Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 2716-2719
Received: 10-05-2019
Accepted: 12-06-2019

Vinay Kumar
Department of Plant Pathology, SVPUA&T, Meerut, Uttar Pradesh, India

Gopal Singh
Department of Plant Pathology, SVPUA&T, Meerut, Uttar Pradesh, India

Satpal Singh
Department of Plant Pathology, SVPUA&T, Meerut, Uttar Pradesh, India

Jay Prkash Kannaujia
Department of Plant Pathology, SVPUA&T, Meerut, Uttar Pradesh, India

Naimish Kumar
Department of Plant Pathology, SVPUA&T, Meerut, Uttar Pradesh, India

Anil dohare
Department of Plant Pathology, SVPUA&T, Meerut, Uttar Pradesh, India

Correspondence
Satpal Singh
Department of Plant Pathology, SVPUA&T, Meerut, Uttar Pradesh, India

Effect of different inorganic and organic additives on spawn growth of two strains (CI-17-04 and CI-17-08) of milky mushroom (*Calocybe indica*)

Vinay Kumar, Gopal Singh, Satpal Singh, Jay Prkash Kannaujia, Naimish Kumar and Anil dohare

Abstract
For Mushroom cultivation spawn is an essential component. Quality spawn has a great effect on the sporophores production. During the mushroom session, productions of quality spawn and its availability is a major challenge for mushroom growers. The present study was conducted with the aim to find out the most favourable inorganic and organic additives for the improvement of spawn growth of two Strains (CI-17-04 and CI-17-08) of Milky Mushroom (*Calocybe indica*). In the present Study different Inorganic and organic additives were mixed as a supplement with wheat grain for spawn production of Milky Mushroom (*Calocybe indica*). The results obtained during the present investigation, in case of inorganic additives maximum mycelial growth (90.00 mm) was found in ferrous sulphate @ 0.50% in Strain CI-17-04 and CI-17-08 while in case of organic additives maximum mycelial growth (90.00 mm) was found in Rice powder @ 1% in both strains. Based on the results obtained, ferrous sulphate and Rice powder would be recommended most effective inorganic and organic additives uses as supplement in Wheat grains for spawn production of *Calocybe indica* Strains (CI-17-04 and CI-17-08).

Keywords: *Calocybe indica*, spawn, rice powder, ferrous sulphate, inorganic and organic additives

Introduction
Mushrooms have been favoured as food by mankind since time immemorial because of their unique flavour and excitingly different taste and nutritional value. Mushroom lies between meat and vegetable. Chinese were the first to do the artificial cultivation of tropical and subtropical mushrooms about one thousand year ago, before the real commercial ventures were started in Europe. The mushrooms comprise of large heterogeneous groups having various shapes, sizes and colours, are quite different in characters, appearance and edibility. Out of more than 2000 edible species, about 300 species belonging to 70 genera are reported from India. Mushroom has been defined as "a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogenous and large enough too seen with naked eye and picked by hand" [1]. milky mushroom (*Calocybe indica*) has become the third commercially grown in India after button and oyster mushrooms [2]. This is a tropical mushroom which can be grown in western plain zone from March-September. This mushroom was first collected in wild form from West Bengal (India) [3]. Nutrient content of fresh milky mushrooms is in gram per 100g. Moisture 87.4% Protein 2.75%, Lipid 0.65%, Fiber 1.63%, Ash 1.28%, and Carbohydrate 6.8%, and nutrient content of dried milky mushrooms is in gram per 100g., Protein 21.4%, Lipid 4.95%, fiber 12.9%, Ash 13.1%, and Carbohydrate 48.5% [4, 5] basically low calorie food (25-30 calorie/100gm fresh weight). Milky Mushroom (*Calocybe indica*) is one of the important mushrooms called as "Kuduk" but popularly known as "Dudhichhata" or white summer mushroom.

Production technology of milky mushroom (*Calocybe indica)* has been introduced which was improved [6]. Cultivation of milky mushroom (*Calocybe indica*) is becoming popular in southern parts of India, for its high temperature optimum (30-35^{o}C) and assumes significance particularly amongst the growers of Kerala due to low cost of production, abundant availability of agro-waste materials and suitability to the agro-climatic conditions prevailing in Kerala. Its production largely depends upon a good casing layer, which will provide the aeration, moisture and anchorage in a slightly alkaline medium for growing fruiting body. The worm cast thus becomes important here as it is highly organic in nature, neutral in reaction, very porous and is having very high water holding capacity which is the pre-requisite of a casing material. Reports are also available that, the spent mushroom substrate is a beneficial product for the enrichment of soils [7]. The present investigation was carried out with objectives to evaluate the beneficial effects of different inorganic and organic supplement for spawn production of Milky Mushroom (*Calocybe indica*).

