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Original Research Article

Isolation and Diagnosis of Dermatophytes of the Hand Tinea and Sensitivity Study to the Antifungals Griseofulvin

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Abstract: The study was conducted during the month of October 2022 until April 2023, with the aim of isolating and diagnosing the fungi that cause skin diseases from patients visiting the dermatology consultation affiliated with Salah al-Din General Hospital who were diagnosed as infected by dermatologists for the period from 1/6/2022 to 1/ 12/2023 For both sexes, aged between 10 and 60 years, by taking skin samples from the area of infection after wiping them with cotton saturated with 70% alcohol for the purpose of sterilization to get rid of any contaminants. Which was diagnosed clinically by a dermatologist in the dermatology consultation at Salah al-Din General Hospital and outpatient clinics. To evaluate the effectiveness of anti-griseofulvin in inhibiting the isolated fungi, the percentage of dermatophyte infections recorded by positive direct microscopic examination was only (88) patients, representing (88%) of the total number of patients. While the number of negative cases by negative direct microscopic examination was (59), or (59%). As for positive laboratory culture results, there were (79) patients, in the rate of (79%). While the number of negative cases by laboratory culture was (21), in the rate of (21%). The fungal genera that cause skin infections, such as *Trychophyton* and *Microsporum*, were isolated, with the genus *Trichophyton* being the most frequent, with 76 isolates belonging to several species, such as *T. mentagrophytes*, with a number of 29 isolates, then the fungus *T. rubrum*, with a number of isolates of 22, then the fungus *T. terreste*, with a number of isolates of 15, and then fungus *T. schoenleinii*, with a number of 10 isolates, while only three isolates of the *M. canis* type were recorded in the genus *Microsporum.* The results of the susceptibility test of the isolated fungi to Griseofulvin showed that the fungus *T. mentagrophytes* is more sensitive to the antichrysofulvin, as the average diameter of inhibition reached 32 mm compared to the control, followed by *T. rubrum*, which had an average diameter of inhibition of 16 mm compared to the control.

Keywords: Fungi, Dermatophytes, Hand tinea, Anti- Griseofulvin.

INTRODUCTION

Dermatophytes are a group of filamentous fungi that cause superficial diseases in humans and interact with the host, causing clinical changes, the contact dermatitis the way to enter the human body through the skin, hair, and nails by invading the stratum corneum of the epidermis and keratinized tissues, which associated with the dermatophytosis. These fungi include three genera: *Trichophyton, Microsporum and Epidermophyton* are examples of infections that develop superficially at the dermis layer where the actions manifest through stimuli and that the immune response takes the form of a rash further from the affected skin [1].

It can be spread easily through contact with infected animals or people, and although the infection is curable and non-invasive, its widespread nature, its treatment cost, and its impact on the psyche of the infected person, as it affects the beauty of the infected person, as it causes a superficial infection called ringworm or tinea, which means cutworms. This name was used for skin infections that begin as a small rash and then spread in the form of a ring [2]. These fungi live in

the moist parts of the body on the stratum carenum and do not infect the mucous membranes and internal tissues of the body except in rare cases, especially in immunosuppressed patients. It is considered one of the major public health problems around the world [3]. There has been a change in the understanding of the dangerous characteristics of these microorganisms since the ancient Greeks identified fungus. As of right now, it is known that in people with strong immune systems, they are frequently not harmful. However, in certain clinical situations, they may function as a contributing factor to the emergence of severe and potentially lethal infections [4].

Fungi cause various diseases in humans and other organisms. Avoiding exposure to moisture for long periods and keeping the affected area dry is important [5]. When they infect the superficial layers of the skin, they because what are called superficial fungal diseases, and these fungi are unable to infect deeper tissues, meaning that the infection is limited to the superficial layer only. The reason is due to several factors, including the location of the infection and the host's immunity [6].

Fungi are eukaryotic organisms, including keratinophilic dermatophytes, that use keratin as a carbon source, thus converting the acidic pH of the skin to a basic pH, which creates an ideal environment for the activation of most keratinolytic proteins [7]. They are usually located in the layers and appendages of the skin and have the ability to invade the outer layer of the skin, the stratum corneum, or other keratinized skin growths derived from the epidermis, such as the skin, hair, and nails [8].

