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THE EFFECT OF *EUCALYPTUS CAMALDULENSIS* D. AQUEOUS EXTRACT ON INCREASING THE PRODUCTIVE AND INHIBITING ABILITY OF SOME POLLUTANT FUNGI IN THE AGRICULTURAL SUBSTRATE AND INCREASING SOME ACTIVE COMPOUNDS IN OYSTER MUSHROOMS.

Hurria H. Al-Joborry[1], Ziena M. Abdul-Qader[2*] and Zainab J. Al-Mousawi[2]

[1]Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.
[2*]Medicinal and Aromatic Plants Research Unit, College of Agricultural Engineering Sciences, University of Baghdad, Iraq.

Abstract

This experiment was aimed to evaluate the effect of *Eucalyptus camaldulensis* D. aqueous extract out of Eucalyptus bark (0, 5%, 10%, 20%) and Eucalyptus leaves (0, 50, 100, 200 g/L) on the ability of production and Inhibition of For some polluted fungi in the agricultural medium and the increase of some active compounds with a medicinal effect in Oyster mushrooms. Results revealed that the aqueous extract of Eucalyptus bark (10%) gave the highest values in total wet yield (350.3 g/kg agriculture medium), Total dry yield (34.73 g/kg agriculture medium), dry matter percent (9.913%), Biological efficiency (70.07%), fruit body average (49.87g) and dried fruit content of protein. Also the application of eucalyptus bark at 20%, eucalyptus leaves at 200 gm/L and eucalyptus leaves at 100 gm/Land the application of eucalyptus cortex at 10% were significantly Inhibited the polluted microorganisms in the agricultural medium (0, 0, 1, 2.67%) respectively, while the application of eucalyptus bark at 20% has significantly increased the fruit content of total phenols and total flavonoids which were peaked at 0.602 and 0.5300 mg/g of dried fruit weight of Oyster mushrooms.

Key words: Mushrooms, Allelopathic plants Extract, Yield, Inhibiting, Active compounds.

Introduction

During the metabolism processes, plants produce secondary compounds that are either toxic or stimulate to the microorganisms. These compounds are extracted from different plants known as allelopathic plants, such as all trees that belong to the Eucalyptus species, which possess a high effectiveness of the chemical antibiotic (Sasikumar *et al.,* 2001 and Singh *et al.,* 2005). Allelochemicals sources are either in leaves, stem, tree phloem, live or dead plants can be classified into terpenes, glucocides, coumanines, aldehyds and phenolic compounds, all of which Eucalyptus leaves are a major source (Khan *et al.,* 2007). Chromatographic analysis showed that the eucalyptus leaf extract contained coumaric acid, gallic acid, hydroxybenzoic acid, syringic acid, gentisic acid and vanillic acid in addition to other laboratory discoveries that proved the eucalyptus leaf extract included total phenols, total flavonids, tannins and alkaloids (Sasikumar *et al.,* 2001 and Habib *et al.,* 2007, and El-Rokiek *et al.,* 2011 and El-Rokiek *et al.,* 2019).

Oyster mushroom is one of the primary analyzed fungi of food and medicinal importance for humans, it is a primary source of protein, carbohydrates, amino acids and mineral elements as well as its content of medicinal materials such as phenols and flavonoids, which are antioxidants that reduce the risk of free radicals in the human body (Gutapa *et al.,* 2017 and Selvamani *et al.,* 2018). The agricultural medium of oyster mushrooms suffers from contamination by microorganisms such as Trichoderma, especially when using the formaldehyde chemical sterilization at (2%), which is preferred to be used in growing countries on the method of thermal sterilization in case of low material costs, which affects the decrease in production, quality and nutritional value of the fruit bodies of the Oyster mushroom (Muslat, 2002

