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Occurrence of thermophilic and thermotolerant mycoflora from *Agaricus bisporus* mushroom compost

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Thermophilic and thermotolerant fungi were isolated from *Agaricus bisporus* compost which was prepared by long method of composting (LMC) at each turning (1st to 4th turning) by dilution plate technique and Waksman direct inoculation method. A sum of 17 and 16 fungi were isolated by dilution plate technique and Waksman direct inoculation method respectively. The isolated fungi were *Absidia corymbifera, Aspergillus fumigatus, A. equatis, A. flavus, A. granulosus, A. nidulans, Aspergillus niger, A. terreus, A. sydowii, A. versicolor, Chaetomium sengalensis, Humicola insolens, H. fuscoatra, Mucor* sp., *Myceliophthora fergusii, Scytalidium thermophilum* and *Thermomyces lanuginosis.* Among isolated fungi *Scytalidium thermophilum, Chaetomium sengalensis* were predominated. Maximum mycoflora were detected at 10^{-3} concentration by serial dilution technique. Similarly, highest count of mycoflora was observed on Czapek Dox agar medium.

Key words: Compost, thermophilic fungi, thermotolerant fungi

INTRODUCTION

The white button mushroom (*Agaricus bisporus*) is cultivated on a composted mixture of straw bedded horse manure, wheat straw, chicken manure and gypsum. The compost is prepared by sequence of aerobic process which is brought about by fermentation of several microorganisms. Among the microorganisms, thermophilic fungi play a key role in preparation of compost. The effect of these fungi on the growth of mushroom mycelia and mushroom yield have been described. —First, they decrease the concentration of ammonia in the compost, which otherwise would counteract the growth of the mushroom mycelium. Second, they immobilize nutrients in a form which are available to the mushroom mycelia. These may have a growth promoting effect on the mushroom mycelia. The effectiveness of thermophilic fungi such as *S. thermophilum* in compost preparation for *A. bisporus* has been demonstrated by previous workers where a 2 fold increase was obtained in the yield of mushrooms on inoculated compost when compared to the pasteurized control. So the aim of the present investigation was to isolate the thermophilic fungi which was present in the compost and prepared by LMC.

MATERIAL AND METHODS

Compost preparation

The compost was prepared by long method using the following ingredients : wheat straw 10 quintals; wheat bran 25 kg; urea 18 kg; gypsum 25 kg. The methodology for compost preparation was followed as per procedure of Mantel *et al*. (1972)

Collection of samples

For the isolation of thermophilic fungi samples were drawn at different stages of composting i.e. 1^{st}, 2^{nd}, 3^{rd} and 4^{th} turning of compost. For collection of compost 10 g compost was drawn from various places likewise in zig-zag pattern and collected in a clean sterilized plastic bags, further they were mixed well by hands and a composite sample was made.

Isolation of thermophilic and thermotolerant fungi

From the collected samples thermophilic and thermotolerant fungi were isolated by 2 methods:(i)

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Dilution plate technique (Apinis, 1963) and (ii) Waksman direct inoculation method (Waksman *et al*, 1939).

From the composite sample 10g compost was aseptically transferred in 250 ml conical flask which had 90 ml distilled sterilized water. The flasks were subsequently shaken on a mechanical shaker for 15 to 20 minutes. Thereafter content was serially diluted as 10^{-3}, 10^{-4} , and 10^{-5}. One ml of each dilution was aseptically transferred on sterilized Petri plates with the help of sterile pipette which had 20g solidified Emerson's modified Yeast Starch Agar (YpSs) medium and incubated at 40±1!. The colonies were observed after 6 days of incubation. Three replications were maintained for each dilution. For this purpose 4 media i.e. Emerson's modified Yeast Starch Agar (YpSs), Czapek Dox Agar (CZA), Yeast Glucose Agar (YGA) and Potato Dextrose Agar (PDA) were taken and 20 ml each medium was poured in sterilized glass Petri dishes. After solidification of medium, a small quantity (0.1g) of compost sample was aseptically sprinkled over on solidify media in dishes. The inoculated plates were incubated at 40±1! and 3 replications were kept for each medium. The observations were recorded after 5 days of incubation.

