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PRODUCTION OF SPAWN WITH HIGH QUALITY FROM NOVEL IRAQI STRAINS OF EDIBLE MUSHROOMS

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Abstract

The results of testing the ability of edible mushrooms to produce some enzymes were varied between two strains, brown *Agaricus bisporus* produced protease, lipase, cellulose and chitinase enzymes. likewise, *Pleurotus ostreatus* produced protease, cellulose and chitinase enzymes. Moreover,the measuring of biomass (dry weight) for two strains after culturing on natural media wool of sheep broth and feather of chicken medium at different concentration from 10g /L to 40 g /L in addition to PDB as control, *Pleurotus ostreatus* gave the highest biomass at the concentration of 10 and 20 g/L on the leaves of sugar plant medium and feather of chicken medium compared to *Agaricus bispous* and control PDB. In quantitative and qualitative analysis of methanolic extract of mycelium powder for both strains using by Gas Chromatography-Mass Spectrum technique (GC-MS). The methanolic extract of the powder mycelium of *Agaricus bisporus* was analyzed to 17 chemical compounds and 15 chemical compounds to *Pleurotus ostreatus* the most common omega - fatty acids such as cis-vaccenic acid, Palmatic acid, Oliec acid and linoleic acid and both strains of edible mushrooms had a high percentage of active compounds In their filtrates mostly were the derivatives of fatty acids and alcohols such as acetol. In an attempt to compare the effect of mycelium sources of two strains of edible mushrooms which were recorded the first time in Iraq-Missangovernorate and belonging to the genus oyster mushroom *Pleurotus ostreatus* and *Agaricus* brown mushroom *Agaricus bisporus*. The results of spawn production were shown after culturing on different culture media the leaves of sugar plant *Stevia rebaudian* medium and eather of chicken medium and wool of sheep medium. The strain of *Pleurotus ostreatus* which was growing on wool medium was found to be suitable for spawn production with short duration completion growth occupied 14 days then followed feather of chicken medium and leaves of sugar plant medium occupied 19 days compared with *Agaricus bisporus.* Which recorded the longest duration completion growth occupied 21 days on the three culture media and the source mycelium of both strains from Potato Dextrose Agar PDA occupied 21 days for *Pleurotus ostreatus* and 24 days for *Agaricus bisporus* and it was found the inoculation of hay medium to produce spawn for each strains using wheat added the extract of *Stevia rebaudian* had reducted in incubation period and the formation of pins to 14 days for *Pleurotus ostreatus* and 19 days for *Agaricus bisporus.*

Key words: spawn production, GC-MS, brown *Agaricus bisporus* (RA999), *Pleurotus ostreatus* (MF065714).

Introduction

Edible mushrooms have been known by humans since ancient times as food and medicine (Silva *et al.,* 2013, Alananbeh, 2014 and Ekhlas *et al*., 2018). They are Heterotrophic organisms have no ability to form organic compounds from carbon dioxide present in the air, Thus, living on organic and decomposing materials, many fungi, especially Basidiomycetes can analyze cellulose into inorganic matter (Kavanagh, 2005). Cellulose is one of the most important components of organic waste accumulated in the environment, which microorganisms analyze as a source of energy in their growth, while the availability of lignin as a source of carbon in the second grade (Radwan, 2002). The degradation of lignin has an environmental and commercial importance in the world because of the enormous quantities of cellulose and lignin which were wasted continuously can be used as basic materials in the preparation of cultural media for the production of edible fungi (Beelman, 2005 and Rukaibaa *et al*., 2017). *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

