

Egyptian Journal of Botany http://ejbo.journals.ekb.eg/



Effect of Some Agricultural Substrates on Production Efficiency of Lentinula edodes (OM432157) and Evaluation of its Vitamins Content

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> THE local strain Lentinula edodes (OM432157), which was discovered for the first time I in the Iraqi environment, was cultivated in the laboratory using nine treatments based on sawdust as the main substrate. A comparative study was done on the nutritional value of the local strain L. edodes (OM432157) in the wild and the cultivated one. It was observed that the highest growth rate of the fungus strain under study was in T8 (1.8 cm) and the lowest growth rate in T5 1.1 (cm). Fungal tissue growth in treatments (T4, and T5) were required (29 days), while in treatment T8 it was required (26 days), and in other remaining treatments it was accomplished in 25 days. The time required for pin heads to form in T5 was faster (17 days), followed by T8 (18 days) and T9 (19 days). The water-soluble vitamins (C, B1, B2) and the fat-soluble vitamins (E, K, D, A) were analyzed by HPLC. It was found that the cultivated mushrooms were a good source of vitamin B2 (6.9mg/100g dw) while wild mushrooms were a source of vitamin B1 (33.3mg/100g dw). The local strain of the cultivated and wild shiitake mushrooms also contained all fat-soluble vitamins, and the highest content of vitamin A was recorded in the cultivated type (19.9mg/100g dw) and the wild (103mg/100g.dw). These results are the first in Iraq, through which we found that the local strain L. edodes (OM432157), has high nutritional value and can be considered ideal supplements for many low-vitamin food items in our diets.

> Keywords: Agricultural waste, Fat-soluble vitamins, Lentinula edodes, Water-soluble vitamins.

Introduction

Mushrooms have been part of the human diet since ancient times. There are more than 2,000 edible species (Kalač, 2016; Li et al., 2021), but only 25 species are widely accepted as daily food worldwide (Valverde et al., 2015; Farhan et al., 2020a). Recently, several species of edible mushrooms have been considered functional foods for possessing enormous nutritional and medicinal values (Kalač, 2013; Haro et al., 2020; Marçal et al. 2021; Ali et al., 2022). Edible mushrooms are collected from their natural environment and have been consumed by humans throughout history. However, especially after the second half of the twentieth century, mushroom cultivation became popular as the number of wild mushrooms began to decrease due to deteriorating environmental conditions and loss of natural resources, so mushrooms cultivated would provide not only food security for developing countries but also sustainable and integrated food systems (Marshall & Nair, 2009; Farhan et al., 2020b).

An important factor in the overall nutritional value of a food is its vitamin content. Vitamins are essential nutrients that must be provided to the body regularly to perform various chemical and physiological functions in the human body (Plozza et al., 2012; Rukaibaa et al., 2020). They are widely distributed in natural food sources and can be easily introduced into diets to meet daily needs. Vitamins are involved in normal metabolic processes and cell regulation and are essential for growth and

[#]Corresponding author email: sunlife88201@yahoo.com Received 22/10/2022; Accepted 15/03/2023 DOI: 10.21608/ejbo.2023.170207.2176 Edited by: Prof. Dr. Neveen M. Khalil, Faculty of Science, Cairo University, Giza 12613, Egypt. ©2023 National Information and Documentation Center (NIDOC)

development. Thus, they are chemicals that the body needs to stay healthy (Glavinic et al., 2017; Zhang et al., 2018). Thirteen types of vitamins play an important role in human nutrition (Eggersdorfer et al., 2012). Based on their solubility, these vitamins can be divided into fat-soluble vitamins and water-soluble vitamins (Waldenstedt, 2006).

Lentinula edodes is an edible mushroom (L. edodes) belonging to the Omphalotaceae family, and is also known as Shiitake and is the second most common edible mushroom globally (Sheng et al., 2021). Shiitake mushrooms are rich in various types of vitamins and are a good source of the B complex. It contains high concentrations of niacin (B3), folate (B9), and riboflavin (B2), while thiamine (B1) and cobalamin (B12) are present in trace amounts. It also contains vitamin C and fatsoluble vitamins (A, E, D). Mushrooms are the only non-animal source of vitamin D (Guillamón et al., 2010; Rathore et al., 2017; Valverde et al., 2015; Rukaibaa et al., 2017).

