E-ISSN: 2788-9297
P-ISSN: 2788-9289
www.agrijournal.org
SAJAS 2024; 4(2): 190-195
Received: 25-08-2024
Accepted: 28-09-2024

Mohammed Sami Farhan
Department of Biology, College of Sciences, University of Tikrit, Iraq

Sarah Omran Rasheed
Ministry of Education, General Directorate of Education, Salah al-Din, Tikrit Education, Iraq

Rand Salwan Numan
Department of Biology, College of Sciences, University of Tikrit, Iraq

Correspondence Author:
Mohammed Sami Farhan
Department of Biology, College of Sciences, University of Tikrit, Iraq

Isolation and diagnosis of fungi and some types of yeasts causing tinea pedis (Athlete's foot) and studying the effect of white vinegar on them

Mohammed Sami Farhan, Sarah Omran Rasheed and Rand Salwan Numan

DOI: https://dx.doi.org/10.22271/27889289.2024.v4.i2c.158

Abstract
The research investigation was carried out through October 2023 to April 2024, aiming to isolate and diagnose the fungi responsible for athlete's foot in patients who consulted dermatologists at Tikrit Teaching Hospital. The participants, ranging in age from 12 to 65 years, included both sexes and had been diagnosed with the condition. Skin samples were collected from the affected areas after sterilization with cotton swabs soaked in 70% alcohol to eliminate contaminants..

To assess the efficacy of white vinegar in suppressing the isolating fungal species, the proportion of skin fungal infections identified through directly microscopic testing was positive for 62 patients, constituting 62% of the total patient cohort, while the negative cases amounted to 59 patients, representing 59%. Positive laboratory testing for results of cultures were observed in 70 patients, constituting 70%, whereas negative cases amounted to 30, representing 30%. The fungal genus that causes skin infections was isolated, Trychophyton, and the two genera, Microsporum and Epidermophyton, were not isolated.

The genus Trichophyton was predominant, comprising 50 isolates from various species, including *T. mentagrophytes*, responsible for tinea pedis, with 21 isolates representing 30%. T. verrucosum followed with 16 isolates, accounting for 22.85%, while *T. rubrum* had 9 isolates, corresponding to 12.85%. The proportion of the fungus T. tonsurans was 5.71 (i.e. a number of 4 isolates), in addition to the isolation of some types of yeasts that infect tinea pedis with a number of isolates of 20, including c.albicans, with a proportion of 17.14% and 12 isolates, followed by C. glabrata yeast, having a percentage about 11.42% and 8 isolates. Current study showed that 49% of the samples of tinea pedis in males were positive, while only 21% of females yielded a favorable outcome on the cultural media. As for the age groups, those infected with tinea pedis, whose ages ranged from 12 to 20 years, were the most susceptible to infection, as their percentage was 50%, while those who ranged in age from 31-40 years, their percentage of infection was 4.28%, and they were the least susceptible to infection.

The results of the effect of white vinegar on both isolated fungi and yeasts showed that all fungal isolates were sensitive to white vinegar at a concentration of 10% and it was the most efficient inhibitor for fungi and yeasts. The results indicated that white vinegar inhibited T. mentagrophyte fungus at a diameter of 10%, measuring 12.5 mm, while at a concentration of 20% it is 32 mm, while at a concentration of 30% it is 40 mm compared to the control, which was 44.5 mm in diameter, while the effect of white vinegar on C. albicans yeast at a concentration of 10% is 10 mm, while at a concentration of 20% it is 13 mm, while at a concentration of 30% it is 18 mm, C. glabrate at a concentration of 10% is 2 mm, while at a concentration of 20% it is 6 mm, while at a concentration of 30% it is 10 mm.

Keywords: Tenia pedis, trichophyton, dermatophytes

Introduction

The comprehension of the perilous attributes of these microbes has evolved since the ancient Greeks recognized fungi. Currently, it is understood that individuals with robust immune systems are often not adversely affected. In specific clinical circumstances, they may serve as an important contributor to the onset of serious and potential fatal infection [1]. A class of fungal illnesses known as dermatophytes are brought on by skin molds. Dermatophytes are known as keratinophilic fungi due to their association with keratinous tissues that form the skin, hair and nails [2]. They can cause an infection called ringworm. Dermatophytes infect keratinous tissues, including nails, hair, and skin in both people and animal, resulting in dermatological conditions.