Resistance to antimicrobial drugs is an inevitable evolutionary process for the microbial world. Although fungal resistance is not equal to bacterial resistance, the economic repercussions of fungal infections are very unfortunate due to the limited availability of antifungal drugs. Reducing the incidence of antifungal resistance is vital to the success of antifungal treatment [9]. Since fungal cells are eukaryotic cells from a biological standpoint and are more similar to mammalian cells, most of the drugs that affect fungi affect the eukaryotic host, and this is a major obstacle to the development of new types of antibiotics. Therefore, the production of antibiotics has been limited to exploiting the difference between mammalian cells and fungi [10].

The threat of antifungal resistance and the challenges involved in developing antifungal drugs have researchers looking for alternative treatments However, increasingly higher doses and longer treatment times have become necessary to achieve effective treatment, so alternative antifungal treatments may be successful with shorter treatment regimens and given the limited number of treatments. Of antifungal drugs, and because prevention with antifungals may lead to the emergence of resistant strains [11]. The fungi from a pathological standpoint, they are mostly to blame for the infections and illnesses that they bring to their hosts [12].

The current study was prepared to include isolating and diagnosing fungi that infect the hand and determining the effectiveness of the antibiotic Griseofulvin on laboratory-isolated fungi.

MATERIALS AND METHODS

Preparing the Culture Media

- **Sabouraud Dextro's Agar with Chloromphenicol & Cyclohiximide (SDACC):-**

It was prepared by dissolving 65 g of sabouraud dextrose agar powder in 1000 ml of water, then shaking well and heating until boiling with a convector, then adding 0.5 g of the antifungal cycloheximide after dissolving it in 10 ml of acetone and 0.05 g of the antibacterial chloramphenicol dissolved in 10 ml. Of 95% ethyl alcohol, sterilize with an oxide, and then distribute it in sterile Petri dishes. Adding antibiotics to the food medium in order to prevent the growth of saprophytic fungi and bacteria, and using the medium to isolate and diagnose skin fungi [13].

- **Corn Meal Agar: -**

The medium was prepared by dissolving 17 grams of the medium in 1000 milliliters of distilled water. The medium was completed by mixing it with the rest of the components and filtering it through the filter. After that, it was sterilized and placed in sterile Petri dishes. The medium served to differentiate the genotypes and the phenotypes of the fungus, *T. mentagrophytes and T. rubrum.*

Rice grain Medium: -

This method entailed the pre-paring of 8 grams of rice with the help of 25 ml of distilled water, sterilization in the autoclave, and the petri dishes used had been sterilized. This medium is used to distinguish between the two types: *M. canis*, and *M. audouinii* are media-sensitive; therefore, the *M. canis* type will be non-culturable on this medium. The second and the most important of them is occurring in the medium where they can grow [14].

- **Mueller Hinton Agar**

It was measuring 1000 ml of the medium 38 g dissolved in distilled water and sterilized by autoclave and then cooled down, put in sterile Petri dishes and then mixed the medium with a fungus to check the sensitivity.

This medium was used to test the sensitivity of fungi isolated from ringworm and to test the sensitivity of the antifungal Griseofulvin at a concentration of 250 mg/ml.

After hardening the medium, a fungal colony with a diameter of 7 mm and 7 days old was placed using a cork auger, and two replicates were made for each concentration, in addition to the control, which was inoculated with the fungus without adding or dissolving any antibiotic in the Mueller-Hinton Agar medium, and all dishes containing the fungi were incubated at a temperature of 28°C. Then, the diameter of the growing colony was measured by averaging the two perpendicular diameters every five days for 15 days.

