OPEN ACCESS Asian Journal of Crop Science

ISSN 1994-7879
DOI: 10.3923/ajcs.2018.168.173

Research Article
Effect of Various Chemicals on the Mycelial Growth and Fruiting Body of Milky Mushroom (*Calocybe indica*)

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Abstract

Background and Objective: Milky mushroom (*Calocybe indica*) has become the centerpiece for commercial utilization all over the India. Milky mushroom is sturdy, fleshy and milky white in color even after flattening. As it grows in hot humid climate hence this mushroom is highly suitable for cultivation in most of the plains of India almost throughout the year. During the present study, effect of different chemicals on radial growth of the *Calocybe indica* was seen. **Materials and Methods:** For this experiment two *Calocybe indica* strains namely APK-2 and CI-6 were used. The sterilized substrates after spawning at 6% filled in polythene bags and kept in spawn running room at 28-32°C. The polythene bags of completely impregnated mycelial growth were sprayed with 11% chemical solution of calcium carbonate, manganese sulphate, potassium sulphate, magnesium sulphate, zinc sulphate and ferrous sulphate before casing. Observations were recorded as total yield (g kg^{-1} dry straw), days for pinhead formation (DFPF), days for first harvesting (DFFH), number of pinhead initiation (NOPI) and average weight of fruiting body (g/FB). **Results:** Results revealed that the highest numbers of fruiting bodies were plucked from strain APK-2 (25.00/bag) with calcium carbonate supplemented treatment and from strain CI-6 (23.33/bag), in control (without supplementation). Maximum yield was harvested significantly well in magnesium sulphate treatment from strains APK-2 and CI-6 (650.00 and 648.33 g kg^{-1} of dry substrate with 65.00 and 64.83% B.E.) followed by calcium carbonate (635.00 and 623.33 g kg^{-1} of dry substrate with 63.50 and 62.33% B.E.), respectively. **Conclusion:** However, maximum average weight per fruit body was recorded significantly well in magnesium sulphate supplemented treatment from strain APK-2 (31.58 g) and similarly from strain CI-6 (37.92 g). These chemical additives are also very cost effective and having no any residual effect on the quality and taste of mushroom.

Key words: Milky mushroom, inorganic additives, mycelial growth, fruiting bodies, production

Citation: Vinit Pratap Singh, Gopal Singh, Brajesh Kumar, Adesh Kumar and Seweta Srivastava, 2018. Effect of various chemicals on the mycelial growth and fruiting body of milky mushroom (*Calocybe indica*). Asian J. Crop Sci., 10: 168-173.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mushrooms are almost fat free proteinaceous edible fungi popular for their pleasant aroma, vitamins of B group and minerals. It generally contains 20-40% proteins on a dry weight basis and no cholesterol[1]. Other than protein, mushrooms are also a good source of non-starchy carbohydrates along with dietary fibre[2]. Mushroom has got a place in all religious books[3]. According to Greek or Roman myth ''Mushroom sprang from a stroke of lightening'' providing strength and energy during battle and Romans also regarded them as "Food of Gods" or "Gods Flesh", which were served only on festive occasions[4]. Mushrooms are carved as exclusive kind of food, since ancient times believed as a product of an interaction between lightning and the earth[5]. Some also considered it as a flower of Ishwar for Hindus or a dish of Jannat for Islamic people. It is also known by various names like Khumbhi, Gobarchatta, Bhumiphor etc., in India. The Hindi term Khumbhi has been originated from Sanskrit word "Ksumpa". The local name "Gobarchatta" is assigned due to its habitat and chhatra like structure. Since some of its species come out by breaking the soil layer so it is also known as "Bhumiphor".

