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# Using Ultraviolet-A Radiation and Antifungals for Treatment of Dermatophytes Isolated from Cattle and Farm Workers

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**Dermatophytes are classified into 3 genera of fungi that commonly involve keratins tissue types; skin, nails and hairs in mammals. Ultraviolet radiation is used in the treatment of these fungi which cause DNA damage leading to inactivation of the pathogenic fungus. The present study aims to evaluate the ultraviolet (UV) radiation influence on the dermatophytes lesion growth that attacks humans and animals. Thirty isolates of dermatophytes were isolated from animals (skin scraping) and humans (skin scraping, hair and nail). Five species were identified viz., Trichophyton mentagrophyte, Trichophyton interdigitale, Trichophyton tonsurans, Trichophyton violaceum and Microsporum canis. UV-A, was found to be more active in delaying the dermatophytes colony growth in some current fungi widespread when combined with antifungal therapy to treat skin superficial fungal diseases, which caused dermatophytes lesion in humans and animals. The present approach involving a combination of antifungal drugs and UV-A to treat apparent dermatophytes diseases could reduce the therapeutic period and dosages which improves patients compliance.**

***Keywords:*** *T. interdigitale, DNA, Ultraviolet-A, antifungal susceptibility, PCR*

## Introduction

Dermatophytes are pathogenic fungi that infect the skin, hair, and nails of humans and animals. The organisms colonize the keratin tissues and cause superficial infections known as dermatophytosis in both animals and humans [1]. The disease course of dermatophytosis is distinctive because there no living tissue is oppressed and the keratinised stratum corneum is simply settled. These fungus and its metabolic products commonly induce an inflammatory and allergic eczema in both animals and humans [2].

The zoophilic (adapted to animals) and geophilic (usually alive in the environment) species are occasionally spread from animals to humans. The anthropophilic specie that is acclimatized to humans may be uncommonly transmitted from persons to animals [3]. The dermatophytes spores (conidia) are the infective stages for humans and animals [4].

Usually, dermatophytes are growing from asexual spores that develop within the fungal hyphae [5]. The dermatophytes infect the developing hair shaft or the skin stratum corneum. These fungi do not usually attack the inactive hairs because, their vital

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and essential nutrients need for growing are deficient or limited [6]. Hyphae of dermatophytes propagate in the hairs and keratin layer of the skin, and ultimately develop infectious arthrospores [7].
Fungal infection of the nail (onychomycosis) is a common disease. Several predisposing heterogeneous factors are also associated with enormous population groups [8]. Onychomycosis is severe health issue and is classified into, superficial white onychomycosis (infection is limited to a pit of the nail surface) and invasive subungual dermatophytes (the lateral, distal or proximal ends of the nail plats are firstly invades then, followed by the underneath of the nail plate) [9]. The distal lateral subungual onychomycosis is the most serious form of the invasive subungual dermatophyte [10].
UV-radiation persuades DNA-damage leading to the inactivation of the pathogenic fungi [11]. UV-A with long wavelength rays has a sluggish influence on the skin [12]. UV-A radiation of long wavelength (315 - 400 nm) seem to be effective in decreasing colony growth of the most prevalent fungi, which caused onychomycosis [13]. Moreover, high doses of UV-A radiation have significant inhibitory effects on dermatophytes [14]. The agar-based disk diffusion (ABDD) method used for diagnosis of dermatophytes is a rapid, easy and a perspective technique [15]. The present work was planned to explore the fungicidal effect and the ability of UV-A radiation to inactivate dermatophyte suspensions growth *in vitro*, and to study the synergistic effect of UV-A radiation with antifungal drugs on locally isolated dermatophyte.

## Materials and Methods

*Case history*

On January and March 2017, dairy buffaloes and cows and farm workers in contact with affected animals that have skin lesions in Dakahlia and Sharqia Governorates. After conforming the animal's skin lesions clinically and laboratory, the farm workers who have extensive lesion all over the body and hands were chosen. The examination revealed that affected animals have main clinical current symptoms which were extensive grey-white crust lesion spread on the body. The disease occurrence prevails at a humid cold to mild weather.

*Collection of samples*

Seventy two samples of skin scraping, hair and nail were collected from infected buffaloes and cows and farm workers in contact with the affected animals which have clinical manifestations of dermatophytosis.

*Isolation and identification of dermatophytes*

Skin scratching, hair samples and nail plate and nail bed fragments were cultivated onto Sabouraud dextrose agar plus cycloheximide and chloramphenicol, Sigma-Aldrich (Germany) and incubated at 30°C for 14 days [16]. Morphological examination of isolated species colony (size, colonies colours and growth rate) were detected and identified. Dermatophyte species were identified microscopically by using 10% KOH and diagnostic methods such as growth on Sabouraud Dextrose Agar with 5% Salt, Bromcresol purple milk solids glucose agar [16].

