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Determination of optimum conditions for the production of the mother culture of the medicinal wild mushroom, *Agaricus bellanniae* isolated from hot Iraqi environment (Baghdad Governorate)

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ABSTRACT

The agricultural wild mushroom, *Agaricus bellanniae*, is one of the new fungi that have been discovered for the first time during July and August 2016 in Baghdad Governorate, Iraq. Due to the lack of research studies on this fungus globally, particularly in Iraq. This study is considered as the first research about determining the optimal conditions for the production of the fungus mother culture. *A. bellanniae* was done by creating a local culture medium suitable for developing wild and edible agricultural fungi. The study is concerned with two aspects, which are: isolation, purification, and diagnosis of the wild mushroom strain under the genus *Agaricus* to obtain a pure isolate characteristic of the fungus *A. bellanniae* registered in the NCBI (MF987843). The second aspect was studying the optimal conditions for preparing the mother culture from the wild mushroom. The study also examined the effect of some environmental factors on the fungus mycelium growth rates, such as the medium concentration and size, pH, and temperature. The results showed that the medium prepared from tree leaves powder achieved the best growth rates for *A. bellanniae*, with a growth rate of 0.77 cm day^{-1}, followed by other media. Also, the best concentration for preparing these media was shown to be 20 g L^{-1}. The best pH that can be adopted at the mycelium production stage for *A. bellanniae*, which achieved the highest growth rate, was at pH 6. The best temperature for the development of the agricultural mushroom, regardless of the media type, was at 30 ^{o}C.

Keywords: *Agaricus bellanniae* mushroom, Mk MF987843, Wild mushroom, Al-Salhiya district, Baghdad Governorate, *Cordia myxa* tree leaves.
Article type: Research Article.

INTRODUCTION

For over two thousand years, edible fungi have been used as food, and people accept to consume it due to their good taste and high nutritional value (Mizuno 2002). The *Agaricus* species are among the most desirable and cultivated species in many countries of the world (Abdul Qader *et al.* 2015; Mizuno 1989; Mizuno *et al.* 1990; Carris *et al.* 2012). In the past years, the production and consumption of agricultural mushrooms were increased. The production percentage of *Agaricus bisporus* took first place with 40% among the rest of the fungi species known worldwide. The fungi species became a desirable food and an alternative option for meat because of many reasons; its high nutritional value as it contains 20-40% protein - dry weight, as well as the similarity of its proteins to the animal meat proteins in terms of quality (Sabri *et al.* 2019; Sabri *et al.* 2020). Besides, it comes in third place after meat and eggs in terms of quantity. The fungi species are considered of high medical importance as it

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contains mineral salts, essential amino acids and vitamins (A, D, B_1, B_2, and B_3 (Heleno *et al.* 2010). Therefore, it is recommended by physicians for patients with heart disease, atherosclerosis, and high blood pressure, as it contains some substances that prevent the growth of cancerous tumours (Farhan *et al.* 2018; Farhan & Chechan 2020). Due to the increase in the world's population and the necessity to provide healthy food in Iraq, the wild agricultural fungi available in Iraq have been of interest because of their high nutritional value. Unfortunately, most of them have not been highlighted by scientific studies. Accordingly, a comprehensive survey was conducted for all the Iraqi governorates to identify the various types of these fungi, specifically the edible fungi types. This study focused on the most important fungi species under the genus *Agaricus* regarding the possibility of collecting, isolating, purifying, and diagnosing them specifically in Baghdad Governorate. Afterward, these species' pure mother culture could be produced with simple local capabilities to be an alternative to the imported mother culture from abroad in hard currency. To achieve this goal, local natural media that combines a low preparation cost and a low pathological micro-organisms infection medium should be an alternative to the traditional commercial media, via using agricultural residues that are available in the environment of Iraq, especially *Cordia myxa* leaves powder. *C. myxa* is one of the subtropical plants cultivated in the central and southern of Iraq, especially in Basra Governorate. It is considered as one of the evergreen trees (Fig. 1) with height reaches 5-7 m (Jamkhande *et al.* 2013; Farhan *et al.* 2020). The plant is known by different names, including *C. myxa*, makhet, dabag, benber, sebstan, megsas (Al Snafi 2016). The leaves and fruits of the *C. myxa* have been used in many different cultures. Its fruits have nutritional and medicinal values because of their excellent content of proteins, carbohydrates, vitamins, minerals, fibre, low fat, and antioxidant content that made it, play important roles as an anti-microorganism, anti-helminths, anti-intestinal infections, anti-diarrhea, anti-arthritis, anti-inflammatory, and a tonic for the liver (Inas *et al.* 2011; Pandey *et al.* 2014). Due to the content of leaves of such trees of phenolic compounds and flavonoids (Afzal *et al.* 2007). They are used in a few countries in wound treatment, ulcers, and getting rid of Trypanosoma (a parasite). The leaves of *C. myxa* are also a major source of Carotenoids attributed to the high antioxidant activity. This led its leaves to be considered one of the basic materials that can be used in the agricultural development field, specifically in the medicinal fungi field, by making it a ready food for the fungi growth, particularly *A. bellanniae*. This food can be used especially in the first stage of production when the mother culture is prepared. The success of the entire production process is linked to the success of this stage. The use of the *C. myxa* leaves could reduce the costs involved with preparing these cultures compared to the traditional method based on the imported commercial medium in hard currency. This type of rare fungus, *A. bellanniae*, was not subjected to such kinds of studies previously.

