ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.pharmascope.org/ijrps

Studies on the evaluation of some strains of *Calocybe indica* P&C for cultivation in Jammu

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Article History:

Received on: 02.04.2019
Revised on: 10.07.2019
Accepted on: 15.07.2019

Keywords:

Jammu,
milky mushroom,
cultivation,
sporophores

ABSTRACT

Calocybe indica, commonly known as milky/summer white mushroom, 'kuduk' or 'dudhi chatta', is a lignocellulolytic, tropical mushroom of Indian origin, which requires a temperature of 30-35°C and a relative humidity of 80-90°C for good growth. It is a new introduction to the domestic mushroom family. There are 40 different species of *C. indica*, out of which four are edible, that is, *C. carnea, C. ionides, C. gambosa and C. indica.* In northern part of our country, very few efforts have been attempted towards the cultivation of *C. indica*. Therefore, the present study was conducted to screen some strains of *C. indica viz.,* DMRO-309, DMRO-319, CI-6, CI-9 and APK-2 procured from Directorate of Mushroom Research, Solan for their growth behaviour, morphometric characters, yield and biological efficiency on wheat and paddy straw for cultivation in Jammu district. On the basis of the results obtained, *C. indica* strain DMRO-309 and APK-2 emerged as the best performers under the climatic conditions of Jammu region.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i3.1494

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INTRODUCTION

Mushroom are conspicuous umbrella-shaped fruiting bodies (sporocarps), which are the sexual reproductive structures of Phylum Ascomycota or Basidiomycota. They are commonly called as macrofungi as their fruiting bodies are large enough to be seen with naked eyes. Mushroom cultivation is an eco-friendly activity, which represents solid-state fermentation, an important microbial technology in which crop residues are converted into valuable food that is rich in protein. In recent years, it has gained a lot of importance due to the increasing demand for high-quality proteins, minerals and vitamins, which can be of direct benefit to human health and fitness. Mushroom are considered to be the highest protein producers per unit area and time due to the utilization of vertical space and short life cycle. In fact, their cultivation under controlled conditions requires less water than any other crop grown in the field and has, therefore, the potential of being a major crop in the coming years (Prakasam, 2012). They are also known as "the ultimate health food" because of their unique nutritional status and were earlier described as "gift from God" by the Romans and the Greeks, who served them only on festive occasions.

Mushroom farming is commonly practiced in more than 100 countries, with an annual turnover rate of 6-7% (Kumar *et al.*, 2013). Studies have shown that in terms of monetary gains, mushroom cultivation can be kept at a third place just after crop and animal husbandry (Prakasam, 2012). In some of the developing countries, this hi-tech venture of mushroom cultivation has a promising scope to meet the nutritional requirements without undue pressure on land. China is the leading producer of mushrooms, growing more than 20 different types of mushrooms at commercial scale, which include some important

ones like *Pleurotus ostreatus*, *Lentinula edodes*, *Auricularia polytricha*, *Flammulina velutipes* and *Agaricus bisporus* (Wu *et al.*, 2013). The second-largest producer of mushroom is the USA, which shares 16% of the world output (Kumar *et al.*, 2013). According to (Prakasam, 2012), three geographical regions- Europe, America and East Asia contribute approximately 96% of the world mushroom production.

In India, cultivation of edible mushrooms started in 1961 with the temperate mushrooms, especially button mushroom (*Agaricus bisporus*). However, our country can make rapid progress in the mushroom industry by cultivating the tropical and subtropical mushrooms. The annual turnover of fresh mushrooms in India is about 1,13,315 tonnes with Punjab, Uttarakhand and Haryana as the leading states in decreasing order (Thakur and Singh, 2014).

Calocybe indica is a lignocellulolytic mushroom, which requires a temperature of 30–35°C and relative humidity of 80 to 90 % for good growth. Therefore, it is an ideal candidate for hot weather cultivation when no other mushroom excepting *Volvariella* species can grow. It has a robust sporophore, attractive colour, sustainable yield, delicious taste, unique texture and excellent shelf life as compared to oyster or button mushroom (Amin *et al.*, 2010). Like the oyster mushroom (*Pleurotus* species), it is capable of growing on a wide range of lignocellulosic substrates. Moreover, its spore content is very low and hence does not cause respiratory allergy problem as the oyster mushroom species do. From J&K state, *Calocybe indica* (CI-3 strain) has been cultivated on a wide range of agrowastes, garden wastes and forest wastes of Jammu division (Chivan and Sumbali, 2016). However, no work has been done on the other available strains of *C. indica*. Therefore, the present study was conducted to screen some strains of *C. indica viz.,* DMRO-309, DMRO-319, CI-6, CI-9 and APK-2 procured from Directorate of Mushroom Research, Solan for their growth behaviour, morphometric characters, yield and biological efficiency on wheat and paddy straw in Jammu.

