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RESEARCH ARTICLE

Occurrence of thermophilic fungal communities and its growth rate on different media and temperatures from available natural substrates

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ABSTRACT
Thermophilic fungi are the chief components of the microflora that develops in heaped masses of plant materials, piles of agricultural and forestry products and other accumulation of organic matter. In this investigation, survey was conducted and collected different compost samples from eight localities of Osmanabad district. Among these localities, Naldurg and Dhoki sites showed highest number of organic substrates, while least in Itkal. Physicochemial properties *i.e.* nitrogen, phosphorus, potassium, total organic matter, total carbon and moisture were analysed from available composts and among them nitrogen was highest in molasses. Isolation of fungi was made from substrates and among five substrates, vermicompost and Farm Yard Manure (FYM) were recorded for highest species richness and % incidence while less in molasses. Among isolated thermophilic fungi, *Aspergillus niger*, *A. flavus*, *Rhizoctonia solani*, *A. fumigatus* and *Rhizopus* sp. found abundant in all the substrates and temperature ranges. Fungal species *A. niger*, *Mucor mucedo*, *A. flavus* and *Pythium* sp. Highest growth on all three media *viz.* Potato dextrose agar (PDA), Czapek's Dox Agar (CZA) and Martins Rose Bengal (MBR) were recorded. *A. niger* and *Rhizopus* sp. were found dominant in tested media and temperature. Generally, the fungi show very little growth at 65 °C. Every temperature gradient showed the growth incidence in all substrates but room temperature (RT) and 35 °C showed richness of fungal incidence. Even some species showed the growth on some substrates at higher temperate (65 °C) to some extent.

Introduction

The response of fungi to temperature varies between the two extremes of obligatorily thermophilic through thermotolerance to psychrophilic species. However, majority of known fungi are mesophiles developing in culture between 5 and 37 °C; the psychrophiles extend below that range of temperature (1). Thermophilic aerobic micro-organisms are physiologically very active and are capable of producing several thermostable enzymes responsible for decomposition of cellulose and to a great extent lignin into simpler compounds. According to its etymological meaning, composting denotes to a bio degradation process of a mixture of substrates carried out by a microbial community composed of various populations in aerobic conditions and in the solid state. It was described the concepts of compost i.e. organic matter is an important component of soil which includes of plant and animal residues that are made up of complex carbohydrates, starch, cellulose, hemicellulose, lignin, protein, fats, organic acids, oils resins etc. (2) Furthermore, compost has a high nutritional value with high concentrations of especially nitrogen, phosphorus and potassium, while the contamination by heavy metals and other toxic substances are very low (3). Agricultural waste products like- animal dung, manure and crop residues are potential sources of plant nutrients and for the complete decomposition of lignocellulosic wastes which involves cellulases, hemicellulases and lignolytic enzymes. Degradation of composts was carried out by huge mixture of bacteria, fungi, insects, worms and other organisms that eat materials and recycle them into new forms (4). Sustainable agricultural practice was found through organic

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composts which can fulfil the crop productivity and human needs along with conservation of available resources without harming the environment (5). Nutrient and organic matter contents of composts have been studied extensively by previous workers (6-8). Similar results were also reported and isolated fungi from different thermogenic places (9). Therefore, the present work has been made to occurrence of thermophilic fungal communities and its growth rate on different media and temperatures from available natural substrates.

Materials and Methods

Substrates collections sites

Study site (Osmanabad district) comes under Marathwada regions of Maharashtra state of India. Five different organic substrates viz. vermicompost, poultry manure, farm yard manure (FYM), cow dung and molasses were collected. Samples were collected from eight different localities *i.e.* Naldurg, Itkal, Murum, Lohara, Dhoki, VashiParanda and Waghdari.