*Molecular identification of dermatophytes*

The DNA strains genomic was achieved using DNA Extraction Kit (Gene JET Genomic-DNA purification Kit, Thermo scientific Co., Lithuania) according to manufacturer's instructions pamphlet. Spectrophotometric determination of the DNA concentration was estimated at 260-230 nm. The PCR readers used for identification of *T. mentagrophyte* and *M. canis* are Forward ITS15´-TCCGTAGGTGAACCTGCGG-3´ and reverse primer ITS4 was5´-TCCTCCGCTTATTGATATG3´. Thermal cycling: The DNA thermal cycler was set according to the following protocol: The initial denaturation cycle proceeds 6 minutes at 95˚C, denaturation cycle (25 cycles) consumed 30 seconds at 95˚C, then 35 cycles of 1 minute at 55˚C and 35 cycles of 1 minute at 72˚C. The final cycle of 10 minutes at 72˚C according to [17]. The amplified PCR yields were electrophoresed in electrophoresis using 1.5% agarose gel and ethidium bromide stain (used as fluorescent dye). The intensified DNA is compared to molecular weight markers (Gene Ruler 100 bp plus DNA ladder) than photographed. The amplified products were purified using Gene JET PCR purification kit (USA) and were sequenced by Chromogen Co., Germany [18]. Every strain was sequenced in both directions, analysed and aligned through Clustal method using DNAstar program, Laser-gene, Wisconsin (USA).

*Determination of the different doses of UV-A light as fungicidal on isolated fungi*

Freshly prepared three isolates (two of *T. mentagrophyte* and one *M. canis)* mycelia clusters were collected and suspended in broth (Sabouraud dextrose) and mycelial suspension were counted

and adjusted to $10^5$ spores/ ml. From each dermatophyte spores suspension, a 10 ml was injected onto a petri dish. UV-A (320-400 nm); 2,5,7,10 & 12 J/cm$^2$. One positive control sample (not exposed to UV-A) was inoculated onto each round. After UV-radiation, the fungicidal effect of UV-A was evaluated by 5 cultures for each fungus which was prepared and incubated at $30^0$C under laboratory conditions.

*Determination of UV-A and antifungal drugs combination on isolated fungi*

Standard concentrate for all antifungal drugs were dissolved in precise solvents and over loaded on blank paper disks (A sterile filter paper of 0.5cm diameter) at the following potencies: Miconazole (10µg/disc), Fluconazole (25µg/disk), Terbinafine (30µg/disk), Itraconazole (8µg/disc) and Griseofulvin (10µg/ disc), Rosco Diagnostica Co., Denmark. The irradiated dermatophyte mycelial suspensions were inoculated on SDA and then antifungal disks were placed on the inoculated agar with a forceps and incubated at $30^0$C for 5-10 days. Then, after growth of the colonies, the inhibition zones were measured and recorded [19&20].

**Results and Discussion**

The results in Table (1) **r**evealed that out of 72 samples (25 animal and 47 human samples) from infected cattle and diseased human, fifty three samples were positive for dermatophytosis by KOH examination (cattle and human). But in culturing on SDA 12 (63.2%), 7 (46.7) were positive for dermatophytes in animals samples (skin scraping and hair), while human samples (skin, hair and nail scraping) were 2 (20%) and 17 (77.3%) positive for dermatophytes, respectively. According to macro-morphological and physiological characters, thirty dermatophytes were isolated from animal (skin scraping) and human (skin scraping, hair and nail) which identified into 4 species, *T. mentagrophyte, T. tonsurans, T. violaceum* and *M. canis*, Table (2). This results comes in the line with Eman-abdeen and El-Diasty [21].

According to obtained data of the UV-A treatments, compared with control samples in Table (3), UV-A radiation can affect colony count changed of *T. interdigitale* and *M. canis* in the culture. Colony count of *T. interdigitale* and *M. canis*decreased as the dose of UV-A radiation increased. Furthermore, a very high doses of UV-A do not inhibit the isolated fungi samples completely [22].

The results recorded in Table (4) showed that Terbinafine, Itraconazole and Griseofulvin were more effective than Fluconazole and Miconazole in inhibiting, *T. interdigitale*, while *M. canis* showed high sensitivity for Griseofulvin and Terbinafine. Current studies indicated that Fluconazole and Miconazole had less effect in control dermatophytes [23]. The combination of UV-A and drug schemes is well recognised in dermatophytes, predominantly in treatment of attack initiated by dermatophytes. Interestingly, treatment of spore suspensions using UV-A combination with antifungal drugs (Fluconazole, Terbinafine, Miconazole, Itraconazole and Griseofulvin) considerably reduced time of exposure necessary to reach virtuous inhibition as compared to UV-A alone [24].