Due to the increasing demand for consumption of shiitake mushrooms, there is a need for data on their nutritional value, as there are several factors that may affect its nutritional contents significantly, such as differences in strain, substrate, environmental conditions, cultivation, and fruiting conditions, mushroom growth stage, and age of the fresh mushroom sample (da Silva, 2016; Siwulski et al., 2020). Vitamins are nutrients that the body of the organism needs, and they are not manufactured inside and have a vital effect (Gökmen et al., 2016)). However, information regarding the contents of vitamins in the shiitake mushroom L. edodes that grows in the Iraqi environment is almost missing or scarce. Therefore, the aim of this study was the sustainable production of the local strain of Shiitake mushroom L. edodes (OM432157) using the widely available agricultural waste in Iraq. Quantitative and qualitative determination of vitamins in the local strain of wild and cultured shiitake L. edodes was done to provide reliable information about this important food source.

Materials and Methods

Reagents

The solvents used as an analytical reagent in HPLC such as acetonitrile and methanol were obtained from Tedia Company, USA. Standard vitamins are obtained from Sigma Chemical Co. (Poole, Dorset).

Egypt. J. Bot. 63, No.3 (2023)

Sample collection

Samples of fruit bodies were obtained from the shiitake mushroom belonging to the strain Lentinula edodes (OM432157), an Iraqi strain obtained in the wild from areas on the outskirts of the capital Baghdad during January and February of 2021. This isolate was recorded for the first time in Iraq. A series of experiments were conducted that included the morphology of the fungus under study. The phenotypic diagnosis of the isolate was confirmed by PCR technique. The DNA chain amplification reaction of the local isolate of the fungus was carried out by using the forward primer ITS1 and the reverse primer ITS4. The product sequence was deposited in the Genbank of the National Center for Biotechnology Information (NCBI).

Cultivation of the strain L. edodes (OM432157)

The strain L. edodes (OM432157) was cultured in the laboratory according to the method carried out by Küçükomuzlu & Pekşen (2005). Nine substrates were prepared based on sawdust as a substrate (Table 1), then the substrates were packed in gaspermeable bags suitable for mushroom production and moistened to a moisture content of 65% using distilled water, sterilized in an autoclave at 121°C for 15h. After sterilization, the substrates were inoculated by sowing 3% of the substrate, during the incubation period the media inside the bags was moistened at a rate of 50-60% and incubated in a normal humidity environment at 24 °C until the mycelium growth was fully completed, while in the fruiting stage, the surrounding humidity was raised to the level 90-95%. Mushroom fruits were obtained and then harvested and dried in a hot air dryer at 40±1°C (Gürgen et al., 2020), which was subsequently used to evaluate their vitamin content.

Standard solutions for vitamins

Vitamin standard stock solutions were prepared according to the method described in the research (Aslam et al., 2008; Ringling & Rychlik, 2013). The standard stock solution (1mg/mL) for each standard vitamin was prepared separately by dissolving 10mg of the standard vitamins individually in 10mL of methanol and stored in the dark at 4°C. Daily buffer solutions were prepared from the stock solution by serial dilution to concentrations of 0.1, 1, 2, 5, and 10mg per liter, respectively. The standard solution was injected into the HPLC, and the peak areas were determined to generate standard curves.