***Author for correspondence :** E-mail : Ziena73mohammed@gmail.com

and Al-Badrany, 2011 and Abdulhadi and Hassan, 2013). Kinge *et al.,* (2016) revealed that the use of eucalyptus tree leaves as the cultivar medium for *P. ostreatus* had a significant effect on growth, yield and reduced the incidence of contamination of the agricultural medium with other types of fungi such as Trichoderma to zero due to the content of eucalyptus leaves of highly effective compounds of antibiotic. Other research has proven that Eucalyptus cortex contains lignin 28.8%, polysaccharides 47%, supersin 1%, ash 2% and natural total monosaccharides 23.2% as well as the following nutrients; nitrogen 0.26%, phosphorus 0.001%, calcium 0.62% and potassium 0.31%, 0.15% Mg as well as High secondary compounds of its content of total phenols, total Flavonoids, total tannin, which is due to it's medical importance as antioxidants (Miranda *et al.,* 2013 and Sartori *et al.,* 2016). After all given above, this research aimed to evaluate the effect of aqueous extract of Eucalyptus leaves and bark in increasing production and the content of fruit bodies, medicinal compounds and reducing the rates of pollution in sterile agricultural medium by the chemical method.

Materials and Methods

This experiment was carried out in fungi production laboratory / Medicinal Plants Research Unit/ college of agricultural engineering sciences/ university of Baghdad during the period of 1/11/2018 to 30/5/2019. A pure isolation of oyster mushroom (*Pleurotus ostreatus* PX22 from Alazrar Albaidha'a Company in Jordan) was obtained. The isolation was activated on a prepared nutrient medium of potatoes Dextrose Agar (PDA), which was placed in Petri dishes with a diameter of 9 cm. Note that this operation was carried out inside a laminar flow. The plates were incubated in a dedicated incubator at a temperature of 25±1°C after the fungal spinning was completed. Fungi was loaded onto boiled and sterilized wheat grains in a special glass bottle of 250 g (Kirbag and Akyuz, 2008 and Oei, 2005) The bottles are incubated at the same incubator and at the same temperature mentioned above. After the fungal yarn growing on wheat grains, 5% was used to pollinate the medium consisting of wet sterilized wheat straw (Muslat, 2002 And Vijay *et al.,* 2002 And Sharma, 1996). Then the agricultural medium was filled in transparent plastic bags with a capacity of 0.500 kg wet straw (Martinez, 1998). Then the pollinated bags were transferred to the incubate room consisting of thermally isolated walls with dimensions of 2.5×4×3 m and the room temperature was installed on 25±2^{0}C using a split type cooling device, With a capacity of 1.5 tons of cooling (Abdulhadi, 2010). After the spinning of the fungi yarn has been completed on all the agricultural medium in the bags (it took 30 days), the incubate room mentioned above was converted into a production room (Kirbag and Akyuz, 2008) By increasing the humidity between 80-90% by using a humidifier, considering the temperature inside the room was 25±2^{0}C (Hassan *et al.,* 2010). Artificial lighting was provided in strength of 400LUX using three florescent lights (Hassan *et al.,* 2010). The temperature and humidity Monitoring controlled by adding a Thermo-hygrograph placed inside the room. The bags were punctured with an equal number of holes on the side of the light and the following experiments were carried out:

1- First experiment : *Eucalyptus camaldulensis* D. leaves extract in order to increase the production ability and to inhibit some polluted fungi in the agricultural medium and increase some of the medically active compounds in the oyster mushroom; The leaves of Eucalyptus were collected from one of the farms on the outskirts of Baghdad before blooming and then were diagnosed in the national herbarium of the Ministry of Agriculture to guarantee that the leaves were belong to *Eucalyptus camaldulensis* D. Then the leaves were washed with water, and dried under the room temperature with continuous stirring in order toavoid the rot. Then, the dried leaves were grinded with a special mill and their powder was kept in plastic boxes in the refrigerator until use. The aqueous extract was prepared according to the method described by Al-Manhel and Niamah (2015). Four concentrations were prepared for the aqueous extract of eucalyptus leaves, which were: 0, 50, 100 and 200 gm per liter of distilled water, then the aqueous extract of eucalyptus leaves was added using a veterinary syringe (capacity of 50 cm^3 per bag). The aqueous extract was injected into the bags at the stage of forming the staples, considering that five bags were used for each concentration, and each bag represents one repetition. The measurements were taken after the fruit bodies reached the required size.