Material and Methods

Experimental site

The experiments were conducted during 2018-2019 in Mushroom Laboratory Department Plant of Pathology, S. V. P. University of Agriculture and Technology, Meerut, UP, India, which is situated on the Western side of the Delhi-Dehradun high way (NH-58) at a distance of 10.0 km away in the north of Meerut city. The district Meerut is situated between 29º 01'N latitude and 77º 45'E longitude at an altitude of 237 meters above the mean sea level.

Establishment of pure culture

Culture of milky mushroom (*Calocybe indica)* strains CI-17-04 and CI-17-08 were obtained from DMR Solan (H.P.) India. Cultures were purified and maintained by single hyphal tip method. For this purpose, the cultures were grown 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 and Petri plates. These were incubated at 28-30 ^{0}C for about a week, again sub cultured on PDA and then stored at room temperature for further use.

Spawn Production and adding of different inorganic and organic supplements

In present study, different inorganic additives, *viz.* ferrous sulphate (0.25% & 0.50%), copper sulphate (0.25% & 0.50%), potassium sulphate (0.25% & 0.50%), zinc sulphate (0.25% & 0.50%) and Organic additives (cereal and pulse) *viz.* Rice powder @ 1%, Barley powder @ 1%, Maize powder @ 1%, Oat powder @ 1%, Pigeon pea powder @ 1%, Chick pea powder @ 1%, Pea powder @ 1% and Black gram powder @ 1%, with the recommended dose of $CaCo_3$and $CaSO_4$ were mixed in wheat grain as supplements. For this study, the spawn was prepared in half litre capacity wide mouthed glass bottles. The grains were cleaned to remove any broken, shrivelled grains either by sieving or winnowing or by hand picking of undesired grains. After this, the grains were soaked overnight in clean water 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 thin layer over a wire net to remove excessive water and enable them to cool about 25-30 ^{0}C. The cooled grains were then mixed with 1.2 percent commercial grade gypsum ($CaSO_4$) and 0.3 percent calcium carbonate ($CaCO_3$). Gypsum prevents the sticking of wheat grains together and calcium carbonate maintains the pH 5.5 - 7.5. The grains were filled up to (100 mm) 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 ^{0}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. Pre sterilized inorganic and organic additives were mixed in bottle under aseptic condition in laminar flow chamber and bottles were inoculated by 9 mm disc in individual bottle [8]. The spawn bottles were incubated without shaking at 28±2 ^{0}C in B.O.D incubator and observations were recorded on 5th, 10th, 15th and 20th day till to completely cover by mycelial growth in bottles [9].

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 [10].

Result and discussion

In the present investigation of different inorganic and organic supplementation for spawn production results shows that:

Effect of different Inorganic additives on spawn production

The results were revealed that among all inorganic additives on the spawn growth of two strains (CI-17-04 and CI-17-08) of milky mushroom (*Calocybe indica)*. In case of Strain CI-17-04 maximum spawn growth (90.00 mm) was observed in ferrous sulphate @ 0.50%, followed by copper sulphate @ 0.50% (86.33 mm). The minimum spawn growth (71.00 mm) was observed in control. While in case of strain CI-17-08 maximum spawn growth (90.00 mm) was observed in Ferrous sulphate @ 0.50%, followed by potassium sulphate @ 0.50% (86.00 mm) and the minimum spawn growth (69.33 mm) was recorded in control as shown in Table 01.

The results were in accordance with the findings of Bani [11] who was suggested that inorganic chemicals significantly increased the growth of strains of *Calocybe indica* (i.e. CI-3, CI-4, and CI-10) as compared to control. Maximum growth was observed in ferrous sulphate (9.00 cm), manganese sulphate (9.00 cm) and calcium sulphate (8.93 cm) supplemented medium in CI-4 strain followed by magnesium sulphate (8.45 cm).

Katiyar [12] result revealed that the maximum spawn growth was observed at 20 day of observation in ferrous sulphate @1% (100mm) fallowed by magnesium sulphate ($MgSO_4$) @ 1% (99.00). Minimum spawn growth in control (no supplementation) 65.00 mm, while the poor results were observed in control.

