

## Agaricus Bisporus Produced by Using Liquid Fertilizer Bacillus sp and Estimation of Protein

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**ABSTRACT:** The present study aimed evaluation of Bacillus sp to increased produce Agaricus bisporus and estimation of protein .The best production of fruiting bodies achieved 440.83 g/5 kg with the compost of palm frond waste after 21 days of harvest, compared with 371.17 g/5 kg from wheat straw, while the use of the Bacillus sp led to increased production rate of 430.00 g/5 kg ( $P > 0.05$ ) compared to the without the use of vaccine 376.94 g/5 kg, and that the best biological efficiency of 26.5% achieved with the palm frond waste compost, followed by an average of wheat straw The best protein content of 20.68% with fruit bodies from the compost of the mixture, compared with 18.27% on fruit bodies from wheat straw, while the use of the vaccine led to an increase in this content by 19.82% compared with 17.64% without the use of the inoculum.

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### INTRODUCTION

Bacillus Sp has various mechanisms that encourage and improve plant growth, as it pollinates crop seeds, and includes the production of hormones to stimulate plant growth in the root zone, including gibberellin, auxin, zeatin, and abscisic acid. Growth stimulation is represented by increasing stem growth and the strength of the root system, which leads to improving water and nutrient uptake and increases the phosphorus available to the plant, which is one of the necessary factors in bio fertilization (Tang0221

B. sp could be a harmless alternative to synthetic fungicides in mushroom production, especially during serious compost green mold outbreaks caused by *T. aggressivum*. Furthermore, the fungicide should be applied alone because an antagonistic reaction was Bacillus bacteria have proven their ability to dissolve insoluble phosphate minerals, increase their readiness or absorption by plant roots, in addition to their ability to produce growth hormones, reduce the harmful effects resulting from salt stress, and produce antibiotics that contribute to protecting plants from pathogens (Al-Dulaimi, 1991). Agaricus bisporus, a white button mushroom, has long been targeted by humans foraging for food (Chang and Miles 2004). Mushrooms have been used as a source of food medicine (Asatiani et al., 2010). It has been used as a dietary food and medicinal supplement in China for over 2000 years. Agaricus bisporus in Europe was cultivated in France in the 17th century (Savoie and Largeteau, 2011). B. strain may potentially be used for biological and integrated treatment in Agaricus cultivation. Soil bacteria can act indirectly by preventing some harmful effects of pathogenic microorganisms in plant development or directly by synthesizing a compound produced by the organism and facilitating the uptake of nutrients in the root region [15].

The study aimed to The effect of using bio-fortification with bacteria to improve the specifications of the medium and increase the productivity of the food fungus A. bisporus

### MATERIALS AND METHODS

The fungus isolates were obtained from the Ministry of Science and Technology and different temperatures in Petri dishes were used Preparation of the filtrate of the fungus Agaricus spp It grew in Distilled water About (broth P.D.) 300 ml for about 28 days at 27 °C, The filtrate of the fungus Agaricus spp was filtered with the help of filter paper, Then the filtrate of the fungus Agaricus spp was kept. The experiment was carried out in my stream, and the client included two parts, the first part was in the laboratory, and it was implemented in the laboratory. Local fungi and biotechnology affiliated with the College of Medical- University of Sumer to study the growth rate of the mycelium of the fungus The solid media and the bioavailability formed in the liquid medium

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of the agricultural media extracts for the study treatments, The second part is the cultivation of the fungus pollen on the farming press to study the period of completion of the growth of the mycelium on the agricultural media.

### **Preparing and fermenting the culture medium**

Preparing the waste (palm fronds and wheat straw) for vegetarian poultry has been completed. Then the process of cutting the plant waste the ingredients were mixed according to the proportions Vedder 1978),. Then, the plant waste was moistened with tap water and left for 18 hours uThe seventh, ninth, thirteenth, and seventeenth days, with moisturizing when needed, re-mixed well, collected in piles and bacillus bacteria vaccine was added at a rate of 106 cell g culture media according to the coefficients the process continued for 21days. after which the media were pasteurized (Beyer, 2003A) until it was completely saturated with water. The excess water was removed and the poultry was added On the second day, the mixtures weA1S1, A2S1, and A3S1 with mixing and stirring carried out on the sixth day

### **Pasteurized and applied to the growing medium**

The pasteurization process was carried out in durable polyethylene and wrapped in heat-resistant bags. They were placed in iron drums with a capacity of 200 liters, to which 30 liters of water were added. Then they were placed on a heat source. An iron stand was placed inside the drums, so that the middle bags would not be immersed in water. The pasteurization process was continued. Then I placed the media bags on top of The carrier and the barrels were closed with an iron cover.

