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Spawn run dynamics of two *Calocybe indica* strains (DMRO-309 and APK-2) on agrowastes

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ABSTRACT

Cultivation of mushroom is an eco-friendly activity, which represents solid state fermentation, an important technology in which agrowastes and other lignocellulosic wastes are converted into valuable protein rich food. Since last few decades, it has gained lot of importance due to increasing demand for high quality proteins, minerals and vitamins, which can directly benefit human health. Mushroom are probably the highest protein producers per unit area and time due to the utilization of vertical space and short life cycle. One such promising mushroom is *Calocybe indica* P&C (milky white mushroom/ summer mushroom) commonly known as 'kuduk' or 'dudhichatta', whose two strains viz., DMRO-309 and APK-2 were cultivated on different agrowastes and their combination and the time period required for complete colonization was evaluated. It was observed that both these strains exhibited statistical differences in the time period taken for colonization of different agrowastes and their combinations. Further, it was observed that *C. indica* strain DMRO-309 was a faster colonizer than APK-2

Keywords: Milky mushroom, *Lyophyllaceae*, Colonization, Substrate, Mycelial growth

INTRODUCTION

Calocybe indica P&C, commonly known as milky white mushroom/summer mushroom, 'kuduk' or 'dudhi chatta', which is a specialty mushroom, belonging to family *Lyophyllaceae* of order *Agaricales* and class *Agaricomycetes*. There are about forty species of *Calocybe* (Suganya and Suriyavathana, 2012), of which around nine species are reported from neotropical regions (Singer, 1977, 1986; Pegler, 1983). This genus has four edible species, which include *C. carnea, C. ionides, C. gambosa* and *C. indica* (Pandey, 1998). It has a robust sporophore, milky white colour, good taste, unique texture, sustainable yield and excellent shelf life as compared to *Agaricus* and *Pleurotus* species (Amin *et al.,* 2010). Like the oyster mushroom (*Pleurotus* species), it is capable of growing on a wide range of lignocellulosic substrates but it needs a casing layer to stimulate fruiting (Atri *et al.,* 2012; Gitte *et al.,* 2014). The average biological efficiency of *C. indica* ranges between 60-90% and further increase can be achieved by adopting good cultivation practices (Senthilnambi *et al.,* 2011). Literature survey reveals that the cultivation of this mushroom is being done in the states like Rajasthan (Doshi and Sharma, 2007), Tamil Nadu (Krishnamurthy *et al.,* 2000; Krishnamoorthy and Bala, 2015), Maharashtra (Navathe *et al.,* 2014) and number of other states of India with tropical climate (Karuppuraj, 2014; Krishnamoorthy and Bala, 2015). In Punjab as well there are reports of its cultivation on local substrates (Atri *et al.,* 2012; Phutela and Phutela, 2012). Out of the total annual turnover of 1,13,315 tonnes of fresh mushrooms produced in India (Thakur and Singh, 2014), milky mushroom is reported to contribute only 3% (Sharma *et al.,* 2017), thereby leaving a lot of scope to work on this mushroom especially in the northern part of India including J&K so as to increase its productivity and contribution towards the overall national average of mushroom production (Chiwan and Sumbali, 2016 a,b).

The investigations presented in this manuscript are on *C. indica* strains (DMRO-309 and APK-2) for complete spawn run on different agro-wastes which has been recommended for cultivation in the warmer regions of the country (Tewari, 2002-2003; 2003). The wild forms of *C. indica* were first reported from Calcutta markets by Purkayastha and Chandra (1974) which was subsequently cultivated by Purkayastha (1982). *C. indica* is a lignocellulolytic mushroom, which requires a temperature of 30-35°C and relative humidity of 80 to 90 per cent for good growth. Therefore, it is an ideal candidate for hot and humid weather conditions.

