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Cultivation technology of *Tricholoma giganteum* on agricultural wastes to promote sustainable agriculture & doubling farmers income

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Abstract

Tricholoma giganteum is the best mushroom for summer season having pleasant taste and good shelf life. The main objective of present study is to develop cultivation technologies for *T. giganteum* by recycling of locally available agricultural wastes to enhance farmer's income. Five local isolates [CIP-12, CIP-17, CIP-18, CIP-19 and CIP-20] of *T. giganteum* were evaluated for yield potential against different parameters and result indicated that PDA (66.55) was the best media for radial growth (mm) of *T. giganteum* at 32 ± 2 °C (66.94) followed by Malt Extract Agar (52.66), Wheat Straw Extract (50.94), Wheat + Paddy Straw Extract (46.83) and Maize Stone Extract (26.11) in case of media, and 35 ± 2 °C (34.66), 38 ± 2 °C (23.27) and 40 ± 2 °C (11.22) in case of temperature. Among all substrates, wheat straw was proved superior and gave maximum yield in terms of per 100 kg dry substrates (57.2kg) followed by Wheat + Paddy Straw (1:1) gave 51.0 kg but there is no fruiting was developed on Maize Stone alone. Among all casing materials Soil + Sand (3:1 ratio) was proved best which gave maximum yield in per 100 kg dry substrates (71.0kg) followed by Spent Mushroom Compost (SMC) + Sand + Soil (1:1:1 ratio) gave 56.2kg and Soil alone gave minimum 50.6kg yield.

Keywords: Casing materials, media, substrates, temperature, *Tricholoma giganteum*

Introduction

Tricholoma giganteum (*Syn-Macrocybe giganteum*), widely distributed in tropics of Asia and Africa. It is pure white in colour resembling the morphology of *Calocybe indica*. It is larger than *Calocybe indica* and fleshier than *Agaricus bisporus*. *Tricholoma giganteum* Masee is a new entry in mushroom industry because there is no any mushroom available for cultivation during summer season except *Calocybe indica* and *Volvariella volvacea*, but due to pungent and bitter taste of *Calocybe indica* and less shelf life of *Volvariella volvacea* are not accepted successfully by the farmers.

In India first time *Tricholoma giganteum* was reported from Tamil Nadu (Prakasam, 2011)^[6] later on total 20 local isolates of *T. giganteum* was reported from different places of Bihar and their potentiality was tested (Dayaram and Kumar, M. 2016)^[3].

Shelf life of *Tricholoma giganteum* is 3-4 days under room temperature and 5-6 days under refrigerated condition. It recorded significantly higher yield with bio efficiency % of 164 to 174% (Prakasam *et al.*, 2011)^[6].

Keeping in view the importance of this mushroom an experiment on “*In vitro* Physico-chemical Studies and Cultivation Technologies of *Tricholoma giganteum* Masee for Yield Potential on Agricultural Wastes” was conducted at RPCAU, Pusa, Samastipur, Bihar.

Materials and Methods

Five local isolates of *Tricholoma giganteum* i.e. CIP-12, CIP-17, CIP-18 CIP-19, CIP-20 and one isolates of *Calocybe indica* used as a control were obtained from Mushroom Centre, FBS&H Department of Microbiology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, maintained on PDA (potato dextrose agar) medium at 30 ± 2 °C temperature and the experiments were conducted in the same department.

Preparation of media other than PDA

Twenty gram Wheat + paddy straw (1:1), 20g maize stone, and, 20g wheat straw was boiled in 1 litre of distilled water for 20-25 minutes separately. The broth was filtered through muslin cloth and 20 g dextrose and 20 g of agar powder was added to each medium and stir the solution until the melting of agar on low flame. At last add a pinch of streptomycin to avoid bacterial growth and maintain the volume to 1 litre.

