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Effect of different sugars and cereals media on mycelial growth of milky mushroom (*Calocybe indica*)

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Abstract

Calocybe indica commonly known as Milky mushroom or Doodhchatta is a robust in size whose pileus is convex initially and become flattened and expanded. It is the third most widely grown mushroom in India. In present investigation, three different sugars at three doses viz. Sucrose, Maltose, Lactose @ 1.5%, 2.5% and 3.5% and different Cereal media viz. potato dextrose agar, wheat dextrose agar, oat dextrose agar, sorghum dextrose agar, maize dextrose agar, and barley dextrose agar media for radial growth of strains of *Calocybe indica* (CI-22-09). In the observation, maximum mycelial growth was found on Sucrose @ 3.5% and Oat dextrose Agar media respectively.

Keywords: Milky mushroom, sugars, cereals, radial growth, maximum growth

Introduction

Mushrooms are delicious, nutritionally rich, medicinally important and non-conventional source of human food. Mushroom is achlorophyllous fungal plant occurring seasonally all over the world in various habitats with quite different characters like shape, size, color, appearance and edibility. Mushroom has been defined as "a macro fungus with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand" (Chang and Miles, 1993) [12]. Dried sporophores of mushroom contain protein, fat, fiber, carbohydrates, soluble sugar, starch and ash. In addition, it has most of the minerals such as Potassium, Sodium, Iron and Calcium. They are also good source of vitamins, especially B complex group but relatively poor in fat soluble vitamins (A, D, E and K). Among B complex vitamins, mushrooms are especially rich in thiamine (B1), riboflavin (B2), niacin and biotin (Chang and Miles, 1999) [11].

Milky mushroom (*Calocybe indica*) is promising mushrooms of the tropical region Out of various mushrooms that may be cultivated on lignin rich agricultural wastes whose wild forms were reported for the first time by Purkayastha and Chandra 1974 [1] from Calcutta markets, and thereafter, attempts were made to domesticate it (Purkayastha, 1979). It is third most largely growing mushroom in India and being tropical in nature, the mushroom is grown commercially in many parts of the country particularly in southern parts of India (Kumar *et al.*, 2014) [14].

Natural occurrence of milky mushroom in plains of Tamil Nadu and Rajasthan has also been reported (Doshi *et al.*, 1989; Krishnamoorthy, 1995) [3,9]. *Calocybe indica* belongs to Phylum: Basidiomycota, Class Agaricomycetes, Order Agaricales and Family Tricholomataceae (Krik *et al.*, 2008) [6]. Nutrient content of fresh milky mushroom is in gram per 100 g. moisture 87.4% protein 2.75%, lipid 0.65%, fiber 1.63%, ash 1.28%, and carbohydrates 6.8%, and nutrient content of dried milky mushroom is in gram per 100 g., protein 21.4%, Ash 13.1%, and Carbohydrate 48.5%. (Sharma and Lall, 2013; Alam *et al.*, 2008) [10, 4] basically low-calorie food (25-30 calorie/100 gm fresh weight). The fruit body contains 12 amino acids (Kumar *et al.*, 2014) [14] and mineral salts viz. copper 0.44 mg/100 g, manganese 0.36 mg/100 g, zinc 0.05 mg/100 g, iron 0.13 mg/100 g, calcium 0.51 mg/100 g, phosphorous 0.38 mg/100 g, potassium 1.35 mg/100 g and sodium 0.21 mg/100 g (Chelladurai *et al.*, 2014) [13].

Even though many attempts had been made to grow *Calocybe indica* while only confined achievement was done in growing the bio-efficiency and productiveness of this mushroom. However, at present limited success has been achieved on its cultivation and productivity therefore, a need was felt to revalidate and production technology on locally available lignocellulosic substrate available in western Uttar Pradesh.

Materials and Methods

Experimental site

The present investigation was conducted in Mushrooms Laboratory, Department of Plant Pathology, Sardar Vallabhbhai Pate University of Agriculture and Technology, Modipuram, Meerut 250110 (Uttar Pradesh). This university is situated on the western side of the Delhi Dehradun highway at a distance of 10.0 km in the north of Meerut City. The Meerut district is situated between 29° 01'N latitude and 77° 45'E longitude at an altitude of 237 meters above the mean sea level.

Establishment of pure culture

Culture of CI-22-09 strain of milky mushroom (*Calocybe indica*) were obtained from DMR Solan (H.P.). 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 °C for about a week, again sub cultured on PDA and then stored at room temperature for further use.

