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Influence of Temperature and Culture Conditions on the Survival of Keratinophilic and Dermatophytic Fungi

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HIGHLIGHTS

- Human pathogenic fungus *Trichophyton tonsurans* has a longer survival period
- Jaipur climatic condition favor the recurrence of keratinophilic and dermatophilic fungi.
- Unsterilized soil samples are potent source of fungal niche.

Abstract: Dermatophytic and keratinophilic fungi are major components of soil microflora. Keratinolytic fungi occur abundantly in the superficial soil layers of landfills and their surroundings. The aim of the present investigation is to evaluate the survival period of these pathogenic and non pathogenic dermatophytes in different culture and temperature conditions. In present research survival period of four dermatophytic and three keratinophilic fungi has been carried out in unsterilized soil, sterilized soil and Sabouraud's dextrose-agar medium at three different temperature conditions. These fungi were collected from soil samples and infected skin samples of tinea patients of Jaipur city. Bait testing (BT) and Direct soil sprinkling (DSS) methods were used for the testing of survival after every fourth month up to two years of the study. Result: Unsterilized soil and SDA medium were found more suitable for survival of all fungi. 11±1 ºC was found to be most suitable temperature condition for all tested fungi up to two years of studies. *T. tonsurans* has a longer survival period among all the test fungi. *T. simii* survived for short period of the time under different conditions.Frequently occurrence of these fungi in different localities of Jaipur district along with longer survival period in different climates concluded that Jaipur city potentially has a high risk for causing cutaneous fungal infections in human and animals.

Keywords: dermatophytes; longevity; soil; temperature; keratinophilic fungi.

INTRODUCTION

The potentially pathogenic keratinophilic fungi and allied geophilic dermatophytic species are widespread worldwide. They are considered primarily to be soil saprophytes [1]. Prevalence and biodiversity of fungi in each soil depend on environmental and nutritional conditions. These fungi abundantly occurred in the superficial soil layer of landfills and distributed worldwide [2-8]. They occur on cornified debris in the soil and

degrade keratin and keratinous material. Therefore they play an important ecological role in decomposing such residue. Since they are ecologically restricted to keratin as substratum, these can be recovered regularly from the soil by hair bait technique of Vanbreuseghem [9]. Keratinophilic fungi are present in the environment with variable distribution patterns that depend on different factors, such as human and or animal presence, which are of fundamental importance [10]. Keratinolytic fungi are a group of microorganisms that are able to decompose keratin remains in the environment and are pathogenic to humans and animals. They occur in several man-made and natural habitats. These fungi exist in communities together with keratinophilic fungi that have weaker affinity to keratin and utilize chiefly the products of its decomposition [11]. The species of keratinophilic fungal group have been divided into three categories according to their natural habitats; anthropophilic, when human being are the natural hosts; zoophilic, when a variety of animals act as natural hosts; geophilic, when the soil is the natural habitats.

Human infections, particularly those involving the skin and mucosal surface constitute a serious problem, especially in subtropical and tropical countries due to their prevailing moisture and temperature regimes. Jaipur has a dry climate in summer temperature exceed even 46 ºC with high humidity in monsoon seasons. These climatic conditions favour the incidence of fungi and consequently the disease [12].

Different climatic and physiological factors show immense role on the growth and sporulation of keratinophilic and dermatophytic fungi. Survival of fungal spore in environmental condition depends on several factors like temperature, environmental light, humidity, pH, climate, organic and inorganic materials, food supplements etc [13]. Information on the longevity of plant pathogenic fungal spores in relation to temperature, moisture content of conidia, humidity of storage condition is available [14-16]. Studies on survival period of keratinophilic fungi are untouched field. Very few reports are available. Long term survival of keratinophilic fungi in sterile soil has been discussed by Grin and Ozegovic [17]. Alteras [18] performed survival studies of keratinophilic fungi in garden soil, forest soil and soils of the plains rich in organic matter. Garg [19] made such studies with regard to sterile soil only. A soil rich in keratin residues constitutes a permanent or occasional reservoir for dermatophytes and other keratinophilic fungi and is a source of potential infection for human and animals. These keratinophilic fungi have attracted the attention of mycologist and dermatologist due to their association with human and animal mycoses. Since the fungi are normally soil inhabiting, it was considered necessary to determine their survival periods in non-sterile and sterile soils and also on Sabouraud's dextrose-agar medium (SDA) on which they were cultured.