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(Jroyse, 2014). *Agaricus bisporus* is the first of the most wide spread edible mushrooms in the world Kavanagh, 2005, Abirami and Ananthi, 2015, Valverde *et al.,* 2015) and has an important effect on the decomposition of lignin through its secretion of Peroxidases and Lacases, which occurs at the stage of forming mycelium in culture medium (Gregori *et al.,* 2007). The selective of grains used in preparation of spawn carefully, should be not broken and free infections and usually washed then treated thermally and dried to become a humidity between 52-48% in order to reduction the bacterial contamination and determination the speed of growth of mushrooms (Oie, 2005). A mixture of $CaCO_3$ and $CaSO_4$ H_2O to improve the texture of the medium through reducing of agglutinations, then sterilized for a sufficient period and then inoculated by mycelium produced from the previous stage and incubated for several weeks at an appropriate temperature until the completion of the growth to get of spawn ready to next stage (fruiting bodies production), spawn should use directly to prevent contamination (Al-Saadawi, 2015) suggest that it is possible to use sawdust at this stage instead of grains to be preserved at a higher temperature and for a longer period without getting contamination or self-degradation because of containing less nutrients than grains, as well as their low cost, due to high carbon content and low nitrogen ratio in sawdust, some studies preferred adding supporting material to sawdust such as Soybean flour, wheat grain, beer yeast, etc. The purpose from this research to get the best medium for growing edible mushrooms to produce spawnin short time in addition to knowledge the active compounds in edible mushrooms.

Materials and Methods

The References of strains of Edible Mushrooms

The current study was done in the Laboratory Biotechnology for Propagation and Production of edible mushrooms at College of Agricultural Engineering Science -Baghdad university-Baghdad- Iraq. 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 (Marthad *et al*., 2019).

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

Test the Ability of Edible Mushrooms to producte of enzymes

1. Sreening lipase production: The sterile medium which contains on substrate (pepton supporting with Tween 80) was prepared and poured in plates. An agar plug (6 mm) was removed from the periphery of 7 days old cultures grown on PDA plates of each species *Pleurotus ostreatu*s and *Agaricus bisporus* then placed in the center culture medium plates on to triplicate plates containing the screening medium, plates were incubated at 35ºC until the fungal growth for 14 days in an incubator. Lipolysis was indicated by the appearance of clear zone of inhibition around the disc of inoculation. (Slikin, 2000).
2. Screening Protease production: The sterile medium skimmed milk agar was poured in plates. An agar plug (6 mm) was removed from the periphery of 7 days old cultures grown on PDA plates of each species *Pleurotus ostreatus* and *Agaricus bisporus* then placed in the center culture medium plates on to triplicate plates containing the screening medium, plates were incubated at 35°C until the fungal growth for 14 days in an incubator. Protease was indicated by the appearance of clear zone of inhibition around the disc of inoculation. (Vijayaraghavan *et al.,* 2013).
3. Screening cellulase production: Carboxy methyle cellulose agar was poured in plates. An agar plug (6 mm) was removed from the periphery of 7 days old cultures grown on PDA plates of each species then placed in the center culture medium plates on to triplicate plates containing the screening medium, plates were incubated at 35°C until the fungal growth for 14 days in an incubator. The detection medium containing a chromogenic substrate (Congo red) was used to screen the edible mushrooms *Pleurotus ostreatu*s and *Agaricus bisporus* for cellulase producing ability and indicated by the appearance of clear zone of inhibition around the disc of inoculation. The detection of producing chitinase by forming clearance zones around the disc of inoculation. (Kasana *et al.,* 2008).
4. Srceening Chitinase Production: Chitin agar was poured in plates. An agar plug (6 mm) was removed from the periphery of 7 days old cultures grown on PDA plates of each species, then placed in the center culture medium plates on to triplicate plates containing the screening medium, plates were incubated at 35°C until the fungal growth for 14 days in an incubator and indicated by the appearance of clear zone of inhibition around the disc of inoculation. (Dhanya *et al.,* 2015).

Determination of Biomass of Edible Mushrooms

Two species of edible mushrooms were cultured in three natural media (feather, leaves of *Stevia rebaudian* Bertoni) at concentrations (1, 5, 10) mg/L in addition to Potato Dextrose Broth (PDB). Test broth for each medium and species were prepared with 250ml Erlenmeyer flasks, filled with 50ml of liquid medium and added one disc in diameter 6mm and final pH was adjusted to 6.5 then incubated for 4 weeks at 35°C and the biomass growth of fungi was observed on fourth week days. A clear biomass mat was obtained by filtration using Whatmman No.2 filter paper inside a biological safety cabinet. The collected biomass was washed twice with distilled water and dried at 45-50°C for 24 hours or until constant dry weight was achieved then the dry weight was estimated gravimetrically mg/L (Lai *et al.,* 2004).