Effect of different sugars on mycelial growth of milky mushroom

Effect of four different sugars was observed in 10 treatments sugar were mixed three doses viz. Sucrose, Maltose, Lactose @ 1.5%, 2.5% and 3.5% for radial growth of strains of *Calocybe indica* (CI-22-09). PDA medium was prepared and required amount of sugar added in the medium before sterilization. Sterilization has been done in autoclave at 121 °C (1.1 kg/cm² pressure) for 20 minutes. The 20 ml medium was poured in each sterilized petri plate and subsequently inoculated with 9 mm disc of seven days old culture. Inoculated plates were incubated at 28-30 °C. Experiment was conducted with three replications of each treatment. The observations of radial growth were taken at each 3days till the colony covered the first full plate.

Effect of different cereal media on mycelial and dry matter growth of (*Calocybe indica*)

For the present investigation six different media and broth (potato dextrose agar, wheat dextrose agar, oat dextrose agar, sorghum dextrose agar, maize dextrose agar, and barley dextrose agar media) were used. Two-hundred-gram oat grains were washed with water 2-3 times and soaked in water for 8 hrs. then boiled with 500 ml distilled water for 20 minutes, until the grains become soft, allowed to cool then, grains were separated and the liquid suspension passed through a muslin cloth. The volume of the extract so obtained was made up to 500 ml by adding distilled water. Twenty-gram agar-agar was melted separately in 500 ml of distilled water and mixed with oat grains extract. The total volume was made up to 1000 ml by adding distilled water.

Maize dextrose agar and Barley dextrose agar media were also prepared by same methods as described above for Wheat and Oat extract agar medium. All the prepared media were sterilized by autoclaving at 121 °C (1.1 kg/cm² pressure) for 20 minutes. The test media were poured into Petri plates and after cooling Petri plates were inoculated with 7 days old culture of *Calocybe indica*. under aseptic condition and incubated at 28±2 °C in B.O.D with three replications of each

treatment. The observations on mycelial growth were recorded until the full Petri plates were covered completely by fungal mycelium.

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.

Result and Discussion

Effect of different sugars on mycelial growth of *Calocybe indica*

To observed the effect of different sugars on the radial mycelial growth of *Calocybe indica* strain (CI-22-09), the Potato Dextrose Agar (PDA) medium was supplemented with sugars supplements viz. Sucrose @ 1.5%, 2.5% and 3.5%, Maltose @ 1.5%, 2.5% and 3.5%, Lactose @ 1.5%, 2.5% and 3.5%, while keeping the control without any supplement as shown in Table-1 and Figure- 1. The result was observed from the experiment revealed at 3rd, 6th, 9th and 12th days of observation for the supplement significantly increased the radial mycelial growth as compared to control. Maximum radial mycelial growth observed at 12th day observation in Sucrose @ 3.5% (90.00 mm) followed by Maltose @ 3.5% (89.00 mm) while, minimum in control (71.83 mm), which is followed by Lactose @ 3.5% (75.16 mm). The results are almost in accordance with the findings of Tanwar (2013) [5] observed that among sugar solutions viz. glucose (1%), maltose (1%) and sucrose (1%) significantly increased the radial growth both strains APK-2, CI-22-09 showed maximum radial with maltose. At 9th day of observation in maltose solution the growth of strain APK-2 was found maximum (full growth) diametric growth of mycelium (9.0 cm) followed by glucose (89 mm) and starch (86.67 mm). Kumar (2013) [2] observed that glucose, maltose and sucrose at the rate of 1% significantly increased the radial growth of two strains of *Calocybe indica* (CI-22-09) as compared to control. While maximum mycelial growths were found in maltose followed by glucose on 3rd, 5th, 7th and 9th day of inoculation in case of both strains of *Calocybe indica* (CI-22-09).

Effect of different cereal media on mycelial growth of *Calocybe indica*

In this experiment six different media viz. Wheat dextrose agar media, Oat dextrose agar media, Sorghum dextrose agar media, Maize dextrose agar media, Barley dextrose agar media and potato dextrose agar media as a control were used for the study of effect on mycelial growth and dry matter weight on two strains (CI-22-09) of milky mushroom (*Calocybe indica*). The observation was recorded on 3th, 6th, 9th and 12th day and shown in table-2 and Figure-2. The results revealed that on 12th day, maximum radial mycelial growth (89.00 mm) was found on Oat dextrose agar media with (7.41 mm/day) growth rate which was significantly higher than all other treatments while it followed by Sorghum dextrose agar media (84.16 mm) with (7.01 mm/day) growth rate. Minimum radial mycelial growth (72.66 mm) was recorded on control with (6.05 mm/day) growth rate which was significantly followed with Maize dextrose agar media (79.33 mm) with (6.61 mm/ day) growth rate. The results

were in accordance with the finding of Singh *et al.* (2009) [7] reported that the *Calocybe indica* grew on all the tested media but maximum linear growth (7.03 cm) was recorded on wheat dextrose agar medium. The next best radial growth was observed on potato dextrose agar medium followed by paddy straw decoction agar medium. The least growth (3.20 cm) was noticed on mustard straw decoction agar medium. Further it

was noticed that growth of fungus on media like gram straw decoction agar, sugarcane bagasse and wheat straw decoction agar was statistically at par. Singh (2012) [8] evaluated five different strains of *Calocybe indica* on eight different media and reported that strain APK-2 showed maximum radial growth (8.97 cm) in PDA media followed MEA and minimum in Wheat extract agar medium.