Solutions and Stains:

- **Potassium Hydroxide Solution:** This solution was prepared by dissolving 10 g of potassium hydroxide in 90 ml of distilled water. To prevent crystallization of the solution and drying of the sample, 10 ml of glycerol was added to it. It was then stored at room temperature and used for the purpose of direct microscopic examination of samples [15].
- **Griseofulvin Stock Solution:** The anti Griseofulvin was used to inhibit the growth of skin fungi. One tablet, at a concentration of 500 mg, was dissolved in 10 ml of acetone, under sterile conditions, and the concentration became 50 mg/ml. The form available in pharmacies, which is in the form of tablets, was used. Acetone was used as a solvent for this antibiotic due to its lack of solubility. This antidote in water and its solubility in acetone [16].
- **Lactophenol Cotton blue Stain**: The dye was prepared by mixing cotton blue dye 0.05 g, phenol crystals 20 g, lactic acid 20 g, glycerol 40 g, and distilled water 20 ml. Then the phenol crystals, which works to kill fungal cells, were dissolved in lactic acid because it works to preserve and clarify fungal structures, and glycerol to prevent the sample from drying out. Adding water and heating it over low heat to dissolve the crystals, then cotton blue dye was added to it, as the dye works to dye the fungal structures to make them easier to distinguish [17].

Collection of Samples:-

100 clinical samples were collected from patients visiting the dermatology consultant affiliated with Salah al-Din General Hospital in the city of Tikrit and some outpatient clinics, where clinical examination of these visitors was conducted with the assistance of the dermatologist.

Samples were obtained after clinical examination of patients attending Salah al-Din General Hospital, and direct microscopic examination was conducted by wiping the affected areas with cotton containing alcohol at a concentration of 70% for the purpose of sterilization, getting rid of bacteria and saprophytic fungi, and removing suspended materials and medications that would obstruct direct microscopic examination. During the following:

- **Skin**: The crusts were collected from the scaly edge of the active border area of the skin because they contain hyphae, which are the fungi responsible for the infection. Then the affected area was scraped off using a sterile surgical blade, then a portion of the crusts was placed on a clean glass slide for the purpose of conducting microscopic examination, and a portion of the crusts was kept. The peels are placed in sterile glass dishes or sterile plastic containers for transfer and cultivation on sabouraud dextrose agar medium.
- **Direct Microscopic examination:** Place a portion of the affected skin peels on a sterile glass slide, then add a drop or two of a 10% potassium hydroxide solution to it, cover the slide with a lid, then heat it gently, avoiding boiling because it leads to the crystallization of potassium hydroxide, and stir it over the flame of a Bunsen lamp to melt the keratinous materials. [18] Then they were left for twenty minutes and gently pressed with a brush. All the prepared glass slides were examined under a microscope using the minimum power of 10x to observe the clusters of crusts taken from the affected skin. Then they were examined at 40x power to see the scattered fungal hyphae and arthrospores [19, 20].

As for the section the other samples were grown on sabouraud dextrose agar medium containing chloramphenicol and cyclohexamide and incubated at a temperature of 25-30C^{o}.

- **Culture of Sample**: Skin peels from the infected person were grown on sabouroud dextrose agar medium containing chloramphenicol and cyclohexamide and incubated at a temperature of 25°C for 14-20 days. The growth of fungal colonies was examined during 2-3 days [21].

Diagnosis of Dermatophytes:-

- **Cultural Characteristics:** After 14-20 days, fungal growth appeared on the surface of the culture medium, and the culture characteristics were examined, which is one of the important methods that must be taken to identify skin fungi, among the first observations, microscopy allows the determination of Fluffy mother. Then we proceeded to a second examination, this time from the reverse side, and the diameter of the colony was measured at the end of the growth [22].

- **Microscopic Examination by Wet Mount Test:** This test was accomplished by putting a drop of Lactophenol mount, cotton blue in a glass slide, picking a portion of the specimen of the fungus using the inoculation needle, mixed it with the dye, spread the sample without pressing it hard on the slide, and cover the slide's surface with the slide cover [23]. The sample was then examined under a microscope using 10X force and then at 40X force observing the fungal hyphae and conidia, their shapes, branches, and different sizes, micro and macro conidia, and the way they are arranged on the fungal hyphae, and observing the arthrospores as well as the chlamydospores [24].

Statistical Analysis:

The study results were statistically analyzed according to Duncan's multinomial test, at a probability level of 5%, and this was implemented using the Excel program in the electronic calculator [25].

RESULTS AND DISCUSSION

100 samples were collected during the period from 6/1/2022 to 12/1/2023 for both sexes, aged between 10 and 60 years, by taking skin samples from the area of infection after wiping them with cotton saturated with 70% alcohol for the purpose of sterilization to get rid of any contaminants.