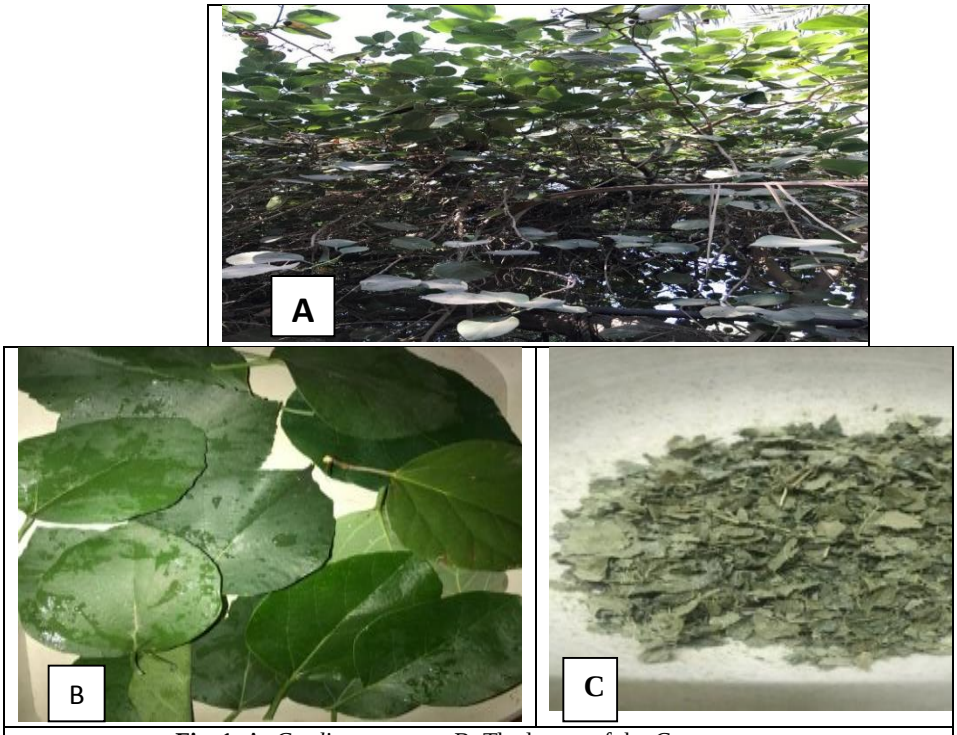


Fig. 1. A: *Cordia myxa* tree; B: The leaves of the *C. myxa* tree;
C: Dried *C. myxa* leaves.

MATERIALS AND METHODS

Samples collection

The study was conducted on the wild agricultural mushroom, belonging to the genus *Agaricus*. The mushroom was collected from the Al-Salhiya area (Baghdad Governorate, Iraq) during July and August 2016 (Figs. 2-3; Chechan *et al.* 2020). The isolation and purification processes have been done in the fungi laboratory and the laboratories of the Food Sciences Department, College of Agricultural Engineering Sciences using the PDA medium (prepared by dissolving 39 g L^{-1} distilled water). This medium was also used to activate and preserve the isolates for comparison.

Fig. 2. Map shows the collection of wild agricultural mushroom, *A. bellanniae* from Al-Salhiya area (Baghdad Governorate, Iraq).

Fig. 3. The wild agricultural mushroom, *A. bellanniae.*

Wild mushroom diagnosis

A. Morphological diagnosis

Morphologically characterized mushroom samples were taken *in situ.* Macrofungi were characterized using coloured field guide books, photographs, monographs, and published work (Stamets & Chilton 1993; Tibuhwa & Kivaisi 2010; Tibuhwa 2012; Owaid *et al.* 2014; Chelela *et al*. 2015; Muslat *et al.* 2020) as well as databases. Conventional characterization was based on the features such as photographs, colour, stalk length, stalk diameter and cap diameter, ecological and host substrate specificity.

B. Molecular identification of a wild mushroom

DNA extraction was done using the equipment listed below:

ZR Fungal / Bacterial DNA MiniPrep ™, Catalogue No. D6005. PreMix was used to duplicate the diagnostic gene ITS4, and the following primers were added (Muslat *et al.* 2020):

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	TCCGTAGGTGAACCTGCGG	63	63 %	650 base pair
Reverse	TCCTCCGCTTATTGATATGC	56	45 %	

The following program was chosen for gene amplification.

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	94 °C	3 min	
2-	Denaturation 2	94 °C	45 sec	35 cycles
3-	Annealing	60 °C	45 sec	
4-	Extension 1	72 °C	45 sec	
5-	Extension 2	72 °C	10 min	
6-	Cooling	4	∞	

Mother culture production

Fruiting bodies of the diagnosed and registered wild white fungus *A. bellanniae* of the strain RWK_2017 were used to produce a pure mother culture using tissue culture in petri dishes containing potato dextrose agar (PDA). Afterward, the petri dishes were transferred to an incubator at a degree of 25 ± 1 °C for three weeks with continuous monitoring to observe the growth of fungal yarn and to exclude the contaminated petri dishes (Chechan *et al.* 2017).

