The Pharma Innovation Journal 2022; 11(6): 1932-1938

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(6): 1932-1938

www.thepharmajournal.com
Received: 02-02-2022
Accepted: 07-05-2022

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Effect of different media on mycelial growth & Chlamydospore production of paddy straw mushroom (*Volvariella volvacea*)

Sharad Shroff, PK Tiwari, AS Kotasthane and R Lakpale

Abstract
Influence of six different Media for mycelial growth & Chlamydospore production of two best high yielding isolates BYT VV-02 & BYT VV-05 of *Volvariella volvacea* was studied and found Malt Extract Media reflects significantly higher Radial Growth, Biomass & Chlamydospore density followed by Potato dextrose Agar, Wheat extract Agar medium, Yeast Mannitol agar medium, while in Czapek's medium with slowest mycelial growth & Poor chlamydospores density is reported followed by Glucose-peptone medium.

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

Introduction
Volvariella volvacea is the most popular edible mushroom species cultivated (Walde *et al.* 2006) [15] and due to its pleasurable taste, it ranks third among essential mushrooms (Ramkumar *et al*. 2012) [11] (Thiribhuvanamala *et al*. 2012) [14] also its rapid growth rate in comparison to other species (Rajapakse 2011) [12]. Other common names for this include paddy straw mushroom, straw mushroom and Chinese mushroom. Cultivation was recorded for the first time in China in 1822 (Chang, 1969) [5]. This mushroom ranks sixth in the global production of cultivated mushrooms and accounts for 5–6% of world production (Buswell and Chen, 2005) [4]. This mushroom can utilize wide range of cellulosic materials and the C: N ratio needed is 40 to 60, quite high in comparison to other cultivated mushrooms. Eastern India comprises North Eastern region (Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Tripura, Sikkim and Assam), West Bengal, part of Bihar, Jharkhand and Odisha has tremendous potential and scope for paddy straw mushroom cultivation due to the easy availability of basic substrate (paddy straw). The high temperature requirement 26 °C to 30 °C for mycelium development and 34 to 37 °C for fructification (Chang ST 1972) [6] relative humidity 70-90% (Bahukandi 1979) [3] also make it a good choice for adoption in round the year cultivation of mushrooms.

Material 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 (Ramasamy, 1972) [12] 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 media

For mycelial growth and biomass production of *Volvariella volvacea*, 6 media *i.e.,* Potato dextrose agar medium, wheat extract medium, Malt extract medium, Yeast- Mannitol medium, Czapek's medium, Glucose-Peptone medium were used to the find out the best suitable media for mycelial growth of 2 isolate (BYT VV-02 and BYT VV-05) of *Volvariella volvacea*. In sterilized petridishes, 20 ml medium was poured and a pinch of streptomycin was added in each medium just before pouring to inhibit the bacterial contamination. After solidification of medium, plates were inoculated centrally with a 5 mm disc of rapidly growing 2 isolates *Volvariella volvacea* mycelium. The inoculated plates were incubated at 32°C and 5 replications were kept for each treatment. The observations were recorded for radial growth when the mycelial growth was reached at periphery of the plates in any treatment. For biomass production, 06 liquid media were taken in 250.0 ml flask and each flask had 100.0 ml medium then they were sterilized. After sterilization, flasks were inoculated by pure culture of 2 isolates of *Volvariella volvacea.* 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 sensitively 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.

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) [7].

Result and Discussion

Effect of Different Media for Isolate (BYT VV-02)