Present investigation deal with longevity of seven keratinophilic and dermatophyic fungi collected from Jaipur city in three different storage temperature and culture conditions.

MATERIAL AND METHODS

Fungal isolates:

For present investigation total 7 fungi were selected which are most frequently reported in Jaipur district [8]. Among these four dermatophytic fungi namely *Trichophyton rubrum, T. tonsurans, Microsporum gypseum* and *T. simii* were isolated from infected skin scrapping of tinea patients, SMS hospital, Jaipur. Remaining three keratinophilic fungi like *Chrysosporium tropicum, Cephaliophora irregularis* and *Gymnoascus ressii* were obtained from soil samples collected from different localities in Jaipur area. Strains were identified by their morphological and physiological characteristics according to the methods described by Conant and coauthors [20] and Forbes and coauthors [21]. Sabouraud's dextrose chloramphenicol agar (Himedia) medium was used for the isolation, purification and maintenance of dermatophytes.

Survival studies:

For the study of the viability or survival of dermatophytic fungi, garden soil was taken for experimental purposes. Selected fungi were tested for their longevity in soil (Sterilized and Unsterilized) and culture medium at three different temperatures that is room temperature (ranging from 16-46 °C in a year), culture room temperature (25±2 °C) and refrigerator temperature (11±1 °C). For the first aspect of the study there was no need of autoclaving the soil. For the second aspect, one liter conical flasks were filled up to three quarters with garden soil and were steam sterilized successively for three days at 20 lb pressure for half an hour. These soils were then transferred to pre-sterilized polythene bags with the help of sterilized spatula. Ten-fifteen days old culture of each test fungi with approximately same diameter were then transferred into each of these bags individually and packed tightly with rubber bands and with proper labels. Each plastic bag was labeled indicating information like type of soil, date of inoculation, name of an organism, date of every four month testing, temperature condition. These bags were then placed in three different temperature conditions for incubation. For the third aspect, the required quantity of SDA was prepared, poured into tubes

and autoclaved. The tubes were also inoculated with a known quantity of inoculum of each test fungus individually and incubated at three different temperature conditions. Definite portions of these inoculated material were taken out aseptically from each bag/tube periodically after every fourth month and tested for viability by direct soil sprinkling method (DSS) and hair bait technique (BT) and the results recorded. '+' sign meant that the fungus was viable and '–' sign meant that the fungus was dead by the time the longevity test was performed. These tests were performed up to VIth testing (24 months).

RESULTS

Survival studies were carried out on seven selected fungi. An experiment was performed after every fourth month of storage through bait testing (BT) and direct soil sprinkling (DSS) methods. A study was conducted up to two years of storage. Data incorporated into Table 1 shows survival studies of *T. tonsurans* in three different culture conditions at three different temperatures. *T. tonsurans* is an anthropophilic fungus causes inflammatory or chronic non-inflammatory finely scaling lesions of skin, nails and scalp. *T. tonsurans* recovered up to sixth testing in both unsterilised soil and SDA media at all three temperature conditions along with 11ºC in sterilised soil condition. In sterilised soil, it was reported up to IIIrd testing only in bait testing method.

Table 1. Longevity testing of *Trichophyton tonsurans.*

Trichophyton tonsurans	**Ist test**		**IInd test**		**IIIrd test**		**IVth test**		**Vth test**		**VIth test**	
	BT	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**
Unsterilised soil												
(i) Room temperature	+	+	+	+	+	+	+	+	+	+	+	+
(ii)Culture room temperature	+	+	+	+	+	+	+	+	+	+	+	+
(iii) 11 ± 1°C	+	+	+	+	+	+	+	+	+	+	+	+
Sterilised soil												
(i) Room temperature	+	+	+	+	+	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	+	-	-	-	-	-	-	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+
SDA medium												
(i) Room temperature	+	+	+	+	+	+	+	+	+	+	+	+
(ii)Culture room temperature	+	+	+	+	+	+	+	+	+	+	+	+
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+

T. rubrum is an anthropophilic fungus become more widely distributed dermatophytes of human beings. During the present investigation this fungus was reported as a most common causative agent of ring worm in Jaipur. Data incorporated into Table 2 represent survival studies of *T. rubrum.* In unsterilised soil all three temperature conditions were found suitable for growth and survival of this fungus up to two years for testing period. DSS method could not recover the fungus at room temperature condition in sixth testing. In SDA medium all three temperature conditions showed survival of the fungus up to two years of testing both in bait testing and direct sprinkling methods. In sterilized soil *T. rubrum* was recovered up to sixth testing in refrigerator temperature and up to IIIrd testing in room temperature and 25± 2ºC temperature conditions while in IVth testing of culture room condition, fungal recurrence was seen only in bait testing method.

