoni) and animal wastes (feathers of chicken, wool of sheep)on the growth rate of edible mushrooms in potato dextrose agar .sterilized in autoclave 121 ºC, 15 lbs/In^2 pressure for 20 minute, then cooled and distributed in sterilized plates then inoculated the plates by placing 7mm in diameter disk from the end of colony in age 7 days of each species of edible mushrooms *Pleurotus ostreatus* and *Agaricus bisporus* then incubated at 35ºC. Six replicates plates were used per treatment then estimated the rate of growth (cm / day).

Effect the pH on Growth Rate of Edible Mushrooms

The effect of different values of pH (5, 5.5, 6, 6.5, 7, 7.5) with different natural media agro-wastes (apple peels, potato peels, banana peels, maize peels, dates seeds, leaves of *Stevia rebaudiana* Bertoni) and animal wastes (feathers of chicken, wool of sheep) on the growth rate of edible mushrooms in potato dextrose agar using a 0.1 NaOH or HCl solution and sterilized in autoclave 121 ºC , 15 lbs/In² pressure for 20 minute, then cooled and distributed in sterilized plates then inoculated the plates by placing 7mm in diameter disk from the end of colony in age 7 days of each species of edible mushrooms *Pleurotus ostreatu*s and *Agaricus bisporus* then incubated at 35ºC, six replicates plates were used per treatment then estimated the rate of growth (cm / day).

Results and Discussion

Effect the Type and Concentration of Natural Medium on Growth Rate of Edible Mushrooms

The results of utilization different natural media which are considered agro-wastes (peels and seeds of plant) and animal wastes such as (feathers of chicken, wool of sheep),the results showed significant differences in averages growth (cm/day) of edible mushrooms *Pleurotus ostreatu*s and *Agaricus bisporus* with the different of type of natural medium as seen in (table 1) which appeared the existence of similar letters in the table indicates that there is no significant difference and that the highest values take the letter a, which is the superior value, and the lowest value is the one that takes the last letter in alphabetical sequences, the best medium for vegetative growth for both species of edible mushrooms were recorded by the animal wastes feathers of chicken, wool of sheep (1.20, 1.18), (1.18, 1.17) then the leaves extract of *Stevia rebaudiana* (1.00, 0.94) for *Pleurotus ostreatu*s and *Agaricus bisporus* respectively. However the rest of agro-wastes media (potato peels, apple peels, banana peels, maize peels and dates seeds)were recorded the less growth rates compared with the animal residues media. this may due to the ability of *Pleurotus ostreatu*s and *Agaricus bisporus* to secret analyzing enzymes such as keratinize enzyme as animal wastes (feather and wool) contain a high percentage of keratin which is a fibrous proteins while cellulase and lignase in agro-wastes contain a high percentage of cellulose and lignine on the possibility of being adopted as a source of carbon by mixing or supporting with other wastes . The utilization of feather and wool as a carbon source and energy to edible mushroom were the first record in Iraq and the world.

Mushrooms require carbon, nitrogen and inorganic compounds as their nutritional sources. The main nutrients are less nitrogen and more carbon so materials containing cellulose, hemicellulose and lignin (i.e., rice and wheat straw, cotton seed hulls, sawdust, waste paper, leaves, and sugarcane residue) can be used as mushroom substrates (Badu *et al*., 2011). *Pleurotus* has the ability to analyze different types of high-content cellulose and cellulose residues because it possesses an enzymatic system capable of analyzing such compounds in organic waste (Manso *et al*., 2011; Rukaibaa *et al*., 2017). Also Gregori and others noted the great role of *Agaricus bisporus* in decomposing of lignin through its secretion of Peroxidases and Lacases, which occurs at the stage of forming mycelium in culture medium (Gregori *et al*., 2017).

Effect the Temperature on Growth Rate of Edible Mushrooms

The effect of temperature on the vegetative growth using by the average growth (cm/day) of edible fleshy mushrooms *Pleurotus ostreatu*s and *Agaricus bisporus* which was used four temperatures (25, 30, 35, 40) ºC in different natural sources media. The results showed in table -2a and -2b which appeared the existence of similar letters in the table indicates that there is no significant difference and that the highest values take the letter a, which is the superior value, and the lowest value is the one that takes the last letter in alphabetical sequences.

