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CARBON DIOXIDE THE GREEN-HOUSE GAS AND MUSHROOM FRUITING

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ABSTRACT :

Mushrooms play a vital role in terrestrial ecosystems, by decomposition processes and nutrient cycling and of plant nutrient uptake. Mushroom has been an important component of the human diet; worldwide cultivation forms a multibillion dollar industry. Hence, mushrooms have a huge impact on ecosystem processes and the terrestrial carbon cycle, as well as have economic importance. Morphologically the edible portion of mushroom is the fruit body. Despite their ecological and economic importance, little is known about the phenology of fruiting bodies. Changes in productivity and phenology of fungal fruit bodies can give clues to changes in fungal activity, but understanding these changes in relation to carbon dioxide concentration is a pending challenge among ecologists and mycologists. Carbon dioxide is the primary green-house gas, responsible for global climate change. Our current study shows that the fruiting of mushroom (Lentinus sajor-caju) depends on the carbon dioxide concentration. At high concentration of carbon dioxide only the stipe (stalk) is formed but not the fruit body. The changes in carbon dioxide concentration alters the mushroom phenology coincide with the global climate change.

KEYWORDS : *global warming, carbon dioxide, phenology, fungi, agarics.*

INTRODUCTION:

Fungi are key components of terrestrial ecosystems as saprotrophs, endophytes, and pathogens and in mycorrhizal associations with plants. As saprotrophs they are the major agents of decomposition of organic matter, releasing CO_2 and mineral nutrients. As mycorrhizal symbionts they are the main suppliers of nutrients to plants, receiving plant carbon derived from photosynthesis in return (Read, 2003; Smith, 2008). Among the fungi, mushrooms have both the importance in the ecosystem as well as have economic importance. The sporocarp of mushroom (fruiting body or fruit body) is a multicellular structure on which spore-producing structures are borne. Pinning is the stage of growth where the cap of the developing mushroom can first be differentiated from the stipe. At the pinning stage, the fruit body is very susceptible to damage. In adverse conditions i.e. environmental changes, many pins will become abort.

Carbon dioxide (CO_2) is a greenhouse gas, which imparts significantly to global warming. The gas CO_2 also affects the growth and fruiting of mushrooms. It has been known for many years that mushrooms have a tendency to grow abnormally long stems and small caps when confined in a limited atmosphere under a glass jar or similar container (Lambert, 1933; Styer, 1933). There is an alteration in the yield of mushrooms with an increment of CO_2 concentration (Loeffen, 1995). High CO_2 concentration inside mushroom houses is one of the major causes of abnormality in fruiting

bodies. An increase of carbon dioxide concentration can decrease cap sizes and increase the length of stipes. However, even stipes is short at CO_2 concentrations of more than 0.5% (Mushroom Growers Handbook). Stipe is the stalk-like structure supporting the cap of a mushroom, composed of sterile hyphal tissue. It can easily and successfully be cultivated on wheat and rice straw, cotton waste and sawdust (Jiskani, 1999). The temperature and relative humidity for the cultivation of these strains were maintained between 12-17°C and 80-85% respectively.

MATERIALS AND METHODS

Fungal Culture Collection

A good quality fungus strain of *Lentinus sajor-caju* was collected from the Laboratory of Dr. Aniruddha Saha, Molecular plant pathology and fungal biotechnology laboratory, Department of Botany, University of North Bengal, West Bengal, for mushroom spawn preparation and mushroom cultivation.

Culture maintenance

Lentinus sajor-caju is grown in vitro on potato dextrose agar medium at 25°C through tissue culture method. The pure culture is maintained at 4-5°C in refrigerator as stock culture.

Mushroom spawn preparation

Mushroom Spawn is produced through standard method. For spawn preparation, 10 kg of wheat grain is boiled in 15 litres of water for 20 minutes and allowed to cool for another 15 minutes without heating to retain moisture content of 48-50%. Then 13.5 g calcium sulphate and 3.5 g calcium carbonate are mixed with 1kg boiled wheat grains. The calcium sulphate prevents sticking the grains together and the pH 6.6 adjusted with calcium carbonate. The grains are filled into 70% space of plastic packets (10x20 cm size), plugged with non-absorbent cotton, wrapped with brown paper and sterilized at 1.54 kg/cm2 for 45-60 minutes. After autoclaving packets are allowed to cool down and shaking to avoid clumping of grains. The packets are inoculated with a pure culture of *Lentinus sajor-caju*. The inoculated packets are incubated at 25°C. About 20 days of incubation, the packets are ready as master spawn (Figure 1).

