

EFFECT OF DIFFERENT CARBON SOURCE ON MYCELIAL GROWTH & CHLAMYDOSPORE PRODUCTION OF PADDY STRAW MUSHROOM(*VOLVARIELLA VOLVACEA*)

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Abstract: *In vitro*, an experiment was conducted to know best carbon source for growth and biomass of two best performing isolates (BYT VV-02 & BYT VV-05) of *Volvariella volvacea* seven carbon source i.e., glucose fructose maltose dextrose sucrose mannitol & Control was studied found that Sucrose was significantly fastest Radial growth & Chlamydospores production followed by Dextrose and Glucose while slowest Radial growth & Chlamydospores production reported in control (without carbon source) followed by Mannitol, Fructose, Maltose & Sorbitol in both isolates of *Volvariella volvacea*.

Keywords: Radial growth, Biomass, Isolates, *Volvariella volvacea*

INTRODUCTION

Mushroom classified as a macro fungus and has a fleshy and distinct spore bearing fruiting body of fungus is a family member of Pluteaceae (Kotl. and Pouz) of class basidiomycetes (Singer, 1986) typically grown above land, or soil or other food substrate. More than 2,000 mushroom species have been observed as edible among 12000 species, but nearly 35 are mostly accepted for consumption and limited species are commercially cultivated and almost 200 wild species are purposed for medical use (Beulah, 2013). Mushrooms are considered a delicacy of high nutritional and functional value and are recognized as a nutraceutical product; they are gone into interest due to their merits such as organoleptic, medicinal properties and economical significance. Mushrooms are being considered as a possible muscle protein replacement due to their high digestibility (Pavel, 2009).

MATERIALS AND METHODS

Source of Material

Two best performing high yielding isolates of *Volvariella volvacea* were obtained from Plant Pathology laboratory of DKS College of Agriculture, Bhatapara, IGKV, Raipur (C.G.)

Site of Experiment:

Experiment was conducted in Plant Pathology Laboratory of DKS College of Agriculture and Research station Alesur, Bhatapara (C.G.).

Maintenance of Pure Cultures

The sub-culture of paddy straw mushroom isolates used in the study was maintained on Potato Dextrose Agar (PDA) medium. In order to maintain the vigour fresh isolations were made from the fruiting bodies every time after 2 to 3 subcultures. Freshly harvested sporophores were swabbed with 95 per cent ethanol.

At the junction of the pileus and stipe, tissue bits were removed aseptically, surface sterilized with 95 per cent ethanol for 20 sec and repeatedly washed in sterile water and placed on PDA medium taken in sterile Petri dishes. The dishes were incubated at 32°C for seven days. Following single hyphal tip method pure cultures were made and stored in PDA slants to carry out further studies. Micrometric observations on the diameter of hyphae and chlamydospores other parameters were observed with the help of image Alpha (Euromax Microscope holland).

Effect of different carbon source

Eight different carbon sources, namely Dextrose, Fructose, glucose, maltose, sucrose, mannitol, Sorbitol & Control were assayed to determine their effect on growth and biomass of *Volvariella volvacea* in place of dextrose each carbon source was substituted in basal medium (PDA) similarly, potato broth was prepared to determine the biomass of *Volvariella volvacea* using different carbon sources. Medium serve as control without adding any carbon source. Five replications were kept for each source of carbon. The petridishes and flasks containing 20 ml medium and 75 ml broth were inoculated with a 5 mm disc of 2 isolates of *Volvariella volvacea* (BYT VV-02 & BYT VV-05) separately and incubated at 32 °C. The observations were taken for radial growth and biomass when mycelial growth was reached at the periphery in any treatment. Each treatment was replicated 5 times. Inoculated flasks were incubated for 15 days at 32°C. Thereafter, mycelium mat was collected on Whatman No. 1 and weighed fresh mycelial weight on electronic balance (with sensitivity 0.01g). For dry weight, mycelium mat was dried for 48 hours in an oven at 60 °C and dry weight was recorded at regular intervals until a constant weight was reached.

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Statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with Six treatments with five replications. The data were analysed by statistical procedure given by (Gomez, K.A. and Gomez, A.A. 1984).