Determining the optimal conditions for the production of the mother culture

The fungus isolations, which was purified from the previous step, were grown on two types of media. The first type was the commercial medium, potato dextrose agar; this was used to stimulate and preserve isolates and compare them. The second type was a group of natural materials from the Iraqi environment, including oats, wheat, barley, corn cobs, broad beans, potato peel, and the *C. myxa* leaf powder. These natural materials were subjected to a set of experiments to determine the optimal conditions for the production of the mother culture of fungus; experiments were done as follow:

Examining the effect of the medium type on the growth rate of fungi

The natural media were dried in an oven at 60 °C, milled well, and distributed into plastic bags. Then, PDA and the natural media were prepared at a concentration of 40 g L^{-1} distilled water. Agar was added to the natural media at a concentration of 15 g L^{-1}. The media components were mixed well, their pH was set at 6.5, and sterilized in an autoclave at 121 °C for 15 min. Thereafter, the media was cooled to 55 °C and distributed in petri dishes with a diameter of 8.5 cm (Mizuno 2002). The prepared dishes were inoculated with a piece of activated mushroom growth on the PDA centre; that piece was placed in the centre of the plate. The dishes were sealed with parafilm. All the steps were carried out in sterile conditions inside vaccination cabins. The dishes were incubated at 25 °C. The fungal growth was monitored until its full growth in the plate, and the growth rate was calculated as follows:

Growth rate (cm / day) = x / y
X= the plate diameter of 8.5 cm
Y= the time required for the fungal growth to reach the plate edge or for the plate to fill with growth (Chechan 2020).

Examining the effect of the medium concentration on the growth rate of fungi

Natural media were prepared using the flour of oats, wheat, barley, corn cobs, broad beans, and potato peel, and *C. myxa* leaf powder at the concentrations of 40, 30, 20, and 10 g L^{-1} distilled water. Agar was added to it at a concentration of 15 g L^{-1} of water and the pH was adjusted to 6.5. The media was sterilized in an autoclave at 121 °C for 15 min. It was cooled and poured into petri dishes 8.5 cm in diameter. The plates were inoculated with a

piece of fungus growth, and the dishes were incubated at 25 °C. The fungal yarn's growth was monitored until its full growth on the plate, and then the growth rate was calculated.

Examining the effect of the pH on the growth rate of fungi:
The effect of pH numbers (5, 5.5, 6, 6.5, and 7) on the growth rate of fungi were studied using flours of oats, wheat, barley, corn cobs, broad beans, and potato peel, and *C. myxa* leaf powder at a concentrations of 20 g L^{-1} distilled water. Then, agar (15 g L^{-1}) was added to distilled water. The pH numbers of the media were adjusted to the abovementioned values. The media were sterilized in an autoclave at 121 °C for 15 min. Afterward, the media were cooled to about 55 °C, distributed in plates, and then the media was inoculated. The growth of mycelium was checked until the plate was full, and then the growth rate was calculated.

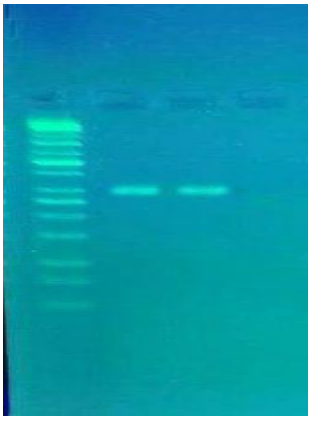
Examining the effect of incubation temperature on the growth rate of fungi
The effect of temperatures of 20, 25, 30, 35, and 40 °C on the growth rate of the studied fungi were examined, and PDA medium was used for comparison. The natural media was prepared at a concentration of 20 g L^{-1} distilled water, and 15 g agar/L distilled water was added to it. The pH of the natural media was set to 6. The dishes were inoculated and incubated at the abovementioned temperatures. The fungal yarn growth was checked until the plate was full and the growth rate was calculated.

Estimation of the mycelium density of *A. bellanniae*
The mushrooms were developed in natural media in ideal conditions, including the flour of oats, wheat, barley, corn cobs, broad beans, and potato peel, and *C. myxa* leaf powder by preparing media at a concentration of 20 g L^{-1} and adjusting the pH at 6 and incubating them at 25 ºC, until the growth was completed in the aforementioned media separately. The fungal yarn was scraped from the media surfaces and transferred to a clean, dry and pre-weighed crucible to estimate the weight of the yarn in a sensitive balance, which counted a value that expresses the growth intensity as in the following equation:

Growth density (g / plate) = y – x.
where x = weight of the Crucible+ the weight of the Mycelium
Y = Weight of the Crucible that is empty.