Figure 1: Steps of Mushroom spawn preparation at mushroom cultivation unit. A- Mixing of chemicals and wheat grain; B- Filling poly bag with spawns substrate; C- Autoclaving substrate; D- Pure fungal culture; E- Inoculation chamber; F and G- Incubator; H- mushroom spawn.

Mushroom Bed Preparation

Mushroom production was done with polyethylene bag technique. In this technique substrate mixed with mushroom spawn and filled up in polyethylene bag. The substrate materials viz. paddy straw, wheat straw, waste papers, sawdust, and coconut husks and leaves were cut into 5-7 cm pieces followed by overnight dip in water with 1% formaldehyde. Then substrates were kept on a slope substratum to remove excess water. Now the drained substrates were mixed with spawn (substrate and spawn in ratio 5 Kg and 700 g respectively) and put in polythene bags of (40 x 60 cm size) up to 4/5th of its capacity, perforated with a needle all over the surface to allow free exchange of gases. The inoculated bags were incubated in the cropping room in dark at the temperature of 25±2°C till the white fungal mycelium mat proliferates. When the substrate was completely covered with white cottony mycelia the polythene bags were removed and kept under white light. For the initiation and subsequent development of fruiting bodies, the temperature and relative humidity inside the cropping room was maintained between 12-17°C and 80-85% respectively. After removing polythene cover beds were sprayed with clean water 3-4 times per day. Within 4-7 days mushroom fruit body flush out as pinhead and it became matured within another 3-5 days (**Figure 2, 3 & 4**).

Figure 2: Mushroom bed preparation. A= wet paddy straw as substrate; B= Mushroom spawn; C-E= Substrate fill up in Poly bag; F= Spawn spreading on substrate; G= Mushroom bed ready for incubation.

Figure 3: Mushroom bed incubation. A= Mushroom bed; B= Mushroom bed covered with white mycelia mat; C= Removing of polythene cover; D-F= Mushroom fruit body appears as pinhead.

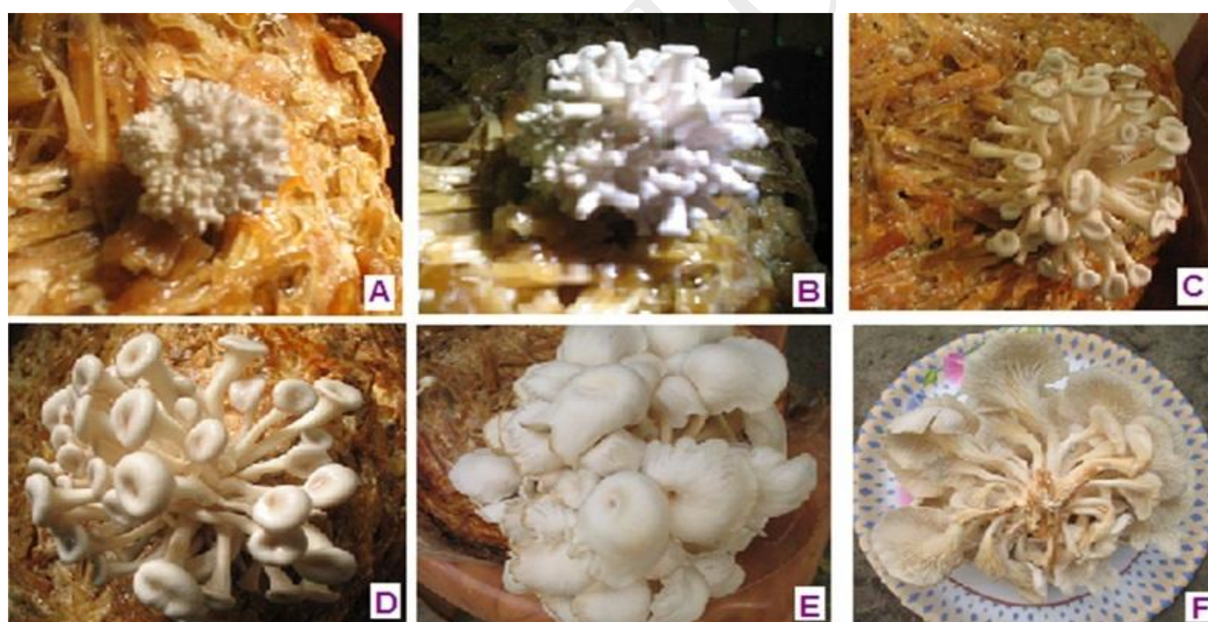

Figure 4: Mushroom as fruit body appearance. A-B= young pin head; C= 3-4 days old pin head; E= Mature mushroom; F- plucked Mushroom on tray.