These types of fungi comprise three genera: Trichophyton, Microsporum, and Epidermophyton, which induce dermatitis in people and animal Trichophyton impacts hair, skin, and nails; Microsporum influences skin and hair; Epidermophyton effects on skin and nails. Categorization is based on the shape of macrospores, microspores and fungal threads, and the color of the surface and background [4].

Tinea Pedis

The principal sites of tinea pedis, an infection caused by fungal are the plantar region of the feet and the interdigital areas. Approximately 72% of individuals will experience tinea pedis at some stage in their lives. Men exhibit a higher susceptibility than women to the development of tinea pedis. Tinea Pedis is among the most prevalent fungus infection globally. This name is given to any chronic infection of the feet, whether the pathogen is bacterial, fungal, or yeast. The infection begins when the skin takes on a white, testaceous color,then turns into itching, cracking, rotting, and peeling in the spaces between the toes. If the condition deteriorates and is not treated, the affected area becomes inflamed. If a bacterial infection occurs with a fungal infection, the rash spreads and is accompanied by severe pain. Infections are often in the moist areas between the toes, and the infection may extend beyond the sole of the foot to reach the palm of the hand. It is sometimes called athlete's foot because athletes often get it. It is caused by human-loving skin fungi, which are rubrum, *T. mentagrophytes*, E. floccosum, and T. violaceum [5].

Due to the restricted availability of antifungal medications, the potential for antifungal prophylaxis to induce resistance of strains, and the difficulties associated with the development of antifungal pharmaceuticals, researchers have sought alternate therapies. Alternative antifungal therapies may prove effective with abbreviated treatment regimens [6]. Affective substances extracted from plants also give better results than the same substance manufactured by chemical methods, which may be accompanied by toxic side effects, indicating the possibility of affctive substances in secondary compounds contributing to enhancing the effective role of the plant [7].

The antibiotic may be unable to penetrate the cell membrane of some microorganisms or the antibiotic that enters the membrane is removed by ion flow pumps, meanwhile, the microorganisms may have acquired resistance that can be taken by chromosomal mutations [8]. One of the alternatives is natural white vinegar which has antifungal properties due to its malic acid content which helps in fighting fungal infections [9]. The present research was conducted with the purpose of assessing the efficacy of natural white vinegar against several fungi and yeasts cultured in the laboratory.

Materials and Methods

Preparation of culture media for fungi

Sabourand Dextro's Agare with Chloromphenicol & Cyclohiximide (SDACC)

Sabouraud Dextrose Agar (SDA)

This medium was utilized for isolating and distinguishing of dermatophytes, made by dissolved 65 g of SDA powder in 1000 ml of distilled water, followed by stirring along with heating to boiling on a hot plate. The medium was subsequently sterilized using autoclaving, after which 0.5 g of the antifungal cycloheximide, dissolved in 10 ml of acetone, and 0.5 g of the antibacterial streptomycin, diluted using distilled water, were incorporated. The medium was shaken well with pH adjustment and then distributed into sterile petri dishes and left to cool, then store in the refrigerator until use [10].

Corn-Meal Agar

This media was utilized to differentiate among two species, *T. rubrum* and *T. mentagrophytes*. The plates containing this media were infected with fungal colony discs measuring 7 mm in diameter, utilizing a cork borer. These plates had been incubating at 30°C during two weeks. The appearance of the red dye gives evidence that the fungus is *T. rubrum*, and the absence of the red dye means that this fungus is *T. mentagrophytes*.

Mueller Hinton Agar

The medium in question was utilized for testing the sensitivity of fungus and yeasts isolated from tinea pedis, as well as to evaluate the sensitive of white vinegar in different concentrations, which are 10, 20% and 30%. It is produced by Emad Laboratory for the Production of Natural Vinegar and Herbal Oils, Mosul, Iraq. After obtaining the results of the inhibition caused by the antibiotics at all levels, the results of the optimal inhibition were taken and it was concluded that the optimal concentrations were as follows:

1- Utilize white vinegar with concentrating of 10% to assess the susceptibility of fungal samples. Upon the solidification of the medium, a 7 mm diameter fungal colony was extracted at 7 days of age using a cork borer. Two replicates were prepared for every concentration, alongside a control inoculating with the fungus without any antidote in the Mueller Hinton Agar medium. Each of the plates containing the fungal culture were incubated at 28 °C, and the diameter of the growing colony was calculated by averaging two perpendicular diameters every 5 days over a period of 15 days.