Jammu division of J&K has tropical to a sub-tropical type of climate with an optimum temperature of 25-35°C and relative humidity of 70-85% existing for most period of the year. The agro-climatic conditions of this division favour cultivation of various agricultural and horticultural crops whose major output is the unusable lignocellulosic waste material having cellulose (30-35%), hemicelluloses (20-30%) and lignin (10-15%) as the predominant components. Only a small fraction of the total waste is utilized for useful applications, leaving behind the unused bulk, which causes environmental problems and incurs disposal cost to the food processors and various agriculturists. Therefore, efforts are being made for the conversion of these waste materials into more profitable products using solid-state fermentation.

MATERIALS AND METHODS

Studies on the evaluation of some strains of *Calocybe indica* (P&C) for cultivation in Jammu were conducted and following materials and methods were used

Source and maintenance of *Calocybe indica* cultures

Pure cultures of 5 strains of milky mushroom (*C. indica* P&C) were procured from Directorate of Mushroom Research, Solan, Himachal Pradesh. These included DMRO-309, DMRO-319, CI-6, CI-9 and APK-2. All the strains were maintained on sterilized PDA (potato dextrose agar) medium and MEA (malt extract agar) medium slants and kept at room temperature during the entire period of investigation. Subsequent culturing was done after every 3 months.

Agrowastes used for the cultivation of *C. indica*

For the cultivation of *C. indica*, two agrowastes, that is, paddy straw and wheat straw were collected from local fields.

Cultivation site and mushroom house

Experiments pertaining to the cultivation of *C. indica* (P&C) were conducted in a mushroom cultivation house located in the Botanical Garden of Department of Botany, University of Jammu, Jammu.

The protocol used for the cultivation of *C. indica* strains

Cultivation of *C. indica* was done by following the process given below:

Preparation of mother spawn

A spawn is a pure fungal culture grown on softened grains in a sterilized condition. During the present study, mother spawn of five strains of *C. indica* (DMRO-309, DMRO-319, CI-6, CI-9 and APK-2) was prepared from pure cultures by following the method of (Munjal, 1973).

Preparation of commercial spawn

It was prepared by putting softened wheat grains in polyethylene bags (9" × 13" -150 gauges) @ 500 g grain/bag. Thereafter, these bags were sterilized at 15 lbs./sq. inch for 2 hours. After cooling, the bags were aseptically inoculated with 10-15 g of mother spawn in a laminar airflow chamber and incubated

at 30±2°C. During the period of incubation, the bags were frequently examined for any type of contamination. Bags exhibiting contamination were immediately discarded, whereas those showing white and uniform mycelial growth covering all the grains were used for experimentation.

Preparation of substrate

Agrowastes like wheat straw, paddy straw, maize stalk, bajra stalk and sorghum stalk were collected from the fields, whereas dehulled maize cobs were collected after threshing. These substrates were finally chopped into small pieces of 5-7 cm and then subjected to hot water treatment.

Hot water treatment

The substrates were soaked individually for 4 hours in a drum containing water. Thereafter, the soaked substrates were boiled in hot water (80-90 ºC) for 40-60 minutes. Excess water was then drained off, and the substrates were spread over sloppy and cemented floor till the moisture content of the substrate remained 60 percent. The water content in the substrate after pasteurization and draining was tested by squeezing between the fingers. The substrate which did not leak out drops of water upon squeezing was considered to be saturated with approximately 60% moisture level and was considered ready for use.

Filling of bags and spawning

Spawning is a technique of introducing spawn into the substrate with the aim of achieving rapid growth for the production of sporophores (fruiting bodies). The cooled substrate was filled in polyethylene bags (12"x18") @ 2 kg wet substrate per bag. Multilayered spawning of the substrate was done alternately. For this, wheat grain based spawn was used @ 6% of dry weight of substrate. After spawning, small holes were made in the polyethylene bags and the lower end corners were cut to drain off the excess water. Thereafter, the necks of the bags were closed with rubber bands.

