Plant Archives Volume 20 No. 2, 2020 pp. 7437-7445 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MORPHOLOGICAL CHARACTERIZATION, MOLECULAR DIAGNOSIS AND ENZYMATIC ACTIVITY OF SOME WILD MUSHROOM IN BAGHDAD PROVINCE, IRAQ

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Abstract

Baghdad province has a rich diversity of wild mushrooms. This trial is the first one to study their properties and characterization. The current study was conducted to distinguish a number of wild mushrooms collected from two locations at Baghdad province from 2017 to 2019. Twelve samples were collected from different area locations to Baghdad province district of Iraq and identified eleven different species. According to the key features (photograph, color, stalk length, stalk diameter, and cap diameter), Indigenous characterization was completed in the field, while different key references such as monographs, manuals, and databases were implemented to do conventional characterization. The wild mushrooms samples were: *Pleurotus fuscus*, *Agaricus bisporus* (white), *Pleurotus floridanus*, *Agaricus nevoi*, *Agaricus subperonatus*, *Agaricus bisporus* (brown), *Pleurotus ostreatus*, *Agaricus heterocystic*, *Agaricus bellanniae*, *Agaricus crocodilinus*, *Agaricus litoralis* and *Pisolithus albus*. Based on the indigenous information eleven fungi samples are to be edible, while *Pisolithus albus* was classified as medicinal fungi. Taxonomic studies of fungi samples according to DNA molecular diagnosis have been shown that the11 species were belonging to 3 genera,3 families, and 2 orders, that to be essential for perfect wild mushrooms classification and to prevent human deaths from the consumption of poisonous ones. The highest enzymatic efficacy at fungi mycelium on this study was to cellulase enzyme from *P. floridanus, Agaricus litoralis, Agaricus crocodilinus,* and *Pisolithus albus*. The highest enzymatic efficacy at fungi mycelium was to protease enzyme from *Agaricus bellanniae*,*Agaricus crocodilinus, Agaricus litoralis*, while was not any efficacy to xylanase enzyme and was noted different efficacy to L-Asparaginase enzyme.

Key words : Baghdad mushroom, wild collection, Fungi identification, fungi enzymatic activity, Wild mushroom.

Introduction

Fungi play a very significant role in our life. They are our greatest friends as well as foes. Most fungi are microscopic, but some (macrofungi) intermittently produce fruiting bodies (sporocarps) that are highly visible. These are commonly called mushrooms or toadstools (Webster and Weber, 2007). Mushrooms are well known as the main food resource and in achieving nutritional security (Tibuhwa, 2012; Chelela *et al.,* 2014; Ekhlas *et al.,* 2018 and Ekhlas *et al.,* 2020). They represent an imperative part of the links in the food web, moreover, their major roles in the environment as pathogens and decomposers and are vital in forest and grassland ecosystems alike. Accordingly, countless are exceptional scavengers in the environment, decomposition dead animal and vegetable material into minimal compounds that turn out to be available to other members of the ecosystem (Webster and Weber, 2007; Rukaibaa et al 2017, and Marthad *et al.,* 2019). Furthermore, the growth of mushrooms in fields and woodlands is very noticeable in damp environments. Where the Baghdad governorate varied ecological conditions like altitude, temperature,

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edaphic factors, etc. Thus, the vegetation of Baghdad province is greatly diversified from its districts. These wide arrays of geomorphology, climatic variations, and vegetation structure make conducive for the luxuriant growth of macrofungi. (Feeney *et al.,* 2014) pointed out that the wild mushroom includes symbiotic, edible mycorrhizal and poisonous that just collected from the wild, where the lack of taxonomic studies on the species of wild mushroom restricted their additional exploitation. Usually, macro morphological characteristics provide further consistent taxonomic facts to delineate most of the species in the genus than micromorphology. However, (Tibuhwa *et al.,* 2010) stated that an analysis of more stable molecular characters is required for the more similar species. Recently in northern and western Iraq, a few kinds of research were carried out an investigation study for some species of wild mushroom, where (Aziz and Toma, 2012) stated an examination of 34 species belonging to 23 genera of basidiomycetes. As well as, (Toma *et al.,* 2013) found in Erbil Governorate of Kurdistan region 44 species of mushrooms belonging to 29 genera that collected and identified from different localities, Besides, 16 species of Basidiomycota, which belong to 16 genera were collected and identified from the same province mentioned previously (Toma *et al.,* 2018). Moreover, 12 genera and species were found, which collected from different localities in Hit city western Iraq (Owaid *et al*., (2014), and identified *Polyporu s*sp. from Fallujah state (Muslat and Owaid, 2015), mostly collected during the rainy season. The goal of this study is to gather a wild fungus, which grows naturally in various fields and gardens at Baghdad province ecological, thereafter identify those samples by using morphological characteristics and the similarity of relevant DNA sequences, as well as exam the specific screening to some enzymes activity. On the other hand, contribute to the overall knowledge of our fungi flora, in Iraq.