Analyses of physico chemical parameters

Different composts were collected from study area and analysed physicochemical characterization. Composts were spread out on a tray for air drying. It was sieved over a 150 mm sieve and used for characterization. Each sample was weighed using digital balance. The samples were oven-dried at a temperature of 110 °C for 24 hrs and reweighed. Moisture and Nitrogen content was determined by the Kjeldahl method (10, 11). The carbon content of compost was determined by titration method as described (12). Phosphorus was determined by Olsens method by using spectrophotometer (13-15). Water soluble and exchangeable potassium was calculated by Ammonium acetate method (16) using Flame photometer. Samples of each heap of compost collected at 5-7 cm depth level and moisture content was determined.

Isolation and identification of thermophilic fungi

Dilution plate technique

The isolation of thermophilic fungi from different substrates was carried out using dilution plate technique (17, 18). Ten gms of sample were transferred to a flask containing 100 ml sterile water. The contents were centrifuged with centrifuge machine for 15 min and then diluted 10^{-3} of 0.5 ml was transferred to sterile petri plates containing different media in triplicates. The pH of medium was adjusted to 6.5 with 0.1N HCl or 0.1N NaOH. Petri plates were incubated in an inverted position at room temperature (RT) and adjusted the temperature in hot air oven at 35 to 65 °C. Pure cultures of isolates were maintained on respective media slants at 4 °C for further study. The percentage of incidence was calculated by employing the following formula (19).

$$\% \text{ Incidence} = \frac{\text{No of colonies of species in all plates}}{\text{Total no of colony of the all the species in all plates}} \times 100$$

Different types of media viz. Potato dextrose agar (PDA) Czapek's Dox Agar (CZA) and Martins Rose Bengal (MBR) agar media were used for collection and isolation of thermophilic fungi. The different topographical characters of the colonies were recorded at regular time intervals. The semi-permanent slides of the isolated fungi were prepared using 1 % cotton blue and lactophenol. Identification of thermophilic fungi was made by referring relevant literature and monographs (20-23). Key to the identification of thermophilic fungi was used according to a published work (24).

Results

Sample collection

Organic substrates were collected from different places of Osmanabad district. Study sites shows different types of compost *viz*. vermicompost, poultry manure, molasses, farm yard manure (FYM), cow dung *etc*. These samples were collected from different eight localities *i.e*. Naldurg (17.82˚N and 76.30˚E), Itkal (17.76˚N and 76.14˚E), Murum (17.96˚N and 76.47˚E), Lohara (17.98˚N and 76.32˚E), Dhoki (18.37˚N and 76.12˚E), Vashi (18.54˚N and 75.78˚E) and Paranda (18.46˚N and 75.65˚E) and Wagdari (17.45˚N and 76.15˚E). Among these localities, Naldurg and Dhoki site showed highest number of organic substrates while least in Itkal (Table 1).

Table 1. Collection of different types of organic substrate (composts) from different study sites.

Sl No.	Place	NL	EL	Organic Substrates				
				PM	VC	FYM	CD	M
1	Naldurg	17.82°	76.30°	--	Yes	-	Yes	Yes
2	Itkal	17.76°	76.14°	Yes	--	--	Yes	--
3	Murum	17.96°	76.47°	--	--	---	Yes	Yes
4	Lohara	17.98°	76.32°	--	--	--	Yes	Yes
5	Dhoki	18.37°	76.12°	--	Yes	--	Yes	Yes
6	Vashi	18.54°	75.78°	--	--	Yes	Yes	--
7	Paranda	18.46°	75.65°	--	Yes	Yes	Yes	--
8	Wagdari	17.45°	76.15°	--	Yes	Yes	Yes	--

NL-North Latitude, EL-East Longitude, VC- Vermicompost, PM-Poultry manure, FYM- Farm yard manure, CD-Cow dung

Physicochemical parameters of substrates

Physicochemical parameters were tested from 5 different sources of available organic substrates such as poultry manure, vermicompost, FYM, cow dung and molasses. Organic fertilizers contain relatively low concentrations of the actual plant nutrients and are not immediately available for plant utilization. Hence, the fortification of organic wastes and their composts as a source of organic nutrients are imperative for sustainable agriculture. Nitrogen content was found highest in molasses (1.12%) than other compost. The lowest value of nitrogen (0.78%) occurs in FYM. In poultry manure, phosphorus and potassium were found very less as compared to other compost while more in VC. Total carbon (%) range is found in between 25.50 to 35.88% in tested compost. Moisture (%) content was found more in cow dung, FYM and molasses while least in poultry manure and VC. Total organic matter (OM) was ranged from 44 to

Table 2. Physico-chemical parameters of different organic compost.