Effect of different media and temperature on radial growth of *T. giganteum*

Five different media i.e. Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Wheat Straw Extract (WEA), Wheat + Paddy Straw Extract in the ratio of 1:1 (W+PSE) and Maize Stone Extract (MSE), medium were evaluated against different isolates of *T. giganteum* and one isolate of *Calocybe indica* (CI-01). Thirty ml of each medium was poured in sterilized Petri dishes. After solidification of medium the plates were centrally inoculated with 7 days old culture of respective strains. For temperature evaluation PDA medium was used and the inoculated plates were kept at 4 temperature i.e., 32 ± 2 °C, 35 ± 2 °C, 38 ± 2 °C and 40 ± 2 °C for incubation. Three replications were maintained for each experiments and the observations were recorded in the form of radial growth at 7 and 14 days after inoculation.

Evaluation of different substrates for yield of *T. giganteum*

Three different locally available agricultural wastes were used as substrates i.e. wheat straw alone, wheat +paddy straw (1:1) and maize stone alone were used to know the suitable substrates for cultivation of *T. giganteum* and *C. indica*. In which first treat the substrates by using hot water treatment method, then spawning was done at the rate of 275-300g spawns (each isolates) per bag (5 kg wet substrates). The observations were recorded for spawn run days, pin head initiation days, and yield (kg/100 kg dry substrates) of *T. giganteum*.

Effect of different casing materials on yield of *T. giganteum*

Various types of casing materials viz., soil + sand (3:1), button mushroom spent compost +sand + soil (1:1:1) and soil alone were used for casing. The observations was recorded for case run days, pin head initiation days and yield (kg/100 kg substrates) of each isolates of *T. giganteum*.

Statistical analysis

Completely randomized design (CRD) and factorial with CRD was used for statistical analysis. All the statistical analysis was performed by using online software OPSTAT. The critical difference (C.D.) was calculated at 5% levels.

Results and Discussion

Effect of different media on radial growth of different strains of *Tricholoma giganteum*

Result indicated that PDA was proved the media for *T. giganteum* best among all followed by MEA (Malt Extract Agar), WSE (Wheat straw Extract Agar), W+PSE (Wheat + Paddy straw Extract Agar), and Maize Stone Extract Agar (MSE) medium.

Significantly maximum radial growth was observed on PDA by CIP-19 (72.33 mm) followed by CIP-12 (70.66 mm), CIP-17 (70.00 mm), CIP-18 (69.33 mm), CI-01 (67.66 mm) whereas the minimum radial growth (49.33 mm) was observed by CIP-20.

Among all poor growth was observed on Maize Stone Extract Agar (MSE) medium in which CIP-19 and CIP-18 gave maximum radial growth i.e. 30.33 mm, followed by CIP-17 (27.66 mm), CIP-12 (24.66 mm), CIP-20 (22.00) and minimum radial growth (21.66) was noticed on CI-01 that is shown in Table 1.

The present finding supports the finding of Singh *et al.*, (2009) and Kerketta, *et al.* (2017) [9, 4], where they reported that *C. indica* grew well on all the tested media but maximum linear growth (7 cm) was recorded on wheat extract agar medium followed by PDA and paddy straw decoction agar medium and maximum mycelial growth of *Caolocybe indica* in culture media potato dextrose agar or malt extract agar and all strain (CI-1, CI-4, CI-522, CI-524 and CI-530) was maximum in PSA (Potato sucrose agar) medium followed by PDA (Potato dextrose agar) medium respectively.

Table 1: Effect of different media on radial growth of different strains of *Tricholoma giganteum* Masee at 7 days

Media	Radial growth of isolates (mm)*						Mean
	CI-01	CIP-12	CIP-17	CIP-18	CIP-19	CIP-20	
PDA	67.66	70.66	70.00	69.33	72.33	49.33	66.55
MEA	59.00	60.33	56.66	56.33	56.66	31.00	52.66
WSE	57.33	58.00	55.66	53.33	52.00	29.33	50.94
W+PSE	53.33	50.33	50.00	49.33	50.33	27.66	46.83
MSE	21.66	24.66	27.66	30.33	30.33	22.00	26.11
Mean	51.80	52.80	52.80	51.73	51.53	31.86	
	Factors		C.D (5%)	SE(d)	SEm±		
	Media		1.943	0.968	0.685		
	Strains		2.128	1.060	0.750		
	Media × Strains		4.759	2.371	1.677		

(*) - Average of three replication.