An investigation was carried out to find out the best suitable medium for growth and biomass of *V. volvacea* (BYT VV-02) and results have been obtained, data are presented in Table 1.0 that BYT VV-02 showed significant differences (65.8 mm to 87.4 mm) in radial growth with respect to different media. The radial growth of BYT VV-02 was significantly higher on Malt extract agar medium (87.4 mm) followed by Potato dextrose Agar medium (86.00 mm), Wheat extract Agar medium (80.0mm), Yeast Mannitol agar medium (77.4 mm), Glucose-peptone medium (72.4 mm), & Czapek's medium (65.8 mm) with slowest growth is reported. Biomass of isolates BYT VV-02 was observed and found significantly more (4.35 g) fresh mycelial weight obtained on Malt extract medium followed by potato dextrose (4.20 g), Yeast-Mannitol (3.18g) & Wheat extract Medium (3.12 g) however significantly less (2.14 g) fresh weight obtains in Czapek's medium followed by Glucose peptone medium (2.82g). while in dry weight of BYT VV-02 isolates was significantly higher on Malt extract agar medium (0.48) and next was Potato dextrose Agar medium (0.40 g) followed by Wheat extract Agar medium (0.31g), Yeast Mannitol agar medium (0.20g), significantly less (0.16g) in by Glucose-peptone medium followed by Czapek's medium (0.18g). Similar findings reported by Ahlawat (2006) [2] malt extract agar medium was the best growth medium for multiplication of paddy straw mushroom.

Table 1: Effect of different media on growth and biomass of isolates (BYT VV-02) of *Volvariella volvacea*

S.N.	Culture Media	Growth (mm)	Fresh weight (g)	Dry weight (g)
1.	Potato dextrose agar medium	86.0	4.20	0.40
2.	Glucose-Peptone medium	72.4	2.82	0.16
3.	Malt extract medium	87.4	4.35	0.48
4.	Yeast- Mannitol medium	77.4	3.18	0.20
5.	Czapek's medium	65.8	2.14	0.18
6.	Wheat extract medium	80.0	3.12	0.31
S.Em±		2.637	0.131	0.012
CD (5%)		7.747	0.385	0.034

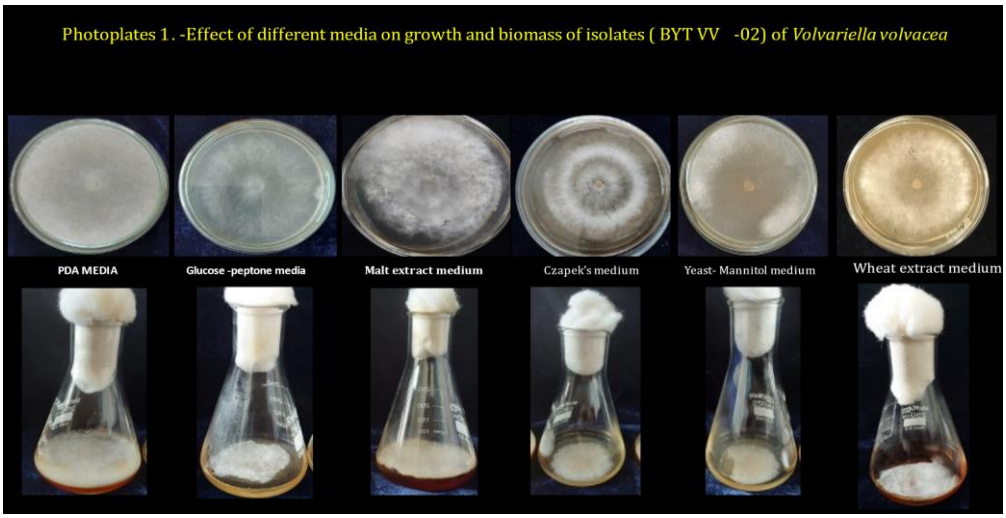

Fig 1: Microscopic characterization of culture on different media is also recorded on different parameter described below

Colony morphology & Aerial mycelium

Colony morphology & Aerial mycelium density of BYT VV-02 isolates of *V. volvacea was* characterized potato dextrose agar medium as a thin transparent irregularly projecting growth with highly dense aerial mycelium while in malt extract agar medium growth is thin fluffy transparent irregularly projecting with highly dense Aerial mycelium. Growth in Glucose peptone Agar medium & Czapek's medium growth was characterized by thin uniformly projecting & thick uniformly projecting with very low aerial mycelium density respectively and Yeast mannitol thin transparent irregularly projecting with very less aerial mycelium reported. And in wheat extract agar medium thick uniformly projecting with dense aerial mycelium is 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 media shown in Table 2.0 Malt Extract media taken significantly less (4.6 days) followed by Potato dextrose agar media (5.2 days), Wheat extract media (6.6 days) and significantly maximum (9.20 days) taken by Czapek's medium followed by yeast mannitol (8.75 days) & Glucose peptone medium (7.20 days).

