Fungi associated with man and women finger nails

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ABSTRACT

Onychomycosis is the fungal infection of nails caused by dermatophytes, non-dermatophyte molds, and yeasts. Onychomycosis is the most common nail disease with the prevalence of 5.5% all over the world. Objective to know the types of fungi that particularly affect the nail area in men and women. Methods. About 25 nail samples were collected, including 13 males and 12 females. These samples were then grown on SDA and DMT medium and incubated at 37 degrees for a period of 2-14 weeks. After that, the samples were diagnosed using dissecting microscope .Results .The percentage of positive samples was (76%) while the percentage of negative samples was (24%).where different species of fungi appeared in both females and males, such as *Cladosporium sp. ,Aspergillus sp. ,Chaetomiums sp.* and *Penicillium sp*. .Conclusions . the prevalence rate of onychomycosis in males was higher than in females.

Keywords: Onychomycosis , fungal infection , nails

1.1 Introduction:

Onychomycosis is the fungal infection of nails caused by dermatophytes, non-dermatophyte molds, and yeasts. Onychomycosis is the most common nail disease with the prevalence of 5.5% all over the world (Lipner and scher, 2018) .where it caused infection approx nearly 2-9% of the general population globally (D.Gill and Marks ,1999) and the mycosis accounts for half of all nail disorders (P.Veer et al , 2007) and one-third of all fungal cutaneous infec- tions (L.A Drake et al ,1999). Although onychomycosis is hardly life threatening, its high prevalence rate and the associated morbidity such as psychosocial effects, occupational discomfort, permanent damage to the nail, spread of the infection to

other persons, and high treatment cost have made it an important public health problem (P.Veer , et al , , 2007).

Onychomycosis can lead to pain, paresthesia, difficulties in daily activities, impaired social interactiom, and low self-esteem (Lipner and scher, 2018). Trauma, tinea pedis, advanced age, diabetes, psoriasis, malignancy and immunosuppression are regarded as risk factors in the etiology of onychomycosis (Lipner and scher ,2018).

Dermatophytes, especially *T. rubrum* and *T.mentagrophytev* are regarded as the most common causative agents in onychomycosis. Non dermatophyte molds including *Scopulariopsis brevicaulis*, *Aspergillus spp , Acremonium, Fusarium spp, Alternaria alternate,* and *Neoscytalidium* are detected in approximately 20% of the patients. Yeasts (*Candida spp*.) are responsible for 10%-20% of cases with onychomycosis (Lipner and scher, 2018). Mixed infectionswith dermatophytes and non-dermatophyte molds in onychomycosis have been rarely reported (Gupta and Nakrieko , 2014).

1.2 Healthy nails:

Healthy nails are shiny,smooth and uniform in consistency on both the surface and free-margin views .They have attached cuticles ,nail folds and nail plates .Healthy nails are not cracked ,rigid , or broken (Draelos , 2013).

1.3 Nail Structure

The nail consists of the nail plate, the nail matrix and the nail bed below it, and the grooves surrounding it(Onumah ,2010).

1. Nail root/matrix-the living part of the nail situated below the cuticle. Mitosis occurs here to produce new nail cells (Feneis and Heinz ,2000). New cells are pushed forward to form the nail plate. They receive nourishment from blood supply and undergoing mitosis. As they dry out and become keratinised, they push towards the nail plate (D. Schoon, Dougles , 2005).
2. Nail plate - Stratum lucidium and corneum. These cells are fully keratinised and dead. There is no blood supply or nerves. It protects the nail bed. It is the visible portion of the nail and terminates at the free edge (de Berker ,2013).
3. Nail bed-Stratum germinativum/spinosum/granulosum. It is a continuation of the root. It is to nourish and protect the nail structure. It has a blood supply and nerve supply. The nail bed has parallel ridges which 'slot in' with corresponding ridges on the under surface of the nail plate (Feneis and Heinz ,2000).
4. Free edge extension of the nail plate which overlaps the hyponychium (Feneis and Heinz ,2000).
5. Hyponychium a portion of skin at the end of the finger underneath the free edge. (de Berker ,2013).
6. Nail walls-folds of skin that overlap the side of the nails (de Berker ,2013).
7. nail folds are soft tissue structures that protect the lateral and proximal edges of the nail plate. The proximal nail fold protects most of the nail matrix from trauma and ultraviolet rays (de Berker ,2013).

