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Original Research Article

Studies on Prevalence of Thermophilic Coprophilous Fungi Isolated from the Dung Samples of Herbivores in Telangana Region

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Abstract: The association concerning thermophilic fungi and herbivore droppings provides an opportunity to study thermophilic coprophilous fungal communities in the microbial environment. Herbivore dung has long been utilized as a model for studying the progression of saprotrophic fungi during decay or disintegration. Naturally, the fungal community associated with herbivore droppings particularly depends on the location, environment especially temperature and rainfall, and also the herbivore diet. In the present investigation, an attempt has been made to study the prevalence of the thermophilic fungi on herbivore droppings at optimum temperature under laboratory conditions. They were set to grow on yeast extract starch agar composition, isolated and identified based on their morphological and physiological characterization followed by purification. An overall 30 species of thermophilic coprophilous fungi representing 17 genera were identified and among them 23 species were found thermophilic and 7 species were thermo tolerant. Furthermore, their percentage of incidence, abundance, and frequency were evaluated by applying standard formulae. Our significant study on prevalence of thermophilic coprophilous fungi from herbivore fresh dung was the first report from Telangana. Telangana state is consisting lot of forest region with varied environmental conditions and as well as consisting more number of animals. Herbivores while grazing on vegetation from surroundings ingest various microbes along with their food including thermophilic fungi. Some spores of the fungi survive through entire gut environment of the animal and released outside along with the animal excreta and germinates at favorable conditions and involves in recycling organic matter by their enzymes activity and contribute in the fertilization of soil surfaces in the environmental biodiversity.

Key words: Dung sample, fungal community, herbivore droppings, Thermophilic coprophilous fungus

Introduction

The thermophilic fungi have a minimum growth temperature at or above 20°C and the maximum extending up to 60-62°C (Maheshwari *et al.,* 2000). The fungi that grow at an optimum temperature between 25-30°C are called mesophiles (Cochrane 1958) and those that can grow at temperatures above 50°C are called either thermophiles or thermotolerant. Johri & Ahmad (1991) and Mouchacca (2000) discussed the importance of these fungi in biotechnology. The variety of fungi that have been recorded in the dung of Cow, Rabbit, Horse and other animals has attracted the attention of several investigators (Bell 1983; Richardson 2001; Ranjith Kumar *et al.*, 2010; Sreelatha *et al.*, 2013). The coprophilous fungi are morphologically and physiologically specialized distinct community and play a vital role in the decomposition and recycling of organic wastes (Richardson, 2008 and Ajmera *et al*., 2020). These are a subgroup of saprophytic fungi that can inhabit in feces or dung most commonly of herbivores (Webster, 1995). Coprophilous fungi have interesting life cycles and morphology. These fungi are found to be vital source for a variety of primary and secondary metabolites of economic importance to the pharmaceutical and agricultural practices (Hein *et al.,* 1998). Interestingly, antagonistic interactions among coprophilous fungi often involve the production of chemical agents by one species that inhibit the growth of another (*Gloer & Truckerbrod, 1988*). Various investigators studied the taxonomy and ecological importance of mesophilic coprophilous fungi. However, much more detailed studies are needed to establish thermophilic coprophilous fungi role in nature. Thermophilic coprophilous fungi are those which can tolerate high temperatures and can also grow on dung (Shanthipriya *et al.,* 2015). These fungi develop thick-walled, pigmented spores that enter the animals while grazing on vegetation, germinate while passing through the gut of an animal breaking their dormancy and excreted in the dung (Bell, 1983; Bell, 2005). In addition to being resistant to the digestive environment of high temperature and pH, the fungi can also enhance the digestion of cellulose components while passing through the gut of animals which is an added advantage (Richardson, 2001; Salar *et al.,* 2007). These fungi spend their entire life on droppings of herbivorous animals. Liberation of these spores will facilitate their reaching distant places and other animals during grazing. Once the dung is voided fungal spores spread on to the surrounding herbage. These fungi form a distinct ecological group and have a selective advantage.

Telangana region being dry with less humidity due to its varied extreme climate such as an average temperature of 25°C (reaching to 48°C) through the major part of the year and less rainfall probably form the ideal for promoting thermophiles. Our previous investigations revealed that the thermophilic fungal propagates are common in compost soil in this region, and appear to increase in abundance with the advance of spring and summer rainfall. In the present investigation, an intensive survey was made to isolate and study the prevalence of the thermophilic coprophilous fungi from the fresh dung samples of different herbivore animals from various places of Telangana region.

Materials and methods

Yeast extract, agar-agar, starch, and all other chemicals were purchased from HiMedia Laboratories Pvt Ltd., Mumbai, India. Fresh dung samples of herbivores such as Cow, Ox, Buffalo, Sheep, Wild sheep, Elephant, Bear and litter of birds including Pigeon, Poultry, etc., were collected from various places in Telangana region and brought to the laboratory in a polythene bag and kept in incubator at 45°C about 12 hrs to activate the growth of the thermophiles present in fresh dung.

Isolation of thermophilic coprophilous fungi

At least 50 samples of each type were analyzed for the presence of these fungi. Ten grams of each sample were taken in to 250 ml Erlenmeyer conical flask containing 100 ml of sterilized distilled water and shaken thoroughly for half an hour. From the supernatant, serial dilutions were made as desired. 500 µl of diluted samples were poured into individual sterilized unpaired Petri dishes aseptically using a sterilized pipette. Sterilized and cooled Yeast extract starch (YES) medium (Yeast extract 5.0 g, Starch 15 g, $MgSO_4$ 0.5 g, K_2HPO_4 1.0 g, agar 20 g, and distilled water 1 liter) was poured in these petri plates by rotating to ensure proper mixing within the medium. Streptomycin was added to the medium to suppress the growth of bacteria and actinomycetes. The Petri plate thus prepared is paired with another empty plate containing filter paper and sealed with adhesive tape to prevent both inside and surface evaporation of the medium. The paired Petri plates thus prepared were incubated for 3-5 days in an incubator at 45± 2ÚC. After completion of the incubation period, the plates were checked for the fungal colonies appearing on the medium (Cooney and Emerson, 1964).

