



Determination of Optimal Temperature and pH for Radial Growth of Some Dermatophyte Species Isolated from Leukemia Patients

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Abstract

The study is concern on determine the effect of different temperatures (25, 28, 30 and 37°C), and different pH values (4.5, 5.5, 6 and 8) on the radial growth (mm) of 15 dermatophyte isolates (*Microsporum canis* 7, *Trichophyton rubrum* 5, *Trichophyton mentagropyhtes* 3). The specimens for the current study were collected from nail infections in patients with different type of leukemia whom admitted at Baghdad Educational Hospital, 7th floor. The result revels that the optimum temperature for radial growth was 30°C then 28°C for all isolates, while the optimum pH for all isolates was 6.

Keywords: Immunocompromised, patients, leukemia, dermatophytes.

تحديد درجة الحرارة والاس الهيدروجيني المثلى للنمو الفطري لبعض عزلات الفطريات الجلديه المعزوله من مرضى اللوكيميا

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الخلاصة:

هدف الدراسة هو تحديد تأثير درجات حراريه مختلفه تتراوح من (25, 28, 30, 37°C), وقيم مختلفه للاس الهيدروجيني(4.5, 5.5, 6 و 8) على التوالي على النمو الفطري ل 15عزلة من الفطريات الجلدية , (*Trichophyton mentagropyhtes* 3 و *Trichophyton rubrum* 5 *Microsporum canis* 7). جميع العينات لهذا البحث جمعت من اطافر مصابة من مرضى اللوكيميا بانواعه المختلفه الراقدين في الطابق السابع/ امراض الدم/ مستشفى بغداد التعليمي. اوضحت النتائج ان درجة الحراره المثلى للنمو الفطري للفطريات الجلديه كانت 30°C ثم 28°C لجميع العزلات, بينما الاس الهيدروجيني الامثل لجميع العزلات كان 6.

Introduction:

Dermatophytes are a group of morphologically and physiologically related molds that cause well-defined infections in vertebrates. The incidence of dermatophytoses has increased over recent years, particularly in immunocompromised patients [1-4].

Dermatophytes have acquired in the evolutionary process the ability to metabolize and subsist upon keratin, a protein resistant to most other organisms. The fungi attack skin, nails, and hair, where keratin is the major structural protein, leading to a wide variety of disease states. [5], dermatophytes include fungi that involve keratinized tissue" hair, skin, nails and mucous membrane ". Dermatophytes species are identified according to the features that produced when they grow on a suitable agar medium, and their classification in to three genera is based on the shape of macro conidia:

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Epidermatophyton (pyriform, rough walls), *Trichophyton* (cylindrical, smooth walls), *Microsporum* (fusiform, rough walls) [6]

Dermatophytes may be described as anthropophilic, zoophilic or geophilic depending upon whether their normal habitat is on man, or an animal, or in the soil. [6].

Anthropophilic dermatophytes are restricted to human hosts and produce a mild or chronic inflammation. This type of infection represents a preference of a parasite or dermatophytes for humans over other animals. [7]. Most common fungi that belong to this group are *T. rubrum*, *T. tonsurans* and *T. violaceum* [6].

Zoophilic dermatophytes occurring primarily in animals may be different transmissible to man and produce human disease; such zoophilic fungi should be considered a possible cause of skin lesions of unclear origin. Several species including *T. verrucosum*, *T. mentagrophytes* and *M. canis* may infect human skin, causing a variety of signs and symptoms. People who have close contact to infested cattle or cats are more often exposed to fungal infections. Certain professions, such as farmers, and children are especially vulnerable. [8]

Geophilic dermatophytes includes 12 dermatologically relevant *Microsporum* species. The most frequent species that cause geophilic dermatophytes infections are *M. canis*, *M. audouinii* and *M. gypseum*. [9]. Taxonomically, dermatophytes includes 39 closely related species belonging to the three main genera; *Epidermatophyton*, *Microsporum* and *Trichophyton* within the family Arthrodermataceae. [10].

One of the most important considerations when evaluating superficial dermatophytes infections is confirming the diagnoses with a laboratory specimen, either by using potassium hydroxide (KOH) 10% preparation for direct examination, or culture by using appropriate culture media

Materials and methods:

Sample collection: During the period that confined between November 2013 to March 2014, 48 specimens (nail clippings) were collected from 105 patients with different types of leukemia who diagnosed clinically by specialist doctors, nail clippings were examined directly by microscopic examination and then they were cultured on plates with SDA medium, from 10-14 days

Isolation of dermatophytes: The total number of dermatophytes isolates was 15 and the rest was diagnosed as opportunistic fungi. The frequency of each dermatophytes species according to the age and gender of leukemia patients is listed as follows: table (1).