Sl. No.	Parameters	Poultry Manure	Vermicompost	FYM	Cow dung	Molasses
1	N (%)	0.84±04	0.90±0.21	0.78±0.12	0.84±0.22	1.12±0.12
2	P (%)	0.07±0.1	0.05±0.01	0.02±0.01	0.16±0.03	0.13±0.01
3	K (%)	0.01±0.001	3.77±0.97	1.42±0.33	4.01±1.01	1.28±0.19
4	Total Carbon (%)	35.09±2.33	27.15±4.45	25.50±6.21	35.88±2.66	34.23±6.33
5	Total OM (%)	60.50±3.11	46.85±5.56	44.00±4.11	61.87±2.22	59.05±9.11
6	Moisture (%)	8.70±2.31	6.95±2.11	28.39±5.23	50.99±7.01	58.97±7.11

Each value is the mean of three replicates, Standard error ±

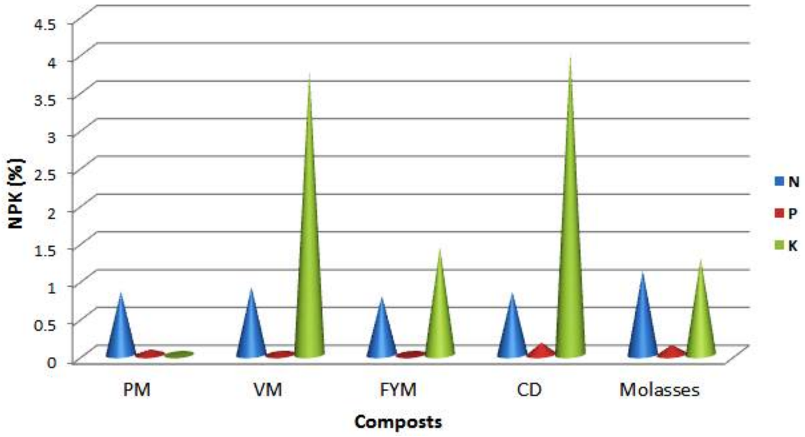

Fig. 1. Percentage of NPK in available composts.
PM-Poultary manure, **VM**-Vermicompost, **FYM**-Farm yard manure, **CD**-Cow dung

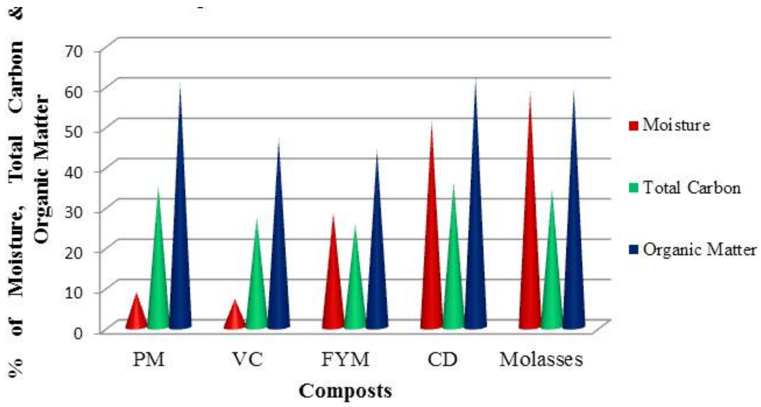

Fig. 2. Percentage of moisture, total carbon & organic matter in available composts.
PM-Poultary manure, **VC**-Vermicompost, **FYM**-Farm yard manure, **CD**-Cow dung.

60.50% in all composts, while the lowest value was found in FYM, while highest in poultry manure (Table 2; Fig. 1 & 2).