Preparation of Methanol Extract of Edible Mushrooms

According to the method of Sathyaprabha and others (Sathyaprabha *et al.*, 2011) with modification 25gm dried biomass powder of *Pleurotus ostreatu*s and *Agaricus bisporus* was taken in a conical flask with 30ml of 95% methanol crushed with respective solvent and stored it for overnight soaking, the flasks were covered with aluminum foil and the mixture was filtered through Whatmman filter paper no.1 and the filtrate concentrated in a rotary evaporator. The methanol was evaporated and the extract was collected and dried until use GC-MS.

Analysis of the Chemical Constituents *Pleurotus ostreatu*s and *Agaricus bisporus* By (GC-MS)

GC-mass chromatography analysis was performed to identify the chemical compounds in biomass (dry weight) extracts of *Pleurotus ostreatu*s and *Agaricus bisporus*. Identification of chemical compounds was done by injecting 2µl of sample into an RT *5 column (30*0.32 nm) of GC-MS model (Perkin Elmer, Clarus 500, USA), helium (3ml/min) was used as a carrier gas. The following temperature gradient program was used (75°C for 2 min followed by an increase from 75 to 175°C at a rate of 50°C per min and finally 7 min at 175°C). The m/z peaks representing mass to charge ratio characteristics of the chemical compounds fractions were compared to those in the mass spectrum library of the corresponding organic compounds. This experiment was conducted in Science and Technology Ministry.

Preparation of Spawn

The grains of wheat were poured in boiling water for 20 minutes then filtrated in sieve until humidity reaches about 50%. Calcium carbonate $CaCO_3$ and $CaSO_4H_2O$ were added to 15 g/kg wet weight of grains, mixed together then distributed in nylon bags by 250 g/bag and blocked by medical cotton and gauze with the present of a plastic ring proved about 10 cm from the beginning of the bag, sterilized bags in autoclave for one hour, cooled to 25°C, then added four pieces of inoculum (2×2 cm) from the center of PDA medium as control and four pieces of inoculum from wool of sheep medium, Feathers of chicken medium and leaves of *Stevia rebaudian* medium for each strains *Pleurotus ostreatus* and *Agaricus bisporus* to test the effectiveness of mycelium source on spawn production. The bags were transferred to the incubator at 25°C with stirring the bags once each a week to prevent grain aggregation and distributed the inoculum equally (Kumari *et al.,* 2017).

Effect of *Stevia rebaudian* Leaves on Spawn Production

The addition of leaves of *Stevia rebaudian* and mycelium growth of *Pleurotus ostreatus* and *Agaricus bisporus* on production of spawn was tested through adding leaves of *Stevia rebaudian* in concentration 40g/kg to wet weight of wheat grains $CaCO_3$ and calcium $CaSO_4H_2O$ were added to the $CaSO_4H2O$ and 15 g/kg wet weight of the grains compared to the same media, but without the addition of leaves of *Stevia rebaudian* powder. The grains were mixed with well-added additives and distributed in nylon bags then blocked by medical

Table 1: Effect of mycelium sources of *Pleurotus ostreatus* and *Agaricus bisporus* on production of spawn.

Type of fungi	Mycelium source	Growth (day)						Duration completion growth
		1	7	14	19	21	24	
Pleurotus ostreatus	PDA	-	++	+++	++++	+++++	/	21
	Wool of sheep	-	+++	+++++	/	/	/	14
	Feathers of chicken	-	+++	++++	+++++	/	/	19
	Leaves of *Stevia rebaudian*	-	+++	++++	+++++	/	/	19
Agaricusbisporus	PDA	-	+	++	++++	++++	+++++	24
	Wool of sheep	-	++	+++	++++	+++++	/	21
	Feathers of chicken	-	++	+++	++++	+++++	/	21
	Leaves of *Stevia rebaudian*	-	++	+++	++++	+++++	/	21

+ growth, - No growth, / time of complete growth.