Days taken for chlamydospore production: Days taken for chlamydospore production by BYT VV-02 isolates of *V. volvacea* in different media shown in Table 2.0 Malt Extract media taken significantly less (8.36 days) followed by Potato dextrose agar media (9.56 days), Wheat extract media (10.20 days) and significantly maximum (12.35 days) taken by Czapek's medium medium followed by yeast mannitol (10.35 days) & Glucose peptone medium (10.25 days).

Chlamydospore density & Colour

Maximum chlamydospores density recorded in Malt Extract media followed by Potato dextrose agar media, & Wheat extract medium minimum density recorded in Czapek's medium followed by yeast mannitol & Glucose peptone medium. Chlamydospores appears light orange in colour grown in different media.

Diameter of Chlamydospores

Diameter of chlamydospore of BYT VV-02 isolates of *V. volvacea* grown in different media shown in Table 2.0 Malt Extract media chlamydospore maximum diameter (26.9 µm) followed by Potato dextrose agar media (25.25µm), & Wheat extract media (24.4 µm) and minimum (22.15 µm) reported in Czapek's medium medium followed by yeast mannitol (23.2 µm days) & Glucose peptone medium (23.6 µm).

Table 2: Microscopic characterization of (BYT VV- 02) isolates growth on different media.

S.N.	Culture Media	Colony morphology	Aerial hyphae	(DTTCPP)	(DTFCP)	Chlamydospores density	Colour of Chlamydospores	Diameter of chlamydospores (µm)	Hyphal Diameter (µm),	No. of Septa per microscopic field	Distance Between Septa (µm),
1.	Potato dextrose agar medium	Thin transparent irregularly projecting	+++	5.20	9.56	+++	Light orange colour	25.25	5.60	5.40	3.20
2.	Glucose-Peptone medium	Thin uniformly projecting	+	7.20	10.25	+	Light orange colour	23.60	4.80	4.50	3.50
3.	Malt extract medium	Thin fluffy transparent irregularly projecting	+++	4.60	8.36	++++	Light orange colour	26.90	6.10	5.60	2.65
4.	Czapek's medium	Thick uniformly projecting	++	9.20	12.35	+	Light orange colour	22.15	4.50	4.50	3.80
5.	Yeast-Mannitol	Thin transparent irregularly projecting	+	8.75	10.35	++	Light orange colour	23.20	4.70	4.90	3.40
6.	Wheat extract medium	Thick uniformly projecting	++	6.60	10.20	+++	Light orange colour	24.40	5.20	5.30	3.30
	CD 5%			0.897	1.187			0.938	0.200	0.571	0.128

Were,

+ to ++++ = less to highly densed (DTTCPP) Days taken to cover 90mm of petriplates (DTFCP) Days taken for chlamydospores Production

Hyphal diameter

Hyphal Diameter of BYT VV-02 isolates of *V. volvacea* grown in different media shown in Table 2.0 significantly maximum diameter (6.10 µm) reported on Malt Extract media followed by Potato dextrose agar media (5.60 µm), Wheat extract media (5.20 µm) and minimum (4.50 µm) was reported in Czapek's medium followed by yeast mannitol (4.70 µm) & Glucose peptone medium (4.80 µm).

Number of septa

Significant difference reported in number of septa of isolate BYT VV-02 in different media shown in Table 2.0 it varied from 4.50 to 5.60 while maximum number of septa reported in 5.60 reported in malt extract & Potato dextrose medium (5.40), wheat extract media (5.30) while minimum (4.50) reported in glucose peptone media & Czapek's medium followed by 4.90 in yeast mannitol media.