Milky mushroom (*Calocybe indica*) has become the third commercially grown mushroom in India after the species of *Agaricus* and *Pleurotus*[6]. *Calocybe indica* belongs to sub-division Basidiomycotina, class Hymenomycetes, order Agaricales and family Tricholomataceae[7]. The dried sporophores of this mushroom contain 17.69% protein, 4.10% fat, 3.40% crude fibre, 64.26% carbohydrates, 4.00% soluble sugar, 2.95% starch and 7.43% ash. In addition to this it has most of the mineral salts such as Potassium, Sodium, Iron and Calcium[8]. Integrating mushroom cultivation in the existing farming systems not only supplements the income of the farmers but also promotes proper recycling of agro-residues thereby improving soil health and promoting organic agriculture. Therefore, there is need to have mushroom which can grow during summer months being longer shelf-life. Milky mushroom (*Calocybe indica*) fits well under these conditions because of its ability to grow at temperature above 30°C, white sporophore, excellent shelf life, high biological efficiency (80-90%) and easier in post-harvest handling[9]. *Calocybe indica* is a relatively new introduction to the world of edible mushrooms from India[10]. It is also known as "Dudh Chhatta" because of its milky white appearance and large sized sporophores or as "white summer mushroom" because of its tropical nature.

So, the main objective of the present study was to evaluate the effect of different chemicals on radial growth of the *Calocybe indica*. For these experiment two strains of *Calocybe indica* namely APK-2 and CI-6 were used.

MATERIALS AND METHODS

The experiments were conducted in Mushroom Laboratory Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India during the year 2015-16. During the experiment the effect of six chemical additives were evaluated for the growth and production of milky mushroom which were provided by the Laboratory of Agricultural Chemistry, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. Analytical and commercial grading of all the chemicals mentioned in Table 1.

Effect of different chemicals on radial growth: The experiment was carried out by using six chemicals viz., ferrous sulphate (1%), manganese sulphate (1%), potassium sulphate (1%), magnesium sulphate (1%), zinc sulphate (1%) and calcium carbonate (1%) mixed in potato dextrose agar (PDA) medium by using poisoned food technique to see the effect of chemicals on the radial growth of the *Calocybe indica*[11]. The PDA medium was prepared and required amount of chemicals were added in the medium after sterilization. The 25 mL medium was poured in each sterilized Petri plate and subsequently inoculated with 9 mm disc of 7 days old culture of strain APK-2 and CI-6. Inoculated plates were incubated at 28±1°C. The observations of radial growth were taken at each 48 h till the colony covered the first full plate.

Effect of different chemicals supplement in substrate on cropping: For this experiment two *Calocybe indica* strains namely APK-2 and CI-6 were used. The sterilized substrates after spawning at 6% filled in polythene bags and kept in spawn running room at 28-32°C. The polythene bags of completely impregnated mycelial growth were sprayed with chemical solution of ferrous sulphate (1%), manganese sulphate (1%), potassium sulphate (1%), magnesium sulphate (1%), zinc sulphate (1%) and calcium carbonate (1%) before casing. Observations were recorded as total yield (g kg^{-1} dry straw), days for pinhead formation (DFPF), days for first harvesting (DFFH), number of pinhead initiation (NOPI) and average weight of fruiting body (g/FB).

Table 1: Grading of chemicals

Chemicals	Grading
Magnesium sulphate ($MgSO_4$)	10% Mg, 13% S
Potassium sulphate (K_2SO_4)	52% K_2O, 16% S
Ferrous sulphate ($FeSO_4$)	20% Fe
Manganese sulphate ($MnSO_4$)	24% Mn
Zinc sulphate ($ZnSO_4$)	22-36% Zn
Calcium carbonate ($CaCO_3$)	40% Ca, 12% C, 48% O

Statistical analysis: Data with appropriate transformations were analyzed with the help of one way analysis of variance table wherever required. The F-value was tested and critical difference (CD) was calculated at 5% of significance for comparing treatment means[12,13].