2- The second experiment: the application of *Eucalyptus camaldulensis* D. tree bark extract in order to increase the productivity and to inhibit the ability of some pollutant fungi in the agricultural medium and some of the medically active compounds in the oyster mushroom; The bark of eucalyptus trees were collected from the same trees which the leaves were collected and then dried under the room temperature conditions to ensure the stability of the weight, then it was ground with a mill of medicinal plants purposes. Aqueous extract was prepared according to the method described by Rustum *et al.,* (2018). Four concentrations of Eucalyptus cortex extract were prepared: 0%, 5%, 10% and 20%. Then the aqueous extract of eucalyptus cortex was added by a 50

cm veterinary syringe by injecting it into the bags at the forming stage. Five bags were used for each concentration, and each bag represented one replicate. The measurements were taken after the fruit bodies reached the required size.

Studied parameters

1- Total yield based on the wet weight of fruit bodies: the yield was collected from each replicate, and it was calculated for each bag in gm/kg wet straw^{-1}.

2- Dry matter percent: 100 gm of fruit bodies were taken from each replicate, before and after the storage, and were cut into small pieces and dried in an electric oven equipped with a fan at a temperature of 60°C until the weight constant (Dundar *et al.,* 2008). The percent of dry matter was calculated according the following equation:

dry matter % = dry weight of fruit bodies / wet weight of fruit bodies ×100.

3- Total dry yield was calculated according to the following equation: Total dry yield = total yield according the wet weight × dry matter percent /100.

4- The production cycle: is the number of days from the first to the last harvest for each replicate containing half a kilogram of dry medium (Al-Badrany, 2010).

5- Rate of fruit bodies weight (gm) = total weight of fruit bodies/number of fruit bodies.

6- Biological efficiency (B.E) was calculated according to the formula described by Vijay *et al.,* (2002).

B.E = wet weight of fruit bodies (gm) / dry weight of agricultural medium (gm) × 100.

7- Medium pollution rate: calculated according to the method described by Abdulhadi and Hassan (2013) represented in the following formula:

% medium pollution rate = the sum of the contaminated spots area in the medium / surface area of the bag × 100.

8- Protein percent in the fruit bodies %: the nitrogen percent was calculated after drying the fruit bodies, then the samples were digested using Micro Kejeldahl device. Then the protein ratio was calculated from the following equation:

% protein = % N × 6.25.

9- Fruits bodies content of total phenols: The phenols in the dry matter were calculated according to the Arnow ¼s method by measuring the optical absorption at a wavelength of 515nm by the spectrophotometer of the chromic complex resulting from the special reactions of the Arnow's Reagent with Orthor Dihydric Phenols and according to the method described by Mahadevan and Sridhar (1986).

10- Fruitbodies content of total flavonoids: Flavonoids were calculated in dry fruit bodies according to the method described by Yidiz *et al.,* (2017) by measuring optical absorption at 415 nm wavelength with a spectrophotometer.

Statistical analysis of the experiments was carried out according to Complete Randomize Design (CRD). The number of replicates in each experiment was 5 replicates (Al-Sahoeke and Waheeb, 1990). Comparisons between the averages were made according to the test of the least significant difference LSD at a 5% probability level by using the statistical software Genstat.

Table 1 revealed the significant differences among the research treatments; the aqueous extract of eucalyptus bark 10% gave the highest total wet and dry yield,and the percent of dry matter in fruit bodies reached 350.3 gm/kg agricultural medium, 34.73 gm/kg agricultural medium, and 9.913% respectively. While the application of eucalyptus leaves extract at 50 gm/L and the control treatment showed a decrease in the total yield of wet and dry fruit bodies reached 249, 247, 23.93, 21.97 g/kg agricultural medium, respectively, also the control treatment recorded the lowest percent of the dry matter reached 8.893%. While the application of aqueous extract of eucalyptus leaves at 100 gm/L, the aqueous treatment of eucalyptus bark at 20%, the aqueous extract of eucalyptus bark at 10% and the aqueous extract of eucalyptus leaves 100 gm/L by giving lowest contamination rate (almost non-existent in some of them),

Table 1: The effect of the aqueous extract of eucalyptus leaves and bark in the total wet and dry yield, the percentage of dry matter in fruit bodies and the rate of inhibition of some fungi affecting the culture medium of the oyster mushroom.