The diagnosis was made clinically by a doctor specializing in dermatology in the dermatology consultant at Salah al-Din General Hospital and outpatient clinics. Through direct microscopic examination and diagnostic examination, the rate of infection with skin fungi was recorded in only (88) patients, in the rate (88%) of the total number of patients, while the number of cases was (59) patients tested negative, in the rate (59%).

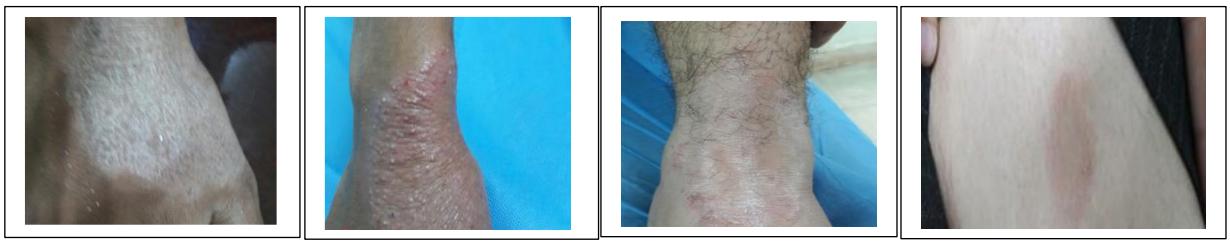
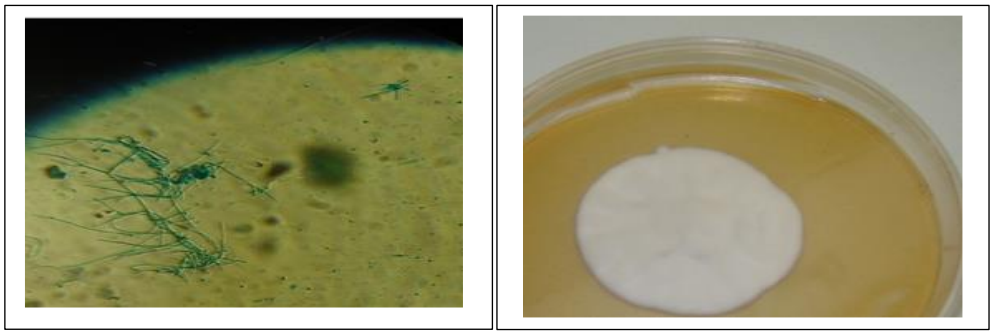


Figure 1: Dermatophytes of ringworm of the hand in some patients

Phenotypic and Microscopic Diagnosis of Dermatophytes:

Fungi isolated from samples of people with tinea capitis were identified by phenotypic characteristics of fungal colonies after growing them on appropriate culture media, such as SDA medium and development at 25°C for 7-14 days, as well as on the microscopic properties after staining with lactophenol dye based on the diagnostic keys for diagnosing fungi (Kidd *et al.,* 2016; Zafar 2017; Carmen2017, the following fungal species have appeared:

T.mentagrophytes: Colonies of this type of fungus appeared after 5-7 days of incubation and were characterized It is flat, white or cream in color, and domed in the middle, while it appears from the opposite side It is yellow, the center of the colony is brown, and microscopic examination shows large conidia microconidia are scepter-shaped, and the microconidia are many in number and circular shape and length of fungal hyphae,as in figure (2) A & B.

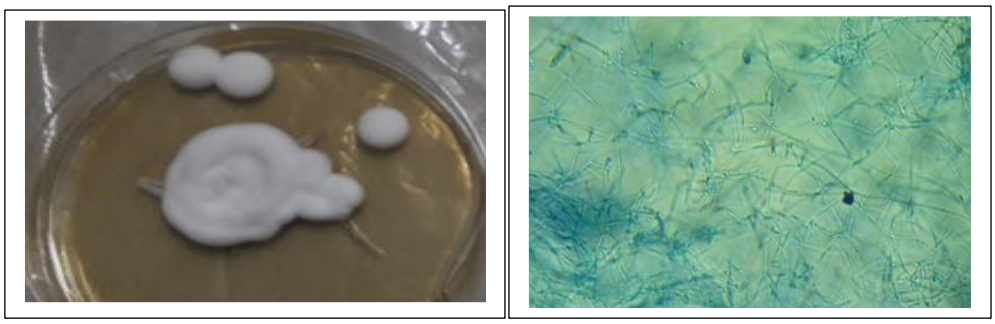


A B

Figure 2: Morphological and microscopic characteristics of the fungus T. mentagrophytes of the colony growing on SDA medium, A- The external appearance from the front; B- For microscopic form under X.40 force