Spawn run

The spawned bags were then kept in the hanging nets placed in the mushroom house, where the temperature of 25-35°C and relative humidity of 80-90 percent were maintained for spawn running. This was done by frequently sprinkling water on the walls, floor and by using mist fan. The spawn run, which refers to the growth of the spawn on the substratum, took 10-15 days. After complete colonization of the substrate by mushroom mycelium, casing was done.

Casing of the bags

It is the process of covering the mycelial colonised substrate with few layers of soil or any other casing material having high water holding capacity, good pore size and neutral pH. After complete spawn run, the bags were cut into two equal halves, and each half was encased with 2-4 cm thick casing material, which consisted of a mixture of FYM. Before use, the casing material was autoclaved at 15 lbs psi for 60 minutes. Further, casing layer was kept moist by sprinkling fresh water as and when required. Three replicates were maintained for each treatment.

Watering and pinhead formation

The cased bags were kept back in the hanging nets at a temperature of 25-35 ºC and relative humidity of 80-90 percent. Watering was done 2-3 times per day by sprinkling the top casing soil in order to maintain moisture. Additionally, a relative humidity of more than 80 percent in the mushroom house was maintained by mist fan. The mushroom house had diffused light conditions due to the blue tarpaulin and a window that was kept partially opened for its aeration. After 8-10 days of casing, numerous needle-shaped pin heads started appearing, and within 6-8 days some of them matured into large-sized sporophores, ready for harvesting.

Harvesting of sporophores

Mature sporophores were harvested when the edges of the cap (pileus) begin to dry or show yellowness. Picking was done by twisting the stipe clockwise and anti-clockwise so that the surrounding young developing mushrooms were not damaged. The sporophore count was recorded for each flush. In all, three flushes were harvested from the same bed at an interval of 10-15 days. Thus, the total cropping period varied from 55-60 days.

Weight and size of sporophores

The freshly harvested sporophores were immediately weighed with the help of an electrical balance (EK8150-13, Zhongshan Camry Manufacturer and Trading Co., Ltd., China) having a sensitivity of 1g and was expressed in grams. The sporophores collected from each bag were also measured for their size, that is, a diameter of the pileus (cap) and length of the stipe (stalk) were recorded with the help of thread and expressed in centimetres and width in millimeters.

Yield and biological efficiency of *C. indica*

The cumulative yield for each substrate and all replicates was recorded by summing up the fresh weight of pickings. Biological efficiency (BE), which is the ability of mushrooms to convert the substrate contents into fruiting bodies was calculated by following (Chang *et al.*, 1981):

Table 1: Growth behaviour of five strains of *Calocybe indica*.

Agrowastes used	*C.indica* strains assessed	Days required for			Total days required for the production of first flush
		Spawn run	Pinhead formation	First flush	
Paddy straw	DMRO-309	$11.67^{a}\pm0.33$	$9.33^{a}\pm0.33$	$9.67^{a}\pm0.88$	30.67
	APK-2	$12.33^{a}\pm0.33$	$10.00^{a}\pm0.58$	$10.33^{b}\pm0.33$	32.66
	DMRO-319	$15.67^{b}\pm0.67$	$12.30^{b}\pm0.88$	$10.16^{b}\pm0.16$	38.13
	CI-6	$17.00^{c}\pm0.58$	$12.67^{b}\pm0.67$	$13.67^{c}\pm0.67$	42.34
	CI-9	$18.33^{d}\pm0.33$	$14.00^{c}\pm0.58$	$14.33^{c}\pm0.67$	46.66
	F- value	63.3	56.4	59.2	-
	P-value	P<0.05	P<0.05	P<0.05	-
Wheat straw	DMRO-309	$13.00^{a}\pm0.58$	$9.67^{a}\pm0.67$	$9.33^{a}\pm0.33$	32.00
	APK-2	$13.67^{a}\pm0.33$	$10.00^{a}\pm0.58$	$10.67^{b}\pm1.20$	34.34
	DMRO-319	$16.33^{b}\pm1.33$	$12.67^{b}\pm0.33$	$12.67^{c}\pm1.20$	41.67
	CI-6	$17.33^{c}\pm0.88$	$13.67^{c}\pm0.33$	$14.00^{d}\pm1.0$	45.00
	CI-9	$19.33^{d}\pm1.45$	$14.67^{d}\pm0.67$	$14.33^{d}\pm1.20$	48.33
	F-value	74.2	59.5	62.3	-
	P-value	P<0.05	P<0.05	P<0.05	-

The values given are mean ± standard error. Fischer's LSD was applied when ANOVA detected asignificant difference (P<0.05) between days required for cultivation by different strains on two agrowastes. Values within a column followed by the same letter do not differ significantly.