Table 2: Effect of addition of Leaves of *Stevia rebaudian* and mycelium growth of *Pleurotu sostreatus* and *Agaricus bisporus* on production of spawn.

Type of fungi		Mycelium source	Growth (day)						Duration completion growth
			1	7	14	19	21	24	
Pleurotus ostreatus	Wheat	PDA	-	++	+++	++++	+++++	/	21
		Leaves of *Stevia rebaudian*	-	+++	++++	+++++	/	/	19
	Wheat	PDA	-	+++	++++	+++++	/	/	19
		Leaves of *Stevia rebaudian*	-	++++	+++++	/	/	/	14
Agaricus bisporus	Wheat	PDA	-	+	++	+++	++++	+++++	24
		Leaves of *Stevia rebaudian*	-	++	+++	++++	+++++	/	21
	Wheat	PDA	-	++	+++	++++	+++++	/	21
		Leaves of *Stevia rebaudian*	-	+++	++++	+++++	/	/	19

+ growth, - No growth, / time of complete growth.

cotton gauze and gauze with the aid of a plastic ring that was fixed about 10 cm from the beginning of the bag, the bags were sterilized at 121°C and 1.5 kg/cm2 for an hour and a half. cooled to 25°C, then added four pieces of inoculum (2×2 cm) from the center of PDA medium medium for each strains *Pleurotus ostreatus* and *Agaricus bisporus*. The bags were transferred to the incubator at 25°C with stirring the bags once each a week to prevent grain aggregation and distributed the inoculum equally.

Results and Discussion

Screening of Enzymes production by Edible Fleshy Mushrooms

Lipase: The results of test the ability of edible fleshy mushrooms for two strains *Pleurotus ostreatu*s and *Agaricus bispous* of analyzing of lipid by production lipase were showed the strain *Agaricus bispous* had the ability to produce lipase enzyme by forming transparent zone around in diameter >15 mm on tween 80 agar and gave positive result, on the other hand the strain *Pleurotus ostreatu*s gave the negative results. see in table 3. Mushrooms produce extracellular enzymes such as chitinase, lipase, cellulase, protease, keratinase and etc. that affect the increase in its nutritional value and degradation of the substrate through enzyme production. but lipases have never been isolated from the basidiomycete fungus *Pleurotus ostreatus* even if many putative lipase coding genes have been automatically annotated in its genome (Riley *et al.,* 2014, Castanera *et al*., 2016).

Table 3: Screening of Enzymes Production ByEdible flesh ushrooms on solid agar. The symbol (+) producer and (-) non producer.

Type of fungi		Type of Enzyme
Agaricus bispous	*Pleurotus ostreatu*s	
+	-	Lipase
+++	+++	Protease
+++	+++	Cellulase
+	+	Chitinase

Protease: The results of test the ability of edible fleshy mushrooms for two strains *Pleurotus ostreatu*s and *Agaricus bispous* of analyzing of protein by production protease on the Skimmed milk agar were showed both straina *Agaricus bispous* and *Pleurotus ostreatu*s had the ability to produce protease enzyme by forming transparent zone around in diameter >15 mm on Skimmed milk agar and gave positive result see in table 3. Proteases play important roles in the physiology of fungi, acting in processes such as germination and sporulation. This enzyme seems to have a close relationship with the lifestyle of fungi, as observed in *Pleurotus and Agaricus* (Cui *et al.,* 2007).

Cellulase: The results of test the ability of edible fleshy mushrooms for two strains *Pleurotus ostreatu*s and *Agaricus bispous* of analyzing of protein by production protease on the Carboxymethyle cellulose agar (CMC) were showed both straina *Agaricus bispous* and *Pleurotus ostreatu*s had the ability to produce protease enzyme by forming transparent zone around in diameter >15 mm on Carboxymethyle cellulose agar (CMC) and gave positive result table 3. This results agreed with the study of (Alananbeh *et al*., 2014) were found the ability of *P.ostreatus* to produce cellulase and also (Wu and jae-shin, 2017) when tested the ability of ten strains of mushrooms onto CMC agar-Congo red plates and produce carboxymethylcellulase therefore in nature most of the mushrooms species grow on soil or wood in nature and have the enzymatic capacity to use cellulose, hemicellulose and other components of lignocellulosic matter as a source of carbon and energy (Sánchez, 2009).

