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IMPACT OF DIFFERENT SUBSTRATES OF SPAWN PRODUCTION AND CALCIUM CARBONATE ON THE GROWTH AND YIELD OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

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ABSTRACT

This experiment was undertaken at mushroom production unit in the college of Agricultural Engineering Sciences, Duhok University, Kurdistan Region-Iraq, during the growing year 2021 and 2022, to investigate the effect of different substrates of spawn production (oat, corn, millet, wheat, canary seed) and calcium carbonate (with or without) on the growth and yield of oyster mushroom (*Pleurotus ostreatus*). The results showed that corn grain was significantly recorded fastest complete colonization of mycelium after (7) days followed by millet, canary seed, oat, and wheat which recorded (9, 10, 12, and 13) days respectively and they were not affected by any pests after 6 months in refrigerator as shelf life of spawn. However, millet and $CaCO_3$ significantly recorded the shortened number of days for the viewing first pinhead, whereas canary seed significantly obtained the higher number of heads per bags, but treatments without using $CaCO_3$ significantly enhanced heads number and head length per bags. Concerning the fruit weight, head diameter and fruit dry weight did not record any significant differences in the single effect of two factors. On the other hand, the interactions between millet or corn grains with using $CaCO_3$ had significant effect on higher head diameter and minimum number of days respectively for the viewing first pinhead per bags.

KYEWORDS: *Pleurotus ostreatus,* Oyster Mushroom, Spawn, Calcium Carbonate, Straw.

INTRODUCTION

Oyster mushroom is an edible mushroom having excellent flavor and tastes. The common name comes from the white shell, which is like appearance of the fruiting body (Ghareeb, 2019). They are classified based on edibility and shape of the fruit body (Ibekwe *et al.*, 2008). *Pleurotus* species are popular and widely grown throughout the world mostly in Asia and Europe owing to their simple and low-cost production technology and higher biological efficiency (Mane *et al.*, 2007). In general, mushrooms are highly being utilized as important food products for their significant role in human health, disease control and nutrition (Chang and Miles, 1989). They are rich source of proteins, vitamins, carbohydrates, and minerals (Ananbeh, 2003), like potassium, phosphorus, calcium, and sodium (Manzi *et al.*, 1999), but poor in fat and calories while rich in proteins, chitin, and vitamins, particularly riboflavin, niacin, B1, B5, B6, C, D, and K (Ahmed *et al.*, 2009).

Cultivating oyster mushroom has extremely increased throughout the world because of their abilities to grow at a wide range of temperature and harvested all year round (Amin *et al.*, 2007), because *ostreatus* is one of the most popular oyster mushroom species that can grow on different agricultural wastes (Sultana *et al.*, 2018), such as wheat straw (Ananbeh and Almomany, 2005). However, growing oyster mushrooms can convert a high percentage of lignocellulosic substrate to fruiting bodies (Sharma *et al.*, 2013). On the other hands, there are other factors that affect mushroom growth includes temperature, moisture percentage, pH and light intensity (Kadiri, and Kehinde, 1999).

Mushrooms can be grown from spawn which is mycelium of mushroom that grow on a different substrate and used like a seed for mushroom production, mainly cereal grains are used for spawn production of mushroom. Hence, to produce spawn production, inoculate a pasteurized medium usually different kinds of grains with the sterile culture of a particular mushroom species, after the culture has grown throughout the medium it is called spawn (Stanley, 2010). However, Hossain, (2018) showed that quality of spawn determines both quality and yield of mushroom cultivation, and different grain substrates such as wheat, barley, millet, maize, oat, and sorghum influenced mycelial growth of *Pleurotus* species for spawn production (Mishra *et al.,* 2018). Nwanze *et al.* (2005) tested the effect of spawn grains such as corn, millet, and wheat on the culture of *Lentinus squarrosulus* mushroom, his results shown that corn spawn induced highest yield and dry weight of fruiting as compared to millet and wheat spawn.

