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USING WATER EXTRACT OF COMMON REED AND JOHNSEN GRASS FLOWERS FOR INCREASING YIELD AND STORAGE LIFE OF KING OYSTER MUSHROOM

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Abstract

The objective of this research was to investigate the effect of nourishing with inflorescence aqueous extracts of reed and Johnsen grass on the productivity of king oyster mushroom under the cold storage conditions. The treatments were implemented at the beginning of the pin formation stage where equal sizes, 50 cc, of the concentrations 0%, 5%, 10% and 20% from each extract were used. Having the fruit bodies picked, they were dried and the phenols and proteins within them were estimated within them. Results showed that nourishing the king mushroom with the influence extract of reed at the concentration of 20% and of Johnson grass at the concentration of 10% increased the production roughly to the double in addition to increasing the biological efficiency of the cultural media; yet, the best between these two treatments was treating the mushroom with 20% of reed inflorescence extract as it recorded the highest weight fruit bodies in addition to an increase in the fresh yield accompanied by an increase in the dry yield and dry matter in the fruit bodies pre and post storage. Moreover, nourishing with the mentioned extracts increased the nutrition value of king mushroom through increasing the protein percentage and phenol content in the fruit bodies of king mushroom.

Key words: King oyster , Nutrition ,Protein, Phenolic compounds, Biological efficiency

Introduction

King oyster mushroom *Pleurotus eryngii* is one of the most important primary decomposer fungi belonging to the oyster fungi group (Obodai *et al.,* 2003) that is characterized by nutritional and medicinal importance due to its fruit body content of a high percentage of protein reaching 53% as well as many vitamins and minerals important for the human body growth (Patel *et al.,* 2012). China is considered the first country that has been able to grow the king mushroom (Hassan *et al.,* 2010). This mushroom is distinguished by its rapid growth and formation of a thick juicy stem with a large-sized fleshy hat with a diameter of more than 15 cm and a fruit body weight reached 70 and 80 g (Hassan *et al.,* 2010; Kirbag and Akyuz, 2008). It grows abundantly on crop residuals such the straw of wheat and rice as well as the residuals of cotton, sugar cane, Johnson grass, and date palm; moreover, this mushroom production greatly responses for adding the mentioned supporters included rice and wheat flour bran to the culturing media (Hassan, 2011; Szarvas, 2011; Al-Badrany, 2010; Hassan *et al.,* 2010; Kirbag and Akyuz, 2008); furthermore, injecting aqueous extract nutrients into the bags containing culturing media during the production stage of the oyster mushroom *Pleurotus ostreatus* increases the yield as these extracts such as the aqueous extract of licorice, black seeds, fava bean powder highly contain nutrients plant and hormones, especially those, contain a proper carbon to nitrogen ratio which is important for this stage (Abdul-Qader *et al.,* 2019; Rustum, 2014; Abdul-Qader *et al.,* 2018). Carrasco *et al.,* (2018) referred that using different herbs residuals as nutritional additives to the culturing media showed clearly significant chemical content in the fruit body, as well as the dry and total yield of fruit bodies, belong to the genus *Pleurotus*. Rustum *et al.,* (2018) found that using an aqueous extract of Johnsen Grass flowers at the concentration of 10% significantly increased the traits of total yield, fruit body weight, biological efficiency and dry yield of the mushroom *P. ostreatus* however, using

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the extract of common reed flowers at the concentration of 20% in injecting way decreased these traits. Given the absence of the previous sufficient studies in Iraq dealing with nourishing the king mushroom with aqueous extract of flowers taken from weeds grown in Iraq, except for a study investigating the effect of different concentrations of licorice on the production and storage capability of the king mushroom (Rustum, 2014), this research aimed to probe the effect of nourishing with the flower extracts of common reed and Johnsen Grass on the king oyster mushroom under the cold storage condition.