Chitinase: The results of test the ability of edible fleshy mushrooms for two strains *Pleurotus ostreatu*s and *Agaricus bispous* of analyzing of protein by production protease on the purified chitin agar were showed both straina *Agaricus bispous* and *Pleurotus ostreatu*s had the ability to produce protease enzyme by forming transparent zone around in diameter >15 mm on

Table 4: GC mass profile of the biomass of *Agaricus bisporus*.

No.	R.t. (min)	Compounds	Molecular formula	M.W. (g/mole)	Area %	R.A. %
1	4.139	Glycerin	C3H8O3	92	0.91	2.48
2	11.921	Cycloheptasiloxane, tetradecamethyl	C14H42O7Si7	518	1.65	1.82
3	14.052	Cyclooctasiloxane, hexadecamethyl	C16H48O8Si8	592	1.55	1.70
4	16.326	Cyclohexasiloxane, dodecamethyl	C12H36O6Si6	444	1.24	2.04
5	17,775	Methyl 14-methylpentadecanoate	C17H34O2	270	3.63	2.15
6	18.367	Palmatic acid	C16H32O2	256	13.24	3.75
7	19.996	9,12-Octadecadienoic acid, methyl ester	C19H34O2	294	3.38	1.85
8	20.063	10-Octadecenoic acid, methyl ester	C19H36O2	296	5.22	1.91
9	20.346	Methyl isoheptadecanoate	C18H36O2	284	1.90	2.02
10	20.661	Cis-Vaccenic acid	C18H34O2	282	43.07	6.52
11	20.868	Cerasynt	C22H44O4	372	5.31	2.55
12	21.990	1,3,3,3-Tetramethyl disiloxanyltris(trimethylsilyl)orthosilicate	C13H40O5Si6	444	1.10	1.88
13	23.910	E,E-2,13-Octadecadien-1-ol	C18H34O	266	2.82	2.57
14	24.227	2-Hexadecar	C19H38O4	330	1.53	2.26
15	24.599	Hexadecamethylheptasiloxane	C16H48O6Si7	532	1.84	2.30
16	25.774	Olein, 2-mono	C21H40O4	356	10.96	3.77
17	26.860	Squalene	C30H50	410	0.65	1.32

RT = Retention time, RA = peak area of each compound / the highest peakaerea *100.

the purified chitin agar and gave positive result see in table 3. This enzyme plays a critical roles in fungal growth and development, in resistance of plants to fungal pathogens and in parasitism of insects by entomopathogenic fungi however (Sufiate *et al*., 2017) demonstrated the first report about the activity of each enzymes chitinase and cellulase which are secreated by *Pleurotus eryngii* and *Pleurotus ostreatus*. against Meloidogynejavanica eggs. as nematicidal action.

Determination of Biomass of Edible Mushrooms at Different Concentrations and Natural Media

The utilization of selective natural media Leaves of *Stevia rebaudian* as agro-wastes medium and feather of chicken as animal wastes medium after obtaining the highest growth rates among the rest of natural media. The results showed in fig. 1 and fig. 2, *Pleurotus ostreatus* was obtained higher biomass dry weight at concentrations 10 g/L and 20 g/L in liquid media of Leaves of *Stevia rebaudian* and feather of chicken media than the stain of *Agaricus bispous* compared with the control potato dextrose broth PDB, in fig. 1 was observed that the higher biomass of dry weight value was obtained using, feather of chicken firstly the biomass of dry weight values for *Pleurotus ostreatus* at concentration (10,20) g/L were recorded (1.20, 1.01) g/Lrespectively. While *Agaricus bispous* was secondly, (0.90, 1.03) g/L respectively and Leaves of *Stevia rebaudian* for *Pleurotus ostreatus* (1.0, 0.88) g/L then *Agaricus bispous* (0.96, 0.90) g/L respectively, these results were matched with the results of vegetative growth on solid media.