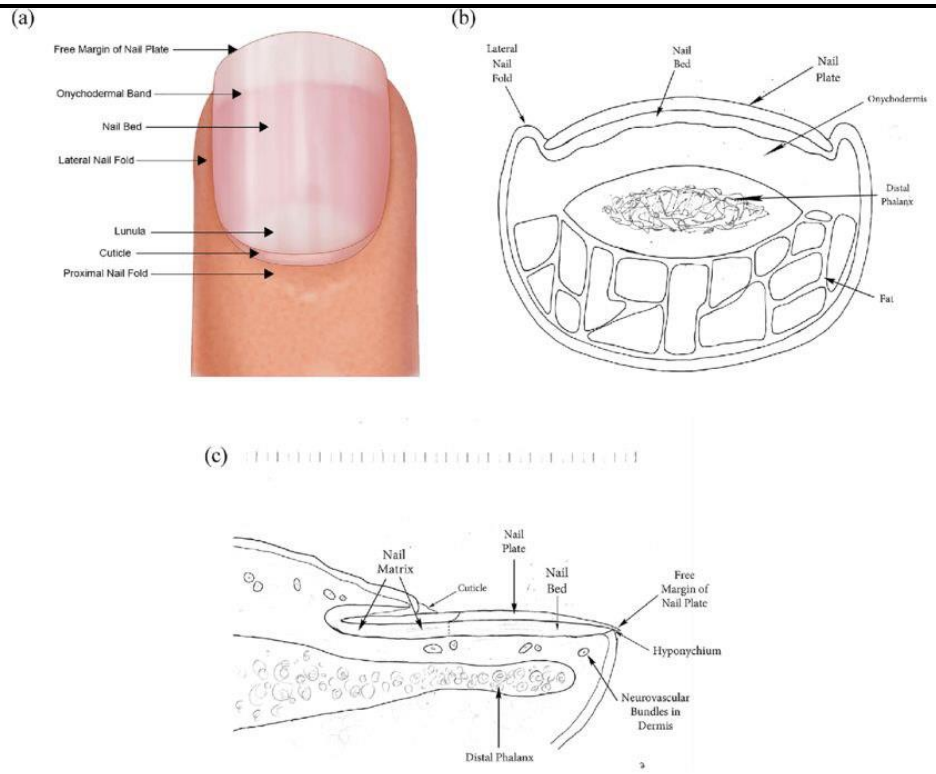


Fig. 1. Healthy nail with normal anatomic structures in place. (a) Surface view (b) Free-margin view. (c) Longitudinal view, the vertical dotted line represents the junction of nail matrix and nail bed ,which corresponds to the distal margin of the lunula .

1.4 Fungal Nail Infections

Fungal nail infections, also known as "onychomycosis," are very common. They may affect up to 14% of the general population. Fungal toenail infections are more common than fungal fingernail infections . Fungal nail infections are caused by many different types of fungi that live in the environment. Small cracks in your nail or the surrounding skin can allow these germs to enter your nail and cause an infection. (Gupta et al 2000)

1.5 CLINICAL MANIFESTATIONS

Typically, onychomycosis presents as a white or yellow- brown discoloration of the nail (Gupta et al 2018). Violaceous, green, and black discoloration of the nail plate have also been ob- served (Thomas et al 2010). Other clinical manifestations include subungual hyperkeratosis, detachment of the nail from the nail bed (onycholysis) and thickening of the nail plate (ony- chauxis) (Piraccini and Alessandrini 2015). Dermatophytoma presenting as linear, single or multiple white, yellow, orange or brown bands on the nail plate is specific for onychomycosis (Thomas et al 2010). In general, toenails are affected seven to ten times more frequently than fingernails (Monteagudo et al 2019). The big toenails are most often affected (Gupta et al 2018) Generally, several toenails are affected and tinea pedis is often present (Fig. 2) (Gupta et al 2019). Also, it is unusual to have more than one fingernail involved without concomitant toenail involvement unless the patient is im- munocompromised or there is a history of trauma (Thomas et al 2010).

Fig.2. Dermatophytoma presenting as a linear, yellow, band on the nail plate of the big toe in a patient with distal lateral subungual onychomycosis .

1.6 Treatment

Fungal nail infections can be difficult to cure, and treatment is most successful when started early. Fungal nail infections typically don't go away on their own, and the best treatment is usually prescription antifungal pills taken by mouth.