Two strains of *T. mentagrophyte* and one strain of *M. Canis* were examined by molecular methods polymerase chain reaction (PCR) with using common primer (ITS) were positive on agarose gel electrophoresis Figure (1).

Molecular approach has been developed as an accurate dermatophytes identification tool compared to morphological basis method. These methods are PCR and ITS fragment length polymorphism [25]. As for molecular identification in the present study, PCR as well as DNA sequencing were used, while PCR technique confirmed the identification of three isolates using ITS 4 region PCR products obtained at 550 bp Figure (2).

Nucleotide analysis in Figure (2&3) showed greater than 98.3% similarity for the entire sequence of the one sequenced strain EGMMM/Egy2016 accession no. KX344464 in comparison of 12 strains of *T. interdigitale*. The sequence similarity for the strains of *T. interdigitale*-ATCC24952, *T. interdigitale*-CBS-558.66, *T. interdigitale*-IHEM1729, *T. interdigitale*-ATCC24957 and *T. interdigitale*-ATCC.MYA-310 were 99.1%, 99.1%, 99.0%, 99.4% and 99.3%. *T. interdigitale*-EGMMM/Egy2016 accession no. KX344464 strain is freely notable compared to those of the other species by the fifteen-base inclusion (TTTCCGTAAGGGGGG) at nucleotides 1–15, and their characteristic nucleotide changes (T,A,A,C,A,A,G,G,G,T) at the next points 13,15,35,201,332,504,598,599,600 and 633. Some of these variations result in amino acid changes.

**Table (1): Occurrence of dermatophytes in examined clinically diagnosed animal and farm workers**

| Samples | Animals | | | | Humans | | | |
|---|---|---|---|---|---|---|---|---|
| | Microscopic examination by using10% KOH | | Culture examination | | Microscopic examination by using10% KOH | | Culture examination | |
| | No. | % | No. | % | No. | % | No. | % |
| **Skin scraping** | 16/19 | 84.2 | 12/19 | 63.2 | 10/15 | 66.7 | 7/15 | 46.7 |
| **Hair** | 2/6 | 33.3 | 0/6 | 0 | 5/10 | 50 | 2/10 | 20 |
| **Nail scraping** | - | - | - | - | 20/22 | 90.9 | 17/22 | 77.3 |
| Total | 18/25 | 72 | 12/25 | 48 | 35/47 | 74.5 | 26/47 | 55.3 |

**Table (2): Dermatophytes isolated from animals and Humans**

| Isolated strain | Humans (farm workers) | Animals |
|---|---|---|
| **Dermatophytes:** | | |
| *Trichophyton mentagrophyte* | 10 | 8 |
| *Trichophyton tonsurans* | 2 | - |
| *Trichophyton violaceum* | 1 | 3 |
| *Microsporumcanis* | 6 | - |
| **Nondermatophytes:** | | |
| Moulds | 4 | 1 |
| Yeasts | 2 | - |

**Table (3): Mean colony count of UV-A on Trichophyton interdigitale and Microsporum canis**

| Microorganism | Mean colony count of UV-A | | | | | |
|---|---|---|---|---|---|---|
| | 0J/cm | 2J/cm | 5J/cm | 7J/cm | 10 J/cm | 12 J/cm |
| ***T. interdigitals*** | 100000 | 89776 | 78954 | 69875 | 63053 | 58962 |
| ***M. canis*** | 100000 | 87400 | 65423 | 45400 | 23056 | 13456 |

**Table (4): Combination treatments with UV-A and antifungal drugs (In *vitro*)**

| Antifungal | *Trichophyton interdigitale* | | | | | *Microsporum canis* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Zone diameter in mm | | | | | | | | | |
| | 2 J/cm | 5 J/cm | 7 J/cm | 10 J/cm | 12 J/cm | 2 J/cm | 5 J/cm | 7 J/cm | 10 J/cm | 12 J/cm |
| Fluconazole | 28 | 30 | | | | 20 | 25 | | | |
| Terbinafine | 35 | 37 | Very sensitive* | | | 30 | 30 | Very sensitive* | | |
| Miconazole | 29 | 30 | | | | 25 | 30 | | | |
| Itraconazole | 27 | 35 | | | | 18 | 25 | | | |
| Griseofulvin | 26 | 31 | | | | 32 | 33 | | | |

*Very sensitives = zone wide not to be detected

**Fig. (1): Single PCR performed with genomic DNA, Lane 1: 100 bp DNA ladder, Lane 2-3 *T. mentagrophyte*, Lane 4: *M. Canis***