RESULTS AND DISCUSSION

For the study of influence of different carbon source two best performing isolates coded as BYT VV-02 & BYT VV-05 local isolates selected amongst collected from different location of Chhattisgarh plain of *Volvariella volvacea* was selected for evaluation described below: -

(BYT VV-02) isolates of *Volvariella volvacea*

Effect of Different carbon source on radial growth and biomass

From the Table-1.0 it was evident that different carbon sources showed significant difference in their growth, fresh and dry mycelial weight of BYT VV-02. In solid media, mycelial growth of BYT VV-02 was significantly more (86.00 mm) noticed in sucrose. However, growth was significantly poorest (35.20 mm) recorded in medium without any carbon source. The basal medium incorporated with

Sorbitol, Dextrose, Glucose, Maltose, Fructose & Mannitol gave 81.40, 71.20, 68.20 65.40, 62.20mm & 56.20 growth of isolate BYT VV-02. In liquid medium, fresh mycelial weight was differing significantly with regards to different carbon sources and significantly higher (4.50g) fresh weight was achieved in liquid medium substituted with Sucrose followed by Dextrose (3.50g) and Glucose (3.40g) which were differ one another. However, lowest (1.80 g) fresh weight was attained in control. In other studied carbon sources fresh mycelia weight was 2.50, 2.60, 2.80 and 3.20g in Mannitol, Maltose, Fructose & sorbitol, respectively. The dry mycelial weight of BYT VV-02 was similar as fresh mycelia weight and it was significantly more (0.42g) recorded in broth substituted by Sucrose and next were Dextrose (0.35g), Glucose (0.32 gm) and Sorbitol (0.30gm) that were differ significantly with each other. While, significantly poorest dry mycelia weight was seen in control (0.06) followed by Mannitol (0.18g), Maltose (0.20g) & Fructose (0.23g) similar results also reported as Carbon source from sucrose to the growth medium encouraged the mycelial growth and chlamydospores production of both *V. volvacea* strain CBE TNAU 1505 and *V. bombycina* strain CBE TNAU 1406.

Table 1. Effect of different carbon sources on radial growth and biomass of isolates (BYT VV-02) of *Volvariella volvacea*

| S.N. | Carbon sources | Growth (mm)* | Fresh weight (g)* | Dry weight (g)* |
|------|----------------|--------------|-------------------|-----------------|
| 1. | Fructose | 62.20 | 2.80 | 0.23 |
| 2. | Glucose | 68.20 | 3.40 | 0.32 |
| 3. | Maltose | 65.40 | 2.60 | 0.20 |
| 4. | Dextrose | 81.40 | 3.50 | 0.35 |
| 5. | Sorbitol | 71.20 | 3.20 | 0.30 |
| 6. | Sucrose | 86.00 | 4.50 | 0.42 |
| 7. | Mannitol | 56.20 | 2.50 | 0.18 |
| 8. | Control | 35.20 | 1.80 | 0.06 |
| | SEm± | 2.301 | 0.124 | 0.010 |
| | CD (5%) | 6.705 | 0.359 | 0.029 |

Microscopic characterization of culture on different carbon source was also recorded on different parameter for BYT VV-02 isolates of *Volvariella volvacea* described below: -

Colony morphology & Aerial mycelium

Shown in Table 2.0 Colony morphology of BYT VV-02 isolates of *V. volvacea* varies with different carbon source characterized by Thick Fluffy growth observed in Sucrose, Dextrose Maltose & Fructose with highly dense to densed aerial mycelium was reported while in glucose & mannitol thin transparent radiating growth with densed aerial mycelium was observed while in Sorbitol thick projecting densed aerial mycelium observed while in control thin transparent without aerial mycelium reported.

