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Rhizobia as Biofertilizer for Oyster Mushroom Cultivation

Gh. Peyvast[1]*, J.A. Olfati[2], A. Kariminia[3] and A. Fallah[4]

[1,2]Department of Horticultural, [3]Department of Soil Sciences,
University of Guilan, Km 12 Rasht-Qazvin Broadcast, Rasht, Iran.
[4]Soil and Water Institute, Tehran, Iran.

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Rhizobium **is a genus of symbiotic N_2-fixing soil bacteria that induce the formation of root nodules of leguminous plants. To evaluate the effect of *Bradyrhizobium* on biological efficiency and quality characteristics of mushroom an experiment was conducted in 2008 with mother spawn preparation. Other than control (T1) Bradyrhizobium added directly to wheat corn (T2), added to pure mycelium and added by pure mycelium and incubated by *Bradyrhizobium* slab at same time (T3).**

Added *Bradyrhizobium* in wheat incubation stage increased positively Ash, Ca, P, K, and protein, and decreased days to primordial initiation in the mushroom samples. Added Bradyrhizobium in Petri culture stage also had an effect of increasing days to primordial initiation, Ca, Mg and protein, but decreasing biological efficiency, Dry matter, Ash, P and K in the mushroom samples. Added *Bradyrhizobium* in both Petri culture stage and wheat incubation stage did not have any significant effect on total nitrogen in the mushroom samples.

Key words: *Bradyrhizobium*, Oyster mushroom, Biological efficiency, Protein content.

Mushrooms have been reported as therapeutic foods, useful in preventing disease such as hypertension, hypercholesterolemia and cancer[2,3]. Oyster mushroom (*Pleurotus ostreatus* var. *sajor caju* [Fr.] Singer) cultivation has increased during the last decade[5,9].

Rhizobium is a genus of symbiotic N_2-fixing soil bacteria that induce the formation of root nodules of leguminous plants. Microbial biofilms are communities of microorganisms attached to biotic or abiotic surfaces in the environment and differentiated to form a complex structure, and to conduct a range of functions. Formation of the biofilms by rhizobia with common soil fungi through mycelial colonization has been observed recently[10]. Mushrooms are conventionally grown with inorganic and organic nutrients added to their culture substrate. In the past, it had been reported using N balance studies that mushroom fungi such as *Pleurotus* spp. can fix N_2 biologically on natural substrates and that this might be quite commonly found among them[2,3]. However, a later study showed that only associations of *Pleurotus* spp. and N_2 fixing

* To whom all correspondence should be addressed.
Tel +98(0)1316690439, Fax: +98(0)1316690281

bacteria (diazotrophs, e.g. Rhizobia) can fix N_2 [4]. Microbial biofilms developed by diazotrophic colonization on the fungal filaments were observed to show nitrogenase activity, and hence increased N accumulation in the hyphae. Recently, N_2-fixing alpha-Proteobacteria was observed to be predominant in bacterial communities in truffles, a type of mushroom found in soil[1]. Further, nitrogenase activity of a developed *Penicillium* spp-bradyrhizobial biofilm was detected using acetylene reduction assay[7]. Here, the *Penicillium* spp. alone did not show the nitrogenase activity. In general, it is reported using N balance studies that fungi such as *Pleurotus* spp. can fix N_2 biologically on natural substrates and that this might be quite commonly found among them[6,8]. Jayasinghearachchi and Seneviratne[7] reported that only associations of fungi (*Pleurotus osteriatus*) and diazotrophs can fix N_2.

This project was undertaken to determine the ability of *Bardyrhizobium* to increase oyster mushroom quality.

MATERIAL AND METHOD

Spawn preparation

Oyster mushroom pure culture, Petri culture and mother spawn was purchased from Keshtpashohan Laboratory in Tehran, Iran. *Bradyrhizobium* elkanii SEMIA 5019 plate developed for soybean that obtain from Tehran (Water and Soil Institute) was used for this study. Petri dishes (10 cm in diameter) were used for biofilm production. To produce biofilm cultures, a 2 × 2 cm yeast manitol agar (YMA) slab that containing *Bradyrhizobium elkanii* was placed in each Petri dish. A small piece (ca. 1 mm2) of mycelial mass of *P. ostreatus* inoculated into the YMA slab. These Petri dishes after 14 days when mycelium grown was completed were used to produce mother spawn.