Treatments	Total wet yield of fruit bodies (gm/kg medium)	Total dry yield of fruit bodies (gm/kg medium)	Dry matter percent in fruit bodies(%)	Medium pollution rate (%)
Distilled water (control)	247	21.97	8.893	32.67
Leaves extract 50 gm/L	249	23.93	9.440	12.33
Leaves extract 100 gm/ L	323.3	31.33	9.700	2.67
Leaves extract 200 gm/ L	263	25.77	9.800	0.00
Bark extract 5%	268.3	26.43	9.853	10.67
Bark extract 10%	350.3	34.73	9.913	1.00
Bark extract 20%	310	30.33	9.793	0.00
L.S.D	22.02	2.15	0.440	6.53

in the agricultural medium of oyster mushrooms, reaching 0%, 0%, 1% and 2.67%, respectively, while the control treatment recorded the highest pollution rate in the agricultural medium, peaked at 32.67% table 1.

Results in table 2 revealed the significant differences among treatments; the application of aqueous extract of eucalyptus leaves at 100 gm/L, the aqueous extract of eucalyptus bark 5%, the aqueous extract of eucalyptus bark 10% by recording the lowest number of days in the production cycle compared to the control treatment, which recorded an increase in the number the days for production cycle reached 39, 36, 35 and 54.67 days, respectively. While the control treatment recorded the lowest value of the harvest yield reached 75g compared to most of experiment treatments, which were recorded a significant increment reached 116.6, 107, 98.6, 97 and 94.4g, respectively. While the application of aqueous extract Eucalyptus bark at 10% gave a significant effect by recording the highest percent of bio-efficiency reached 70.07% compared to the application of the aqueous extract of Eucalyptus bark at 5% and aqueous extract of eucalyptus leaves 100 g/L and the eucalyptus leaves extract 50 g/L and the control treatment which all were recorded a decrement in the percent of biological efficiency reached 53.67, 52.60, 49.87 and 49.40%, respectively table 2.

Table 2: Effect of aqueous extract of eucalyptus leaves and bark on the production cycle (day), harvest yield rate (gm), and biological efficiency (%) of Oyster mushrooms.

Treatments	Production cycle (day)	Harvest yield rate (%)	Biological efficiency (%)
Distilled water (control)	54.67	75.0	49.40
Leaves extract 50 gm/L	46.67	97.0	49.87
Leaves extract 100 gm/ L	40.67	98.6	64.67
Leaves extract 200 gm/ L	36.00	116.6	52.60
Bark extract 5%	44.33	88.9	53.67
Bark extract 10%	39.00	107.0	70.07
Bark extract 20%	35.00	94.4	62.00
L.S.D	7.61	29.02	4.40

Table 3: Effect of aqueous extract of Eucalyptus leaves and bark on the dried fruit bodies content of protein (%), total phenols (mg/ gm) and total flavonoids (mg/gm) of oyster mushrooms.

Treatments	Dried fruit bodies content of protein (%)	Dried fruit bodies content of total phenols (mg/gm)	Dried fruit bodies content of total flavonoids (mg/gm)
Distilled water (control)	19.67	0.367	0.2023
Leaves extract 50 gm/L	20.17	0.386	0.2840
Leaves extract 100 gm/ L	23.33	0.476	0.3387
Leaves extract 200 gm/ L	20.67	0.530	0.4143
Bark extract 5%	19.67	0.397	0.2933
Bark extract 10%	21.50	0.489	0.3970
Bark extract 20%	20.67	0.602	0.5300
L.S.D	2.54	0.0985	0.05664

Results and Discussion

Results in table 3 showed a significant differences among the treatments. The application with the aqueous extract of eucalyptus leaves at 100 g/L and the aqueous extract of the eucalyptus bark 10% were recorded the highest percent of protein in the dried fruit bodies reached 23.33% and 21.50%, respectively, compared to All the treatments, which were recorded the lowest percent of protein in the dried bodies reached 20.67, 20.17, 20.17, 19.67, 19.67%, respectively. While the aqueous treatment of eucalyptus bark at 20% has recorded the highest content of fruit bodies of total phenols and total flavonoids peaked at 0.602 and 0.5300 mg/g respectively. While the application of aqueous extract of eucalyptus leaves at 50 g/L and the control treatment were recorded a significant decrease in the content of dried fruit bodies of total phenols reached 0.386 and 0.367 mg/g, respectively. The control treatment also recorded a decrease in the content of dried fruit bodies of the total flavonoids reached 0.2023 mg/g table 3.