The operation lasts for 8 hours on the first day, 6 hours on the second day, and 3 hours on the third day. Celsius containing an oil heater, sprayed the floors and moved the floors with 40 tires to a special room at a temperature of sterilized water when needed, stirring, and ventilation were carried out using a vacuum cleaner. The temperature was reduced to 27 degrees Celsius after the smell of ammonia completely disappeared.I left the culture media to cool, then I transferred it to the boxes and then became Ready for agriculture (Kivaisi 2007)

### **Spawning Method**

The cultivation was carried out in the production room, where the room was Sterilized with 38% formalin by diluting 40ml in 1 liter of distilled water for 48 hours, after which the ventilation process was carried out the cultivation medium was added inside cork boxes with a length of 40,0 cm , a width of 25.7 cm and a height of 19.0 cm and included 36 replicates , with 6 replicate for each mixture . the medium was 15 cm high . the cultivation was carried out by spawning the seeds(spawn ) at a rate of 2% of the dry weight and was mixed with medium using the ruffling in method covering it with polyethylene to maintain humidity and incubation at a temperature of 25 c was provided using an oil heater until the hyphae appeared and were completed in the entire medium to by ready for adding the covering layer(Royse,2008,1976,Qasim )

### **Casing Layer**

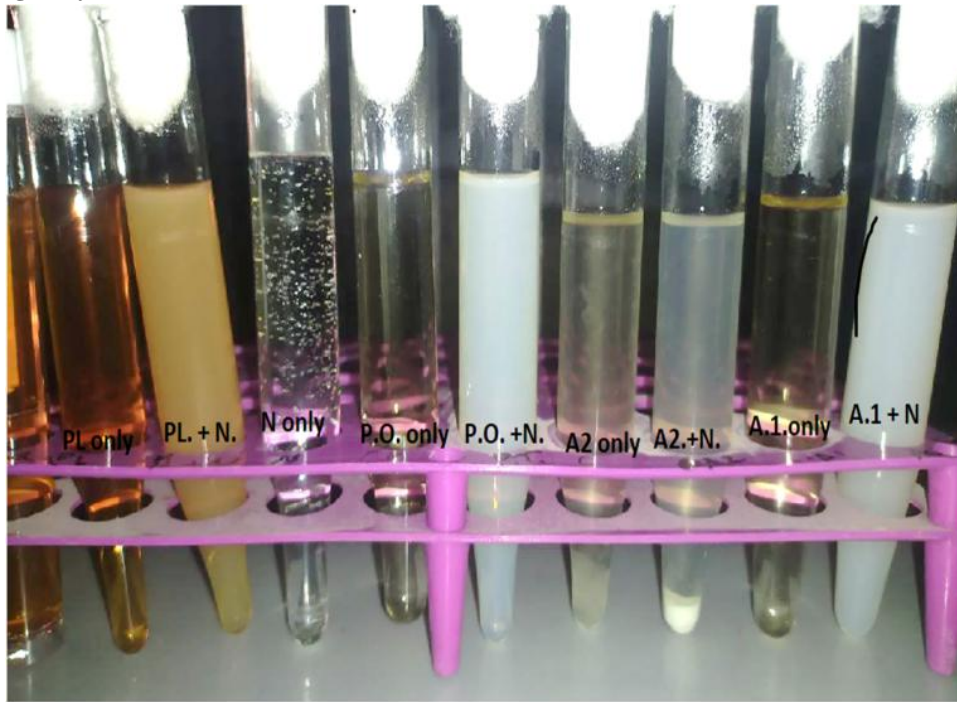
Peatmos will be used 100% in preparing the covering layer, and its number pH is 7.5 and the salt level is 1 gm/organic substance, with a percentage of 95.99 of the dry weight and a percentage of npk 14:16:18 It contains some necessary elements. Such as iron, copper, zinc, manganese, molybdenum, and boron, and the process of wetting with water until it becomes The peat moss came together in a cohesive mass when taken by hand and was sterilized using 38% formalin and left It was used to cover the cultivation medium after it had been sealed until the smell of formalin disappeared (Carroll, 1989). It was used to cover the cultivation medium after completion of Mycelium growth in a thin layer 2.5 cm thick, 38% formalin, and left Harvasting Serving operations were carried out until the fruiting bodies began to appear In the second week, the size of the fruiting bodies of the edible mushrooms increased until they reached the button stage.From which the fruiting bodies were harvested by holding the mushroom cap at the beginning (Button stage).

Using a small knife - and by rotating the leg slightly - the lower part of the leg is cut off - the finger ( - ) and the index finger. Sharp, and there are plastic containers prepared to place the fruiting bodies for each treatment, and they are taken for it. Measurement, and after completing the harvesting process, the holes formed as a result of the required harvesting are covered. Using mulching soil to maintain the productivity of food mushrooms

### **Statistical analysis**

The data were collected and analyzed statistically and their rates were compared using (LSD). The correlation coefficients between the characteristics of the fruiting bodies formed on the culture media were also measured.

