Optimal Conditions for the Production of Mother Culture for Cultivated Iraqi Edible Mushroom *Lentinula edodes* RSR strain (Shiitake Mushroom)

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Abstract

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This study was conducted to determine the optimal conditions of using different agro-natural wastes to develop a local culture media suitable for producing mother cultures of the wild Shiitake Iraqi strain mushroom *Lentinula edodes* RSR, by using different natural media composed of wheat, oats, barley, corn cobs, green peas, chickpeas, wheat bran, rice bran, barley bran, bumper leaves, moringa leaves, sawdust and whey cheese. In addition to potato dextrose agar (PDA) used for comparison at 10 to 40 g/L concentration. The optimal concentration of the medium produced was 20 g/L, and the best pH for mycelium development of this strain was 6.5 at 23ºC under dark conditions. The best mycelial growth and density obtained was on wheat flour agar media.

Keywords: Agro-wastes, shitake cultivation, mushroom, environmental factors.

Introduction

Lentinula edodes (Shiitake mushroom) is a popular edible and medicinal mushroom, the second most important cultivated edible mushroom in the world. Until the 1980s. Japan was the world's largest producer of shiitake mushrooms, which were traditionally grown in logs. However, China is currently the world's leading producer of shiitake mushrooms, accounting for more than 95% of total production, and this species has overtaken the white button mushroom *Agaricus bisporus* as the most commonly cultivated, accounting for 22% of total production (Royse *et al.*, 2017). Mushrooms are becoming increasingly essential in human diets around the world, due to its high nutritional and therapeutic value, and low calories content (Ajonina and Tatah, 2012; Alananbeh *et al*., 2014). In addition, they are richness in vitamin B complex and vitamin D. Therefore, it has bio therapeutic applications such as antitumor, anticancer and antiviral activities due to the presence of lentinan (Jin *et al*., 2020; Ren *et al*., 2018; Wang *et al*., 2017). Cultivation and modification programs for mother culture's production through increasing the methods of mushroom production in order to increase its qualities, sawdust and field crops such as wheat, barley, rice, and others, as well as leaves and stalks of millet, maize, banana in addition to yellow corn, and rice husks, are the major materials used in its production. In experiments on shiitake mushroom production, hazelnut husk, wheat straw, wheat bran, and beech sawdust have all been examined (Puri *et al*., 2011; Özçelik and Pekşen, 2007), eucalyptus sawdust, rice bran, wheat bran, soybean bran (Casaril *et al.*, 2011), and chickpea straw, corn stalk, alfalfa hay, sunflower head residue (Atila, 2019), wheat stalk, wheat bran, peanut shell, corn cob and grapevine pruning waste (Baktemur *et al*., 2020) were also used.

The purpose of this research was to identify the best natural media and define optimal conditions of temperature, pH, and nutrients for growing edible mushroom *Lentinula edodes* RSR strain (shiitake mushroom) using environment-friendly, locally available resources.

Materials and Methods

Sample collection

Wild cultivated mushroom *Lentinula edodes* (RSR strain, genus Lentinus (higher Basidiomycetes) samples were collected from Maysan province-Iraq, during winter to autumn in season 2017-2018. This mushroom was propagated using two different types of media. Several agricultural and industrial wastes of natural materials were selected for the preparation of the mother culture and compared with the standard PDA medium to determine the optimal conditions for production of mother culture of cultivated *Lentinula edodes* RSR strain (Shiitake Iraqi mushroom).

Media preparation

The medium Potato dextrose agar (PDA) was prepared according to the manufacturer's instructions by dissolving 39 grams of PDA powder in 1000 ml of distilled water, adjusting the pH to 5.5, and sterilized the medium in the autoclave at 121°C and 15 lbs/In^2 pressure for 20 minutes. This medium was used to activate and cultivate Shiitake edible mushrooms.