Table 1 : Effect of the type of medium at a concentration of 40 g/L in the growth rate of *Pleurotus ostreatus* and *Agaricus bisporus* with pH 6.5 and 25ºC

Growth rate (cm/day)		Type of Medium
Agaricus bisporus	***Pleurotus ostreatus***	
0.50 d	0.61 e	PDA
0.50 d	0.61 e	Potato peels
0.50 d	0.61 e	Apple peels
0.65 c	0.71 d	Banana peels
0.65 c	0.71 d	Maize peels
0.65 c	0.77 c	Dates seeds
1.18 a	1.20 a	Feathers of chicken
1.17 a	1.18 a	Wool of sheep
0.94 b	1.00 b	Leaves of *Stevia rebaudiana*
0.02762	0.02358	LSD 0.05

Table 2a : Effect of temperature on the growth rate of *Pleurotus ostreatus* in the selected media at a concentration of 40 g/L, pH 6.5

Medium mean	Temp.				Type of Medium
	40	**35**	**30**	**25**	
0.61	0.57	0.64	0.65	0.60	PDA
0.65 e	0.57	0.71	0.71	0.61	Potato peels
0.58 f	0.50 k	0.57 j	0.65 h	0.62 i	Apple peels
0.75 d	0.61 i	0.85 e	0.85 e	0.71 g	Banana peels
0.66 e	0.61 i	0.61 i	0.71 g	0.71 g	Maize peels
0.78 c	0.65 h	0.85 e	0.85 e	0.77 f	Dates seeds
1.22 a	0.94 d	1.42 a	1.42 a	1.10 c	Feathers of chicken
1.22 a	0.94 d	1.42 a	1.42 a	1.10 c	Wool of sheep
1.05 b	0.85 e	1.21 b	1.20 b	0.94 d	Leaves of *Stevia rebaudiana*
	0.69 d	0.92 b	0.94 a	0.79 c	Temp. Mean
Medium = 0.01258 Temp. = 0.00839 Medium x Temp. = 0.02516					LSD 0.05

Table 2b : Effect of temperature on the growth rate of *Agaricus bisporus* in the selected media at a concentration of 40 g/L, pH 6.5.

Medium mean	Temp.				Type of Medium
	40	**35**	**30**	**25**	
0.53 f	0.50 j	0.57 h	0.57 h	0.50 j	PDA
0.52 f	0.47 k	0.57 h	0.57 h	0.50 j	Potato peels
0.53 f	0.50 j	0.57 h	0.57 h	0.50 j	Apple peels
0.66 d	0.57 h	0.71 f	0.71 f	0.65 g	Banana peels
0.60 e	0.53 i	0.57 h	0.65 g	0.65 g	Maize peels
0.66 d	0.57 h	0.71 f	0.71 f	0.65 g	Dates seeds
1.09 b	0.85 e	1.21 b	1.21 b	1.10 c	Feathers of chicken
1.22 a	0.94 d	1.42 a	1.42 a	1.10 c	Wool of sheep
1.05 c	0.85 e	1.21b	1.21 b	0.94 d	Leaves of *Stevia rebaudiana*
	0.64 d	0.83 b	0.84 a	0.73 c	Temp. Mean
Medium = 0.01140 Temp. = 0.00760 Medium x Temp. = 0.02280					LSD 0.05

The optimal temperatures were recorded 30 ºC and 35 ºC in both edible mushrooms for all the different natural media. In *Pleurotus ostreatus* appeared a similar pattern in vegetative growth at temperatures 25 ºC, 30 ºC and 30 ºC in the best media leather of chicken, wool of sheep then Leaves of *Stevia rebaudiana* the average growth are ranged for three temperatures 25 ºC, 30ºC and 30ºC (1,01, 1.42, 1.42); (1.01, 1.42, 0.94) then (0.94, 1.21, 1.21) for three natural sources media respectively while the rest of natural sources of media were varied among potato peels medium, date seeds medium, maize peels medium then apple and banana peels media in contrast with temperatures 40ºC the averages growth were decreased this due to the ability of oyster mushroom to growth in wide spectrum of temperatures which explains the growth of this fungus and naturally in temperate, tropical and subtropical regions of the world (Ibekwe *et al*., 2008).

These results agreed with other studies (Tsing, 2015) and (Rukaibaa *et al*., 2017). In Table 2b *Agaricus bisporus* gave faster growth of mycelium in wool of sheep and leather of chicken than, potato peels medium, date seeds medium, maize peels medium then apple and banana peels media at temperature 25 ºC, 30 ºC and 35 ºC the best average growth

were recorded in two natural media, wool of sheep and leather of chicken medium (1.10,1.21,1.21); (1.10,1.42,1.42) at 25 ºC, 30 ºC, 35 ºC respectively, however the average growth became slow with increasing of temperatures these results confirmed about the optimum temperatures for average growth of *Agaricus bisporus* on culture medium ranged between 24 ºC to 35ºC (18).