Picture 1 Preparing daily work culture.





Picture 2 A ,B,C Preparing media from mixtures of palm fronds and wheat straw

### **Mushroom mycelium growth rate, biomass and protein content.**

The effect of Three concentrations of palm frond extract on the growth rate of food fungus hyphae in solid PSA medium and measuring the dry weight of biomass formed in liquid PS medium and its protein content were studied with the use of potato extract medium without adding

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The results of this study show that the palm frond treatment achieved the best results in mushroom mycelium growth, biomass and protein content. The results of the study showed a significant effect on the growth rate of white nutritious pancakes on solid media, achieved a significant effect, 2.53, 2.60 and the measurement coefficient gave a growth rate of 2.00.

The date palm fronds treatment showed a significant increase in the content of the biomass of proteins, which achieved the highest protein content in the biomass formed in the liquid medium, amounting to 26.25%. The standard treatment recorded a protein content of 18.75%.

**Chemical properties of culture media**

**Table 1 Nitrogen and carbon content in culture media in fermentation process**

no	treatment	C%	N%	CIN	PH	EC
1	A1	40.6	1.94	20.92	6.21	1.25
2	A2	46.2	2.10	22.00	7.31	1.61
3	A3	45.6	2.04	22.36	7.33	1.70
4	A4	44.3	2.18	20.32	7.18	1.23
5	A5	44.2	2.20	20.9	7.21	1.34

A1=conrol A2= mixtur without inoculum A3= palam frond without inoculm A4= palm frond with inocum A5= mixture with inoculm

The results in Table 1 showed that the treatment T2 (50% wheat straw + 50% date palm fronds without treatment) was kept in carbon content and outperformed all treatments as it gave 46.2% compared to the s standard treatment T0 (wheat straw) of carbon and was 45.6 The nitrogen content of the medium is affected by the parameters of the palm frond used in preparing the medium, and the treatment A3 was superior by giving a higher nitrogen value of 2.20%, than the nitrogen content of the standard treatment A1, which was 1.96%. The study evaluated the impact of different treatments on the electrical conductivity values of the prepared media. The highest electrical conductivity values recorded were 1.70 and 1.61 dS/m for treatments A2 and A3, respectively. In contrast, treatment A1 resulted in produced an electrical conductivity value of 1.25 dS/m. Treatment A4 The lowest electrical conductivity value in the sample is 1.23 dS/m.

The pH value is important in the decomposition process and its effect on the growth of food fungi. The highest pH value was achieved with palm fronds at a rate of 7.33, followed by the medium of the mixture (50% wheat straw and 50% cane) and the medium of standard wheat straw at a rate of 7.31 and 6.21 respectively

The use of the inoculum showed a slight increase in the pH value of 7.21 compared to 7.18 without the use of the vaccine.

**Production of fruiting bodies of A. bisporus**

**Productivity of A. bisporus For every 5 kg of soft culture medium for a period of 21 day**

**Table 1 -Productivity of A. bisporus For every 5 kg of soft culture medium for a period of 21 day**

Average	A3	A2	A1	Treatment
376.78	453.33	325.33	351.67	N0 B0
377.11	348.00	445.33	338.00	N11
B0 average 376.94	400.67	385.33	344.83	A*B average
35.65	36.14	47.21	23.60	N0 B1
38.52	43.17	43.84	28.57	N1
Average B1 37.09	39.66	45.52	26.09	A*B laverage
Average N	42.13	46.12	25.17	A average
34.74	37.95	44.86	21.42	A*N0 average
40.87	46.30	47.39	28.93	A*N laverage

LSD P> 0.05 A=4.156, B=3.394 , N=3.394, AB=5.878, AN=5.878, SG= 4.799, ABN=8.313

showed that the best production was achieved at a inoculummedium and the use of cultureThe interaction between the type of with palm frond medium with pollen. The mixture medium using the inoculum and wheat frond medium kg5 / 496.33grate of while the lowest , 400.67 and 397.50 g/5kg and percentage of 23.88% and 24.86% respectively. mixed with pollen at a rate of medium without using inoculum at percentage of of wheat straw of 344.83 g/5kg with standard rate production was at a

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which are associated with 43.93% and a significant difference at ( $P > 0.05$ ) This result agrees with Cao et al (2022) *Bacillus* mushrooms during various phases of substrate preparation and mushroom growth. Representatives of these genera are degrading enzymes and are dominant in different periods depending on lignin characterized by different temperature optima of promoting activity of many of them the amount of scientific research conditions. The protective and mushroom growth [According to the findings of Liu et al (2015) treatment with *B. velezensis* B145 significantly increased the yield of white button mushroom. During our farm experiments.