Identification of thermophilic and thermotolerant fungi

Colonies were identified with the help of with the standard manual and key given by Salar (2018) and microphotographs were taken by Nikon make microscope at 10X, 40X and 100X.

RESULTS AND DISCUSSION

Occurrence of thermophilic and thermotolerant mycoflora during different turnings of composting At dilution 10^{-3}

Thermophilic and thermotolerant mycoflora during different turning schedule of white button mushroom compost preparation was under taken and data are presented in Table 1 and Fig.1.

From Table 1 it can be seen that total number of thermophilic and thermotolerant mycoflora colonies per plate (90.0 mm) greatly varied with respect to different turning schedule of compost preparation. In all, 17 mycoflora i.e. *Absidia corymbifera, Aspergillus fumigatus, A. equatis, A. flavus, A. granulosus, A. nidulans, Aspergillus niger, A. terreus, A. sydowii, A. versicolor, Chaetomium sengalensis, Humicola insolens, H. fuscoatra, Mucor* sp., *Myceliophthora fergusii, Scytalidium thermophilum, Thermomyces lanuginosis,* were isolated at different turning schedule of compost preparation of white button mushroom. Among the isolated mycoflora, maximum (31.67) colonies of *S. thermophilum* was observed per plate whereas *A. sydowii* and *A. flavus* showed minimum (0.33) colonies. At 3rd and 4th turning 29.33 and 26.99 colonies per plate recorded while it was comparatively less (11.66) recorded at 2nd turning and 1st turning (19.68).

A sum of 11 mycoflora belonging to 6 genera were found to be associated at 1st turning of compost. Of these, *S. thermophilum* had highest (5.67) number of colonies while *A. terreus* showed lowest (0.33) number of colonies. The number of colonies of other mycoflora were in between 0.67-2.67 of total colonies.

At 2nd turning, 12 fungi were isolated of 6 genera. Among then, *S. thermophilum* showed highest (3.33) colonies whereas lowest (0.33) were *A. niger, A. sydowii, A. equatis* and *A. flavus.* Colonies of other mycoflora were found to vary from 0.67-2.00.

On 3rd turning, 11 fungi were found associated with compost, of which *S. thermophilum* count was maximum (10.33) and next were *C. sengalensis, H. insolens* (4.67). However *Mucor* sp.*, A. nidulans, A. niger* showed least (0.33) number of colonies. At schedule of 4th turning, 9 mycoflora were detected which belonging to 7 genera . Among the detected mycoflora, highest (12.33) number of colonies was noticed with *S. thermophilum* and it was lowest (0.33) with *A. fumigatus, A. nidulans* and *A. equatis.* A new fungus *M. fergusii* was isolated and had 0.67 colonies per plate. Number of colonies of other mycoflora was in between 1.00-2.33.

At dilution 10^{-4}

Table 2 reveals that thermophilic and thermotolerant mycoflora colonies per plate (90.0 mm) varied greatly with respect to different turning schedule of compost preparation . At dilution 10^{-4} , 15 fungi i.e. *Aspergillus equatis, A. flavus, A. fumigatus, A. nidulans, A. niger, A. terreus, A.*

Table 1: Occurrence of thermophilic and thermotolerant mycoflora at (CFU×10^3/ml)

Mycoflora	TURNING I*	TURNING II*	TURNING III*	TURNING IV*	Total mycoflora
Absidia corymbifera	2.67	0.67	1.67	0.67	5.67
Aspergillus equatis	-	0.33	-	0.67	1.00
A. flavus	-	0.33	-	-	0.33
A. fumigatus	1.67	1.00	3.00	-	5.67
A. granulosus	0.67	1.00	-	-	1.67
A. niger	2.00	0.33	0.33	-	2.67
A. nidulans	2.33	1.00	0.33	-	3.67
A. sydowii	0.00	0.33	-	-	0.33
A. terreus	0.33	0.67	-	0.33	1.33
A. versicolor	0.67	-	0.67	-	1.33
C. senegalensis	1.00	2.00	6.33	6.33	15.67
H. insolens	1.00	-	4.67	3.33	9.00
H. fuscoatra	-	-	1.00	1.67	2.67
Mucor sp.	1.67	0.67	0.33	0.33	3.00
M. fergusii	-	-	0.67	-	0.67
S. thermophilum	5.67	3.33	10.33	12.33	31.67
T. lanuginosis	-	-	-	1.33	1.33
Total	19.68	11.66	29.33	26.99	