Materials and Methods

Survey study made to identify some mushrooms at two locations of Baghdad province, Iraq, during May/ 2017 to 2019 with the purpose of finding the mushrooms. The study area is located at western of Baghdad and city center respectively, namely the old location of agriculture college- Abu Ghurayb and Al-Salihia, Alawi al-Hillah district at 33.30° N and 44.15° E with an annual average rainfall of 150 mm and 32 meters elevation above the sea level (Fig. 1). However, these district climates either dry or semi-dry categorized by less than 100 mm of rain /year with extreme evaporation degrees (MOE, 2012). Furthermore, The collected mushroom was grown in late spring, from May to September at the end of the summer season; therefore little mushrooms could be growing until the fruiting stage with a climate similar to semi-desert (Owaid *et al.,* 2014).

Samples collection and characterization

Samples of wild mushrooms were collected from two sites, firstly Abu Ghurayb and secondly Al-Salihia (fields or public gardens and from trees) during May/ 2017 to 2019. According to Verbeken and Buyck (2002).

1. Twelve samples of mushroom fruit bodies were observed, then they are photographed and collected from their geographical location and kept in a box for storage until they reach laboratory for identification, while morphological (macro) features were applied for the wild mushrooms field observation and characterization. Morphologically characterized by mushrooms samples

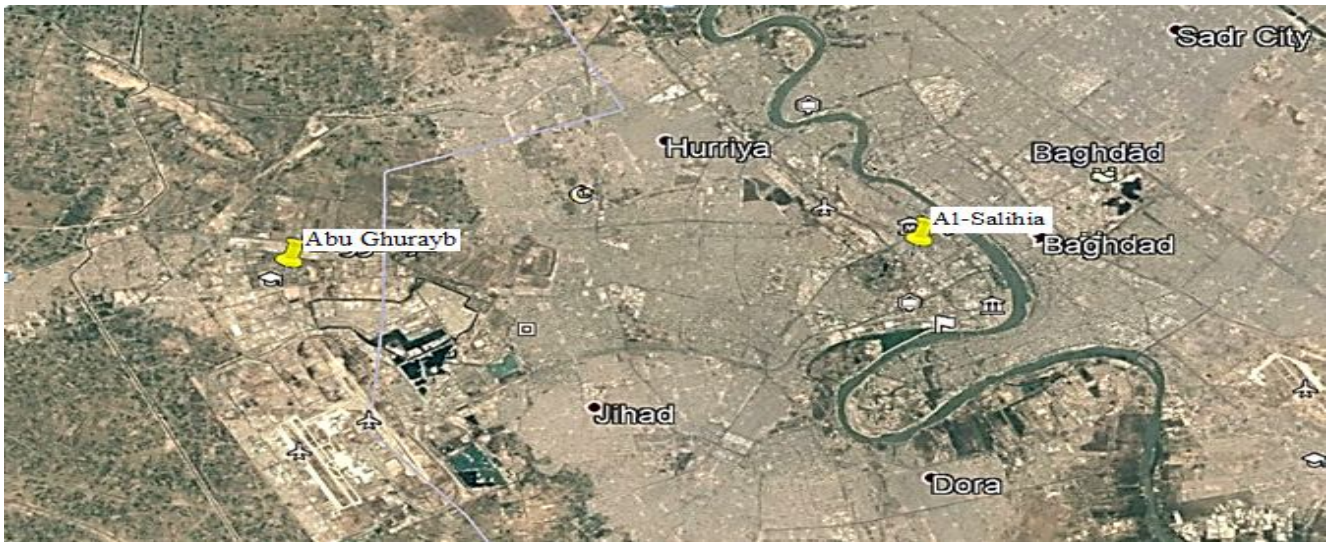

Fig. 1: Samples collected location.

were taken in situ., where colored field guide books, database photographs, monographs, and published work were used for macrofungi characterization u (Chelela *et al.,* 2015; Owaid *et al.,* 2014; Stamets, 1993; Tibuhwa, 2012; Tibuhwa and Kivaisi 2010). Moreover, the conventional description depended on features such as color, photograph stalk length, stalk diameter and cap diameter, ecological, and host substrate specificity.

