EXPLOITATION OF THERMOPHILIC FUNGI IN COMPOST PRODUCTION FOR WHITE BUTTON MUSHROOM (*AGARICUS BISPORUS*) CULTIVATION – A REVIEW

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ABSTRACT

Compost production is the most important and integral part of button mushroom cultivation and its quality parameters mainly determine success or failure of a crop. It is a product of fermentation brought about by the activities of thermophilic organisms and among them fungi especially, *Scytalidium thermophilum, Humicola insolens* and *H. grisea* play a decisive role in bringing about the selectivity and productivity of the compost. In a pursuit to improve the present day composting procedures using thermophilic fungi and also to prepare compost in most environment friendly manner in shortest possible time, series of experiments were conducted at Directorate of Mushroom Research, Solan, India. Role of thermophilic fungi especially that of *H. insolens* was thoroughly investigated. Four different strains of *H. insolens* were isolated from different parts of India through molecular characterization. Based on many physiological, enzymatic and under *in vitro* studies, I-1 and X-21 strains of *H. insolens* and *S. thermophilum*, respectively were short listed as potent strains for their further exploitation in compost production. Artificial inoculation of these fungi on zero day under long method of composting (LMC) not only brought down the composting period to 20 days but also *S. thermophilum* significantly increased the button mushroom yield. Inoculation of these fungi under short method of composting period (phase-II) could be brought down from seven to five days. These two fungi and their combinations were further utilized in improving the environment friendly indoor composting (Anglo-Dutch method). High conversion of compounding mixture to compost was found in inoculated treatments with increased yield over control. Total composting operation lasted for 13 days. Highest degradation of cellulose, hemicellulose and carbon was observed in inoculated pile, which contributed in good spawn run and highest yield.

Keywords: compost, thermophilic fungi, indoor composting, *Humicola insolens, Scytalidium thermophilum*

INTRODUCTION

Compost production is the most important and integral part of *Agaricus bisporus* (white button mushroom) cultivation. It is a product of fermentation brought about by the variety of organisms including bacteria, actinomycetes and fungi. These organisms convert and degrade the straw to form lignin humus complex and also convert soluble form of nitrogen into microbial cell substances [1-3]. This decomposed straw along with microbial biomass both become a source of organic and inorganic nutrition for the mushroom mycelium [4]. The process of composting is governed by a carefully ordered changing population of organisms [5]. Further, these mycoflora also play a key role towards selectivity and conditioning of the compost and make the growth of competitor microorganisms more difficult [6-8]. Compost if properly prepared, *A. bisporus* can only successfully grow in it at the practical exclusion of the competing organisms [9]. Various kinds of flora encountered during whole process of composting have different role to play. Mesophilc flora present in the initial process of composting bring about the biodegradation of the straw and other ingredients which results in heat energy resulting in establishment of thermophilic flora in the compost which later on govern the whole process of composting. Of the thermophilic fungi generated in the compost during the course of composting, *Scytalidium thermophilum, H. insolens, Humicola grisea* and *Chaetomium thermophile* are of utmost importance as they are responsible for nutrition of *A.bisporus* and faster composting process [10-12].

Work on isolation and role of thermophilic fungi in compost production started at the National Centre almost 2 decade back. Majority of those studies were based on isolation of different thermophilic flora across the country, from different compost piles having different N values. Molecular characterization of different thermophilc fungi, their role in composting

process, etc. Special emphasis was given to *S. thermophilum* in its role in composting process. All these studies were reported from time to time in different journals which were finally reviewed [12-15].

H. insolens is reported to be an ideal agent in compost preparation and hasten the composting process [16, 17]. It secretes various cellulolytic as well as lignolytic enzymes to degrade the compounding mixture of compost. Increased growth of *A. bisporus* mycelium in presence of this fungus is also reported [18]. Cellulolytic role of *H. insolens* is well defined in terms of Xylanase [19], â-glucosidase [20] but its lignolytic activity is only tangentially known. Stanek [6] indicated that *Humicola* spp. produce vitamins and amino acids which can be utilized by *A. bisporus* for its growth. No systematic studies on the role of this fungus on compost production for button mushroom however, have been reported so far in spite of the fact that this fungus remains a choice of interest due to its abundant occurrence in compost at various stages of its preparation.

In a pursuit to improve the present day composting procedures and also to prepare compost in most environment friendly manner in shortest possible time series of experiments were conducted at ICAR-Directorate of Mushroom Research, Solan, India from 2007 onwards. Role of thermophilic organisms and especially that of *H. insolens* was thoroughly investigated [21, 22, 23, 24, 25, 26, 27, 28, 29]. A thesis on *H. insolens* was also approved [30]. Significant findings there on and especially that of *H. insolens* are presented in this communication.

Isolation of *Humicola insolens*

Primary objective of the study was to isolate varied diversity of *H. insolens* across the country. We could isolate *H. insolens* from 51 samples collected from Haryana, Himachal Pradesh, Jharkhand, Maharashtra, Meghalaya, Punjab, Rajasthan, Tamil Nadu and Uttar Pradesh. Based on morphological characters these were placed in twelve groups (I-1 to I-12). I-1 isolate was distributed in samples collected from Haryana, Himachal Pradesh, Punjab, Rajasthan and Uttar Pradesh states. I-2 isolate was isolated from Maharashtra and Tamil Nadu states. I-3 isolate from Haryana, Himachal Pradesh, Punjab and Uttar Pradesh. I-4 from Haryana, Jharkhand, and Uttar Pradesh. I-5 from Haryana and Himachal Pradesh. I-6 from Meghalaya only. I-7 was isolated from Haryana and Uttar Pradesh, I-8 from Himachal Pradesh and Uttar Pradesh, I-9 from Haryana and Uttar Pradesh. I-10 from Himachal Pradesh and Maharashtra and I-11 and I-12 Haryana and Punjab. Dominance of I-1 was maximum across the country followed by I-3. Frequency of occurrence of *H. insolens* was 51%. *S. thermophilum* was another fungus which was most dominantly isolated from all most all the samples (55% dominance) (Fig.-1). This fungus also showed lots of variations with respect to its morphology and was grouped on the basis of molecular characterization in seven groups (S-1 to X-21). Other thermophilic fungi isolated were *H. grisea*, *A. fumigatus*, *T. lanuginosus*, *H. fuscoatra*, *S. thermophile*, *S. chlorianum* and *T. duponti*. Most of them are known thermophilic taxa [31].