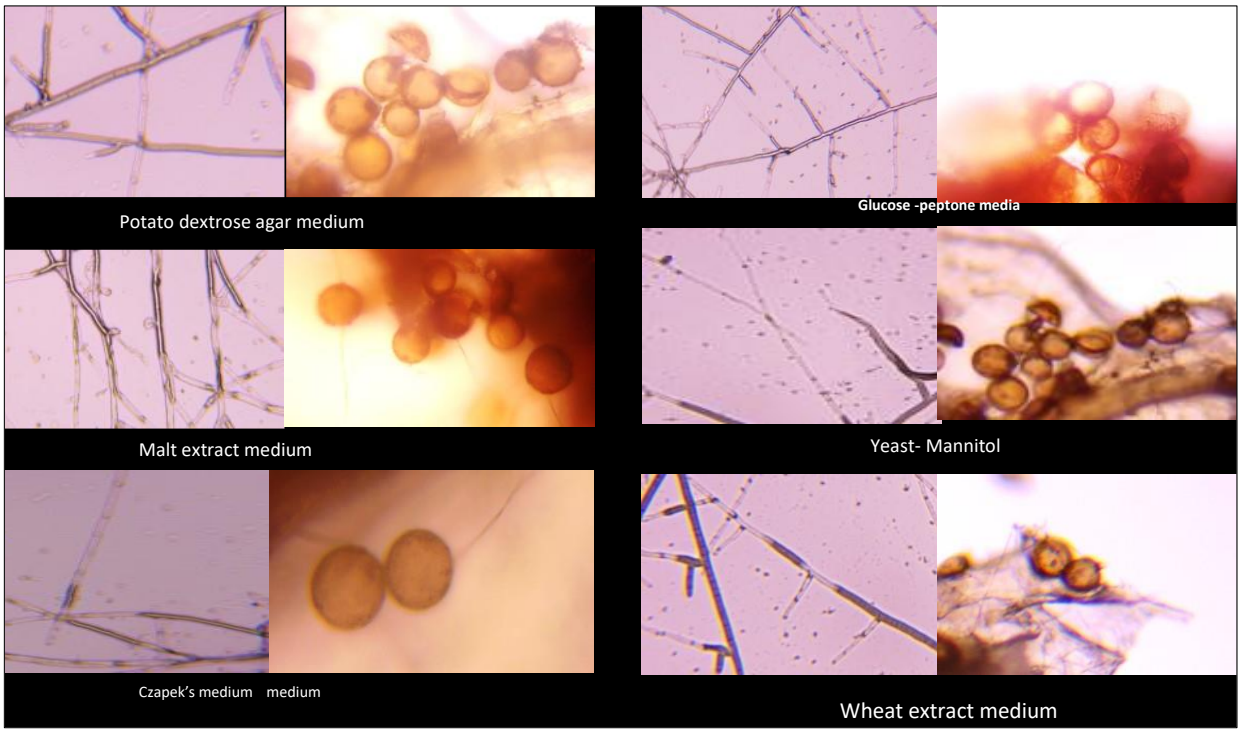

Fig 2: Microscopic characterization of (BYT VV-02) isolates growth on different media.

Distance between septa

Among different media distance between septa measured shown in Table 2.0 it varied from 2.65 µm to 3.80 µm minimum (2.65 µm) distance reported in Malt extract Agar medium followed by Potato Dextrose Agar medium (3.20 µm) & wheat extract medium (3.40 µm) and significantly maximum (3.80µm) observed in Czapek's medium followed by Glucose petone medium (3.50 µm) & Yeast Mannitol medium (3.40 µm).

Effect of Different Media for Isolate (BYT VV-05) of *Volvariella volvacea*

An investigation was carried out to find out the best suitable medium for growth and biomass of *V. volvacea* (BYT VV-05) and results have been obtained, data are presented in Table 3.0, BYT VV-05 showed significant differences (55.80 mm to 84.25 mm) in radial growth with respect to different media. The radial growth of BYT VV-05 was significantly higher on Malt extract agar medium (84.25 mm) followed by Potato dextrose Agar medium (82.10 mm), Wheat extract Agar medium (70 mm), Yeast Mannitol agar medium (69.70 mm), Glucose-peptone medium (67.20 mm), & Czapek's medium (55.80 mm) with slowest growth was reported. Significantly more (3.80 g) fresh mycelial weight of BYT VV-05 isolate was obtained on Malt extract medium followed by potato dextrose (3.50 g), Wheat extract Medium (2.90 g) & Yeast-Mannitol (2.55 g) however significantly less 1.45 g) fresh weight obtains in Czapek's medium followed by Glucose peptone medium (1.90 g). While in dry weight of BYT VV-05 isolates was significantly higher on Malt extract agar medium (0.370) and next was Potato dextrose Agar medium (0.340 g) followed by Wheat extract Agar medium (0.250 g), Yeast Mannitol agar medium (0.210 g), significantly less (0.120 g) in by Czapek's medium followed by Glucose-peptone medium (0.190 g). Similar results reported by Nasim *et al.*, (2001) [9] The discs (0.5 cm diameter) from actively growing cultures plates of mushroom were planted on fresh media plates of Malt extract agar medium (MEA) and Potato dextrose agar medium (PDA). An increase in diameter of culture disc was recorded daily. The result of mycelia growth of Paddy straw mushroom maximum in containing MEA medium and slowest in PDA. Similar Ahlawat (2006) [2] reported malt extract agar medium was the best growth medium for multiplication of paddy straw mushroom Similar findings also reported by Sharma *et al.*, (2019) [13] Among five liquid media studied, malt extract was found to be the best medium for the growth of all the strains of *V. volvacea* both in solid as well as in liquid phase.

