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Impact of different additives on spawn production of different strains of lion's mane mushroom (*Hericum Spp.*)

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Abstract

Medicinal mushrooms have bioactive compounds with therapeutic properties. Lion's Mane Mushroom is found throughout the northern hemisphere in Europe, Asia, and North America. The present investigation was conducted with the aim to determine the effect of organic and inorganic additive on mycelium growth (spawn) of Lion's mane mushroom (*Hericum Spp.*). In the present study organic additive viz. pulses flour (Pigeon pea) and cereal flour (Rice flour) @2% and inorganic additive (MgSO₄) were mixed @1% as a supplement with wheat grains for spawn production of six different strains of *Hericum*. The results obtained during the present investigation, in the spawn production maximum mycelial growth (90.00 mm) was found in strain He-02 in the organic additive Pigeon pea flour and also in cereal flour (Rice flour) on 20th days, while maximum mycelial growth (90.00 mm) was also found in strain He-02 when MgSO₄ was mix in wheat grains for spawn production of six strains of *Hericum* as inorganic supplements. Based on the results obtained, for spawn production of Lion's mane mushroom (*Hericum Spp.*) strains He-02, by organic and inorganic supplementation, Pigeon pea flour and rice flour while in case of inorganic additive MgSO₄ would be recommended most effective supplement in wheat grains for strain He-02.

Keywords: Additives, spawn, mushroom *Hericum Spp.*

Introduction

Mushrooms are delicious, nutritionally rich, medicinally important and non-conventional source of human food. Mushroom occurs seasonally throughout the world in various environments with very different characteristics, such as form, size, colour, appearance and edibility.

Lion's mane mushroom, is one of the most important medicinal mushroom, also bearded tooth which produce beautiful fruiting bodies, described as a mass of fragile icicle like spines suspended from either a branched supporting framework or from a tough, un-branched cushion of tissue (Arora 1986) [2].

The Lion's Mane mushroom (*Hericum erinaceus*) was first cultivated in the 1960's, and is now widely cultivated as medicinal mushroom in many countries. This mushroom generally have short stalks and form a whitish cluster of downward cascading spines which can commonly be found on dead woods or living trees in Asia, Europe, and North America (Ginns J. 1985) [5].

In Japan, *Hericum erinaceus* is known as "Yamabushitake", Yamabushi literally means "mountain priest". In China, the mushroom goes by the name "Houtou", which means "monkey head". This mushroom is also known as "Lion's Mane", "Monkey's Mushroom", "Bear's Head", "Hog's Head Fungus", "White Beard", "Old Man's Beard", "Pom Pom" and "Bearded Tooth" in other parts of the world (Thongbai *et al.* 2015) [16].

Lion's Mane mushroom contains 32 aroma substances. The dominant compound was 1-octen-3-ol, which accounted for 56–60% total content of aroma substances. Lion's Mane mushroom has a relatively high nutritional value. Lion's Mane mushroom fruiting bodies contain 57 per cent carbohydrates, 3.52 per cent fats, 7.81 per cent fibre, 22.3 per cent protein and 9.35 per cent dry matter ash. Moreover, the following soluble sugars were also found arabinose at 127.17 mg/g, glucose at 11.35 mg/g, mannitol at 12.98 mg/g, inositol at 1.43 mg/g and trehalose at 9.71 mg/g dry matter (Mau J. L. *et al.* 2001) [12].

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It was reported that preparations from its fruiting bodies and mycelia are useful in treating gastric ulcers and used anti-cancer, anti-hypertensive, hypolipidemic, neuronal disease protecting activities. This mushroom has also been used in the orient as an edible and popular folk medicine to treat various human diseases (Yang and Jong, 1989; Chang and Miles, 1989; Ahn, 1992; Mizuno, 1995; Wasser and Weis, 1999) [18, 4, 1, 13, 17].

Material and Methods

Experimental site

Present investigations were performed during 2019-2020 at Mushroom Laboratory, Pathology Department, S. V. P. Agriculture and Technology University, Meerut, UP, India, located on the western side of the Delhi-Dehradun highway (NH-58) at a distance of 10.0 km north of Meerut city. The Meerut district is located between latitude 29° 01'N and longitude 77° 45'E at 237 meters above mean sea level.

Establishment of pure culture

The culture of six strains of *Herichium* was purified and maintained by single hyphal tip method. For this purpose, the culture was grown in sterilized Petri plates on Potato Dextrose Agar Medium (PDA) for 8-10 days. Single branched hyphae from the periphery of the growing colony were marked under low power (10x) in the compound microscope and transferred to PDA slants. These tubes were incubated at 18-20°C for about a week, again sub cultured on PDA and then stored in a refrigerator at 5-10°C for further use.