Table 1: Effect of different Inorganic additives on spawn growth of two strains (CI-17-04 & CI-17-08) of milky mushroom (*Calocybe indica*).

S. No.	Inorganic additives	Spawn growth (mm)									
		5th Day		10th day		15th day		20th day			
		Growth (mm)		Growth (mm)		Growth (mm)		Growth (mm)		Growth rate (mm/day)	
		CI-17-04	CI-17-08	CI-17-04	CI-17-08	CI-17-04	CI-17-08	CI-17-04	CI-17-08	CI-17-04	CI-17-08
1.	Ferrous sulphate @ 0.25%	20.33	23.00	40.33	42.33	63.00	59.00	88.66	87.33	4.43	4.35
2.	Ferrous sulphate @ 0.50%	21.00	25.33	42.67	46.66	65.66	60.66	90.00	90.00	4.50	4.50
3.	Copper sulphate @ 0.25	17.00	18.33	36.33	34.33	58.33	51.33	84.66	82.66	4.23	4.10
4.	Copper sulphate @ 0.50%	19.00	19.00	38.33	38.66	61.00	52.66	86.33	83.66	4.31	4.18
5.	Potassium sulphate @ 0.25%	13.33	16.33	30.00	33.66	60.66	47.00	77.33	85.66	3.86	4.28
6.	Potassium sulphate @ 0.50%	14.33	17.33	32.33	37.33	52.00	51.00	79.33	86.00	3.96	4.30
7.	Zinc sulphate @ 0.25%	15.00	20.33	35.66	40.33	53.00	56.00	81.66	79.00	4.08	3.85
8.	Zinc sulphate @ 0.50%	16,33	22.00	39.66	41.33	57.66	57.00	83.33	81.33	4.16	4.30
9.	Control	11.33	14.66	28.33	28.00	41.00	37.33	71.00	69.33	3.55	3.45

	CD at 5%	1.69	1.72	1.48	2.49	2.25	1.94	2.90	1.52	-	-
	SE (m)	0.56	0.57	0.49	0.83	0.75	0.64	0.96	0.50	-	-

Effect of different Organic additives (cereals and pulses) on spawn growth

The results indicated that the maximum spawn growth was observed in Rice powder @ 1% (90.00 mm), which was followed by Pigeon pea powder @ 1% (85.66 mm) and minimum spawn growth (71.33 mm) was observed in control in Strain CI-17-04. While In case of strain CI-17-08 Maximum spawn growth (90.00 mm) was observed in Rice powder @ 1% which was followed by Maize powder @ 1%, (86.66 mm) and minimum spawn growth (74.00 mm) was recorded in the control as shown in Table 02.

The results were in accordance with the findings of Kumar [13] result revealed that maximum growth from the strain CI-6 while in case of CI-4, the maximum growth was observed in wheat, Bajra and maize grain. Maximum time was observed in rice grain for complete mycelial growth in both strains CI-6 and CI-4, respectively. Among the grain spawn, bajra grain spawn significantly took minimum DFSR from the strain CI-6. Maximum time was taken for full spawn run by rice grain while maximum DFPF and DFFH, it was noticed in rice grain spawn in both strains CI-6 and CI-4. Maximum yield was recorded from two strains CI-6 and CI-4 with spawned wheat grain + gram husks and maximum average weight per sporophore was recorded in oat grain spawn from strain CI-6 and in rice + gram husk from CI-4.

Senthilnambi [14] conducted an experiment to find the suitability of different grains as spawn substrates of *C. indica*. The results revealed the supremacy of sorghum grains as the most suitable substrate for early spawn run, which took only 13.7 days for hundred percent mycelial growths. The yield and number of buttons harvested were found maximum in the sorghum grain spawn followed by ragi grain spawn. The maize grain substrates took 19 days for complete spawn run and recorded low yield when compared to other spawn substrates. The days for pin head formation and first harvest of the crop were earlier in case of sorghum grain spawn followed by ragi spawn.

Table 2: Effect of different Organic additives (Cereal & Pulses) on the spawn growth of two strains (CI-17-04 & CI-17-08) milky mushroom (*Calocybe indica*).