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Alignment Report of EGMMM-Egy2016 - ITS gene with other dermatophytes

Majority                                  TTTCCGTA--GGTGAACCTGCGGAAGGATCATTAGCGCGCAGGCCGGAGGCTGGCCCCCC
                                          ---------+---------+---------+---------+---------+---------+
                                                  10        20        30        40        50        60
                                          ---------+---------+---------+---------+---------+---------+
EGMMM-Egy2016                             TTTCCGTAAGGGGGGACCTGCGGAAGGATCATTAGCGCGCAGGCCGGAGGCTGGCCCCCC   60
Trichophyton_interdigitale-RCPF1301       ........--..T.A.............................................   58
Trichophyton_interdigitale-ATCC24952      ........--..T.A.............................................   58
Trichophyton_mentagrophytes-ATCC.MYA-44   ........--..T.A.............................................   58
Trichophyton_krajdenii-UAMH8546           ........--..T.A.............................................   58
Trichophyton_interdigitale-ATCC.MYA-310   ........--..T.A...................A.........................   58
Trichophyton_interdigitale.IHEM1729       ........--..T.A.............................................   58
Trichophyton_interdigitale-CBS-558.66     ........--..T.A..-..........................................   57
Trichophyton_interdigitale-ATCC24957      ........--..T.A...................A.........................   58
Trichophyton_mentagrophytes               ........--..T.A...................A.........................   58
Trichophyton_interdigitale-strain-CBS42   ........--..T.A...................A.........................   58
Trichophyton_cf.mentagrophytes-ATCC4695   ........--..T.A...................A.........................   58
Trichophyton_interdigitale-SSU            ........--..T.A...................A.........................   58

Majority                                  ACGATAGGGCCAAACGTCCGTCAGGGGTGAGCAGATGTGCGCCGGCCGTACCGCCCCATT
                                          ---------+---------+---------+---------+---------+---------+
                                                  70        80        90       100       110       120
                                          ---------+---------+---------+---------+---------+---------+
EGMMM-Egy2016                             ACGATAGGGCCAAACGTCCGTCAGGGGTGAGCAGATGTGCGCCGGCCGTACCGCCCCATT   120
Trichophyton_interdigitale-RCPF1301       ............................................................   118
Trichophyton_interdigitale-ATCC24952      ............................................................   118
Trichophyton_mentagrophytes-ATCC.MYA-44   ............................................................   118
Trichophyton_krajdenii-UAMH8546           ............................................................   118
Trichophyton_interdigitale-ATCC.MYA-310   ............................................................   118
Trichophyton_interdigitale.IHEM1729       ............................................................   118
Trichophyton_interdigitale-CBS-558.66     ............................................................   117
Trichophyton_interdigitale-ATCC24957      ............................................................   118
Trichophyton_mentagrophytes               ............................................................   118
Trichophyton_interdigitale-strain-CBS42   ..........................-.................................   117
Trichophyton_cf.mentagrophytes-ATCC4695   ............................................................   118
Trichophyton_interdigitale-SSU            ............................................................   118

Majority                                  CTTGTCTACATTACTCGGTTGCCTCGGCGGGCCGCGCTCTCCCAGGAGAGCCGTTCGGCG
                                          ---------+---------+---------+---------+---------+---------+
                                                 130       140       150       160       170       180
                                          ---------+---------+---------+---------+---------+---------+
EGMMM-Egy2016                             CTTGTCTACATTACTCGGTTGCCTCGGCGGGCCGCGCTCTCCCAGGAGAGCCGTTCGGCG   180
Trichophyton_interdigitale-RCPF1301       ............................................................   178
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Trichophyton_interdigitale-ATCC24952          ............................................................   178
Trichophyton_mentagrophytes-ATCC.MYA-44       ............................................................   178
Trichophyton_krajdenii-UAMH8546               ............................................................   178
Trichophyton_interdigitale-ATCC.MYA-310       ............................................................   178
Trichophyton_interdigitale.IHEM1729           ............................................................   178
Trichophyton_interdigitale-CBS-558.66         ............................................................   177
Trichophyton_interdigitale-ATCC24957          ........C...................................................   178
Trichophyton_mentagrophytes                   ........C...................................................   178
Trichophyton_interdigitale-strain-CBS42       ............................................................   177
Trichophyton_cf.mentagrophytes-ATCC4695       ........C................................T..................   178
Trichophyton_interdigitale-SSU                ........C................................T..................   178

