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GROWTH REQUIREMENTS OF *PLEUROTUS OSTREATUS* AND ANTAGONISTIC POTENTIAL AGAINST *RHIZOCTONIA SOLANI*

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ABSTRACT

A study was conducted in Laboratory of Botany/Department of Plant Protection/College of Agriculture/University of Kufa for the period from 10/2/2019 to 1/7/2020. The aimed to identify changes in the nature and speed of growth of the oyster mushroom due to exposure to various environmental factors, including the type of nutrient medium, temperature, pH, and salinity (EC) and their interactions. The results showed that the growth of the fungus was significantly different with different food media, temperature, pH levels, lightness and salinity. The best nutritional medium for the growth of the fungus was the medium of wheat hay followed by barley hay medium and sawdust medium after 10 days incubation. The fungus has failed to thrive on the alfalfa medium. Regardless of the type of medium, 30C° and pH 6 was the best growing condition. The oyster mushroom showed a high antagonism against pathogenic *R.solani*, especially at 20C° and 25C°. The results showed that the oyster mushrooms are sensitive to medium salinity (EC). The highest fungal radial growth (8.5cm) was recorded in PDA medium treated with distilled water, followed by PDA at EC 2dS/m which resulted in 8cm fungal radial growth. However, the oyster mushroom completely failed to grow on PDA with EC values of 14 and 16 dS/m.

Keywords: mushroom, radial growth, *P. ostreatus*, plant residues, *R. solani*

Introduction

The mushroom edible is a heterotrophic microorganism with a saprophytic livelihood that breaks down and decomposes the basic materials present in their environment or that are grown on them from plant and organic waste (Volk and Lvors 2001). Breeding fungi on agricultural and organic wastes is one of the beneficial treatments to reduce environmental pollution resulting from the accumulation of agricultural waste compared to burning waste, which increases air pollution with CO_2 gas. The growth of fungal yarn on these residues increases its protein content and reduces the carbon / nitrogen ratio, the ratio N / C, as well as increases the synthesis of the dissolving enzymes of the organic matter, including Lignase, Peroxidase, Laccase, and Cellulase (Elisashvili *et al.*, 2003). The oyster mushroom, Pleurotus ostreatus, is a food fungus that has medicinal benefits because it contains effective compounds, including antimicrobial agents, fungi and toxins (Oei, 2002 and 2005).

The fungus produces antiviral substances, bacteria, and fungi and their toxins, such as the 1-octen-3-ol, which is an anti-bacterial compound, which is found in the fruiting body, and the 4-Methoxy benzaldehyde, which is present in the mycelium, and they are among the volatile compounds (Shu-Ting and Philip, 2004). The fungus also has an enzymatic ability to break down toxic substances in the medium in which it grows (Persky *et al.*, 2002 and Lacina *et al.*, 2003). In addition to its ability as an anti-parasite, including the plant parasitic nematode (Chase *et al.*, 2003).

Species belonging to the oyster mushroom genus are characterized by high growth potential, colonization and rapid growth of mycelium and production on different cultivation media under different environmental conditions (Kong, 2004).

At present, the oyster mushroom ranks second after the white mushroom Agaricus bisporus, with 25% of the global production of food fungi (OECD), (2008. The mushroom is unique for its high nutritional value as it is rich in protein, which constitutes 40-20% of dry weight (Ahmed *et al.*, 2009). Oyster mushrooms have a complex enzyme system that enables them to grow on various types of plant residues with a high content of lignin and cellulose, such as wheat straw, rice straw, yellow corn moss, date palm waste, and weed residues, including halves and cane (Hassan, 2011).

Materials and Methods

Effect of medium type on *P. ostreatus* growth at different temperatures

Suitability of five types of plant residues including sawdust, wheat hay, barley hay, rice husck, corncon and alfalfa residues was assessed for growing oyster mushrooms according to Sharma (2006). The plant residues under study were washed with distilled water, dried in the oven at 40 m, grinding 1 kg of each type, and then moistened with water at a ratio of 1 liter / kg for 12 hours. Then the excess water was removed and the medium was transferred to graduated cylinders of 100 ml, which were closed with medical cotton

and autoclaved for 20 minutes. After cooling, each cylinder was inoculated with a 5 mm tablet of oyster mushrooms taken from the edge of a fresh mushroom culture, incubated at 25, 30 or 35C with three replications for each treatment. the distance traveled by the mycelium (cm) down the medium was measured after 10 days of inoculation. The daily growth rate of mycelium was calculated.