Solutions and stains

KOH solution was used for direct microscopic examination of samples. To make this solution, 10 g of KOH were dissolved in 90 mm of distilled water according to the instructions. After adding 10 m of glycerol to the solution, it was then kept at room temperature. This was done in order to prevent the solution from crystallizing and the sample from drying out. One of these is the Lactophenol Blue Stain that is found on cotton. In order to make it simpler to differentiate between different fungal structures, the dye acts to stain them. A mixture consisting of 0.05 grams of cotton blue dye, 20 grams of phenol crystals, 20 grams of lactic acid, 40 grams of glycerol, and 20 millimeters of distilled water was used to get the desired color. Subsequently, the crystals of phenol, which are capable of killing fungi cells, were dissolved in lactic acid, which contributes to the preservation and clarification of the fungal structures, and glycerol, which serves in preventing the samples from dried out. Following the addition of water, the mixture was cooked over a low heat in order for the dissolution the crystals. After that, the cotton blue dye was incorporated into it [11].

Methods of work

Collection of Samples

100 samples collected following a clinical assessment of patients at Tikrit Teaching Hospital. A direct microscopy was performed by swabbing the affected foot area with cotton soaked in 70% alcohol to sterilize the site, eliminate bacteria as well as saprophytic fungi, and remove any suspended particles and medicinal products that could hinder the microscopic analysis. The scales were obtained from the skin's active border, as it harbors fungal hyphae responsible for the illness, The infected region was cleaned utilizing a sterile surgical blade, and a piece of the scaling was put on a clean glass plate for microscopic analysis. A portion of the scales is maintained in sterile glass plates or sterile plastic containers for transfer and culture on Sabouraud Dextrose Agar media.

Direct microscopic analysis for the samples

A portion of the infected skin's scales was positioned on a sterile glass slide, followed by the addition of one or two drops of 10% KOH solution. It was then covered with a cover slip and gently heated, taking care to avoid boiling, as this causes crystallization of KOH. The slide was subsequently passed over a Bunsen lamp flaming to dissolve the keratinous substances (Szepietowsk and Schwartz, 2005). Subsequently, it was allowed to rest for 20 minutes and was lightly pushed to facilitate spreading. All prepared glass slides were scrutinized under a microscope at a minimum magnification of 10x for examining the clusters of scales obtained from the diseased skin, followed by examination at 40x to visualize the dispersed hyphae of fungi and arthrosporous. The remaining section of the samples was cultivated on SDA supplemented with chloramphenicol and cycloheximide, and kept at 25-30 degrees Celsius [12].

Culture of Sample

Skin scales were taken from the affected foot region and subsequently cultured on Sabouraud Dextrose Agar media supplemented with chloramphenicol and cycloheximide, then incubating at 25 °C during 15 to 20 days, with growth assessed every 48-72 day [13].

Dermatophytes Diagnostics

Culture Features

Following a period of between 15 and 20 days and the emergence of growth of fungi on the culture medium's surface, the cultural properties are assessed, which is a crucial method for identifying dermatophytes, which includes the incubation period and the shape of the colony, whether it is sunken or raised, as well as its color and texture, whether it is powdery, cottony or fluffy. The assessment is conducted from the reverse side, then the colony's diameter is assessed once growth ceases [14].

Microscopic examining for colonies

Wet mount test

The test involved applying a drop of cotton blue lactophenol dye onto a slide of glass, subsequently taking a segment of fungal hyphae from the colonies using a sterile needle, combining it together with the dye, and then covering the slide with a cover slip while using gentle pressure to disperse the sample (Forobes, *et al*., 1998). These sample was analyzed microscopically at 10X and subsequently at 40X magnification, focusing on the hyphae of fungus and conidia, including their morphology, branching, and varying sizes of micro and macro conidia, as well as their arrangement on the hyphae, along with the observation of arthrospores and chlamydospores [15].