Table 2. Longevity testing of *Trichophyton rubrum*

Trichophyton rubrum	**Ist test**		**IInd test**		**IIIrd test**		**IVth test**		**Vth test**		**VIth test**	
	BT	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**
Unsterilised soil												
(i) Room temperature	+	+	+	+	+	+	+	+	+	+	+	-
(ii)Culture room temperature	+	+	+	+	+	+	+	+	+	+	+	+
(iii) 11 ± 1°C	+	+	+	+	+	+	+	+	+	+	+	+
Sterilised soil												
(i) Room temperature	+	+	+	+	+	+	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	+	+	+	-	-	-	-	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+
SDA medium												
(i) Room temperature	+	+	+	+	+	+	+	+	+	+	-	-
(ii)Culture room temperature	+	+	+	+	+	+	+	+	+	+	+	+
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+

T. simii has shorter survival period. It survived up to two years only in refrigerator in all three culture conditions (Table 3). While in room temperature and culture room temperature, it survived only up to six months in all culture conditions. Only bait testing was found positive in IIIrd testing in the unsterilized soil condition also. *M. gypseum* survived up to two years in all culture conditions at 11ºC and culture room temperatures. At room temperature, it survived up to IVth testing in SDA and sterilised soil. In unsterilised soil *M. gypeum* was reported up to Vth testing through the bait testing method (Table 4).

Table 3. Longevity testing of *Trichophyton simii.*

Trichophyton simii	**Ist test**		**IInd test**		**IIIrd test**		**IVth test**		**Vth test**		**VIth test**	
	BT	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**
Unsterilised soil												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	+	-	-	-	-	-	-	-
(iii) 11 ± 1°C	+	+	+	+	+	+	+	+	+	+	+	+
Sterilised soil												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+
SDA medium												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+

Table 4. Longevity testing of *Microsporum gypseum*

Microsporum gypseum	**Ist test**		**IInd test**		**IIIrd test**		**IVth test**		**Vth test**		**VIth test**	
	BT	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**
Unsterilised soil												
(i) Room temperature	+	+	+	+	+	+	+	+	+	-	-	-
(ii)Culture room temperature	+	+	+	+	+	+	+	+	+	+	+	+
(iii) 11 ± 1°C	+	+	+	+	+	+	+	+	+	+	+	+
Sterilised soil												
(i) Room temperature	+	+	+	+	+	+	+	+	-	-	-	-
(ii)Culture room temperature	+	+	+	+	+	+	+	+	+	+	+	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+
SDA medium												
(i) Room temperature	+	+	+	+	+	+	+	+	-	-	-	-
(ii)Culture room temperature	+	+	+	+	+	+	+	+	+	+	+	+
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+

Table 5,6 showed the survival period of *G. reessii* and *Cephaliophora irregularis*. At 11ºC temperature both fungi survived up to sixth testing in all culture conditions. At room temperature, *G. reessii* survived up to IInd testing in all three culture conditions and while *C. irregularis* in SDA medium. Likewise *C. tropicum* survived up to sixth testing at 11ºC in all three culture conditions and culture room temperature in unsterilized soil (Table 7). While at room temperature fungus recovered up to IInd testing. At 25± 2ºC temperature *C. tropicum* survived up to IVth testing in bait testing methods.

Table 5. Longevity testing of *Gymnoascus ressii.*

Gymnoascus ressii	**Ist test**		**IInd test**		**IIIrd test**		**IVth test**		**Vth test**		**VIth test**	
	BT	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**
Unsterilised soil												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	-	+	-	-	-	-	-	-
(iii) 11 ± 1°C	+	+	+	+	+	+	+	+	+	+	+	+
Sterilised soil												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+
SDA medium												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	-	+	-	-	-	-	-	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+

Table 6. Longevity testing of *Cephaliophora irregularis.*

Cephaliophora irregularis	Ist test		IInd test		IIIrd test		IVth test		Vth test		VIth test	
	BT	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**
Unsterilised soil												
(i) Room temperature	+	+	+	+	-	+	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	-	+	-	-	-	-	-	-
(iii) 11 ± 1°C	+	+	+	+	+	+	+	+	+	+	+	+
Sterilised soil												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	-	+	-	-	-	-	-	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+
SDA medium												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+