As regard 14 days it was observed that all isolates except CIP-20 covered the media surface and measured 90 mm radial growth but in case of CIP-20 it was observed 81.66 mm radial growth. However in other media i.e. Malt Extract Agar medium it was measured upto 80.00 mm except CIP-20 (Table 2).

Table 2: Effect of different media on radial growth of different strains of *Tricholoma giganteum* Masee at 14 days

Media	Radial growth of isolates (mm)*						Mean
	CI-01	CLP-12	CIP-17	CIP-18	CIP-19	CIP-20	
PDA	90.00	90.00	90.00	90.00	90.00	81.66	88.61
MEA	81.00	81.66	82.00	80.00	80.00	50.66	75.88
WSE	75.33	74.33	79.33	77.33	75.00	47.00	71.38
W+PSE	69.00	66.33	71.00	72.33	74.66	35.33	64.77
MSE	35.66	34.66	42.66	36.66	39.00	31.00	36.61
Mean	70.20	69.40	73.00	71.26	71.73	49.13	
	Factors		C.D (5%)	SE(d)	SEm±		
	Media		2.059	1.026	0.726		
	Strains		2.256	1.124	0.795		
	Media × Strains		5.044	2.514	1.777		

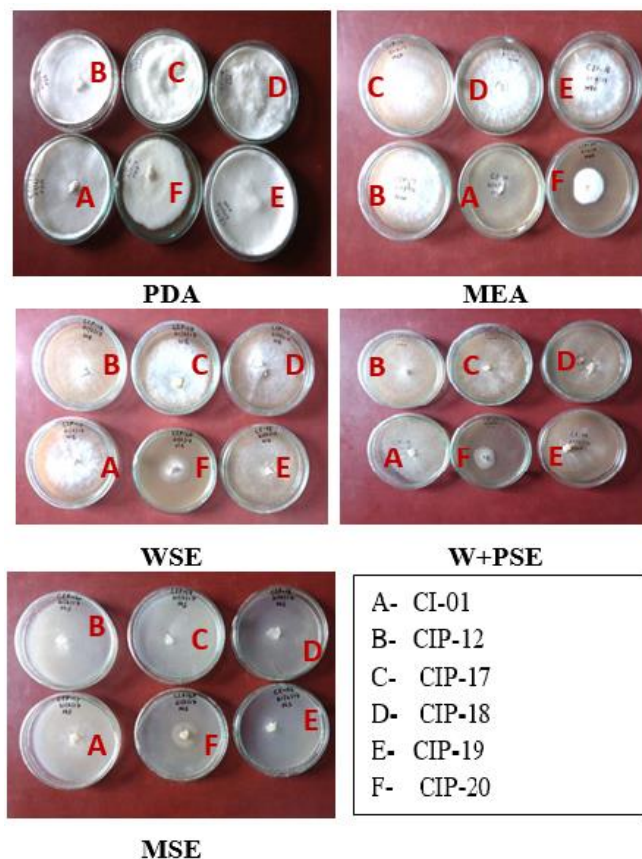


Fig 1: Effect of different media on radial growth of different isolates of *Tricholoma giganteum* Masee at 14 days

Effect of different temperature on radial growth of *Tricholoma giganteum* Masee.

Results revealed that, among different temperature 32 ± 2 °C was most suitable for all strains of *T. giganteum* Masee which yielded maximum radial growth i.e. 72.66 mm in CIP-19, followed by CIP-12 (71.33 mm), CIP-17 (70.66 mm), CIP-18 (70.00 mm), CI-01 (68.33 mm) and minimum radial growth was recorded in CIP-20 (48.66 mm) after 7 days, and all strains attain 90.00 mm except CIP-20 which gives 82.33 mm after 14 days of inoculation at 32 ± 2 °C.