Natural medium was used as a solid medium for growth of edible mushrooms *Lentinula edodes* and included two types of natural media obtained from the local market of Baghdad city. The Agro-wastes included wheat flour, barley flour, oat flour, corn flour, green peas flour, chickpeas flour, bumper leaves (*Cordia dichotoma*), Moringa *(Moringa*

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oleifera) leaves, corn cob, rice bran, wheat bran, barley bran and sawdust. Whereas, the industrial natural products included whey cheese, agro-wastes of bumper leaves, moringa leaves, corn cob, rice bran, wheat bran, barley bran and sawdust. All these materials were heat-dried in an oven at 60ºC and well ground to produce a powder and then placed in plastic bags, sealed well and then kept in the refrigerator until use. The ingredients of the medium were mixed well in a glass beaker by using a heater with a magnetic stirrer. The medium pH was adjusted to 6.5 then sterilized in the autoclave at 121ºC for 15 minutes, then cooled to 55ºC, and distributed in a 9 cm Petri plates. The prepared plates were inoculated with a 5mm diameter disk from the colony edge of 7 days old culture of Shiitake mushroom and the dishes were then tightly wrapped with parafilm. The plates were incubated at 25ºC, and mycelium was observed until full development in the dish (Chechan *et al*., 2017; Sabri *et al.,* 2019).

Production of mother culture

Fruiting bodies of identified mushroom *Lentinula edodes* RSR strain were used for producing pure mother culture in Petri dishes containing PDA medium. The dishes were then transferred to an incubator at 25±1ºC for three weeks with continuous monitoring for mycelium growth and the removal of contaminated dishes (Hussein, 2020; Sabri *et al.,* 2019;).

Effect of media concentration on the growth rate of mother culture of shitake mushroom

The natural flour media of each of wheat, oats, barley, corncobs, green peas, chickpeas with bumper leaves, moringa leaves, rice bran, wheat bran, barley bran, sawdust and whey cheese were prepared at concentrations of 10, 20, 30 and 40 g/L of distilled water with 15 g/L agar agar and the pH was adjusted to 6.5, then sterilized in an autoclave at 121ºC and 15 lbs/In^2 pressure for 20 minutes, then cooled and placed in 8.5 cm diameter Petri plates. The plates were then inoculated with a piece of mycelium growth, and then incubated at a temperature of 25°C, where the mycelium growth was monitored until it covered the whole dish. The growth rate was then calculated based on the following equation (Amar, 2017; Chechan, 2020):

$$\text{Growth rate (cm/day)} = x/y$$

Where: x= dish diameter of 8.5 cm, y= the time in days required for fungal growth to reach the edge of the dish.

Optimization of growth conditions for mother culture of shitake mushroom

Acidity (pH) - The effect of pH (6.0, 6.5, 7.0 and 7.5) on the growth rate of shiitakes mycelium was investigated in the flour medium of each of oats, barley, corn cobs, green peas, chickpeas, bumper leaves, moringa leaves, rice bran, wheat bran, barley bran, sawdust and whey cheese at a concentration of 20 g/L distilled water containing 15 g agar agar, and the pH of the media was adjusted. The medium was then sterilized in an autoclave (121ºC for 20 minutes at 15 lbs/in^2 pressure), followed by cooling to around 55ºC before placing in petri plates and inoculation with the mushroom culture. Finally, the mycelium growth was monitored until it fully covered the plate, and the growth rate was calculated.

Temperature - The effect of different temperatures (21, 23, 25 and 27ºC) on the growth rate of shiitake mushroom grown using different selected media were investigated in comparison with PDA medium. The natural media was incubated at the mentioned temperatures, and the mycelium growth was monitored until the plate was fully covered, then the growth rate was calculated. Media were prepared at a concentration of 20 g/L, and the pH was adjusted to 6.5.

Light and darkness - The influence of presence or absence of light on the growth rate of the shiitake mushroom was investigated using different selected media as compared with the PDA medium. The natural media was prepared with a concentration 20g/L then incubated at 23 ºC, and the pH was adjusted to 6.5. The mycelium development was observed until the dish was fully covered, then the growth rate was calculated.