S.N0.	Organic additives	Spawn growth (mm)									
		5th Day		10th day		15th day		20th day			
		Growth (mm)		Growth (mm)		Growth (mm)		Growth (mm)		Growth rate (mm/day)	
		CI-17-04	CI-17-08	CI-17-04	CI-17-08	CI-17-04	CI-17-08	CI-17-04	CI-17-08	CI-17-04	CI-17-08
1.	Rice powder @ 1%	20.33	25.00	40.33	44.66	63.00	64.66	90.00	90.00	4.50	4.50
2.	Barley powder @ 1%	21.00	16.66	42.67	33.66	65.66	52.66	74.66	79.00	3.73	3.98
3.	Maize powder @ 1%	17.00	22.00	36.33	42.00	58.33	62.00	88.33	86.66	4.41	4.33
4.	Oat powder @ 1%	19.00	18.33	38.33	35.66	61.00	57.00	78.66	81.33	3.93	4.06
5.	Pigeon pea powder @ 1%	13.33	19.00	30.00	41.00	60.00	61.66	85.66	89.33	4.28	4.46
6.	Chick pea powder @ 1%	14.33	20.33	32.33	37.66	52.00	59.00	82.00	83.00	4.10	4.15
7.	Pea powder @ 1%	15.00	17.00	35.66	35.00	53.00	55.66	76.00	85.33	3.80	4.26
8.	Black gram powder @ 1%	16.33	21.33	39.66	40.33	57.66	62.66	84.00	80.66	4.20	4.03
9.	Control	11.33	13.66	28.33	29.33	41.00	50.00	71.33	74.00	3.56	3.70
	CD at 5%	1.69	2.05	1.69	1.91	2.25	2.83	2.74	1.91	-	-
	SE (m)	0.56	0.68	0.56	0.63	0.75	0.83	0.91	0.63	-	-

Conclusion

Based on the obtained result it can be conclude that, in case of different Inorganic additives maximum spawn growth was observed in ferrous sulphate @ 0.50% in both strains. While in case of different Organic additives, maximum spawn growth was observed in Rice powder @ 1% in both strains CI-17-04 and CI-17-08.

Reference

1. Chang ST, Miles PG. Mushroom biology a new discipline. The Mycologist, (UK), 1993.
2. Chadha KL, Sharma SR. Mushroom research in India: History, Infrastructure and Achievements, Advances in Horticulture. 1995; 13:1-12.
3. Purkayastha RP, Chandra A. New species of edible mushroom from India. Trans. Br. Mycol. Soc. 1974; 62:415-418.
4. Alam N, Amin R, Khan A, Ara I, Shim MJ, Lee MW *et al*. Nutritional analysis of cultivated mushrooms in bangladesh – *Pleurotus Oystrus, Pleurotus sajor caju, Pleurotus florida* and *Calocybe indica. Mycobiology*, 2008; 23(4):228-232.
5. Sharma SK, Lall AM. Non - enzymatic antioxidant expression and nutritional composition of Calocybe *indica* under different organic supplementations. Journal of cell and tissue research. 2013; 13(1):3541-3544.
6. Purkayastha RP, Nayak D. Effects of substrates and spawn density on the production and protein content of fruit body of *Calocybe indica*. Mush. Jou. 1977; 52:132-138.
7. Mishra KK, Pandey V. Impact of physico-chemical properties of casing soil on sporophore development and yield of white summer mushroom, *Calocybe indica* p&c Department of Plant Pathology, College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar - 263 145, Uttarakhand, INDIA, 2007.
8. Dlamini BE, Earnshaw DM, Masarirambi MT. Growth and yield response of Oyster mushroom (*Pleurotus ostreatus*) grown on locally available substrates. Curr. Res. J Biol. Sci. 2012; 4(5):623-629.
9. Sinder JW. Mushroom spawn and methods of making, U.S. Patent. 1932; 1:86.
10. Steel RGD, Torrie JH, Dickey DA. Principles and Procedures of Statistics. A biometrical approach. 3rd Ed., McGraw Hill Book Co., USA, 1997, 1-198.
11. Bani D, Munjal RL. Response of Summer White Mushroom to Supplementation of Cultivation Substrate *Calocybe indica* Asian J Exp. Biol. Sci. 2010; 2(4):766-768.

12. Katiyar S. studies on different chemicals on spawn and casing for production of milky mushroom (*Calocybe indica*), Master Thesis S.V.P.U.A.T, Modipuram Meerut, 2018.
13. Kumar R, Singh G, Mishra P. Effect of inorganic supplements on growth and yield of different strains of milky mushroom 2010; 42(3):332-335.
14. Senthilnambi D, Balabaskar P, Eswaran A. Impact of different spawn Substrates on yield of *Calocybe indica.* African Journal of Agricultural Research. 2011; 6(12):3946-3948.