(*)-Average of three replication

Table 2: Occurrence of thermophilic and thermotolerant mycoflora at (CFU×10^4/ml)

Mycoflora	TURNING I*	TURNING II*	TURNING III*	TURNING IV*	Total mycoflora
Aspergillus equatis	-	0.33	-	0.33	0.67
A. flavus	1.00	-	-	-	1.00
A. fumigatus	1.33	-	-	0.33	1.67
A. niger	4.00	-	0.67	-	4.67
A. nidulans	2.33	-	-	0.33	2.67
A. sydowii	0.33	-	0.67	-	1.00
A. terreus	0.67	-	-	0.67	1.33
A. versicolor	-	-	-	1.33	1.33
C. sengalensis	1.00	2.33	2.33	2.33	8.00
H. insolens	1.00	1.67	1.33	2.00	6.00
H. fuscoatra	0.67	1.33	2.67	1.00	5.67
Mucor sp.	2.00	0.67	-	-	2.67
M. fergusii	-	-	-	1.33	1.33
S. thermophilum	4.00	3.00	2.00	5.33	14.33
T. lanuginosis	-	0.33	-	-	0.33
Total	18.33	9.66	9.67	14.98	

(*)-Average of three replications

versicolor, A. sydowii, C. sengalensis, H. insolens, H. fuscoatra, Mucor sp.*, M. fergusii, S. thermophilum, T. lanuginosis,* were isolated. Among the isolated mycoflora, *S. thermophilum* showed maximum (14.33) colonies whereas *T. lanuginosis* had minimum (0.33) colonies per plate. At different

Table 3: Occurrence of thermophilic and thermotolerant mycoflora at (CFU×10^5/ml)

Mycoflora	TURNING I*	TURNING II*	TURNING III*	TURNING IV*	Total mycoflora
Absidia corymbifera	-	-	-	0.33	0.33
Aspergillus equatis	-	-	0.33	0.67	1.00
A. flavus	-	-	1.00	-	1.00
A. fumigatus	-	-	-	1.67	1.67
A. niger	-	-	0.33	-	0.33
A. nidulans	0.33	0.33	1.00	-	1.67
A. sydowii	0.33	0.33	-	-	0.67
A. terreus	-	-	-	0.33	0.33
C. sengalensis	0.33	2.00	2.67	1.67	6.67
H. insolens	0.33	2.67	1.00	1.00	5.00
H. fuscoatra	-	2.67	1.33	3.00	7.00
Mucor sp.	-	-	0.33	-	0.33
M. fergusii	-	-	-	0.33	0.33
S. thermophilum	2.00	0.33	4.33	5.67	12.33
T. lanuginosis	-	0.33	0.00	1.33	1.67
Total	3.32	8.66	12.32	16.00	

(*)-Average of three replication

turning scheduled, maximum (18.33) colonies were recorded at 1st turning while least noticed obtained at 2nd and 3rd turning (9.66 and 9.67 respectively). At 4th turning 14.98 colonies were recorded per plate.

Among the isolated mycoflora, 11 mycoflora belonging to 6 genera were found associated at 1st turning. Of these, *S. thermophilum* and *A. niger* had maximum (4.00) colonies per plate while *A. sydowii* showed least (0.33) count. The colonies of other mycoflora were varied from 0.67-2.33.

At 2nd turning, only 7 fungi were found associated with compost of 6 genera. Among them, *S. thermophilum* showed highest (3.00) colonies whereas lowest (0.33) was obtained with *T. lanuginosis* and *A. equatis.* The total number of colonies of other mycoflora in between from 0.67-2.33. At 3rd turning, 6 fungi that belongs to 4 genera were detected from compost, of which *H. fuscoatra* gave maximum (2.67) colonies per plate. However *A. sydowii,* and *A. niger* yielded minimum (0.67) colonies per plate.