2. Fruit bodies were taken to the fleshy mushroom lab. At the college of Agricultural Engineering Sciences of Baghdad University, for its subculture development to accomplish the fungi samples molecular diagnosis and specific screening to some enzymes. The macrofungi nomenclature was according to CABI bioscience databases (CABI, 2015) and (Kirk and Ansell, 1992) while Scientific names were also recognized by the 'Index fungorum'. Collected information data were summarized and shown in table 1.

Molecular diagnosis and Specific screening to enzymes activity

The fungi molecular diagnosis made by 18S with ITS1 and ITS4 primers usage.

Primer	Sequence
Forward	CCGTAGGTGAACCTGCGG
Reverse	CCTCCGCTTATTGATATGC

After PCR reaction samples results were electrophoresis on agarose gel 1.5% and send to Macrogene Co. Who given us nucleotides sequence for each isolate, this sequence inter in blast program to finds the similarity between sequences of the isolates, while all isolates recorded in NCBI.

The specific screening to some enzymes activity like Amylase, Protease, Xylanase, L-Asparaginase, Cellulase according to Ashutosh *et al.,* 2014 and Marthad *et al.,* 2020 as follows :

a. Amylase activity

Starch agar media was used with all mushroom mycelium to screened the extracellular amylase activity. where a 1% iodine solution was used to flood the noticeable amount of the growth in plates. A clear zone around the growth of mycelial was recorded for the activity of starch hydrolysis

b. Protease activity

The examination of protease producing organism was carried out using Gelatin agar media, in which center inoculation was done, plates were flooded with the reagent that containing 20% HCl and 15% $HgCl_2$ after the incubation of 10 days.

c. Xylanase activity

In order to the screening of Xylanase activity in mushroom mycelium, a medium containing xylan was used, where 0.1% Congo red was used to flood the noticeable amount of the growth in plates. Then, incubated for 30 minutes and washed with 1M NaCl, where it was observed a clear zone formation for the plates of xylanase enzyme production

d. L-Asparaginase activity

A 1% L-asparagine was used as a medium to screen of L- Asparaginase activity after flooding the mycelial growth plate with Nessler s reagent, where L-asparagine acted as an active ingredient. Plates showed pink coloration after the addition was recorded as extracellular L- asparaginase producer.

e. Cellulase activity

0.5% sodium salt of carboxymethyl cellulose was used as a medium for the tests, once the plates of mycelial colonization were flooded with 0.2% of congo red solution (0.2%), then they washed with 1M solution of NaCl followed by the incubation period of 15 minutes.

f. Lipase activity

The lipase activity was calculated according to (Slifkin, 2000) procedure, where it was prepared from the following ingredients (5g NaCl, 0.1g $CaCl_2$, and 10g peptone) in 1 L of distilled water. Subsequently, the pH was adjusted to 5.5 using 5ml Tween-80 and the medium was sterilized in an autoclave (121°C and under 15 lbs/ In^2 pressure) for 20 minutes, to detect the lipase production.

Results and Discussion

Results indicate that: Mushrooms samples collected were subjected in eleven Basidiomycota macrofungal species belonging to 3 genera (Pleurotus, Agaricus, and Pisolithus), 3 families (Pleurotaceae, Agaricaceae, and Sclerodermataceae) and 2 orders (Agaricales and Boletales). Our recorded fungal species are saprotrophic in habitats. They were appearing around seasons that due to the habitat of the mushroom genus, soil and climate of Baghdad district, and this district soil type is loam sand, which branch of the Euphrates River passes beside first location (old location of agriculture college) Abu Ghurayb and the Tiger River pass beside secondary location Al-Salihia. The climate of the Baghdad zone is extremely hot with lower humidity. Usually, rainfall occurs from December to April during the winter and spring months in most parts of Iraq, with 16°C as average day temperatures dropping at night to 2°C. In contrast, the weather is dry and hot to extremely hot during summer,

with an air temperature increases to a maximum of 45-50 °C during July and August, with a shade temperature is over 43°C yet dropping at night to 26°C (Jaradat, 2002).

