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ORIGINAL ARTICLE

THE ROLE OF LOCALLY PRODUCED CASING MATERIALS AND EXTRACTS COMPARED TO IMPORTED AND THEIR EFFECT ON THE PRODUCTIVE QUALITIES AND BIO-EFFICIENCY OF WHITE BUTTON MUSHROOM (*AGARICUS BISPORUS*)

Hosham Kareem Ibrahim AL-Dulayme[1]*, Idham Ali Abed[2] and Dhafer Fakri Al-Rawi[1]

[1]College of Education for pure Science-University of Anbar, Iraq.
[2]Center of Desert Studies-University of Anbar, Iraq
E-mail : hoshankareem9@gmail.com

Abstract: The experiment was carried out in cooperation between the Faculty of Education for Pure Sciences, Department of Biology and the Center for Desert Studies - University of Anbar to investigate the role of locally prepared casing materials and extracts and their effect on the productive qualities and biological efficiency of white button mushroom *Agaricus bisporus*. In this study, 4 types of Casing soil assimilates were used. They are : T1 used as a control agent (imported soil) and T2 prepared locally from reed peat, palm roots, coal, limestone, dolomite, T3 composed of river sand, peat moss and T4 consisting of spent mushroom substrate, river sand and peat moss and 4 types of extracts organic (t2), mineral extract (t3), organic and mineral mixture (t4). T2 casing soil was characterized by the highest total fruit yield rate 1n stages (1, 2, 3 and 4) amount to 48.771 kg m^2 and the highest biological efficiency rate reached 72.253% with blend T2t2 and highest cap diameter of 47.97 mm compared to other treatments, While the highest cap rate of 19.96, 19.54 and 19.36 mm was achieved with T4, T3 and T2 titration coefficients respectively. The highest rate of stem diameter was 10.76, 10.56 and 10.15 mm achieved with T1, T2 and T4 respectively. It was found that the t2 extract gave the highest rate of cap diameter, thickness 52.26 and 21.08 mm, respectively, and the highest rate of fruiting bodies production at 2, 3 and 4 reached 31.000 kg -2 compared with other extracts. It was also found that the use of t4 and t2 extracts gave the highest rate of leg diameter of 10.65 and 10.64 mm respectively. Due to the significant results achieved by local treatment T2 when compared with the imported treatment T1 used as control, it is recommended to prepare them for use as a successful alternative for the purpose of reducing the cost of producing domestic mushrooms.

Keywords: Casing layers, Extracts, Productive traits of fleshy fungi, *A. bisporus*

1. Introduction

A. bisporus occupies the first place among the world's most important edible mushrooms in terms of economic importance, as it accounts for 80% of other edible mushroom is cultivated [Elias (2008)]. The fleshy fungus has been used as food sources for humans since ancient times, as it is eaten by humans for its delicious taste and nutritional value, as well as being of high economic importance in addition to their rapid life cycle and the possibility of producing it throughout the year. It is a rich source of proteins containing all essential amino acids that are important to a human body [Owaid *et al.* (2017)]. Its proteins resemble animal meat proteins in quality and rank third after meat and eggs in quantity [Atila *et al.* (2017)]. *A. bisporus* returns to the heterotrophic organisms. It does not make its own food and gets it applied to organic materials and tissues, which is equipped by agriculture media called it the compost. This medium plays an essential role in the production of edible mushrooms [Rashid (2019)]. *A. bisporus* cultivation requires the coverage of compost after the growth of the fungal mycelium is completed by using the casing layer as a prerequisite for the formation of the fruit bodies, and should have a high ability to absorb and retain water, It is characterized by good dissociation strength and high gas exchange capacity, pH between 6.8 and 7.5 [Rashid *et al.* (2018a)], and it plays an important role in maintaining the colonized mycelium-

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derived compost from dehydration and isolating it from direct contact with the outer ocean [Jubair and Abdel Hadi (2013)]. Failure to select suitable Casing soil leads to failure to form the fruit bodies [Noble *et al*. (2003)]. The process of producing mushrooms in Iraq is still on a limited scale because of the lack of permanent local sources of casing soil and importation in hard currency leads to increased production costs. Due to the importance of *A. bisporus* food, health and environmental importance and the importance of casing soil in the quantity and quality of production so it is necessary to study the possibility of providing local coverage soil with good quality and low cost, in addition to study the possibility of renewing the components of the compost as a result of depletion after the second harvest by addition of biodegradable materials with low density and high porosity to try to control production conditions and late cycles of production.