Days taken to cover 90 mm of Petriplates

Days taken to cover 90mm of petriplates by BYT VV-02 isolates of *V. volvacea* in different carbon source shown in table 2.0 Sucrose taken significantly less (4.9 days) followed by Dextrose (5.6 days), glucose (5.8 days) and significantly maximum (12.35 days) taken by control followed by Maltose (11.35 days), Mannitol (7.5 days), Sorbitol (6.3 days) & Fructose (6.2 days)

Days taken for chlamydospore production

Days taken for chlamydospore production of petriplates by BYT VV-02 isolates of *V. volvacea* in different carbon source shown in table 2.0 Sucrose taken significantly less (9.80 days) followed by Dextrose (11.25 days), Glucose (11.40 days) and significantly maximum (17.50 days) taken by control followed by Maltose (16.45 days), Mannitol (13.26 days), Sorbitol (12.60 days) & Fructose (12.35 days)

Chlamyospore density & Colour

Maximum chlamyospores density recorded in Sucrose followed by Dextrose, Glucose and significantly less in control followed by Maltose, Mannitol, Sorbitol & Fructose mostly light orange colour chlamyospores recorded.

Diameter of Chlamyospores

Diameter of chlamyospore of BYT VV-02 isolates of *V. voluacea* grown in different carbon source shown in table 2.0 it varied between 22.30 to 27.35 μm in diameter significantly maximum 27.35 μm reported in Sucrose followed by Dextrose (26.20 μm) & Glucose (25.80 μm) minimum (22.30 μm) recorded in control followed by Maltose (23.00 μm), Sorbitol (23.70 μm), fructose (24.70 μm) & Mannitol (25.60 μm).

Hyphal diameter

Hyphal Diameter of BYT VV-02 isolates of *V. voluacea* grown in different carbon source shown in table 2.0 it varied between 4.10 to 6.20 μm in diameter significantly maximum 6.20 μm reported in Sucrose followed by Dextrose (5.30 μm) & Glucose

(4.80 μm) minimum (4.10 μm) recorded in control followed by Maltose (4.2 μm), Sorbitol (4.5 μm), fructose (4.5 μm) & Mannitol (5.10 μm).

Number of septa

Significant difference reported in number of septa of isolate BYT VV-02 grown in different carbon source shown in table 2.0 it varied between 2.6 to 4.6 μm in diameter significantly maximum 4.6 reported in Sucrose followed by Dextrose (4.5) & Glucose (3.9) minimum (2.6) recorded in control followed by Maltose (3.2), Mannitol (3.4). Sorbitol (3.6), & fructose (4.1).