Pleurotus spp.

Pleurotus spp. were classified primary decomposers, that meaning they grow on a wider array of substrates than species from many other groups, primordia will not form lower than 16-18°C (60-65° F) where high-temperature tolerant mushroom. Our collected samples were white color for *Pleurotus fuscus* (its photo was lost), overlaps between brown to yellow for Oyster mushroom *Pleurotus floridanus* and *Pleurotus ostreatus* was gray, sample photo No.1,2 and 7 respectively, were collected from Abu Ghurayb area table1, which were grown on a stalk of dead trees or near roots in two locations. The color of the Oyster mushrooms spans the rainbow: blue, white, brown, gray, pink, and golden. *Pleurotus ostreatus* is the most diverse varieties from temperate climates. (Stamets, 1993).The humidity, air temperature, compact material, and fresh air are considered as the main environmental factors that affect the stalk height, diameter and capsize in mushroom (AMGA, 2004). Their caps were wavy, convex to flattened, shaped somewhat like an oyster shell, white, gray, purple, and fleshy, while the stalk is smallest and usually off-center or may be absent. Oyster mushrooms grow in groups from the fallen logs or bark of trees (Telander, 2012).

Agaricus spp.

Agaricus spp. were classified as secondary decomposers, that meaning they need a selectively substrate to Grow (Stamets,1993). Agaricus genus belonging Agaricaceae family and Agaricales Order, Basidiomycota Phylum, Basidiomycetes Class, Fungi Kingdom. Our collected samples were divided into two groups, firstly from the Abu Ghurayb area samples Numbers. were 3, 4, 5and 6 respectively, and secondary from Al-Salihia location samples Numbers were 8, 9, 10, and 11 respectively. Their caps convex, rapidly flattening, flatten, or fibrillose, and they were called white buttons *that include light brown Agaricus nevoi*, dark brown *Agaricus subperonatus*,brown *Agaricus bisporus*, white *Agaricus bisporus*. As well as, white to brown *Agaricus heterocystic*, white to brownish *Agaricus bellannia*, white *Agaricus corocodilinus* and white *Agaricus litoralis*, photo samples No. 3,4,5,6,8,9,10, and 11 respectively, and their characteristics listed on Table 1. Finally, it grows on overfull earth, even throughout the asphalt, in parks, footpaths, nearby trees, and gardens in cities, frequently soiled with particles of earth. (Polese, 2005).

Pisolithus albus

Pisolithus albus a new record or maybe the first time collected for Iraq. This genus is belonging to the Fungi Kingdom, Basidiomycota Division, Agaricomycetes Class, Boletales Order, and Sclerodermataceae family. Sample of this fungi was found grown under *Eucalyptus* sp. tree, at Al-Salihia distract as egg-shaped matt with dark brown color, and it was content thickly dark brown powder.(photo,12).

The fungi molecular diagnosis made by 18S with ITS1 and ITS4 primers usage.After PCR reaction samples results were electrophoresis on agarose gel 1.5% and send to Macrogene Co. While Macrogene Co repeated us a nucleotides sequence for each isolate, this sequence inter in blast program to finds the similarity between sequences of the isolates (Fig. 5).

Result of PCR amplification of total genomic DNA of all fungal samples for this study recorded in NCBI at researchers' name, as a new isolate for those fungi, and recorded the first time in Baghdad province of Iraq. While Global items gave by NCBI shown in table 2 for the researcher's benefits.

Specific screening to some enzyme activity results

Pleurotus floridanus

Pleurotus ostreatus

Fig. 2: Photo of oyster mushroom fruit bodies.

Fig. 3: Photos of white button mushroom fruit bodies samples 3,4,5,6,8,9,10,11 respectively.