The other Petri dishes were maintained without the *Bradyrhizobium* inoculation.

Sterilized-plastic bottles containing processed wheat grains were incubated separately with pure mycelium alone (Treatment 1) of American oyster mushroom (*Pleurotus ostreatus*), which is the conventional practice, or the pure mycelium together with a 2 × 2 cm yeast manitol agar (YMA) slab containing *Bradyrhizobium elkanii*, a rhizobial strain used for soybean biofertilizers (Treatment 2) and in another treatment plastic bottles containing processed wheat grains were incubated by pure mycelium and incubated by *Bradyrhizobium* slab at same time (Treatment 3). All incubated bottle maintain at 25 °C until mycelium grown was completed and then was used for spawn preparation followed by Singh and Singh method[9].

Oyster mushroom cultivation

The rice straw (*Oryza sativa* L. var. Alikazemi) was obtained locally, and had been stored approximately 6 months. The straw was chopped into small pieces (1-2 cm), weighed, and soaked in water overnight. Extra water present in the substrates was drained and the substrates were spread on clean blotting paper and air-dried for 15 min to remove excess water. Substrate was pasteurized by boiling for 30 min in water. A sample of substrate was weighed before and after drying in an oven at 60°C for 2 days to determine dry matter content. Total nitrogen, potassium, and ash (%) were measured. Amounts of substrates (3000 g) with 85% moisture were mixed with 10% spawn (wet wt/wet wt). Inoculated substrates were placed in 50 × 35 cm polythene bags. Bags were tightly closed and pin holes (1/100 cm^2) made through the bags for drainage. Bags were kept in a spawn running room at 25 ± 1°C in the dark until primordia formed. After primordia formation, large holes were made in the polythene bags to allow normal development of fruiting bodies. Bags were kept at 22 ± 1°C with a 12-h photoperiod (1500-2000 lux) and 85-90% relative humidity. Adequate ventilation was provided to prevent increased CO_2 concentration in the room. Mushrooms were manually harvested 3 days after primordia initiation.

Traits measurement and data analysis

Biological efficiencies (BE) were calculated from ratios of weight (kg) of fresh mushrooms harvested·kg^{-1} to dry weight of substrates. The mushrooms from each replication were oven dried at 60°C until it showed a constant weight. Dry weight was then recorded. Total nitrogen and protein were determined in samples of 0.5 g dry weight by the Kjeldhal method using concentrated H_2SO_4, K_2SO_4, and $CuSO_4$ to digest

the sample[3]. Potassium was measured by flame photometry (AT-8000-Gold supplier-Shanghai), total Ca, Mg and P content in ash were measured with a spectrophotometer (Jenway-6405 UV/Vis-Essex-UK) at 570, 510 630 nm respectively. A completely randomized experimental design with 10 replications (2×5) was used. Data were analyzed using SAS (ver. 9, SAS, Inc., Cary, N.C.). The Tukey test was performed to separate means.

RESULTS AND DISCUSSION

Added *Bradyrhizobium* in wheat incubation stage increased Ash, Ca, P, K, and protein, and decreasing days to primordial initiation in the mushroom samples significantly (Table 1). Added *Bradyrhizobium* in Petri culture stage also had the effect of increasing days to primordial initiation, Ca, Mg and protein, but decreasing biological efficiency, Dry matter, Ash, P and K in the mushroom samples (Table 1). No significant differences were found by adding of *Bradyrhizobium* in both Petri culture stage and wheat incubation stage on total nitrogen in the mushroom samples (Table 1). Formation of the biofilms by rhizobia with common soil fungi through mycelial colonization has been observed recently by Seneviratne and Jayasinghearachchi[9], in our research we found significant effect of *Bradyrhizobium* on mushroom total protein.