RESULTS AND DISCUSSION

Isolates diagnosis for the fungus selected
The fungus *Agaricus bellanniae* belongs to *Basidi* fungi of the order *Agaricales* belonging to the family *Agaricaceae*, which is included in the edible fungi. It is classified from the thrush fungi, which grows in late summer or early autumn among weeds in the form of pieces, arcs, or gin rings, and is distributed in the great eastern plains of Illinois in America (Muslat *et al.* 2020). This type is characterized by its short, convex, bell-shaped hat at the beginning, and then it widens later to become flat with an inverted convex into the inside of the hat from the middle at maturity stage. The hat diameter is 3.5-8 cm; it contains a scale with yellowish-brown colour, unlike the colour of the hat, which is distinguished by its colour pale brown. The centre of the hat is in a dark colour and begins to fluctuate in colour upon maturity. The edge of the hat is soft with no bumps. The nostrils are free at the leg region and are short and congested at the beginning of being white, and then they become pink at the heart of the hat. The stalk ranges between 3.5-7 cm and a diameter of 5-12 mm, and it is similar and begins to widen at the base. The stalk becomes brown when it gets older and sometimes pink when bumps appear at the base. The flesh is white and unchanged when cut, and the spores are dark brown. This diagnosis relied on the gene ITS4 gene. Firstly, the fungus genetic material was extracted and checked for purity, and then amplified by PCR using the abovementioned gene. One package appeared, representing the whole genome extracted from the fungus. ITS4 gene amplification was done using the mentioned primers and the prepared program for this purpose. Fig. 4 shows gel electrophoresis of DNA extracted from fungal isolates; a single bind appeared at the site 600 - 700 bases and in two replicates (Chechan *et al.* 2020). This bind was sent to Macrogene Co., South Korea to show the sequence of nitrogenous bases. Upon analysing these results in the BLAST program, it was found that the isolate under study was related to *A. bellanniae*, and the isolate matched with an isolate that had been registered in the GenBank with the code NR145001.1. The strain under-study was recorded in the GenBank with the searchers' names as a new isolate of *A. bellanniae* and was given the international code MF987843. Note that this strain of fungus is being diagnosed for the first time in Iraq and the Middle East.



M F1F2

Fig. 4. Electrophoresis of the amplification product of the ITS4 gene showing the location of the segment between the molecular weight 600-700 nitrogenous bases.

Table 1 and Fig. 5 show the locations of the fungus *A. bellanniae* in the countries of the world, and the absence of any strain of this fungus was previously recorded either in Iraq or in the Middle East region. However, it was only recorded in the United States of America through three diagnostic studies in Northern America. It was coded in the US National Centre for Biotechnology Information (NCBI) with the code NR145001.1, KJ877783.1, and KJ877782.1 (Mizuno 2002), in addition to other similar types recorded in Canada, France, Ukraine, and China.

Table 1. Compatibility ratio for nitrogenous bases for the isolate *Agaricus bellanniae* RWK_2017 with ten isolates registered in the GenBank.

No.	Accession	Country	Source	Compatibility
1	ID: NR_145001.1	USA: Pennsylvania	*Agaricus bellanniae*	96%
2	ID: KJ877782.1	USA: Pennsylvania	*Agaricus bellanniae*	96%
3	ID: KJ877783.1	USA: Illinois	*Agaricus bellanniae*	96%
4	ID: JF727867.1	France	*Agaricus litoralis*	94%
5	ID: JF797190.1	France	*Agaricus lanipes*	94%
6	ID: KT951353.1	China	*Agaricuslitoraloides*	93%
7	ID: AY484686.1	USA	*Agaricus macrocarpus*	93%
8	ID: KJ847463.1	Canada: Ontario	*Agaricus crocodilinus*	93%
9	ID: AJ884640.1	Ukraine	*Agaricus romagnesii*	93%
10	ID: KF937296.1	Colombia	*Agaricus campestris*	93%

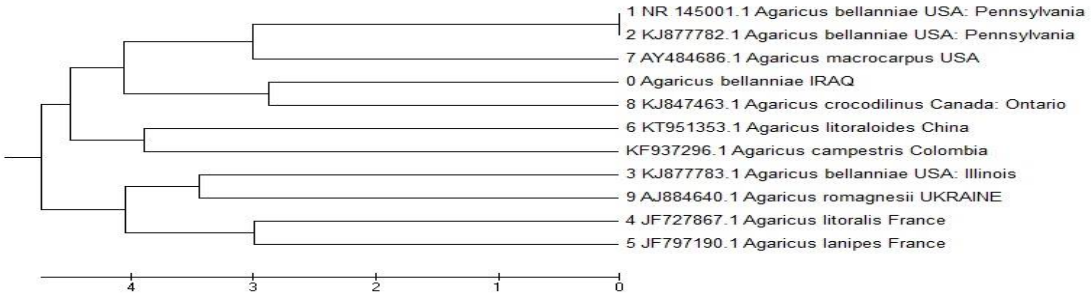


Fig. 5. Genetic relationship tree of a local isolate of the fungus understudy with several fungous strains of the same genus in the NCBI GenBank.

Determining the optimum conditions for preparing the mother culture of *A. bellanniae*