Effect of temperature and pH on the growth of *P. ostreatus* on P.D.A.

interaction effect of temperatures 20, 25, 30, and 35 C and pH of 5, 6, 7, 8 and 9 on *P.ostreatus* growth was evaluated. Sterile PDA medium was distributed into three 500 mL conical flasks at 300 mL medium per flask and adjusted the pH of the media to 8 and 9 using 0.2 molar Phosphate buffer and to 5 and 6 using buffer sodium acetate at a concentration of 0.2 molar. After autoclaving, the media were poured into 9 cm Petri dishes and 12 plates per pH. Each plate was then inoculated with a 1 cm diameter disc taken from the edge of a four-day-old P.ostreatus colony grown on PDA. Plates of each pH were divided into four groups (three replicates) and incubated at 20, 25, 30 or 35 pm for seven days. Then the radial growth diameter of the fungus was calculated every 24 hours.

Antagonism of *P. ostreatus* against *R.solani* at different temperature and pH

The dual culture method (Ligocka *et al.*, 2002) was used to test P.ostreatus antagonism to Rhizoctonia solani in Petri dishes on sterile PDA medium. The test was carried out at four pH, 5, 6, 7 and 8 for the medium and incubated at 20, 25, 30 or 30 C, with three replications for each temperature. The colonies' diameters were measured after 7 days of incubation. Antagonism level was estimated according to a 5 degree scale (Bell *et al.*, 1982) where 1= the antigonstic fumgal covering the entire plate, 2= the antigonstic covering 3/4 of the plate area, 3= 50% of the plate area is covered by the antigonstic or by the pathogenic fungus, 4= the pathogenic fungus covering 3/4 of the plate and 5= the pathogenic fungus covering the entire plate.

Results and Discussion

Effect of medium type on *P. ostreatus* growth at different temperatures

It is noted from the results (Table 1) that the highest growth of the oyster mushroom was on the prepared medium of hay, which was 8.4 cm after 10 days of incubation followed by the two mediums of barley hay and sawdust with a growth rate of 6.9 and 6.3 cm, respectively, while the mushrooms did not grow on alfalfa residues medium. The increase in the growth rate of the oyster mushrooms on the wheat hay medium is due to the nutritional contents of the medium including proteins, carbohydrates and mineral elements important for fungal growth and can be consumed by the fungus. The mushroom failed to grow on the alfalfa medium. This is mostly due to the high salinity content in the alfalfa medium that prevented the fungus from benefiting from the mineral elements. The results also showed (Table1) that the highest fungal growth was recorded at 30C° in all media after 10 days, and that any deviation from the temperature of 30C° causes a decrease in the growth rate of the oyster mushroom. In general, the highest growth rate of the fungus was recorded in the wheat hay medium of where incubated at 30C°, followed by barley hay medium at the same temperature.

Temperature is one of the main factors in the production of oyster fungi at all the breeding stages (spinning stage, seeding stage and fruiting stage) which is between 20 to 30C° (Hoa, 2015). The optimum temperatures for the growth of microorganisms are determined by the optimum conditions for performing cell reactions in which the enzymes participate. The rapid decline in growth rates when the temperature is raised above the optimum may be due to the imbalance of enzymatic activities, as well as dissolved substances in the medium stops through the cytoplasmic membrane when temperature is below the minimum requirement (Al-Saad, 1990; Tanner, 1997).