Effect the pH on Growth Rate of Edible Mushrooms

The effect of pH on the vegetative growth using by growth rate (cm /day) of edible fleshy mushrooms *Pleurotus ostreatus* and *Agaricus bisporus* which were used four pH values (6, 6.5, 7, 7.5) in different natural sources media . the results as seen in table -3a and -3b which appeared the existence of similar letters in the table indicates that there is no significant difference and that the highest values take the letter a, which is the superior value, and the lowest value is the one that takes the last letter in alphabetical sequences.The optimum pH values were at 6 and 6.5 in all the different natural media for both fleshy edible mushrooms but the best media which were recorded the higher growth rate leather of chicken, wool of sheep then Leaves of *Stevia rebaudiana* the average growth are ranged for pH 6,6.5 and 7 in *Pleurotus ostreatus* (1.40,1.42,1.39); (1.40,1.43,1.39) and (1.20, 1.21, 1.18) and in *Agaricus bisporus* (1.19, 1.21, 1.19); (1.40, 1.42, 1.39) and (1.20, 1.17, 1.17) for three natural sources media respectively. The rest of natural sources of media were varied in growth rate with increasing of pH values. In study of (Rukaibaa et al,2017)noted The best pH that can be used at the *Pleurotus ostreatus* and *Pleurotus eryngii* were pH 6.5 and 7 compared with the decreasing of pH. However in table 4-4 the results of *Agaricus bisporus* varied between the average of vegetative growth and the optimum was at pH 6.5 then the growth rate decreased at pH 5, 7, 7.5. The optimal pH level *Agaricus bisporus* for mycelium growth according to scientific research (Mauracher *et al*., 2014) were ranged from 6.7 to 7.7. The different of the means of vegetative growth of fungi with the different of pH values due to the effect of free hydrogen ions on the work of the cytoplasmic membranes, enzyme activity, nutrient in the medium, and the mechanism of its transition to the cell.

Table 3a : Effect of pH values on the growth rate of *Pleurotus ostreatu*s in the selected media at a concentration of 40 g /L , Temp.35ºC

Medium mean	pH				Type of Medium
	7.5	7	6.5	6	
0.61 e	0.59 op	0.60 nop	0.65 l	0.61 mno	PDA
0.67 d	0.69 jk	0.68 k	0.71 j	0.63 lm	Potato peels
0.61 e	0.58 p	0.61 mn	0.65 l	0.62 mn	Apple peels
0.81 c	0.78 i	0.83 gh	0.85 g	0.81 h	Banana peels
0.58 f	0.55 q	0.58 p	0.61 m	0.60 no	Maize peels
0.81 c	0.78 i	0.81 h	0.85 g	0.83 gh	Dates seeds
1.37 a	1.30 c	1.39 b	1.42 a	1.40 ab	Feathers of chicken
1.37 a	1.30 c	1.39 b	1.42 a	1.40 ab	Wool of sheep
1.17 b	1.10 f	1.18 e	1.21 d	1.20 de	Leaves of *Stevia rebaudiana*
	0.85 d	0.89 c	0.93 a	0.90 b	PH. Mean
Medium = 0.01381 PH. = 0.00921 Medium x PH. = 0.02762					LSD 0.05

Table 3b : Effect of pH values on the growth rate of *Agaricus bisporus* in the selected media at a concentration of 40 g /L , Temp.35ºC

Medium mean	PH.				Type of Medium
	7.5	7	6.5	6	
0.53 f	0.49 o	0.54 n	0.57 m	0.55mn	PDA
0.53 f	0.50 o	0.55mn	0.57 m	0.50 o	Potato peels
0.54 f	0.50 o	0.54 n	0.57 m	0.55mn	Apple peels
0.67 d	0.64 k	0.68 j	0.71 i	0.68 j	Banana peels
0.54 f	0.50 o	0.55mn	0.57 m	0.55mn	Maize peels
0.65 e	0.61 l	0.64 k	0.71 i	0.66jk	Dates seeds
1.18 b	1.16 g	1.19ef	1.21 d	1.19ef	Feathers of chicken
1.37 a	1.30 c	1.39 b	1.42 a	1.40ab	Wool of sheep
1.16 c	1.10 h	1.17fg	1.20 de	1.20 de	Leaves of *Stevia rebaudiana*
	0.75 c	0.80 b	0.83 a	0.80 b	PH. Mean
Medium = 0.01091 PH. = 0.00727 Medium x PH. = 0.02181					LSD 0.05