Table 2: Morphometric characters of the sporophores produced by *Calocybe indica* strains.

Agrowastes used	*C.indica* strains assessed	Number of sporophores per bag	Range of sporophore weight (g)	Range of pileus diameter (cm)	Range of stipe length (cm)	Range of stipe width (mm)
Paddy straw	DMRO-309	$14.67^{b}\pm0.88$	28-392	6-17	6-18	20-42
	APK-2	$19.47^{a}\pm1.45$	15-235	5-12	6-14	14-39
	DMRO-319	$7.00^{c}\pm0.15$	16-120	4-10	4-9	12-34
	CI-6	$5.67^{d}\pm1.20$	15-104	4-8	4-10	12-29
	CI-9	$2.67^{e}\pm0.67$	7-92	3-7	4-8	10-22
	F-value	48.2	-	-	-	-
	P-value	P<0.05	-	-	-	-
Wheat straw	DMRO-309	$17.67^{b}\pm1.45$	19-320	5-15	6-14	17-40
	APK-2	$21.33^{a}\pm1.20$	14-208	4-10	6-10	14-38
	DMRO-319	$6.60^{c}\pm1.52$	16-115	4-7	5-13	12-31
	CI-6	$5.33^{d}\pm0.45$	17-103	4-6	4-10	11-27
	CI-9	2.33e±0.33	12-74	2-6	3-8	10-20
	F-value	52.1	-	-	-	-
	P-value	P<0.05	-	-	-	-

The values given are mean ± standard error. Fischer's LSD was applied when ANOVA detected a significant difference (P<0.05) between the type of strain used and a number of sporophores produced on two agrowastes. Values within a column followed by the same letter do not differ significantly.

Table 3: Yield and biological efficiency of different strains of *Calocybe indica.*

Agrowastes used	*C. indica* strains assessed	Yield g/500g of dry substrate	Biological efficiency (%)
Paddy straw	DMRO-309	$448.07^{a}\pm2.58$	89.61
	APK2	$435.13^{b}\pm2.31$	87.02
	DMRO-319	$393.21^{c}\pm2.16$	78.64
	CI-6	$374.36^{d}\pm0.88$	74.87
	CI-9	$344.37^{e}\pm2.79$	68.87
	F-value	21.45	-
	P-value	P<0.05	-
Wheat straw	DMRO-309	$435.86^{a}\pm1.23$	87.17
	APK2	$415.32^{b}\pm0.96$	83.06
	DMRO-319	$385.19^{c}\pm1.12$	77.04
	CI-6	$370.67^{d}\pm0.78$	74.13
	CI-9	$336.03^{e}\pm0.49$	66.60
	F-value	15.21	-
	P-value	P<0.05	-

The values given are mean±standard error. Fischer's LSD was applied when ANOVA detected a significant difference (P<0.05) between the type of strain used and yield. Values within a column followed by the same letter do not differ significantly.

Biological efficiency (%)=

$$\frac{Fresh\ weight\ of\ mushroom\ yield}{Dry\ weight\ of\ substrate\ used} \times 100$$

Statistical analysis

The data were analysed using analysis of variance (ANOVA) on variables such as growth behaviour of different strains on two standard agrowastes (straw of wheat and paddy); different morphometric characters of five strains on these tested substrates; yield and biological efficiency of these strains on these tested substrates. Analysis of variance and other statistical analysis were done using SPSS software package (Version 18.0).

RESULTS AND DISCUSSION

During the present investigation, five strains of *Calocybe indica* P&C *viz.*, DMRO-309, DMRO-319, CI-6, CI-9 and APK-2 were screened for their growth behaviour during cultivation, morphometric characters of the sporophores, their yield potential and biological efficiency on two different agrowastes *viz.*, paddy straw and wheat straw, which are commonly available in Jammu district.