Identification of thermophilic coprophilous fungi

The isolated fungal colonies were then studied to reveal the Microscopic features of strains such as hyphae, conidiophores, branching conidia, pigmentation etc. were determined using a Olympus make microscope (X40) followed by standard manuals (Cooney and Emerson, 19649; Mouchacca, 1997; Barnett *et al.,* 1972; Von Arx and Muller, 1975; Ellis 1971; Subramanian, 1971; Domesch *et al.,* 1980) and photograph of the microscopic images was taken through optical device. The isolated fungi were further purified by subculturing on slants of the YES medium. The fungal growth was analyzed at two different temperatures and classified them into thermophilic and thermo tolerant according to their ability to grow at 45ÚC and 27ÚC temperatures respectively.

Evaluation of thermophilic coprophilous fungi

The number of colonies of each species appearing on a media contained Petri plate was counted. The percentage of incidence, percentage of frequency and percentage of abundance were calculated by using following formulae.

$$\text{Percentage of Incidence} = \frac{\text{Number of colonies of a species in all the plate}}{\text{number of colonies of all the species in all the plates}} \times 100$$

$$\text{Percentage of Frequency} = \frac{\text{Number of observations in which a species appeared}}{\text{Total number of observations}} \times 100$$

$$\text{Percentage of Abundance} = \frac{\text{Total number of colonies of a species in all the observations}}{\text{Total number of colonies in all observations}} \times 100$$

Results

Altogether 340 samples were collected from 17 different sources of domestic herbivore animals (7), zoo animals (5) and birds droplets (5) samples analyzed for thermophilic coprophilous fungi. They were more abundant in a variety of environments probably because of their ability to use a wide variety of substrates.

A total of 140 dung samples collected from 7 types of domestic herbivore animals (Cow, Ox, Buffalo, Horse, Goat, Sheep, and Wild Sheep) analyzed for the presence of thermophilic coprophilous fungi revealed 24 species representing 15 genera. However, the percentage of incidence, frequency, and abundance varied significantly and the data has been presented in Table 1. A total of 100 dung samples of five zoo herbivore animals like Bear, Deer, Elephant, Rabbit and Zoo dump were collected and analyzed for the incidence of thermophilic coprophilous fungi and the results has been presented in Table 2 reveals that a total of 19 species representing 13 genera were recorded. A total of 100 dung samples of 5 types of bird droplets including duck, parrot, peacock, pigeon, and Poultry were analyzed for the presence of thermophilic coprophilous fungi. A total of 15 species representing 7 genera was recorded and calculated their percentage of incidence; frequency and abundance has been given in Table 3.

Out of 30 species representing 17 genera isolated from 340 dung samples of herbivore animals (12 types) and bird's droplets (5 types) has been presented in Table 4. They were grouped in to thermophilic and thermo tolerant based on temperature requirements and results presented in Table 5. Out of 30 species representing 17 genera, 23 species were found thermophilic, while 7 species were found thermo tolerant.

Some of the microscopic features of isolated thermophilic coprophilous fungi and their microscopic images were given in figure 1. The isolated fungal colonies were identified by microscopic features of strains followed by standard manuals and photograph of the microscopic images was taken through optical device (eye piece).

A) **Coprinus annuloporus** : On YES medium at 35-45ÚC the thick cream and spongy like colonies were observed. After 4 days of incubation, the total petri dish occupied with cream spongy and dust like spores were observed. Basidia clavate, two-spored. Cheilocys-tidia absent. Pleurocystidia absent. Veil made up of (sub) globose to clavate-elongate, with a thin, smooth wall, and narrow, hyaline hyphae.

B) **Humicola fuscoatra**: On YES agar medium growth observed at above 45ÚC conidia of two kinds, alueriosporus and phialosporus. Aleurioconidia is formed directly or sterigmata comprehensive from creeping. Hyphae pale brown, globose, Subglobose. Phialoconidia bore on simple, erect conidiophores (Phialides), catenulate, hyaline, long-ovate apiculate at one end.

Table 1. Incidence, frequency and abundance of thermophilic coprophilous fungi in different domestic herbivore animal dung