Spawn Production and adding of different additive

In the present study, two different type additives viz. Organic additives (rice flour, pulse flour) and inorganic additives (MgSO₄) were mixed with two different doses @ 2% and 1% with wheat grains respectively. For this study, the spawn was prepared in half litre capacity glass bottles. The grains were washed by sieving or winnowing or by hand picking undesired grains to remove any damaged, shrivelling grains. After that the grains were soaked in clean water overnight and then washed. They were boiled in water for 15 minutes taking care that grains should not split but remain slightly hard after boiling. The boiled grains were spread in a thin layer over a wire net to remove excess water and enable them to cool about 22-25°C.

The cooled grains were then mixed with 1.2 percent commercial grade gypsum (CaSO₄) and 0.3 percent calcium carbonate (CaCO₃). Gypsum prevents the sticking of wheat grains together and calcium carbonate maintains the pH 5.5 - 7.5. The grains were filled up to 2/3rd of its capacity in the bottle in three replicates. The bottles were plugged with non-absorbent cotton and covered with butter paper.

These bottles were then sterilized at 121°C (15 lbs pressure) for 2 hours on two consecutive days. Sterilized bottles were taken out from the autoclave, while still hot and were shaken to avoid clumping of grains. Sterilized bottles were inoculated with few 9mm diameter disc of a vigorously grown 7 days old culture of Lion's Mane mushroom. Before the inoculation pre balanced by electric balance and sterilized by autoclave (10 lb pressure for 15 minutes) organic additives rice flour, pulse flour (pigeon pea) while inorganic additives (MgSO₄) were mixed in a bottle after sterilization under an aseptic condition in the laminar flow chamber. The spawn bottles were incubated without shaking at 20±1°C in B.O.D incubator after inoculation of 7th days old culture of *Herichium* and

observations were recorded on 5th, 10th, 15th and 20th day till to completely cover by mycelial growth in bottles.

Statistical analysis

The Complete randomized design (CRD) was applied and the data thus obtained were analyzed statistically. Analysis of variance (ANOVA) technique and critical difference (CD) was calculated at five percent level of significance for comparison with other treatment (Gomez and Gomez 1996) [6].

Result and Discussion

In the present investigation, different additives were used for spawn production and results were observed as follows.

Effect of organic additive on spawn's production

In the present study six strains of *Herichium* were grown on different additive mix in wheat grains viz. Rice flour with @ 2% for the evaluation of organic additive effect on spawn production Table-1 and Fig-1. The results revealed that on 20th day, maximum mycelial growth (90 mm) was observed in strain of He-02 which was significantly superior to other strains and followed by strain He-08 (83.66 mm). The minimum mycelial growth (76.33 mm) was observed in strain He-07 which was statistically lower than all other strains.

Kumar (2019) [10] also reported that organic additives (cereal) were mixed as a supplement with wheat grain for spawn production of Milky Mushroom (*Calocybe indica*) and the results obtained during the investigation, organic additives maximum mycelial growth was found in Rice powder @ 1%.

The results were in accordance with the findings of Ko *et al.* (2005) [9] tested different supplements (rice flour, wheat bran, barley bran, Chinese cabbage, egg shell, and soybean powder) were found to be suitable for the mycelial growth of all the tested species. In mycelia growth, soybean powder was the best supplement for *Herichium americanum*, *Herichium coralloides*, and *Herichium erinaceum* while barley bran was the best for *Herichium alpestre*, *Herichium laciniatum*, and *Herichium erinaceus*. For *Herichium abietis*, rice flour and Chinese cabbage was the best.

And for the evaluation of organic additive on *Herichium*, six strains were also grown on pulses flour mix in wheat grains viz. Pigeon pea flour with @ 2% Table-2 and Fig-2. For this study six strains viz. He-01, He-02, He-04, He-05, He-07 and He-08 were used. The results revealed that on 20th day, maximum mycelial growth (90 mm) was observed in strain of He-02 which was statistically similar with strains He-08, He-07 and He-04 with growth (89.33 mm), (87.66 mm) and (87.33 mm) respectively and statistically superior than others. The minimum mycelial growth (76.00 mm) was observed in strain He-05 which was statistically lower than all other strains.

Kumar (2019) [10] also studies on two strains (CI-17-04 and CI-17-08) of *Calocybe indica* and found in case of Strain CI-17-04 maximum growth was observed in Rice powder @ 1. It was followed by Pigeon pea powder @ 1%, which was statically similar to Black gram powder @ 1%. The minimum growth was observed in control.