Treat- ments	Substrates		
T1	White sawdust + wheat bran		
T2	Red sawdust + wheat bran		
Т3	Bark mulch + wheat bran		
T4	White sawdust + wheat bran + rice bowl		
T5	Red sawdust + wheat bran + rice bowl		
T6	Sawdust + cane + wheat bran + rice bowl		
T7	Sawdust + cane + wheat bran + rice husk + champagne		
T8	Sawdust + cane + wheat bran + rice husk + champagne		
Т9	Sawdust + corn cobs + wheat bran		

TABLE 1. Types of treatments used in mushroom cultivation

Determination water-soluble vitamins

The vitamin B group was extracted according to the (AOAC, 1990) method. Mushroom powder (2g) was placed in 25mL of H_2SO_4 (0.1N) solution and incubated for 30min at 121°C. Then adjust the pH of the sample to 6.5 and 4.5 using 2.5M of sodium acetate adding acetate Taka diastase enzyme and storing the preparation at 35°C until the next day. The mixture was then filtered through a Whatman No. 4 filter, diluted the filtrate with 50 mL of pure water, and filtered again through a millipore filter (0.45µm). Twenty µL of the filtrate was injected into the HPLC and then the vitamin B content was quantified against the vitamin B standards.

Vitamin C was extracted according to the method described by Babarinde & Fabunmi (2009) with some modifications. Mushroom powder (10g) was mixed and homogenized using an extraction buffer containing metaphosphoric acid (0.3M) and acetic acid (1.4M). The mixture was placed in a conical flask and shaken at 10,000 rpm for 15min. The mixture was then filtered through a Whatman filter No. 4. Vitamin C was then determined by a titration method by preparing an ascorbic acid standard by dissolving 100 mg of L-ascorbic acid in a solution of metaphosphoric acid (0.3M) / acetic acid (1.4M) at a final concentration of 0.1mg/mL.

Determination of fat-soluble vitamins (D, E, K, A)

Mushroom powder (10g) was mixed with 1g of pyro Gallic acid, 70mL of ethanol, and 30mL (50%) of KOH, stirred, and re-condensed for 40 min using a water bath ($50\pm2^{\circ}$ C) (Jun et al.,

2007; Aumaporn., 2009). Then extracted three times using different concentrations of ether (50mL, 30mL, and 20mL). Double-distilled water was used to neutralize the extract that was dried using anhydrous sodium sulfate. Furthermore, the extract was concentrated to about 5mL using a water bath (50 \pm 2°C), diluted to 10mL with methanol, filtered using a 0.45µm membrane, and the sample was ready for HPLC analysis. Twenty µL of samples were directly injected into an HPLC column. The fat-soluble vitamins were identified by comparing their retention periods with those of the original standards.

Statistical Analysis

The statistical program Statistical Analysis System -SAS (2018) was used in data analysis to study the effect of different coefficients on the studied traits according to a complete randomized design (CRD-Completely Randomized Design), one way or two ways according to the factors studied, and the significant differences between the averages were compared by testing the least significant difference (Least Significant Difference-LSD).

Results and Discussion

Molecular identification

The PCR technique was used to diagnose the selected local isolate from the genus *Lentinula*. The DNA was isolated from the mycelium of the fungus under study. The sequences of the nitrogenous bases of the amplification products were determined after they were sent to Macrogen. The results, after being analyzed by the BLAST program at the National Center for Biotechnology Information and compared with the available information, showed that there is a match with a percentage of 99% between this isolate and the strains of the fungus belonging to the species *edodes*, accordingly, the isolate under study returned to the fungus *Lentinula edodes*, and the accession number was OM432157.

Mushroom cultivation

a) Daily growth rate

Nine treatments were tested using different types of media. Results in Table 2 showed that treatment T8 recorded the highest daily growth rate for shiitake strain, which amounted to 1.8 cm/day, followed by treatments T7, and T3 with a growth rate of 1.5 cm/day, then treatments T1, T2, and T4 with a growth rate 1.4 cm/day, and treatment T6 with a growth rate 1.3cm/day, while

treatments T9, T5 recorded the lowest growth rate compared to the rest of the treatments, reaching 1.1cm/day (Fig. 1). These results are consistent with that reported by López-Rodríguez et al. (2008), who reported growth values ranging from 0.76 - 1.12cm per day.