	Incidence %								
Name of the Fungi	Cow	Ox	Buffalo	Horse	Goat	Sheep	Wild sheep	Frequency%	Abundance%
Acremonium thermophilum	_	_	_	1.6	13.52	_	_	28. 50	2
Aspergillus fumigates	_	11	22.3	13.2	_	_	_	42. 8	6.6
A. terries	_	_	9.3	_	_	12.2	_	28. 5	3.06
A. niger	10.3	_	_	_	_	_	_	14. 2	1.4
Cheatomium v. caprophile	_	_	_	_	_	2.3	_	14. 2	0.32
Humicola gresia	15.4	20.2	_	_	7.8	17.5	19	71. 4	11.3
H. fuscoatra	6.9	_	_	_	_	5.6	_	28. 5	1.7
H. insolence	11.3	19	16.6	29.16	_	3.6	_	71. 4	11
Malbranchea cinnamomea	20.5	10.7	_	_	16.6	6.9	21.6	71. 4	10.7
M. pulchuella	7.6	6.3	22.9	_	10.6	7.4	31.3	85. 7	12
Myriococcum albomyces	_	_	_	12.3		4.4	_	28. 5	2.3
Penicillium dupontis	_	_	_	_	9.4	16.2	_	28. 5	3.6
Rhizomucor miehei	_	_	_	21.5	_	_	_	14. 2	3
R. pusillus	6.4	_	_	_	10.6	5.4	7.4	57. 0	4.2
R. arrhizus	_	_	_	15.7	_	_	_	14. 2	2.2
R. microspores	_	_	_	_	11.2	_	_	14. 2	1.5
R. piriformis	_	2.1	2	_	1	1.5	_	57. 0	0.93
Scytalidium thermophilum									
Myceliophthora thermophila	14.3	_	_	_	_	_	5.3	28. 5	2.7
(=Sporotrichum thermopilum)	_	_	_	_	_	3.7	_	14. 2	0.72
Talaromyces duponti	_	_	_	_	21.2	2.6	_	28. 5	3.3
Thermoascus arantiacus	5.3	_	n_	_	_	_	_	14. 2	0.74
Thermomyces lanuginosus	3.6	14.7	12.4	_	_	_	15.6	14. 2	6.5
Thielavia terrestris	_	_	_	_	_	13.4	_	14. 2	1.9
Torula thermophila	5.4	15.7	17.2	7.4	_	3.4	_	71. 4	6.9
Sterile mycelium	_	_	_	5.6	_	3.4	_	28. 5	1.2

Table 2. Incidence, frequency and abundance of thermophilic coprophilous fungi in different herbivore animals of zoo

		% of Incidence					
Name of the Fungi	Bear	Deer	Elephant	Rabbit	Zoo dump	Frequency %	Abundance %
Acremonium thermophilum	_	18.5	_	10	_	40	5.6
Aspergillus fumigatus	15.4	_	_	14	_	40	5.8
A. flavus	_	14.5	7.4	_	18	60	7.8
A. terreus	12	_	15.6	_	_	40	5.4
A. niger	14	_	_	27	_	40	8
Cheatomium v. caprophile	_	_	11.3	_	10	40	4.2
Coprinus annuloporus	_	_	6.5	_	_	20	1.2
Humicola gresia	_	30.1	16	_	8	60	10
H. insolence	11	_	10	_	6	60	5.3
Malbranchea cinnamomea	_	12	6.9	_	_	40	3.7
M. pulchella	20	6	_	_	7	60	6.5
Myriococcum albomyces	5	_	6.3	_	_	40	2.2
Penicillium duponti	_	_	_	_	5	20	0.9
Rhizomucor pusillus	7.3	_	_	_	20	20	5.4
R. arrhizus	_	21	_	_	_	20	4.1
R. microspores							
Myceliophthora thermophila	_	_	_	39	6	40	8.9
(=Sporotrichum thermopilum)	_	_	5.7	_	_	20	1.1
Thermomyces lanuginosus	5.4	_	9.3	_	12	60	5.2
Torula thermophila	10	_	5	_	_	40	5
Sterile mycelium	_	_	_	10	9	40	3.7

Table 3. Incidence, frequency and abundance of thermophilic coprophilous fungi in different bird droplets

			Incidence %				
Name of the Fungi	Duck	Parrot	Peacock	Pigeon	Poultry	Frequency %	Abundance %
Acremonium thermophilum	12.4	_	_	_	_	40	7.1
Aspergillus fumigatus	_	_	25.9	18	_	40	8.8
A. flavus	29.1	15	_	_	_	40	8.8
A. terries	_	12.5	_	_	_	60	9.9
A. niger	36.8	_	_	15.1	_	40	10.4
Crysosporium fergusii	_	_	_	_	11	20	3.5
Humicola gresia	_	_	_	_	31.9	20	6.4
H. stelleta	_	_	_	_	27.1	20	5.4
Malbranchea cinnamomea	_	_	_	_	17.5	20	3.5
M. pulchuella	_	_		_	5.6	20	1.12
Myriococcum albomyces	_	_	_	12.6	_	20	2.5
Rhizo mucorpusillus	13.5	_	_	26.1	16.3	60	11.2
R. arrhizus	16.7	46.3	35.7	_	_	60	19.8
R. microspores	_	4.3	19.6	17.1	_	60	8.2
R. rhizopodiformis	_	_	_	21.3	_	20	4.2

Table 4. Different thermophilic and thermotolerant coprophilous fungi from different herbivores dung.