Different types of wastes have been suggested as prospective nutritional sources for growth of edible mushrooms with low costs. The due to success of leaves *Stevia rebaudian* as a natural medium for growth of edible mushrooms to exist of high sweet compounds steviol glucosides specially steviosides which contains from three

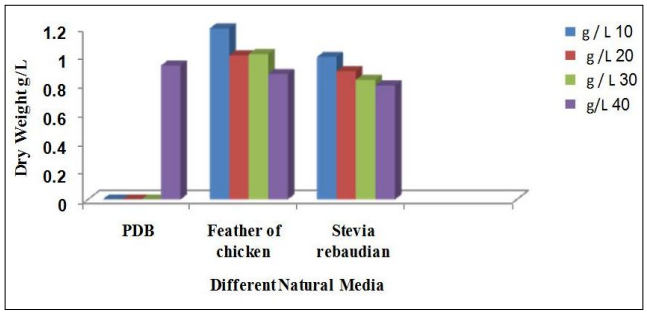

Fig. 1: Effect of the concentration of natural medium on biomass (dry weight) of *Pleurotus ostreatus* in liquid Media.

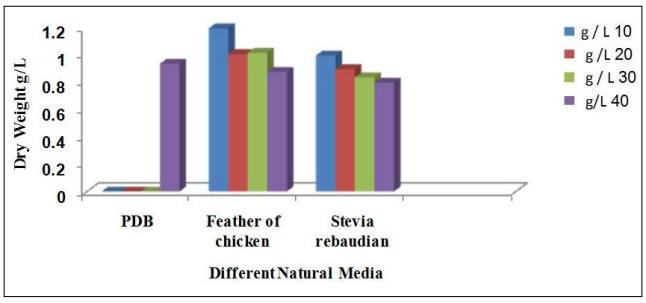

Fig. 2: Effect of the concentration of natural medium on biomass (dry weight) of *Agaricus bispous* in liquid Media

Table 5: GC mass profile of the biomass of *Pleurotus ostreatus.*

No.	R.t. (min)	Compounds	Molecular formula	M.W. (g/mole)	Area %	R.A. %
1	13.934	1-Butoxy-3,3,3-trimethyl-1-[(trimethyl)oxy]disiloxanyl	C19H54O7Si7	590	2.84	1.69
2	16.056	N-(Trifluoroacetyl)-N,O,O;O-tetrakis(trimethylsilyl)norepinphrine	C22H42F3NO4Si4	553	3.23	1.91
3	17.880	Dodecamethylcyclohexasiloxane	C12H36O6Si6	444	2.17	1.80
4	18.945	Methyl14-methylpentadecanoate	C17H34O2	270	2.16	1.87
5	19.253	4-Methyldocosane	C23H48	324	2.85	2.04
6	19.373	Palmitic acid	C16H32O2	256	12.87	2.54
7	19.509	1-Isopropoxy-3,3,3-trimethyl-1-[(trimethylsilyl)oxy]disil	C18H52O7Si7	576	2.18	1.79
8	20.683	9,12-Octadecadienoic acid,methyl ester	C19H34O2	294	4.06	1.68
9	21.186	Pentadecanoic acid	C15H30O2	226	44.08	4.87
10	21.363	Arachic acid	C20H40O2	312	5.37	2.03
11	23.571	Octadecamethylcyclononasiloxane	C18H54O9Si9	666	2.59	2.11
12	24.109	Linoleic acid	C18H32O2	280	2.75	2.51
13	24.723	Cyclodecasiloxane,eicosamethyl	C20H60O10Si10	740	2.94	2.17
14	25.868	Glycerol2-monooleate	C21H40O4	356	7.68	2.82
15	26.871	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane	C16H50O7Si8	578	2.22	2.07

RT = Retention time, RA = peak area of each compound / the highest peakaerea *100.

molecules glucose, steviol and glycan that are considered as a carbon source and energy for growth of edible mushrooms *Pleurotus ostreatu*s and *Agaricus bispous*, while the chemical components of feather has high ratio of keratin (Pemba and Sharangi, 2016).