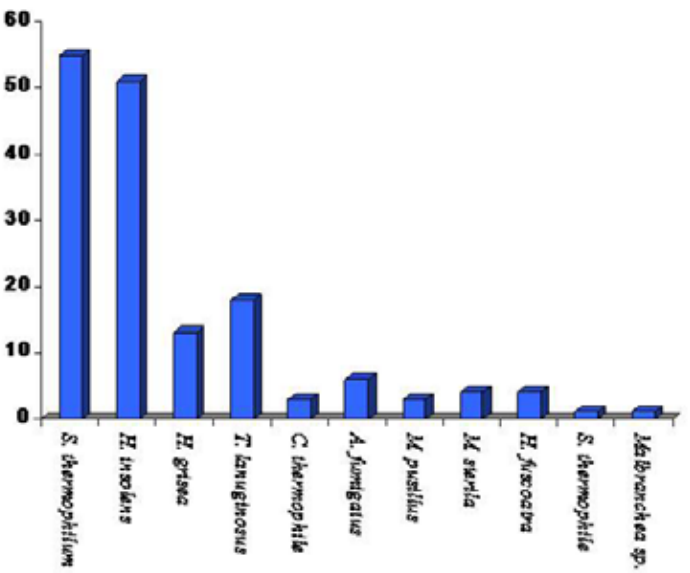

Figure 1. Relative dominance (% frequency) of *H. insolens* with respect to other thermophilic fungi

Molecular identification and characterization of *H. insolens* isolates

To identify the 12 isolates at genus level PCR amplification of Internal Transcribed Spacer was done by using ITS 1 and ITS 4 primers. Amplified region of ITS of twelve isolates showed clear band between 500-600 bp (Fig. 2). No intra-species diversity could be detected as far as ITS lengths of 5.8S r-DNA region were concern. Since, 5.8S gene is highly conserved at genus level, it could be confirmed that all twelve isolates belonged to the same genus. The isolates were

sequenced using ITS-1 and ITS-4 universal primers and BLAST against website of NCBI confirmed the identity of the strains as *H. insolens* . Although the BLAST have shown more similarity of the isolates with *S. thermophilum*, but the consideration in BLAST such as expect value, differences in the initial sequences numbers between subject and query, and missing values helped the confirm the identity as *H. insolens*. The reason for giving more similarity might be due to the close taxonomic position of *S. thermophilum* with *H. insolens* and more n values in the *H. insolens* sequences. However, RAPD analysis of 12 strains showed the presence of 4 different strains of this fungus (Fig. 3) These strains were quite different from *H. insolens* strains reported earlier [32].

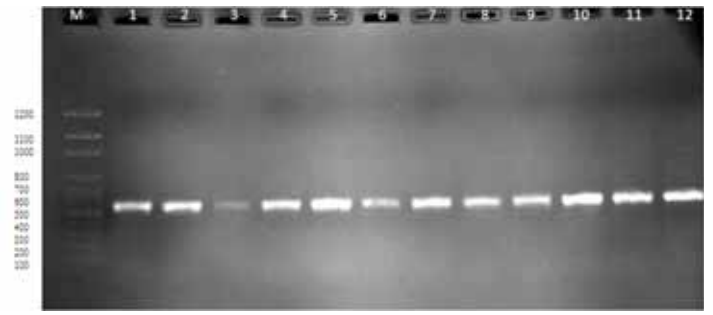

Figure 2. ITS profile of different isolates of *H. insolens*

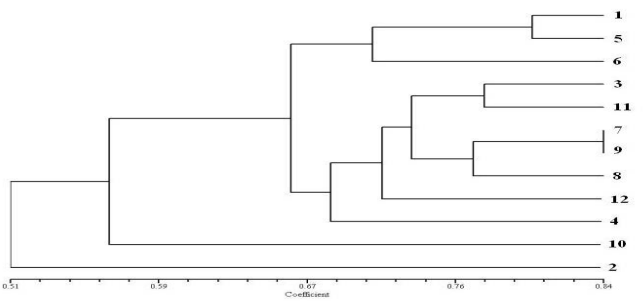

Figure 3. Phylogram of *H. insolens* isolates showing four different groups

Physiological and interaction studies

In present investigation four *H. insolens* strains initially characterized by molecular techniques were taken for various physiological and interaction studies. Initially all the strains were grown on different media to screen suitable medium for these strains and also to shortlist the potent strain which shows best performance/growth as compared to others. Best growth and mycelial weight of all strains was observed in malt extract followed by yeast and compost extract (Fig. 4). PDA was not a preferred medium for *H. insolens*. Strain-1 showed best growth compared to all other strains. Growth of *H. insolens* on different pH was studied on MEA and on different agricultural residue (wheat straw, wheat bran, cotton seed cake, cotton seed meal) along with different compost formulations (Table-1) to shortlist the best agricultural residue and formulation used in compost production for *A. bisporus*. Under this study best growth of all the strains was found in cotton seed meal followed by wheat bran and wheat straw at 7.0-8.0 pH (Fig. 5). Strain-1 exhibited the highest growth among all the strains on all agricultural residues at different pH. All the four strains preferred most of the formulations for their growth. Highest growth was observed in strain-1 in formulation 1, 2, 5 and 6, followed by strain-2 in formulation- 2 at different pH ranges (5.0-8.0).After screening different strains on different media and pH, study was conducted to evaluate the cardinal temperature for different strains.

It was found that best growth of different strains was observed in the range of 45-55 °C. Slow growth was recorded at 25-35°C (Table 2). Present data obtained are very similar to what has been reported in the literature [31, 33-35].

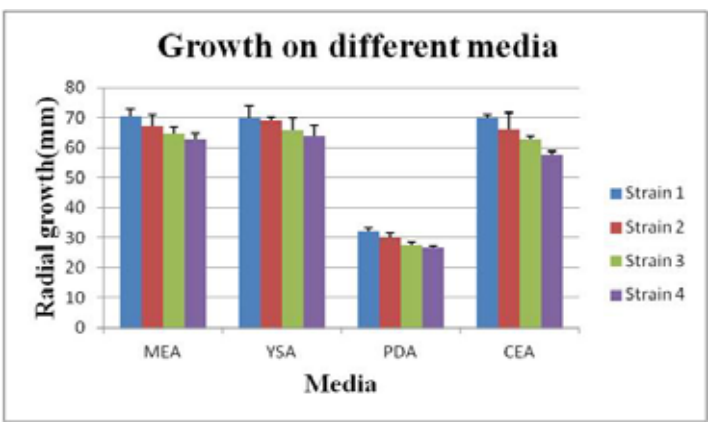

Figure 4. Showing growth of *H. insolens* strains on different media

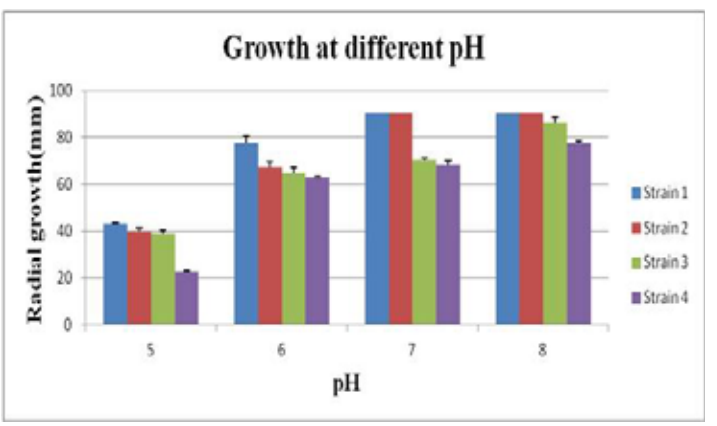

Figure 5. Showing growth of *H. insolens* strains on different pH levels in chicken manure based formulation