Name of the Fungi	TT	TP	Substrate
Acremonium thermophilum	+	_	Goat, horse, deer, rabbit, duck, parrot.
Aspergillus fumigates	+	_	Horse, bear, ox, buffalo, pigeon.
A. flavus	+	_	Zoo dump, duck, parrot.
A. niger	+	_	Cow, pigeon, duck.
A. nidulans	+	_	Cow, sheep.
A. terries	+	+	buffalo, sheep, elephant, bear.
Cheatomium v. coprophilia	_	+	Elephant, sheep, zoo dump.
Cheatomium thermophile	_	+	Sheep.
Chrysosporium fergusii	_	+	Poultry.
Coprinus annuloporus	+	+	Elephant.
Humicola gresia	_	+	Cow, goat, ox, wild sheep, elephant, zoo dump, poultry.
H. fuscoatra	_	+	Sheep, cow.
H. insolence	_	+	Ox, horse, buffalo, elephant, cow, zoo dump.
H. stelleta	_	+	Poultry.
Malbranchea cinnamomea	_	+	Cow, ox, sheep, wild sheep, goat, elephant, bear, deer, poultry.
M. pulchuella	_	+	Cow, ox, buffalo, goat, sheep, wild sheep, poultry,
Myriococcum albomyces	_	+	Sheep, horse, bear, elephant, pigeon.
Penicillium duponti	_	+	Sheep, goat, zoo dump.
Rhizo mucormiehei	_	+	Horse.
R. pusillus	_	+	Cow, goat, sheep, wild sheep, poultry, pigeon, duck.
R. arrhizus	_	+	Horse, deer, duck, parrot, peacock, pigeon.
R. microspores	_	+	Goat, zoo dump, rabbit, duck, peacock, pigeon, parrot.
R. rhizopodiformis	_	+	Ox, buffalo, goat, sheep, pigeon.
Scytalidium thermophilum	_	+	Cow, wild sheep.
Sporotrichum thermopilum	_	+	Sheep, elephant.
Talaromyces duponti	_	+	Goat, sheep.
Thermoscus uranticus	_	+	Cow.
Thermomyces lanuginosus	_	+	Cow, sheep, ox, buffalo, wild sheep, bear, elephant, zoo dump.
Thielavia terrestris	_	+	Sheep.
Torula thermophila	_	+	Cow, ox, buffalo, horse, sheep, bear, elephant.
Sterile mycelium	_	+	Sheep, horse, rabbit, zoo dump.

Table 5. Temperature relationship between thermophilic and thermotolerant coprophilous fungi

	Temperature (°C)						
Name of the Fungi	25	30	35	40	45	50	55
Acremonium thermophilum	+	+	+	+	_	_	_
Aspergillus fumigates	+	+	+	+	+	_	_
A. flavus	+	+	+	+	_	_	_
A. niger	+	+	+	+	_	_	_
A. nidulans	+	+	+	+	_	_	_
A. terries	+	+	+	+	_	_	_
Cheatomium v. coprophilia	_	_	_	+	+	_	_
Cheatomium thermophile	_	_	_	+	+	_	_
Chrysosporium fergusii	_	_	_	+	+	_	_
Coprinus annuloporus	_	_	+	+	+	_	_
Humicola gresia	_	_	_	_	+	+	_
H. fuscoatra	_	_	_	_	+	+	+
H. insolence	_	_	_	_	+	+	_
H. stelleta	_	_	_	_	+	+	+
Malbranchea cinnamomea	_	_	_	_	+	+	+
M. pulchuella	_	_	+	+	+	+	_
Myriococcum albomyces	_	_	_	_	+	+	_
Penicillium duponti	_	_	_	_	+	+	+
Rhizo mucormiehei	_	_	_	+	+	+	+
R. pusillus	_	_	_	+	+	+	+
R. arrhizus	_	_	_	+	+	+	+
R. microspores	_	_	_	+	+	+	_
R. rhizopodiformis	_	_	_	+	+	+	_
Scytalidium thermophilum	_	_	_	+	+	+	
Sporotrichum thermopilum	_	_	_	+	+	+	+
Talaromyces duponti	_	_	_	+	+	+	+
Thermoscus uranticus	_	_	_	+	+	+	_
Thermomyces lanuginosus	_	_	_	+	+	+	+
Thielavia terrestris	_	_	_	+	+	+	+
Torula thermophila	_	_	_	+	+	+	+
Sterile mycelium	_	_	_	+	+	+	+

C) **Humicola insolens:** On the YES agar medium at 40ÚC the thin white colonies increase in diameter at the rate of 2cm per day, and evidence of sporulation can be seen after forty-eight hours. The first indication of the spore production consists of scattered swellings or groups of swelling along with the hyphae. Probably most common is the production of intercalary spores, which may be produced singly or in pairs and occasionally in short chains. The aleuriospores are generally globose, occasionally there occur larger, intercalary spindle-shaped spores. The color of the colony depends upon the age of the spores. Young cultures are white, but as the spores become fully mature the entire colony turns grayish-brown because of a mixture of brown spores and persistent grayish hyphae. This grayish brown color of mature colonies is a consistent character and is in contrast to the jet black colony produced by *H.insolens.*

D) ***Humicola grisea*** : At incubation temperature of 35- 50 ÚC on YES agar medium, growth is very rapid. In thirty-six hours the colony will completely cover the surface of the agar in a small Petri dish. The colorless hyphae are rather fine, rarely exceeding in width, and produce a thin prostate mycelium which is white in the initial stages of growth, very quickly the center of the colony becomes grayish as the aleuriospores mature and in two days the entire colony appears dark gray. By the end of the third day, the colony has lost the gray color and has become dull-black.

E) ***Humicola stellata*** : *H.stellata* grows and sporulates well; forming a dark colony, on YES agar medium. It grows more slowly, forms a pale colony, sporulates sparsely, and produces colorless chlamydospores on the hyphae. *H.stellata* grows well at 37ÚC and produced a dull black colony. Microscopic examination showed the colorless, septate hyphae. The aleuriospores, which are borne singly on the tips of the aleuriophores, are subglobose and colorless when young, but become dark brown and stellate with maturity.