2. Materials and Methods

The *Agaricus bisporus* cultivation included some stages:

First : Preparation of the Compost

The Compost attended at the Center for Desert Studies-Anbar University, which included two steps depending on Bahi (1984).

Phase 1 (fermentation): This phase began to moisten and mix the components of wheat straw (550 - 500 kg), chicken manure (300 kg), calcium sulphate $CaSO_4$ (40 kg), and urea (5 kg) collected and organized in the form of stacked length of 170 cm and height 120 cm and width of 120 cm to maintain the activity of microorganisms necessary for the analysis of the components of the medium and continued fermentation 23 days, on the day 24 the compost is transferred to the pasteurization.

Phase 2 (Pasteurization and Softening compost for pollination): After the fermentation process has been completed, the composts were transferred to pasteurization room equipped with a hot water steam pump. The pasteurization process takes 8 days. On the first day, the temperature of the components of compost was raised to 60-58m for 6 hours and then reduced by 2°C every day. On the seventh day, it was reduced to 48°C. The room was fully ventilated until the temperature is reached to 25-30°C, after then compost was ready for pollination [Hassan *et al*. (2002)].

Second: Spawn production

A. bisporus A15 was imported from Al-Wadq Company for Mushroom Production in Baghdad / Iraq. The fungal pollination was prepared according to Oei (2005) method with some modifications by developing the mycelium on the sterile wheat seeds, boiling the seeds in boiling water for 15 minutes with flipping continuous, Remove excess water and spreading the seeds on a clean cloth. Add 5g of calcium carbonate ($CaCO_3$) and 10g of calcium sulphate ($CaSO_4$) per kilogram of cereals based on wet weight [Elias (2008)]. The seeds were distributed in clean bottles capacity 1 liter reality 250g per bottle and then sterilized in the Autoclave and left to cool and pollination with pieces Agar contains mycelium mushrooms A. bisporus A15, Mix the contents of the bottle well to ensure distribution and incubate at 25°C for 21 days with shake once a week, and save after mycelium colonization at 4°C until use [Qaisi *et al*. (2016)].

Third: Agriculture Spawning

The pollination was added between the layers of compost by a percentage of 1.52-%. The mixture was homogenized after then placed in a square Plastic bags measuring 60 cm length 40 cm width, 20 cm high by 20 kg per bag, incubated at 25°C and humidity up to 85% until the emergence of the mycelium and its spread over the whole medium of agriculture [Alalaf (2012)].

Fourth: Preparation of the casing layer and its addition

Four types of casing soil for the purpose of the production process prepared are given below:

1- T1 Soil coverage Bvb Substrate Dutch origin, component 90% Black Peat and 10% CaCO3 pH between 7.0 to 7.6. This soil was prepared in cooperation with Al-Wadq for Fungal Production, Baghdad, Iraq, and was used directly and 100% as a comparative treatment without sterilization.

2- T2 was attended from a mixture of materials representing 35% of the reed peat (obtained through the use of a heavy drilling machine on depths distributed from 1 to 3 meters and with an age of more than 80 years), 35% palm peat (Obtained from the trunks of palm trees removed the leg and the age of more than 60 years), 18% coal, 10% limestone and dolomite, 2% agricultural gypsum, mix the mixture well and take a sample to estimate the qualities.

3- T3 Local soil attended from a mixture of local peat moss and river sand from the strand of the Euphrates River and by 1:1 (48-48%), with calcium carbonate at 4%. Mix the mixture well and take a sample to estimate the qualities.

4- T4 attended from mixing 50% of the Spent Mushroom Substrate with 20% washed river sand, 25% local peat moss, 5% $CaCO_3$ and calcium sulphate. Mix the mixture well and take a sample to estimate the qualities.

The components of each soil were mixed on a clean concrete floor with plastic sheeting. Each treatment was wet by adding water with mixed and stirring for a moisture content of up to 70%. Each treatment was packed in thermoplastic bags with a weight of 8 kg and was transferred for sterilization by Autoclave at 121°C for one hour. Then soil was left to cool at room temperature until 25°C and was added to the compost after incubation period of 5 cm thickness and 12 refiner for each treatment.