T.rubrum:- Colonies of this fungus appeared when grown on SDA medium on the fifth day and were of the same type, The front is white and cream in color, fluffy and slightly raised in the middle, while the back is white. Dark yellow, and

microscopic examination showed the presence of divided hyphae and the presence of large conidia Macroconidia are rod-shaped and elongated, and Microconidia are numerous It has a teardrop shape, as in Figure (3) A & B.



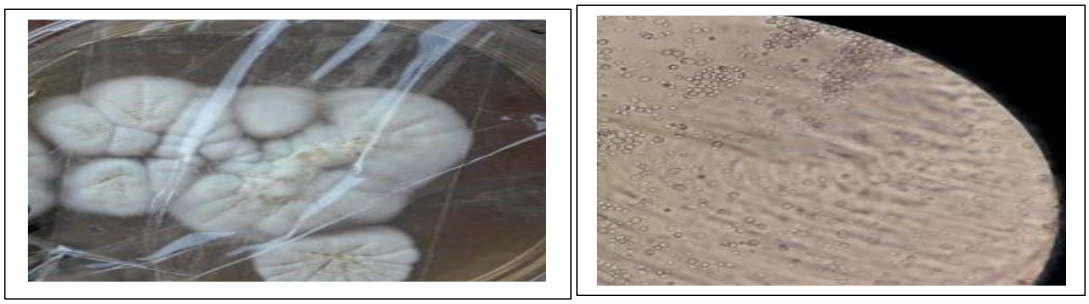
A B

Figure 3: Phenotypic and microscopic characteristics of the fungus *T. rubrum* from the colony growing on SDA medium, A- The external appearance from the front; B- For microscopic form under X.40 force

T. terrestre:

Trichophyton terrestre exhibits moderate growth at 25ºC, maturing in about 8 days. Colonies expanded in diameter rather slowly. Colonies were off-white to light in colour. Reverse appeared yellowish to ochraceous, or even slightly reddish in colour. Texture was felty to powdery the isolate presented here developed a pale to golden yellow exudate on prolonged incubation.

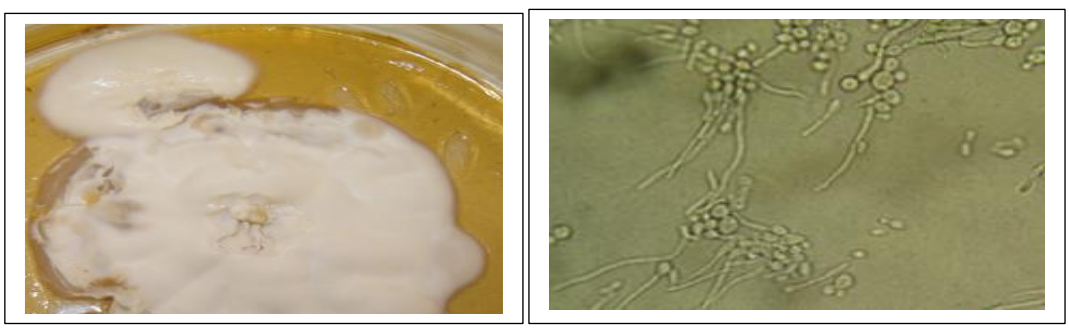
Microscopically, *T. terrestre* produces hyaline (transparent, non-pigmented) and separated hyphae. Microconidia are teardrop to slightly club shaped. There may not be a clear distinction between what may be called small or large conidia. (i.e. the two are not clearly differentiated.) Microconidia (and macroconidia) show a truncated base or basal scar at the point of attachment.