F) ***Malbranchea cinnamomea*** : Colonies on YES agar medium at 45ÚC are robust, dense, thick, smooth with a few outward radiating folds, velvety with coarse, creamy yellow tufts of hyphae, sulfur yellow. The medium turns black from diffused pigment. Vegetative hyphae hyaline, later becoming yellowish-brown with racket hyphae. Arthroconidia have borne on curved, lateral branches arising from

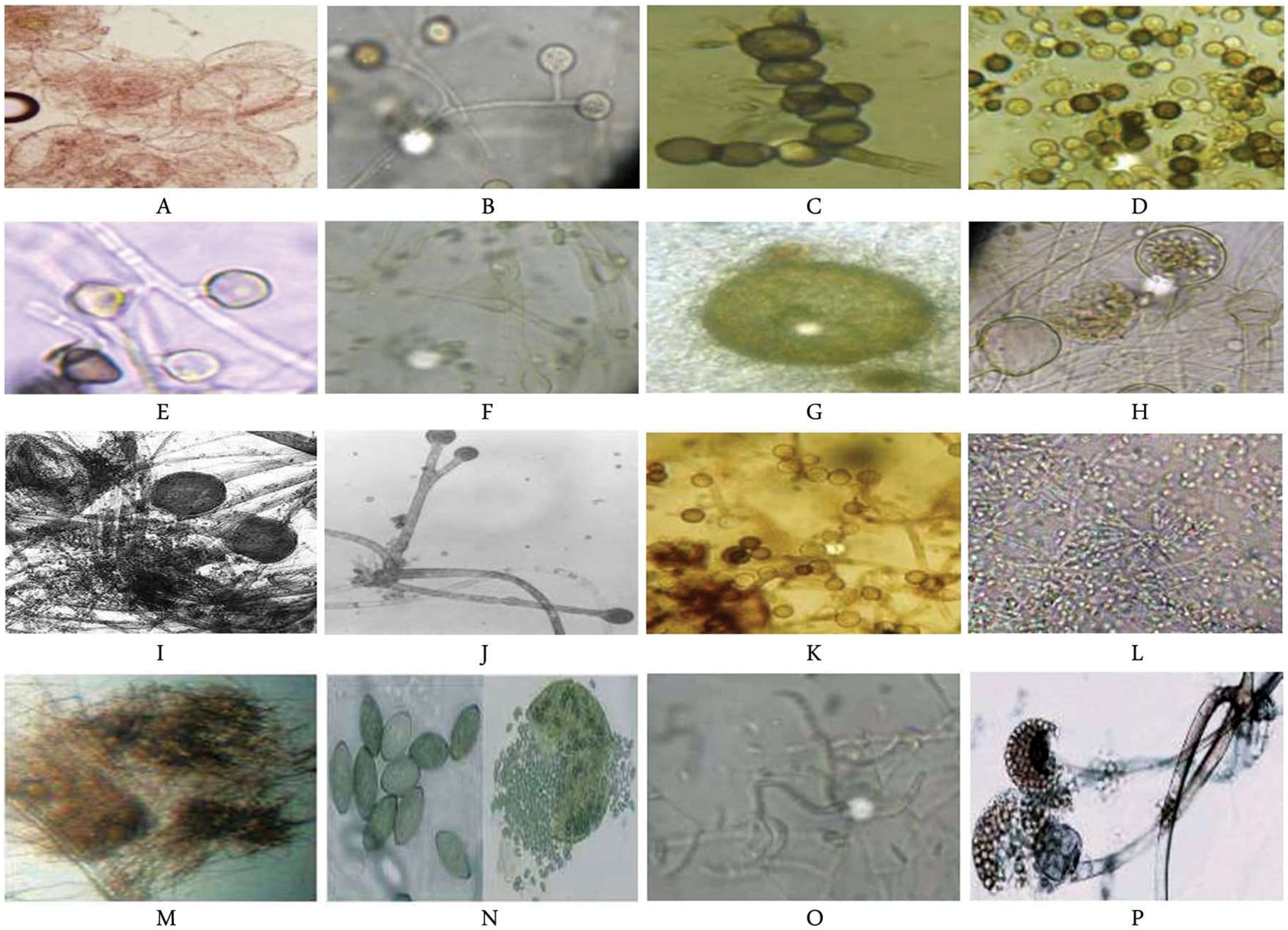

Fig. 1. A. *Coprinus annuloporus* (Elephant dung) B. *Humicola juscoatra* (Cow and Sheep dung) C. *Humicola insolens* (Buffalo, Horse, Elephant, Goat, Elephant, Bear, Deer, Ox, *Horse,* and Poultry dung) D. *Humicola grisea* (Cow, Ox, Wild Sheep, Poultry) E. *Humicola stellata* (poultry) F. *Malbranchea cinnamomea* (Goat, Sheep, Wild Sheep, Elephant, Bear) G. Myricoccum *albomyces* (Sheep, Horse, Bear, Elephant, and Pigeon dung) H. *Rhizomucor miehei* (Horse dung) I. *Rhizopus arrhizus* (Horse, Deer, Duck, Parrot, Peacock, and Pigeon) J. *Rhizopus rhizopodijormis* (Ox, Buffalo, Goat, Sheep, and Pigeon) K. *Scytalidium thermophilum* (Cow, Wild Sheep dung) L. *Penicillium duponti* (Goat dung) M. *Thielavia terrestris* (Sheep dung) N. *Thermoascus aurantiacus* (Isolated from cow dung) O. *Malbranchea pulchella* (Cow, Ox) P. *Rhizopus microspotus* (Rabbit dung)

broader vegetative hyphae. Conidia cylindrical, often curved, thick-walled, attached hyaline frill from the outer hypha wall of the separating empty cell, hyaline.