Oral antifungal therapy should be prescribed only after confirmation of fungal infection. Oral terbinafine is typically the first-line treatment for confirmed onychomycosis. The treatment course is generally 6 weeks for fingernails and 12 weeks for toenails (Lipner and Scher, 2014).Topical antifungal agents can be used but are often ineffective . Systemic azoles can also be used. Surgical debridement or removal of the affected nail is also a consideration for cases that are resistant to antifungals, and laser treatments for onychomycosis appear to be a promising area for future study (Gupta et al 2013). but Even after treatment, fungal nail infections can come back. This is more common in people who have conditions like diabetes that make them more likely to get a fungal nail infection.

1.7 Classification of Dermatophytes :

dermatophytes are previously classified as Deutromycetes Depending on the methods of asexual reproduction, since sexual reproduction has not yet been discovered, and it is distinguished by its composition Macroconidia and Microconidia (Ichhpnjani and Bhatia ،1994) While it was classified by (Hibbett et al 2007) into

Kingdom: Fungi
Phylum: Ascomycota
Sub phylum: Pezizomycotina
Class: Eurotiomycetes
Sub class: Eurotimycetidae
Order: Onygenales
Family: Arthrodermatacea

2.1 Materials And Methods:

This chapter included the use of devices and tools. It was used for preparing media and sterilization, and also for diagnosing samples. The table below shows the tools and devices used during the work.

Table 1: Devices and tools used in this study

Devices and tools	Country
Autoclave	China
Incubator	China
Sensitive balance	China

Light microscope	China
Slides	China
Disposable petri dishes	China
Sterile swab stick	China
Flasks	China

2.2 Preparation the Medium

A :Sabouraud Dextrose Agar with Chloramphenicol :Prepare according to manufacturer's instructions.This is done by dissolving 65 g of Sabouraud Dextrose Agar with Chloramphenicol (1×250 capsule) in 1000 ml of distilled water

B: Dermatophyte test medium (DTM) :Prepare according to manufacturer's instructions. This is done by dissolving 40.2 g of dermatophyte test medium in 1000 ml of distilled water. Use this medium to distinguish skin fungi from others.

2.3 Sterilization :

Use the Autoclave to sterilize the culture media at a temperature of 121°C and a pressure of 15 pounds for 20 minutes, except for the CHROMagar Candida medium .

2.4 Sample collection :

About 25 nail samples were collected. These samples included 13 males and 12 females. The sample is collected after sterilizing the nails with 70% alcohol to reduce contamination. After that, the nails are cut and kept in sterile dishes, with each dish marked with the person's information from The name, age and type of the sample, as well as the time of collecting the sample so that it can be grown later in the media.

Table 2: Distribution of samples according to age and sex

Age group	No. of people		Total
	Male	Female	
7-10	1	0	1
20-30	5	4	9
31-40	5	5	10
41-65	2	3	5

2.5 Sample culture :

The sample was cultured on two different media, First was SDA suppplemented with chloramphenicol,to prevent bacterial growth and Cycloheximide as antifungal agent . The secound medium was DMT.This was done using Forceps and bunsen burner. The forceps were sterilized by bunsen burner until red, then cooled at one end of the medium. After that, a sample was taken and cultured in the dish. This process was done in a sterile place and near a bunsen burnerand durring these process you should be wear gloves and a mask in order to reduce possible contamination from the air. After completing the culture, the dish was marked with the patient's information, as well as the date the sample was cultured, and it was incubated at 37 degrees for a period of 2-14 weeks until growth appears in the dish.

2.6 Morphological identification :

The dishes were examined after three days of incubation , and were continued to be monitored periodically to monitor growth, especially for fungi that appear late. Morphological examination of