In despite of that, calcium carbonate ($CaCo_3$) is used in cultivation of mushroom to enhance pH of substrate (Wajid Khan *et al*., 2013), Although mycelium of mushroom obtains nutrients from substrate at specific level of pH (Sarker *et al*., 2007). Thus, rapid mycelial growth of *Pleurotus* species takes place at pH between (6.4 to 7.8) (Iqbal and Shah, 1989). Therefore, the aims of the study are to determine the effect of different kinds of spawn grains (oat, corn, millet, wheat, canary seed) and calcium carbonate on the growth and yield of oyster mushroom (*Pleurotus ostreatus*).

MATERIALS AND METHODS

The experiment was carried out in the mushroom production unit at College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region-Iraq during the year 2021 to 2022. The study was arranged in a complete randomized design (CRD) with 10 treatments, 3 replications (10X3) and 30 experimental units. Also, the data was recorded according to Duncan's test at (5%) level, and the data was analysed by (SAS, 2007) program.

Spawn preparation

One kilogram of five different kinds of grains includes (canary seed, oat, wheat, millet, and corn) which individually immersed in hot water for 24 hours, and let overnight to reducing moisture near 50% then added enough amount of calcium carbonate and calcium phosphate to adjusting pH and grain texture. After that, 225 g of every type of grains added in conical flask that volume (500 ml) and sterilized by autoclave at 15 lbs pressure and 121°C for 90 minutes. Concerning, each type of grains has 3 conical flasks. However, for grains isolation, pure cultures of mycelium were obtained in the previous research that titled "Influence of glucose and peptone on the mycelial growth of oyster mushroom (*Pleurotus ostreatus*)" (Edo, 2021). Finally, under aseptic conditions the pure mycelium in the petri dishes (90 x 15) mm cutting off into two half and one half transferred to each conical flask that contains 225 g of grains, and incubated at temperature 28°C for mycelium colonization of oyster mushroom (Hoa and Wang, 2015) for 21 days.

Calcium carbonate

Calcium carbonate ($CaCO_3$) factor consists of two levels, involving, (0 and 500 g) per 25 kg of wet straw for pH adjusting between 7 to 8. In this research the bags of wheat straw immersed in water for 48 hours to get enough amount of water then boiled the straw in hot water for 30 minutes, after that, split straw into two parts each part contains (25 kg) of wet straw then mixed 500 g of $CaCO_3$ (chalk) to one part and other part let without ($CaCO_3$). Finally, 110 g of spawn mixed with 1600 g of sterilized straw were filled in the polyethylene bags, which incubated at 25 ºC and more than 75% moisture in the growing room.

Measurements

- **Mycelium colonization:** complete colonization of mycelium in each conical flask was visually recorded daily by the naked eye.
- **Shelf life of spawn:** spawn placed in refrigerator at (4 ºC) for six months.
- **First pinhead (days):** recorded after observing first pinhead from spawn run.
- **Number of fruit (No.):** single fruit for each head counted but only for the first flush (harvest).
- **Head length (cm):** was measured using roller from the base of the main stem to the peak of cap.
- **Head diameter (cm):** head cap diameter was measured by using roller.
- **Fruit weight (g):** only first flush (harvest) was measured for fruiting weight.
- **Fruit dry weight (g):** 50 g of fresh fruits for each experimental unit placed in oven at 72 ºC until the weight was stabilised.

RESULTS

1. Mycelium colonization

Figure (1) registered that corn grain was significantly recorded complete colonization of mycelium after (7) days followed by millet, canary seed, oat, and wheat which recorded (9, 10, 12, and 13) days respectively.