**Fruiting bodies after harvest**



**fruiting bodies in the production room**

### **Bio-efficiency of fruiting body production**

Depending on the type of culture medium used, the best biological efficiency was achieved at a rate of 265% with slender fronds, followed by wheat straw medium and then the mixture medium at 25.8 and 22.9% respectively.

The use of bacterial inoculation showed the best bio efficiency at a rate of 26.3, while it decreased 23.8% without using inoculation by 10.5%.

The interaction between the type of medium and the use of the inoculation showed that the best biological efficiency was achieved at a rate of 29.3 with the palm frond medium using the inoculation followed by the wheat straw using inoculation at a rate of 28.8 while the lowest biological efficiency was 20.8 with mixture medium without using inoculation Table 2

These results are in agreement with the results of (. Muslat, M. M. (2002)) as the protected basidiomycetes possess the enzyme urease, which is responsible for tolerating urea.

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The effect of the treatments used in this study on the growth of *Agaricus bisporus* mycelium may be explained by the fact that the treatments used in it help date palms with wheat straw provide the growth requirements of the mycelium of carbon in the forms that the mycelium can represent and benefit from in

Table 2 Percentage of biological efficiency in the production of food mushroom

*A. bisporus*

Average B*N	A3	A2	A1	Treatment
23.7	28.0	19.9	23.2	N0 B0
239	22.1	27.3	22.3	N1
B0 Average 23.8	251	23.6	227	A*B1 average
29.1	26.5	26.6	34.2	N0 B1
23.6	15.1	32.1	23.5	N1
AverageB1 26.3	20.8	29.3	28.8	A*B laverage
AverageN	22.9	265	25.8	A average
26.4	27.3	23.3	28.7	A*N0 average
23.7	18.6	29.7	22.9	A*N laverage

LSD  $P > 0.05$  A=7.65, B=6.24, G=6.24, AB =10.82 , AN=10.82, BN= 8.83 , ABN=15.30

Protein content of the fruiting bodies of *A. bisporus*

The use of a bacterial vaccine at a concentration of 106 cells/g showed that the best rate

The protein content of 19.82% was significantly higher○

( $P > 0.05$ ) compared to not using the bacterial vaccine .17.64%

Table 3 shows that the three media (fronds, straw mixture) significantly differed in the percentage of protein in the fruiting bodies. The reason for the difference in the percentage of protein in the fruiting bodies between the agricultural media may be due to the difference in their nitrogen content, noting that the nitrogen content of straw does not exceed 0.3% (Beyer, 2007), as the nitrogen content of the fronds is several times that of the straw.

This Result agree with (Ahlawat and Vijay (2010)(influence of growing conditions and substrate choice. It may very well be that one mushroom produces a lot of protein ,This illustrates the importance of microorganisms, including bacteria, in the life cycle of *A. bisporus*; they create a selective substrate in which this mushroom-forming fungus can thrive.Together, substrate production involves a complex microbial community, during which the 'self-heating' stage in PI, resulting from high microbial metabolic activity, causes a shift from mesophilic to thermophilic bacteria. Interestingly.

Average B*N	A3	A2	A1	Treatment
16.960	19.030	15.620	16.230	N0 B0
18.317	19.990	17.150	17.810	N1
B0 Average 17.638	19.510	16.385	17.020	A*B average
19.250	21.530	17.150	19.070	N0 S1
20.387	22.180	19.030	19.950	N1
AverageB1 19.818	21.855	18.090	19.510	A*B laverage
Average N	20.682	17.237	18.265	A average
18.105	20.280	16.385	17.650	A*N0 average
19.352	21.085	18.090	18.880	A*N1 laverage

## CONCLUSION

In this study, the use of bacillus inoculum increased the yield of *Agaricus sp* and total protein-protein complex content, while also reducing the time to harvest. Bacillus is considered safe and can utilize inexpensive nutrition sources as substrates for fermentation, thereby reducing costs. As a significant gram-positive bacteria heterotrophic bacterium, Bacillus has numerous

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important applications in fermentation biological manufacturing, and environmental protection. These applications include biological nitrogen removal from wastewater and biological fertilizer to inhibit NH<sub>3</sub> volatilization and reduce nitrogen loss in agroecosystems (Y. Gu, X. 2018, B.o. Sun, Z. 2020).

Carbon and Nitrogen were elements responsible for the growth while Nitrogen was essential in the protein content of the mushroom. Protein content in edible mushrooms ranged from 6.60 to 36.87 g/100 with an average value of 23.80 g/100 g dry weight

Bacillus is extensively used as a biological control agent in agricultural fields including in the button mushroom culture, *Agaricus bisporus*. Its ability to efficiently colonize and persist in crops. In addition, it is equipped with an antimicrobial arsenal enabling it to fight *Bacillus* species that are found naturally in mushroom casing and are generally regarded as safe), All these features allow *B. sp* to be highly competitive in the environment and make it a particularly efficient biocontrol agent.

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