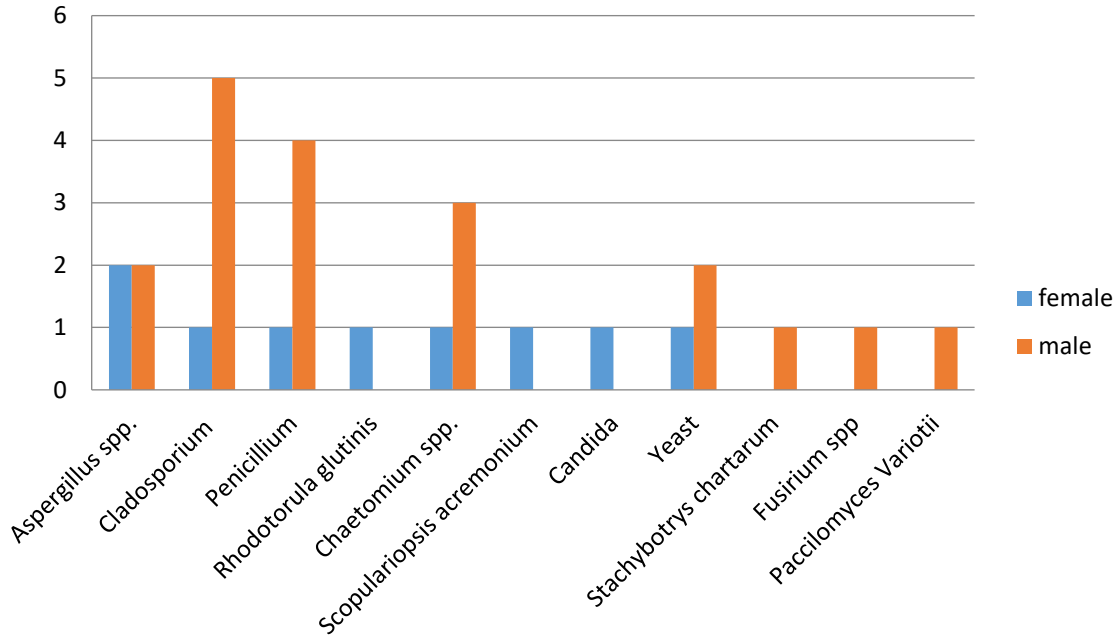
growing cultures is used by dissecting microscope to seen the distinctive characteristics of colonies such as shape, edge, texture, and different reproductive structures (Ellis, 1976,1971;Seifert et al., 2011; Ellis et al., 2007; de Hoog et al., 2000).

3.1Results:-

The Results ,show that percentage of positive samples was (76%) while the percentage of negative samples was (24%).where different species of fungi appeared in both females and males, such as *Cladosporium sp. ,Aspergillus sp. ,Chaetomiums sp.* and *Penicillium sp.*

Table 3 : Number and percentage of positive and negative nail fungal infections for males and females.

Sex	NO. of samples	Number & % of positive results	Number & % of negative results
Males	13	11(57.89%)	2(33.33%)
Females	12	8(42.10%)	4(66.66%)
Total	25	19(76%)	6(24%)



Graph 1. This graph represents the fungi that appeared in both females and males .

The total number of fungal isolates that was isolated from nails samples from male and female paticipants were 29 belong to different species as shown in table 4 we identified 14 fungal specie, belonging to 9 genera. Among these species, the most frequent was cladosporium spp. (20.68%).
Followed by *Aspergilus spp. and Chaetomium spp .The percentage of Cladosporium spp , Penicillium spp and Chaetomium spp* in males was higher than in female samples.
Among the reported species, the participants had *Aspergillus fumigatus ,Aspergillus flavus ,Aspergillus Niger ,Chaetomiune atrobueum ,Chaetomiune bostrychodes , Stachybotrys Chartarum , Paccilomyces Variotii , Scopulariopsis acremonium ,Rhodotrulo glutinis , fusarium spp ,titanium Spp ,Pinicilium spp.*

Table 4: The total number of fungal isolated from male and female

Genus	Types of isolation	NO	%
Aspergilus	*Aspergillus fumigatus*	1	3.44
	Aspergillus Niger	3	10.34
	Aspergillus flavus	2	6.90

Chaetomium	*Chaetomium atrobuenm*	2	6.90
	Chaetomiune bostrychodes	2	6.90
Other genera	*Cladosporium spp*	6	20.68
	Pinicilium spp	5	17.24
	Stachybotrys Chartarum	1	3.45
	Paccilomyces Variotii	1	3.45
	Scopulaviopsis acremoning	1	3.45
	Rhodotrulo glutinis	1	3.45
	fusarium spp	1	3.45
	Candida spp	1	3.45
	Yeast	2	6.90
Total		29	100