Incidence of thermophilic fungal growth on different temperatures and media

Isolated fungi are grown at different temperatures *i.e.* RT, 35, 45, 55 & 65 °C on the different media. Growth was observed on all temperature gradients and media, except 65 °C (Table 3). Generally; the fungi show very little growth at 65 °C. The optimum growth of the fungi was observed between RT and 35 °C. Twenty five fungal species representing 19 genera were isolated from organic substrates (Table 4). In case of PDA medium, the highest incidence was found at RT and 35 °C. *Aspergillus niger* and *Rhizoctonia* sp. (79.3 and 70.6%) respectively followed by *Humicola insolens* while lowest incidence occurred in *Mucor mucedo, Myceliopthora thermophila* and *Cladospora thermophila* at 65 °C. Martins Rose Bengal (MBR) medium was respond highest incidence of *Mucormiehei* (65.3%) at RT while *A. niger* and *C. thermophila* were found lowest incidence. In case of Czapak Dox Agar (CZA) medium, *Aspergillus flavus* (72.6%) at 35 °C and *A. niger* (69.5%) at RT was found highest incidence while least in *A. niger* at 65 °C.

Growth rate of thermophilic fungi at higher temperature

The impacts of temperature on growths rely upon numerous components, including the family, species and strain of the organism, the measure of accessible water, sorts of supplements and numerous other natural elements. Obviously, temperature was likewise a significant factor. In our examination on temperature connections for the parasites has been done at three unique temperatures *viz*. 45, 55 and 65 °C. It was seen that at 45 °C development rate of *Af, Afl, Hi, R, Mt, Pc, Ct* and *C. thermophilium* was maximum while at same temperature development rate of *Mm, P Pb, Aa, Fo, G* was minimum. At the equivalent, 45 °C development rate of *Hl, Pd* and *Tv* was found medium subsequently there was no growth rate on *Ar, At, Mmi, Rs, Th* and *C*. At 55 °C development rate of *Af, Hl* and *Pc* was minimum while *Afl, Hi, R, Mt, Ct* and *C. thermophilium* was

Table 3. Thermophilic fungal diversity and growth rate at higher temperature

Temp (^{0}C) → Fungi ↓	Higher Temperature (^{0}C)					
	Growth Rate	45	Growth Rate	55	Growth Rate	65
Aspergillus fumigatus	Maximum	+++	Minimum	+	No	-
Aspergillus niger	Maximum	+++	Medium	++	No	-
Aspergillus flavus	Maximum	+++	No	-	Minimum	+
Aspergillus rapens	No	-	No	-	No	-
Aspergillus tamari	No	-	No	-	No	-
Mucor mucedo	Minimum	+	No	-	No	-
Mucor miehei	No	-	No	-	Minimum	+
Humicola insolens	Maximum	+++	Medium	++	No	-
Humicola lanuginosa	Medium	++	Minimum	+	No	-
Pythium sp.	Minimum	+	No	-	No	-
Rhizotonia solani	No	-	No	-	No	-
Rhizoctonia sp.	Maximum	+++	Medium	++	Minimum	-
Myceliophthora thermophila	Maximum	+++	Medium	++	No	+
Penicillium cinnmoni	Maximum	+++	Minimum	+	Minimum	-
Penicillium duponti	Medium	++	No	-	No	-
Penicillium befeldianum	Minimum	+	No	-	No	-
Alternaria alternata	Minimum	+	No	-	No	-
Fusarium oxysporun	Minimum	+	No	-	No	-
Trichoderma viride	Medium	++	No	-	No	-
Trichoderma harzianum	No	-	No	-	No	-
Cladospora thermophila	Maximum	+++	Medium	++	Minimum	+
Cladosporum sp.	No	-	No	-	No	-
Geotrichum candidum	Minimum	+	No	-	No	-
Chaetomium thermophilum	Maximum	+++	Medium	++	Minimum	+
Torula sp.	Minimum	+	No	-	No	-

medium. It was seen that at 55 °C there was no development rate of *An, Ar, At, Mm, Mmi, P, Rs, Pd, Pb, Aa, Fo, Tv, Tk, C* and *G*. At 65 °C development rate of *Afl, Mmi, R, Pc, Ct* and *C. thermophilium* was least. Furthermore, at a similar temperature there was no development rate of *Af, An, Ar, At, Mm, Hi, Hl, Rs, Mt, Pd, Pb, Aa, Fo, Tv, Th, C* and *G* (Table 3).