Estimation of the mycelium growth density of *Lentinula edodes* in natural media

The mushroom RSR strain under study were grown in natural media which mentioned previously under optimal conditions by preparing the media at a concentration of 20 g/L and the pH was adjusted to 6.5 and incubated at 23ºC until plates full growth in each of the media tested. The mycelium was removed from the media and transferred to a clean, dry, pre-weighted plate to estimate the final mycelium weight using a sensitive scale for each medium, a value that expresses growth density as in the following equation (Amar, 2017):

$$\text{Growth Density (g/dish)} = X - Y$$

where X= weight of the plate + weight of the mycelium for each media, and Y= weight of the empty plate.

Statistical Analysis

The experimental design used was the randomized complete block design (RCBD), and the results obtained was statistically analyzed using the Genestat Discovery 3 program. Mean values were compared using the LSD test at P= 0.05.

Results and Discussion

Effect of media concentration on the growth rate of mother culture of shitake mushroom

A number of agricultural wastes were selected for the preparation fourteen types of natural media of the mother culture and compared with the standard medium potato dextrose agar (PDA) commonly used to culture fungi. Results obtained summarized in Table 1 demonstrate that the optimum growth rate was at a concentration of 20 g/L in the following media: barley flour agar (0.430 cm/day), followed by oat flour agar (0.425 cm/day), wheat bran agar (0.425 cm/day), wheat flour agar (0.423 cm/day), rice bran agar (0.422 cm/day), corn cob agar (0.410 cm/day), barley bran agar (0.404 cm/day), bumper leaves (0.391 cm/day), moringa leaves (0.388 cm/day), whey cheese (0.353 cm/day), and sawdust agar gave the lowest growth rate (0.223 cm/day) as compared with the standard media (PDA) 0.283 cm/day with significant differences at P=0.05. Shiitake

mushroom (*Lentinula edodes*) produces lignocellulosic enzymes which facilitate the use of agricultural raw materials as substrate. The increase in growth rate in the cereals flour agar may be due to the nutritional effect of the media prepared such as carbohydrates, proteins, amino acids, and vitamins. Cereals of the Gramineae family such as barley grains contain high levels of most of the nutritious compounds especially polysaccharides. It is also rich in lysine, arginine, aspartic acid, and alanine and other amino acid (Biel *et al.,* 2020; Joo Lee *et al*., 2014). Barley grains contain major phenolic acid ferulic acid, as well as synaptic and p-coumaric acids in free form, oligomeric flavonoids such prodelphinidin (B3), catechin, and procyanidin (B2) (Irakli *et al.,* 2020). Baghel (2012) reported similar results with malt extract as the most effective for mycelium growth of *L edodes with* highest average mushroom yield obtained on substrates with 20 % rice bran. According to Khandakar *et al*. (2008), six edible mushroom species: *Pleurotus ostreatus, Pleurotus highking, Pleurotus geesteranus, Pleurotus eryngii, Lentinus edodes and Hypsizygus tessulatus* grew well on wheat extract agar and potato dextrose agar media. Results of this study showed that the concentration 20 g/L produced the highest growth rate, whereas the concentration of 10 g/L gave the lowest growth rate, and such concentration was selected to study the effects of growing conditions such as pH, temperature, light and darkness on mushroom growth as compared to the standard PDA medium.

Optimization of growth conditions for mother culture of shitake mushroom

Acidity (pH) - The mycelium growth of shitake mushroom gave the best growth rate at concentration of 20 g/L (barley flour, wheat flour, rice bran, oat flour, corn cob, wheat bran, barley bran, glue berry, moringa leaves and whey cheese agar) and grown at different pH values (6, 6.5, 7 and 7.5) (Table 2). The best mycelium growth rate and yield was produced at pH=6.5. The media which led to optimal growth was barley bran, rice bran, wheat bran, oat flour, wheat flour and barley flour. The growth rate of the remaining natural media varied with increasing pH values. Three strains of *Lentinula edodes* were reported to grow throughout a wide pH range (Baghel, 2012; Quaicoe *et al.,* 2014). Furthermore, other workers (Razeghi yadak *et al.,* 2009) indicated that every strain of *Lentinula edodes* has special pH optimum which depends on several factors.