Table 1. Medium composition (compost formulations)

	Formulations					
Substrate	**F-I (g)**	**F-II (g)**	**F-III (g)**	**F-IV (g)**	**F-V (g)**	**F-VI (g)**
Wheat Straw	250.0	250.0	250.0	250.0	250.0	250.0
Chicken manure	125.0	125.0	125.0	125.0	62.5	100.0
Wheat bran	_	_	_	_	18.75	25.0
Cotton seed meal	50.0	_	_	_	_	_
Cotton seed cake + Cotton seed meal	_	50.0			18.75	_
Soya bean meal	_	_	_	50.0		_
Nutri	_	_	50.0	_	_	_
Gypsum	19.0	2.5	9.0	18.75	25.0	7.5
Urea	2.5	2.25	2.5	2.5	_	3.75

Table 2. Growth of four strains of *H. insolens* at different temperature

	Radial growth (mm) of *H. insolens* at different temperature (°C)			
Strains	**25**	**35**	**45**	**55**
I-1	22.33	43.66	70.33	89.16
I-2	16.83	43.66	67.16	70.83
I-3	16.83	33.50	64.83	68.33
I-4	9.6	20.83	64.83	68.33
$CD_{0.05}$	2.93	2.34	2.08	2.61

Dual culture studies between various strains and *A. bisporus* and other mesophilic fungi indicated that strain-1 increased the growth of *A. bisporus* while reduction in its growth was observed with rest three strains. When *A. bisporus* growth was again observed after 15 days it was found that it completely parasitized *H. insolens* and possibly utilized it as nutrient source. Increased growth of *A. bisporus* in dual culture with *H. insolens* could be due to the ability of *A. bisporus* to degrade the mycelium of this strain [36-38] or utilization of vitamins and amino acids produced by this strain of *H. insolens* [6]. Vijay [8, 39] also presented similar observations. Study therefore, gave indication that large population of this fungus if present in the compost may provide extra nutrition to the *A. bisporus* mycelium later, when such compost is spawned. Artificial inoculation of this fungus in the compost may also prove beneficial. Therefore, this strain was further chosen for field studies on improving the composting processes presently in vogue in the country and also to develop new composting techniques. Almost similar trend with respect to culture filtrates of four *H. insolens* strains was observed on *A. bisporus*. As regards to dual culture and culture filtrates studies on other mesophilic organisms, it was observed that strain-2 inhibited the growth of *Fusarium accuminatum* profoundly (Table 3).

This fungus is usually found in chicken manure, wheat bran and other stored materials commonly used in the compost. It is usually associated with damping off type symptoms on button mushroom pinheads. Strain-2 if present in the compost at spawn run and at cropping stage may inhibit the growth of this fungus thereby reducing the symptoms. Strain -1 inhibited the growth of *Chrysospermum luteum* and *Papulaspora bysinna* markedly both of which are strong competitors of button mushroom mycelium and are the causal agents of yellow mould disease and brown plaster mould respectively. Both these moulds are more prevalent in long method compost where thermophilic population is always at its low ebb. Such composts therefore, are more prone to attack by different competitors. Surprisingly other *H. insolens* strains increased the

Table 3. Growth (mm) and % inhibition of different mesophiles in culture filtrates of different strains of *Humicola insolens*

	Growth(mm) (% inhibition)			
***H. insolens* Strains**	***Chaetomium luteum***	***Fusarium accuminatum***	***Papulaspora byssinia***	***Agaricus bisporus***
I- 1	8.50 (-73.84)	48.00 (+6.66)	15.50 (-59.74)	27.50 (+22.22)
I- 2	34.50 (+6.15)	8.50 (-81.11)	40.00 (+3.89)	18.00 (-20.00)
I- 3	34.00 (+4.61)	48.00 (+6.66)	40.00 (+3.89)	18.00 (-20.00)
I- 4	35.50 (+9.23)	50.00 (+11.11)	42.00 (+9.09)	12.50 (-44.44)
Control (Fresh medium)	32.50 (0.00)	45.00 (0.00)	38.50 (0.00)	22.5 (0.00)

growth of these competitors. Study indicated that strain-1 of *H. insolens* is very superior to other strains with many beneficial attributes.

Production of extra-cellular enzymes by different strains of *Humicola insolens*

In the present investigation degradation of hemicellulose, cellulose and lignin at various pH levels was measured in terms on xylanase, exo-cellulase, endocellulase, β-glucosidase and laccase on different agricultural residues and on different compost formulations (Table-1). All these enzymes play an important role in degrading the polymeric components of agricultural residues in monomeric form or sugars which later can easily be utilized by different thermophilc fungi and *H. insolens* in particular. The highest activity of all the cellulolytic and lignolytic enzymes was recorded at pH 8.0 in all agricultural residues and formulations by all the strains.

Best xylanase, β-glucosidase, FPase, activity was observed in wheat bran followed by cotton seed meal and wheat straw when tried on different agriculture residues. Best CMCase activity was observed in cotton seed meal followed by cotton seed cake. Hence, high degradation of cellulose was observed in wheat bran as compared to other agricultural residues followed by cotton seed meal. Maheswari *et al.* [40] also reported the best cellulolytic activity of *H. insolens* in wheat bran. Other agricultural residues showed more or less similar trend. This indicated that *H. insolens* has high titer of xylanase enzyme in the medium and maximum activity was observed in neutral to alkaline medium in increasing order.

Next to the xylanase, high β-glucosidase activity was observed in different strains of *H. insolens* in wheat bran followed by wheat straw and the highest activity was observed at pH 8.0 by all the strains and best one was observed in strain-1. Flavio *et al*. [20] also reported best β-glucosidase activity of *H. insolens* at pH 8.0-9.0 which confirms our findings. Other celluloytic activities were less as compared to xylanase and β-glucosidase and highest of which was observed at pH 8.0 by all the strains in all the residues and formulations and best was observed in strain-1. Similar trend was found in different formulations, exhibiting the highest cellulolytic and lignolytic activity in formulation-6 (F-6) having wheat bran in medium followed by formulation-5 and 2 (F-5 and F-2) having cotton seed meal and cotton seed cake. This indicated that supplementation of medium with nitrogen compounds increases the laccase activity. The highest activity of cellulolytic and lignolytic enzymes was observed in strain-1 followed by strain-2 and 3. Least activity was observed in strain-4. Further wheat bran followed by wheat straw was best utilized by these strains and formulation (F-6) containing wheat bran supported maximum liberation of these enzymes. Therefore, strain-1 of this fungus along with formulation F-6 was chosen for further studies.