The effect of the medium type on the growth rate of *A. bellanniae*

It is obvious from Fig. 6 that the medium prepared from the *Cordia myxa* leaf powder, achieved the best growth rates for the studied *A. bellanniae*. The growth rate of the fungus in the centre of the leaves of the *C. myxa* plant was 0.566 cm day^{-1} compared to 0.404 cm day^{-1} for the commercial medium PDA, followed by the other media: 0.472, 0.472, 0.472, 0.386, 0.472, 0.386 and 0.566 for the centre of the flour of *C. myxa* respectively. Accordingly, the prepared media from oats, wheat, barley, sorghum, legumes, potato peelings, and *Cordia myxa* leaves were used in later stages of study with PDA medium for comparison. The increase in growth rates on the centre of the *C. myxa* leaves is due to the nutritional effect of the medium, which was prepared from it because of its necessary active compounds for growth and easily absorbed materials. The reason for this fact is that the effective absorption capacity of these nutrients is greater than their molecular synthesis energy when fed with the *C. myxa* leaf extract after completing the incubation phase and before the fruiting bodies of *A. bellanniae* are formed. This process enables them to use more energy to increase the growth rates of these fungi. Also, the studies of Al-Snafi (2016) showed that *C. myxa* leaves contain phenolic compounds and flavonoids, which made them inducible media to increase growth rates compared to other natural media not containing these substances. According to the current study, this large number of chemicals and food in the *C. myxa* leaf powder could be a ready food for agricultural mushrooms, which are considered as medicinal leaves, and also *A. bellanniae* as a medicinal fungus. As a result, when medicinal mushroom fed on the components of a medicinal plant, the concentration of the chemical compound that has medicinal and therapeutic importance in the edible fungus *A. bellanniae* may be increased, noting that the effect of the powder of the *C. myxa* leaves on the growth and production of *A. bellanniae* or any other fungi has not been studied previously. Poluboyarinov *et al.* (2005) reported that the growth rate of the fungal yarn *A. bisporus* increased by 4.2 mm day^{-1} compared to PDA at a rate of 3.5 mm day^{-1} by adding selenium compounds at a concentration of 10 g L^{-1} to the nutrient medium. Dodileva & Korpatch (1989) also reported that the concentration of organic compounds in media of wool powder or animal skins reflected positively on mycelium's growth rates. The period required for the growth completion on the plate with a diameter of 9 cm, decreased from 5 to 4 days compared to the growth completion in PDA. Dodileva (1985) also indicated that using media with boiled concentrated plant residues, with agar, beef bones powder, and fish powder, surpassed PDA regarding the growth rate. The growth rate was 10 mm day^{-1} compared to 8.6 mm day^{-1} exhibiting a difference of two days from the period of completion of growth on a plate. Elias (2008) also reported that oat extract reduced the average duration of growth of the *A. bisporus* from 18.1 days to 13.5 days compared to PDA, while the growth rate of the fungus itself increased from 5.5 mm day^{-1} to 6.7 mm day^{-1}.

Hoa & Wan (2015) found that PDA and YDA (Yam- dextrose agar; the yam is a vegetable crop resemble potatoes) are suitable media for the growth of all species of the genus *Pleurotus* due to containing polysaccharides and proteins. It also showed that sweet potato-dextrose, yeast extract, and yam-dextrose - were all suitable for the growth of the fungus *Pleurotus ostreatus*.

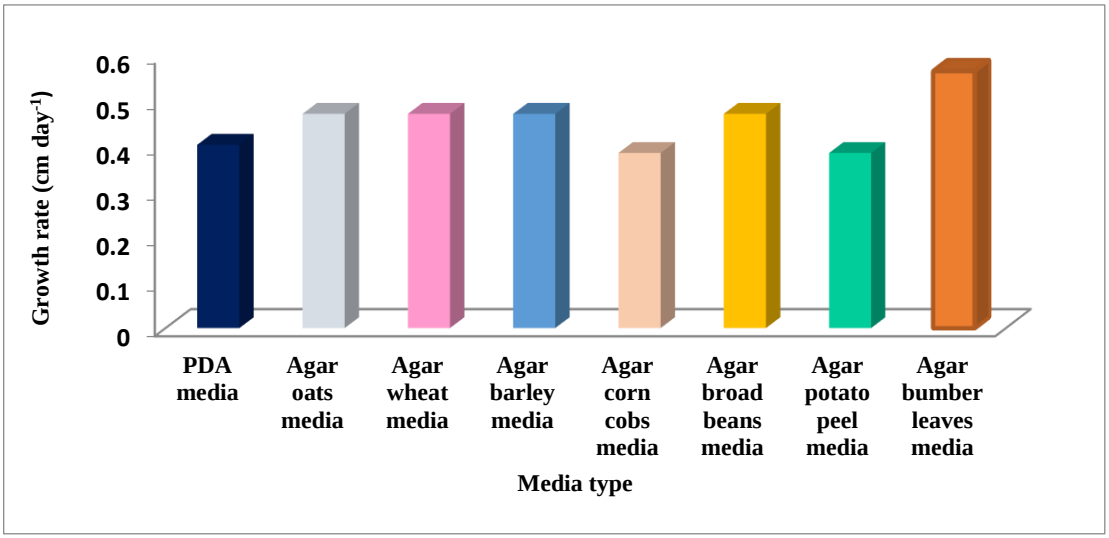


Fig. 6. The effect of the medium type on the growth rate of the wild mushroom, *A. bellanniae* with a concentration of 40 g L^{-1}, pH 6.5 and temperature 25 °C.

The effect of medium concentration on the growth rate of *A. bellanniae*

Fig. 7 shows the effect of different concentrations from natural media represented by the flour of oats, wheat, barley, corn cobs, broad beans, and potato peel, and the *C. myxa* leaf powder which ranged between 10-40 g L^{-1} on the growth rate of the wild agricultural fungus, *A. bellanniae*. The best growth rate of the fungus was achieved when the concentrations of the seven media mentioned above were 20 g L^{-1}, reaching 0.531, 0.531, 0.531, 0.425 0.531, 0.404, 0.607 cm day^{-1}, respectively, while decreased to 0.472, 0.472, 0.472, 0.326, 0.472, 0.354 and 0.531 cm day^{-1}, at 10 g L^{-1} respectively. Therefore, a concentration of 20 g L^{-1} was adopted to prepare these media in the following stages of the study.