Discussion

The present investigation supports earlier findings, as compare to soil compost has relatively low nitrogen content between 0.5 to 2.0 %, that is slowly mineralized in soil (25, 26). It was reported that the vermicompost had lower pH, total organic carbon (TOC), organic matter (OM) and carbon/nitrogen ratio (C/N ratio) but higher electrical conductivity (EC), nitrogen, phosphorous and potassium (NPK) content than the raw substrate and the heavy metal content in vermicompost was higher than that of raw substrates (27). Nitrogen content is found highest in molasses than other compost; these results are in agreement with those obtained and found that the total nitrogen rate ranged from 0.99 to 2.01% (28). In poultry manure, phosphorus and potassium are found very less as compared to other compost, these results are in agreement with the results obtained and found that the C/N ratio ranged from 15:1 to 20:1 and it was ideal for ready-to-use compost (29). Moisture (%) content was found more in cow dung; these results are in agreement with the optimum value of total organic matter higher than 10% (30).

It was reported that, bagasse as a source of thermophilic fungi (31) and the baled or heaped bagasse made development of thermophilic microorganisms (32). The occurrence of *Humicola lanaginosus* in the manure was also reported (33). It was reported the occurrence of *Cheatomium thermophile varcoprophile* on goat dung (34). Recently, it was isolated 22 thermophilic fungi belonging to 8 genera on different substrates from different region of Dharwad (Karnataka State) (35). The thermophile *T. lanuginosus* was one of the most common fungi occurring in soil (36). Fungal consortium of *Aspergillus* and *Humicola* sp., and actinomycetes, especially Streptomyces had been earlier used for the conversion of nutrient rich compost (37). It was reported that microorganisms isolated and characterized from the above composts include the species of fungi *viz*., *Aspergillus, Trichoderma, Mucor, Penicillium, Alternaria, Cladosporium, Monilia, Helminthosporium, Coccidioides, Scedosporium,* actinomycete *viz*., *Nocardia* and bacteria *viz*., *Bacillus, Lactobacilli, Micrococcus, Pseudomonas, Clostridium*. Of these isolates, members of the genus *Aspergillus* were most prevalent (38%) followed by *Bacillus* comprising of 20% of the total microbial isolates (38). It was reported that there were seven different types of thermophilic fungi from forest leaf litters and tested on YpSs Emerson agar medium and analyzed the radial growth on variable temperatures (30-55 °C) (39). It was reported that the isolation of 20 species belonging to 7 genera on different substrates collected from different places of Osmanabad district

Table 4. Percentage of Incidence of thermophilic fungi from different culture media and temperatures.