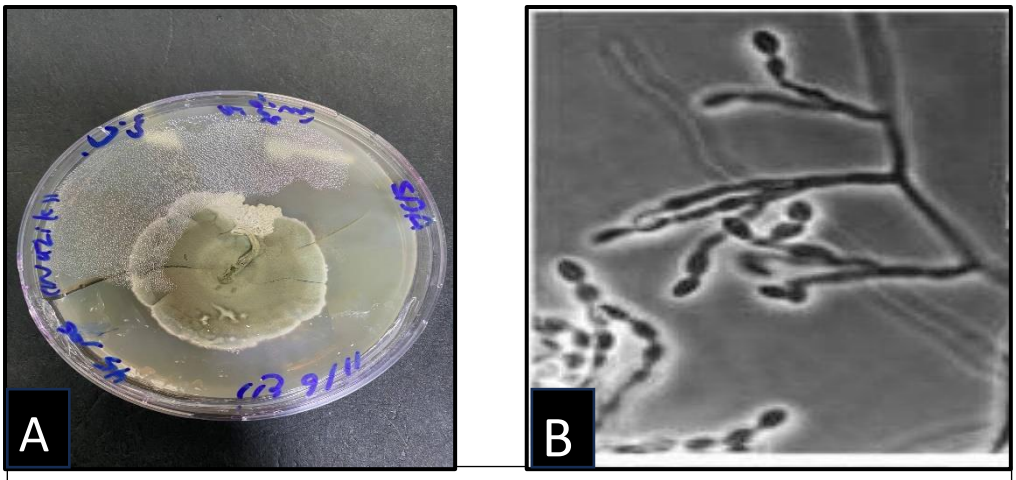


Fig 3.A : Morphology of *Scopulariopsis acremonium* on Sabouraud Dextrose Agar with Chloramphenico B: Conidiophore and chain conidia of *Scopulariopsis acremonium*

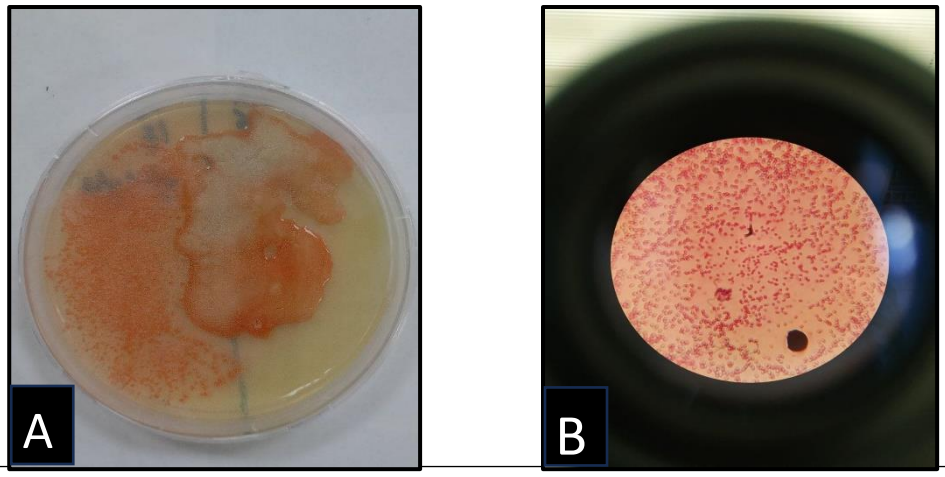


Fig 4. A : Morphology of *Rhodotrulo glutinis* on Sabouraud Dextrose Agar with Chloramphenicol, B: *Rhodotrulo glutinis* under microscope