Production of *Agaricus bisporus* compost using *Scytalidium thermophilum* and *H. insolens* under *in vitro* conditions

In the present studies seven different strains of *S. thermophilum* and four that of *H. insolens* were evaluated for their ability to convert agricultural residues to button mushroom compost under *in vitro* conditions in BOD incubators by providing exact conditions of composting. Main aim of the study was to select the suitable strains of both these fungi so that they could be inoculated in the compounding mixture for large scale compost production in the field for production of compost in shortest possible time without causing any environment pollution. All the strains were able to convert substrate

into compost both in unsterilized and sterilized substrates under *in vitro* conditions in seven days time. In both the sets mixture of *S. thermophilum* (X-21 strain) and *H. insolens* (I-1 strain) performed exceedingly well as evident by various biological and physico-chemical parameters including spawn run and yield. Sterilized substrate performed better compared to unsterilized substrate (Table 4). Large number of representative strains which were inoculated in the compost were isolated in sterilized set as compared to unsterilized set. This was obvious as unsterilized substrate harbored other fungi like *A. fumigatus* and *M. pusillus* which reduced the extent of proliferation of representative strains in unsterilized treatments. Presence of these unwanted fungi later hampered the *A. bisporus* spawn run also. Several workers also suggested the role of these thermophilic fungi in making compost selective and nutritive for *A. bisporus* [3, 6, 10, 35, 41]. Less ammonia

Table 4. Physical and biological parameters of the composts prepared by different thermophilic fungi after 7 days of incubation (sterilized substrate)

Strains	Temp. C	pH	Wt. Loss%	Colour	Ammonia ppm	cfu/g(10^4)	Dominant Flora• (No of colonies)
				S .thermophilum			
S-1	41.2	7.8	47.65	+++	7.0	52.00	1(38.3)>7 (13.6)
S-2	41.9	7.6	47.62	+++	7.0	53.66	1(36.0)>7 (15.6)
S-3	39.2	7.8	45.65	++	6.5	46.00	1(27.6)>7 (15.0)
S-4	39.1	7.8	47.78	+++	6.0	37.76	1 (28.6)>5(7.3)
S-5	39.1	7.8	47.65	+++	6.0	38.16	1(25.3)>9 (11.6)
S-6	39.2	7.6	47.82	+++	6.0	30.46	1(21.0)>8 (6.6)
X-21	37.6	7.5	55.67	++++	5.5	65.00	1(55.6)>7 (8.3)
				H. insolens			
I-1	37.1	7.6	40.72	++++	5.5	60.33	2(52.6)>8 (7.0)
I-2	44.9	7.8	32.67	+++	6.0	40.66	2(32.0)>7(8.3)
I-3	43.5	7.8	33.19	+++	6.0	42.33	2(32.3)>9 (8.3)
I-4	44.3	7.8	33.92	++	6.5	32.00	2(23.3)>7 (6.6)
				Control*			
C-1	46.6	8.8	25.21	++	8.0	15.33	1(6.6)>12 (6.6)
C-2	43.8	7.6	43.62	++++	3.5	77.00	1(46.6)>2(30.3)
C-3	40.3	8.9	24.02	+++	8.0	45.00	1 (23.3)> 2(15.6)
C-4	20.5	8.7	9.30	+	12.5	10.00	6 (8.3)>10 (1.6)

*C-1 (uninoculated substrate incubated at 47±1C), C-2 (Substrate inoculated with X-21 and I-1 strains), C-3 (Substrate inoculated with pasteurized compost @ 20g/1.5Kg. compost) and C-4 (uninoculated substrate incubated at room temperature 25±1C)

*Dominant flora *1. S. thermophilum 2. H. insolens 5. C. thermophile 6. A. fumigatus, 7. S. thermophile 8, T. duponti 9. T. lanuginosus 10. T. viride 12. M. pusillus*

emission was observed in consortium and other inoculated treatments. C-3 treatment which was inoculated with pasteurized compost also performed well but it showed the presence of thermophilic fungi other then X-21 and I-1 strains which were not as effective as these two fungi and hence high ammonia levels were observed in this treatment coupled with slow and poor spawn run. Degradation of compost was measured in terms of NDF, ADF, hemicellulose, cellulose, carbon, nitrogen and ADL which defined the maturity of compost and are also known as compost maturity indices. It was found that best degradation in all of these parameters was found in C-2 and X-21 treatments followed by I-1treatment. More degradation of these components was found in sterilized set (Table 5).

Table 5. Physico-chemical analysis of compost prepared by different thermophilic fungi

Strains	NDF%	ADF%	Hc %	ADL%	Cell. %	C %	N %	C/N
				Scytalidium thermophilum				
S-1	56.25 (14.46)	50.18 (14.60)	6.07 (13.28)	20.15 (+9.39)	20.65 (12.68)	39.59 (2.60)	1.73 (1.70)	22.84 (1.08)
S-2	55.76 (15.20)	49.11 (16.42)	6.65 (5.00)	22.16 (+20.30)	20.54 (13.15)	38.45 (5.41)	1.72 (2.27)	22.35 (3.20)
S-3	54.87 (16.56)	48.11 (18.12)	6.76 (3.42)	20.67 (+12.21)	20.66 (12.64)	39.55 (2.70)	1.75 (0.56)	22.60 (2.12)
S-4	54.67 (16.86)	48.65 (17.20)	6.02 (14.00)	21.65 (+17.53)	19.65 (16.91)	37.86 (6.86)	1.75 (0.56)	21.63 (6.32
S-5	55.67 (15.34)	50.65 (13.80)	5.02 (28.28)	20.67 (+12.21)	19.78 (16.36)	38.66 (4.89)	1.76 (0.00)	21.96 (4.89
S-6	57.65 (12.33)	50.70 (13.71)	6.95 (0.71)	20.65 (+12.10)	19.65 (16.91)	38.57 (5.11)	1.72 (2.27)	22.42 (10.52)
X-21	48.60 (26.09)	43.84 (25.39)	4.76 (32.00)	18.21 (1.14)	17.62 (25.49)	36.78 (9.52)	1.78 (+1.13)	20.66 (10.52)
				Humicola insolens				
I-1	48.76 (25.85)	45.76 (22.12)	3.00 (57.14)	18.67 (+1.35)	20.12 (14.92)	37.65 (7.38)	1.78 (+1.13)	21.71 (5.97)
I-2	56.75 (13.70)	52.87 (10.02)	3.88 (44.57)	22.76 (+22.34)	20.65 (12.68)	38.67 (4.87)	1.73 (1.70)	21.77 (5.71)
I-3	54.76 (16.72)	51.65 (12.10)	3.11 (55.57)	21.78 (+18.24)	20.62 (12.81)	40.28 (0.91)	1.78 (+1.13)	22.62 (2.03)
I-4	57.32 (12.83)	52.65 (10.39)	4.67 (33.28)	23.76 (+28.99)	21.65 (8.45)	38.75 (4.67)	1.78 (+1.13)	22.89 (0.86)
				Control				
C-1	65.76	58.76	7.00	18.42	23.65	40.65	1.76	23.09
C-2	38.65(41.22)	36.52(37.84)	2.13(69.57)	20.76(+12.70)	17.76(24.90)	33.62(17.29)	1.78(+1.13)	18.88(18.23)
C-3	65.55(0.31)	57.65(1.88)	7.90(+12.85)	25.75(+39.73)	22.75(3.80)	38.65(4.92)	1.72(2.27)	22.47(2.68)
C-4	70.66(+7.45)	62.76(+6.80)	7.90(+12.85)	22.76(+23.56)	24.76(+4.69)	38.76(4.64)	1.57(10.79)	24.65(+6.75)