Temperature - Temperature is an important factor in the vegetative growth of the edible mushroom *Lentinula edodes* RSR strain. Results obtained in this study (Table 3) indicated that the optimal temperature which gave the highest growth rate of shiitake mycelium was at 23 ºC for all the different media used. The best growth rate was noted for wheat flour agar media followed by rice bran agar, wheat bran agar, barley bran agar and oat flour agar. The minimum growth rate was recorded for whey cheese agar at different temperatures (21, 23, 25 and 27ºC) and averaged 0.387, 0.492, 0.387 and 0.377 cm/day, respectively, which agrees with Turković (2015) who reported that low temperature slows down the growth, and the mycelium grows relatively quickly when incubated at 16-23ºC. At the same time, Quaicoe *et al.* (2014) investigated the effects of media and temperature on the growth of three strains of *Lentinula edodes* and discovered that the optimal temperature for the development of all three strains was at 25ºC on five different natural media: maize, rice, millet, sorghum meal agar, and potatoes dextrose agar (PDA).

Table 1. Effect of medium composition at a concentration of 10, 20, 30 and 40 g/L on the growth rate (cm/day) of *Lentinula edodes* at pH 6.5 and temperature of 25ºC.

	Concentrations (g/L)				
Medium	**10**	**20**	**30**	**40**	**Mean**
Potato dextrose agar (control)	0.200 o	0.283 m	0.321 hijkl	0.338 ghij	0.285 d
Wheat flour agar	0.392 cd	0.423 ab	0.408 abcd	0.392 cde	0.403 a
Barely flour agar	0.389 d	0.430 a	0.400 abcde	0.395 bcde	0.403 a
Rice bran agar	0.390 d	0.422 abc	0.400 abcde	0.399 bcde	0.403 a
Oat flour agar	0.392 cd	0.425 ab	0.400 abcde	0.395 bcde	0.403 a
Corn flour agar	0.318 j	0.345 g	0.345 ghij	0.350 fghi	0.339 c
Green peas flour agar	0.232 n	0.351 fgh	0.386 de	0.349 fghi	0.330 c
Chickpeas flour agar	0.284 m	0.306 klm	0.354 fg	0.350 fghi	0.324 c
Bumper agar	0.355 fg	0.391 d	0.388 de	0.348 fghij	0.370 b
Moringa leaves agar	0.335 ghijk	0.388 d	0.350 fghi	0.349 fghi	0.355 b
Corncob agar	0.320 ijkl	0.410 abcd	0.386 de	0.351 fgh	0.367 b
Wheat bran agar	0.377 ef	0.425 ab	0.402 abcde	0.392 cde	0.399 a
Barley bran agar	0.376 ef	0.404 abcde	0.402 abcde	0.376 ef	0.389 a
Sawdust agar	0.198 o	0.223 no	0.298 lm	0.341 fghij	0.265 e
Whey cheese agar	0.301 lm	0.353 fg	0.233 n	0.278 m	0.291 d
Mean	0.324 c	0.372 a	0.365 ab	0.360 b	

LSD at P=0.05 for medium = 0.015, for concentration = 0.007, for medium x concentration = 0.030
Values followed by the same letters in the same row or column are not significantly different at P=0.05

Table 2. Effect of pH on the growth rate of *Lentinula edodes* RSR strain in selected media at concentration of 20 g/L.