Distance between septa

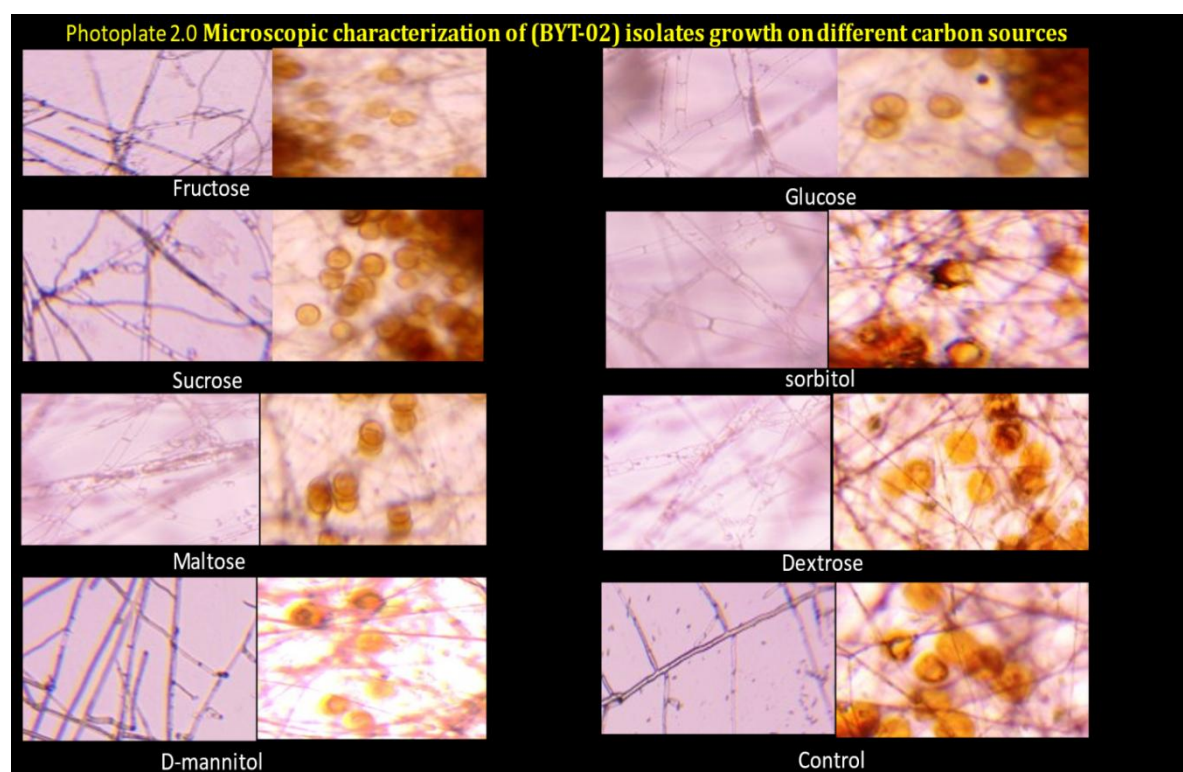
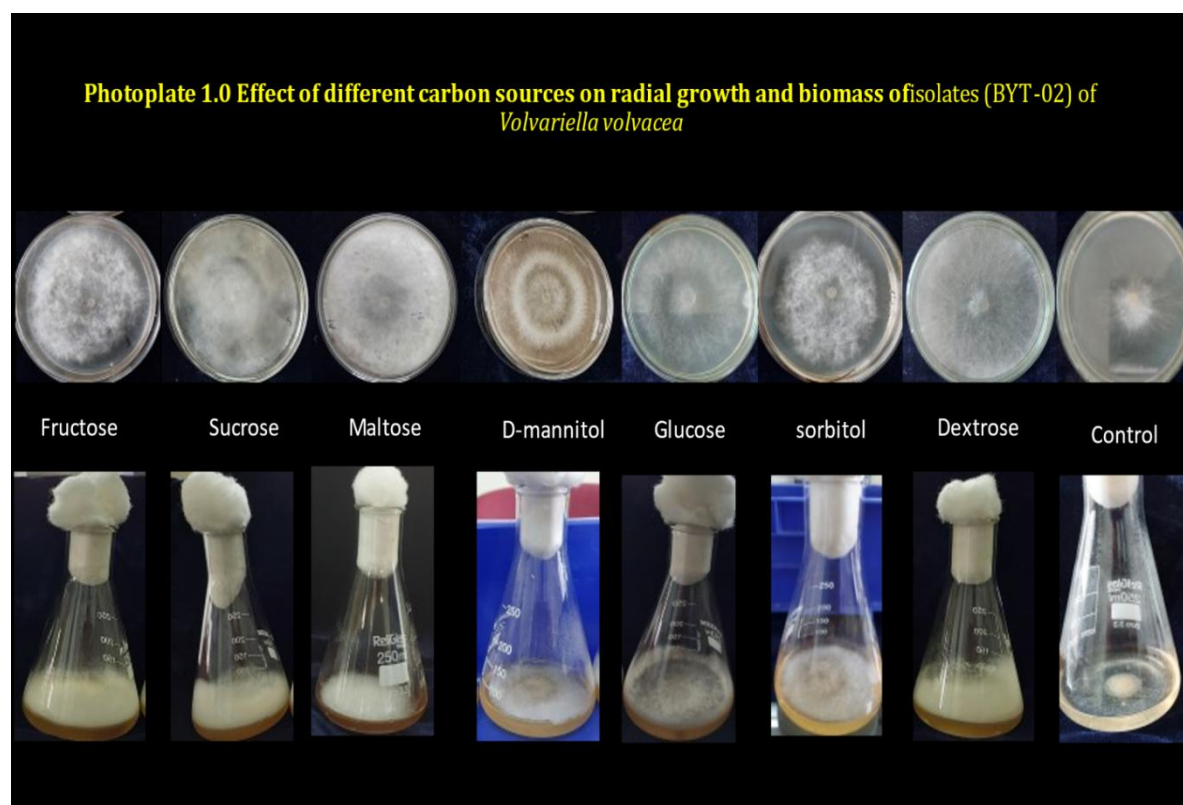
Among different media distance between septa of isolate BYT VV-02 grown in different carbon source shown in table 2.0 it varied between 2.5 to 4.1 μm in diameter significantly minimum 2.5 μm reported in Sucrose followed by Dextrose (2.6 μm) & Glucose (3.4 μm) maximum (4.10 μm) recorded in control followed by Maltose (3.8 μm), Sorbitol (3.6 μm), fructose (3.4 μm) & Mannitol (3.1 μm).