Figures in parenthesis are % increase or decrease as compared to control (C-1)

Sterilization break the polymer of cellulose, hemicelluloses and lignin compounds which were easily accessible to the thermophilic fungi making compost favorable and selective for the growth of *A. bisporus* which resulted in higher yield (Table 6). Thus, from the study it was concluded that mixed inoculum of X-21, and I-1 was best as compared to other treatments. These strains were therefore, further evaluated in the field on large scale for improving the composting process with particular reference to environment controlled composting.

Studies on improvements in *Agaricus bisporus* compost using thermophilic fungi

In India seasonal growers prepare their compost employing long method which gives low yields and also pollutes the atmosphere. Many environment controlled units still employ short method which gives satisfactory yields however, phase-I phase of such compost emits lots of malodorous gases and thus creates environment pollution. This method of composting has become unpopular with the commercial units mainly due to environment related issues and gradually is being replaced by almost environment friendly indoor composting process [42]. In this process compost is prepared totally under enclosed structures (bunkers+ tunnel) thus avoiding pollution to a greater extent. Further, compost by such method is prepared in 12 days time without much loss of the ingredients with higher productivity.

Main aim of the present studies was to improve the present day composting procedures (long, short and indoor methods) with the help of potent thermophilic fungi (I-1 & I-3 strains of *H. insolens* and X-21 strain of *S. thermophilum)* to reduce period of composting, obtained higher yields, more compost per unit weight of ingredients taken and environment friendliness. These two fungi and their strains were used as artificial inoculants in above three kinds of compost and findings there on are communicated.

Table 6. Spawn run and yield of unsterilized and sterilized set

	Unsterilized set				Sterilized set				
Treatments	**Days taken for spawn run**	**Condition of spawn run***	**Yield g/kg compost**	**Incidence of competitor fungi**	**Treatments**	**Days taken for spawn run**	**Condition of spawn run***	**Yield g/kg compost**	**Incidence of competitor fungi**
				Scytalidium thermophilum strains					
S-1	8.0	++	68.3	+	S-1	7.0	+++	100.0	-
S-2	8.0	++	74.3	+	S-2	7.0	+++	120.0	-
S-3	8.0	++	67.0	++	S-3	7.0	+++	95.0	-
S-4	8.0	++	40.0	++	S-4	7.0	+++	105.0	-
S-5	8.0	++	85.0	++	S-5	7.0	+++	125.0	-
S-6	8.0	+++	100.0	+	S-6	7.0	+++	130.0	-
X-21	7.0	++++	130.0	-	X-21	6.0	+++++	160.0	-
				Humicola insolens strains					
I-1	7.0	++++	105.0	+	I-1	6.0	+++++	155.0	-
I-2	7.0	+++	85.0	++	I-2	6.0	++++	125.0	-
I-3	8.0	++	45.0	++	I-3	8.0	++++	100.0	-
I-4	8.0	+	20.0	+++	I-4	8.0	+++	85.0	-
				Control sets					
C-1	10.0	+	90.0	+++	C-1	9.0	++	120.0	-
C-2	6.0	+++++	155.0	-	C-2	6.0	+++++	170.0	-
C-3	9.0	+++	105.0	+	C-3	8.0	++++	145.0	-
C-4	No spawn run	-	0.0	++++	C-4	10.0	++	70.0	++++
CD(p=0.05)			23.39						

*+ poor, ++ Average, +++ good, ++++ very good, +++++ excellent

Long method of composting (LMC)

Improvements in the yield and shortening the duration of LMC was attempted by inoculating potent strains of *S. thermophilium* (X-21), *H. insolens* (I-1 and I-3) and their consortium in compost at zero day. Data obtained indicated that composting period can be brought down from 28 to 20 days in inoculated treatments as compared to control. Significantly higher yield was obtained in compost inoculated with X-21 treatment (Table 7). Control treatment (uninoculated) harboured the heavy infestation of *Coprinius* sp. and *P. bysinna* resulting in lowest yield. Comparative results of each treatment were analyzed by measuring various physico-chemical factors such as temperature, pH, moisture %, C/N ratio, cellulose, hemicellulose, etc. at regular intervals of composting. Diversity of thermophilic mycoflora was also studied in each treatment at various stages of compost preparation. Heavy population of inoculated fungus/ fungi was isolated at spawning in respective treatments.

Table 7. Yield performance of *A. bisporus* in long method composts

Pile No.	Treatments	Ammonic conc. (ppm)	Days taken for spawn run	Condition of spawn run	Total compost produced	Days taken for Ist harvest	Yield kg/q compost	Competitor fungi
1	*H.insolens* (I-1)	8.0	13.0	++	550	35.0	11.92	-
2	*H.insolens* (I-3)	9.0	13.0	++	510	35.0	12.96	-
3	*S.thermophilum*(X-21)	7.0	12.0	+++	580	35.0	16.16	-
4	Consortium(I-1+I-3+X-21)	8.0	12.0	++	630	34.0	12.34	-
5	Control(uninoculated)	14.0	15.0	+	540	36.0	10.44	**
$CD_{0.05}$							5.43	

******Occurrence of *Coprinus* sp. and *Papulaspora bysinna* in control treatment during spawn run
+good, ++ very good, +++ excellent