Table 3: Effect of different media on growth and biomass of isolates (BYT VV-05) of *Volvariella volvacea*

S.N.	Media,	Growth (mm)	Fresh weight (g)	Dry weight (g)
1.	Potato dextrose agar medium	82.10	3.50	0.340
2.	Glucose-Peptone medium	67.20	1.90	0.150
3.	Malt extract medium	84.25	3.80	0.370
4.	Yeast- Mannitol medium	69.75	2.55	0.210
5.	Czapek's medium	55.80	1.45	0.120
6.	Wheat extract medium	70.00	2.90	0.250
S.Em±		3.612	0.111	0.010
CD (5%)		10.604	0.327	0.028

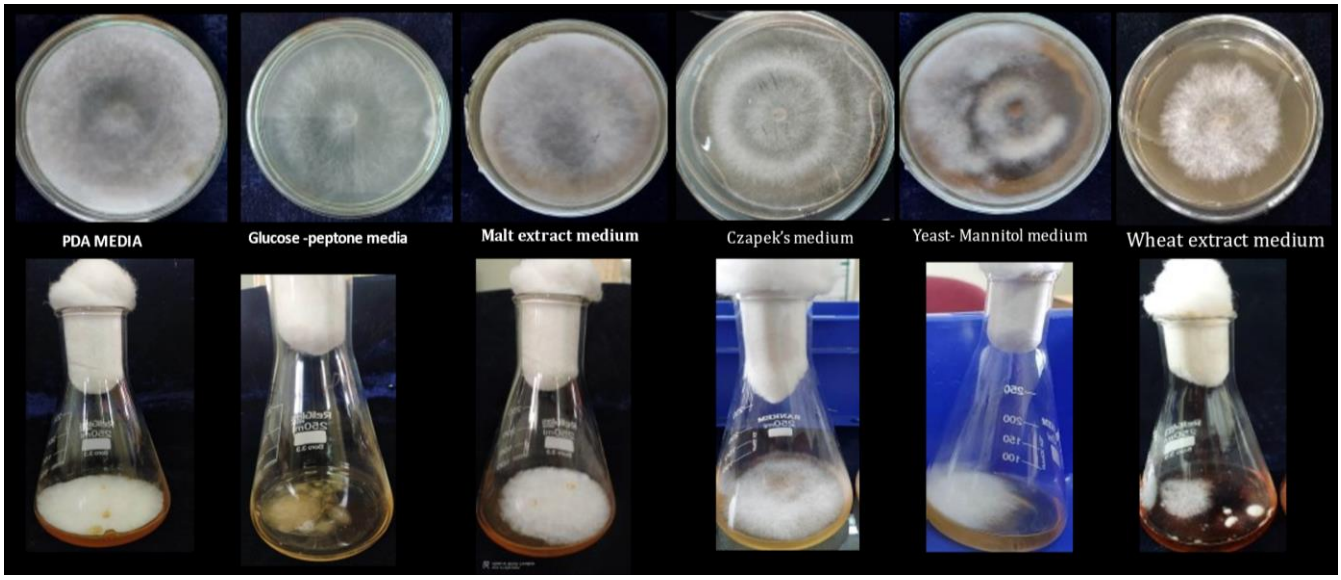

Fig 3: Effect of different media on growth and biomass of isolates (BYT-05) of *Volvariella volvacea*