TABLE 2. Daily growth rate of shiitake mushroomstrain on culture media and for differenttreatments

Treatments	Growth rate (cm/day)
T1	1.4 a
T2	1.4 a
Т3	1.5 a
T4	1.4 a
T5	1.1 b
T6	1.3 a
Τ7	1.5 a
Т8	1.8 c
Т9	1.1 b

Different letters mean statistically significant differences among the samples. The significance level was set as $(P \le 0.05)$



Fig.1. The daily growth rate of the local shiitake mushroom strain according to the nine treatments

b) Incubation stage

The results shown in Table 3 indicate that the growth of the fungal tissue in the two treatments T4 and T5 required a higher incubation period than the remaining treatments, which amounted to 29 days, followed by treatment T8, where the growth of the fungal tissue in this treatment required 26 days, while the growth of the fungal tissue was completed in the remaining treatments within 25 days. Ayodele & Okhuoya (2007) indicated that the reinforcement of the basic substrates (sawdust) with agricultural and industrial residues can

enhance the fungal growth and reduce the period required to complete growth.

TABLE 3.	The	incubat	ion	stag	e requir	ed to co	omp	lete
	the	growth	of	the	fungal	tissue	on	the
	med	lium for	the	nin	e differe	ent trea	tme	ents

Treatments	Incubation period(days)
T1	25 a
T2	25 a
Т3	25 a
T4	29 b
T5	29 b
T6	25 a
Τ7	25 a
Т8	26 a
Т9	25 a

Different letters mean statistically significant differences among the samples. The significance level was set as ($P \le 0.05$)

c) Pin formation stage

Table 4 shows the beginning of the emergence of primary sprouts (pins) in the fifth treatment T5 during 17 days and this is evidence of its efficiency, followed by treatment T8 in a period of 18 days and the ninth treatment T9 during 19 days. As for the remaining treatments, the pins formation was delayed up to 26 days in T7, 25 days in T2 and T4, 28 days in T6, and 33 days in T1. Several studies point to the fact that the chemical and physical properties of the substrates affect the nature of mushroom production and affect the growth of the fungus (Suwannarach et al., 2022), and thus the formation of pins in a shorter period (Onyango et al., 2011).

TABLE 4. Number of days required for the beginning of the emergence of the primary buds (the stage of the pin)

Treatments	Pins formation stage (in days)
T1	33 a
T2	25 b
Т3	22 b
T4	25 b
T5	17 c
Т6	28 d
Τ7	26 d
Т8	18 c
Т9	19 c

Different letters mean statistically significant differences among the samples. The significance level was set as $(P \leq 0.05)$

d) Fruiting stage

Based on the results of the previous stage, three treatments (T5, T8, and T9) were selected according to their high efficiency of growth during the incubation stage and the formation of pins. Figure 2 summarizes the results, which indicate the formation of fruiting bodies in a typical growth period (7 days) in treatments T5 and T9, while the production period in the eighth treatment was 9 days (Fig. 3). The result is consistent with the characteristics of L. edodes fungus since it can produce enzymes such as laccase and carboxymethylcellulose, which hydrolyze lignin, cellulose, and hemicellulose, converting them into simple sugars used for their growth (Gomes-da-Costa et al., 2008; Siwulski et al., 2020). The difference in the production of edible mushrooms in different culture media may be due to the abilities of these media to supply the edible mushrooms with nutritional and environmental requirements, due to their different contents of cellulose, hemicellulose, lignin, and other compounds (Moonmoon et al., 2011; De Andrade et al., 2013; Abdullah et al., 2022). Most studies indicated that the efficiency of mushroom production is closely related to the nutritional composition of the growing substrate (Hoa et al., 2015; Sassine et al., 2021) and the type of supplement added (Parola et al., 2017). The results of our study are in line with the findings of Kumar et al. (2022) who found that adding any amount of additive to the substrate showed an improvement in the quality and yield of mushrooms.

Quantitative and qualitative analysis of vitamins in the local strain L. edodes (OM432157) cultivated and wild type

Determination water-soluble vitamins The results shown in Table 5 are the qualitative measurement of the water-soluble vitamins C, B1, B2 of the local strain of shiitake mushroom (OM432157) *L. edodes* grown in the laboratory (Fig. 4) and compared with the wild of the same strain (Fig. 5).