From Jammu division (UT of Jammu and Kashmir), *Calocybe indica* strain CI-3 has been cultivated on a wide range of lignocellulosic wastes such as agrowastes, forest wastes and garden wastes of Jammu division (Chiwan and Sumbali, 2016 a,b). Later, five other strains *viz.,* DMRO-309, APK-2, DMRO-319, CI-6 and CI-9 were also screened for their growth behaviour on two agrowastes (paddy straw and wheat straw) for selection of the two best strains for cultivation in Jammu division (Shrikhandia and Sumbali, 2019). In the present communication, an attempt has been made to evaluate the growth behaviour of the select strains DMRO-309 and APK-2 during spawn run on varied agro-wastes and their combinations.

MATERIALS AND METHODS

Studies on the time taken for spawn run by two strains of *Calocybe indica viz.,* DMRO-309 and APK-2 on different agro-wastes and their combinations (1:1) were conducted from June 2015 to September 2017. For these studies, a low cost mushroom cultivation house measuring 30 feet in length, 12 feet in width and 8 feet in height was fabricated using timber pillars, bamboo, wire net, tarpaulin and nylon ropes.

Source and maintenance of cultures

Pure cultures of *C. indica* strains DMRO-309 and APK-2 were procured from ICAR-Directorate of Mushroom Research, Chambaghat, Solan, Himachal Pradesh. Both the strains were maintained on sterilized potato dextrose agar medium and malt extract agar medium slants, which were kept at room temperature during the period of investigation. Subsequent culturing was done after every three months.

Agrowastes used for the cultivation

For cultivation of *C. indica* strains six agro-wastes, namely

wheat straw, paddy straw, maize stalk, dehulled maize cobs, sorghum stalk and bajra stalk collected from the local fields of Jammu were used individually and in seven 1:1 combinations each of wheat straw and paddy straw, paddy straw and maize stalk, wheat straw and maize stalk, paddy straw and dehulled maize cobs, wheat straw and dehulled maize cobs, maize stalk and dehulled maize cobs and sorghum stalk and bajra stalk.

Preparation of spawn

Cleaned whole wheat grains were boiled for 15-20 minutes so as to bring the grain moisture level between 45-50%. After hand picking the damaged grains, the intact boiled grains are air dried for 25-30 minutes. Subsequently, the boiled grains are spread on a blotting paper/ wire gauze mesh and then mixed with 2% gypsum and 4% calcium carbonate so as to separate these from one another and adjust the pH at desired level. After filling the grains in polyethylene bags, these are sterilized at 15 p.s.i. for 30 minutes. After cooling, the bags were aseptically inoculated with mother spawn (10-15 g) and incubated at 30±2°C. The bags were frequently examined for any type of contamination and those showing such signs were immediately discarded. The healthy spawn bags showing 45-50% white and uniform mycelia growth covering on the grains were used for experimentation. Usually, the process takes 20-23 days for spawn to get ready.

Preparation of substrate

In the laboratory the collected agrowastes were chopped into small pieces (5-7cm) and then subjected to hot water treatment at 80-90 ºC for 40-60 min. The water was then drained off and the moistened agrowaste was spread over sloppy cemented floor till the moisture content reached 60 per cent.

Filling of bags and spawning

Spawning of the substrate was done by using spawn @ 6% of dry weight of the substrate. After spawning, the substrate filled bags were punctured at different places, followed by cutting of the lower end corners of substrate filled bags to drain-off the excess water. Thereafter, the necks of the bags were closed with rubber bands and placed in the hanging nets of the mushroom house, where temperature of 25-35°C and relative humidity of 80-90 per cent were maintained for spawn run. It took 10-15 days to completely colonize the substrate bags.

Statistical analysis

The data was analysed using analysis of variance (ANOVA) on variables such as types of agrowastes and type of strain used on period of spawn run using SPSS software package (Version18.0).