At 4th turning, 10 mycoflora were isolated which belonging to 5 genera and highest (5.33) colonies was noticed with *S. thermophilum* whereas *A. fumigatus* and *A. equatis* gave lowest (0.33) colonies per plate and a new fungi *M. fergusii* was also isolated. The number of colonies of other mycoflora were varied in between 0.67-2.33.

At dilution 10^{-5}

Data presented in Table 3 clearly indicates that total number of thermophilic and thermotolerant mycoflora colonies per plate (90.0 mm) varied greatly with respect to different turning schedule of composting preparation. In all, 15 fungi i.e. *A. corymbifera, A. equatis, A. flavus, A. fumigatus, A. niger, A. nidulans, A. sydowii, A. terreus, C. sengalensis, H. insolens, H. fuscoatra, Mucor* sp., *M. fergusii, S. thermophilum, T. lanuginosis,* were isolated at dilution 10^{-5} during different turning schedule of compost preparation of white button mushroom.

Among the isolated mycoflora, *S. thermophilum* had maximum (12.33) colonies whereas *A. corymbifera*, *M. fergusii, A. niger, A. terreus* and *Mucor* sp., showed minimum (0.33) colonies per plate.

Comparatively more (16.00) of thermophilic and thermotolerant fungi were found at 4th turning as compared to other turning schedule whereas it was at (8.66) recorded at schedule of 1st turning next were 2nd (8.26) and 3rd turning (12.30). At 1st turning 6 mycoflora belonging to 4 genera were found associated with compost. Of these, *S. thermophilum* had highest (2.00) colonies while *C. sengalensis, H. insolens, A. nidulans, A. sydowii* showed least (0.33) number of colonies per plate. On 2nd turning 7 fungi were isolated which belongs to 5 genera. Among them, *H. insolens* and *H. fuscoatra* had highest (2.67) colonies whereas *A. nidulans, A. sydowii, S. thermophilum, T. lanuginosis* and *C. sengalensis* showed (2.00) colonies.

On 3rd turning, 10 fungi belongs to 6 genera were detected from compost of which *S. thermophilum* gave maximum (4.33) of colonies and next were *C. sengalensis* (2.67). However, *Mucor* sp., *A. equatis, A. niger* showed least (0.33) number of colonies. At schedule of 4th turning,10 mycoflora were detected which belongs to 7 genera and highest (5.67) number of colonies was noticed with *S. thermophilum* while lowest (0.33) observed with *A. corymbifera, M. fergusii and A. terreus.* At this stage of turning other mycoflora was in between 3.00-1.67.

Table 4: Colonies of thermophilic and thermotolerant fungi at different dilution

Mycoflora	Number of colonies			
	10^{-3}/ml	10^{-4}/ml	10^{-5}/ml	Total
Absidia corymbifera	5.67	-	0.33	6.00
Aspergillus equatis	1.00	0.67	1.00	2.67
A. flavus	0.33	1.00	1.00	2.33
A. fumigatus	5.67	1.67	1.67	9.01
A. granulosus	1.67	-	-	1.67
A. niger	2.67	4.67	0.33	7.67
A. nidulans	3.67	2.67	1.67	8.01
A. sydowii	0.33	1.00	0.67	2.00
A. terreus	1.33	1.33	0.33	2.99
A. versicolor	1.33	1.33	-	2.66
C. sengalensis	15.67	8.00	6.67	30.34
H. insolens	9.00	6.00	5.00	20.00
H. fuscoatra	2.67	5.67	7.00	15.34
Mucor sp.	3.00	2.67	0.33	6.00
M. fergusii	0.67	1.33	0.33	2.33
S. thermophilum	31.67	14.33	12.33	58.33
T. lanuginosis	1.33	0.33	1.67	3.33
Total	87.68	52.67	40.33	180.68

From the Table 4 it was clear that the number of colonies per plate greatly varied with respect to different dilution. Among the different dilution, maximum (87.68) colonies were recorded at 10^{-3}/ ml and it were gradually decreases as increases the dilution at 10^{-4} and 10^{-5} per ml (52.67 and 40.33) per plate. In the isolated mycoflora, *S. thermophilium* showed highest (58.33) colonies whereas it was lowest (1.67) noted with *A. granulosus*. The colonies of other mycoflora were in between 2.33-30.34 per plate.