Findings and Discussion

The study analyzed 100 samples from both sexes, aged 12 to 60 years, collected from the affected foot region after sterilization using cotton swabs soaked in 70% alcohol to eliminate impurities.

Direct microscopic examination revealed that 62 patients, constituting 62% of the total, were positive for skin fungal infection, while 59 patients, representing 59%, were negative. Positive laboratory results for cultures were seen in 70 patients, constituting 70%, whereas the negative cases totaled 30, representing 30%. The manifestation of certain negative outcomes in direct microscopic analysis may stem from an inadequate sample size that is insufficient to yield a positive result, or from an error in sample collection, as it may not be obtained from the periphery of the infection but rather coming from the central region, which has developed local immunity and is devoid of dermatophytes [16].

The emergence of negatively outcomes in lab cultures can be attributed to errors in sample storage methods, as samples are kept in moisture-retaining containers that promote the growth of fungi present in the original sample during culturing. Consequently, this may lead to a lack of positive culture results, or it may stem from the culturing technique and the medium used for growing employed. Additionally, some patients utilize topical therapies indiscriminately without medical consultation, potentially compromising the viability of skin fungus and their subsequent growth in culture [17].

Fungal genus Trichophyton causing skin infections was isolated, and the two genera, Microsporum and Epidermophyton, were not isolated, The genus Trichophyton became the more prevalent, with 50 isolates from several species, including *T. mentagrophytes*, which accounted for 21 isolates, then T. verrucosum with 16 isolates, then *T. rubrum* with 9 isolates, then T. tonsurans with 4 isolates. The study showed the dominance of the Trichophyton genus and the two genera Microsporum and Epidermophyton were not isolated, as The genus Trichophyton became the most prevalent, with 50 isolates, as it infects the nails, hair, and skin, whereas the genus Microsporum mostly infects the hair and skin, and in rare cases it may also inffect the nails, while Epidermophyton may infect the skin and nails but not the hair (10). The findings indicated that the prevalence of infection rate among males surpassed that of females, as the male population was 49 and females 21 infections out of the total number Total.

Table 1: Shows the percentage of isolated fungal isolates.

Clinical form	Number	percentage	Males	percentage	Females	Percentage
T. mentagrophy tes	21	30%	16	32.65%	5	23.80%
T. verrucosum	16	22.85%	12	24.48%	4	19.04%
T.rubrum	9	12.85%	8	16.32%	1	4.76%
T.tonsurans	4	5.71%	2	4.08%	2	9.52%
c.albicans	12	17.14%	6	12.24%	6	28.57%
c.glabrate	8	11.42%	5	10.20%	3	14.28%
Total	70	100%	49	100%	21	100%

The present research revealed that the *T. mentagrophytes* fungus, responsible for tinea pedis, was identified in 30 isolates, followed by T. verrucosum, with a percentage of (22.85) isolates, followed by *T. rubrum*, with a percentage of (12.85) isolates, while T. tonsurans was found to be (5.71) isolates, in addition to the isolation of some types of yeasts that infect tinea pedis, with a number of isolates of 20, including c. albicans, with a percentage of (17.14) isolates, followed by c. glabrate, with a percentage of (11.42) isolates, followed by (8) isolates.

The present investigation revealed 49% of male samples for tinea pedis tested positive, however just 21% for female samples yielded a positive finding on the cultivation. This outcome aligns with several research indicating that guys are prone to foot fungal infections. Some scientists indicated that this is due to males being more susceptible to infection and is a consequence of foot perspiration due to prolonged shoe wear and lack of sunlight as well as ventilation during sport. Males are more likely to be infected with athlete's foot due to poor hygiene, in addition for directing communication among them, which leads to the spread of infections between individuals and through contact with infected people or their belongings (such as swimwear, sportswear) or through contaminated materials or contact with infected people and contact with infected animals such as cats, dogs and cattle, and the use of tools contaminated with fungal spores and towels from a sick individual to another healthy people.

Females are often infected with tinea pedis due to their constant contact with water and chemicals, such as detergents and nail polish [18]. Infections occur in the moist areas between the toes and may extend from the sole of the foot to the palm of the hand. It is sometimes called athlete’s foot because athletes get it [19].