Singh *et al.* (2015) [15] also evaluate different organic additive pulses flour and found maximum mycelial growth in 2% pigeon pea flour additive grains on 20th days and the mycelium was thick then other pulse flour and minimum growth was found in control (no organic additive) on 20th days.

The results were in accordance with the findings of Ramabadran *et al.* (2000) [14] used the various substrates for spawn production, partially-filled paddy grains (PFPg) and sorghum grains were rapidly colonized by *P. ostreus* horse gram flour 3% was found to be highly favorable.

Effect of Inorganic additive on spawn's production

For the evaluation of inorganic additive effect on *Hericium*, six strains were grown on inorganic additive mix in wheat grains viz. MgSO₄ with @ 2% as shown in Table-3 and Fig-3. Observation was recorded on 5th, 10th, 15th and 20th days. The results revealed that on 20th day, maximum mycelial growth (90 mm) was observed in strain of He-02 which was significantly superior to all other strains and it followed by strain He-08 (84.00 mm). The minimum mycelial growth (67.00 mm) was observed in strain He-01, which was statistically lower than all other strains. The results were in accordance with the findings of Bani *et al.* (2010) [3] found

that inorganic chemicals significantly increased the mycelium growth of strains of *C. indica* (i.e. CI-3, CI-4, and CI-10) as compared to control. Maximum radial growth was observed on 8th day of observation in ferrous sulphate, manganese sulphate and calcium sulphate supplemented medium in CI-4 strain followed by magnesium sulphate. Kumar *et al.* (2010) [11] also reported the maximum radial growth observed in ferrous sulphate, manganese sulphate, copper sulphate supplemented medium in CI-6 strain followed by magnesium sulphate. Gupta (2015) [7] also reported that, Maximum radial mycelia growth was observed at 9th week observations in potassium sulphate in strain APK-2 followed by Magnesium sulphate and calcium carbonate and found minimum in control (no supplementation). Katiyar (2018) [8] also observed the maximum spawn growth at 20 day of observation in ferrous sulphate @1% followed by magnesium sulphate (MgSO₄) @ 1% and minimum spawn growth in control (no supplementation).

Table 1: Effect of Cereal additive (Rice flour) on the spawn growth of different strains of *Hericium* spp.

Strain	Spawn growth (mm)				Growth rate (mm/day)
	5 th day	10 th day	15 th day	20 th day	
He-01	13.00	20.66	49.66	83.33	4.16
He-02	20.00	35.33	57.66	90.00	4.50
He-04	13.00	23.66	49.33	82.00	4.10
He-05	12.66	20.33	49.00	81.66	4.08
He-07	12.66	20.00	43.33	76.33	3.81
He-08	15.33	28.33	56.33	83.66	4.18
CD at 5%	2.20	3.64	2.64	2.96	-
SE(m)	0.70	1.17	0.85	0.95	-

Average of three Replications

Table 2: Effect of Pulse additive (Pigeon pea flour) on the spawn growth of different strains of *Hericium* spp.

Strains	Spawn growth (mm)				Growth rate (mm/day)
	5 th day	10 th day	15 th day	20 th day	
He-01	18.33	36.33	59.33	82.33	4.11
He-02	28.33	48.00	63.66	90.00	4.50
He-04	20.00	37.66	61.00	87.33	4.36
He-05	13.00	34.66	53.33	76.00	3.80
He-07	20.00	39.33	61.66	87.66	4.38
He-08	20.66	38.33	63.00	89.33	4.46
CD at 5%	3.11	2.81	4.04	2.68	-
SE(m)	1.00	0.93	1.29	0.86	-

Average of three Replications

Table 3: Effect of Inorganic additive (MgSO₄) on the spawn growth of different strains of *Hericium* spp.

Strains	Spawn growth (mm)				Growth rate (mm/day)
	5 th day	10 th day	15 th day	20 th day	
He-01	12.66	20.66	40.33	67.00	3.35
He-02	23.33	39.33	67.00	90.00	4.5
He-04	13.00	22.66	44.00	72.00	3.60
He-05	14.00	24.00	48.33	75.33	3.76
He-07	15.66	26.33	53.66	78.00	3.90
He-08	20.33	37.33	61.66	84.00	4.20
CD at 5%	2.96	2.74	2.47	1.84	-
SE(m)	0.95	0.88	0.79	0.59	-

Average of three Replications

Fig 1: Effect of Cereal additive (Rice flour) on the spawn growth of different strains of *Hericium* spp.

Fig 2: Effect of Pulse additive (Pigeon pea flour) on the spawn growth of different strains of *Hericium* spp.

Fig 3: Effect of Inorganic additive (MgSo₄) on the spawn growth of different strains of *Hericium* spp.

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