Materials and Methods

The experiments were implemented in the Medicinal and Food Fungi Production Project/College of Agriculture Engineering/ University of Baghdad during the period from 1/2/2012 to 30/6/ 2013. Pure isolation of the king oyster mushroom *Pleurotus eryngii* was obtained from the JANCOA Institute in the People's Republic of China. The isolation was activated on WFA media comprised of Agar and wheat flour at the rate of 1:1 poured in Petri dishes with a diameter of 9 cm inside a sterile cabinet laminar flow. The Petri dishes were incubated in a dedicated incubator at a temperature of 25±1°C. After the fungal mycelium growth was completed, it was loaded on boiled disinfected wheat grains placed in glass jars of 250g capacity (Kirbag and Akyuz, 2008; Oei, 2005). The jars were incubated at the same incubator at the same temperature degree mention above. Having the mycelia on the wheat grains completed, the rate of 5% was cultured on media comprised 20% of flour bran added to the wet sterilized wheat straw (Muslat, 2002; Vijay *et al.,* 2002; Vijay and Sharma, 1996) packed in transparent plastic bags sized1 kg capacity (Martinez. 1998). The inoculated bags were transferred to a thermally isolated incubation chamber sized 3×4×2.5 m where the temperature was fixed on 25±2°C using a split unit air-conditioner powered 1.5 tons (Abdulhadi, 2010). After 30 days needed for the mycelia to be completed on all media bags, the incubation chamber was converted into a growth chamber (Kirbag and Akyuz, 2008) through increasing the moisture to 80-90% with a humidifier yet the temperature was still 25±2°C. The temperature and relative moisture were monitored with thermo-hygrograph placed inside the chamber. The bags were punched with an equal number of holes to the light facing side and then the following experiments were carried out:

1- First experiment: using the flower extract of common reed (*Phragmites communis*) to increase the king oyster mushroom production

Having the reed inflorescences were collected and dried, four aqueous extract concentrations, 0%, 5%, 10% and 20%, were prepared according to the method described by Rustum *et al.,* (2018). A veterinary syringe with a capacity of 50 cm^3 was used for adding the aqueous extracts as an injection to the bags during the pin formation stage. Each concentration involved five bags representing five replicates. Then, the data were collected when the fruit bodies reached the required size.

2- Second experiment: using the flower extract of Johnson grass (*Sorghum halepense*) to increase the king oyster mushroom

The Johnson grass inflorescences were collected and dried. The aqueous extract was prepared as mentioned in the first experiment for reed and the same concentrations, 0%, 5%, 10% and 20%, as well as concerning the treatment implementation and data collection.

3- Third experiment: effect of the inflorescence extracts of reed and Johnson grass on the storage capability of the king oyster mushroom

The fruit bodies produced from the first and second experiments were picked to measure the fresh weight. From the first and second experiments, 200g of homogeneous fruit bodies were taken individually and placed in plastic containers prepared for this purpose. Then they were wrapped with transparent plastic film lids, brand falcon Abdulhadi, 2010) knowing that the number of replicates in the experimental treatments was five represented by one container of each. Next, they were stored in refrigerated incubators prepared for this purpose at a temperature of $\pm 2^0$C for three weeks. Having the storage period ended, the required measurements were taken.

1- Total yield based on the fresh weight of the fruit bodies: it was measured in each replicate and calculated for each bag based on $g.kg^{-1}$ moist straw.

2- Dry matter percentage: from each replicate, 100g of the fruit bodies were taken pre and post storage and chipped into small pieces. Then they were dried with a fan- equipped electric oven at a temperature of 60°C until the weight stability (Dundar, 2008). After that, the dry matter percentage was calculated according to the following equation: Dry matter% = dry weight of the fruit bodies/ the fresh weight × 100

3- Biological efficiency (B.F): it is the media ability for producing the largest amount of fruit bodies (Ahmed *et al.,* 2009; Vijay *et al.,* 2002) that was calculated

according to the following equation:

B.F% = fresh weight of the fruit bodies (g)/dry weight of the culture media (g)×100.

4- Dry yield = total dry yield based on fresh weight×dry matter percentage/100

5- Average weight of the fruit body (g) = total weight of the fruit bodies/number of the bodies.

6- Protein percentage (%) pre and post storage:

The percentage of nitrogen within the fruit bodies was estimated after the fruit bodies had been dried and ground. Then they were digested with Micro Kejeldahl. After that, the protein percentage was calculated according to the following equation:

Protein %= N%25

7- Weight loss post percentage storage (%): it was calculated according to the following equitation:

Weight loss % = (fruit bodies' weight pre storage - fruit bodies' weight post storage/fruit bodies' weight pre storage) × 100

8- Spoilage percentage post storage (%): it is the percentage of fruit bodies inadequate for marketing and calculated according to the following equation:

Spoilage percentage post storage (%) = (weight of the spoiled fruit bodies/ total fruit bodies) x 100