Composting period was reduced to 20 days compared to present 28 days normally taken when LMC is employed. Though control set also supported fairly good spawn run but many a bags showed heavy incidence of *Coprinus* sp. an indicator mould suggestive of free ammonia in such composts resulting in the lowest yield in the experiment. Incidence of *P. bysinna* was also observed in few bags in this treatment. No *Coprinus* growth was observed in thermophiles inoculated piles suggestive of the fact that no free ammonia was available in such composts indicating that it was fully converted into microbial proteins by inoculated fungi where their population was maximum in such piles compared to control pile. Above finding suggest that presence of specific fungi in large number is responsible for final selectivity of the compost. The potency of X-21 and I-1 strains was therefore, well established in the field conditions as well evidenced with increased yield obtained using these two fungi. Role of *S. thermophilum* in compost production and nutrition of button mushroom has earlier been outlined by several workers under short/indoor methods of composting [10-11, 40, 42]. Straatsma [10] hypothesized that *S. thermophilum* increases the growth of *A. bisporus* by unknown mechanism. In the present investigation we also found early spawn run and highest yield in this treatment. This finding can go a long way to the seasonal growers who prepare compost by such method as they will be saving time, labour, energy and money by reducing the composting period. Growers will also get more selective compost with higher yields as compared to 28 days of composting.

Short method of composting

Here efforts were made to prepare compost by short method by artificially inoculating the compounding mixture with potent strains *(*X-21 & I-1) on day zero. Main aim of the study was to shorten the duration of composting and also to increase in yield in such compost. Here we kept the compost in the tunnel for five days only against the seven days normally kept as a standard protocol. We could complete the phase –II operation in five days particularly in inoculated composts as evidenced by low ammonia level (< 5.00 ppm) detected in such composts at spawning. On the contrary control treatment showed higher ammonia (9.00 ppm). This treatment though showed good spawn run but incidence of *Coprinus* sp. was observed in many bags. This treatment therefore offered lowest yield. Quick disappearance of ammonia from inoculated piles was attributed to the presence large population of S -7 and I-1 strains in respective treatments at spawning. Above finding suggest that these two strains can convert ammonia into microbial proteins at a rapid pace and can make the compost selective for the growth of *A. bisporus* in shortest possible time causing little environment pollution [17, 35]. Though phase–II period in inoculated treatments was reduced to five days but these treatments could not increased the yield significantly (Table 8).

Degradation of compost was also measured. More degradation of cellulose and hemicelluloses was observed in inoculated piles which was almost same in I-1 and X-21 treatments and also in consortium of these fungi. Least degradation was observed in control treatment. Higher degradation of these organic matter in the treated sets resulted in high quality compost

Table 8. Yield performance of *A. bisporus* on short method composts

Pile No.	Treatments	Colour of compost	Ammonia (ppm) at spawning	Days taken for spawan run	Condition of spawn run	Conversion ratio	Total compost produced	Total yield (kg/q compost)
1	*H.insolens* (I-1)	Dark brown	3.00	15	+++	2.87	420	15.00
2	*S.thermophilum* (X-21)	Dark brown	5.00	14	++++	2.99	430	15.50
3	Consortium (I-1+X-21)	Dark brown	5.00	15	+++	2.03	500	13.89
4	Control(uninoculated)	Brown	9.00	16	++	2.02	470	12.90
$CD_{0.05}$								3.46

++ good, +++ very good, ++++ excellent

with high yield as compared to control**.** Although increase in lignin content was exhibited in all inoculated and control treatments during spawning.

Indoor composting

Laws governing pollution are becoming stringent day by day in the country and many of the units producing compost by long and short methods are facing environmental issues, as such composting procedures pollute the atmosphere and create nuisances to the residents residing nearby. In a pursuit to lessen the environment pollution, Vijay [42] standardized a technology for express composting using a combination of INRA and Anglo Dutch methods. In this technology author completed phase-I in specially built phase-I tunnels (bunkers) where compost was fermented under aerobic conditions by providing artificial aeration through blowers. Temperature was manipulated inside the bunkers in such a fashion which was congenial for the growth of thermophilic fungi. Natural thermophilic population present in the compost was utilized in completing the process in as less in 12-14 days time. This technology is being adopted by several units across the country successfully, however, it could not mitigate environment pollution completely. We have successfully reported the use of X-21 and I-1 strains in LMC and SMC in shortening the duration and also increasing the yield. Therefore, it was thought prudent to utilize these two fungal strains in this technology also for its further refinement with an ultimate objective to lessen the environment pollution, period of composting, raw materials losses, ease in material handling and higher productivity. Series of experiments were conducted to refine this technology results obtained in brief are presented below.

Anglo Dutch method

The first experiment conducted in this series was on the improvement in the already standardized Anglo Dutch technique (cold process). Here we inoculated the compost with X-21 and I-1 strains and found that in this technique these inoculants were not much effective and marginally higher yield in treated compost was observed. Population dynamics of inoculated piles revealed the progressive increase of inoculated fungi from 3rd day to spawning (maximum population). Low count of other fungi was noticed (Table 9)

Table 9. Population dynamics of thermophilic fungi in inoculated compost at various intervals

	Population dynamics of thermophillic fungi on		
Organisms	**+3 day**	**+ 6 day**	**At spawning**
	cfu/g of compost		
H. insolens (I-1)	3.6	5.6	8.0
S. thermophilum (X-21)	8.3	7.30	11.6
S. thermophilum	3.3	4.6	6.0
A. fumigates	4.3	3.2	2.6
T. lanuginosus	0.0	0.66	0.0
H. fuscoatra	0.33	0./0	0.0

In control set four fungi were isolated and similar trend as reported above was observed here too, however, their population was low compared to inoculated treatments (Table 10). Further strains of *S. thermophilum* and *H. insolens* isolated were different than I-1 and X-21.

Table 10. Population dynamics of thermophilic fungi in uninoculated (control) compost

Organisms	Population dynamics of thermophillic fungi on		
	+3 day	+ 6 day	At spawning
	cfu/g of compost		
H. insolens	2.6	4.6	8.00
S. thermophilum	6.5	9.33	12.3
A. fumigates	7.3	5.6	0.0
H. fuscoatra	0.33	0.33	0.0

Both the composts showed almost similar trend with respect to cfu and different thermophilic fungi. This fact can be attributed to the conditions that here compost was fermented at 45-52 °C in the phase–I tunnel which was most congenial for the growth of thermophilic flora. Given these conditions test fungi present in the treated lots and ingredients habouring thermophilic fungi in control set grew rapidly in equal proportions and almost similar trend was observed in both sets. Marginal increase in yield in treated set can be attributed to the presence of X-21 & I-1 in inoculated compost which are known to increase the yield as evidenced in experiments conducted on LMC and SMC (not significant increase).