In order to compare the means of various entries, calculating the critical difference (CD) by the following formula:

$$\text{Critical difference (CD)} = SE \times 't'$$

where, SE is standard error of the difference of the treatment means to be compared and:

$$SE = (2\ _{Mse}/r)^{1/2}$$

RESULTS AND DISCUSSION

Effect of different chemicals on radial growth: It was evident from the data presented in Table 2 and Fig. 1 that the chemicals viz., ferrous sulphate (1%), potassium sulphate (1%), magnesium sulphate (1%) and calcium carbonate (1%) significantly increased the radial growth of two strains of *C. indica* (i.e., APK-2 and CI-6) as compared to control. Maximum radial growth was observed on 9th day of observation in magnesium sulphate (9.00 cm), potassium sulphate (8.92 cm) and calcium carbonate (7.92 cm) supplemented medium in APK-2 strain followed by ferrous sulphate (7.78 cm). Similarly in case of CI-6 maximum radial growth on 9th day observed in magnesium sulphate (9.00 cm), potassium sulphate (8.78 cm) and calcium carbonate (8.22 cm) supplemented medium followed by ferrous sulphate (7.52 cm). The poor growth was recorded in strains APK-2 (3.78 cm) and CI-6 (2.95 cm) in zinc sulphate supplemented medium.

The results are almost in accordance with the findings of many scientists who studied the effect of various source of carbon (brown sugar, fructose, lactose, glucose, sucrose, starch and maltose), nitrogen [wheat bran, yeast cream, beef cream, peptone, $(NH_4)_2SO_4$, NH_4Cl and $(NH_4)_2CO_3$] and inorganic salts (K_2SO_4, $MgSO_4$, $CaSO_4$, $MnSO_4$ and $FeSO_4$) on the mycelial growth of *P. Ostreatus*[14]. The mycelial growth was more pronounced with brown sugar as the carbon source, wheat bran as the nitrogen source and $MgSO_4$ as the inorganic salts source. The most suitable culture medium for *P. ostreatus* consisted of various nutrient sources, including 3% wheat bran, 1% brown sugar, 1% $MgSO_4$ and 10 mg vitamin B1 per litre[12]. The chemicals viz., salicylic acid (0.1%), ferrous sulphate (1%), copper sulphate (1%), manganese sulphate (1%), potassium sulphate (1%), magnesium sulphate (1%), zinc sulphate (1%) and calcium carbonate (1%) significantly increased the radial growth of the five strains of *Calocybe indica* (i.e., CI-3, CI-4, CI-5, CI-6 and CI-10) as compared to control. Maximum radial growth was observed at 6th day's observations in ferrous sulphate, manganese sulphate and copper sulphate (9.00, 9.00 and 8.93 cm) in CI-6 strain followed by magnesium sulphate (8.45 cm)[15].

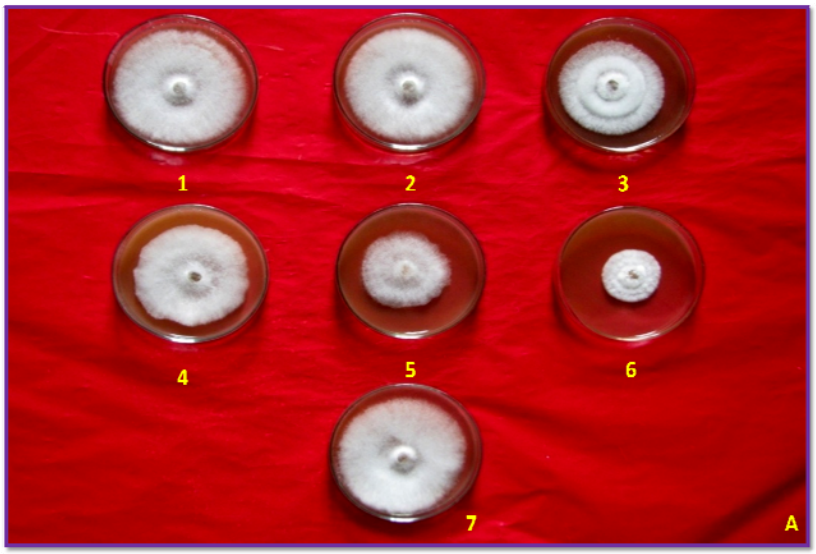

Fig. 1: Effect of different chemicals on radial growth of *Calocybe indica* (1: $MgSO_4$, 2: K_2SO_4, 3: $FeSO_4$, 4: $CaCO_3$, 5: $MnSO_4$, 6: $ZnSO_4$ and 7: Control)