Phenolic material content in the fruit bodies pre and post storage: the phenols in the dry matter were estimated according to Arnow's method involving measuring the light absorption at the wavelength 515 nm with a complex spectrophotometer as a result of the reaction of the Arnow's Reagent indicator with the Orthor Dihydric Phenols. The Orthor Dihydric Phenols were estimated from the standard curve of the pure phenol, Catechol ($C_6H_4(OH)_2$) where several concentrations were used to prepare the standard curve so that it was higher than the highest sample value and less than the lowest one (Mahadevan and Sridhar, 1986). The three experiments were statistically analyzed according to the Completely Randomized Design comprised 5 replicates (Al-Sahoeke and Waheeb, 1990) and the means were compared relying upon the least significant difference (LSD) at the probability level of 5% using the statistic software SAS (2012).

Results

The inflorescence extract effect of Johnson grass and common reed on the total yield (g), fruit body weight (g), biological efficiency (%) and fruit body dry yield (g) of the king oyster mushroom (*P. eryngii*)

Table 1 shows significant differences between the study treatments. The treatment of Johnson grass extract at the concentration of 10% was superior giving the highest total yield, biological efficiency, and dry yield that were 960.80g, 96.28% and 90.30g respectively compared to the control treatment and the treatment of concentration 20% which recorded a decrease in theses trait values to 655.20 and 608.40g, 65.62 and 62.04%, and 64.42 and 61.82g respectively. On the other hand, the concentration 20% recorded the highest fruit weight averaged 43.60g compared to the control treatment where the weight of the fruit bodies reduced to 27.60g.

The inflorescence extract effect of Johnson grass and common reed on the protein and phenol percentages pre and post cold storage of the king oyster mushroom (*P. eryngii*)'s fruit bodies

Table 2 illustrates the superiority of reed inflorescence extract recording the highest protein percentage in the fruit bodies pre and post storage reaching 30.18% and 26.98% respectively compared to the control treatment that recorded 19.30% and 17.32% respectively. The treatments of inflorescence extracts of Johnson grass at the concentration of 10%, Johnson grass at the concentration of 20% and Johnson grass at the concentration of 20% recorded the highest phenol content in the fruit bodies reached 0.41, 0.41, 0.42, 0.35, 0.34, and 0.34 mg.g^{-1} respectively. The content in the fruit bodies pre storage noticeably decreased at the treatments of 5% reed inflorescence extract, control treatment, 5% Johnson grass extract and 10% reed inflorescence extract recording 0.33, 0.36, 0.37 and 0.37mg.g^{-1} respectively, while the phenol content in fruit bodies post storage decreased at the treatments of control, 5% Johnson grass extract, 5% reed inflorescence extract and 10% reed

Table 1: Effect of reed and Johnson grass inflorescence extracts on the total yield (g), fruit body weight (g), biological efficiency (%) and dry yield (g) of the king oyster mushroom (*P. eryngii*).

Treatments	Total yield (g)	Fruit body weight (g)	B.F (%)	Dry weight (g)
Control (water only)	655.20	27.60	65.62	64.42
Johnson grass inflorescence extract 5%	780.40	40.00	74.28	76.92
Johnson grass inflorescence extract 10%	960.80	41.20	96.28	90.30
Johnson grass inflorescence extract 20%	837.60	36.20	81.38	84.58
Reed inflorescence extract 5%	756.00	41.00	77.60	77.62
Reed inflorescence extract 10%	743.80	41.00	78.08	71.10
Reed inflorescence extract 20%	608.40	43.60	62.04	61.82
LSD 5%	80.38	6.86	8.21	11.95

Table 2: Effect of reed and Johnson grass inflorescence extracts on the protein and phenol percentage in the fruit bodies, pre and post storage of the king oyster mushroom (*P.eryngii*).

Treatments	Protein percentage storage (%)		Total phenol content storage ($mg.l^{-1}$)	
	pre	post	pre	post
Control (water only)	19.30	17.32	0.36	0.25
Johnson grass inflorescence extract 5%	22.56	20.60	0.37	0.25
Johnson grass inflorescence extract 10%	24.34	22.12	0.41	0.35
Johnson grass inflorescence extract 20%	24.82	22.70	0.42	0.34
Reed inflorescence extract 5%	26.80	23.62	0.34	0.23
Reed inflorescence extract 10%	27.72	23.88	0.37	0.23
Reed inflorescence extract 20%	30.18	26.98	0.41	0.34
LSD 5%	1.30	1.55	0.03	0.03

Table 3: Effect of reed and Johnson grass inflorescence extracts on the dry matter and the physiological spoilage percentage of the fruit bodies, pre and post the cold storage of the king oyster mushroom (*P.eryngii*).