Increasing the temperature after 32 ± 2 °C decreases the radial growth. Among all temperature regime (32 ± 2 °C, 35 ± 2 °C, 38 ± 2 °C and 40 ± 2 °C), 32 ± 2 °C was found most suitable for the mycelial growth of *T. giganteum* including control (CI-01) followed by 35 ± 2 °C, 38 ± 2 °C & and very poor mycelial growth was observed at 40 ± 2 °C after 7 and 14 days of inoculation, which is presented by Table- 3 and 4.

Similar trend of radial growth of *Calocybe indica* strain was observed by Varshney (2007) [12] where he reported temperature requirement from 25 to 35°C for mycelial growth of *Calocybe indica*. All strains of *Calocybe indica* showed maximum mycelial growth at 28°C followed by 32°C and minimum at 20°C,

Similarly Singh *et al.*, (2015) [11] among all strains (i.e. APK-2, CI-6, CI-8, CI-9, and CI-10) of *Calocybe indica* showed maximum mycelial growth at 30°C followed by 27°C and minimum at 21°C on 3rd, 5th, 7th and 9th day's observations. At 30°C temperature on 9th day's strain APK-2 showed maximum radial growth (full growth) of mycelium (9.0 cm). Shukla *et al.*, (2014) [8] also supported mycelial growth of six strains (CI-5, CI-6, CI-7, CI-8, CI-9, and CI-10) of *Calocybe indica*. He found that maximum mycelial growth at 35 °C at

4th, 6th, and 8th day.

Table 3: Effect of different temperature on radial growth of *Tricholoma giganteum* Masee at 7 days

Temperature	Radial growth of isolates (mm)*						Mean
	CI-01	CIP-12	CIP-17	CIP-18	CIP-19	CIP-20	
32 ± 2 °C	68.33	71.33	70.66	70.00	72.66	48.66	66.94
35 ± 2 °C	35.66	39.33	35.66	35.33	40.00	22.00	34.66
38 ± 2 °C	23.33	22.33	23.33	24.00	25.00	21.66	23.27
40 ± 2 °C	11.66	11.00	10.66	11.66	12.00	10.33	11.22
Mean	34.75	36.00	35.08	35.25	37.41	25.66	
	Factors		C.D (5%)	SE(d)	SEm±		
	Temperature		1.481	0.734	0.519		
	Strains		1.814	0.898	0.635		
	Temperature × Strains		3.629	1.797	1.271		

(*) - Average of three replications.

Table 4: Effect of different temperature on radial growth of *Tricholoma giganteum* Masee at 14 days

Temperature	Radial growth of isolates (mm)*						Mean
	CI-01	CIP-12	CIP-17	CIP-18	CIP-19	CIP-20	
32 ± 2 °C	90.00	90.00	90.00	90.00	90.00	82.33	88.72
35 ± 2 °C	63.00	66.33	65.66	65.66	66.33	36.00	60.50
38 ± 2 °C	47.00	47.00	47.00	46.00	48.66	33.33	44.83
40 ± 2 °C	14.00	13.66	14.33	15.00	14.33	13.66	14.16
Mean	53.50	54.25	54.25	54.16	54.83	41.33	
	Factors		C.D (5%)	SE(d)	SEm±		
	Temperature		1.731	0.857	0.606		
	Strains		2.120	1.050	0.742		
	Temperature × Strains		4.241	2.100	1.485		