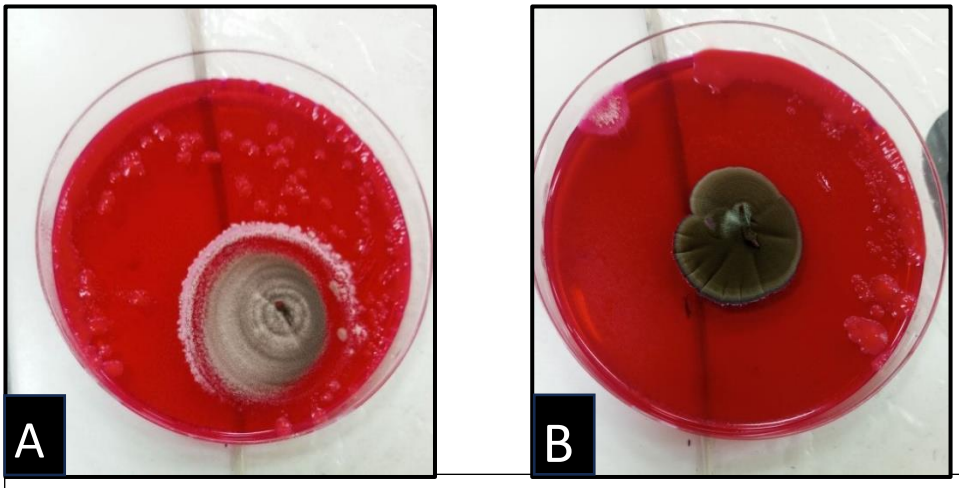


Fig 5.A : *Pinicilium* spp. , B *Cladosporium* spp. on Dermatophyte test medium

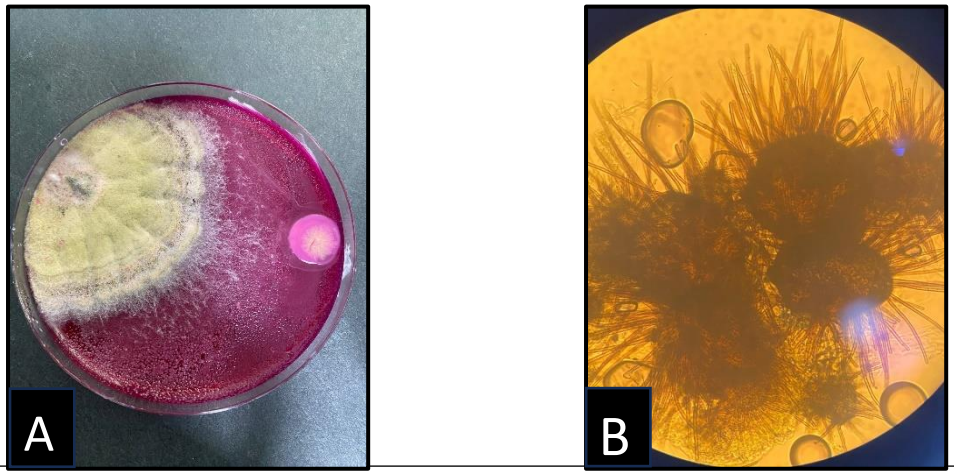


Fig 6. : A :Morphology of *Chaetomium atrobuenm* on Dermatophyte test medium*m*, B :Perithecia of *Chaetomium atrobuenm* under light microscope.

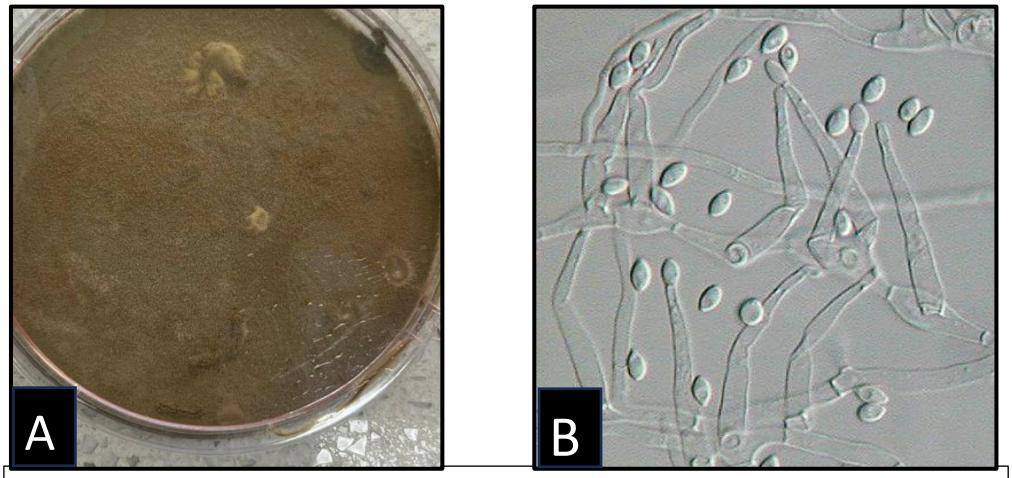


Fig 7.A : A:Morphology of *Paccilomyces Variotii* on Sabouraud Dextrose Agar with Chloramphenicol ., B: Conidiophores and conidia of *Paecilomyces variotii* under microscope