Treatments	Dry matter storage (%)		Physiological spoilage storage ($mg.l^{-1}$)	
	pre	post	pre	post
Control (water only)	9.40	6.60	12.00	7.60
Johnson grass inflorescence extract 5%	9.80	7.60	12.20	5.40
Johnson grass inflorescence extract 10%	9.80	7.60	12.20	7.40
Johnson grass inflorescence extract 20%	9.80	7.60	12.20	7.60
Reed inflorescence extract 5%	9.60	7.20	9.40	8.20
Reed inflorescence extract 10%	9.80	7.60	9.40	8.60
Reed inflorescence extract 20%	10.00	7.80	9.40	8.60
LSD 5%	N.S	N.S	N.S	2.61

inflorescence extract recording 0.25, 0.25, 0.23 and 0.23 $mg.g^{-1}$ respectively.

Inflorescence extract effect of Johnson grass and common reed on dry matter percentage physiological spoilage pre and post cold storage of the king oyster mushroom (*P.eryngii*)'s fruit bodies

Table 3 demonstrates that the difference between the study treatments was insignificant in the traits of the dry matter percentage and physiological spoilage pre and post storage. However, it was observed that the treatment of Johnson grass extract at the concentration of 5% was superior recording the lowest weight loss percentage post the cold storage that not exceeded 5.40% compared to the inflorescence extract treatments of the reed at the concentrations of 20%, 10% and 5%which recording higher loss percentage reached 8.20 %, 8.60% and 8.60% respectively.

Discussion

Several pieces of research concluded that using plant extracts, for instance, a licorice extract, as nourishment either in a spray or injection form for the oyster mushroom *P.ostreatus* increases the total yield and biological efficiency significantly due to their content of plant hormones especially the gibberellin. Moreover, using the Johnson grass extract at the concentration of 20% due to its high content of proteins increased the total and dry yield as well as the biological efficiency of the mushroom *P.ostreatus* (Abdulhadi, 2011; Rustum *et al.,* 2018). The superiority of the treatment of inflorescence extract 10% recording the highest total yield, biological efficiency, and dry yield table 1 may be attributed to the high content of proteins in the extract that helps to increase the dry matter accumulation within the fruit bodies as protein constitutes one of the dry matter ingredients (Dundar *et al.,* 2008; Kirbag and Akyiz, 2008) indicating that the conversion efficiency of the mycelia increased with using the inflorescence extract at the concentration of 10%. The reason behind the decrease in the total yield, the dry yield and the biological efficiency in the treatment of 20% of the reed inflorescence extract may be the presence of a type of hormones or growth inhibiter reducing the biological efficiency that reflected on the total yield table 1. The superiority obtained by the concentration 20% of the reed inflorescence extract recording the highest protein percentage in the fruit bodies pre and post storage may be due to the richness of extract in substances helping to preserve the protein content in the fruit bodies pre and post storage table 2 that is confirmed by the treatment of reed inflorescence extract which was the best among all treatment. It recorded the highest dry matter percentage pre and post storage even though the difference between was insignificant table 2. The superiority of the two treatments, 10% reed inflorescence extract and 20% Johnson grass inflorescence extract in preserving the phenol content in the fruit bodies is due to that the extracts contain the high content of phenols (Faraji and Jame, 2016) table 2. Phenols are considered antioxidants giving them medical and nutritional importance as they increase the body's immunity and its ability in fighting cancerous diseases

(Wang *et al.,* 2001; Rustum, 2010).

Conclusion

We conclude from this study that nourishing the king mushroom with the extract of reed inflorescence at the concentration of 20% or Johnson grass inflorescence at the concentration of 10% increase production to about double in addition to increasing the biological efficiency of the cultural media using for mushroom production; however, the best of them is the treatment of the inflorescence extract of reed at the concentration of 20% since it recorded the highest weight of the fruit bodies and increased the fresh yield accompanied by an increase in the dry yield and the dry matter percentage within the fruit bodies pre and post storage. This result is important nutritionally and economically because a high proportion of nutritional fungi are sold dried for use in soups and added in a crushed form to the children's food or used in the food industry as dietary alternatives rich in protein.

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