The correlation among tinea pedis with the age of patients

According to age groups, those infected with athlete's foot between the ages of 12 and 20 were the most susceptible to infection, with a percentage of 50%, while those between the ages of 31-40 had a percentage of 4.28%, the least susceptible to infection.

Table 2: Number and percentage of the correlation among tinea pedis with the age of patients

Age Group (12 years to 60 years	Number	Percentage
12-20	35	50%
21-30	7	10%
31-40	3	4.28%
41-50	10	14.28%
51-60	15	21. 42%
Total	70	100%

The greater frequency of tinea pedis in children compared to adults may be ascribed to the child's immune status due to the absence of saturated fatty acids that provide natural protection against fungal diseases. However, at puberty, the effectiveness of the sebaceous glands begins at this stage and the effectiveness of antifungal saturated fatty acids increases during puberty. Hence, it is necessary to enhance cognitive and health awareness among students in schools. This is done by establishing basic education and guidance units in schools, which include attention to personal hygiene and foot hygiene after playing and sweating, avoiding the exchange of socks and not sharing towels, as ringworm germs are transmitted indirectly through the needs of the infected person who falls and peels the skin more than direct contact with the infected person's body. As athlete's foot is a global disease and there are many cases of infection in all countries of the world, and it is widespread in areas with a moderate climate such as tropical regions, because people in tropical regions do not wear shoes due to the hot weather. As adults are more susceptible to infection than children and it is a result of foot sweating resulting from prolonged shoe usage and lack of exposure the foot to sunlight as well as ventilation [20].

Effectiveness of white vinegar against isolated fungi and yeasts that cause athlete's foot

The diameters of the expanding fungal colonies was utilized to quantify the efficacy of antifungal agents in inhibiting these species; a reduction in colony diameter indicated enhanced inhibitory capacity, whereas an increasing in diameter suggested diminished efficacy of the antifungal material. The study's results indicated substantial variations (p<0.05) among the materials regarding their inhibitory effects on the most prevalent fungus species. The results indicated that white vinegar at a concentration of 10% inhibited the T. mentagrophyte fungus with a diameter of 12.5 mm. while at a concentration of 20% it is 32 mm, while at a concentration of 30% it is 40 mm compared to the control, which was 44.5 mm in diameter.

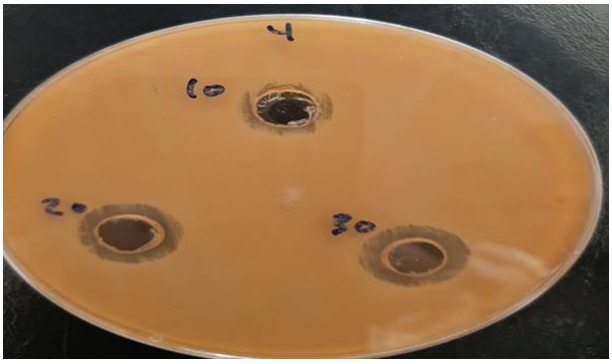

Fig 1: Shows the effect of white vinegar on T. mentagrophyte fungus.

While the effect of white vinegar on C.albicans yeast at a concentration of 10% was 10 mm, at a concentration of 20% it was 13 mm, and at a concentration of 30% it was 18 mm.

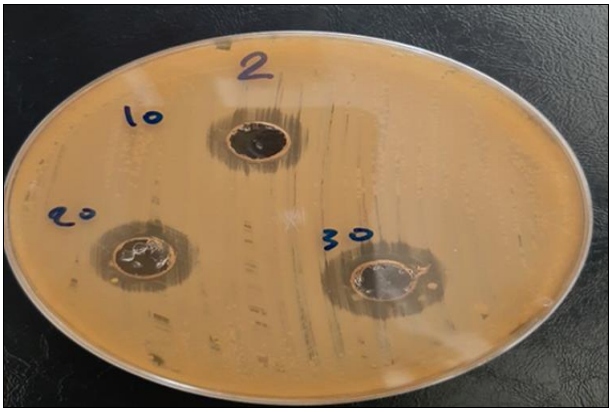

Fig 2: Shows the effect of white vinegar on C.albicans yeast.

C. glabrate at a concentration of 10% is 2 mm, while at a concentration of 20% it is 6 mm, while at a concentration of 30% it is 10 mm.