Fifth: Preparation of the extracts of casing soil components

After the collection of the materials used in the preparation of the casing layer, (peat roots, date palm roots, limestone and dolomite), the components were grinded with an electric grinding machine to convert them into very small soil-like soil. The various components were soaked in water for one hour, then washed and squeezed using a cloth. The extracts were then sterilized by filtration and collected in closed bottles of 1-5 liters, there were three types of extracts:

Organic extract t2: composed of peat roots and palm peat

Mineral extract t3: composed of limestone and dolomite

Extract of t4: mixture of organic and mineral

These extracts were kept in dark places until they were used as Reinforced materials medium and cover material after the first phase of the fruit bodies.

Sixth: Add the extracts of casing soil after the first harvest and the nature of its effect on the quantity and type of fruit bodies.

The extracts were added to the casing soil by spraying. Three types of extracts (t2, t3 and t4) were used by adding 1 ml of extract to 4 ml of filtered water, a total of 9 replicates were treated in each treatment of the extracts and 3 were selected for control with treated water (t1). The concentration of the extracts after the second and third harvest was increased by 1: 3 and 1: 2 in order and added in the same way. Polyethylene bags were placed as a buffer between each repeater before addition to prevent overlapping of the extracts with each other, and were added two times in the morning and evening using spray machine.

3. Measurements of the harvest

Estimated number of phenotypic harvest which represented the diameter of the cap and thickness, and diameter of the leg by taking the rate of 5 bodies fruit Non-open cap medium-sized and the longitudinal fruit body was cut using a knife. These traits were measured for fruit bodies Using a numeric prefix (the verni ruler). The number of fruit body and the quotient of one harvest and the total value of each one was repeated during the production cycle.

Biological Efficiency (B.E): It was estimated according to the method described by Royse (1985).

Experimental Design: The data were collected and analyzed statistically according to Sahuki and Wahib (1990).

4. Results and Discussion

Effect of the type of Casing layer and extracts in the cap diameter (mm) of harvesting 2, 3 and 4 mm

The results shown in Table 1 show that the highest rate of cap diameter in harvest (2, 3 and 4) was achieved with the use of T2 covering soil rate of diameter reached 47.97 mm followed results of T1 and T3 soil treatments at 46.65 and 45.67 mm respectively with a significant difference and recorded the treatment T4 the lowest rate of this status was 44.38 mm. It was also found that the use of the treatment of extract t2 gave the best rate of cap diameter of the genes (2, 3 and 4) reached 52.26 mm compared with the treatment t1, which was left without adding any extract, The lowest value was recorded 40.93 mm. A significant effect was also found between the type of Casing layers and the extracts used. The T4 t2 combination showed the highest cap diameter of 57.31 mm followed by the T2 t2 combination of 54.07 mm while the T4 t3 combination recorded the lowest rate of 38.65 mm.

Effect of type of Casing layer and extracts in thickness of Cap (mm) of harvesting 2, 3 and 4

The results shown in Table 2 show that the thickness

Table 1: Effect of the type of Casing layer and extracts in the cap diameter (mm) of harvesting 2, 3 and 4.

Rate	T4	T3	T2	T1	T/t
40.93	40.06	41.76	41.64	40.26	1t
52.26	57.31	47.47	54.07	50.21	2t
43.95	38.65	46.62	45.60	44.96	3t
47.53	41.52	46.84	50.60	51.19	4t
	44.38	45.67	47.97	46.65	Rate
T=0.972		t=0.972	Tt=1.945		LSD 5%

of the cap of the Harvesting (2, 3, and 4) in treatment T4, T3 and T2 is rapprochement. The table shows that the highest rate of this status reached 19.96, 19.54 and 19.36 mm in T4, T3 and T2 respectively. Measurement to the treatment T1, which had the lowest rate reached 18.49 mm. It was also found that the use of t2 extract gave the highest rate of Cap thickness, reached 21.08 mm compared to the t4 extract, which achieved the lowest rate of this status, reached 17.92 mm. The same table shows that the overlap between T4 Casing layer and t2 support source gave the higher of the Cap thickness reached 21.59 mm compared to the overlap between the T1 Casing layer and the t3 support source, which has registered the lowest rate reached to 17.33 mm.