Majority                                      AGCCTCTCTTTAGTGGCTAAACGCTGGACCGCGCCCGCCGGAGGACAGACGCAAAAAAAT
                                              ---------+---------+---------+---------+---------+---------+
                                                      190       200       210       220       230       240
                                              ---------+---------+---------+---------+---------+---------+
EGMMM-Egy2016                                 AGCCTCTCTTTAGTGGCTAAACGCTGGACCGCGCCCGCCGGAGGACAGACGCAAAAAAAT   240
Trichophyton_interdigitale-RCPF1301           ............................................................   238
Trichophyton_interdigitale-ATCC24952          ............................................................   238
Trichophyton_mentagrophytes-ATCC.MYA-44       ............................................................   238
Trichophyton_krajdenii-UAMH8546               ............................................................   238
Trichophyton_interdigitale-ATCC.MYA-310       ............................................................   238
Trichophyton_interdigitale.IHEM1729           ............................................................   238
Trichophyton_interdigitale-CBS-558.66         ............................................................   237
Trichophyton_interdigitale-ATCC24957          ............................................................   238
Trichophyton_mentagrophytes                   ............................................................   238
Trichophyton_interdigitale-strain-CBS42       ............................................................   237
Trichophyton_cf.mentagrophytes-ATCC4695       ..................C.........................................   238
Trichophyton_interdigitale-SSU                ..................C.........................................   238

Majority                                      TCTTTCAGAAGAGCTGTCAGTCTGAGCGTTAGCAAGCAAAAATCAGTTAAAACTTTCAAC
                                              ---------+---------+---------+---------+---------+---------+
                                                      250       260       270       280       290       300
                                              ---------+---------+---------+---------+---------+---------+
EGMMM-Egy2016                                 TCTTTCAGAAGAGCTGTCAGTCTGAGCGTTAGCAAGCAAAAATCAGTTAAAACTTTCAAC   300
Trichophyton_interdigitale-RCPF1301           ............................................................   298
Trichophyton_interdigitale-ATCC24952          ............................................................   298
Trichophyton_mentagrophytes-ATCC.MYA-44       ............................................................   298
Trichophyton_krajdenii-UAMH8546               ............................................................   298
Trichophyton_interdigitale-ATCC.MYA-310       ............................................................   298
Trichophyton_interdigitale.IHEM1729           ............................................................   298
Trichophyton_interdigitale-CBS-558.66         ............................................................   297
Trichophyton_interdigitale-ATCC24957          ............................................................   298
Trichophyton_mentagrophytes                   ............................................................   298
Trichophyton_interdigitale-strain-CBS42       ............................................................   297
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Trichophyton_cf.mentagrophytes-ATCC4695 ............................................................   298
Trichophyton_interdigitale-SSU          ............................................................   298

Majority                                AACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA
                                        ---------+---------+---------+---------+---------+---------+
                                                310       320       330       340       350       360
                                        ---------+---------+---------+---------+---------+---------+
EGMMM-Egy2016                           AACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGA   360
Trichophyton_interdigitale-RCPF1301     ............................................................   358
Trichophyton_interdigitale-ATCC24952    ............................................................   358
Trichophyton_mentagrophytes-ATCC.MYA-44 ............................................................   358
Trichophyton_krajdenii-UAMH8546         ............................................................   358
Trichophyton_interdigitale-ATCC.MYA-310 ............................................................   358
Trichophyton_interdigitale.IHEM1729     ............................................................   358
Trichophyton_interdigitale-CBS-558.66   ............................................................   357
Trichophyton_interdigitale-ATCC24957    ............................................................   358
Trichophyton_mentagrophytes             ............................................................   358
Trichophyton_interdigitale-strain-CBS42 ............................................................   357
Trichophyton_cf.mentagrophytes-ATCC4695 ............................................................   358
Trichophyton_interdigitale-SSU          ..............................A.............................   358

Majority                                ATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCG
                                        ---------+---------+---------+---------+---------+---------+
                                                370       380       390       400       410       420
                                        ---------+---------+---------+---------+---------+---------+
EGMMM-Egy2016                           ATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCCTGGCATTCCG   420
Trichophyton_interdigitale-RCPF1301     ............................................................   418
Trichophyton_interdigitale-ATCC24952    ............................................................   418
Trichophyton_mentagrophytes-ATCC.MYA-44 ............................................................   418
Trichophyton_krajdenii-UAMH8546         ............................................................   418
Trichophyton_interdigitale-ATCC.MYA-310 ............................................................   418
Trichophyton_interdigitale.IHEM1729     ............................................................   418
Trichophyton_interdigitale-CBS-558.66   ............................................................   417
Trichophyton_interdigitale-ATCC24957    ............................................................   418
Trichophyton_mentagrophytes             ............................................................   418
Trichophyton_interdigitale-strain-CBS42 ............................................................   417
Trichophyton_cf.mentagrophytes-ATCC4695 ............................................................   418
Trichophyton_interdigitale-SSU          ............................................................   418