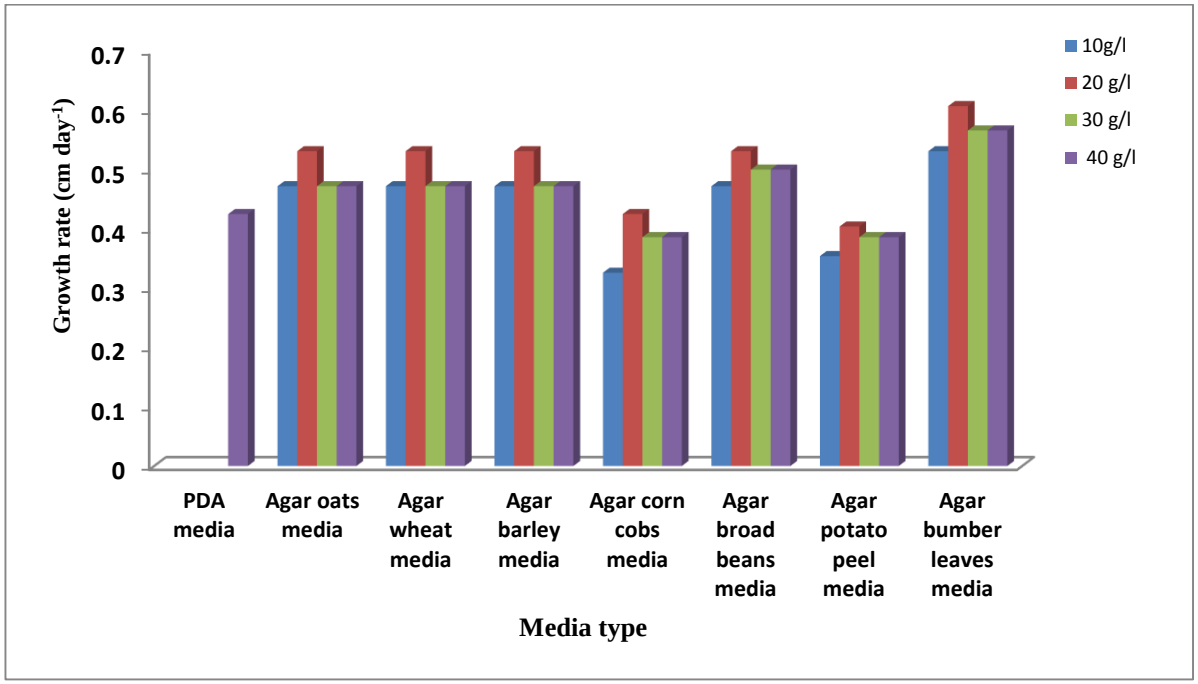


Fig. 7. The effect of medium concentration on the growth rate of the wild mushroom, *A. bellanniae* at a pH of 6.5 and a temperature of 25 °C.

Effect of pH on the growth rate of *A. bellanniae*

Fig. 8 shows the effect of the pH on the growth rate of the wild mushroom *A. bellanniae* in the natural media at a concentration of 20 g L^{-1} and a temperature of 25 ºC. PDA was used for comparison. It has been proven through this experiment that the food fungus under study was highly sensitive to the change of the pH of the media in which it grew, especially in the stages of mycelium formation. It is also found that the best pH that can be adopted at the stage of mycelium preparing was 6, noting that the growth rate decreased when raising the pH to 6.5 compared to lowering the pH from 5.

The best growth rate of mushrooms was achieved on the seven selected natural media represented by the flour of oats, wheat, barley, corn cobs, broad beans, and potato peel, and the *C. myxa* leaf powder at concentrations of 20 g L^{-1} and pH 6. The growth rates at the mentioned factors were 0.654, 0425, 0.566, 0.425, 0.531, 0531 and 0.53 cm day^{-1} respectively. At pH 6.5, the growth rates decreased to 0.607, 0.404, 0.531, 0.425, 0.531, 0.531, 0.53 and 0.425 cm day^{-1}, respectively, while the growth rates at pH 5 reached 0.531, 0.386, 0.425. 0.354, 0.34, 0.326, 0.327 and 0.327 cm day^{-1}. For the following stages of the study, the media were prepared at PH 6. The previous studies reported differences in determining the optimal pH of fungi at the stage of fungal growth. Gabriel (2004) indicated that the optimum pH for mycelium growth for *Pleurotus* sp. was 6.5, and mycelium growth completely stopped when the pH was 4. The PH value of over 6.5 may help accelerate the formation of mycelium, however, the fruiting bodies would be deformed or abnormal.

Siwulski *et al.* (2011) reported that the optimal pH for the mycelium growth of *Mycogone perniciosa* was 5.5, while that of *Verticillium fungicola* was 6.5. The reason behind the differences in growth rates according to different pH levels was attributed to the effect of free hydrogen ions on the functioning of the cytoplasmic membranes, enzymes activity, the readiness of nutrients in the medium, and the mechanism of their transport to the cell.