Effect of type of Casing layer and extracts in the leg diameter rate(mm) of harvesting 2, 3 and 4

The results of the statistical analysis rapprochement the results of the leg diameter rate of Harvesting (2, 3 and 4) at the level of 5% were significant in T1, T2 and T4, as shown in Table 3. The table showed that the highest rate of stem diameter reached 10.76, 10.56 and 10.15 mm in T1, T2 and T4, respectively, Measurement to the treatment T3, which achieved the lowest rate reached 9.78 mm. It was also found that the use of extracts t4 and t2 gave the highest rate of leg diameter reached to 10.65 and 10.64 mm and decreased the rate of this characteristic at treatment t1 without adding the extracts to 9.93 mm. A significant effect was found between the layers of coverage and the extracts used.

Table 2: Effect of type of Casing layer and extracts in thickness of Cap (mm) of harvesting 2, 3 and 4

Rate	T4	T3	T2	T1	T/t
19.50	21.00	19.33	18.98	18.69	1t
21.08	21.59	21.47	20.57	20.69	2t
18.85	19.17	19.62	19.31	17.33	3t
17.92	18.10	17.75	18.58	17.25	4t
	19.96	19.54	19.36	18.49	Rate
T=0.801		t=0.801	Tt = 1.653		LSD 5%

Table 3: Effect of type of Casing layer and extracts in the leg diameter rate (mm) of harvesting 2, 3 and 4

Rate	T4	T3	T2	T1	T/t
9.93	9.58	9.53	10.41	10.23	1t
10.64	10.38	10.10	10.94	11.14	2t
10.02	10.56	9.31	9.26	10.96	3t
10.65	10.08	10.21	11.63	10.71	4t
	10.15	9.78	10.56	10.76	Rate
T=0.725		t=0.692	Tt=1.451		LSD 5%

T2t4 showed the highest rate reached 11.63 mm, while the T2 t3 combination recorded the lowest rate reached 9.26 mm.

Maybe the reason for the superior treatment of T2 in the three successive harvesting after the first cut was due to the synthetic tissue of this locally prepared layer in addition to its physical and chemical properties, providing the optimal conditions to growth A. bisporus compared to other treatments. The diameter of the cap in harvests 2 and 3 due to the presence of positive correlation between the number of fruit bodies and cap diameter was 0.616 and 0.635 respectively. The results shown in Table 1 indicate the superiority of the organic extract t2 obtained from reed plum and palm roots after grinding and washing in giving the best rate of cap diameter of the 2, 3 and 4, which reached 52.26 mm, The reason for excellence is due to its good organic nutrient content, which plays an important role in increasing the biomass of the fungus [Jubair and Abdel Hadi (2013)]. The overlap in the T4 t2 combination of the Spent Mushroom Substrate mixed with the Peat moss and supplemented with organic extract t2 increased the cap diameter, This is due to the fact that the organic extract is rich in nutrients compared to the Poor Casing layer of organic matter [Nadi *et al*. (2015]. Interference has been a part of the nutritional requirements that have been exhausted during successive harvesting. The reduction in cap diameter may be due to the use of t3 supported (extracted from dolomite powder and limestone after burning and washing) in the second harvesting because its components contain high concentration of mineral elements, especially Mg, which causes a delay in production or reduce it by half because of this element of toxicity on the fungal mycelium. The high PH values and electrical conductivity recorded for the components involved in the composition of this extract, which amounted to (9.12, 9.67 DS m^{-1} limestone) and (12 DS m^{-1}, 7.36-dolomite) has negatively affected on the rate of cap diameter through the appearance in distorted shape, which reflected the delay in production and

Fig. 1: Effect of t3 extract on the appearance characteristicsof *A. bisporus*

reduced by half [Zied *et al*. (2012)] as shown in Fig. 1.