Table 1: Effect of different sugars on mycelial growth of *Calocybe indica*

Treatment	Mycelial Growth (Mm)				Growth Rate (Mm/Day)
	3 rd Day	6 th Day	9 th Day	12 th Day	
PDA+ Sucrose @ 1.5%	13.50	33.60	56.00	79.83	6.65
PDA+ Sucrose @ 2.5%	13.66	35.00	60.33	84.00	7.00
PDA+ Sucrose @ 3.5%	14.50	36.66	62.83	90.00	7.50
PDA+ Maltose @ 1.5%	12.33	33.50	57.16	80.00	6.66
PDA+ Maltose @ 2.5%	13.16	34.66	60.33	85.66	7.13
PDA+ Maltose @ 3.5%	13.16	36.83	62.83	89.00	7.41
PDA+ Lactose @ 1.5%	13.16	34.83	59.16	82.66	6.88
PDA+ Lactose @ 2.5%	12.33	31.50	54.83	78.83	6.56
PDA+ Lactose @ 3.5%	11.66	28.66	51.66	75.16	6.26
PDA (control)	11.16	25.50	47.66	71.83	5.98
CD at 5%	1.22	1.29	1.44	1.11	-
SE (m)	0.42	0.44	0.50	0.31	-

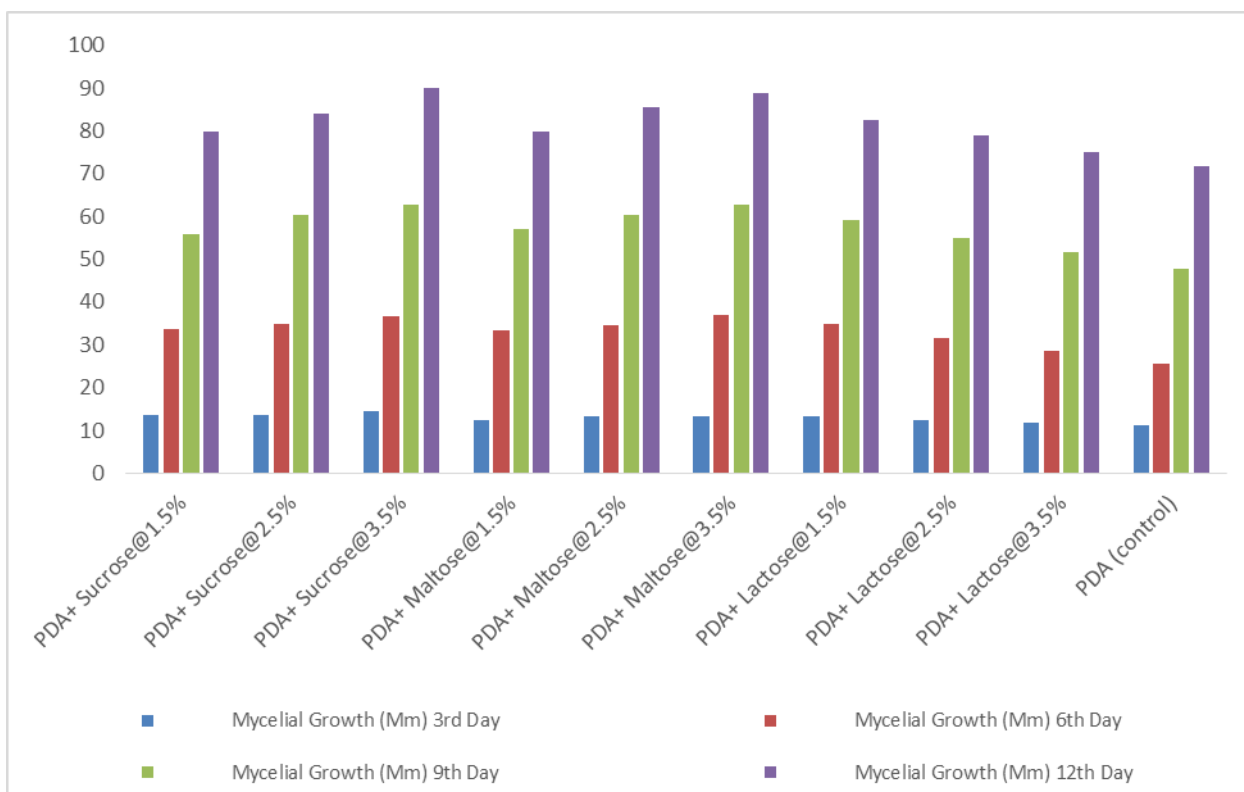


Fig 1: Effect of different sugars on mycelial growth of *Calocybe indica*

Table 2: Effect of different cereal media on mycelial growth of *Calocybe indica*