Microscopic characterization of culture on different media is also recorded on different parameter described below: -
Colony morphology & Aerial mycelium
Shown in Table 4.0 Colony morphology of BYT VV-05 isolates of *V. volvacea* characterized in different growth medium in potato dextrose agar medium characterised by thin transparent irregularly projecting growth with highly dense aerial mycelium growth is reported similarly in malt extract agar medium growth is thin fluffy transparent irregularly projecting with highly dense Aerial mycelium. While in Glucose peptone Agar medium & Czapek's medium growth is characterized by thin uniformly projecting & thick uniformly projecting with very low aerial mycelium density reported and on Yeast mannitol thin transparent irregularly projecting with very less aerial mycelium reported. While in wheat extract agar medium thick uniformly projecting with dense aerial mycelium is 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 media shown in table 3 Malt Extract media taken significantly less (4.4 days) followed by Potato dextrose agar media (5.3 days), Wheat extract media (6.4 days) and significantly maximum (9.20 days) taken by Czapek's medium followed by yeast mannitol (9.0 days) & Glucose peptone medium (7.6 days).

Days taken for chlamydospore production
Days taken for chlamydospore production by BYT VV-05 isolates of *V. volvacea* in different media shown in Table 4.0 Malt Extract media taken significantly less (7.9 days) followed by Potato dextrose agar media (9.8 days), Wheat extract media (10.00 days) and significantly maximum (12.10 days) taken by Czapek's medium followed by yeast mannitol (10.70 days) & Glucose peptone medium (10.80 days).

Chlamydospore density & Colour
Maximum chlamydospores density recorded in Malt Extract media followed by Potato dextrose agar media, & Wheat extract medium minimum density recorded in Czapek's medium followed by yeast mannitol & Glucose peptone medium. Chlamydospores appears light orange in colour grown in different media.

Diameter of Chlamydospores
Diameter of chlamydospore of BYT VV-05 isolates of *V. volvacea* grown in different media shown in Table 4.0 Malt Extract media chlamydospore maximum diameter (26.6 µm) followed by Potato dextrose agar media (25.50µm), & Wheat extract media (24.5 µm) and minimum (21.90 µm) reported in Czapek's medium followed by yeast mannitol (22.70 µm days) & Glucose peptone medium (23.8 µm).

Hyphal diameter
Hyphal Diameter of BYT VV-05 isolates of *V. volvacea* grown in different media shown in Table 4.0 significantly Maximum diameter (5.80 µm) was reported in Malt Extract media followed by Potato dextrose agar media (5.70 µm), Wheat extract media (5.40 µm) and Minimum (4.60 µm) reported in Czapek's medium followed by yeast mannitol (4.80 µm days) & Glucose peptone medium (5.00 µm).

Table 4: Microscopic characterization of (BYT VV-05) isolates growth on different media

S.N.	Culture Media	Colony morphology	Aerial hyphae	(DTTCPP)	(DTFCP)	Chlamydospores density	Colour of Chlamydospores	Diameter of chlamydospores (µm)	Hyphal Diameter (µm),	No. of Septa per microscopic field	Distance Between Septa
1.	Potato dextrose agar medium	Thin transparent irregularly projecting	+++	5.3	9.8	+++	Light orange colour	25.5	5.7	5.6	3.2
2.	Glucose-Peptone medium	Thin uniformly projecting	+	7.6	10.8	+	Light orange colour	23.8	5.0	4.3	3.5
3.	Malt extract medium	Thin fluffy transparent irregularly projecting	+++	4.4	7.9	+++	Light orange colour	26.6	5.8	5.9	2.6
4.	Czapek's medium	Thick uniformly projecting	+	9.2	12.1	+	Light orange colour	21.9	4.6	4.4	3.9
5.	Yeast-Mannitol	Thin transparent irregularly projecting	++	9.0	10.7	++	Light orange colour	22.7	4.8	5.0	3.4
6.	Wheat extract medium	Thick uniformly projecting	++	6.4	10.0	++	Light orange colour	24.5	5.4	5.2	3.3
	CD 5%			0.848	1.137			2.746	0.590	0.577	0.379

Where,
+ To ++++ = less to highly densed (DTTCPP) Days taken to cover 90mm of petriplates (DTFCP) Days taken for chlamydospores Production

Number of septa
Significant difference reported in number of septa of isolate BYT VV-05 in different media shown in Table 4.0 it varied from 4.40 to 5.90 while maximum number of septa reported in 5.90 reported in malt extract & Potato dextrose medium (5.60), wheat extract media (5.20) while minimum 4.30 & 4.40 reported in glucose peptone media & Czapek's medium respectively followed by 5.00 in yeast mannitol media.