INRA method

Second experiment conducted was on improvement in the INRA technology [43]. Contrary to Anglo Dutch method, compost is fermented at 75-80 °C in bunkers in this technology. Since most of the thermophilic fungi are eliminated at higher temperature artificial inoculation of compost with thermophilic fungi becomes imperative. In the present investigation we inoculated the compost with the potent strains (X-21 & I-1) at beginning of phase-II. Population dynamics indicated almost complete dominance of inoculated fungi in treated set (Table 11) .

Table 11. Population dynamics of thermophilic fungi in inoculated compost

Organisms	cfu/g of compost		
	+3 day	+ 6 day	At spawning
	Inoculated		
H. insolens(I-1)	0.0	3.3	6.0
S. thermophilum (X-21)	0.0	4.6	15.3
S. thermophilum	0.66	1.3	0.33
A. fumigates	3.3	2.3	0.66
T. lanuginosus	1.0	0.66	0.0
	Control (ininoculated)		
S. thermophilum	1.33	0.66	6.66
A. fumigatus	2.3	2.6	1.3

Treated compost gave very high productivity compared to control (Table 12). Low yield in control set was the function of absence of test fungi and very low population of *S. thermophilum* in particular which is responsible for conditioning of compost and nutrition of button mushroom [35].

Table 12. Yield obtained with indoor compost (hot process)

Treatments	Moisture %	pH	N% at spawning	Days taken for spawn run	Condition of spawn run	Conversion ratio	Total yield (kg)	Yield kg/q. compost
Consortium	65.3	7.45	2.01	12	++++	2.90	237.8	16.40
Control	64.2	7.50	2.14	15	++	3.10	190.65	12.30

Total Indoor Compost Technology (TICT)

In both the experiments discussed above, composting process was completed in 12-13 days time and phase–I was completed in bunkers where slight environmental pollution was noticed. Main aim of the present studies was to prepare the compost in most environment friendly manner without any pollution so that a perfect composting technology is passed on to the commercial units which are presently reeling under the environment related issues and many are at the verge of closing. In the third experiment we completely bypassed the phase-I in bunkers and entire composting process was completed in phase-II tunnel itself except for the slight period of wetting and flipping in composting yard. Strains X-21 & I-1 were used as the inoculants (3 % on fresh weight of the straw). Entire composting period lasted for 10 days only.

Methodology followed

- 0 day: Wheat straw was properly wetted (75% moisture). Later all the ingredients were thoroughly mixed into it along with half of inoculum (1.5% on dry wt. basis). Heap was made thereafter
- +1 day: break opened the heap, water added if required, mixed the ingredients properly, again made a heap (temp around 58-60 ^{o}C)
- +2 day: Again turned the compost ingredients and made a heap (temp 62-65 ^{o}C)
- +3 day: -do- (temp around 70 ^{o}C)
- +4 day: Transferred the entire mass to the phase-II tunnel after adding rest of inoculum (1.5%) and equalized at 45-48^{o}C
- +5 day: Maintained the compost temp at 45-52 ^{o}C (PPC)
- +6 day: - do –
- +7day: Kill at 59 ^{o}C for 6 hours through self-heat generation of heat. later (POPC) at 48-52 ^{o}C
- +8 day: Post pasteurization conditioning (POPC) continue
- +9 day: POPC
- +10 day: POPC and cooled down at night
- +11day: Spawn

Minimum of 10 % fresh air during entire operation, even during kill was maintained.

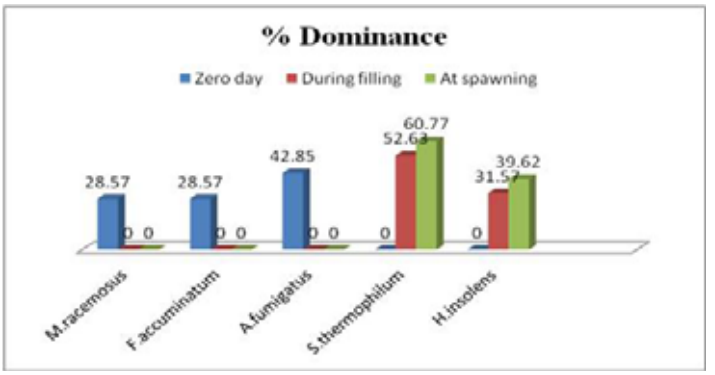

Figure 4. % dominance of thermophilic fungi

During filling most dominating fungus was *S. thermophilum* having dominance of 52.63 followed by *H.insolens* (31.57%). Not a single colony of mesophilic fungi isolated at zero day was noticed at filling. During spawning or after phase-II operation similar results were observed with high dominance of *S. thermophilum* followed by *H. insolens* (Fig. 4).

Satisfactory yields were obtained with such technology especially in the treated set (Table 13). We termed this technique as Total Indoor Compost Technology (TICT). Here degradation of ingredients by thermophiles leads to final selectivity of compost and making compost more nutritive for *A. bisporus*. Further in this technology degradation of organic compounds was more as compared to bunker + tunnel technology and less emission of ammonia was observed causing minimal pollution and more conversion of raw material to valuable compost. Duration of composting reduced from 14-16 days in bunker technology to 10-11 days with more output of compost and satisfactorily yields. Steam requirement during phase-II was nil. This technique has since been validated several times at DMR and also at selected All India Co ordinate Research Projects on Mushrooms and also at farmer's field giving satisfactory yields.

Table 13. Yield data in TICT compost

Trial Name	Treatment	Compost colour	Days taken for spawn	Condition of spawn run	Days taken for Ist harvest	Convertion ratio	Total compost produced (kg)	Total yield (kg)	Yield kg/ 100 kg compost
TICT	Consortium (I-1+X-21)	Dark brown	13	++++	31	2.96	1480	226.44	15.30
	Control	brown	15	+++	33	3.10	1550	204.60	13.20

Modified Indoor Composting Technology

The fourth and last experiment conducted in the series was on further improvement in the TICT. We termed this technique as Modified Total Indoor Composting Technology (MICT). Compost prepared above using various techniques (three experiments) was successful and inoculation of compost with X-21 and I-1 strains always gave superior product with higher yield. In the last experiment we have developed a total indoor composting technique (TICT), however, since in this technique substrate was fermented out doors for couple of days, slight pollution was observed. We wanted to avoid this even so that a technology is perfected with zero pollution. We further wanted to cut down the time of composting from 11-12 days to 8 days so that commercial farms may take more crop of button mushroom.