(*) - Average of three replication

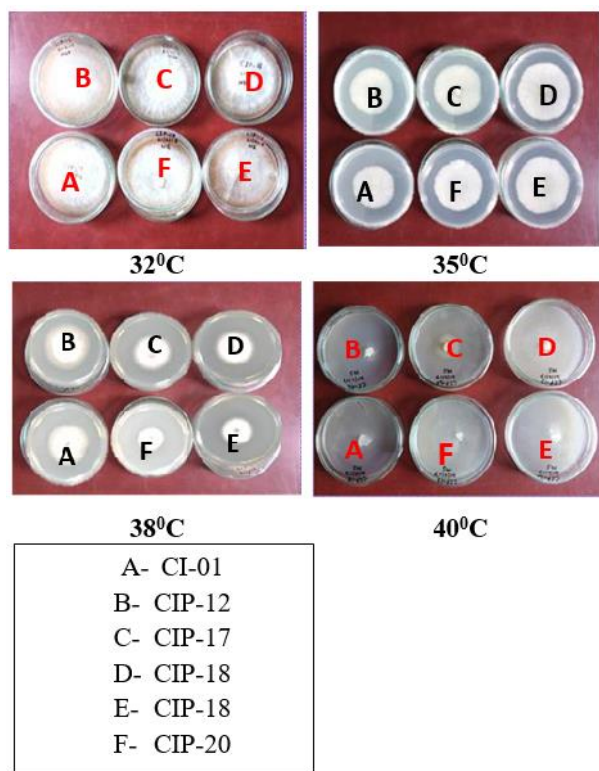


Fig 2: Effect of different temperature on radial growth of different isolates of *Tricholoma giganteum* Massee at 7 days.

Evaluation of different substrate for yield potential of *T. giganteum* Massee.

Among three locally available agricultural wastes used as substrates, result indicated that wheat straw was proved best gave maximum yield in terms of per 100 kg dry substrates by CIP-18 (59.4 kg), followed by CIP-19 (59 kg), CIP-20 (58 kg), CIP-17 (57.6 kg), CIP-12 (56.2 kg) and minimum yield was obtained by CI-01 (53.8 kg).

As regards wheat + paddy straw (1:1) substrates the maximum yield was observed in CIP-17 (52 kg) followed by CIP-20 (51.6 kg), CIP-12 (51.2 kg), CIP-18 (51 kg), CIP-19 (50.6 kg), and minimum yield was obtained by CI-01 (50.2 kg) presented by Table-5.

On maize stone very poor mycelial growth was observed but there is no fruiting was developed.

The present findings corroborate the results of Rawal *et al.*, (2014) [7] where he tested three different combinations of substrates viz., wheat straw + paddy straw (1:1), wheat straw + paddy straw (1:2), wheat straw + paddy straw (2:1) along with wheat straw and paddy straw alone for yield performance of *Calocybe indica*. The wheat straw alone substrate significantly took minimum days for spawn run; pinhead formation as well as 1st harvest followed by wheat straw + paddy straw (2:1), and wheat straw + paddy straw (1:1).

Present finding is in confirmation of finding of Vijay Kumar *et al.*, (2014) [13] conducted an experiment to find out the efficacy of different substrates. Among the six different substrates, wheat straw substrates was superior which recorded minimum days for spawn run, pinhead formation and for first harvest with highest no. of fruiting bodies, highest diameter, maximum yield and highest biological efficiency. Paddy straw was the next best superior substrate for cultivation of milky mushroom Similarly Kumar *et al.*, (2015) [5] tested different agricultural wastes alone or in combination on yield of two strains (CI-6 and CI-4) of *C. indica*. Minimum time was recorded for spawn run in wheat straw (WS) substrates alone, while days for pinhead formation and days for first harvesting was observed in the combination of WS+PS (paddy straw) substrates with the ration of 2:1 in strain CI-4 and CI-6, respectively. Maximum yield per kg dry substrates was harvested in case of CI-4 with WS+BS (Brassica straw) (1:2) and CI-6 with WS+BS (2:1) combination. However, maximum average weight per fruit body was recorded in WS+BS (1:1) combination from strain CI-4 and CI-6. Singh, *et al.*, (2017) [10] for cultivation of *Calocybe sp.* (DMRO-600) mushroom, five locally available substrates in pure form and in combinations with wheat straw were evaluated. Out of these, wheat straw substrate gave highest yield (1052.50 g), maximum number of fruiting bodies (40.75), early spawn run (21.50 days) along with early first harvest (33.25 days), followed by wheat straw + paddy straw (932.50 g), paddy straw (841.25 g), wheat straw + sugarcane bagasse (840.56 g), sugarcane bagasse (825.0 g), maize straw (703.75 g), wheat straw + maize straw (596.25 g), wheat straw + dehulled maize cobs (543.75 g) and dehulled maize cobs (503.75 g).