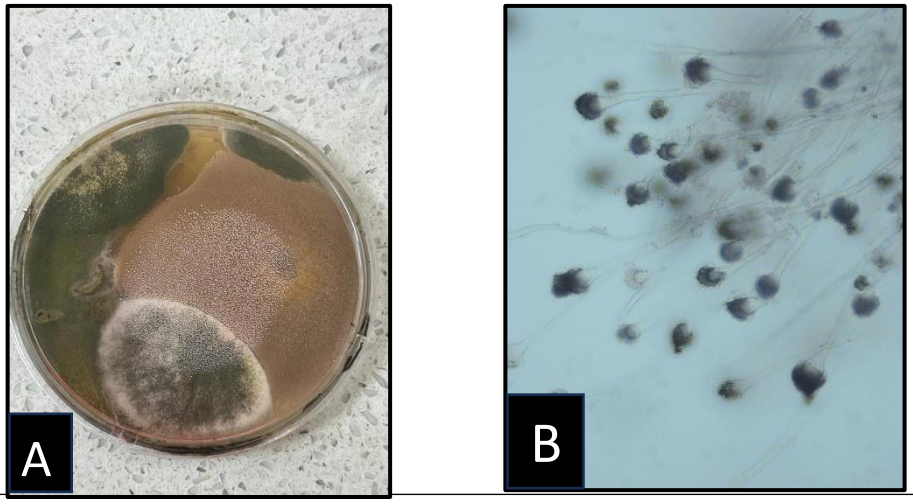


Fig 8. : A : Morphology of *Aspergillus fumigatus* on Sabouraud Dextrose Agar with Chloramphenicol , B: Conidiophores and conidia of *Aspergillus fumigatus* under microscope .

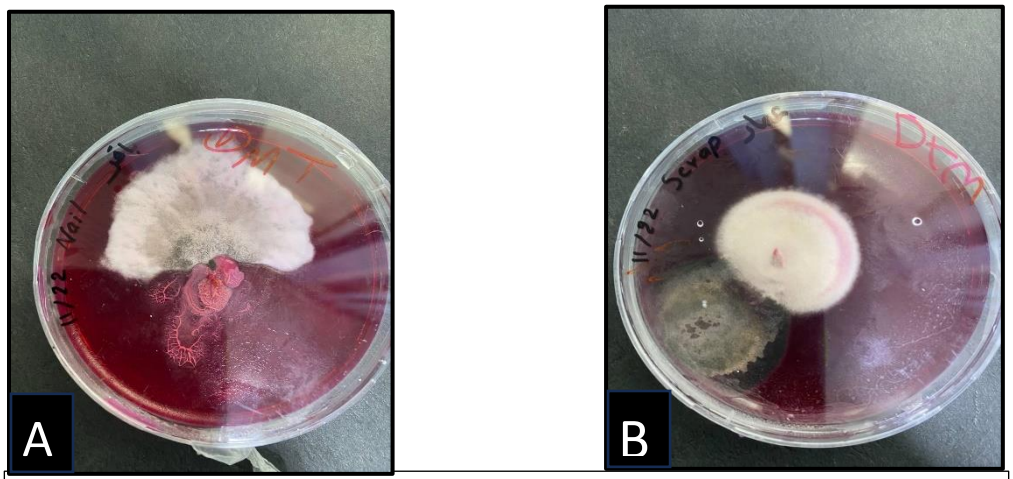


Fig 9 : A :Morpholgy of *Chaetomiune bostrychodes* on Dermatophyte test medium, B :Morphology of *Stachybotrys Chartarum* and *Fusirium* spp on Dermatophyte test medium