Treatment	Mycelial Growth (mm)				Growth rate (mm/day)
	3 rd day	6 th day	9 th day	12 th day	
WDA	14.5	39.16	58.00	80.83	6.73
ODA	16.66	39.33	61.16	89.00	7.41
SDA	15.83	39.5	61.50	84.16	7.01
MDA	1.16	35.33	55.83	79.33	6.61
BDA	16.00	36.50	57.50	82.66	6.68
PDA	12.00	27.50	48.00	72.66	6.05
CD at 5%	0.86	1.15	1.35	1.14	-
SE (m)	0.28	0.39	0.45	0.38	-

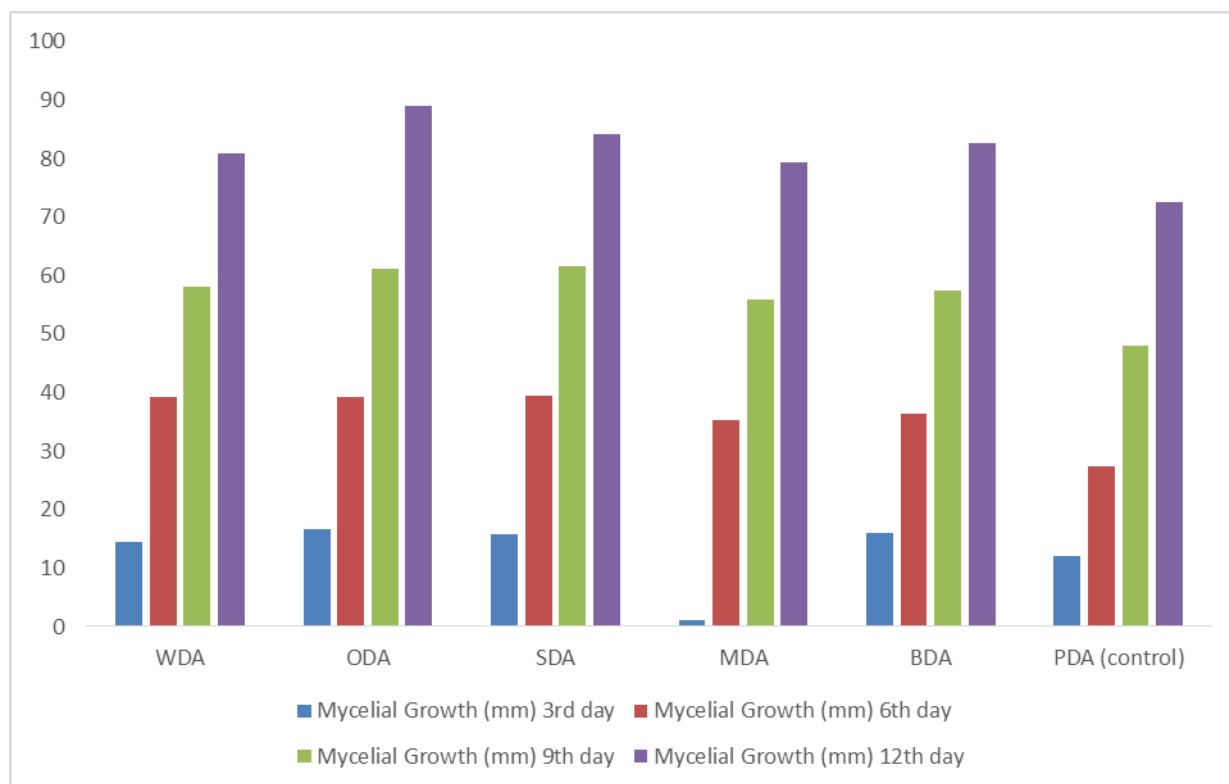


Fig 2: Effect of different cereal media on mycelial growth of *Calocybe indica*

Conclusion

Among 10 treatments in which sugars were mixed in three doses viz. Sucrose, Maltose, Lactose @ 1.5%, 2.5% and 3.5% for radial growth of strains of *Calocybe indica* (CI-22-09), media supplementation with Sucrose @ 3.5% was found best while, among different cereal media, Oat dextrose Agar media was found best.

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