The experiments in current study were restricted to the most frequent species which include the following species: *M. canis* 7 isolates, *T. rubrum* 5 isolates and *T. mentagrophytes* 3 isolates.

Determination of optimal temperature for radial growth of dermatophytes:

Temperature affects whole dynamic events in the cell directly through its impact on the genetic material, enzymes, and lipids of the cell membrane and then its impact on speed of growth rate [11]. All the vital activities in the cell are affected by heat and the determination of optimum temperature for fungal growth is occur by a range of factors, which affect all cellular reactions involving; enzymes and proteins [12].

Determination of optimal pH for radial growth of dermatophytes:

Micro-organisms normally regulate the pH concentration within the cells according to the external pH concentration by ion exchange method across cell membrane, the regulation of internal pH in the cell occurs by transport system of protons through cytoplasmic membrane, as well as, ion exchange H^+ / K^+ , both ways require ATP as a source of energy, and helps the regulation of pH in the cells of micro-organisms which prefer neutral pH (neutrophils) [12].

Statistical analysis:

The statistical test which used in this study was the ANOVA test, $p < 0.05$.

For the 1st experiment, statistical analysis had shown significant difference at a level of probability $P < 0.05$ between different degrees of temperature which were used to measure the diameter of radial growth of fungal colonies for the following species, *T. mentagrophyte*, *T. rubrum* and *M. canis*. For the 2nd experiment, statistical analysis had shown significant difference at a level of probability $P < 0.05$ between different pH concentrations which were used to measure the diameter of radial growth of fungal colonies for the same species.

Results and discussion:**Table 1-** Fungal isolates from infected nails with dermatophytes species.

Age group of patients	Fungus name	Male	Percentage %	Female	Percentage %	Total No.	Total Percentage
17-34	<i>M. canis</i>	3	20	4	26.67	7	46.67
35- 52	<i>T. rubrum</i>	2	13.33	3	20	5	33.33
53-80	<i>T. mentagrophyte</i>	1	6.67	2	13.33	3	20
	Summation	6	40	9	60	15	100

There are numerous species of anthropophilic dermatophytes, including *T. rubrum*, *T. mentagrophytes* var. *interdigitale*, and zoophilic dermatophytes like *M. canis* has been involved occasionally in immunosuppressed patients [13]. The incidence of dermatophytoses has increased over recent years, particularly in immunocompromised patients [2, 14]. The increase in frequency of these infections has been attributed to several factors, including high-dose chemotherapy, oropharyngeal-mucositis and prophylactic therapy [15]. All the above findings are agreed with this study.

The number and percentage of infected individuals had been registered in table 1, according to their genders and age. Statistical analysis had shown significant difference at a level of probability P (< 0.05) between study and control groups related to genders and age.

It had been found that the age groups (17-25, 44-52) years were the most susceptible ages for fungal infection in females (study group), and the age (17-25) the most susceptible ages for fungal infection in males. This result was agreed with other researchers including [16- 17], who concluded that Females are more susceptible for fungal infections than Males. And disagreed with [18] who concluded that there was no significant difference in infection between males & females.

The first experience (Determination of optimal temperature) had shown that the optimum temperature for radial growth of *T. mentagrophyte* was 30°C, 28°C, optimum temperature for radial growth of *T. rubrum* was 30°C, and finally for *M. canis* was 37°C figure 1.