The reason for the superiority of treatment T4 may be attributed to the best cap thickness at a significant level of 5% compared with the other to used Spent Mushroom Substrate in its composition, which plays an important role in increasing the soil's ability to retain water and ventilation and increase its permeability [Uzun (2004)]. While the reason of superior between treatment T4 and extract t2 and overlap between them in giving the best Cap thickness to its superiority in giving the best diameter cap, In addition to the number of the fruit bodies of this layer decreased during harvesting (2, 3 and 4) respectively, at a rate of (441.72, 363.01 and 313.64) body m^{-2}. The reason of lower thickness of the Cap with the T1 t3 combination may be due to the use of t3 extract during the second Harvesting only, which recorded the lowest rate of cap diameter as well, This is confirmed by the correlation between the thickness of the Cap and Cap diameter in Fairy 2, which amounted to 0.750, The decrease was also due to the fact that the T1 treatment gave an increase in the number of fruit bodies compared to the other Casing layer during the Fairy (2, 3 and 4) respectively, at a rate of (359.10, 625.09 and 94.04) body m^{-2}.

The reason for the superiority of T1 and T2 to give in Give the best rate of fungal was attributed to the best leg diameter and efficiency in the quality [Siqueira *et al*. (2009)]. The correlation results between the phenotypic characteristics of the body showed a positive relationship between the diameter of the stem and the

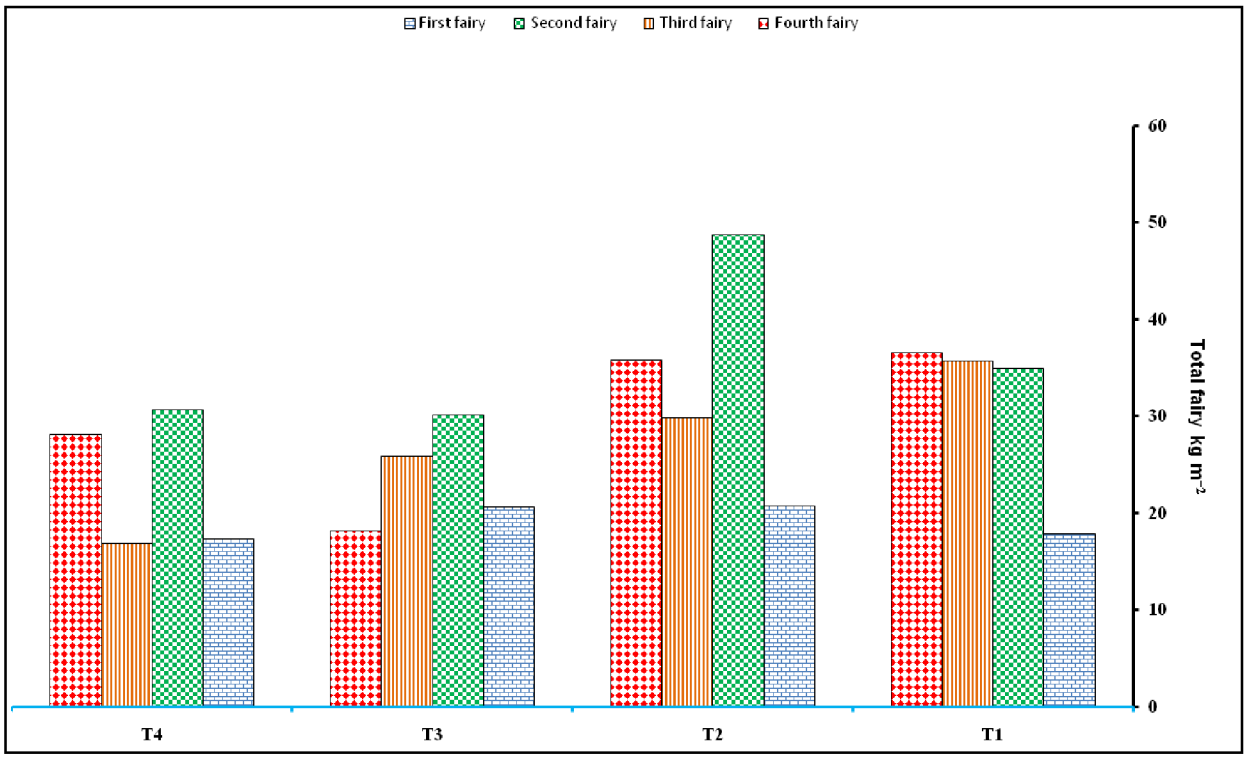

Fig. 2: Effect of Casing layer and extracts in the rate of the total sum of the fruit bodies (kg m^{-2}) in the first fairy with the total sum of the fairies 2, 3 and 4 fruits.