Growth behaviour during cultivation

Data given inTable 1 depicts the time required for complete spawn run, pinhead formation and first harvest. A perusal of data shows that among the five different strains of *C. indica* evaluated, two strains (DMRO-309 and APK-2) were found to have very fast spawn run on both the tested substrates and statistically did not differ significantly. *C. indica* strain DMRO-309 took 11.67 and 13 days for complete colonization on paddy straw and wheat straw respectively, whereas strain APK-2 colonized paddy straw and wheat straw completely in 12.33 and 13.67 days respectively. Earlier, Krishnamoorthy and Muthusamy (1997); Rawal and Doshi (2014); Singh *et al.* (2018) have also found similar results with APK-2 strain while growing it on different agrowastes in other parts of the country. In contrast to the present results, some researchers have even observed requirement of as high as 32 days for complete spawn run by APK-2 and different CI strains of *C. indica* on some other types of lignocellulosic wastes (Krishnamoorthy and Muthusamy, 1997; Krishnamoorthy *et al.*, 2000; Nagaratna and Mallesha, 2007; Singh *et al.*, 2009; Kaur *et al.*, 2011; Vijaykumar *et al.*, 2014; Krishnamoorthy and Balan, 2014; Chivan and Sumbali, 2016; Singh *et al.*, 2018). The other three tested strains of *C. indica* (DMRO-319, CI-6 and CI-9) took more time (approximately 15 to 19 days) to colonize the tested substrates and showed significant differences (Table 1). However, literature shows no work on the cultivation of *C. indica* DMRO-309 strain but few other CI strains of *C. indica* like CI-1, CI-2, CI-3, CI-4, CI-5, CI-6, CI-7, CI-8, CI-9, CI-10, CI-13, CI-14, CI-15, CI-16, CI-18 and CI-524 have been reported to show early colonization on these two standard agrowastes (Singh *et al.*, 2009; Kaur *et al.*, 2011; Kumar *et al.*, 2011; Senthilnambi *et al.*, 2011; Dhakad *et al.*, 2015; Chivan and Sumbali, 2016; Singh *et al.*, 2018) .

Table 1 also shows that apart from substrate colonization, even the pinhead formation after casing was initiated early in DMRO-309 and APK-2, which took 9.33 and 10 days on paddy straw and 9.67 and 10 days on wheat straw respectively. Statistically, the difference was insignificant. However, the other tested strains exhibited delayed pinhead initiation on these substrates requiring approximately 12-14 days (Table 1). Likewise, DMRO-309 and APK-2 also exhibited early sporophore formation, which were ready for harvesting after 9.67 days (DMRO-309) and 10.33 days (APK-2) on paddy straw and after 9.33 days (DMRO-309) and 10.67 days (APK-2) on wheat straw. The other three tested strains (DMRO-319, CI-6 and CI-9) showed delayed but significant statistical differences in sporophore maturation (Table 1).

The nonsignificant statistical difference with respect to total days required for the production of the first flush of a crop by DMRO-309 and APK-2 was observed (Table 1). Both these strains took minimum time period on both the agrowastes. However, the other three strains exhibited statistical differences and delay in the total days required for crop production, which ranged from 38.13-48.33 days on the tested agrowastes. Recently, Alsowadi and Alhomam, 2019 also observed that *C. indica* APK-2 strain is a not only a fast colonizer *in vivo* but is also a rapid biomass producer *in vitro* conditions at a temperature of 30ºC. Few other researchers have observed early pinhead emergence and sporophore maturation even in case of different CI strains of *C. indica* grown on other agrowastes and their mixed combinations (Singh *et al.*, 2009; Chivan and Sumbali, 2016; Singh *et al.*, 2018). It is possible that variations detected in cropping period on different agrowastes may be due to the environmental variations (temperature, humidity and light arrangements) or due to specific nutritional requirements of the cultivated mushroom (Khanna and Garcha, 1981) . In the present investigation, diffuse blue light, humidity above 80% and temperature of 30±2 °C was used throughout the cultivation programme, which probably enhanced the rate of the growth process and reduced the time period of cultivation.