G) ***Myricoccum albomyces*** : Colonies on YES medium at 45ÚC, appear as white in early stages becoming grayish-black by age and finally grayish-black extended into the medium. Two distinct types of hyphae are perceptible. Aerial hyphae constricted at the septa, forming branched, and chain-like series of cylindrical or oval thick-walled cells that break apart easily. Ascocarp superficial, dark brown scattered or gregarious, globose, glabrous wall, non-ostiloate, 150-250 µm in diameter. Asci pyriform when young, at maturity irregularly oblong, 8-spored, ascus membranous, simple, very thin. Ascospores single-celled, smooth, dark brown, globose, with a single apiculus, irregularly distributed in the ascus.

H) ***Rhizomucor miehei*** : Heavy colonies bearing numerous sporangia appear on the Yeast extract starch medium at 45-48 ÚC. Colonies occur, at first white, subsequently changes gray-brown to beigebrown. Sporangiospores are nearly spherical, colorless. Zygospres in abundance, subspherical, reddish-brown or yellow when young, blackish at maturity produced on homothallic mycelium.

I) *Rhizopus arrhizus* : On Yeast extract starch agar media growth occurs at 40°C colonies are initially woolly and white, subsequently becoming gray and then developing small black dots in the mycelium which are mature sporangia. Within 2 to 3 days growth is very rapid filling the tube or Petri dish but fails to grow above 45 °C. Hyphae are hyaline, broad and contain ribbon-like irregular branches, hyphae are initially aseptate later changed to sparsely septate. Sporangiophores and rhizoids are borne from creeping aerial hyphae referred to as stolons. Sporangiophores are produced in small groups called as globose sporangia with ellipsoidal columellae. For the production of zygospores species requiring compatible mating (heterothallic).

J) *Rhizopus rhizopodiformis* : Growth very rapid at 45 ÚC on YES media filling a 90 mm Petri dish in 36 hours colony white at first later grayish black. Vegetative hyphae hyaline, 3-7 μm in diameter, reduced stolons. Rhizoids are pale brown in color. Sporangiophores are arisen singly or in groups from the nodal regions. Sporangia black, spherical, smooth, sporangiospores pale brown, spherical.

K) *Scytalidium thermophilum* : Colonies on YES at 45 ÚC are white at first but later turns through grayish to jet-black as spore maturation proceeds. Hyphae is colorless, flat, branched, septate. Conidiogenous cells are small and conidia are dark-brown in color, smooth-walled, generally globose to oval produced, and developed in chains on hyphal branches.

L) *Penicillium duponti* : Mycelium is composed of branched hypahe, growth is fairly rapid at optimum temperatures of 45-50ÚC. Dark brown drops of liquid exudates are sometimes formed in the central areas of agar cultures. The growth is always white at the start and then generally develops some rather dull shade of vary. Considerably, Conidia are borne in long tangled chains that separate readily from the phialides.

M) *Thielavia terrestris* : Colonies grows rapidly at 42 ÚC with aerial mycelium composed of colourless, regularly septate, broad hyphae with swollen cells, usually smooth, black, composed of thick-walled, dark brown and irregularly interwoven hyphal cells, Asci clavate, stalked, Ascospores.

N) *Rhizopus microspores*: Growth at 45ÚC is extremely rapid colonies cottony, turf high, at maturity grayish black, vegetative mycelium hyaline. Rhizoids pale brown. Sporangiophores unbranched.

O) *Malbranchea pulchella* : Colonies on YES at 47 ÚC are robust, dense, thick smooth or with few outward radiating folds, creamy yellow tufts of hyphae, sulfur yellow. The medium turns dark-brown or black due to diffused pigment. Hyphae, hyaline, later becoming yellowish brown with prominent racket hypahe, arthroconidia borne on curved or loosely coiled lateral branches arising from vegetative hyphae.

P) *Thermoascus aurantiacus*: The growth is very rapid at 45ÚC and extends outward from a central inoculum to reach the periphery of 10 cm Petridish in two or three days. At first the hyphae are largely within the sub-stratum or closely spread over the surface often reaching the Petri dish lid. The culture at this stage is nearly pure white or very pale gray-buff. Microscopic examination of such cultures reveals the dactyloid conidiophores that arise as terminal branches of these aerial hyphae. Hyphae colorless, septate, spores erect, oval.