Effect Different Carbon sources on Growth Rate of Edible Mushrooms

Fungi can grow in different carbon substrates as a carbon and energy source. In Table-4a and -4b which appeared the existence of similar letters in the table indicates that there is no significant difference and that the highest values take the letter a, which is the superior value, and the lowest value is the one that takes the last letter in alphabetical sequences. The results showed the effect of different

concentrations of two type of different natural media agro-wastes (peels and seeds of plant) and animal wastes such as (feathers of chicken, wool of sheep) which were ranged from 10 to 40 gm/Lafter fixation of environmental suitable conditions pH and temperature, in table the results showed significant differences in averages growth (cm/day) of edible mushrooms *Pleurotus ostreatus* and *Agaricus bisporus* with the different concentration and type of natural medium, the best medium for vegetative growth for both species of edible mushrooms were recorded by wool of sheep, feather of chicken, leaves of *Stevia rebaudiana* of then dates seeds at the best concentrations 10 gm/L and 20 gm/L the of growth rate of *Pleurotus ostreatus* were (1.57,1.55), (1.50, 1.50), (1.42, 1.39) then (1.19, 1.11) and *Agaricus bispous* (1.50, 1.49), (1.35, 1.30), (1.36, 1.30) then (0.85, 0.81) respectively. The rest of concentrations 30 gm/L and 40 gm/L were recorded less growth rates. However the rest of natural media were varied with different concentration this reflects the good content of protein and carbohydrate as well as the mineral elements important to grow these edible mushrooms . It should be noted that numerous of researchers have studied the effect of the different concentration of media on growth rate of fungi including the study by (Rukaibaa *et al*., 2018), which found that the best concentration of *Ceratophylum demeresm* powder in growth rate of *Pleurotus ostreatus, Fusarium moniliforme* and *Trichoderma harzianum* is 100 g / L. Also (10) shown that the media prepared from cumin and onion powders have achieved best growth rate for the fungi under study *Pleurotus ostreatus* and *Pleurotus eryngii* which was 1.42 cm/ day. The best concentration of preparation of these medium was 20 g/L.

Table 4a : Effect of Concentration of medium on growth rate of *Pleurotus ostreatus* in the Selected Media with pH 6.5 and 35 ° C.

Medium mean	Conc.				Type of Medium
	40	30	20	10	
0.56 ef	0.68 jklmno	0.54 lmnop	0.50 op	0.53 mnop	Potato peels
0.53 f	0.60 klmno	0.51 nop	0.49 p	0.50 op	Apple peels
0.62 de	0.80 hij	0.65 jklmnop	0.53 mnop	0.51 nop	Banana peels
0.66 d	0.71 ijkl	0.68 jklmn	0.60 klmnop	0.67 jklmno	Maize peels
1.02 c	0.90 gh	1.03 fg	1.13 ef	1.02 fg	Dates seeds
1.41 b	1.42 abcd	1.45 ab	1.50 a	1.27 cde	Feathers of chicken
1.50 a	1.43 abc	1.52 a	1.55 a	1.52 a	Wool of sheep
1.35 b	1.25 de	1.32 bcd	1.39 abcd	1.43 abc	Leaves of *Stevia rebaudiana*
	0.97	0.96	0.96	0.93	Conc. Mean
Medium = 0.0880 Conc. = N. S. Medium x Conc. = 0.1759					LSD 0.05

Table 4b : Effect of Concentration of medium on growth rate of *Agaricus bisporus* in the Selected Media with pH 6.5 and 35 ° C.

Medium mean	Conc.				Type of Medium
	40	30	20	10	
0.51 g	0.57 n	0.55 n	0.50 o	0.44 p	Potato peels
0.66 d	0.57 n	0.71 k	0.71 k	0.65 l	Apple peels
0.56 f	0.71 k	0.61 m	0.52 o	0.40 q	Banana peels
0.59 e	0.64 l	0.65 l	0.55 n	0.52 o	Maize peels
0.78 c	0.71 k	0.75 j	0.81 i	0.85 h	Dates seeds
1.28 b	1.20 g	1.29 e	1.30 e	1.35 d	Feathers of chicken
1.46 a	1.42 c	1.45 b	1.49 a	1.50 a	Wool of sheep
1.27 b	1.20 g	1.25 f	1.30 e	1.36 d	Leaves of *Stevia rebaudiana*
	0.87 d	0.90 a	0.89 b	0.88 c	Conc. Mean
Medium = 0.01246 Conc. = 0.00881 Medium x Conc. = 0.02492					LSD 0.05

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