Analysis of the Chemical Constituents *Pleurotus ostreatu*s and *Agaricus bispous* By (GC-MS)

After cultivation of edible flesh mushrooms in liquid medium potato dextrose broth for 30 days. The GC-MS chromatography of biomass mycelium powder of *Agaricus bisporus* was extracted with 95% methanol to the seventeen peaks of the compounds detected was shown in table 4. Peaks were determined to be Glycerin, Cycloheptasiloxane, tetradecamethyl, Cyclooctasiloxane, hexadecamethyl, Methyl 14-methylpentadecanoate, Palmitic acid, 9,12-Octadecadienoic acid, methyl ester, 10-Octadecenoic acid, methyl ester, Methyl isoheptadecanoate, Cis-Vaccenic acid, Cerasynt, 1, 3, 3, 3-Tetramethyl disiloxanyltris (trimethylsilyl) orthosilicate, E,E-2, 13-Octadecadien-1-ol, 2-Hexadecar, Olein, 2-mono, Spinacen from this table fatty acids are the predominant compound in the structures of dry mycelium of *Agaricus bisporus* such as cis-vaccenic acid, is an omega-7 fatty acid, then Palmatic acid is a common saturated fatty acid and predominant fatty acids in chemical structures of mushroom then Olein, 2-mono derives from oleic acid.

Table 6: GC-Mass analyzing of filtrate of *Agaricus bisporus.*

No.	R.t. (min)	Compounds	Molecular formula	M.W. (g/mole)	Area %	R.A. %
1	17.343	Hexadecanoic acid	C16H32O2	256	22.35	3.36
2	19.148	9-Hexadecenoic acid	C16H30O2	254	56.66	3.29
3	22.106	Z,Z-4,16-Octadecadien -1-ol acetate	C20H36O2	308	20.99	2.54

RT = Retention time, RA = peak area of each compound / the highest peakaerea *100.

While the biomass mycelium powder of *Pleurotus ostreatu*s was extracted with 95% methanol to the fifteen peaks of the compounds detected was shown in table 5. Peaks were determined to be 1-Butoxy-3,3,3-trimethyl-1-[(trimethyl) oxy] disiloxanyl, N-(Trifluoroacetyl)-N,O,O,O-tetrakis (trimethylsilyl) norepinephrine, Dodecamethyl cyclohexasiloxane, Methyl14-methylpentadecanoate, 4-Methyldocosane, Palmitic acid, 1-Isopropoxy-3,3,3-trimethyl-1-[(trimethylsilyl) oxy] disil, 9,12-Octadecadienoic acid, methyl ester,(6Z)-6-pentadecen-1-ol,Arachic acid, Octadeamethyl cyclononasiloxanc, 17-Octadecynoic acid, Cyclodecasiloxane, eicosamethyl, Glycerol2-monooleate, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15, Hexadecamethyloctasiloxa, (6Z)-6-pentadecen-1-ol a fatty alcohol is a predominant then palmatic acid.

These results agreed with (Goyal*et al.,* 2015) that revealed the mushrooms were found to be rich in polyunsaturated fatty acids (>75%), linoleic acid being the most predominant one in both the mushrooms. Among saturated fatty acids, palmitic acid was the major fatty acid. No significant differences were found in saturated and unsaturated fatty acid contents of the two mushrooms.

Both *Agaricus bisporus* and *Pleurotus sajorcaju*. and Badu and others (Badu *et al.,* 2011) showed the Fatty acid composition varied among six wild mushroom species *Boletus reticulatus, Flammulinavelutipes var.*

Table 7: GC-Mass analyzing of filtrate of *Agaricus bisporus.*

No.	R.t. (min)	Compounds	Molecular formula	M.W. (g/mole)	Area %	R.A. %
1	2.077	Acetol	C3H6O2	74	8.12	1.13
2	17.348	Hexadecanoic acid	C16H32O2	256	10.50	2.11
3	19.153	9-Hexadecenoic acid	C16H30O2	254	48.81	3.41
4	22.100	Brassidic acid	C22H42O2	338	9.62	2.09
5	23.867	Olealdehyde	C18H34O	266	22.95	3.26

RT = Retention time, RA = peak area of each compound / the highest peakaerea *100.