Majority                                GGGGGCATGCCTGTTCGAGCGTCATTTCAGCCCC-TCAAGCCCGGCTTGTGTGATGGACG
                                        ---------+---------+---------+---------+---------+---------+
                                                430       440       450       460       470       480
                                        ---------+---------+---------+---------+---------+---------+
EGMMM-Egy2016                           GGGGGCATGCCTGTTCGAGCGTCATTTCAGCCCC-TCAAGCCCGGCTTGTGTGATGGACG   479
Trichophyton_interdigitale-RCPF1301     ..................................-.........................   477
```

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Trichophyton_interdigitale-ATCC24952          ..................................-.........................   477
Trichophyton_mentagrophytes-ATCC.MYA-44       ..................................-.........................   477
Trichophyton_krajdenii-UAMH8546               ..................................-.........................   477
Trichophyton_interdigitale-ATCC.MYA-310       ..................................-.........................   477
Trichophyton_interdigitale.IHEM1729           ..................................-.........................   477
Trichophyton_interdigitale-CBS-558.66         ..................................-.........................   476
Trichophyton_interdigitale-ATCC24957          ..................................-.........................   477
Trichophyton_mentagrophytes                   ..................................-.........................   477
Trichophyton_interdigitale-strain-CBS42       ..................................-.........................   476
Trichophyton_cf.mentagrophytes-ATCC4695       ..................................C.........................   478
Trichophyton_interdigitale-SSU                ..................................-.........................   477

Majority                                      ACCGTCCGGCGCCCCCGTCTTTGGGGGTGCGGGACGCGCCCGAAAAGCAGTGGCCAGGCC
                                              ---------+---------+---------+---------+---------+---------+
                                                      490       500       510       520       530       540
                                              ---------+---------+---------+---------+---------+---------+

EGMMM-Egy2016                                 ACCGTCCGGCGCCCCCGTCTTTGGGGGTGCGGGACGCGCCCGAAAAGCAGTGGCCAGGCC   539
Trichophyton_interdigitale-RCPF1301           ............................................................   537
Trichophyton_interdigitale-ATCC24952          ............................................................   537
Trichophyton_mentagrophytes-ATCC.MYA-44       ............................................................   537
Trichophyton_krajdenii-UAMH8546               ............................................................   537
Trichophyton_interdigitale-ATCC.MYA-310       ............................................................   537
Trichophyton_interdigitale.IHEM1729           ............................................................   537
Trichophyton_interdigitale-CBS-558.66         ............................................................   536
Trichophyton_interdigitale-ATCC24957          ............................................................   537
Trichophyton_mentagrophytes                   ............................................................   537
Trichophyton_interdigitale-strain-CBS42       ............................................................   536
Trichophyton_cf.mentagrophytes-ATCC4695       .T..........................................................   538
Trichophyton_interdigitale-SSU                ........................A...................................   537

Majority                                      GCGATTCCGGCTTCCTAGGCGAATGGGCAACAAACCAGCGCCTCCAGGACCGGCCGCCC-
                                              ---------+---------+---------+---------+---------+---------+
                                                      550       560       570       580       590       600
                                              ---------+---------+---------+---------+---------+---------+

EGMMM-Egy2016                                 GCGATTCCGGCTTCCTAGGCGAATGGGCAACAAACCAGCGCCTCCAGGACCGGCCGCCC-   598
Trichophyton_interdigitale-RCPF1301           ...........................................................-   596
Trichophyton_interdigitale-ATCC24952          ...........................................................-   596
Trichophyton_mentagrophytes-ATCC.MYA-44       ...........................................................-   596
Trichophyton_krajdenii-UAMH8546               ...........................................................-   596
Trichophyton_interdigitale-ATCC.MYA-310       ...........................................................-   596
Trichophyton_interdigitale.IHEM1729           ...........................................................-   596
Trichophyton_interdigitale-CBS-558.66         ...........................................................-   595
Trichophyton_interdigitale-ATCC24957          ...........................................................-   596
Trichophyton_mentagrophytes                   ...........................................................-   596
Trichophyton_interdigitale-strain-CBS42       .........................................................GGG   596
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```
Trichophyton_cf.mentagrophytes-ATCC4695 ..........................................................-      597
Trichophyton_interdigitale-SSU          ..........................................................-      596

Majority                                TGGCCTCAAAATCTGTTTTATACTTATCAGGTTGACCTCGGATCAGGTAGGGATACCCGC
                                        ---------+---------+---------+---------+---------+---------+
                                                610       620       630       640       650       660
                                        ---------+---------+---------+---------+---------+---------+
EGMMM-Egy2016                           TGGCCTCAAAATCTGTTTTATACTTATCAGGT-GACCTCGGATCAGGTAGGGATACCCGC      657
Trichophyton_interdigitale-RCPF1301     ................................T...........................      656
Trichophyton_interdigitale-ATCC24952    ................................T...........................      656
Trichophyton_mentagrophytes-ATCC.MYA-44 ................................T...........................      656
Trichophyton_krajdenii-UAMH8546         ................................T...........................      656
Trichophyton_interdigitale-ATCC.MYA-310 ................................T...........................      656
Trichophyton_interdigitale.IHEM1729     ................................T...........................      656
Trichophyton_interdigitale-CBS-558.66   ................................T...........................      655
Trichophyton_interdigitale-ATCC24957    ................................T...........................      656
Trichophyton_mentagrophytes             ................................T...........................      656
Trichophyton_interdigitale-strain-CBS42 ................................T...........................      656
Trichophyton_cf.mentagrophytes-ATCC4695 ................................T...........................      657
Trichophyton_interdigitale-SSU          ................................T...........................      656

Majority                                TGAACTTAAGCATAT
                                        ---------+-----
                                                670
                                        ---------+-----
EGMMM-Egy2016                           TGAACTTAAGCATAT                                                   672
Trichophyton_interdigitale-RCPF1301     ...............                                                   671
Trichophyton_interdigitale-ATCC24952    ...............                                                   671
Trichophyton_mentagrophytes-ATCC.MYA-44 ...............                                                   671
Trichophyton_krajdenii-UAMH8546         ...............                                                   671
Trichophyton_interdigitale-ATCC.MYA-310 ...............                                                   671
Trichophyton_interdigitale.IHEM1729     .....C.........                                                   671
Trichophyton_interdigitale-CBS-558.66   ...............                                                   670
Trichophyton_interdigitale-ATCC24957    ...............                                                   671
Trichophyton_mentagrophytes             ...............                                                   671
Trichophyton_interdigitale-strain-CBS42 ...............                                                   671
Trichophyton_cf.mentagrophytes-ATCC4695 ...............                                                   672
Trichophyton_interdigitale-SSU          ...............                                                   671
```