Table 2. Microscopic characterization of (BYT-02) isolates growth on different carbon sources

| S.N. | Carbon sources | Colony morphology | Aerial hyphae | (DTTCP) | (DTFCP) | Chlamyospores density | Colour of Chlamyospores | Diameter of chlamyospores (μm) | Hyphal Diameter (μm), | Number of Septa microscopic field | Distance Between Septa to (μm), |
|------|----------------|----------------------------|---------------|---------|---------|-----------------------|-------------------------|---|------------------------------------|-----------------------------------|--|
| 1. | Fructose | Thick fluffy | ++ | 6.2 | 12.35 | ++ | Light orange colour | 24.70 | 4.50 | 4.1 | 3.4 |
| 2. | Glucose | Thin transparent radiating | +++ | 5.8 | 11.40 | ++ | Light orange colour | 25.80 | 4.80 | 3.9 | 2.9 |
| 3. | Mannitol | Thick fluffy | + | 11.35 | 16.45 | + | Light orange colour | 23.00 | 4.20 | 3.2 | 3.8 |
| 4. | Dextrose | Thick fluffy | +++ | 5.6 | 11.25 | +++ | Light orange colour | 26.20 | 5.30 | 4.5 | 2.6 |
| 5. | Sorbitol | Thick projecting | +++ | 6.3 | 12.60 | ++ | Light orange colour | 23.70 | 4.50 | 3.6 | 3.6 |
| 6. | Sucrose | Thick fluffy | ++++ | 4.9 | 9.80 | ++++ | Light orange colour | 27.35 | 6.20 | 4.6 | 2.5 |
| 7. | Maltose | Thin Transparent radiating | ++ | 7.5 | 13.26 | ++ | Light orange colour | 25.60 | 5.10 | 3.4 | 3.1 |
| 8. | Control | Thin transparent | - | 12.25 | 17.50 | - | Light orange colour | 22.30 | 4.10 | 2.6 | 4.1 |
| | SEm \pm | | | 0.292 | 0.548 | | | 0.948 | 0.187 | 0.144 | 0.119 |
| | CD (5%) | | | 0.844 | 1.585 | | | 2.745 | 0.540 | 0.416 | 0.345 |

Were,

- + Poor chlamyospore formation
- ++ Moderate chlamyospore formation
- +++ High chlamyospore formation
- ++++ Very high chlamyospore formation
- (DTTCP) Days taken to cover 90mm of petriplates
- (DTFCP) Days taken for chlamyospores Production



BYT VV-05 isolates of *Volvariella volvacea*
Effect of different carbon source on radial growth and biomass

From the Table-3.0 it was evident that different carbon sources showed significant difference in their growth, fresh and dry mycelial weight of BYT VV-05. In solid media, mycelial growth of BYT VV-05

was significantly more (85.30 mm) noticed in Sucrose followed by Dextrose (83.10mm), Glucose (69.10mm). However, growth was significantly poorest (34.90 mm) recorded in medium without any carbon source or control. The basal medium incorporated with Sorbitol, Maltose, Fructose & Mannitol gave 69.10mm, 68.00mm, 65.10mm &

59.10mm growth of isolate BYT VV-05. In liquid medium, fresh mycelial weight was differing significantly with regards to different carbon sources and significantly higher (4.30g) fresh weight was achieved in liquid medium substituted with Sucrose followed by Dextrose (3.80g) and Glucose (3.50g) which were differ one another. However, lowest (1.54 g) fresh weight was attained in control. In other studied carbon sources fresh mycelia weight was 2.60g, 2.70g, 2.75g and 3.40g in Mannitol, Maltose,

Fructose & Sorbitol, respectively. The dry mycelial weight of BYT VV-05 was similar as fresh mycelia weight and it was significantly more (0.42g) recorded in broth substituted by Sucrose and next were Dextrose (0.39g), Glucose (0.35 gm) and Sorbitol (0.26 gm) that were differ significantly with each other. While, significantly poorest dry mycelia weight was seen in control (0.18) followed by Mannitol (0.24g), Maltose (0.28g) & Fructose (0.30 g).