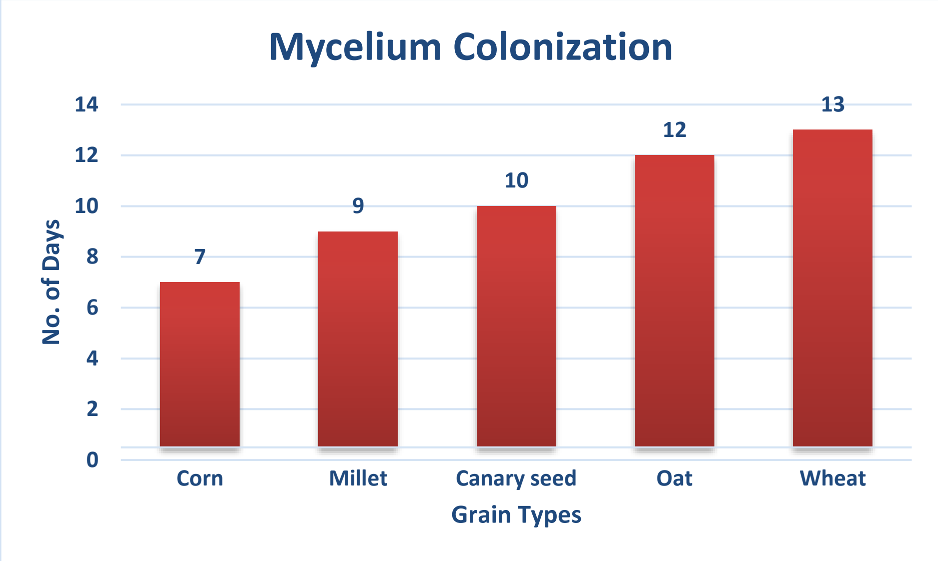

Fig.(1): Fully colonization of mycelium growth after inoculation (oyster spawn).

2. Shelf life of spawn

After complete colonization of grains in conical flask were stored in refrigerator for six months for testing spawn shelf life, meaning that how much mycelium will stay viable and testing of spawn for grown, whereas the grain did not affected by any pests as shown in the figure (2). Then the spawn grown in straw for guarantee success and testing of rest parameters. However, this spawn was mother spawn (F1).

Fig.(2):- Grain types of oyster mushroom in refrigerator after 6 months at 4 ºC.

3. First pinhead per days

The results in table (1) shows that there was a significant difference between $CaCo_3$ levels, the minimum number of days were observed for the first pinhead of oyster mushroom when $CaCo_3$ is used, which is (18.07) days compared to (20.73) days for not using $CaCo_3$. Also, grain types significantly shortened number of days for the viewing first pinhead, which millet obtained (18.00) days, and wheat record (21.33) days. The binary interactions between $CaCo_3$ levels and grain types shows a significant effect on first pinhead and the best results are observed with using $CaCo_3$ and millet grain that had (15.67) days compared to wheat grain which recorded (21.33) days for the observing first pinhead.

Table (1): Effect of $CaCo_3$, grain types and their interaction on the number of days for the first pinhead observing of oyster mushroom.

$CaCo_3$ Levels	Grains type					Effect of $CaCo_3$
	Corn	Canary	Oats	Wheat	Millet	
Without $CaCo_3$	20.67 ^{bc}	20.33 ^{bc}	21.00 ^{bc}	21.33 ^{c}	20.33 ^{bc}	20.73 ^{b}
With $CaCo_3$	17.67 ^{ab}	19.00 ^{bc}	17.67 ^{ab}	20.33 ^{bc}	15.67 ^{a}	18.07 ^{a}
Effect of Grains	19.17 ^{ab}	19.67 ^{ab}	19.33 ^{ab}	20.83 ^{b}	18.00 ^{a}	

* Means with same letter for each factor and interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

4. Number of heads per bag

Data in table (2) reveled that there is a significant increase in number of heads per bag without applying $CaCo_3$, at rate (6.47) compared to applying $CaCo_3$ (4.20). The grain types significantly affected on this parameter, the highest number (6.67) was recorded by using Canary grain. The interaction between $CaCo_3$ level and Grain types achieve a maximum value, without applying $CaCo_3$ and Canary grain at rate (9) heads per bag, compared to other means.

Table (2): Effect of $CaCo_3$, grain types and their interaction on the number of heads per bag of oyster mushroom.

$CaCo_3$ Levels	Grains type					Effect of $CaCo_3$
	Corn	Canary	Oats	Wheat	Millet	
Without $CaCo_3$	7.33 ^{ab}	9.00 ^{a}	6.00 ^{bc}	3.00 ^{d}	7.00 ^{ab}	6.47 ^{a}
With $CaCo_3$	2.33 ^{d}	4.33 ^{cd}	5.67 ^{bc}	5.67 ^{bc}	3.00 ^{d}	4.20 ^{b}
Effect of Grains	4.83 ^{b}	6.67 ^{a}	5.83 ^{ab}	4.33 ^{b}	5.00 ^{b}	

* Means with same letter for each factor and interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

5. Fruit weight per bag

The data presented in table (3) shows that there were no significant differences between treatments in the fruit weight per bag when applying $CaCo_3$ level and grain types. Also, the binary interaction between $CaCo_3$ level and grain types have no significant effect on fruit weight per bag.