Our results showed significant contents of vitamins B2 and B1 in the strain of wild and cultivated shiitake mushrooms (Table 5). The cultivated mushrooms are a good source of vitamin B2 (6.9mg/100g DW) and constituted 98% of the total water-soluble vitamins. This percentage is higher than that generally found in vegetables, cheese, and eggs, and higher than what Mattila (2001) obtained (0.15mg/100g.dw). While it contained somewhat lower amounts of Thymine (vitamin B1, 0.09mg/100g.dw) and vitamin C (0.04mg/100g.dw) (Table 5, Fig. 5). This result was very similar to the values recorded for vitamins in previous research (Mattila, 2001; Caglarirmak, 2007).

When comparing these results with the original wild mushrooms (that were used for comparison), we found that wild mushrooms, unlike cultivated mushrooms, are a good source of vitamin B1 and recorded the highest content of this vitamin, reached to 33.3mg/100g.dw and constituted 80% of the total soluble vitamins in the water. While its content of vitamin B2 was moderately reached 7.7mg/100g.dw and constituted 18% of the total water-soluble vitamins. In general, the values of B1 and B2 for cultivated and wild mushrooms in the current research were higher than the values recorded in previous research by Mattila (2001) and Ribeiro et al. (2009). We note that both types of mushrooms recorded the lowest level of vitamin C, which was 0.2mg/100g.dw in wild mushrooms and 0.04mg/100g.dw in cultivated mushrooms (Table 5 and Figs. 4, 5).



Fig. 2. Number of days required for the formation of fruit bodies on the media of the four treatments



Fig. 3. The different stages of cultivation of the local breed *L. edodes* (OM432157) (a-Bag cultivation stage, b-Fungal tissue growth stage, c- Pins formation stage, d- Pins elongation stage)

TABLE 5. Comparison of the content of the local strain L	L. edodes (OM432157) cultivated and wild mushrooms of
water-soluble vitamins (µg / 100g)	

Vitamins	Wild mushroom mg/ 100g	Locally cultivated mushroom (OM432157) <i>L. edodes</i> mg/100g
Vitamin C	0.28 a (0.67%)	0.04 b (0.56 %)
Vitamin B2 Riboflavin	7.73 c (18.74%)	6.93 d (98.01%)
Vitamin B1 Thymine	33.21 e (80.54%)	0.09 f (1.27 %)
Total	41.23 g	7.07 h

The different letters along the rows mean that there are significant differences between wild and cultivated mushrooms. Values were compared using a significance level ($P \le 0.05$).







Fig. 5. Quantitative and qualitative analysis of water-soluble vitamins in the local strain *L. edodes* (OM432157) of wild shiitake mushrooms

According to Mattila (2001), there is disagreement about vitamin C contents and therefore some research studies have not mentioned vitamin C values; While other studies reported very high values, ranging from 14.68mg/100g dw to 25mg/100g dw (Mattila et al., 2002; Caglarirmak, 2007).

Determination of fat-soluble

Figures 6, 7 and Table 6 summarize the percentage of fat-soluble vitamins, which include vitamins (E, D3, K, A). Studies often indicate that edible mushrooms contain fewer fat-soluble vitamins than water-soluble vitamins (Heleno et al., 2012; Naeem et al., 2020). Several biological functions in the body have been associated with fat-soluble vitamins (Lounder et al., 2017; Makarova et al., 2017). In the current study, it was observed that the local strain of L. edodes (OM432157) cultivated mushroom recorded the highest content of vitamin A, which amounted to 19.9mg/100g.dw and constituted about 75% of the total fat-soluble vitamins, thus similar to wild L. edodes (OM432157), which recorded the highest content of vitamin A (103mg/100g.dw) and constituted 62% of the total vitamins. Very low levels of vitamin A have been reported in previous studies (<3.1mg/100g dry weight) and are often below the quantitative limit (Yang et al., 2002). The quantities of vitamin A that we find in the wild and cultivated local breed are much higher than the vitamin content in animal and vegetable food known to contain a large amount of vitamin A, including butter (0.59mg/100g), cheese (0.39mg/100g), eggs (0.28), salmon (0.041mg/100 g) and milk (0.04mg/100g) (Souci et al., 2000).