A B

Figure 4: Phenotypic and microscopic characteristics of the fungus *T. terrestre* from the colony growing on SDA medium, A- The external appearance from the front; B- For microscopic form under X.40 force

T. schoenleinii-: Colonies of this fungus appeared growing on SDA medium after 5-7 days of incubation Disc-shaped, cottony, white in color, yellow on the opposite side, appearing underneath The microscope is in the form of hives branching into two branches and divided by chandelier-like partitions, but no presence appears For large and small conidia, as shown in Figure (5) A & B.



A B

Figure 5: Phenotypic and microscopic characteristics of the fungus T. rubrum from the colony growing on SDA mediu, A- The external appearance from the front; B- For microscopic form under X.40 force

M.canis:- This fungus colonies are when washed on SDA medium on the third day appeared Yellowish white in color, the surface of the colony was cottony and the fungal hyphae are raise from the middle to the top. The bottom side of the colony is yellow-orange in color, and under microscopic analysis it provides the presence of Macro conidia which are numerous and spindle-shaped conidia, and the Micro conidia that are few in number it is shown in Figure (6) A & B.

A B

Figure 6: Phenotypic and microscopic characteristics of the fungus T. rubrum from the colony growing on SDA medium, A- The external appearance from the front; B- For microscopic form under X.40 force

The results of laboratory culture of samples collected from 100 cases of infection with ringworm of the hand revealed the diagnosis of fungi in (79) cases of infection, as these cases showed fungal growth on the culture medium and showed the presence of fungi belonging to the genera *Trichophyton* and *Microsporum* only, and the genus *Epidermophyton* was not isolated, and this result is consistent with what was found, this result is consistent with [26, 27].

As for positive laboratory culture results, there were (79) patients, in the rate (79%). It has been shown from our current study that the fungus *T.mentagrophytes*, which caused ringworm of the hand, was detected by (36.70%) with a number of (29) isolates, then the fungus *T.rubrum* with a rate of (27.84%) and with a number of 22 isolates, then the fungus *T.terrestre* with a number of 15 isolates and a rate of (18.98). %) As for the fungus *T. schoenleinii*, the percentage was (12.65%, with a number of 10 isolates, and the fungus *M.canis* was with three isolates, with a percentage of (3.79%), as is clear in Figure (7). While the number of negative cases by laboratory culture appeared (21), a rate of (21%).

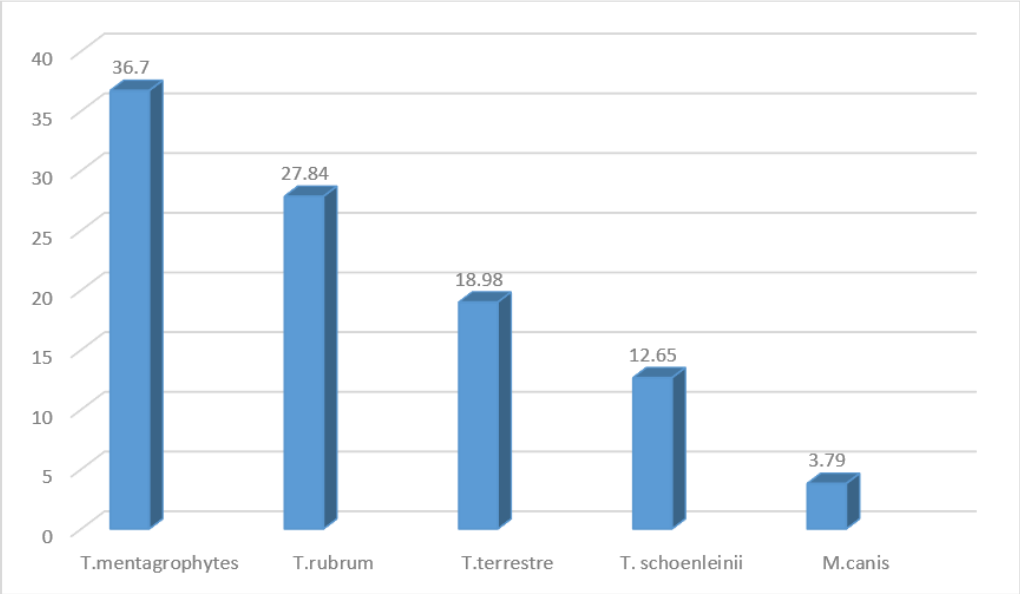


Figure 7: Fungal species isolated during the study

Some of the results that show up negatively in direct microscopy are because the sample is taken in small amounts and not enough to show a good outcome, or the sample is taken from the injury corner instead of the central part in which the fungus is high and spreads more [28].