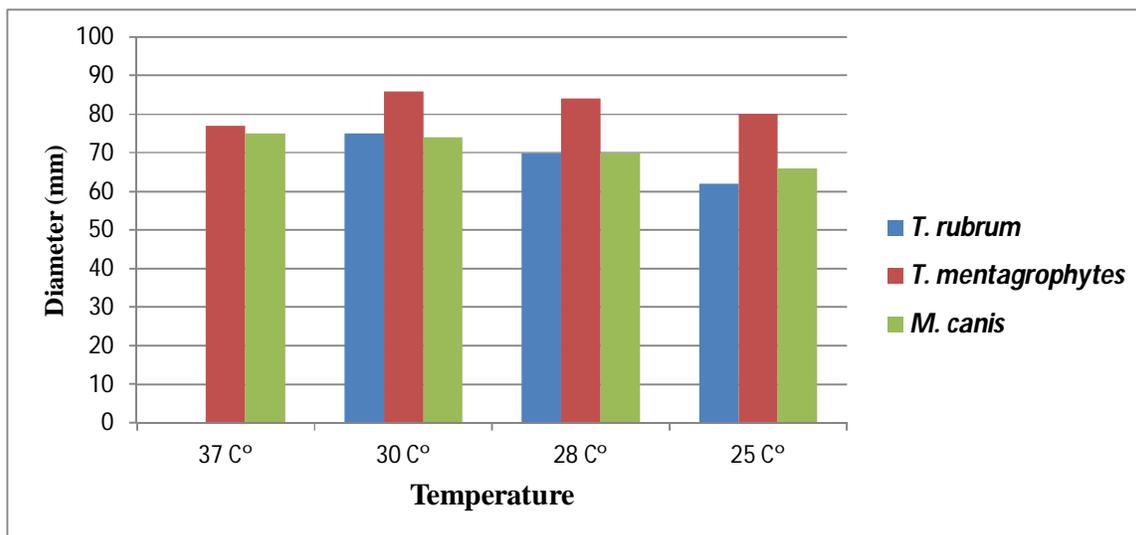


Figure 1- The effect of different temperatures on the radial growth of dermatophytes colonies on SDA medium, for (10-14 days).

The findings were described in this study represent the average of three replicates +/- standard error. The results of this study were agreed with [19] who stated that the optimum temperature for growth of dermatophytes ranged (25-33)°C since the current result is within the range. Also there was agreement

with [20] who concluded that the optimum temperature for growth of dermatophytes was ranged (30, 33)⁰C, and there was agreement with [21] who concluded that mycelial growth is little at 37⁰C, and the sporulation is optimum at 25-30⁰C. The results of this study were disagreed with [22] who found that there were no differences in the effect of temperatures (30, 35)⁰C on the growth of dermatophytes associated with the genus *Trichophyton*.

The second experience (Determination of optimal pH) had shown that the optimum pH for radial growth of *T. mentagrophyte*, *T. rubrum*, and finally for *M. canis* was 6 figure 2.

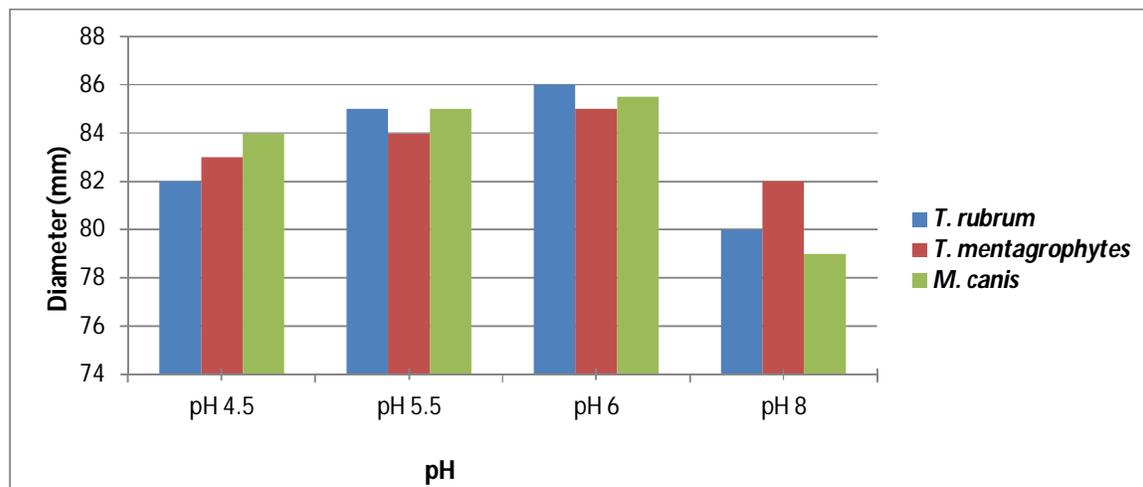


Figure 2- The effect of different pH values on the radial growth of dermatophytes colonies on SDA medium, for (10-14) days.

The different pH values affect the vital activities, which are necessary for fungal growth. The optimum pH for growth is varied with various fungal species; the pH suitable for the *in vitro* growth of fungi is optimum at pH 5 or 6 [23] This result was agreed with the findings of this study, also there was agreement with [20] who concluded that the optimum pH for growth was 6. It was clear from the results that the diameter of colonies for all the fungal isolates were decline when pH concentration is raised to 8, this observation was agreed with [24] who found that the length of hyphae of some fungi became shorter when pH concentration was increased from 6 to 8.

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