Since vitamin E has antioxidant properties (Selvi et al., 2007), its production may be higher when mushrooms are under stress conditions. Therefore, the controlled production of mushrooms (cultivated) may not allow the production of large amounts of tocopherol, as well as the sensitivity of vitamin E to heat and light. Therefore, we found that wild L. edodes (OM432157) outperformed the cultivated mushrooms and contained a higher percentage of vitamin E (55.6mg/100g) and constituted 34% compared to the cultivated mushrooms (3.08mg/100g.dw) and formed (12%) of the total fat-soluble vitamins. There is a dearth of information about the vitamin E content in edible wild mushroom varieties. However, the values obtained in this study are higher than the previous available studies indicating a significant decrease in the vitamin content in mushrooms (Afiukwa et al., 2013),

Studies have proven the importance of vitamin K as a vital and functional component that possesses antioxidant capabilities (Rahman et al., 2018), and it is an essential cofactor in the synthesis of blood clotting factors, and its deficiency leads to bleeding. Our findings in this study indicate that the content of the cultured mushrooms of vitamin K was 1.8mg/100g.dw versus 4.4mg/100g.dw for the wild mushrooms.



Fig. 6. Quantitative and qualitative analysis of fat-soluble vitamins in the local strain L. edodes (OM432157)



Fig. 7. Quantitative and qualitative analysis of fat-soluble vitamins in the cultivated local strain L. edodes (OM432157)

TABLE 6. Comparison of the content of the local strain *L. edodes* (OM432157) cultivated and wild of fat-soluble vitamins (µg/100g)

Vitamins	Wild mushroom mg/100g	Locally cultivated mushroom (OM432157) <i>L. edodes</i> mg/100g
Vitamin E	55.55 a (33.74%)	3.08 b (12.08%)
Vitamin D3	1.00 c (0.60 %)	0.65 c (2.55%)
Vitamin K	4.45 d (2.70%)	1.84 e (7.22%)
Vitamin A	103.61 f (62.94%)	19.91 g (78.13%)
Total	164.61 h	25.48 i

The different letters along the rows mean that there are significant differences between wild and cultivated mushrooms. Values were compared using significance level ($P \le 0.05$)

There are two main types of vitamin D they are vitamin D2, which is made by plants and not produced by the human body, and vitamin D3, which can be taken from animal sources. Most studies on shiitake content focused on vitamin D from type D2 and did not focus on type D3, despite its biological and medical importance (Mattila, 2001; Dimopoulou et al., 2022). Therefore, in this research, we investigated the amount of vitamin D3 in the local strain of the cultivated shiitake mushroom and compared it with the wild and found that both types of the cultivated and wild L. edodes strain (OM432157) contained similar levels of vitamin D3 which amounted to 1.0mg/100g.dw and 0.6mg/100g.dw, respectively (Table 6 and Figs. 6, 7).

Conclusion

In the current study, we focused on cultivating the local strain L. edodes (OM432157) in the laboratory which was obtained from the Iraqi environment and was discovered for the first time in Iraq, then determining the water- and fat-soluble vitamins in the local strain L. edodes (OM432157) wild and cultivated in the laboratory. This is due to the absence of previous studies on the wild strains that grow in the Iraqi environment and their comparison with those grown in the laboratory. The experimental results proved that the three treatments that were evaluated are suitable for the production of the local strain of shiitake mushroom. This local strain is an excellent source of vitamins such as vitamin A, which is rarely detected in mushrooms, as well as vitamins K and E, in addition to vitamins B1, B2, C, and D. The presence of vitamins in the human diet is very important. Therefore, the obtained data shed light on the potential of the local strain L. edodes (OM432157) wild and cultivated as a source of food compounds, and contribute to raising awareness for preserving Iraqi wild fungi resources and developing their production technology.