In the present study, a total number of 6 genera respectively *Chaetomium sengalensis, Humicola insolens, H. fuscoatra, Myceliophthora fergusii, Scytalidium thermophilum, Thermomyces lanuginosis* species were isolated from button mushroom compost at 1st ,2nd ,3rd and 4th turning.

The above results are in line with the results of other worhers (Pathak, 2014; Saha, 2003; Lyons,2000) and obtained thermophilic and thermotolerant fungi from beginning of composting at different stages of turning scheduled.

Occurrence of thermophilic and thermotolerant fungi detected by Waksman's direct inoculation method

An experiment was laid down to know the effect of different media on occurrence of thermophilic and thermotolerant fungi in compost prepared for white button mushroom cultivation. Four media were used i.e. Potato Dextrose Agar media (PDA), yeast glucose agar (YGA), Emerson's modified yeast starch agar media (YpSs), Czapek dox agar (CZA) media were taken for this purpose and data are depicted in Table 5.

Data present in Table 5 indicates that 16 fungi i.e. *Absidia corymbifera, A. equatis, Aspergillus fumigatus, A. granulosus, A. nidulans, A. sydowii, A. niger, A. terreus, A. versicolor, C. sengalensis, H. insolens, H. fuscoatra, Mucor* sp., *M. fergusii, S. thermophilum* and *T. lanuginosis,.* were detected on different media and *S. thermophilum* showed frequency found in *A. equatis* (1.69%). Yeast glucose media (YGA) plates were also been observed with maximum frequency noted on *C.*