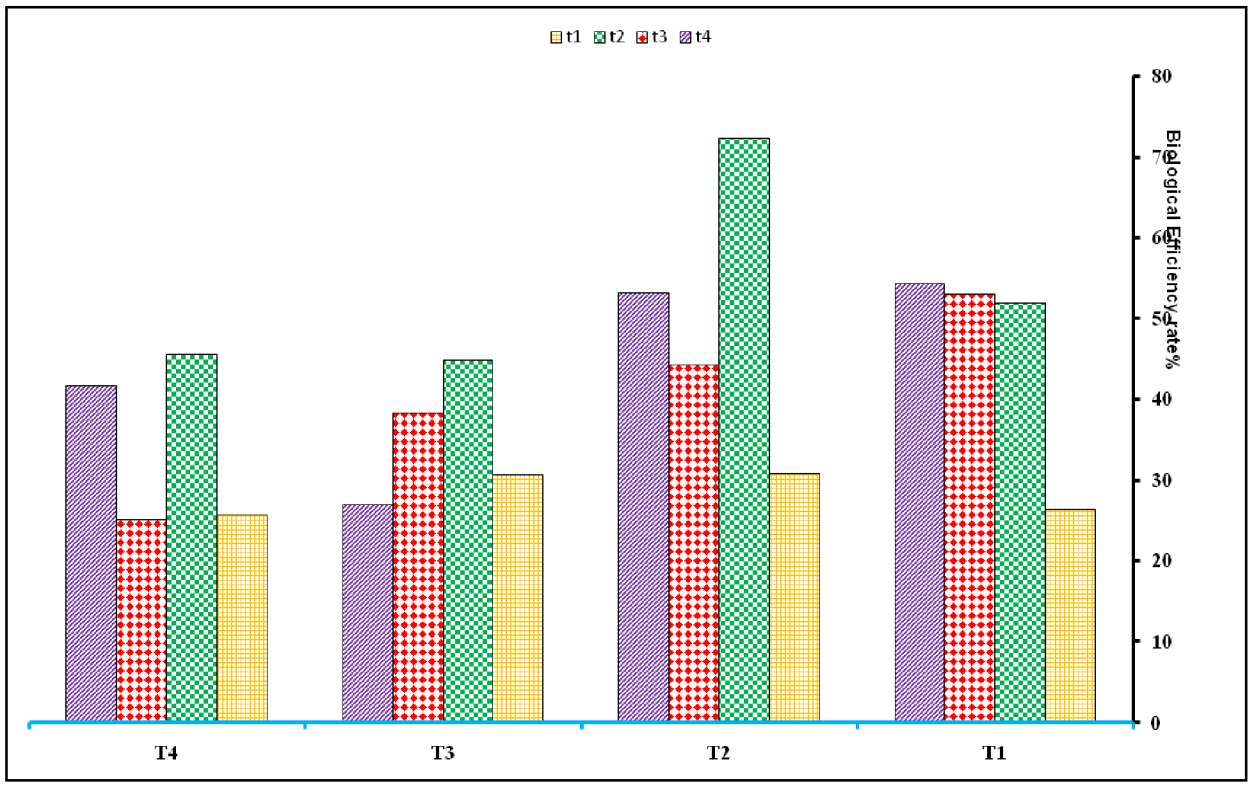

Fig. 3: Effect of the treatments used and extracted in the rate of the biological efficiency (%) of the fruit bodies of *A.bisporus*

diameter of the cap in the second fairy was 0.525. While The reason for the lower diameter of the stem with T3 treatment (consisting of sand and peat moss) during fairies 2, 3 and 4 to increase the pressure in the late fairies on the culture media by the sand as a result of decomposition of the organic Peat moss and inability to provide the growth requirements for fungi, In addition, the increase in the number of fruit body in the T3 casing layer during fairies (2, 3 and 4) respectively, at an rate reached (354.95, 551.65 and 145.80) body m^{-2} contributed to reduce the rate of leg diameter. While the stem diameter reduction with the T2 t3 combination was due to the use of t3 extract, which recorded the lowest diameter and cap thickness, In addition, the increase in the number of fruit body in the T2 layer, prepared locally during the fairies (2, 3 and 4) respectively, at a rate of (382.53, 534.60 and 350.54) body m^{-2} contributed to reduce leg diameter.