**Fig. (2): Nucleotides sequences alignment of Trichophyton interdigital**

Se[illegible] pair distances of Untitled ClustalW (Slow/Accurate, IUB)
[illegible]sda[illegible] [illegible]e 07, 2016 5:09 PM

Percent Identity (upper triangle) / Divergence (lower triangle)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ■ | 99.7 | 99.7 | 99.7 | 99.7 | 99.6 | 99.6 | 99.7 | 99.4 | 99.4 | 99.3 | 99.0 | 98.8 | 1 | EGMMM-Egy2016 |
| 2 | 0.3 | ■ | 100.0 | 100.0 | 100.0 | 99.9 | 99.9 | 100.0 | 99.7 | 99.7 | 99.6 | 99.3 | 99.1 | 2 | Trichophyton_interdigitale-RCPF1301 |
| 3 | 0.3 | 0.0 | ■ | 100.0 | 100.0 | 99.9 | 99.9 | 100.0 | 99.7 | 99.7 | 99.6 | 99.3 | 99.1 | 3 | Trichophyton_interdigitale-ATCC24952 |
| 4 | 0.3 | 0.0 | 0.0 | ■ | 100.0 | 99.9 | 99.9 | 100.0 | 99.7 | 99.7 | 99.6 | 99.3 | 99.1 | 4 | Trichophyton_mentagrophytes-ATCC.MYA-44 |
| 5 | 0.3 | 0.0 | 0.0 | 0.0 | ■ | 99.9 | 99.9 | 100.0 | 99.7 | 99.7 | 99.6 | 99.3 | 99.1 | 5 | Trichophyton_krajdenii-UAMH8546 |
| 6 | 0.4 | 0.1 | 0.1 | 0.1 | 0.1 | ■ | 99.7 | 99.8 | 99.9 | 99.9 | 99.7 | 99.4 | 99.3 | 6 | Trichophyton_interdigitale-ATCC.MYA-310 |
| 7 | 0.4 | 0.1 | 0.1 | 0.1 | 0.1 | 0.3 | ■ | 99.9 | 99.6 | 99.6 | 99.4 | 99.1 | 99.0 | 7 | Trichophyton_interdigitale.IHEM1729 |
| 8 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | ■ | 99.7 | 99.7 | 99.6 | 99.3 | 99.1 | 8 | Trichophyton_interdigitale-CBS-558.66 |
| 9 | 0.6 | 0.3 | 0.3 | 0.3 | 0.3 | 0.1 | 0.4 | 0.3 | ■ | 100.0 | 99.6 | 99.6 | 99.4 | 9 | Trichophyton_interdigitale-ATCC24957 |
| 10 | 0.6 | 0.3 | 0.3 | 0.3 | 0.3 | 0.1 | 0.4 | 0.3 | 0.0 | ■ | 99.6 | 99.6 | 99.4 | 10 | Trichophyton_mentagrophytes |
| 11 | 0.8 | 0.4 | 0.4 | 0.4 | 0.4 | 0.3 | 0.6 | 0.4 | 0.4 | 0.4 | ■ | 99.1 | 99.0 | 11 | Trichophyton_interdigitale-strain-CBS42 |
| 12 | 1.1 | 0.7 | 0.7 | 0.7 | 0.7 | 0.6 | 0.9 | 0.8 | 0.4 | 0.4 | 0.9 | ■ | 99.6 | 12 | Trichophyton_cf.mentagrophytes-ATCC4695 |
| 13 | 1.2 | 0.9 | 0.9 | 0.9 | 0.9 | 0.7 | 1.1 | 0.9 | 0.6 | 0.6 | 1.1 | 0.4 | ■ | 13 | Trichophyton_interdigitale-SSU |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | | |

**Fig. (3): Nucleotides identity of *Trichophyton interdigitale* sequences**

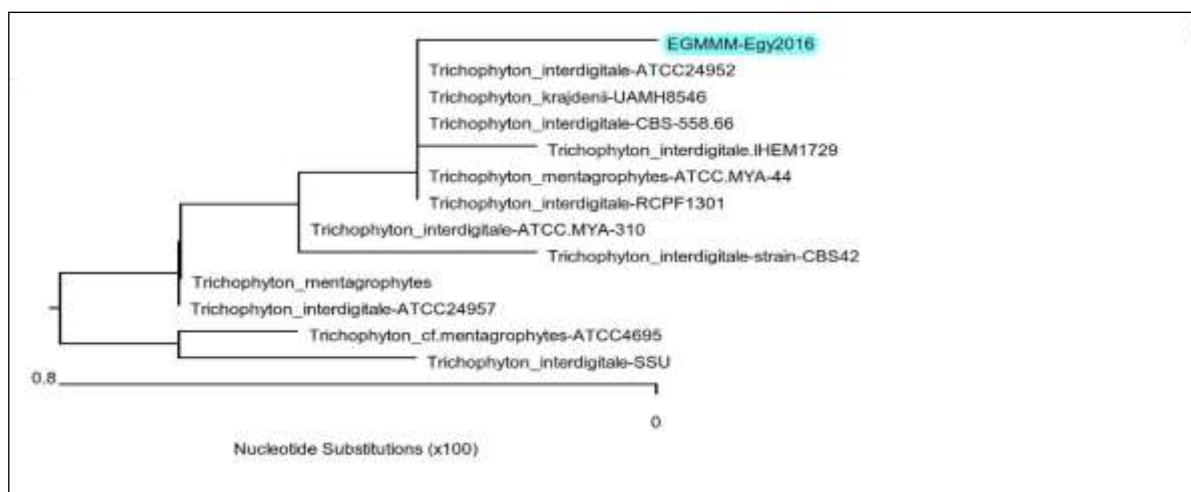


**Fig. (4): Phylogenetic tree of nucleotide sequences of *Trichophyton interdigitale***

DNA sequences for one isolate from human source (*T. mentagrophytes*) revealed identification of *T. mentagrophytes complex* which displayed higher similarities with the type strain *T.interdigitale* RCPF1301 compared with the neotype strain *Trichophyton cf mentagrophytes*-ATCC46950 (Figure 3), but the strain for *T.interdigitale* isolated from humans (isolate number "EGMMM/Egy2016" had a resemblance of 100 % with the sequence of the *T. interdigitale* strain RCPF1301. The obtained sequences were deposited at NCBI under accession no. (Gene bank accession number: KX344464). Phylogenetic trees were generated based on nucleotide sequence and the predicted amino acids sequences as shown in Figure (4). The topology of the neighbour-joining tree of the sequence is almost the same as that constructed from the nucleotide sequence.

## Conclusions

UV-A is efficient in lessening colony growing in some predominant fungi, which caused dermatophytosis in humans and animals. UV-A is well alternate to the present antifungal treatments for superficial fungal diseases, but in the proposed approach, the combination of antifungal drug and UV-A to treat fungal diseases provides effectiveness in decreasing treatment period and drug doses to improve patient compliance. Use of DNA-based methods is considered a significant assistance in monitoring progress in dermatophytes in the environment.

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