Temp (°C) → Fungi ↓	CULTURE MEDIA														
	PDA					MBR					CZA				
	RT	35	45	55	65	RT	35	45	55	65	RT	35	45	55	65
Af	57.5	49.2	40.3	29.6	0	48.9	50.2	0	0	0	31.9	19.0	16.4	0	0
An	79.3	39.9	21.1	0	0	59.3	45.2	28.5	17.2	13.4	69.5	50.3	31.2	17.2	10.9
Afl	62.1	64.5	39.5	24.5	12.5	35.8	29.3	16.7	0	0	65.9	72.6	51.7	17.2	0
Ar	31.6	14.9	0	0	0	42.1	34.8	15.6	13.0	0	24.6	29.5	0	0	0
At	0	0	0	0	0	21.6	15.3	0	0	0	0	0	0	0	0
Mm	16.5	12.0	0	0	0	49.5	53.8	37.5	16.5	0	34.2	36.5	15.9	0	0
Mmi	29.3	14.2	0	0	11.5	65.3	45.5	0	0	0	0	0	0	0	0
Hi	68.9	65.5	46.8	19.8	0	0	42.8	19.2	0	0	0	0	0	0	0
Hl	36.4	30.2	18.5	15.2	0	0	24.2	20.1	0	0	0	0	0	0	0
P	0	50.6	45.5	0	0	0	24.6	21.5	14.1	0	21.4	15.6	14.5	13.5	0
Rs	0	0	0	0	0	14.5	0	0	0	0	21.6	0	0	0	0
Rm	69.5	70.6	48.6	19.5	0	0	0	0	0	0	0	0	0	0	0
Mt	52.3	50.3	35.7	24.5	14.4	51.3	48.7	24.6	16.4	0	0	0	0	0	0
Pc	34.8	30.8	0	20.5	0	17.3	23.7	0	0	0	35.1	24.8	14.7	0	0
Pd	42.6	21.5	11.1	0	0	0	15.6	0	0	0	42.8	35.9	19.2	14.0	0
Pb	0	0	0	0	0	25.8	12.5	0	0	0	42.7	0	21.9	17.1	0
Aa	0	0	0	0	0	32.0	24.8	19.5	0	0	0	0	0	0	0
Fo	0	0	0	0	0	32.1	30.2	16.5	0	0	0	-0	0	0	0
Tv	29.2	25.9	19.5	0	0	32.4	30.5	49.5	25.6	0	15.6	0	0	18.2	0
Th	18.2	0	12.8	0	0	0	43.5	19.2	0	0	0	0	0	0	0
Ct	56.9	63.9	48.7	40.7	14.4	40.1	35.2	28.2	15.0	11.9	0	0	0	0	0
C	65.2	50.4	0	0	0	15.9	16.5	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0	0	0	31.5	24.8	0	0	0
Cha	35.4	25.4	23.9	19.4	0	46.5	39.4	21.0	17.01	0	24.6	16.4	0	0	0
T	33.2	24.11	0	0	0	20.11	16.11	12.11	0	0	14.11	9.22	0	0	0

Legands: ***RT***-Room temperature, ***Af***-*Aspergillus fumigatus*, ***An***-*Aspergillus niger*, ***Afl***-*Aspergillus flavus*, ***Ar***-*Aspergillus rapens*, ***At***- *Aspergillus tamari*, ***Mm***-*Mucor mucedo*, ***Mmi***-*Mucor miehei*, ***Hi***-*Humicola insolens*, ***Hl***- *Humicola lanuginosa*, ***P***-*Pythium sp*, , ***Rs***-*Rhizotonia solani*, ***R***-*Rhizoctonia sp*, ***Mt***- *Myceliophthora thermophila*, ***Ct***-*Cladospora thermophila*, ***Pc***-*Penicillium cinnmoni*, ***Pd***- *Penicillium duponti*, ***Pb***-*Penicillium befeldianum*, ***Aa***-*Alternaria alternata*, ***Fo***-*Fusarium oxysporun*, ***Tv***-*Trichoderma viride*, ***Th***-*Trihoderma harzianum*, ***C***-*Cladosporum sp*, ***G***-*Geotrichum candidum*, ***Cha***-*Chaetomium* ***Rm*** *Rhizopus microcarps*,***T***-*Torula* sp.,**PDA**-Potato Dextrose Agar, **CZA**- Czapek's Dox Agar and **MBR**- Martins Rose Bengal.

and *Aspergillus fumigatus* was present nearly in all of the sources (40) *Aspergillus* sp., was associated with different composts has been reported (41, 42). Study was conducted on municipal waste with microbial colonies like bacteria, fungi and actinomycetes in large numbers and temperature plays an important role in their growth during composting (43).

Conclusion

From the present investigation, it is concluded that the thermophiles prefer to colonize organic substrates. However, colonization of these fungi varied with different substrates and temperature level. In all 25 species representing 19 genera were isolated. Thermo tolerant fungi of *Aspergillus niger* and *A. flavus* were constantly associated and found common. A positive correlation was observed between percentage of incidence and temperature gradient level.

Authors' contribution

All the authors contributed equally to the work presented in this paper.

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Competing Interests

The authors declared that they have no conflict of interest.

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