indicate that: The highest enzymatic efficacy at fungi mycelium was to cellulase enzyme from *Pleurotus floridanus*, *Agaricus crocodilinus*, *Agaricus litoralis* and *Pisolithus albus*, while highest enzymatic efficacy was to protease enzyme from *Agaricus bellanniae*,*Agaricus crocodilinus* and *Agaricus litoralis*. Whilst mycelium of all these species were not any efficacy to xylanase enzyme. On the other hand, the fungi tested appeared different efficacy to L-Asparaginase enzyme. The fungus which had a good activity for amylase were *Pleurotus ostreatus*, *Agaricus nevoi*, *Agaricus crocodilinus*, *Agaricus litoralis* and *Pisolithus albus*, for protease was *A. bisporus* (brown) and *Pisolithus albus*, for L-Asparaginase was only *Agaricus bellanniae*, for cellulase were *Pleurotus fuscus*, *Agaricus bellanniae* and *Agaricus subperonatus*. The fungus had a moderate activity for amylase were *Pleurotus floridanus*, *Agaricus bellanniae* and *Agaricus subperonatus*, where for protease were *Pleurotus floridanus*, *Agaricus arizonicus* and *Agaricus bitorquis* and for L-Asparaginase were *Agaricus crocodilinus* and *Agaricus litoralis*, for cellulase were *Agaricus heterocystic* and *Agaricus nevoi*, and for lipase were *Pleurotus fuscus*, *Pleurotus ostreatus*, *Agaricus heterocystic*, *Agaricus nevoi*, *Agaricus crocodilinus*, *Agaricus litoralis* and

Fig. 4: Photo of Pisolithus albus fruit bodies sample No.12.

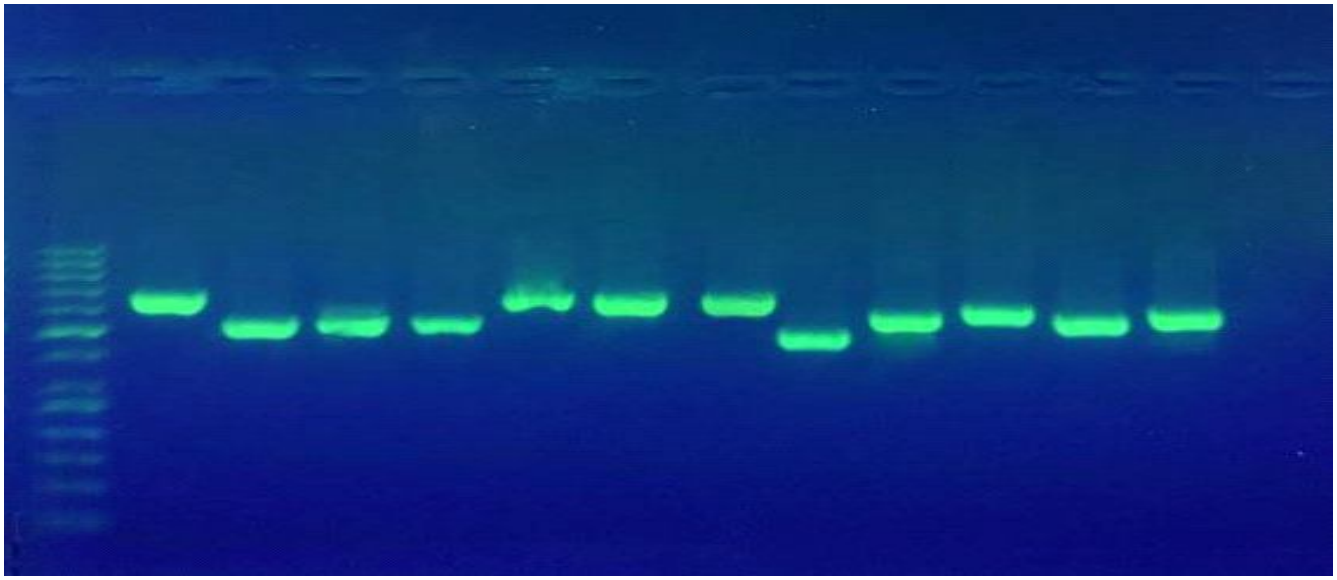

Fig. 5: RAPD profiles in different studied fungi samples (1-12) on agarose gel for amplification results by PCR method to gen rRNA 18S.

Table 1: Characterizations of Baghdad wild mushroom fruit bodies samples.