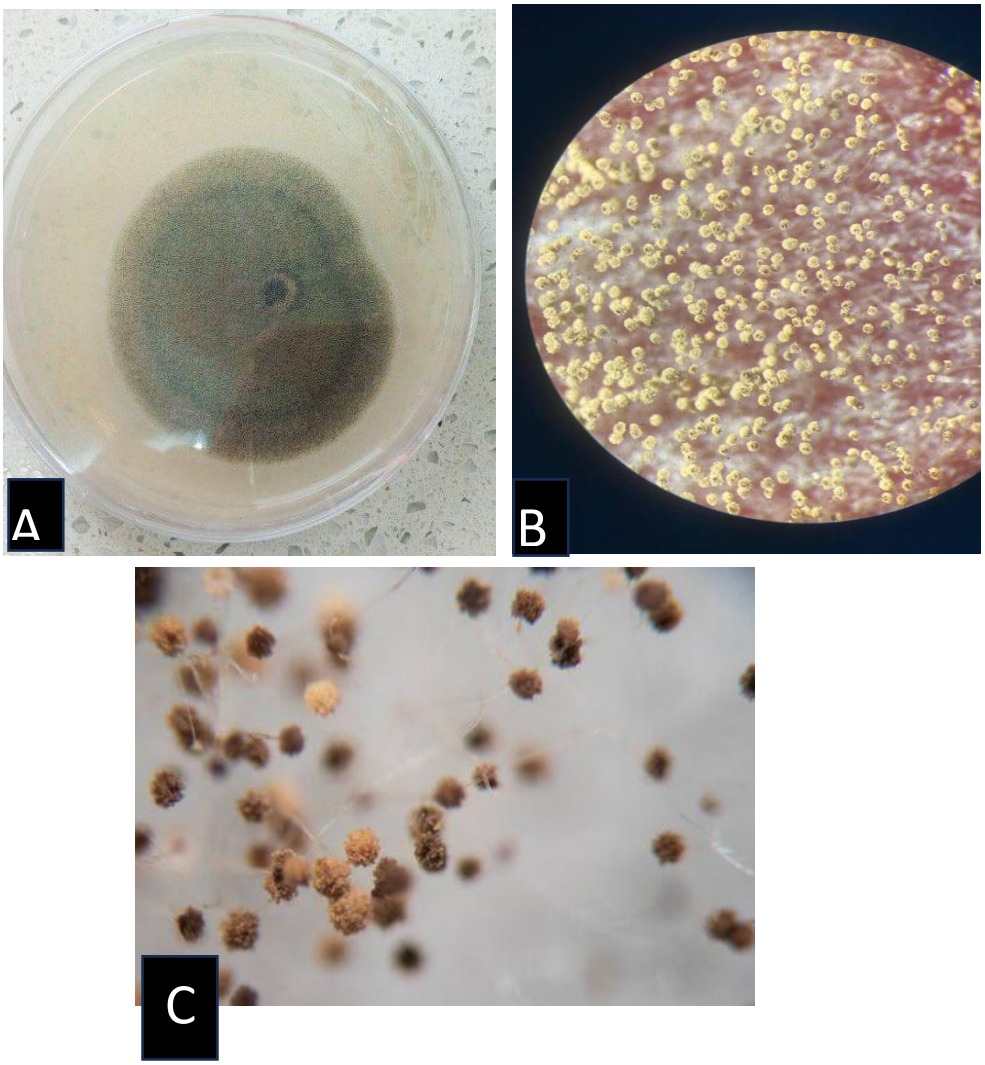


Fig.10 A: Morphology of *Aspergillus Niger* and *Aspergillus flavus* on Sabouraud Dextrose Agar with Chloramphenicol B: head of *Aspergillus flavus* under microscope. C : head of *Aspergillus Niger* under microscope.

3.2Discussion

Onychomycosis represents one of the most common nail diseases. Difficult to bear by some patients, onychomycosis is one of the most frequent reasons for consulting by lack of uniformity and group-control a mycological centre. However, frequency of onychomycosis is miss appreciable.In any case, all the studies concur in the opinion that onychomycosis has been in constant progression over the last twenty years. Rarely observed in children, frequent in adults, onychomycosis principally affects particularly the elderly (DChabasse,2003).The study showed that the most frequent group was *Cladosporium spp* and *Penicillium spp*. Then followed by *Chaetomium spp*. And *Aspergilus spp*. which is

not consistent with the study (Martinelli et al 2017) It was found that the highest infection rate was *Aspergilus spp.* Follow it *Pinicilium spp.* and *Cladosporium spp.* In our study males were affected more than females and that agree with the results of other research such as (Ahuja ,2011) . There are mixed reports about the prevalence of onychomycosis regarding gender(Brilhante et al 2005) . Gender-related back- grounds have been responsible for such variations. Among these, trauma caused as the result of outdoor activities in males and hand wet work in females are the major predis- posing risk factors for the development of onychomycosis. The age group between 20_30 has been the most affected and this does not agree with (Arun et al 2006) where found The commonest affected age group was 21-40 years.

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