RESULTS AND DISCUSSION

During the present investigation, the two select strains (DMRO-309 and APK-2) of *Calocybe indica* were evaluated for complete spawn run on straw of paddy and wheat, stalk and dehulled cobs of maize, stalk of sorghum and bajra.

It was observed that substrate colonization in all the substrate bags started from third day onwards. Perusal of data presented in table 1 shows that both the strains exhibited statistical differences in the time period taken for colonization of different substrates and their combinations. When grown on single type of agro-waste, *C. indica* DMRO-309 took minimum time period of 9.33 days for complete spawn run on paddy straw, followed in increasing order on wheat straw (11.45 days). Spawn run by DMRO-309 required more days on the stalk and dehulled cobs of maize and showed nonsignificant differences. Further, nonsignificant differences were observed on the stalks of sorghum and bajra with the requirement of maximum time period of 19.63 and 20.15 days respectively for complete colonization by DMRO-309 (Table 1). This is the first attempt on the cultivation of DMRO-309 at Jammu but earlier Singh *et al.* (2017) conducted investigations on DMRO-600 at Faizabad (U.P.) and reported requirement of upto 27 days for complete spawn run on different agro-wastes.

In comparison to DMRO-309, the other select strain APK-2 was observed to be a slow colonizer. As depicted in table 1, APK-2 also took minimum time (11.67 days) to colonize paddy straw, followed in increasing order on wheat straw (13.56 days). Similar, results were obtained by Karuppuraj *et al.* (2014) who reported requirement of 10-11 days for complete spawn run by APK-2 on paddy straw and other agro-wastes from Chennai (Tamil Nadu). Few other workers have also reported that strain APK-2 requires less time for complete colonization of agro-wastes (Krishnamoorthy, 2003; Krishnamoorthy and Amutha, 2007). However, Rawal and Doshi (2014) and Selvaraju *et al.* (2015) observed requirement of 14 and 19 days respectively by APK-2 for complete spawn run on paddy straw. During the present investigation, APK-2 showed requirement of moderate time period (approximately 16 days) for colonization of maize stalk and de-hulled maize cobs, whereas delayed spawn run was observed on the stalks of sorghum and bajra (Table 1). Differences in the time period shown by the two select strains (DMRO-309 and APK-2) in colonizing different substrates may be due to the differences in their enzymatic activity, which are required for degrading substrate components.

Data presented in table 1 also reveals moderate to delayed spawn run on different agrowastes when used in combinations. The seven combinations in 1:1 ratio included that of paddy straw and wheat straw; paddy straw and maize stalk; wheat straw and maize stalk; paddy straw and de-hulled maize cobs; wheat straw and dehulled maize cobs; maize stalk and de-hulled maize cobs; sorghum stalk and bajra stalk. On the substrate combinations also, DMRO-309 showed faster colonization and significant differences in days required for complete spawn run (Figure 1). Minimum time of 11.67 days was taken for spawn run by DMRO-309 on the substrate combination of paddy straw and wheat straw, whereas maximum time (23.15) days was taken on the combination of sorghum stalk and bajra stalk. Singh *et al.* (2017) while conducting study on DMRO-600 also observed requirement of as high as 22-24 days for complete mycelial growth on different substrate combinations of wheat straw, paddy straw, maize straw and de-hulled maize cobs in the ratio 1:1.

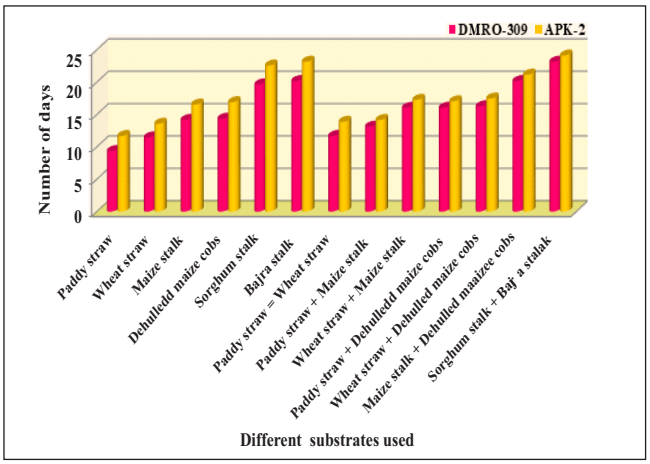

Fig. 1: Number of days taken by DMRO-309 and APK-2 strains of *Calocybe indica* for spawn run on different agro-wastes and their combinations.