Distance between septa: Among different media distance between septa of isolate BYT VV-05 measured shown in Table 4.0 it varied from 2.60 µm to 3.90 µm. Minimum (2.60 µm) distance reported in Malt extract Agar medium followed by Potato Dextrose Agar medium (3.20 µm) & wheat extract medium (3.30 µm) and significantly maximum (3.90µm) observed in Czapek's medium followed by Glucose peptone medium (3.50 µm) & Yeast Mannitol medium (3.40 µm).

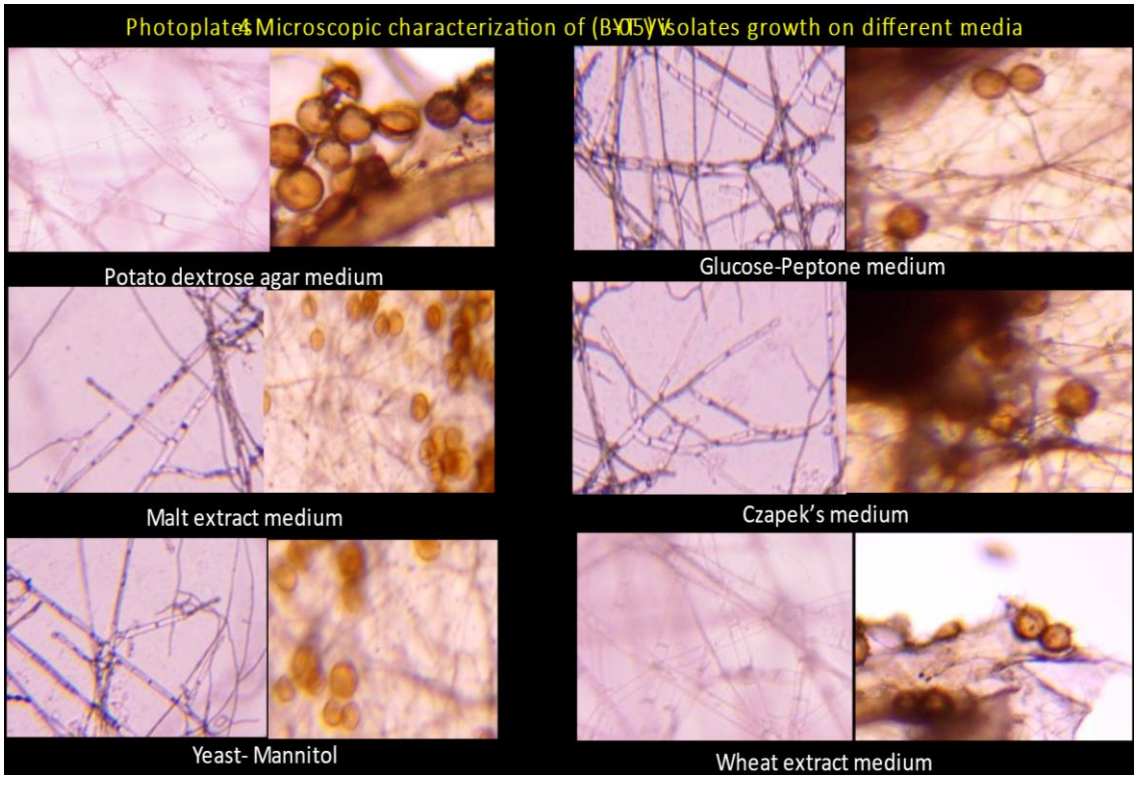

Fig 4: Microscopic characterization of (BYT-05) isolates growth on different media

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