No.	Scientific name	Location	Fruit bodies Colour	Cap diameter	Stalk length	Stalk diameter
1	*Pleurotus fuscus*	Abu Ghurayb-Baghdad province Iraq	White	6-8 cm	3-5 cm	1.8 cm
2	*Pleurotus floridanus*		Brown to yellow	13.0 cm	6.0 cm	1.5 cm
3	*Agaricus nevoi*		Light-brown	2-5 cm	2.5cm	1.2cm
4	*Agaricus subperonatus*		Dark brown	6.0 cm	4.0 cm	1.7 cm
5	*Agaricus bisporus*		White	5.0 cm	4.0 cm	2.0 cm
6	*Agaricus bisporus*		Brown	9.0 cm	3.0 cm	1.8 cm
7	*Pleurotus ostreatus*		Gray	6.0 cm	3.5 cm	2.0 cm
8	*Agaricus heterocystic*	Al-Salihia Baghdad province Iraq	White to brown	4-6 cm	3-6 cm	1-0.8 cm
9	*Agaricus bellanniae*		White to brown	0.2-0.6 cm	3-5cm	0.5-0.8cm
10	*Agaricus crocodilinus*		White	3.0 cm	3.0 cm	1-1.1 cm
11	*Agaricus litoralis*		White	5.0 cm	2.0 cm	1.5 cm
12	*Pisolithus albus*		Egg-shaped with dark brown colour was content thickly dark brown powder.			

Table 2: Nucleotides numerous and Isolate items of wild fungi isolates after recorded in NCBI.

Isolate No.	Nucleotides numerous	Isolate name according blast program	Isolate item after recorder in NCBI	Isolate country name recorded in NCBI
R1	622	*Pleurotus fuscus*	MH443283	Abu Ghurayb-Baghdad province -Iraq
R2	210	*Pleurotus floridanus*	MK967460	
R3	624	*Agaricus nevoi*	MH443278	
R4	634	*Agaricus subperonatus*	MH443277	
R5	581	*Agaricus bisporus* (White)	MK967453	
R6	660	*Agaricus bisporus* (Brown)	MK967423	
R7	540	*Pleurotus ostreatus*	MF065715	
R8	542	*Agaricus heterocystic*	MG719865	Al-Salihia-Baghdad province - Iraq
R9	636	*Agaricus bellanniae*	MF987843	
R10	691	*Agaricus crocodilinus*	MK156342	
R11	684	*Agaricus litoralis*	MK156341	
R12	381	*Pisolithus albus*	MK967456	

Table 3: Shown extracellular enzymatic activity for wild fungi isolates mycelium.

No.	Species	Amylase	Protease	Xylanase	L-Asparaginase	Cellulase	Lipase
1	*Pleurotus fuscus*	+	+	-	-	+++	++
2	*Pleurotus ostreatu*	+++	+	-	+	++	++
3	*Pleurotus floridanus*	++	++	-	+	++++	+
4	*Agaricus bellanniae*	++	++++	-	+++	+++	+
5	*Agaricus heterocystic*	+	+	-	-	++	++
6	*Agaricus nevoi*	+++	+	-	+	++	++
7	*Agaricus subperonatus*	++	++	-	+	+++	+
8	*A. bisporus (white)*	_	+	-	-	+	+
9	*A. bisporus (brown)*	+	+++	-	-	+	+
10	*Agaricus crocodilinus*	+++	++++	-	++	++++	++
11	*Agaricus litoralis*	+++	++++	-	++	++++	++
12	*Pisolithus albus*	+++	+++	-	+	++++	++

Note: (++++): very good activity, (+++): good activity, (++): moderate activity, (+): poor activity, (-): No activity.

Pisolithus albus. While, fungi had poor enzyme activity for Amylase were *Pleurotus fuscus*, *Agaricus heterocystic* and *A.bisporus* (brown), for Protease were *Pleurotus fuscus*, *Pleurotus ostreatus*, *Agaricus heterocystic, Agaricus nevoi* and *A. bisporus* (white), for L-Asparaginase were *P. ostreatu*, *P. floridanus*, *Agaricus nevoi*, *Agaricus subperonatus* and *Pisolithus albus*, for cellulase were *A. bisporus* (white) and *A. bisporus* (brown), and for lipase were *Pleurotus floridanus*, *Agaricus bellanniae,Agaricus subperonatus*, *Agaricus bisporus* (white) and *Agaricus bisporus* (brown) respectively, (Table 3).

The present study indicates that enzymes activities of the fungi mycelium are dependent on the genus and species nature and confirms that *Pleurotus spp*. are primary decomposers and white-rot basidiomycete belongs to the sub-class of ligninolytic microorganisms that which generate, manganese, laccases, peroxidases, amylase, cellulase, protease and pectinase (Palmieri *et al.,* 2001; Rashad and Abdou 2001; Fan *et al.,* 2008; Abdou, 2003; Rashad *et al.,* 2009). Where *Agaricus spp.* are secondary decomposers required to the selective substrate, partially decomposed to grow (Volk and Ivors, 2001).

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