Morphometric characters of the sporophores

Morphometric characters of the sporophores of tested strains of *C. indica* have been given in Table 2. Perusal of data indicates a wide variation in the number of sporophores per bag (2.33-21.33), sporophore weight (7g-392g), pileus diameter (2-17 cm), stipe length (3-18 cm) and stipe width (10-42 mm). Among the tested strains, DMRO-309 and APK-2 were the best both in terms of growth behaviour as well as morphometric characters. Similar results have been obtained by few other workers during cultivation of APK-2 and different CI strains of *C. indica* in north-western and southern parts of India (Krishnamoorthy and Muthusamy, 1997; Tandon and Sharma, 2006; Bhatt *et al.*, 2007; Singh *et al.*, 2009; Kaur *et al.*, 2011; Kumar *et al.*, 2011; Selvaraju *et al.*, 2015; Dhakad *et al.*, 2015). However, cultivation of strain DMRO-309 is being attempted for the first time and was found to show good growth behaviour and morphometric characters of the sporophores (Table 1 &Table 2).

Yield potential and biological efficiency

Results presented in Table 3 show significant differences in the sporophore yield of all the tested strains of *C. indica* on both the agrowastes that were utilized. Maximum sporophore production of 448.07 g and 435.86 g per 500g of the dry substrate was achieved by spawning DMRO-309 in paddy straw and wheat straw bags respectively. This strain also showed the highest biological efficiency (89.61%) on paddy straw, followed in decreasing order (87.17%) on wheat straw (Figure 1). The other strain, APK-2 strain proved to be less efficient than DMRO-309 as it produced 435.13g and 415.32g of sporophores per 500g of dry substrate and yielded a biological efficiency of 87.02% and 83.06% on paddy straw and wheat straw respectively (Table 3). Rest of the three strains *viz.*, DMRO-319, CI-6 and CI-9 gave moderate yield and biological efficiency of 393.21g (78.64%), 374.36g (74.87%) and 344.37g (68.87%) on paddy straw and 385.19g (77.04%), 370.67g (74.13%) and 336.03g (66.60%) on wheat straw respectively (Table 3).

The results obtained in the present study for APK-2 strain are in accordance with the findings of many other researchers who also observed high yield and high biological efficiency of APK-2 on these standard agrowastes as compared to other lignocellulosic substrates (Krishnamoorthy and Muthusamy, 1997; Rawal and Doshi, 2014; Selvaraju *et al.*, 2015). However, Singh *et al.* (2018) while cultivating APK-2 strain on wheat straw observed only 53.33% biological efficiency, which could be increased by adding different chemicals as supplements. Similarily, Krishnamoorthy (2014) reported significantly higher yield performance ranging from 601.60g to 817.50g per 500g of the dry substrate on chemically treated paddy straw in five mushroom farms of Tamil Nadu, thus exhibiting biological efficiency as high as 120.32% to 163.50%.

Cultivation of *C. indica* DMRO-309 and DMRO-319 was attempted for the first time and they showed significant differences in the yield performance,