Discussion

The Present work notably carried out on the herbivore dung samples for an ecological group of fungi at elevated temperatures and their incidence was calculated. The results of Table. 1 reveal that the *Malbranchea cinnamomea* was with the highest percentage of incidence followed by *Humicola gresia, Scytalidium thermophilum*, *H. insolense* and *A. niger* were recorded from cow dung in descending order. *Thermomyces lanuginosus* followed by *Thermoascus uranticus* and *Torula thermophila* were recorded with the lowest incidence, while *M. pulchella, H. fuscoatra* and *R. pusillus* were observed with a moderate percentage of incidences in Cow dung sample. *H. gresia* followed by *H. insolense*, *Torula thermophila* and *T. lanuginosus* were recorded with the highest percentage of incidence in Ox dung samples. *R. rhizopodiformis and M. pulchella* were recorded with the lowest incidence, while *A. fumigatus* and *M. cinnamomea* were recorded with a moderate percentage of incidence in Ox dung samples. Buffalo dung samples showed the highest percentage of incidence with *M. pulchella* and *A. fumigatus*, while *Rhizomucor miehei* recorded the lowest percentage of incidence. *Torula thermophila*, *H. insolense, Thermomyces lanuginosus* and *A. terreus* were recorded with an intermediate percentage of incidence. *H. insolense* followed by *R. miehei* showed the highest percentage of incidence in horse dung samples, while *Acremonium thermophilum* and *T. thermophila* were with the lowest incident. *R. arrhizus* followed by *A. fumigatus* and *Myriococcum albomyces* were recorded with an intermediate percentage of incidence in Horse dung samples. Goat dung samples showed the highest percentage of incidence with *Talaromyces duponti*, whereas *R. rhizopodiformis* and *H. gresia* were recorded with the least incidence. *M. cinnamomea* followed by *A. thermophilum, R. microspores, R. pusillus, M. pulchella* and *Penicillium duponti* were recorded with a moderate percentage of incidence. Sheep dung showed the highest percentage of incidence with *H. gresia* and *P. duponti*. *R. rhizopodiformis* followed by *Cheatomium v. coprophile* and *T. duponti* were recorded with the lowest percentage of incidence, while *Thielavia terrestris*, *A. terreus, M. pulchella* and rest of the fungi are recorded with an intermediate percentage of incidence in Sheep dung samples. Wild Sheep dung samples showed the highest percentage of incidence with *M. pulchella,* while *M. cinnamomea*, *H. gresia* and *Thermomyces lanuginosus* were recorded with a moderate percentage of incidence. *Scytalidium thermophilum* and *R. pusillus* were recorded with the lowest incidence in wild Sheep dung samples. Among Cow, Ox, Buffalo, Horse, Goat, Sheep, and wild Sheep dung samples, wild Sheep dung showed the highest percentage of incidence with *M. pulchella* followed by *H. insolense* in Horse dung samples. *M. pulchella* was recorded with the highest percentage of frequency followed by *H. gresia, H. insolense, M. cinnamomea*, and *T. thermophila* were significantly similar in their percentage of frequency. The highest percentage of abundance was recorded with *M. pulchella* followed by *H. gresia*, *H. insolense* and *M. cinnamomea* in decreasing order. Least abundance was recorded with *Myceliophthora thermophila* followed by *Thermoascus aurantiacus* and *R. rhizopodiformis.* Only single thermo tolerant fungus was recorded in Cow, Ox, Sheep and Goat dung. No thermo tolerant fungi occurred in the dung sample of wild Sheep. *M. pulchella* was with the highest percentage of frequency and abundance among thermophilic coprophilous fungi from 7 domestic herbivores animals. Interestingly among all *M. pulchella* incidence occurred in all domestic animals dung except Horse dung.

Table 2 reveals that a total of 19 species representing 13 genera were recorded in Bear, Deer, Elephant, Rabbit, and zoo dump samples. However, they differed in their percentage of incidence, frequency and abundance. The highest incidence recorded by *M. pulchella* followed by *A. fumigatus. Myriococcum albomyces* and *Thermomyces lanuginosus* were recorded with the lowest percentage of incidence, while *A. niger, A. terrius, H. insolense, Torula thermophila* and *R. pusillus* were recorded with an intermediate incidence in bear waste samples. *H. gresia* followed by *R. arrhizus* was recorded with the highest incidence in deer waste samples. Least incidence was recorded by *M. pulchuella,* while *A. thermophilum* followed by *A. flavus* and *M. cinnamomea* were recorded with an intermediate percentage of incidence in deer waste samples. *H. gresia* and *A. terrius* were recorded with the highest incidence, while *Torulla thermophila* and *S. thermophilum* with the least incidence in elephant dung samples. Rest of the fungi with an intermediate percentage of incidence. Rabbit waste samples showed the highest percentage of incidence with *R.*

microspores followed by *A. niger*. The lowest incidence was recorded by *A. thermophilum,* while the rest of fungi with moderate incidence. Zoo dump samples were recorded with the highest percentage of incidence with *R. pusillus* and *A. flavus*. *H. insolense* and *R. microspores* with the lowest incidence, whereas the rest of the fungi with a moderate percentage of incidence. A. flavus, *H. insolense, H. gresia, M. pulchuella* and *Thermomyces lanuginosus* were recorded with the highest percentage of similar frequency but differ in their incidence and abundance. Least percentage of frequency recorded by *C. annuloporus, P. duponti*, *R. pusillus* and *S. thermophilum;* significantly similar in their percentage of frequency, while differs in their incidence. The rest of the fungi recorded intermediate in their percentage of frequency. *H. gresia* followed by *R. microspores* and *A. niger* were recorded with the highest abundance. Lowest abundance was recorded by *P. duponti* followed by *S. thermophilum* and *Coprinus annuloporus.* Elephant dung supported good growth of thermophilic coprophilous fungi followed by Zoo dump and bear dung source. Deer and rabbit dung supported the intermediate degree of growth of thermophilic coprophilous fungi.

Table 3 reveals that the excreta of poultry and pigeon supported a maximum number of thermophilic coprophilous fungi. Excreta of the Duck, Parrot and Peacock have supported the intermediate percentage of thermophilic coprophilous fungi. *A. niger* followed by *A. flavus* were occurred with the highest incidence, followed by *Rhizomucor arrhizus*, *R. pusillus*, and *Acremonium thermophilum* in descending order in Duck excreta samples. Droplets of parrot showed the highest incidence of *Rhizomucor arrhizus* followed by *A. flavus, A. terreus* and *R. microspores* in decreasing order. *Rhizomucor arrhizus* was recorded with the highest percentage of incidence followed by *A. fumigatus* and *R. microspores* in droplets of peacock samples. *R. pusillus* followed by *R. rhizopodiformis* showed the highest percentage of incidence in pigeon droplets, while the rest of the fungi showed intermediate incidence. Poultry waste showed the highest incidence of *H. gresia* followed by *H. stelleta*, while *M. pulchuella* was recorded with the lowest incidence and rest of the fungi with intermediate incidence. *R. pusillus* followed by *R. arrhizus, R. microspores* and *A. terrius* showed the highest percentage of similar frequency. *R. rhizopodiformis, Myriococcum albomyces, M. pulchuella, Malbranchea cinnamomea, H. stelleta, Humicola gresia* and *Crysosporium fergusii* were recorded with the lowest frequency which is significantly similar. Rest of the fungi recorded with moderate frequency. *Rhizomucor arrhizus* followed by *R. pusillus* and *A. niger* were recorded with the highest percentage of abundance. *Myriococcum albomyces* and *Crysosporium fergusii* showed the lowest abundance, while the rest of the fungi showed moderate abundance.