Effect of Casing layer and extracts in the rate of the total sum of the fruit bodies (kg m^{-2}) in the first fairy with the total sum of the fairies 2, 3 and 4 fruits

It is noted from Fig. 2 that the highest total rate of fruit body yield fairies 1, 2, 3 and 4 was achieved with treatment T2 at a production rate of 48.771 kg m^{-2} followed by the productivity of T1 and T3 with a production rate of 36.654 and 30.234 kg m^{-2} respectively While reached the lowest rate of this character in the production cycle was 16.934 kg m^{-2} with the treatment T4.

The reason for the increase in fungus yield was due to the correlation of the yield with the capacity and the ability of the materials used to prepare the soils as well as their water retention and good ventilation [Mohan (1994)], in addition to their moisture content and Extent of homogeneity of the depth of casing soil when added [Radwan (2002)]. While the reason for the superiority of the treatment T2 by recording the highest yield rate during the production cycle reached 135.288kg m^{-2} compared to the total output of the control treatment T1 and other treatment T3 and T4 to the fact that they have physical, chemical and biological characteristics, as well as their texture and suitability to the requirements of the mycelium growth stage and the influence of other physical factors such as the temperature that induce the formation of fruit bodies and provide the environmental requirements of temperature, humidity and ventilation, which prepare conditions for the growth of mushrooms as well as maintain its strength through the fairies sequentially compared to the other Casing soil [Zied *et al*. (2011)]. The reason for the increase

yield when use of extract t2 during the production cycle may be due to water soluble substances found in the extract, which contributed to the increase of the yield food fungus *A. bisporus* because it has the ability to analyze these materials to raw materials and use in the feed. The reason for the decrease in the rate of production per square meter (for the total) based on wet weight in the treatment T4 may be due to the fact that these soil lose their properties in fairies 3 and 4, so the formation of pin or not due to the degradation of organic matter in their components and depletion in fairies 1 and 2 and survival the sand at a high rate of 20-30% which causes the imbalance of water conservation within the soil components and decline to the culture media below it, which causes a weakness of mycelium in producing of the primers of the fruit bodies. In addition to lack of ventilation and their inability to support the primers of fruit body to develop [Olle *et al*. (2012)], the reason can be attributed to increase the concentration of soluble salts in the casing layer due to the increased concentration of the support and therefore the lack of absorption of water and nutrients [Pecchia and Beyer (2013)].

Effect of the treatments used and extracted in the rate of the biological efficiency (%) of the fruit bodies of *A. bisporus*

The results of Fig. 3 showed that the differences in the composition of the casing soil significantly affected on the rate of biological efficiency of fruit production. The highest efficiency rate was 72.253% with the T2 t2 combination, followed by the T1 t4 combination with an efficiency rate of 54.302% and 53.112% 45.471% and 725.08% with combinations T2 t4, T4 t2 and T4 t3 respectively.

The reason for the high value of biological efficiency in the used agriculture medium is due to increase in the amount yield based on wet weight in the T2 t2 and T1 t4 combinations, which was positively reflected in increased bio-efficiency. Schisler (1982) noted that the bio-efficiency of the world's mushroom farms is poor if 30-50%, medium between 50-70%, good between 70-90% and excellent 100% or more [Jabr *et al*. (2008)]. The biological efficiency is directly related to the type of fungus strain, nutrients in the agricultural medium, growth conditions, cover layer and extracts used to increase production in the T1 and T2 casing soil during the production stages, which reflected positively on the increase in bio-efficiency rate [Upadhyay *et al*. (2003)]. The reason for the decrease in the biological efficiency of the production of A. bisporus in the T3 treatment during fairies 3 and 4 and in the T4 treatment during fairy 3 only may be due to the different casing soil components and their ability to provide suitable conditions in addition to the different combinations of supported added at different concentrations, which led to the weakening of the process of the formation of fruit bodies and this reflected negatively on production and thus reduced bio-efficiency [Boddy *et al*. (2008)].

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