Table 1 : Effect of medium type and incubation temperature on growth of oyster mushroom after 10 days of inoculation

Medium type	Incubation temperature C°				Average
	20	25	30	35	
Saw dust	6	6.5	7.5	5	6.3
Wheat hay	8	8.5	10	7	8.4
Barley hay	4	8	9	6.5	6.9
Rice hay	3	5	6	3.5	4.4
Rice husk	1.5	2.5	3	1	2
Corn cons	5	5,5	6	4	5
Alfalfa	—	—	—	—	—
Average	4.6	6.1	6.9	4.5	
L.S.D. ($P \leq 0.05$)	Temperature=0.31 Media=0.55 Interaction=0.95				

Antagonism of *P. ostreatus* against *R. solani* at different temperatures and pH levels

Table (2) shows the sensitivity of the oyster mushroom growth to the change in temperature and pH. The table also shows that the best anti-fungal ability against the pathogen *R. Solani* was at 20 C° with a first-degree antagonism, in which the oyster fungus covered the entire plate and did not allow the pathogen *R. solani* to grow, followed by the 25 C° with first degree of antagonism in all the pH under study except for the pH 8 in which the two thirds of the plate was colonized by the oyster mushroom while the rest was covered by the pathogen *R. solani*. The results also showed a decrease in antagonistic ability of oyster fungus as the temperature increased. At 35 C° the pathogen covered two thirds of the plate at the expense of the oyster mushroom which colonized only the other third of the plate at all pH levels except for the

pH 8 where the plate was completely covered with the pathogenic *R. solani*. These results are consistent with previous results, as the pH of 6-5 is the optimum for the growth of pathogenic and non-pathogenic isolates of *R. solani* (Agrios, 2010). The effect of environmental factors, such as temperature and pH, on the growth of *P.ostreatus* was reflected in its antagonism to *R. solani*. Changes in temperature and pH have affected fungal growth speed and activity, influencing fungal ability in competing and parasitizing other fungal pathogens, or through the secretion of many enzymes and antibiotics that cause killing of host cells or limit their growth and spread (Kredics *et al.*, 2003). Also, some environmental factors favorable to the growth of *P. ostreatus* may, at te same time, be inhibitory and unfavorable to the pathogen (Hunter and Bodman, 2000). The difference in growth rates concurring with the difference in the pH is usually attributed to the effect of free hydrogen ions.

Table 2 : *P. ostreatus* antagonism to *R. solani* at different temperatures and pH values

pH	Incubation conditions (Temperature C^o)	Antagonism index (1-5)
5	20 C^o	1
	25 C^o	1
	30 C^o	2
	35 C^o	4
6	20 C^o	1
	25 C^o	1
	30 C^o	2
	35 C^o	3
7	20 C^o	1
	25 C^o	1
	30 C^o	3
	35 C^o	4
8	20 C^o	1
	25 C^o	2
	30 C^o	3
	35 C^o	5

Antagonism of *P. ostreatus* estimated based on a 5 degree scale where 1= the plate is completely covered with antagonistic fungus *P. ostreatus*, 2= the antagonistic fungus covers 3/4 of the plate area, 3= 50% of the plate area is covered by the antagonistic or by the pathogenic fungus, 4= the pathogenic fungus covering 3/4 of the plate and 5= the entire plate is covered by the pathogenic fungus *R. solani*. on the functioning of the cytoplasmic membranes, the activity of enzymes, the readiness of nutrients and the mechanism of their transport to the cell.

The effect medium salinity (EC) on *P. ostreatus* growth

The results shown in Table (3) indicate that the growth of *Pleurotus ostreatus* was negatively affected by increasing the salinity concentration (EC) of the P.D.A growth medium. The highest radial growth of the oyster mushrooms was in the treatment of distilled water (8.50 cm), followed by the PDA WITH EC of 2 dS/m with a radial growth of 8 cm. In the other salinity concentrations, the growth of the fungus decreased gradually with increasing the EC values in the nutrient PDA. The minimum radial growth of the fungus was 1.25 cm at 12 dS/m, while the fungal growth was not detected at 14 and 16 dS/m.

Table 3 : Effect of P.D.A medium salinity (EC) on radial growth of *Pleurotus ostreatus*

EC (dS/m)	DW	2	4	6	8	10	12	14	16	L.S.D.$_{(P\leq 0.05)}$
Fungal radial growth (cm)	**8.50**	**8.00**	**7.25**	**6.00**	**4.00**	**2.50**	**1.25**	**0**	**0**	**0.92**

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