The above results show that natural white vinegar works well as a natural antifungal to reduce the use of antibiotics manufactured against skin fungi, as a high percentage of them are not guaranteed to be effective, in addition to the emergence of generations of fungi that are anti-fungal, in addition to their ability to tolerate high concentrations. In light of the available sources, studies on the adoption of natural products such as white vinegar extracts have shown inhibitory effectiveness against isolated fungi and yeasts without side effects when compared to antibiotics. This is due to the fact that natural white vinegar has antifungal properties due to its content of malic acid, which helps in combating fungal infections [21].

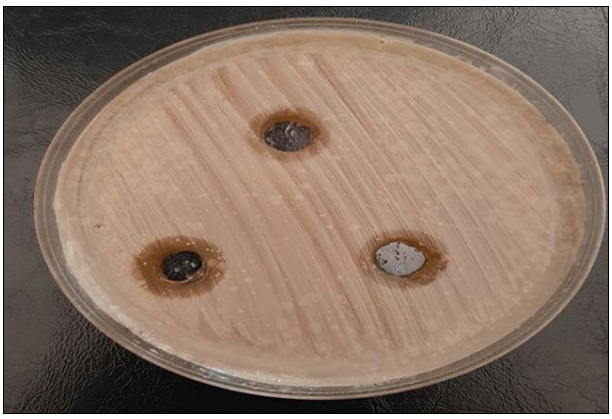

Fig 3: Shows the effect of white vinegar on C. glabrate yeast.

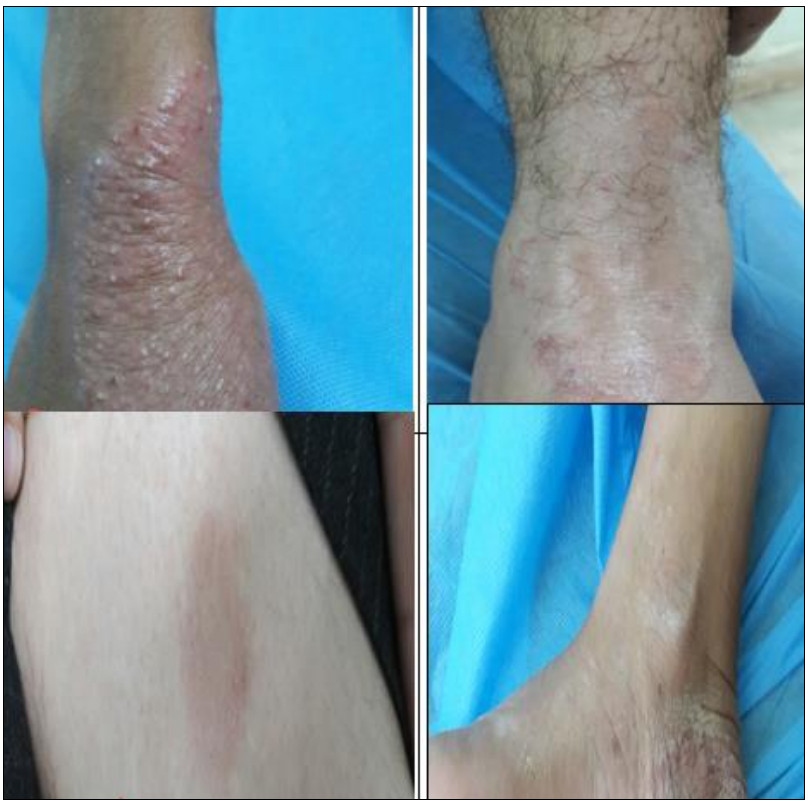

Fig 4: Shows tinea pedis

Conclusion

This study found a significant prevalence of skin fungal infections, with 62% of patients testing positive via direct microscopic examination and 70% showing positive culture results. The predominant fungal genus isolated was *Trichophyton*, with *T. mentagrophytes* being the most common species. Negative results in direct microscopy and cultures may be attributed to factors such as inadequate sample collection or storage errors. Additionally, indiscriminate use of topical therapies could have affected fungal growth in cultures. The study also revealed a higher infection rate in males compared to females, emphasizing the need for improved diagnostic practices and timely treatment.