Conclusion

An extensive and intensive survey of different dung samples of herbivorous animals of various places in Telangana region revealed the presence of a variety of thermophilic coprophilous mycoflora. Our present investigation suggests the most attractive attribute of these thermophilic coprophilous fungi from a biotechnology point of view is that they produce enzymes (Shanthipriya *et al.,* 2015 and Shanthipriya *et al.,* 2020) capable of catalyzing biochemical reactions at a temperature markedly higher than those of conventional organisms and are more stable at harsh environment thus can prolong the shelf life of commercial products.

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References

Ajmera Shanthipriya, Sana Shanawaz and Sivadevuni Girisham. 2020. Studies on Decomposition of Banana Leaf and Mixture of Cattle Dung and Urine by Thermophilic Coprophilous Fungi. Nature Environment and Pollution technology. 19 (2): 815-823.

Barnett HLand Hunter BB. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company, Minneapolis, Minnesota.

Bell A . 1983. Dung Fungi: An illustrated guide to coprophilous fungi in New Zealand, Wellington, Victoria University Press.

Bell A. 2005. An Illustrated Guide to the coprophilous Ascomycetes of Australia. CBS, Utrecht, the Netherlands.

Cochrane VW. 1958. Physiology of fungi XiiI Wiley, New York. Pp: 524.

Cooney DC. and Emerson R. 1964. In: Thermophilic fungus: An account of their Biology, Activities and Classification. Freeman WH and San Francisco. 1-188.

Domesch KH, Games H and Heidi AT. 1980. Compendium of soil fungi. Academic press. London. 1: 66-194.

Ellis MB. 1971. Dematacious Hyphomycetes: 59. CAB IMI Kew Surrey. England

Gloer JB and Truckenbrod SM. 1988. Interference competition among coprophilous fungi-production of (+)-isoepoxydon by *Poronia punctata,* Appl. Environ. Microbiol. 54: 861-864.

Hein SM, Gloer JB, Koster B and Arugosin F. 1998. A new antifungal metabolite from the coprophilous fungus Ascodesmis sphaerospora. J Nat Prod. 61: 1566-7.

Johri BN and Ahmad S. 1991. Thermophilic fungi at cross roads of biotechnological development, In: Fungi and Biotechnology: Recent Advances (ed. H.C. Dube). Vedams Books, New Delhi, India. Pp. 45-73.

Maheshwari R, Bharadwaj G and Bhat MK. 2000. Thermophilic fungi: their physiology and enzymes, Microbiol. Mol. Biol. Rev. 64: 461-488.

Mouchacca J. 1997. Thermophilic fungi: Biodiversity and taxonomic status. Cryptogamie Mycologie. 18(1): 19-69.

Ranjith Kumar R, Sreelatha B, Girisham S and Reddy SM. 2010. Incidence of thermophilic fungi from different substrates in Andhra pradesh (India). Int. J. of Pharma and Biosci. 1: 1-6

Richardson MJ. 2001. Coprophilous Fungi from Brazil. Brazilian Archives of Biology and Technology. 44(3): 283-289.

Richardson MJ. 2008. Records of coprophilous fungi from the Lesser Antilles and Puerto Rico. Caribbean J. Sci. 44: 206-214.

Salar RK and Aneja KR. 2007. Thermophilic fungi: Taxonomy and Biogeography. J. Agri. Technol. 3: 77-107.

Shanthipriya A, Koteswara Rao V, Sreelatha B, Girisham S and Reddy SM. 2015. Effect of Nutritional Factors on Growth and L-asparaginase Production by Three Thermophilic Caprophilous Fungi. Current Research in Microbiology and Biotechnology. 3(4): 707-712.

Shanthipriya Ajmera, Koteswara Rao V, Ranipadmini V, Ramchander Merugu and Sivadevuni Girisham. 2019. Isolation and Molecular Characterization of Thermophilic Coprophilous Fungus *Malbranchea cinnamomea* GSMBKU from Goat Dung. Journal of Pure and Applied Microbiology. 13(4): 2227-2233.

Shanthipriya Ajmera, Vasavi Dathar P, Shivakumar Singh and Girisham S. 2020. Production and optimization of Cellulases by thermophilic coprophilous fungus *Malbranchea Cinnamomea.* International Journal of Botany Studies. 5(4): 116-120.

Sreelatha B, Shanthipriya A and Girisham S. 2013. Incidence of thermophilic fungi in different dung samples of Warangal district of A.P. Int. J. Pharm. Biol. Sc. 3: 355-359.

Subramanian CV. 1971. Hyphomycetes. I.C.A.R. Publication. New Delhi. Pp. 930.

Von Arx JA and Muller E. 1975. In A re-evaluation of the Bi-tunicate ascomycates with keys to families and genera. Studies in Mycology: Institute of the Royal Netherlands. Acad. Sci and Lett. Baarn. 9: 1-159.

Webster J. 1995. Fungal Ecology. London. Chapman and Hall.