The Oyster mushroom *P. ostreatus* has a multi-lateral enzyme system, which enables it to grow and feed on different types of agricultural media such as the nutrients that are mixed with the agricultural medium or the extracts that given by injection (Kim and Kwon, 2004 And Al-badrany, 2010 And Abdul-Qader *et al.,* 2019). The significant effect of the aqueous extract of eucalyptus bark at 10% was revealed by giving the highest values in total wet and dry yield and the dry matter percent of the fruiting bodies of the oyster mushroom table 1 it also recorded the lowest number of days of the production cycle and the highest rate of the harvest yield and the highest percent of biological efficiency (% BE) table 2, it may be due to the eucalyptus bark of chemical compounds, especially nitrogen, carbon and monosaccharides (Miranda *et al.,* 2013). The oyster mushroom requires organic carbon for nutrition because it unable to manufacture chlorophyll as it is in higher plants because it does not contain chlorophyll and that the best sources of carbon necessary for the growth and production of oyster mushrooms are monosaccharides including glucose and fructose (Abdulhadi, 2012 and Ghorai *et al.,* 2009 and

Hardar and Arazi, 1986), Fungal require nitrogen during its growth phase on the agricultural medium, which leads to a decrease in its content in the agricultural medium (the agricultural medium's nitrogen content ranges between 0.5-0.8%). The nitrogen is consumed in the formation process of fruit bodies, which leads to a decrement in its percent in the agricultural medium after the first harvest, indicating the medium need for this nutrient to ensure the formation of fruit bodies (Upadhyay *et al.,* 2002).

Many studies were found that supporting the agricultural medium after the end of the sitting process with different sources of organic nitrogen and organic carbon contributes to increase the production characteristics and the fruit bodies content of the protein and the total phenolic materials (Abdul-Qader *et al.,* 2019 and Abdulhadi, 2012). The significant effect of applying the aqueous extract of the eucalyptus bark 20%, the aqueous extract of eucalyptus leaves 100 g/L and the aqueous extract of the eucalyptus bark 10% were due to the high content of these extracts, whether from the leaves or the bark of the secondary compounds such as total phenols and total flavonoids with high effectiveness of antibiotic to the microorganisms that are in the agricultural medium and that are not completely eliminated during the chemical sterilization process of the agricultural medium (Kinge *et al.,* 2016), This is confirmed by the results reached in table 3. The reason for the significant effect of applying the eucalyptus leaves extract 100 g/L by recording the highest percent of dried fruit bodies content of protein, is due to the high leaf content of nitrogen and amino acids (Oliveira *et al.,* 2017 and Journet and Cochrane, 1978). Eucalyptus bark extract at 20% recorded the highest concentration of total phenols and total flavonoids in dried fruit bodies table 3 and that was due to the high eucalyptus bark content of these active compounds and this has proven by many studies (Sartori *et al.,* 2016).

Conclusion

According to the results obtained in this research, it was approved that the oyster mushroom nutrition after the end of the incubation phase with the aqueous extract of eucalyptus forage at a concentration of 10% has significantly increased both the wet and dry total yield, the ratio of the dry matter, the biological efficiency and the percent of fruit body and the fruit body content of protein. While the application of aqueous extract of eucalyptus bark at 20% has significantly increased the fruit bodies content of total phenols and total flavonoids reached 0.602 and 0.5300 mg/g of the weight of dried fruit bodies of the oyster mushroom, which explains the significant increment of applying aqueous extract of the eucalyptus bark at 20%, the Eucalyptus leaves extract at 200 g/L, the aqueous extract of eucalyptus bark at 10% and the eucalyptus leaves extract at 100 g/L in inhibition of some contaminated fungi in the agricultural medium (0, 0, 1, 2.67%) respectively due to the active compounds content that contributing on inhibiting the growth of fungi that contaminated agricultural medium.

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