Table 1. Effect of *Bradyrhizobium* on measured characteristics

Treatments	Days to primordial initiation	Biological efficiency (Kg fresh mushroom/Kg Dry substrate)	Dry matter (%)	Ash (% in FM)
T1:Control (without *Bradyrhizobium*)	25.00[b]	84.00[a]	9.2[a]	1.8[b]
T2:*Bradyrhizoubium* in wheat incubation	24.33[b]	91.33[a]	12[a]	4.14[a]
T3: Use of *Bradyrhizobium* in Petri culture preparation	33.33[a]	30.33[b]	7.6[a]	0.37[b]

Table 1. Continue

Treatments	Ca (mg. 100 g DM)	Mg (mg. 100 g DM)	P (mg. 100 g DM)	K (mg. 100 g DM)
T1:Control(without *Bradyrhizobium*)	56.40b	112.96a	393.9a	5933.3ab
T2: *Bradyrhizoubium* in wheat incubation	65.67a	84.22a	406.9a	6933.3a
T3: Use of *Bradyrhizobium* in Petri culture preparation	67.30a	115.49a	287.2a	5133.3b

Table 1. Continue

Treatments	N (% in DM)	Protein (% in DM)
T1:Control(without *Bradyrhizobium*)	3.99a	24.91b
T2: *Bradyrhizoubium* in wheat incubation	4.44a	31.08a
T3: Use of *Bradyrhizobium* in Petri culture preparation	4.82a	30.09ab

CONCLUSIONS

The pure mycelium together with *B. elkanii* increased ash, Ca, P, K and protein contents by 128%, 16%, 3%, 17% and 24%, respectively, compared to the pure mycelium alone. It also increased biological efficiency and dry matter content of mushroom by 10% and 30%, respectively, and decreased time to primordial initiation by ca. 7 days. Therefore, the use of rhizobia as biofertilizers in mushroom cultivation seems to be a very promising method of producing a higher yield of minerals and protein-rich mushroom more efficiently, which should be further developed for other types of mushrooms in the future. This will also increase the profit margin of the present mushroom industry.

REFERENCES

1. Barberi, E., C. Guidi., J. Bertaux., P. Frey-Klett., J. Garbaye., P. Ceccaroli., R. Saltarelli., A. Zambonelli and V. Stocchi. *Environ. Microbiol,* 2007; **9:** 2234-2246.
2. Bobek, P. and S. Galbavy. Hypercholesterolenic and antiatherogenic effect of oyster mushroom (*Pleurotus ostereatus*) in rabbit. *Nahrung*, 1999; **43**(5), 339-342.
3. Bobek, P., L. Ozdyn, and L. Kuniak. The effect of oyster mushroom (*Pleurotus ostereatus*) ethanolic extract and extraction residues on cholesterol levels in serum lipoproteins and liver of rat. *Nahrung*, 1995; **39,** 98-99.
4. Bremner. J.M. and M.A. Tabatabai. Use of an ammonia electrode for determination of ammonium in Kjeldhal analysis of soils; Commun. *Soil Sci. Plant Anal*. 1972; **3:** 159-165.
5. Chang, S.T. World production of cultivated and medicinal mushrooms in 1997 with emphasis on Lentinus edodes (Berk) Sing, China. *Int. J. Med. Mush*. 1999; **1:** 291-300.
6. Ginterová. A and Maxianová. A. The balance of nitrogen and composition of proteins in *Pleurotus ostreatus* grown on natural substrates. *Folia Microbiol*. (Prague) 1975; **20:** 246-250.
7. Jayasinghearachchi. H.S and Seneviratne. G. Can mushrooms fix atmospheric nitrogen? *J. Biosci.* 2004; **29**(3): 293-296.
8. Rangaswami. G., Kandaswami. T.K. and Ramasamy. K. *Pleurotus sajor-caju* (Fr.) Singer, a protein rich nitrogen fixing mushroom fungus. *Curr. Sci*. 1975; **44:** 403-404.
9. Royse, D.J. Influence of spawn rate and commercial delayed release nutrient levels on *Pleurotus cornucopiae* (oyster mushroom) yield, size and time to production. *Appl. Microbiol. Biotechnol.* 2002; **58:** 527-531.
10. Seneviratne. G. and Jayasinghearachchi. H.S. Mycelial colonization by *Bradyrhizobia* and *Azorhizobia*. *J. Biosci.* 2003; **28:** 243-247.
11. Singh. R. and U.C. Singh. Modern mushroom cultivation. Jodhpur, *Agrobios*, 2005; 229.