Table (3): Effect of CaCo3 level, grain types and their interaction on the fruit weight per bag of oyster mushroom.

$CaCo_3$ Levels	Grains type					Effect of $CaCo_3$
	Corn	Canary	Oats	Wheat	Millet	
Without $CaCo_3$	244.13 ^{a}	206.65 ^{a}	192.88 ^{a}	218.10 ^{a}	175.40 ^{a}	207.43 ^{a}
With $CaCo_3$	279.86 ^{a}	221.28 ^{a}	238.09 ^{a}	257.06 ^{a}	219.55 ^{a}	243.17 ^{a}
Effect of Grains	262.00 ^{a}	213.96 ^{a}	215.49 ^{a}	237.58 ^{a}	197.48 ^{a}	

* Means with same letter for each factor and interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

6. Head length per bag

Table (4) revealed that the treatment without calcium carbonate gave highest value (10.23 cm) of head length per bags. However, the maximum head length of oyster mushroom (10.53 cm), obtained from the wheat grains, and the minimum head length (8.71 cm) was obtained in millet grains. Regarding the dual interactions between $CaCo_3$ and grain types, noticed that without using $CaCo_3$ with corn or wheat grains significantly increased (11.18 and 11.08) cm respectively of head length per bags, and the lower length (8.08 cm) of head (fruit) per bags was observed in millet as shown in the table (4).

Table (4): Effect of $CaCo_3$, grain types and their interactions on the head length of oyster mushroom.

$CaCo_3$ Levels	Grains type					Effect of $CaCo_3$
	Corn	Canary	Oats	Wheat	Millet	
Without $CaCo_3$	11.18 ^{a}	9.99 ^{ab}	9.58 ^{ab}	11.08 ^{a}	9.34 ^{ab}	10.23 ^{a}
With $CaCo_3$	8.63 ^{b}	9.33 ^{ab}	9.30 ^{ab}	9.99 ^{ab}	8.08 ^{b}	9.07 ^{b}
Effect of Grains	9.91 ^{ab}	9.66 ^{ab}	9.44 ^{ab}	10.53 ^{a}	8.71 ^{b}	

* Means with same letter for each factor and interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

7. Head diameter per bag

Table (5) shows that both levels of $CaCo_3$ and all types of grains did not obtain significant differences in the diameter of head in oyster mushrooms. Whereas there is a significant difference observed in the double interactions of $CaCo_3$ and grains type, when oyster substrate treated by $CaCo_3$ and mixed with corn grains recorded significant head diameter, which gave (15.94 cm) per bags.

Table (5): Effect of $CaCo_3$, grain types and their interactions on the head diameter of oyster mushroom.

$CaCo_3$ Levels	Grains type					Effect of $CaCo_3$
	Corn	Canary	Oats	Wheat	Millet	
Without $CaCo_3$	10.72 ^{b}	12.56 ^{ab}	12.09 ^{b}	13.65 ^{ab}	11.01 ^{b}	12.00 ^{a}
With $CaCo_3$	15.94 ^{a}	11.77 ^{b}	13.38 ^{ab}	12.64 ^{ab}	11.32 ^{b}	13.01 ^{a}
Effect of Grains	13.33 ^{a}	12.16 ^{a}	12.73 ^{a}	13.15 ^{a}	11.17 ^{a}	

* Means with same letter for each factor and interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

8. Fruit dry weight

Tabulated data in table (6) observed that both $CaCo_3$ and grains spawn had no significant effect on the fruit dry weight of oyster mushroom. However, the same table clarified that the twin interactions between two factors under the study also had no significant effect on the fruit dry weight of oyster mushroom.

Table (6): Effect of $CaCo_3$, grain types and their interactions on the fruit dry weight of oyster mushroom.