The appearance of negative results by laboratory culture is an error in the method of storing the sample until planting, as it is stored in containers that retain moisture, which helps in the growth of saprophytic fungi, which reduces

the appearance of the original sample upon culture, and thus the absence of a positive culture result [29], or it may be the reason. Is that some patients resort to using topical treatments randomly without consulting a doctor, which may lead to affecting the vitality of skin fungi and their failure to grow after transplantation [30] or the reason may be due to the method of planting and the culture medium.

Dermatophytes and their Distribution by Sex:-

The results showed that the infection rate among males was higher than that of females, as the number of males was 50, representing 63.2%, and 29 females, representing 36.7% of the total number.

Table 1: Fungal species isolated during the study and their distribution by sex

Isolated Fungi	Number	percentage	Male	percentage	Female	percentage
T.mentagrophytes	29	36.70	20	40	9	31.03
T.rubrum	22	27.84	16	32	6	20.68
T.terrestre	15	18.98	5	10	10	34.48
T. schoenleinii	10	12.65	7	14	3	10.34
M.canis	3	3.79	2	4	1	3.44
Total	**79**	**100**	**50**	**100**	**29**	**100**

The distribution gender disability demonstrates similar pattern with those studies by [31-34], whereas, it is in contrast with the studies done by [35-37], their results show that females infection is higher than that of males, and the likely cause is because the rate of infection is different between the two sex at their respective sites of infection [38], however, the reason for the discrepancy between the sexes may be attributed to the difference in the degree of exposure to causative factors between males and females, as males are more distinguished activity vitality and movement, as there is a lot of movement and movement between different geographical areas and a lot of contact with each other among them, whether during play or direct contact with infected pets it provides opportunistic conditions for disease to occur and makes them a suitable environment for the growth of skin fungi if they are transmitted to them [39] .

It has been indicated that this situation could return short hair in males compared to females this facilitates the access of fungal spores to the hair follicle, and the frequent frequency of males to barbers are the cause of ringworm, often due to shaving tools contaminated with spores of this type of fungus that can easily spread and cause infection [40].

Infection According to Age Groups

Figure (8) shows that the number of infections ranges according to age groups, with the highest percentage being 40.5% in the age group between 41-50 years, then followed by the age group 31-40 years, reaching 30.37%. While the lowest percentage appeared in the age group of 10-20 years, which amounted to 6.32%, then it was followed by the age group of 51-60 years, amounting to 8.86%, while the age group of 21-30 years occupied the rate of 11 samples, with a rate of 13.92%.