	pH				
Medium	**6**	**6.5**	**7**	**7.5**	**Mean**
Potato dextrose agar	0.332 m	0.391 ilj	0.371 k	0.360 kl	0.363 g
Wheat flour agar	0.422 efg	0.498 ab	0.456 c	0.385 j	0.440 c
Barely flour agar	0.429 de	0.489 b	0.458 c	0.399 hilj	0.443 bc
Rice bran agar	0.422 efg	0.500 ab	0.452 c	0.414 fg	0.447 b
Oat flour agar	0.447 c	0.499 a	0.434 d	0.410 gh	0.447 b
Corn cob agar	0.410 gh	0.455 c	0.423 ef	0.399 hilj	0.421 d
Wheat bran agar	0.425 de	0.499 ab	0.433 d	0.420 efg	0.444 bc
Barely bran agar	0.429 de	0.502 a	0.489 b	0.401 hi	0.455 a
Bumper leaves agar	0.393 ilj	0.411 gh	0.399 i	0.323 n	0.382 f
Moringa leaves agar	0.388 lj	0.422 efg	0.410 gh	0.349 l	0.392 e
Whey cheese agar	0.351 l	0.387 lj	0.300 o	0.292 o	0.333 h
Mean	0.404 c	0.459 a	0.420 b	0.377 d	

$LSD_{0.05}$ for Medium = 0.006, pH = 0.003, Medium x pH =0.012
Values followed by the same letters in the same row or column are not significantly different at P=0.05

Table 3. Effect of Temperature on the growth rate of *Lentinula edodes* RSR strain in selected media at concentration of 20 g/L and pH of 6.5.

	Temperature °C				
Medium	**21**	**23**	**25**	**27**	**Mean**
Potato dextrose agar	0.389 s	0.448 n	0.390 s	0.388 s	0.403 j
Wheat flour agar	0.498 f	0.654 a	0.500 f	0.488 hi	0.535 a
Barely flour agar	0.488 hi	0.523 d	0.489 hi	0.478 j	0.494 d
Rice bran agar	0.488 hi	0.599 b	0.499 f	0.461 l	0.511 b
Oat flour agar	0.389 s	0.511 e	0.500 f	0.487 hi	0.471 f
Corn cob agar	0.389 s	0.486 i	0.455 m	0.406 r	0.434 h
Wheat bran agar	0.466 k	0.589 c	0.486 i	0.401 r	0.485 e
Barely bran agar	0.487 hi	0.524 d	0.502 f	0.498 f	0.502 c
Bumper leaves agar	0.420 p	0.433 o	0.412 q	0.389 s	0.413 i
Moringa leaves agar	0.457 lm	0.499 f	0.422 p	0.420 p	0.449 g
Whey cheese agar	0.387 s	0.492 gh	0.387 s	0.377 t	0.411 i
Mean	0.441 c	0.523 a	0.458 b	0.435 d	

$LSD_{0.05}$ for Medium = 0.002, Temperature = 0.001, Medium X Temperature = 0.005
Values followed by the same letters in the same row or column are not significantly different at P=0.05.

Furthermore, Medany (2011) reported that the optimum temperature for mycelial growth of *L. edodes* appeared to be 25ºC. In addition, Hassegawa *et al*. (2005) reported that the optimum growth temperature for *Lentinula. edodes* was 20ºC in yeast extract mannitol broth with rice bran, in contrary with Dulay *et al*. (2021) who found that 14 native *Lentinus* isolates preferred to grow below 30ºC as the optimum temperature, an indication that they were tropical mushroom.

Light and Darkness- All natural sources media were tested under light and darkness in addition to the appropriate conditions identified in the previous stages (concentration 20g/L, pH 6.5, Temperature 23ºC). The results obtained showed that the highest growth rate was obtained under continuous darkness for all tested media was 0.772 cm/day for wheat flour agar media, and the lowest was 0.469 cm/day for the bumper leaves agar media as compared to 0.499 cm/day for the standard PDA medium (Table 5). Similar results were obtained by several workers (Baghel, 2012; Quaicoe *et al.*, 2014; Tapingkae, 2005).

Estimation of the growth density of shitake mushroom mother culture grown on different media

The mycelium density in different natural media was evaluated based on the weight of the growing mycelium in the media at the end of the incubation period (Table 4). There were significant differences in mycelium growth density among the different media used, with wheat flour agar medium gave the highest growth density (1.102 gm/plate), whereas the whey cheese agar medium gave the lowest density (0.283 g/plate) compared with PDA (0.500 g/plate).