Competing interests The authors report no conflicts of interest regarding this work.

Authors' contributions: Ekhlas M. Farhan, Proposed the idea of this study, material preparation, Collection, isolation, identification and registration of a local strain *Lentinula edodes* in NCBI, Cultivation of the strain *L. edodes*, designing the experimental work, interpreting the data, and writing the manuscript. Rukaibaa A. Chechan; supervised and review the manuscript. All authors read and approved the manuscript.

Ethics approval: Not applicable

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تاثير الركيزه الزراعية على كفاءة انتاج السلالة المحلية لفطر الشيتاكي L. edodes (OM432157) في المختبر وتقييم محتواه من الفيتامينات القابلة للذوبان في الماء والقابلة للذوبان في الدهون

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تم زراعة السلالة المحلية (CM432157) L. edodes (OM432157) البرية المكتشفة لاول مرة في البيئة العراقية في المختبر باستخدام تسعة معاملات على أساس نشارة الخشب كركيز و اساسية . مع اجراء دراسة مقارنة حول القيمة الغذائية للسلالة المحلية (OM432157) L. edodes (البرية و المزروعة في المختبر . لوحظ ان اعلى معدل نمو للفظر في T1 المرية و المزروعة في المختبر . لوحظ ان اعلى معدل نمو لسلالة الفظر قيد الدراسة في T3) 18. سم(واقل معدل نمو للفطر في T5) سم(. تطلب نمو النسيج الفطري في ما 11 (روعة في المختبر . لوحظ ان اعلى معدل نمو لسلالة الفطر قيد الدراسة في T8) 18. سم(واقل معدل نمو للفطر في T5 11) سمر . تطلب نمو النسيج الفطري في المعاملات (T4, T5) (29 يوم) ، وفي المعاملة T8 (20 يوم)، في حين تطلب نمو النسيج الفطري في المعاملات (T4, T5) (29 يوم) ، وفي المعاملة T8 (20 يوم)، في حين تطلب نمو النسيج الفطري في المعاملات (T4, T5) (29 يوم) ، وفي المعاملة T8 (20 يوم)، في حين تطلب نمو النسيج الفطري في المعاملات (T4, T5) (29 يوم) ، وفي المعاملة T6 (20 يوم)، في حين تطلب نمو النسيج الفطري في المعاملات (T4, T5) (29 يوم) ، وفي المعاملة T6 (20 يوم)، في حين تطلب نمو النسيج الفطري في المعاملات (T4, T5) (29 يوم) ، وفي المعاملة T6 (20 يوم)، في حين كان الفقين الوقت اللازم لتكون رؤوس الدبوس في T5 أسرع بشكل ملحوظ الفلوري في المعاملات الباقية 25 يوم). ولي النيتامينات الذائبة بالماء (B1, B2) و النويتامينات القابلة للذوبان في الدهون (C, B1, B2) و السرة مصدر الفيتامينات الذائبة بالماء (B1, B2). كما احتوت القابلة للذوبان في الدهون (C, B1, B2) و السري مصدر الفيتامينات القابلة الذوبان في الدهون، وسجلت اعلى السلالة المحلية للدوبان في الدهون (C, B1, B2) و السري (D9, D9, D9) و السرو ع (D9, D9, D9) و البري (D9, D9) و السري وصدن الفيتامينات القابلة الذوبان في الدهون، وسجلت اعلى محتوى من فيتامينات القابلة الدوبان في الدهون، وسجلت اعلى السلالة المحلية و الدهون (D9, D9, D9) و السري (D9, D9, D9) و السري (D9, D9) و السري وصدي الفرر البري (D9, D9) و النوع المزروع (D9, D9, D9) و البري (D9, D9) و البري (D9, D9) و النوع المزروع (D9, D9, D9) و البري (D9, D9) و النوع المزروع (D9, D9, D9) و العروى و (D9, D9) و البرول (D9, D9) و و و و و0) و العروى (D9, D9) و و و و و