Further in the above technologies substrate was inoculated with test fungi as such (unsterilized) and hence, recovery of the inoculated fungi from the respective inoculated lots was only to the tune of 50-60 per cent and besides these other thermophilic fungi also came into play and they along with I-1 and X-21 strains also participated in the composting process. Role of test fungi (I-1 and X-21) in compost production could not therefore be fully justified. To nullify such effect wetted substrate in the present investigation was first sterilized/ pasteurized by live steam for 6-8 hours at 75 ^{o}C in the pasteurization tunnel so that colonies of other thermophilic fungi being harboured by substrate are almost killed. Then inoculations were done in such substrate justifying the full potential of X-21 and I-1 strains [29]. *In vitro* experiments reported in the present review also gave an indication that if sterilized substrate is inoculated with test fungi then they degrade it at faster pace due to softening of the straw and liberation of cellulosic components in the medium. Such treatment also gave quick spawn run and higher yields.

In this technique required conditioning and pasteurization temperature was achieved through self generation of heat due to rapid multiplication of test fungi in the inoculated piles as evidenced by their huge count in respective piles at spawning (Table 15) (tunnels at DMR usually require steam).

Table 14. Population dynamics of thermophilic microorganisms

Population Dynamics	Av. Colony count (X 10^4)	Dominant fungi
Mixing (Phase 0)	4.0	*A. fumigatus*
After sterilization (Phase I)	2.6	*S. thermophilum* (1.0), *A. fumigatus* (1.6)
During spawning (after Phase II) T-1 *H. insolens*(I-1)	12.33	*H. insolens*
T-2 *S. thermophilum*(X-21)	12.66	*S. thermophilum*
T-3 Consortium(I-1+X-21)	14.66	*S. thermophilum* (10.0), *H. insolens*,(4.66)
T-4 Pasteurized compost	13.33	*S. thermophilum* (9.0), *H. insolens* (3.33)
T-5 Control(uninoculated)	8.33	*S. thermophilum*

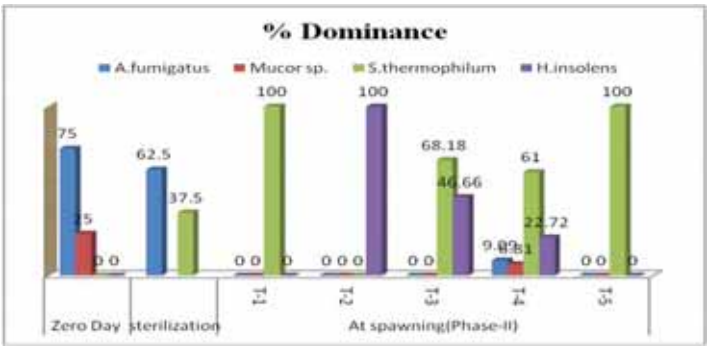

Fig. 5. % Dominance of thermophilic fungi

Population dynamics data showed that only inoculated fungus/fungi were isolated in the respective treatments which indicated that they only participated in the composting process (Table 14 and Fig.5). Thermophilic fungi particularly *S. thermophilum* and *H. insolens* are responsible of faster composting process [12]. Artificial inoculation of phase-I compost with *S. thermophilum* is reported to enhance the composting operation and further higher button mushroom yield has been reported by such treatment [44]

Similarily ability of *H. insolens* in faster composting process and early disappearance of ammonia from compost has been reported [45]. In the present investigation compost was free from ammonia inside the tunnel in as less in seven days time presumably with the combined effect of the thermophilic flora without being subjected to phase-I. Compost was ready in 6 days time as evidenced by sweet smell. Since the compost was prepared solely in phase-II tunnel under fully aerobic conditions, no environment pollution was observed in this technique. Only little of the ammonia generated which disbursed in the atmosphere making compost in most eco friendly manner which is the need of the hour.

Comparative results of each treatment were also analyzed by measuring various physio-chemical factors (Table 15). Highest reduction in C:N ratio, hemicellulose and cellulose were observed in consortium treatment which corroborated well with highest yield obtained in this treatment (Table 16). Least reduction of cellulose, hemicellulose and C:N ratio was observed in control which resulted in lowest yield.

Excellent compost was obtained after termination of the composting phase. There was no smell of ammonia in any of the treatments. It was brown to dark brown in colour with full growth of inoculated fungus/ fungi. Spawn run was completed in 12 days time in all the treatments including control and it was rated as excellent ++++. No barren patch of compost was observed in any of the 652 bags spawned for the trial and it was milky white in colour. Very heavy first flush was obtained in treated compost (around 10 % conversion). Highest yield of 19.96 kg mushrooms / 100 kg compost was achieved in consortium treatment followed by 19.06 kg in *H. insolens* treatment. Control yielded 16.10 kg mushrooms mainly due to poor second and third flush compared to other treatments (Table 16.).

Table 15. Physico-chemical characteristics of compost prepared with thermophilic fungi

Treatment	Moisture%	pH	C %	N %	C/N	Cell %	NDF%	ADF%	Hc.%	ADL%	Colour
Before filling (Phase- 0)	73	7.8	51.62	1.68	30.74	36.00	69.64	48.00	21.64	7.87	Pale Yellow
After sterilization (Phase- I)	65	7.8	47.71	1.64	29.28	28.00	67.00	50.00	17.00	8.50	Brown
During spawning (Phase- II) T-1 *H. insolens* (I-1)	60	7.3	37.12	1.72	21.56	22.66	50.33	47.33	2.78	20.15	Dark brown
T-2 *S. thermophilum* (X-21)	60	7.5	37.12	1.70	21.83	23.66	55.66	50.66	4.98	25.33	Dark brown
T-3 Consortium (I-1+X-21)	57	7.3	33.12	1.68	19.70	20.33	50.66	47.66	2.73	18.11	Dark brown
T-4 Pasteurized compost	60	7.3	38.86	1.90	20.44	23.33	55.66	51.66	6.66	26.66	Dark brown
T5 Control (uninoculated)	60	7.3	39.44	1.68	23.48	23.33	57.66	51.66	6.00	29.16	Brown

Table 16. Button mushroom yield in compost prepared with indoor compost method

S.No.	Treatment	Days taken for spawn	Condition of spawn run	Days taken for Ist harvest	Convention ratio	Total yield kg/ q compost
1	*H. insolens* (I-1)	12.0	++++	36.0	3.36	19.06
2	*S. thermophilum* (X-21)	12 .0	++++	36.0	2.84	18.60
3	Consortium (I-1+X-21)	12 .0	++++	36.0	3.01	19.96
4	Pasteurized compost	12 .0	++++	36.0	2.89	16.42
5	Control (uninoculated)	12 .0	++++	36.0	3.12	16.10
CD0.05						2.50

++++ excellent

New technique improved the consistency of compost quality, more compost per unit wt. of ingredients taken, higher yields with no or minimum environment pollution. Such technique will be a boon for those who are facing environment related issues and they will be able to produce productive compost in as less in eight days time with the help of thermophilic fungi short listed and reported in this communication. Cultures of these potent strains have been deposited at NBAIM, Mau (UP) for their preservation and also at DMR, Solan, India.

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