Table 2: Effect of different chemicals on the radial growth of *Calocybe indica* strain APK-2 and CI-6 in PDA media

		3rd Day		5th Day		7th Day		9th Day			
Chemicals	Dose (%)	APK-2	CI-6	APK-2	CI-6	APK-2	CI-6	APK-2	Increase/decrease (%)	CI-6	Increase/decrease (%)
$MgSO_4$	1.0	2.88	2.98	5.02	5.27	7.28	7.27	9.00	17.34	9.00	23.28
K_2SO_4	1.0	2.87	2.82	5.02	4.82	7.13	6.57	8.92	16.92	8.78	20.27
$FeSO_4$	1.0	2.20	2.22	4.23	4.05	6.10	5.60	7.78	1.43	7.52	3.01
$CaCO_3$	1.0	2.75	2.82	4.45	4.47	6.27	6.43	7.92	3.25	8.22	12.60
$MnSO_4$	1.0	2.15	2.07	3.73	3.68	5.23	5.22	6.73	-12.25	6.78	-7.12
$ZnSO_4$	1.0	0.98	0.90	1.72	1.37	2.72	2.18	3.78	-50.71	2.95	-59.59
Control	-	2.00	2.52	4.02	4.10	6.45	5.92	7.67	-	7.30	-

CD at 5% Chemical: 0.2446, Strains: 0.1307, Chemical Strains: 0.346, Average of three replications

Effect of different chemical supplements on yield: The results in Table 3 and 4 indicated that incorporation of the chemical supplements increased the yield and number of sporophores of both strains of *Calocybe indica*. Wheat straw substrate supplemented with different chemicals took less period for pinhead formation and first harvesting while produced more number of pin head initiation and higher yield in case of both strains APK-2 and CI-6 as compared to control (without supplementation). The magnesium sulphate supplemented treatment showed least days for pinhead formation (15.33 and 14.00 days) and for first harvesting (18.33 and 23.33 days) in both strains APK-2 and CI-6 as compared to control, respectively. Maximum number of pinhead was recorded in treatment supplemented with magnesium sulphate and potassium sulphate (both 49.67/bag) in APK-2 and they were non-significant while in case of CI-6 supplemented with magnesium sulphate (62.00/bag) followed by potassium sulphate (58.67/bag) which were significantly superior in overall treatments.

Maximum numbers of fruiting bodies were harvested from strain APK-2 (25.00/bag) with calcium carbonate supplemented treatment and from strain CI-6 (23.33/bag), in control (without supplementation). Maximum yield was harvested significantly well in magnesium sulphate treatment from strains APK-2 and CI-6 (650.00 and 648.33 g kg^{-1} of dry substrate with 65.00 and 64.83% BE) followed by calcium carbonate (635.00 and 623.33 g kg^{-1} of dry substrate with 63.50 and 62.33% BE) as compared to control (533.33 and 475.00 g kg^{-1} of dry substrate with 53.33 and 47.50% BE), respectively. However, maximum average weight per fruit body was recorded significantly well in magnesium sulphate supplemented treatment from strain APK-2 (31.58 g) and similarly from strain CI-6 (37.92 g), respectively.

Kumar *et al.*[16] recorded comparatively similar results that is minimum time was observed for spawn run in supplemented with magnesium sulphate followed by salicylic acid and manganese sulphate in strains CI-6 and CI-4, which were significantly superior than without supplemented treatment. The salicylic acid supplemented treatment was significantly showed least days for pinhead formation and magnesium sulphate for first harvesting in both strains CI-6 and CI-4 as compared to control. Maximum numbers of pinhead initiation were showed in treatment supplemented with magnesium sulphate in CI-6 followed by ferrous sulphate and supplemented with manganese sulphate followed by calcium carbonate in CI-4. Maximum yield was harvested significantly well in magnesium sulphate treatment from strains CI-6 and CI-4 followed by calcium carbonate as compared to control[16].