Table 5: Evaluation of different substrate for yield potential of *Tricholoma giganteum* Massee

Substrate	Days for spawn run*						Mean	Sporophore count/bag*						Mean	Yield kg/100kg substrate*						Mean
	CI-01	CIP-12	CIP-17	CIP-18	CIP-19	CIP-20		CI-01	CIP-12	CIP-17	CIP-18	CIP-19	CIP-20		CI-01	CIP-12	CIP-17	CIP-18	CIP-19	CIP-20	
Wheat straw	13	12	11	12	13	11	12.00	14	17	15	16	15	16	15.50	53.8	56.2	57.6	59.4	59.0	58.0	57.2
Wheat + paddy straw (1:1)	15	13	14	13	12	14	13.50	12	13	14	12	11	13	12.50	50.2	51.2	52.0	51.0	50.6	51.6	51.0
Mean	14	12.5	12.5	12.5	12.5	12.5		13	15	14.5	14	13	14.5		52.0	53.6	54.8	55.2	54.8	54.8	
	C.D (5%)		SE(d)		SEm±			C.D (5%)		SE(d)		SEm±			C.D (5%)		SE(d)		SEm±		
Substrate	0.488		0.234		0.165			0.402		0.192		0.136			0.043		0.020		0.014		
Strains	0.846		0.405		0.286			0.696		0.333		0.236			0.074		0.035		0.025		
Substrates × Strains	1.196		0.573		0.405			0.984		0.471		0.333			0.105		0.050		0.035		

(*) - Average of three replications.

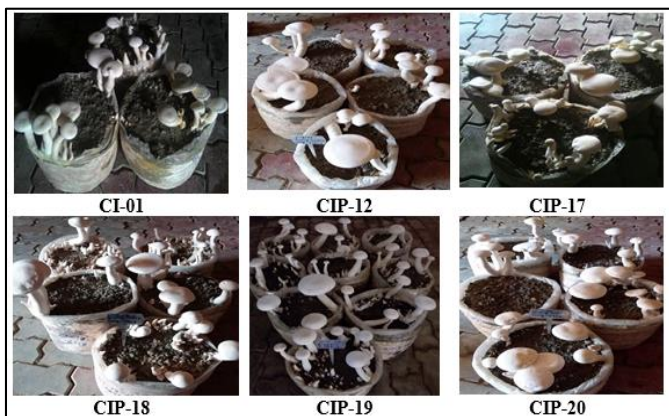
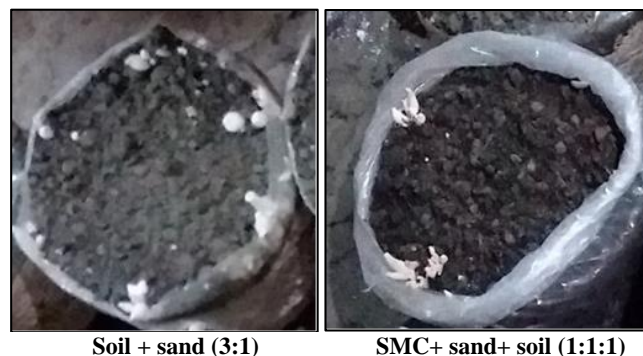


Fig 3: Sporophore development of different strains on wheat straw



Fig 4: Sporophore development of different strains on wheat straw +paddy straw (1:1)

(50.2 kg), CIP-17 (49.8 kg) and minimum yield was obtained by CI-01 (49 kg)/100kg substrates presented by Table 6. Similar trend was observed by Ashrafi *et al.*, (2017) [2] conducted the production trial of milky white mushroom with five casing mixture viz. Loam soil + Sand (3:1), SMS compost + Sand (3:1), Loam soil + SMS compost + Sand (2:1:1), Loam soil + SMS compost + FYM (1:1:1) and SMS compost + FYM (1:1). Best result was obtained from SMC + Sand (3:1) followed by Soil + SMC + FYM (1:1:1) produced statistically similar yield, but SMC and SMC + Sand (3:1) took shorter time to harvest than Soil + SMC + FYM (1:1:1). So, SMC or SMC + Sand (3:1) can be recommended to use as casing material. 3:1) and soil + FYM (1:1:1) produced statistically similar yield.