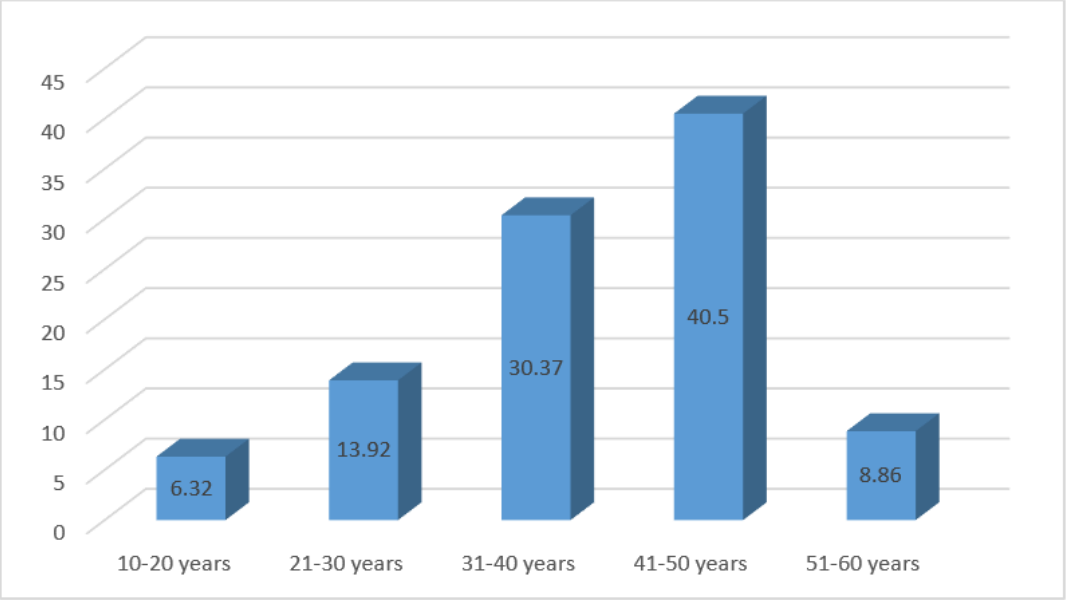


Figure 8: The relationship between ringworm infection and the patient's age

The cause of the infection may be due to lack of attention to personal hygiene and incomplete mental awareness poor health awareness and failure to apply proper health conditions, or to the child's immune status, Direct contact between children contributes to transmitting the infection among them [41].

Biological Activity of the Antifungal Crisofulvin Against Isolated Dermatophytes Causing Tinea Hand

The diameter of the growing colony of fungal species was used to express the ability of antifungal substances to inhibit those species. The smaller the diameter of the colony growth is evidence of an increase in the ability of the inhibitory substance, and vice versa in the case of increasing the diameter of the colony. The results of the study showed that there were significant differences at ($P<0.05$) between the concentrations used in their inhibitory effect against fungal species.

It was observed from the results that the *T. mentagrophyte* fungus was inhibited by the anti-griseofulvin with a diameter of 32 mm compared to the control, which had a diameter of 44.5 mm, while *T. rubrum* was inhibited with a diameter of 16 mm compared to the control, which had a diameter of 52 mm, while the average diameter of inhibition in *T.terrestre* 8 mm compared to the control, which had a diameter of 32 mm, while *T. schoenleinii* was inhibited by a diameter of 38 mm compared to the control, which had a diameter of 56.5 mm. As for the fungus *M. canis,* it was inhibited with a diameter of 12 mm compared to the control, which had a diameter of 39.5 mm. As in a table (2).

The effectiveness of this antibiotic is due to the fact that Griseofulvin interacts with tubulin proteins, from which microtubules polymerize, changing their shape and inhibiting the assembly of tubulins during spindle formation [42]. It also affects microtubules inside the cytoplasm, the latter of which participate in transporting compounds within the cell. It is believed that this interaction results in a disturbance in the transport of components [43]. The newly manufactured cell wall thus works to inhibit the construction of the cell wall of the fungus, and leads Griseofulvin to defects in DNA synthesis and thus inhibits the construction of proteins inside the cell [44].

Antifungals are limited due to toxicity, drug interactions, and the emergence of resistance to fungi, which causes increased rates of morbidity and mortality [45]. Although crisofulvin is the main antifungal agent, it is given orally to treat skin fungi caused by *Trichophyton*, *Microsporum*, and *Epidermophyton*, and is used in the treatment of ringworm. The body in many countries. This drug is produced from the fungus Penicllium grseofulvin. It inhibits the growth of fungi by binding to microtubules responsible for the formation of mitotic spindles and leads to impaired development of the cell wall [46].

Table 2

Isolated Fungi	Inhibition diameter (mm)	Control (mm)
T.mentagrophytes	32±0.05e	44.5±0.05c
T.rubrum	16±0.05f	52±0.05ab
T.terrestre	8±0.05g	32±0.05e
T. schoenleinii	38±0.05d	56.5 ±0.05 a
M.canis	12±0.05fg	39.5±0.05cd

CONCLUSION

It was concluded from the results that the fungus *T.mentagrophytes* came first, followed by the fungus *T.rubrum* in infecting patients with ringworm, and the infection rate in males was higher than in females, and the age group 41-50 years was more susceptible to infection.

As for the antifungal effect of crisofulvin the fungus *T.mentagrophytes* ranked first, followed by the fungus *T.rubrum* in inhibition, as the average diameter of inhibition reached 32 and 16 mm, respectively, compared to the control rate.

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