References

1. Farhan MS, Abdullah BA, Mamdwooh AE, Numan RS. Review of virulence factors in Candida. J Res Appl Sci

Biotechnol. 2024;3(2):75-82.
2. Rasheed SO, Farhan MS, Oubid SH. Isolation and diagnosis of skin fungi that cause tinea capitis in children. Eur Sci Methodol J. 2024;2(5):167-76.
3. Upadhyay V, Kumar A, Singh AK, Pandey J. Epidemiological characterization of dermatophytes at a tertiary care hospital in Eastern Uttar Pradesh, India. Curr Med Mycol. 2019;5(1):[Page numbers missing].
4. Taghipour S, Abastabar M, Piri F, Aboualigalehdari E, Jabbari MR, Zarrinfar H, *et al*. Diversity of geophilic dermatophyte species in the soils of Iran; the significant preponderance of *Nannizzia fulva*. J Fungi. 2021;7(5):345.
5. Numan RS, Farhan MS, Abdullah BA, Mamdwooh AE, Abdulla SN. Review of the clinical types of dermatophytes. J Res Appl Sci Biotechnol. 2024;3(3):104-12.
6. Kumar MB, Gulati N, Chander J, Singla N, Bhalla M, Nayyar C, *et al*. Species distribution and antifungal susceptibility profile of dermatophytes from a tertiary care centre in North India. J Lab Physicians; c2022. [Volume and issue missing].
7. Ngegba P, Musa PD. Fungicidal effect of three plant extracts in control of four phytopathogenic fungi of tomato (*Lycopersicum esculentum* L.) fruit rot. Int J Environ Agric Biotechnol. 2018;3(1):239-44.
8. Casadevall A. Fungal diseases in the 21st century: the near and far horizons. Pathog Immun. 2018;3(2):183.
9. Tyagi S. Fungal pathogenicity and diseases in humans: a review. J Pharm Phytochem. 2016;5(6):192-93.
10. Al-abide NM, Mezher MA, Mahde Shugran AH. Identification of chemical content and biological efficacy of *Brassicaceae* family and their effect on the growth of *Candida albicans* yeast isolated from various pathological infections. EurAsian J Biosci. 2020;14(2):[Page numbers missing].
11. Karaman BF, Topal SG, Aksungur VL, Ünal İ, İlkit M. Successive potassium hydroxide testing for improved diagnosis of tinea pedis. Cutis. 2017;100(2):110-4.
12. Ely JW, Rosenfeld S, Seabury SM. Diagnosis and management of tinea infections. Am Fam Physician. 2014;90(10):702-10.
13. Ellabib MS, Khalifa ZM. Dermatophytes and other fungi associated with skin mycoses in Tripoli, Libya. Ann Saudi Med. 2001;21(3-4):193-5.
14. Henshaw E, Olasode A. Prevalence of skin infections, infestations, and papular urticaria among adolescents in secondary schools in Calabar, Nigeria. Ghana Med J. 2019;53(4):287.
15. Kwon-Chun KJ, Bennett JE. Medical mycology. Rev Inst Med Trop Sao Paulo. 1992;34(6):504.
16. Milne LJ. Fungi. In: Collee JG, Marmion BP, Fraser AG, Simmons A, editors. Practical microbiology. 14th ed. Singapore: Longman; c1996. p. 695–717.
17. Collee J, Frase A, Marmion B, Simon A. Makie and McGartney practical medical microbiology. 14th ed. New York: Churchill Livingstone; c1996. p. 978.
18. Ulland TK, Ferguson PJ, Sutterwala FS. Evasion of inflammasome activation by microbial pathogens. J Clin Invest. 2015;125:469-77.
19. Hawksworth DL, Lücking R. Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectrum. 2017, 5(4).
DOI: 10.1128/microbiolspec.FUNK-0052-2016.
20. Iwasa K, Ogawa K, Azukizawa H, Tanabe H, Iwanaga T, Anzawa K. Revival of favus in Japan caused by *Trichophyton schoenleinii*. J Dermatol; c2019. https://doi.org/10.1111/1346-8138.14804.
21. Martinez-Rossi NM, Bitencourt TA, Peres NT, Lang EA, Gomes EV, Quaresemin NR, *et al*. Dermatophyte resistance to antifungal drugs: Mechanisms and prospects. Front Microbiol. 2018;9:1108.