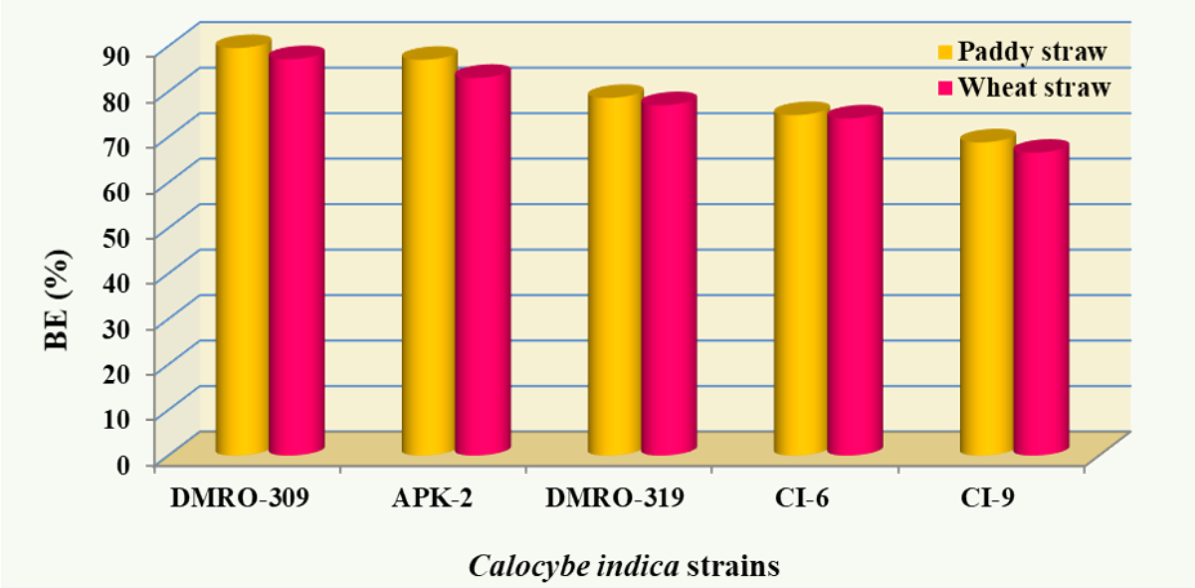

Figure 1: Biological efficiency (BE%) of different strains of *Calocybe indica* on the straw of paddy and wheat.

whereas biological efficiency was recorded to be more than 77% on the tested agrowastes (Table 3). Earlier, Singh *et al.* (2017) while cultivating a wild *Calocybe* species (DMRO-600) obtained a biological efficiency of 70% and 64% on wheat straw and a combination of wheat and paddy straw (1:1) respectively.

Results presented in Table 3 reveal moderate yield and biological efficiency of approximately 74% by *C. indica* CI-6. In contrast to our findings, NRCM (2006) reported that CI-6 strain gave average biological efficiency of 42.34% when grown in different centres of the country. The present investigation also indicates that among the five tested strains, the least biological efficiency was shown by CI-9 (Figure 1). Earlier, Kaur *et al.* (2011) also observed that among the evaluated nine strains of *C. indica* (CI- 1to CI-7, CI-9 and APK-2), least biological efficiency of 47.17% on wheat straw was shown by CI-9. Significant differences in the yield performance and biological efficiency shown by different strains of *C. indica* may be attributed to the differences in their enzymatic activity required for effective colonization of the substrate and later production of sporophores. The ability of a mushroom to digest and absorb the nutrients from the substrate through enzymatic activity is considered to be the main factor for selection of mushroom strains (Rajarathnam *et al.*, 1992).

Microbial contamination of mushroom beds is almost inevitable. While conducting studies on the cultivation of *C. indica*, frequent contamination of substrate bags by different antagonistic microorganisms was observed during spawn run stage and sporophore formation stage in case of strain DMRO-319, CI-6 and CI-9. The substrate bags having these strains were found to be frequently contaminated with *Trichoderma*, *Aspergillus* and *Coprinus* species at spawn run stage, which maybe because of the weak enzymatic activity of these strains, due to which substrate components were not colonized quickly. This causes delayed spawn run with reduced sporophore production and yield and provides sufficient time to the antagonists to grow on the substrate bags. In contrast, no contamination or very little contamination by antagonistic microbes was observed in the substrate beds spawned with strain DMRO-309 and strain APK-2. This may be attributed to their good lignocellulolytic enzymatic activity, which is required for fast colonization of the substrates, thereby providing very less time to the contaminating agents for growth and further spread. Literature also reveals negative effects of the antagonistic microbes causing frequent contamination of the substrate beds during spawn run and thus leading to either complete loss or reduced crop yield (Salam *et al.*, 2004; Sarmah *et al.*, 2006; Singh *et al.*, 2010). Therefore, from the results of the present investigation, it can be concluded that *C. indica* strain DMRO-309 and APK-2 were the best performers under the climatic conditions of Jammu and may be exploited for commercial cultivation.

CONCLUSIONS

It can be concluded from the present study that preferential colonization of substrates and sporophore

yield exhibited by a particular strain may depend upon a number of factors like percent lignocellulosic composition of substrate material, which consists of cellulose, hemicelluloses, lignin and other phenolics that either favour or hinder the activity of mycelial growth. Secondly, proper packaging of the substrate is important for the retention of moisture in the substrate beds, which results in proper mycelial growth. Thirdly, the biodegradative potential of mycelium to grow on a particular substrate lies in its ability to degrade it, which in turn is decided by the repertoire of lignocellulolytic enzymes it possesses.

ACKNOWLEDGEMENT

The authors are highly thankful to the Head, Department of Botany, University of Jammu, J&K for providing lab facilities and to UGC-SAP-II for providing financial assistance for carrying out this research work.

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