$CaCo_3$ Levels	Grains type					Effect of $CaCo_3$
	Corn	Canary	Oats	Wheat	Millet	
Without $CaCo_3$	4.56 ^{a}	3.81 ^{a}	4.74 ^{a}	4.49 ^{a}	3.29 ^{a}	4.18 ^{a}
With $CaCo_3$	3.98 ^{a}	4.44 ^{a}	4.18 ^{a}	3.32 ^{a}	3.03 ^{a}	3.79 ^{a}
Effect of Grains	4.27 ^{a}	4.12 ^{a}	4.46 ^{a}	3.91 ^{a}	3.16 ^{a}	

* Means with same letter for each factor and interaction are not significantly different at 5% level based on Duncan's Multiple Rang Test.

DISCUSSIONS

The results shows that corn grain was the best spawn for complete colonization of oyster mycelium growth due to large size of corn grains and have large pore space, hence, increased oxygen concentration causes to increased mycelial growth this is confirmed by (Mbogoh, *et al.*, 2011 and Kenealy, and Dietrich, 2004) who reported that mycelial growth increased with increasing oxygen concentration within spawn grain substrates. Also corn have more nutrients for mycelial growth than smaller grains (Mottaghi, 2006). Then followed by millet because millet grains are best carbohydrate source for mycelium propagation (Kumbhar, 2012).

However, oyster mushroom (*Pleurotus ostreatus*) growth and yield characters significantly influenced by different substrates of spawn and calcium carbonate. Different substrates of spawn significantly effect on some characters of oyster mushroom, which was number of days for the first pinhead and number of heads per bag as illustrated in tables (1 and 2), may be this was happened as a result of the surface area of grain, meaning that the smaller grain have more surface area than the larger grain which resulted in faster inoculation (mycelium growth inside the straw) of substrates. The same results were reported by (Mamiro and Royse, 2008) which showed that smaller grains have a greater number of inoculation points per kg than larger grains or may be as a result of temperature and humidity as reported by Shah *et al.* (2004) which recorded that spawn run and pinhead formation were observed at 25 ◦C.

Also head length of oyster mushroom significantly affected by spawn types and the best results was recorded by wheat grain, as shown in table (4), this result may be due to the size of grain where the bigger grains have a greater food reserve (Elliot, 1985) and can sustain the mycelium for longer periods of time during stress (Fritsche, 1988), Thus, different kinds of spawn may influence on the growth and productivity (Pathmashini *et al*., 2008). However, calcium carbonate positively influenced in the number of days for the first pinhead as showed in table (1) this result is agreed with (Khan *et al*., 2013) which showed that cotton waste containing 2% lime was proved one of the best for cultivation of oyster mushroom compared to other variables of lime which are 0%, 4% and 6%, that significantly effect on the number of days taken for initiation of pinheads. Whereas calcium carbonate does not effect on the other parameters which are number of heads per bag, fruit weight per bag, head length, head diameter and fruit dry weight.

CONCLUSIONS

In conclusion, the study results shows that growing oyster mushroom were the best when mixed calcium carbonate with its substrate. Also, millet grains cause early harvesting of oyster mushroom compared to other types of grains. Concerning the singular influences of grains and $CaCO_3$ did not recorded any significant changes in the fruit weight, fruit (head) diameter and fruit dry weight. However, the binary interactions between millet and corn with using $CaCO_3$ significantly influenced on head diameter and shortened period of days for the viewing first pinhead respectively. Also corn grain was significantly affected on complete colonization of mycelium followed by millet, canary seed, oat, and wheat. Whereas grain types did not affect by shelf life of oyster spawn after 6 months in refrigerator.

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تأثير الأوساط المختلفة لإنتاج البذار وتأثير كربونات الكالسيوم على نمو وإنتاج فطر المحاري (*Pleurotus ostreatus*)