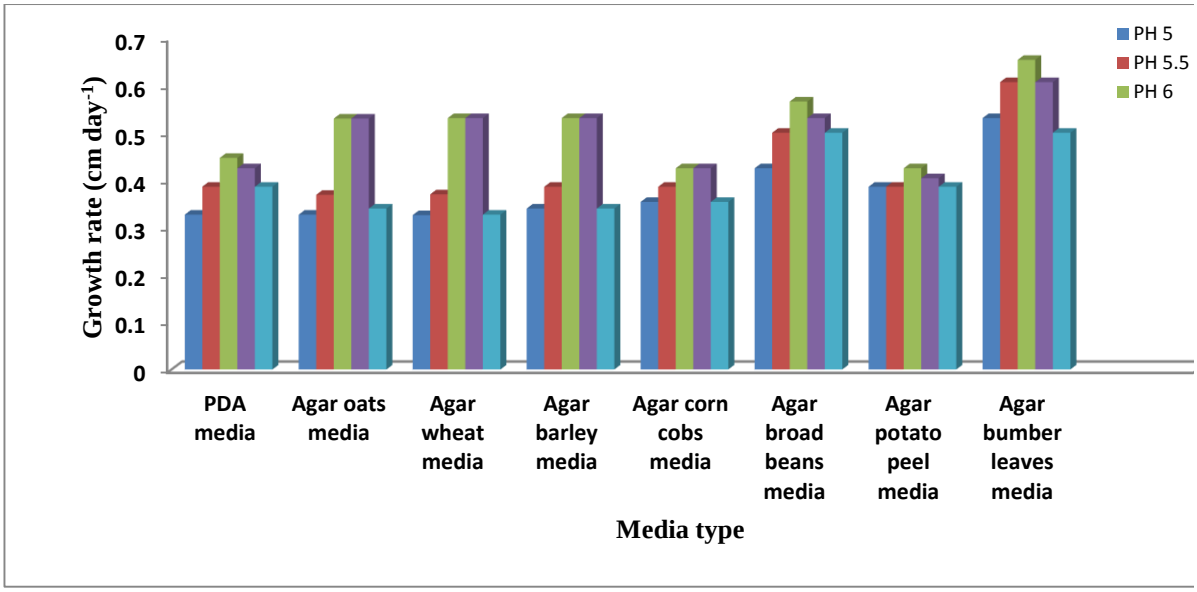


Fig. 8. The effect of the pH on the growth rate of wild mushrooms, *A. bellanniae* in natural media at a concentration of 20 g L^{-1} and a temperature of 25 ° C.

The effect of temperature on the growth rate of *A. bellanniae*

Fig. 9 shows the effect of temperature on the growth rate of *A. bellanniae*. Four temperature degrees were used in this study including 25, 30, 35 and 40 ºC. The natural media were pollinated with the fungus at the abovementioned temperatures and then were incubated. The growth rate of oyster fungi for all the natural media showed consistency at the abovementioned temperatures in general. The best temperature for the development of these fungi was 30 °C regardless of the medium type. The fungi growth rate in this study reached 0.77 cm day^{-1} per day at the centre of the *C. myxa* leaves and 0.425 cm day^{-1} on potato peel powder. It was observed during the study that any deviation from 30 °C upon the development of this type of fungi causes significant decrease in the growth rate in all the natural media.

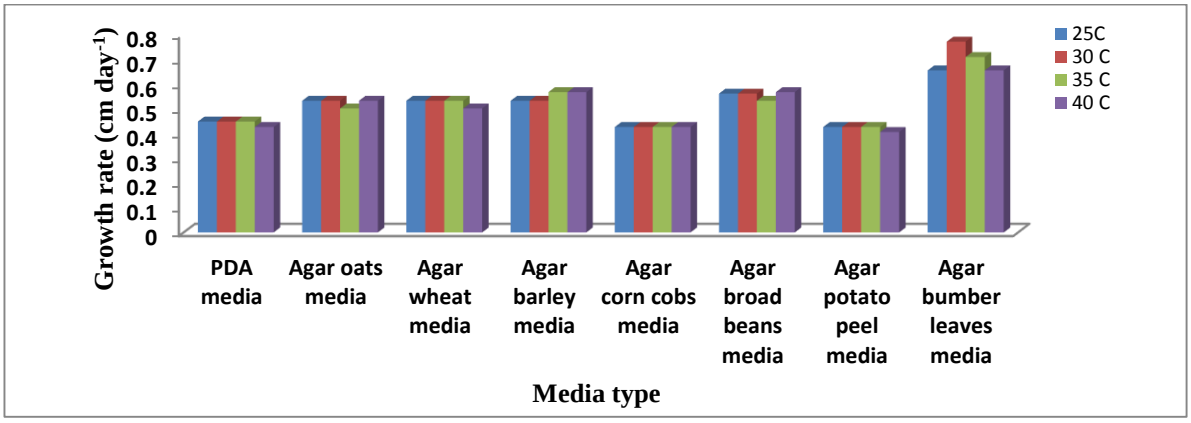


Fig. 9. The effect of temperature on the growth rate of the wild fungi, *A. bellanniae* on natural media at a concentration of 20 g L^{-1} and pH 6.