RESULT

Biological efficiency

Observations during period of spawn run, appearance of pinhead, maturation of fruiting bodies were recorded up to 4th flush. Fresh weights of mature fruit bodies were also recorded up to 4th flush to calculate the total yield and corresponding biological efficiency. Total yield was calculated as the fresh weight of mushrooms harvested up to 3rd flush per 300 g of dry substrate used for mushroom cultivation. Biological efficiency (B.E.) was determined by the ratio of fresh weight (g) of mushrooms up to 4th flush to dry weight

(g) of substrate and expressed as percentage (Table 1). Wheat straw and Paddy straw were found more effective yield performance of *Lentinus sajor-caju* in compare to others substrates used.

$$\text{Biological efficiency} = \frac{\text{Fresh weight (g) of mushrooms harvested}}{\text{Dry weight (g) of substrate}} \times 100$$

Table 1: Yield performance of *Lentinus sajor-caju* species on different substrates.

Dry weight of Substrate	Production (Fresh weight)			Total yield	Biological efficiency
	1st Flush	2nd Flush	3rd Flush		
Paddy straw (300 g)	234.00	184.00	26.00	444.00	148.00
Wheat straw (300 g)	216.00	158	79.00	453.00	151.00
Waste papers (300 g)	187.00	83.00	22.00	292.00	97.33
Saw dust (300 g)	188.00	45.00	25.00	258.00	86.00
Coconut husk-leave (300 g)	182.00	53.00	26.00	261.00	87.00

Mushroom yielding on different substrates

Mushroom spawn running on different substrates (2 Kg substrate mixed up with 250 g Spawn), fruit body appearance as pinhead and maturation of fruiting bodies were recorded (Table 2).

Table 2: Days for completion of spawn running, pinhead formation and fruiting body formation of *Lentinus* species on different substrates.

Substrate	Spawn running (Days)	Pinhead formation (Days)	Fruiting body formation (Days)
Paddy straw	21-24	26-28	29-32
Wheat straw	24-27	31-37	39-42
Waste papers	22-27	29-32	34-37
Saw dust	24-27	30-35	38-42
Coconut leaves	29-32	36-40	44-48

CO_2 released and its effect during growth of *L. sajor-caju*

Effect of Carbon dioxide on yield of *Lentinus sajor-caju* was studied. In this experiment five mushroom beds were kept in a closed glass chamber for spawn running, pinhead formation and fruit body formation. Carbon dioxide released during different stages of *L. sajor-caju* growth and its effects on yielding of fruit body were measured by Portable Gas Detectors (USA) and regular observation respectively. Growth stages are graphically represented in Figure 6.

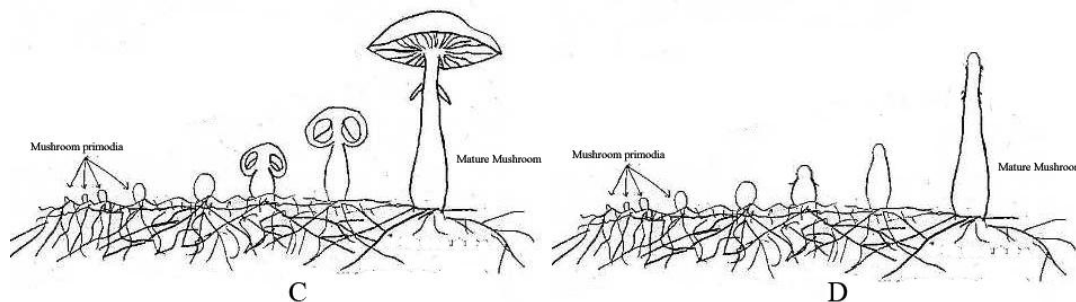

Figure 5: Effect of CO_2 on the growth and development of fruit body of *Lentinus sajor-caju*. (A) Normal concentration of CO_2 with normal fruitbody. (B) High concentration of CO_2 with abnormal fruitbody. Schematic diagram of mushroom showing growth and development (C) Normal concentration of CO_2 with normal fruitbody. (D) High concentration of CO_2 with abnormal fruitbody.