Table 3. Effect of different carbon sources on radial growth and biomass of isolates (BYT VV-05) of *Volvariella volvacea*.

| S.N. | Carbon sources | Growth (mm)* | Fresh weight (g)* | Dry weight (g)* |
|------|----------------|--------------|-------------------|-----------------|
| 1. | Fructose | 65.10 | 2.75 | 0.30 |
| 2. | Glucose | 77.30 | 3.50 | 0.35 |
| 3. | Maltose | 68.00 | 2.70 | 0.28 |
| 4. | Dextrose | 83.10 | 3.80 | 0.39 |
| 5. | Sorbitol | 69.10 | 3.40 | 0.26 |
| 6. | Sucrose | 85.30 | 4.30 | 0.42 |
| 7. | Mannitol | 59.10 | 2.60 | 0.24 |
| 8. | Control | 34.90 | 1.54 | 0.18 |
| | SEm± | 2.415 | 0.132 | 0.012 |
| | CD (5%) | 6.990 | 0.383 | 0.036 |

Similar finding also reported by Rangasamy (1956), who had tested various carbon sources to induce the growth and morphogenesis of *V. Diplasia* and revealed that the highest biomass production was from starch supplemented broth followed by sucrose. Also supported by Jyothi and Anitha (2014) accustomed sucrose rich substrates such as sugar cane baggase along with paddy straw (1:1) to increase the yield of *Volvariella* sp.

Microscopic characterization of culture of different carbon source can also be recorded in different parameters for BYT VV-05 isolates of *Volvariella volvacea* described below: -

Colony morphology & Aerial mycelium

Shown in Table 4.0 Colony morphology of BYT VV-05 isolates of *V. volvacea* characterized as Thick Fluffy growth observed in Sucrose, Dextrose Maltose & Fructose with highly dense to dense aerial mycelium is reported while in glucose & mannitol thin transparent radiating growth with dense aerial mycelium was observed & Sorbitol thick projecting dense aerial mycelium observed while in control thin transparent without aerial mycelium reported.

Days taken to cover 90 mm of Petriplates

Days taken to cover 90mm of petriplates by BYT VV-05 isolates of *V. volvacea* in different carbon source shown in table 4.18 Sucrose taken significantly less (4.7 days) followed by Dextrose (5.2 days), Glucose (6.3 days) and significantly maximum (12.7 days) taken by Control followed by Maltose (11.80 days), Mannitol (7.2 days), Sorbitol (6.5 days) & Fructose (6.1 days)

Days taken for chlamydospore production

Days taken for chlamydospore production of petriplates by BYT VV-05 isolates of *V. volvacea* in different carbon source shown in table 4.18 Sucrose taken significantly less (9.20 days) followed by Dextrose (10.30 days), Glucose (10.70 days) and significantly maximum (18.20 days) taken by control followed by Maltose (17.10 days), Fructose (12.80 days) Mannitol (12.00 days), & Sorbitol (11.70 days).

Chlamydospore density & Colour

Maximum chlamydospores density recorded in Sucrose followed by Dextrose, Glucose and significantly less in control followed by Maltose, Mannitol, Sorbitol & Fructose mostly light orange colour chlamydospores recorded.

Diameter of Chlamydospores

Diameter of chlamydospore of BYT VV-05 isolates of *V. volvacea* grown in different carbon source shown in table 4.18 it varied between 21.30 to 26.40 µm in diameter significantly maximum 26.40 µm reported in Sucrose followed by Dextrose (26.00 µm) & Glucose (24.90 µm) minimum (21.20 µm) recorded in control followed by Maltose (23.90µm), Sorbitol (24.50 µm), fructose (25.30 µm) & Mannitol (25.60 µm).

Hypal diameter

Hypal Diameter of BYT VV-05 isolates of *V. volvacea* grown in different carbon source shown in table 4.18 it varied between 2.30 to 5.90 µm in diameter significantly maximum 5.9 µm reported in Sucrose followed by Dextrose (5.50 µm) & Glucose

(5.30 μm) & minimum (2.30 μm) recorded in control followed by Maltose (3.8 μm), Sorbitol (4.6 μm), fructose (4.8 μm) & Mannitol (5.10 μm).