Table 3: Effect of different chemicals on spawn run, cropping period and yield of *Calocybe indica* using APK-2 strain

Chemicals	DFPF	DFFH	NOPI	NOFB	Yield (g kg^{-1} dry substrate)	Average weight (g/FB)	Biological efficiency (%)
Magnesium sulphate ($MgSO_4$)	15.33	18.33	49.67	20.67	650.000	31.580	65.00
Potassium sulphate (K_2SO_4)	16.67	25.33	49.67	22.67	563.330	24.870	56.33
Ferrous sulphate ($FeSO_4$)	19.67	24.67	46.33	23.33	608.330	26.070	60.83
Manganese sulphate ($MnSO_4$)	18.00	22.33	41.67	21.33	548.330	25.760	54.83
Zinc sulphate ($ZnSO_4$)	15.67	19.67	40.00	20.33	578.330	28.440	57.83
Calcium carbonate ($CaCO_3$)	16.33	21.33	45.33	25.00	635.000	25.410	63.50
Control	21.33	26.00	39.00	21.33	533.330	25.030	53.33
CD at 5%	1.890	2.217	NS	2.113	45.162	2.651	-
SE (d)	0.873	1.024	4.067	0.976	20.855	1.224	-

Average of three replications, DFPF: Days for pinhead formation, DFFH: Days for first harvesting, NOPI: Number of pinhead initiation, NOFB: Number of fruiting body

Table 4:- Effect of different chemicals on spawn run, cropping period and yield of *Calocybe indica* using CI-6 strain

Chemicals	DFPF	DFFH	NOPI	NOFB	Yield (g kg^{-1} dry substrate)	Average weight (g/FB)	Biological efficiency (%)
Magnesium sulphate ($MgSO_4$)	14.00	23.33	62.00	17.33	648.330	37.920	64.83
Potassium sulphate (K_2SO_4)	17.00	26.33	58.67	21.00	495.000	23.670	49.50
Ferrous sulphate ($FeSO_4$)	19.00	26.00	56.33	20.67	585.000	28.330	58.50
Manganese sulphate ($MnSO_4$)	17.33	25.00	52.33	20.33	521.670	25.760	52.16
Zinc sulphate ($ZnSO_4$)	18.00	24.00	49.33	17.00	536.670	31.790	53.67
Calcium carbonate ($CaCO_3$)	14.67	25.00	55.33	22.33	623.330	27.980	62.33
Control	22.33	28.00	46.33	23.33	475.000	20.460	47.50
CD at 5%	2.217	1.967	6.089	3.182	22.745	4.881	-
SE (d)	1.024	0.909	2.812	1.469	10.503	2.254	-

Average of three replications, DFPF: Days for pinhead formation, DFFH: Days for first harvesting, NOPI: Number of pinhead initiation, NOFB: Number of fruiting body

CONCLUSION

During the present experiment, maximum numbers of fruiting bodies were harvested from strain APK-2 with calcium carbonate supplemented treatment and from strain CI-6, in control (without supplementation). Maximum yield was harvested significantly well in magnesium sulphate treatment from strains APK-2 and CI-6 followed by calcium carbonate, respectively. However, maximum average weight per fruit body was recorded significantly well in magnesium sulphate supplemented treatment from strain APK-2 and similarly from strain CI-6.

In view of the presented results, it can be concluded that the yield, yield contributing characteristics and biological efficiency in the supplemented sets increased as compared to the unsupplemented control set except zinc sulphate but the utilization of magnesium sulphate supplemented substrate is promising for the enhanced production of milky mushroom (*Calocybe indica*). The addition of chemical additives to substrate significantly increased the mycelia extension, density and yield of *Calocybe indica*.

SIGNIFICANCE STATEMENT

Salient findings of this study is that the addition of these inorganic additives in the substrate will furnished significant increment in the yield of milky mushroom because these additives are cost effective for the poor farmers and having no any residual effect on the quality and taste of mushroom.

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