velutipes, Lactariussalmonicolor, Pleurotus ostreatus, Polyporussquamosus and *Russulaanthracina*) the unsaturated fatty acids were at higher concentrations than saturated fatty acids. (In general, approximately 70% of fatty acids were the same in six species. In addition, results appeared that mushrooms were rich in polyunsaturated fatty acids, as well as in study of Rukaa and zinea on the analyzing of chemical structures of *Agaricus bisporus* using by GC. Linoleic acid was the predominant among the other compounds. The filtrates of the two strains of edible flesh mushrooms *Agaricus bisporus* and *Pleurotus ostreatus* were extracted and subjected to screening of different chemical compounds by Gas Chromatography-Mass Spectrum technique, according to the results various active compounds are presented in *Agaricus bisporus* when compared with *Pleurotus ostreatu*s The active compounds in filtrate of *Agaricus bisporus* are Palmitic acid (also known as hexadecanoic acid) is a fatty acid that is found naturally in animals and plants and also can be created in laboratory settings. Palmitic acid is widely used in a variety of applications, including personal care products and cosmetics and Ethyl linoleate is a long-chain fatty acid ethyl ester resulting from the formal condensation of the carboxy group of linoleic acid with the hydroxy group of ethanol as seen in table 6.

The filtrate of *Pleurotus ostreatu*s the chemical compound were Acetol is an organic chemical consisting of a primary alcohol substituent on acetone, the fatty acid Hexadecanoic acid and its derivatives then Brassidic acid unsaturated trans fatty acid and Olealdehyde table 7.

Preparation of spawn

The results were shown in table 1, the production of spawn on wheat grains after inoculation by two strains of edible mushrooms *Agaricus bispous* and *Pleurotus ostreatu*s that were grown on PDA medium, Wool of sheep medium and feather of chicken medium. The growth intensity was monitored for 21 days or until completion, with the aim of determining the best source mycelium among the three, it is noted that the signs of growth on the surface of grain inside the bags begin to appear after 24 hours from inoculation on all media the mycelium of *Pleurotus ostreatu*s source which grow on wool of sheep medium was exceeded on the rest media, the duration completion growth was recorded 14 days then the mycelium source from feather of chicken and leaves of *Stevia rebaudian* were 19 days the duration completion growth compared with the production of spawn from *Agaricus bispou*s which were recorded the longest duration of growth was 21 days in all treatments the variation between the source of mycelium and type of strain may be due to the chemical structure of wool and feather which involve of keratin and different enzyme activity of both strains.

Effect of adding leaves of *Stevia rebaudian* and mycelium source of *Pleurotus ostreatus* and *Agaricus bispous* in production Spawn

The results of adding leaves of *Stevia rebaudian* on wheat grains were shown in table 2 after inoculation by two strains *Pleurotus ostreatus* and *Agaricus bispous*one was produced on PDA and the other on the leaves of *Stevia rebaudian* medium and the intensity of growth was monitored until completion on the medium, in order to determine the effect of the treatment of wheat grain with leaves of *Stevia rebaudian* in spawn preparation. It was observed that the density of growth on wheat with leaves of *Stevia rebaudian* exceeded in both strains, the duration completion growth was recorded 19 days and 14 for *mycelium source of Pleurotus ostreatus* from PDA medium and leaves of *Stevia rebaudian* medium and 21 and 19 days for mycelium source of *Agaricus bispous* from PDA medium and leaves of *Stevia rebaudian* medium compared with wheat only without adding of leaves of *Stevia rebaudian* to wheat grains.

The success of the growth of all replicates added to the leaves of *Stevia rebaudian* gave a perception of the possibility of increasing the growth of mycelium on this medium and to resist the contaminants that could face the development process on that medium. This may be due to the addition of leaves of *Stevia rebaudian* to wheat grains, which resulted in the provision of ready sources of nutrients during the growth of mycelium and gave a great energy and supplied nutrients requirements for a longer period than those provided by the same medium without the addition of supports, likewise the leaves of *Stevia rebaudian* are enrich in proteins and fatty acid in addition to active compounds such as phenols and polysaccharides, that may due to support production spawn stage and protection of wheat grains from microbial contamination (kumari *et al.,* 2017).

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