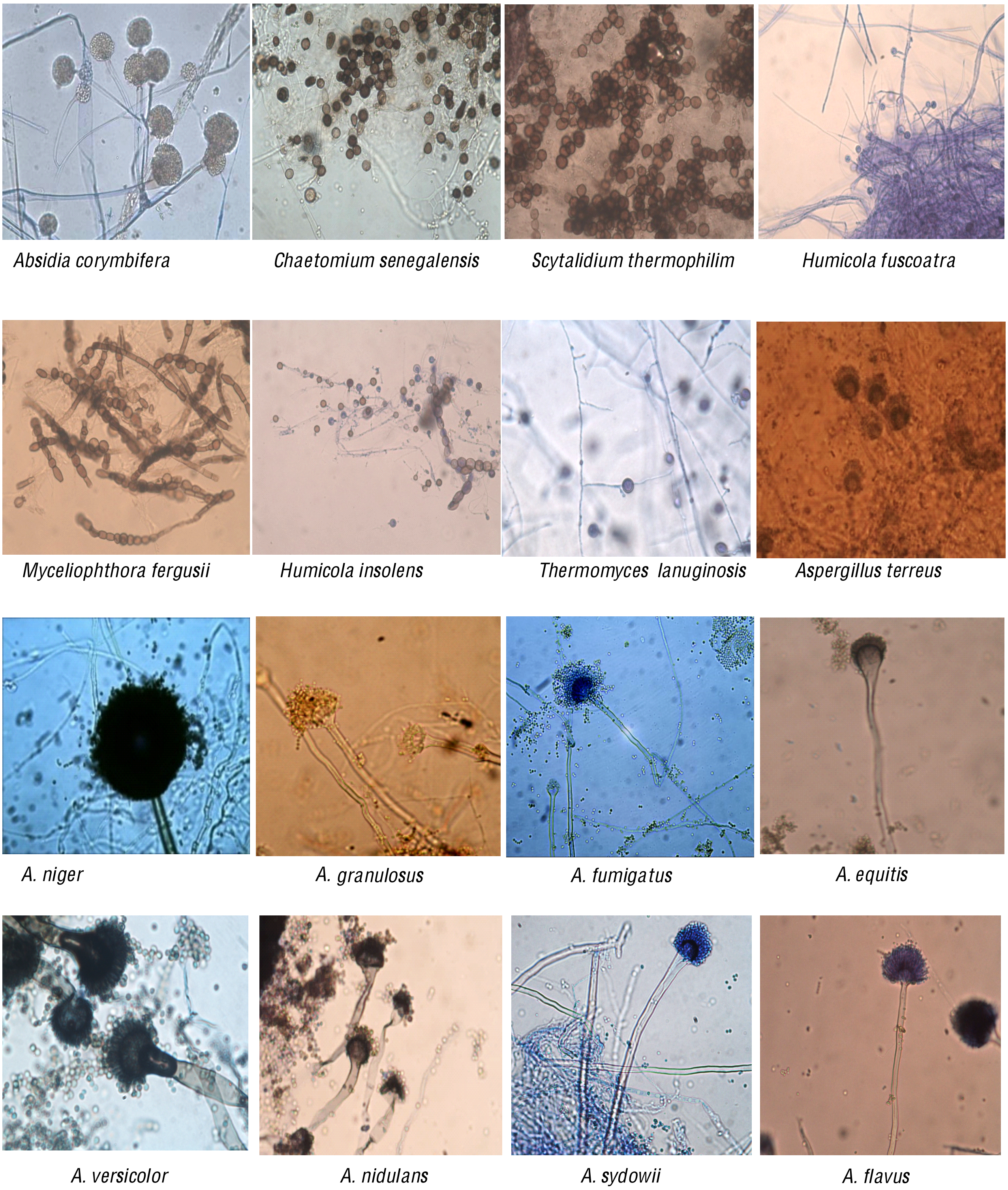

Fig. 1 : Isolated thermophilic and thermotolerant mycoflora

sengalensis (17.77%) and minimum frequency observed in *A. corymbifera, M. fergusii, Mucor* sp. In this method, highest frequency was noted on czapek dox Agar (99.97%) followed by Yeast glucose media (91.07%) then Emerson's modified yeast starch agar media (89.12%) and least frequency found in Potato dextrose agar (79.60%). Overall highest total mycoflora was observed in *S. thermophilum* (61.32%).

Table 5 . Occurrence of thermophilic fungi and thermotolerant fungi by Waksman's direct inoculation method on different media

	Frequency of microorganisms isolated (%)				
Mycoflora	YpSs	CZA	PDA	YGA	Total mycoflora
Absidia corymbifera	6.2	6.72	**25.42**	2.22	40.56
Aspergillus equatis	-	-	1.69	-	1.69
A. flavus	-	-	-	-	-
A. fumigatus	-	4.2	3.38	-	7.58
A. granulosus	3.87	4.2	8.47	8.88	25.42
A. niger	10.85	9.24	22.03	13.33	55.45
A. nidulans	0.77	-	6.77	-	7.54
A. sydowii	-	2.52	-	-	2.52
A. terreus	-	1.68	-	-	1.68
A. versicolor	-	-	3.38	-	3.38
Chaetomium sengalensis	9.3	7.56	-	**17.77**	34.63
Humicola insolens	18.6	14.28	5.08	11.11	49.07
H. fuscoatra	13.95	14.28	-	13.33	41.56
Mucor sp.	-	2.52	-	2.22	4.74
Myceliophthora fergusii	-	-	-	2.22	2.22
Scytalidium thermophilum	22.48	27.73	-	11.11	61.32
Thermomyces lanuginosis	3.1	5.04	3.38	8.88	20.4
TOTAL FREQUENCY (%)	89.12	99.97	79.6	91.07	359.76

(*)-Average of three replication;Emerson's modified Yeast Starch Agar-YpSs , Czapek Dox Agar- CZA , Potato dextrose agar-PDA,Yeast Glucose Agar- YGA

In present study Czapek agar medium gave highest colonies . CZA medium suitable for isolation of thermophilic and thermotolerant fungi. Sharma *et al.* (2015) also suggested CZA for isolation of thermophilic fungi.

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