الخلاصة

أجريت هذه الدراسة في وحدة إنتاج الفطر في كلية علوم الهندسة الزراعية، جامعة دهوك، أقليم كردستان العراق، خلال سنة 2021 و 2022 ، لدراسة تأثير الاوساط المختلفة لإنتاج بذار الفطر (الشوفان، الذرة، الدخن، القمح، وبذور الكناري) وتأثير كربونات الكالسيوم على نمو وحاصل فطر المحاري (*Pleurotus ostreatus*). بينت النتائج أن حبوب الذرة سجلت بشكل معنوي استعمار كامل للميسيليوم بعد (7) أيام يليها الدخن وبذور الكناري والشوفان والقمح التي سجلت (9 ، 10 ، 12 ، 13) يوما على التوالي، ولم يتلف أي من أنواع البذور بعد 6 أشهر في الثلاجة كعمر تخزيني. بالنسبة للدخن و كاربونات الكالسيوم ($CaCO_3$) أثرت بشكل معنوي على عدد الأيام المختصرة لأول رأس الفطر، بينما حصلت بذور الكناري على عدد أكبر من الرؤوس لكل كيس، أما المعاملات بدون استخدام كربونات الكالسيوم $CaCO_3$ تسببت بزيادة كبيرة في عدد الرؤوس وطول الرأس لكل كيس. وكذلك فيما يتعلق بالتأثير الفردي للعاملين لم تسجل أي فروق معنوية في وزن الثمرة وقطر الرأس و وزن الجاف للثمار. من ناحية أخرى أعطت التأثيرات المزدوجة بين حبوب الدخن والذرة مع استخدام كربونات الكالسيوم قطرًا كبيرًا للرأس وعددًا أدنى من الأيام على التوالي لرأس الفطر لكل كيس.

الكلمات المفتاحية: *Pleurotus ostreatus*، فطر المحاري، القش، كربونات الكالسيوم، سباون.

كارتێكرنا ناوەندێن جیاواز یێن بەرهەم ئینانا توڤی وكارتێكرنا كاربوناتا كالسیوم لسەر گەشە و بەرهەمێ كڤاركا سەدەفی (Pleurotus ostreatus)

پوختە

ئەڤ ڤەكولینە یا هاتیە ئەنجام دان ل یەكا بەرهەم ئینانا كڤاركا ل كولیژا زانستێن ئەندازیاریا چاندنێ، زانكویا دهوك، هەرێما كوردستانێ، عیراق، ل سالا (2021 - 2022). ژ بو تاقیكرنا كارتێكرنا ناوەندێن جیاواز یێن بەرهەم ئینانا توڤێ كڤاركا (شوفان ، گەنموك ، گارس ، گەنم و خارنا بالندێ كناری) و كارتێكرنا كاربوناتا كالسیوم لسەر گەشەو بەرهەمێ كڤاركا سەدەفی (Pleurotus ostreatus). ئەنجاما دیار كرن كو بكار ئینانا گەنموكا رێژا بەلاڤبونا مایسلیومی یا تمامبویی پشتی بورینا (7) روژا، و پشتی وێ گارس و خارنا بالندێ كناری و شوفان و گەنم دهێن كو ئەڤ ئەنجام یێن تومار كرین (9، 10، 12 ، 13) ل دیڤئێكدا، و چ جورێن توڤا ژناڤ نەچون پشتی (6) هەیڤا د سەلاجێ دا وەك ژیێ كوگەهكرنێ. سەبارەت گارس و كاربوناتا كالسیوم (CaCO3) كارتێكرنەكا بەرچاڤ كرە سەر ژمارا روژا بو دەركەفتنا ئێكەم سەرێ كڤاركێ. ژلایەكێ دیڤە خارنا بالندێ كناری بلندترین ژمارا سەركێن كڤاركا تومار كرن بو هەر كیسەكی. هەروەسا ئەو هوكارێن بێ بكارئینانا كاربوناتا كالسیوم (CaCO3) بونە ئەگەرێ زێدەبونا ژمارا سەركێن كڤاركا و درێژاهیا سەركا بو هەر كیسەكی بو بلندترین ئاست. سەبارەت كارتێكرنا تاك یا هەر هوكارەكی چ كارتێكرنێن بەرچاڤ تومار نەكرن د كێشا كڤاركا دا و تیرەیا كڤاركا و كێشا هشك یا كڤاركا. ژ لایەكێ دیڤە كارتێكرنێن جوت دناڤبەرا گارسی یان گەنموكا دگەل بكارئینانا كاربوناتا كالسیوم بونە ئەگەرێ زێدەبونا تیرەیێ سەركێن كڤاركا و كێمبونا روژێن پێدڤی بو دەركەتنا وان.

پەیڤێن دەستپێكی: Pleurotus ostreatus ، كڤاركا سەدەفی ، قەسەل ، كاربوناتا كالسیوم ، سباون.