APK-2 strain of *C. indica* also exhibited similar trend on different agro-waste combinations but it took more days for spawn run than taken by strain DMRO-309 (Fig. 1). Minimum time period for spawn run by APK-2 strain was observed on the 1:1 combination of paddy straw and wheat straw (approx. 13.87 days), whereas maximum time (24.11 days) was taken on 1:1 mixture of sorghum and bajra stalks (Table 1). Earlier, Rawal and Doshi (2014) while working with APK-2 observed that 15-18 days were required for spawn run on different combinations of paddy straw and wheat straw. Even, Krishnamoorthy (2003) concluded that the conventional substrates like paddy straw, wheat straw and sorghum stalks were colonized more quickly by the milky white mushroom fungus compared to non-conventional substrates (black gram hay, soybean hay, maize stalks, finger millet, etc.)

On the combination of paddy straw and wheat straw, both the strains of *C. indica* took minimum time period for complete spawn run, followed in increasing order by paddy straw and maize stalk, wheat straw and maize stalk, paddy straw and dehulled maize cobs, wheat straw and de-hulled maize cobs, maize stalk, de-hulled maize cobs and sorghum stalk and bajra stalk. This may probably be due to improper packaging of these substrates on account of their dissimilar sizes, which may create air spaces resulting in the loss of necessary moisture content, which is very necessary for rapid colonization of substrates.

Table 1: Time period required by DMRO-309 and APK-2 strains of *Calocybe indica* for spawn run on different agro-wastes and their combinations.

Agro-wastes used	Total days required for spawn run by	
	DMRO-309	APK-2
Paddy straw	09.33±0.33[a]	11.67±0.67[a]
Wheat straw	11.45±0.16[b]	13.56±0.33[b]
Maize stalk	14.15±0.26[c]	16.53±0.14[c]
Dehulled maize cobs	14.33±1.02[c]	16.90±0.23[c]
Sorghum stalk	19.63±0.56[d]	22.56±0.11[d]
Bajra stalk	20.15±0.41[e]	23.15±1.02[e]
F-value	53.5	66.1
P-value	P<0.05	
Paddy straw+ Wheat straw	11.67±0.33[a]	13.87±1.03[a]
Paddy straw + Maize stalk	13.11±0.16[b]	14.14±0.52[b]
Wheat straw + Maize stalk	16.01±0.26[c]	17.25±1.21[c]
Paddy straw+Dehulled maize cobs	16.03±1.02[c]	17.03±1.02[c]
Wheat straw+Dehulled maize cobs	16.25±0.56[c]	17.46±0.16[c]
Maize stalk+Dehulled maize cobs	20.15±0.41[d]	21.15±0.22[d]
Sorghum stalk+Bajra stalk	23.15±0.41[e]	24.11±0.13[e]
F-value	64.2	71.1
P-value	P<0.05	

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

CONCLUSION

From the results of the present study, it can be concluded that both the strains exhibited statistical differences in the time period taken for colonization of different substrates and their combinations. However, strain DMRO-309 expressed fast substrate colonization activity as compared to APK- 2, which may be attributed to strainal differences and varied substrate compositions. Further, both the stains showed fast spawn run on single type of substrate than their 1:1 combinations. This may be due to proper packaging of substrate when used singly than in combinations, which is important for the retention of moisture in the substrate beds for proper mycelial growth.

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