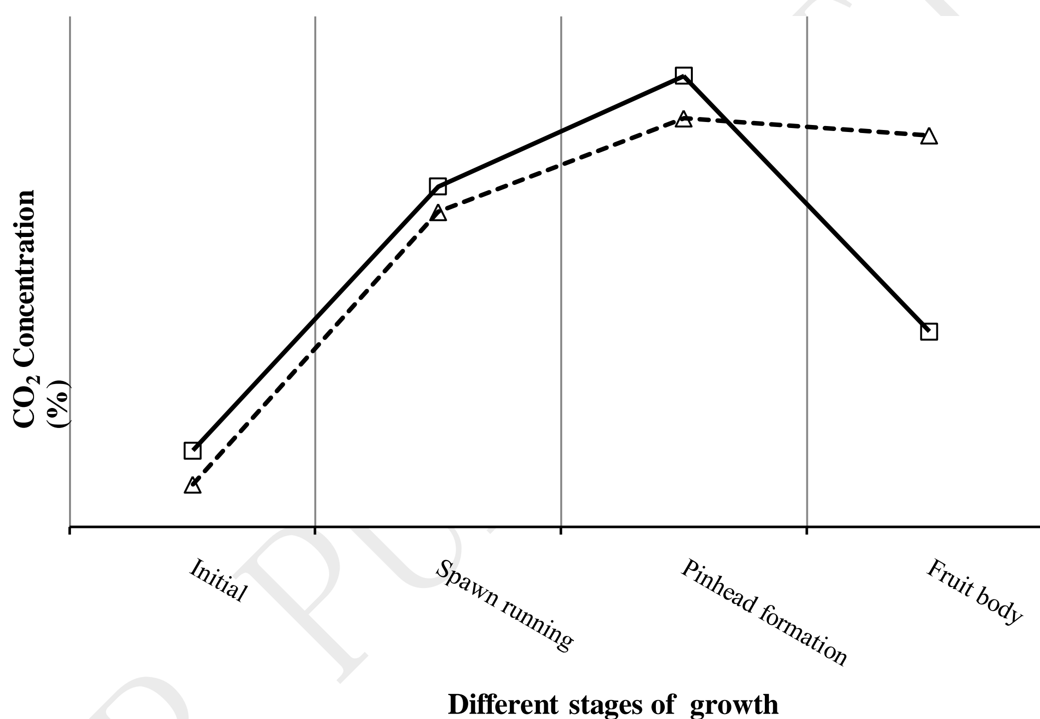

Figure 6: CO_2 released and its effect on different stages of *Lentinus sajor-caju* growth and development. Dotted line with triangle indicates spawn running, pinhead formation and stipe elongation; Solid line with square indicates spawn running, pinhead formation and pileus formation.

Discussion

After the harvest of wheat grain, the agriculture waste straw remains a problem for the producers since it is not utilized by the cattle as fodder. The straw is burnt in the field, causing huge pollution and heat is generated a source for global warming. We have used the discarded wheat straw as the substrate for mushroom cultivation. Our result shows that the mushroom *Lentinus sajor-caju* grows well on wheat straw with the biological efficiency of 151, highest in case of the wheat straw when compared with the other traditional substrates.

Our data reveals that at high CO_2 concentration only stipe develops and the pileus or caps fails to grow as previously reported. For a long time, it has been known that conditions of limited aeration alter the developmental processes of certain fungi (Hawker, 1950). Although CO_2 has been suggested as the active

principle responsible for the inhibition of sporangial formation of *Choanephora cucurbitarum* (Barnett and Lilly, 1955) and for the abnormal growth and development of mushrooms (Lambert, 1933), other factors may be involved (Mader, 1943; Stoller, 1952). The experiments of Plunkett (1956) indicated that arrested fruiting can be reversed by the inclusion of alkali in the case of *Collybia velutipes*, but not with *Polyporus brumalis*. Arrested fruiting in sealed chambers is reversed by the inclusion of KOH, soda lime, or Ascarite, all of which are known to combine chemically with CO_2 (Niederpruem, 1963).

Is it only the environmental factor CO_2 or there are some internal factors i.e. hormonal, genetic in the fruiting of mushroom. Therefore, studying the role of plant hormones on the growth and fruiting of mushroom will be a good approach to understand the development of fruiting in mushroom. Search for the genes related to the initiation and development of fruit body could enlighten in the molecular path of fruit body formation and its development.

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