Number of septa

Significant difference reported in number of septa of isolate BYT VV-05 grown in different carbon source

shown in table 4.18 it varied between 2.7 to 4.1 μm in diameter significantly Minimum (2.7) reported in Sucrose followed by Dextrose (3.1) & Glucose (3.4) & Maximum (4.2) recorded in control followed by Maltose (4.3), Mannitol (4.1), Sorbitol (4.0), & Fructose (3.8).

Table 4. Microscopic characterization of (BYT VV-05) isolates growth on different carbon sources

| S.N. | Carbon sources | Colony morphology | Aerial hyphae | (DTTCCP) | (DTFCP) | Chlamydo spores density | Colour of Chlamydo spores | Diameter of chlamydo spores (μm) | of Hyphal Diameter (μm), | Number Of Septa per microscopic field | Distance Between Septa to (μm), |
|-----------|----------------|--------------------------------------|---------------|----------|---------|-------------------------|---------------------------|---|---------------------------------------|---------------------------------------|--|
| 1. | Fructose | Thick fluffy | ++ | 6.1 | 12.80 | + | Light orange colour | 25.3 | 4.8 | 3.8 | 3.0 |
| 2. | Glucose | Thin transparent radiating | +++ | 6.3 | 10.70 | ++ | Light orange colour | 24.9 | 5.3 | 3.4 | 2.9 |
| 3. | Mannitol | Thick fluffy | + | 11.8 | 17.10 | + | Light orange colour | 23.9 | 3.8 | 4.3 | 3.8 |
| 4. | Dextrose | Thick fluffy | +++ | 5.2 | 10.30 | +++ | Light orange colour | 26.0 | 5.5 | 3.1 | 2.8 |
| 5. | Sorbitol | Thick projecting | +++ | 6.5 | 11.70 | + | Light orange colour | 24.5 | 4.6 | 4.0 | 3.2 |
| 6. | Sucrose | Thick fluffy | ++++ | 4.7 | 9.20 | ++++ | Light orange colour | 26.4 | 5.9 | 2.7 | 2.2 |
| 7. | Maltose | Thin Transparent uniformly radiating | ++ | 7.2 | 12.00 | + | Light orange colour | 23.2 | 5.1 | 4.1 | 3.0 |
| 8. | Control | Thin transparent | - | 12.7 | 18.20 | + | Light orange colour | 21.2 | 2.3 | 4.2 | 4.0 |
| SEm \pm | | | | 0.374 | 0.548 | | | 0.932 | 0.185 | 0.140 | 0.118 |
| CD (5%) | | | | 1.083 | 1.585 | | | 2.698 | 0.536 | 0.406 | 0.342 |