Table 7. Longevity testing of *Chrysosporium tropicum.*

Chrysosporium tropicum	Ist test		IInd test		IIIrd test		IVth test		Vth test		VIth test	
	BT	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**	**BT**	**DSS**
Unsterilised soil												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	+	+	+	+	+	+	+	+
(iii) 11 ± 1°C	+	+	+	+	+	+	+	+	+	+	+	+
Sterilised soil												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	+	-	+	-	-	-	-	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+
SDA medium												
(i) Room temperature	+	+	+	+	-	-	-	-	-	-	-	-
(ii)Culture room temperature	+	+	+	+	+	-	+	-	-	-	-	-
(iii) 11±1°C	+	+	+	+	+	+	+	+	+	+	+	+

DISCUSSION

Survival studies were carried out on seven most commonly reported dermatophytic and keratinophilic fungi of Jaipur city. Majority of human and animal pathogenic fungi are existing in soil as soil saprophytes and gain entrance in host through inhalation, penetration, abrasion and implantation. Jaipur or Pink city which also known as Paris of India, is the capital of Rajasthan. These fungi were reported frequently during last 25-30 years of survey studies of Jaipur city [5,12,22-24]. *T. rubrum* and *T. tonsurans* are anthropophilic fungi causing dermatophytosis in human beings frequently reported from tinea patients of Jaipur [12]. Climatic conditions during monsoon season favour the growth and sporulation of these fungi. During present studies all most all fungal spores showed viability up to two years at 11 ± 1 °C temperature in all the three storage conditions. At low temperature condition rate of fungal deterioration get reduce. At this temperature, the fungi stored after sub-culturing, so it was found that low temperature condition favoured the longevity of fungi in different soil types and media. During comparative analysis of sterilised and unsterilised soil for fungal survival, it was interesting to observed that unsterilised soil was found to be more suitable for all tested fungi as compared to sterilised soil. *M. gypseum* reported up to sixth testing in culture room condition and up to fifth testing in room temperature condition. *M. gypseum* frequently occurs wherever keratinaceous material is deposited [7-8,25]. Therefore *M. gypseum* appears to have more general distribution pattern in soil. While *C. tropicum* survived up to forth testing through bait testing technique. DSS method was failed to recovered both in third and forth testing. *C. tropicum* was reported as most predominant keratinophilic fungi of Jaipur district. This fungus was reported almost all habitats around the Jaipur. Deshmuch and Verekar [26] also reported frequent distribution of *M. gypseum* and *C. tropicum* in Maharashtra. The present observations agree with the opinion of Alteras [18] that the test fungi can survive for longer periods in soil that is unsterilised as compared to sterilised soil where there are no micro-organisms and the organic content also gets destroyed by sterilisation of soil, making it difficult for the micro-organism to live for longer period in sterilised soil. Grin and Ozegovic [17] showed that anthropophilic and zoophilic fungi are normally lysed and destroyed by micro-organisms present in the natural soil. In striking contrast to their observation, the present studies showed that dermatophytes and related keratinophilic fungi, when placed on unsterilised soils, survive and proliferate there with ease. *T. tonsurans* showed a longer survival period followed by *T. rubrum*. Both fungi

were collected from infected skin scrapping of tinea patients. These anthropophilic strains showed a longer survival period in soil conditions. According to Deshmukh [27] soil inhabiting keratinophilic fungi like *M. gypseum*, *Chrysosporium* species and *Trichophyton* group fungi are generally stable and survive in soil up to 7 year without any deterioration. Soil culture method is very good technique for the long time preservation of *M. gypseum.*

Cephaliophora irregularis is commonly isolated from Rajasthan university campus soil [5]. This fungus survived up to two year only at 11ºC temperature. At room temperature and culture room temperature on SDA it survived only up to eight months. While in sterilized and unsterilized conditions, it recovered upto IIIrd testing only through bait testing.

CONCLUSION

The frequently occurrence of these fungi in different localities of Jaipur district along with a longer survival period in different climates concluded that Jaipur city potentially has a high threat for causing cutaneous fungal infections in humans and animals and could be considered as a source of these infections. There longer survival and infection rate suggested modification and adaptability of fungal pathogen with diverse climatic conditions of Jaipur. Fungi also acquire regulatory and sensory mechanism for encourage continued existence under the most recent environmental circumstances.

Conflict of Interest: Author declare no conflict of interest.

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