It was observed during the study that the growth of *A. bellanniae* on the selected seven natural media, represented by the flour of oats, wheat, barley, corn cobs, broad beans, potato peel, and the *C. myxa* leaf powders were not only distinguished by the speed of their growth estimated in cm day^{-1} which was done by measuring the mycelium elongation in the colonies but instead is characterized by its high density compared to PDA. The fungus mycelium density in the media as mentioned above was estimated depending on the yarn's weight growing in the media after the end of the incubation period specified for the fungus. According to Figs. 10 and 11, significant differences were observed at the level of 1% in fungal mycelium growth density. *C. myxa* leaf powder agar was the best media

in giving the highest density level, reached 1.66 g. At the same time, potato peel powder agar gave the lowest density (0.33 g). This is due to the contents of the *C. myxa* leaf agar including a large number of chemicals and food stuffs that can be considered as prepared food for the edible fungi under study. This result indicates the degree of suitability of *C. myxa* leaf powder agar medium in the production of mycelium from wild agricultural mushrooms and growth rate indication as well. The speed of growth and its intensity will provide an economic benefit for agricultural mushroom producers in reducing the time factor in terms of the number of tissue pieces that can be obtained from growing the fungus in these two media (*C. myxa* leaf and potato peel powder agars).

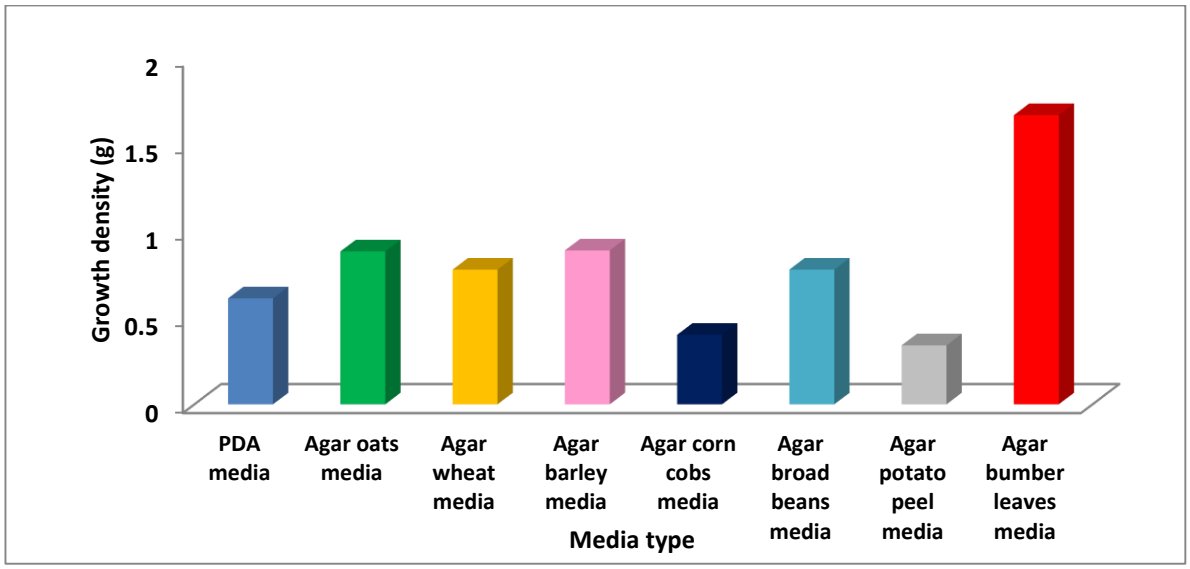
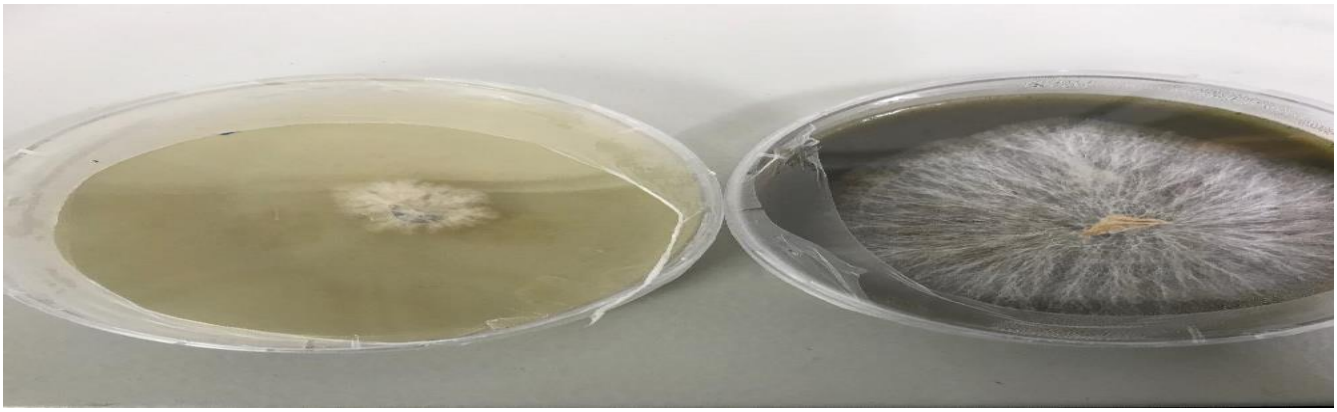


Fig. 10. The density of the wild fungus, *A. bellanniae* in natural media estimated on mycelium weight compared to PDA.



Commercial medium (PDA) **Nature media prepared from *C. myxa* leaf powder**

Fig. 11. Mycelium growth for the strain *A. bellanniae* on PDA and natural media at optimum conditions for the production of the mother culture at a concentration of 20 g L^{-1}, 30 ºC , and pH 6.

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