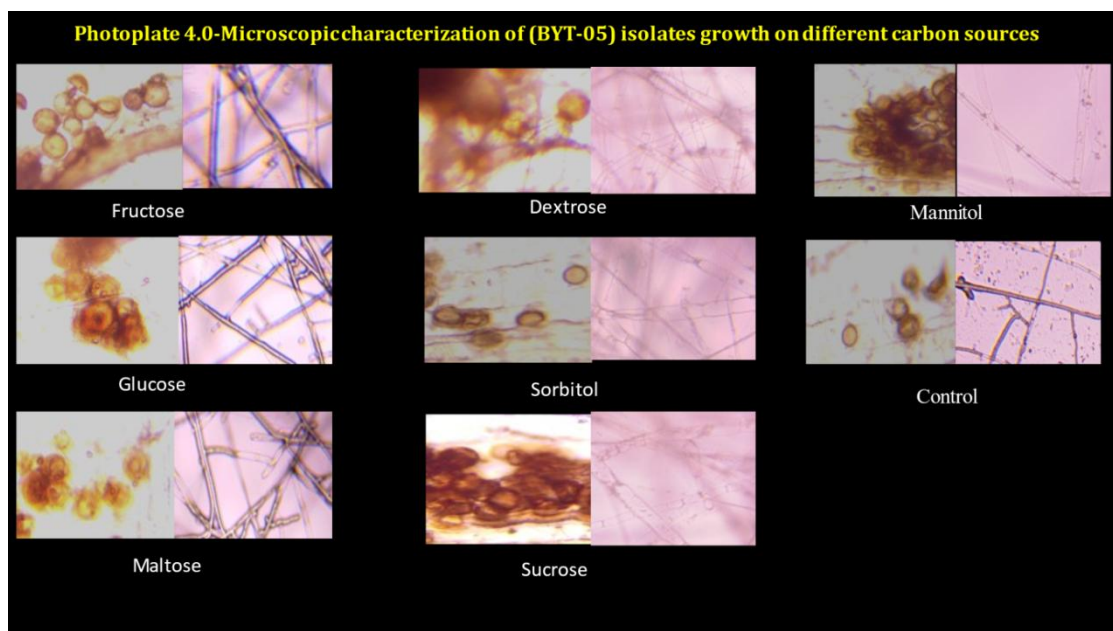
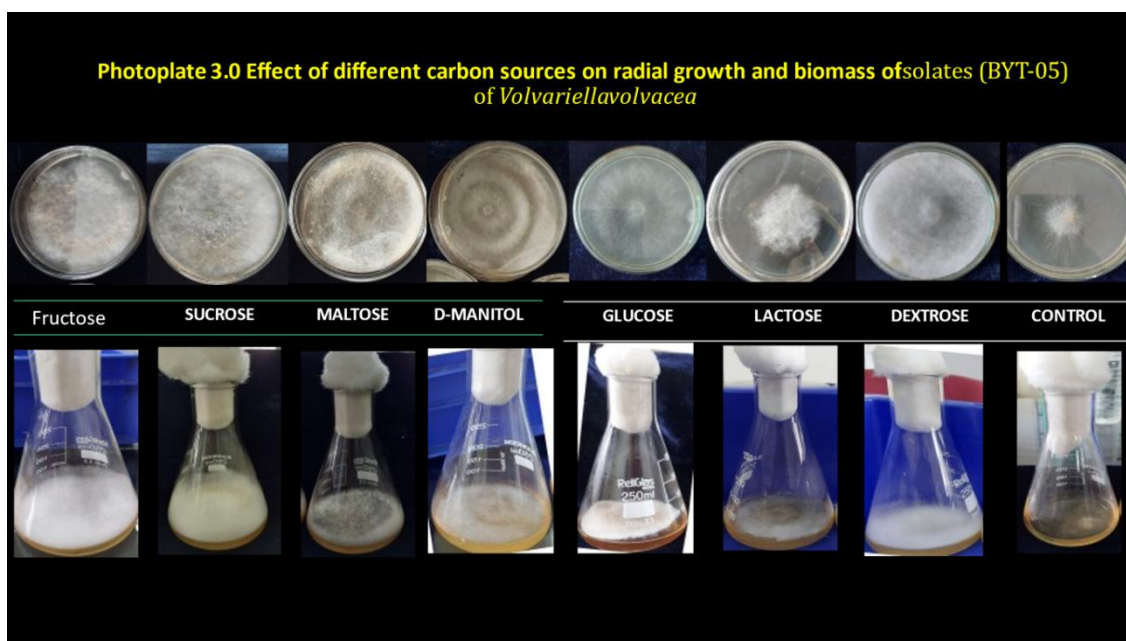
Were,

- + Poor chlamydo spore formation
 - ++ Moderate chlamydo spore formation
 - +++ High chlamydo spore formation
 - ++++ Very high chlamydo spore formation
- (DTTCCP) Days taken to cover 90mm of petriplates
(DTFCP) Days taken for chlamydo spores Production

Distance between septa

Among different media distance between septa of isolate BYT VV-05 grown in different carbon source shown in table 4.0 it varied between 2.2 to 4.1 μm in diameter significantly minimum 2.2 μm reported in

Sucrose followed by Dextrose (2.8 μm) & Glucose (2.9 μm) maximum (4.00 μm) recorded in control followed by Maltose (3.8 μm), Sorbitol (3.2 μm), fructose (3.0 μm) & Mannitol (3.0 μm).



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