Soil + sand (3:1) **SMC+ sand+ soil (1:1:1)**



Soil

Fig 5: Different casing materials

Effect of different casing materials on yield of *T. giganteum*

Out of three casing materials, Soil + Sand (3:1 ratio) gave maximum yield in terms of per 100 kg dry substrates was obtained by CIP-17 and CIP-19 (72.8 kg) followed by CIP-20 (72.4kg), CIP-18 (71.8 kg), CIP-12 (70.8 kg), CI-01 (66 kg). In case of Spent Mushroom Compost(SMC) + Sand + Soil (1:1:1), maximum yield was obtained by CIP-19 (57.2 kg) followed by CIP-20 (56.8 kg), CIP-17 (56.7 kg), CIP-12 (56 kg), CIP-18 (55.4 kg) and lowest yield was obtained by CI-01 (54.8 kg). Out of three casing material lowest yield was obtained on soil alone. In which maximum yield was obtained by CIP-18 (51.6 kg), followed by CIP-12 and CIP-19 gave 51.4 kg, CIP-20

Table 6: Effect of different casing materials on yield of *T. giganteum*

Substrate	Days for case run*							Mea n	Pin head initiation days*							Mea n	Yield kg/5kg substrate*							Mea n								
	CI-01	CIP-12	CIP-17	CIP-18	CIP-19	CIP-20	CI-01		CIP-12	CIP-17	CIP-18	CIP-19	CIP-20	CI-01	CIP-12		CIP-17	CIP-18	CIP-19	CIP-20												
Sand + Soil (3:1)	28	25	25	24	24	23	24.83	35	32	33	32	34	31	32.83	66.0	70.8	72.8	71.8	72.8	72.4	71.0											
SMC + Sand + Soil (1:1:1)	30	28	27	28	26	26	27.50	38	36	35	36	33	35	35.50	54.8	56.0	56.7	55.4	57.2	56.8	56.2											
Soil	35	33	34	33	34	32	33.50	42	40	41	39	38	40	40.00	49.0	51.4	49.8	51.6	51.4	50.2	50.6											
Mean	31	28.6	28.6	28.3	28	27		38.3	36.0	36.3	35.6	35.0	35.3		56.6	59.4	59.8	59.6	60.4	59.8												
C.D (5%)	SE(d)			SEm±				C.D (5%)			SE(d)				SEm±																	
Substrate	0.321			0.57				0.623			0.305				0.216				0.051			0.025				0.018						
Strains	0.454			0.222				0.157			0.882				0.432				0.305				0.072			0.035				0.025		
Substrates × Strains	0.786			0.385				0.272			1.527			0.748				0.529				0.125			0.061				0.043			

(*) - Average of three replications.
SMC – Spent Mushroom Compost

Conclusion

The conclusion of present studies is to find out suitable temperature (32 ± 2 °C) and media (PDA) for mycelial growth and to develop cultivation technologies for *Tricholoma giganteum* by recycling of agricultural wastes to enhance farmer's income because this mushroom can be grown upto 38-40°C temperature. Result indicated that wheat straw was proved best substrate, and gave maximum by CIP-18 (59.4 kg per 100 kg dry substrates), followed by wheat + paddy straw (1:1) gave (51.0kg) but on maize stone there is no sporophore developed. Out of three casing materials Sand + Soil (3:1) was the best, and gave maximum yield 71.0kg followed by SMC + Sand + Soil (1:1:1) gave 56.2kg and lowest yield was obtained by soil alone (50.6kg).

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