Table 4. The Growth Density of *Lentinula edodes* mushroom when grown on different natural media.

Medium	Mean growth density (gm/plate)
Potato dextrose agar	0.500 i
Wheat flour agar	1.102 a
Barely flour agar	0.994 b
Rice bran agar	0.981 c
Oat flour agar	0.912 d
Corn cob agar	0.900 f
Wheat bran agar	0.905 e
Barely bran agar	0.886 g
Bumper leaves agar	0.791 h
Moringa leaves agar	0.787 h
Whey cheese agar	0.283 j
$LSD_{0.05}$	0.004

Values followed by the same letters in the same column are not significantly different at P=0.05

It can be concluded from this study that wheat flour agar, barley flour agar, rice bran agar, barley bran agar at 20 g/L concentration were the best natural media prepared from agro-wastes which led to high growth rate of cultivation of *L. edodes* RSR strain, the optimum temperature was 23ºC, the best pH was 6.5, and the best growth rate was under continuous dark conditions. In addition, the best mycelial growth and density was obtained on wheat flour agar media.

Table 5. Effect of light and darkness on the growth rate of *Lentinula edodes* RSR strain at 20 g/L concentration, pH 6.5 and temperature of 23°C.

	Illumination		
Medium	Light	Dark	Mean
Potato dextrose agar	0.447 k	0.499 hi	0.473 e
Wheat flour agar	0.654 c	0.772 a	0.713 a
Barely flour agar	0.522 fg	0.652 c	0.587 c
Rice bran agar	0.599 d	0.710 b	0.654 b
Oat flour agar	0.507 gh	0.652 c	0.579 c
Corncob agar	0.488 i	0.530 f	0.509 d
Wheat bran agar	0.588 d	0.771 a	0.679 b
Barely bran agar	0.522 fg	0.702 b	0.612 c
Bumper leaves agar	0.420 l	0.469 j	0.444 f
Moringa leaves agar	0.457 jk	0.552 e	0.504 de
Mean	0.520 b	0.631 a	

$LSD_{0.05}$ for Medium = 0.034, Light = 0.015, Medium x Light = 0.048

الملخص

صاحب، رشا سلام، شذى علي شفيق ورقيباء علي جيجان. 2022. الظروف المثلى لاستخدام المخلفات الزراعية الطبيعية المختلفة لتطوير وسط استزراع محلي مناسب لإنتاج المزارع الأم للفطر شيتاكي البري العراقي *Lentinula edodes* (السلالة RSR). مجلة وقاية النبات العربية، 40(4): 356–361. https://doi.org/10.22268/AJPP-40.4.356361

أجريت هذه الدراسة لتحديد الظروف المثلى لاستخدام المخلفات الزراعية الطبيعية المختلفة لتطوير وسط استزراع محلي مناسب لإنتاج المزارع الأم لسلالة الفطر شيتاكي البري العراقي *Lentinula edodes* RSR، حيث تمّ استخدام أوساط طبيعية مختلفة مكوّنة من القمح، الشوفان، الشعير، كيزان الذرة، البازلاء الخضراء، الحمص، نخالة القمح، نخالة الرز، نخالة الشعير، أوراق البمبر، أوراق المورينغا، نشارة الخشب وجبن مصل اللبن. كما استخدم الوسط الغذائي أجار ديكستروز البطاطا/البطاطس للمقارنة بتركيز 10 إلى 40 غ/ليتر. وجد أن أفضل تركيز لتحضير هذه الأوساط هو 20غ/ ليتر عند درجة حرارة 23 °س ورقم هيدروجيني 6.5 وفي ظروف مظلمة. وتمّ الحصول على أفضل كثافة فطرية عند استخدام وسط دقيق القمح، والتي يمكن اعتمادها في إنتاج الغزل الفطري لهذه السلالة.

كلمات مفتاحية: مخلفات زراعية، استزراع فطر شيتاكي، الفطر الزراعي، عوامل بيئية.

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