

Occurrence And Etiology Of *Tinea*  
*Versicolor* And *Tinea Pedis* In Jordan

— by

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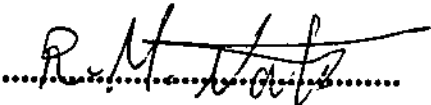

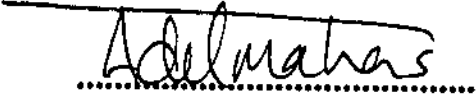
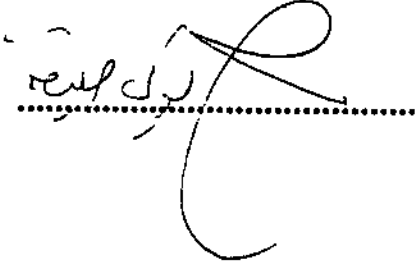
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**Dedication**

TO MY FAMILY

WITH LOVE

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## Abstract

### Occurrence And Etiology of *Tinea pedis* And *Tinea versicolor* in Jordan

by

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Dermatophytes are group of fungi that attack only the keratinized tissues, skin, nails and hair causing dermatophytosis (tineas). This study deals with only two types of different superficial fungal diseases; *Tinea pedis* and *Tinea versicolor*. The study includes an investigation on the relation between the incidence of the two diseases and age, sex, place of living, underlying diseases, animals contact, bathing type of under wear, shoes, and an investigation of the etiological agent in cases of *Tinea pedis*.

The study revealed the occurrence of 233 cases of *Tinea pedis* and *Tinea versicolor* among patients who visited the Dermatology Clinics of the majour Hospital in Amman area and certain prisons, during the period of June 96 to May 97. One hundred and sixty nine cases were reported from the Dermatological Clinics in Medical Hussein Center and Queen Alia Hospital, and 64 cases from the prisoners in Gafgafa, Jwaydeh, Beerein and military prisoners. Determination of etiologic agents were made by direct microscopic examination of samples taken from the patients using (25%) KoH and by culturing on Sabouraud dextrose agar medium, Dermatophyte test medium and Chrom agar.

*Tinea pedis* accounted for 81 cases (34.8%). This disease was more common among elderly people of more than 40 years old, who accounted for 33 cases (40.7%), moreover males accounted for 53 cases (65.4%).

Diabetic patients were found to account for 8 cases (9.9%). The study also revealed no significant relationship between the disease and animal contact. Amman area accounted for 27 cases (38.6%) of this disease. However most of cases were reported during May 97 twenty five cases (30.9%), shoe type may have effect on the disease incidence.

*Candida* species were isolated from the majority of the cases 44 cases. (54.3%). *Candida albicans* was isolated from 26 cases (32.1%) followed by *Candida krusei* 6 cases (7.4%), other unknown *Candida* species 11 cases (13.6%) and *Hansenula* Species 1 case (1.2%); all of these were found to be associated with the intertriginous type of infection; on the other hand, *Trichophyton rubrum* was isolated from 5 cases (6.2%) and was associated with the hyperkeratotic type; followed by *Epidermophyton floccosum* which accounted for 3 cases (11.1 %); *Penicillium* sp. which was isolated from 4 cases (4.9%); *Cladosporium* sp. from 3 cases (3.7%) and *Alternaria* sp. from 2 cases (2.5%).

In comparison, *Tinea versicolor* was found in 152 cases (65.2%) of the patients. The disease was more common among individuals of 21 to 30 years old who accounted for 92 cases (60.5%). Males were affected more than females 124 cases (81.6%) and the disease was more common among military patients 53 cases (53.6%). Patients with blood pressure were found to accounted for 2 cases (1.3%).

The study did not indicate any significant relation between the infected individuals and animals contact. Amman area accounted for 25 cases of this disease (25.3%). Highest incidence of the disease was reported during July 96 which accounted for 40 cases (26.3%) of the.

to internal wear and bathing the study revealed no significant relationship between them and the disease incidence. Direct microscopic examination revealed the presence of *malassezia furfur*; the etiologic agent of *Tinea versicolor*.

# INTRODUCTION

Human superficial infection with fungi dermatophytosis are of world wide distribution in both sexes and among all ages. Very scant study was made on such infections in Jordan. Shehabi *et al.*, 1985, reported that 2.7% of *Tinea capitis* among 4860 children living in various urban and suburban areas of Jordan to have *Tinea capitis* he also reported causative agents were *Trichophyton schoenlenii*, *Microsporum canis* and *Trichophyton violacum*. However a more thorough study on dermatophytosis in Jordan was made by Al Ali (1995) on king Hussein Medical center patients. This study indicated the presence of six types of tinea, with a distribution rate as follows *Tinea versicolor* (33.3%) *Tinea pedis*(29%), *Tinea cruris* (11.3%), *Tinea unguium* (11.3%), *Tinea corporis* (9.3%) and *Tinea capitis* (6%). She also reported their etiologic agents and the frequency of their occurrences as follows; *Trichophyton mentagrophyte* (47%); *Trichophyton rubrum* (22%); *Epidermophyton floccosum* (19%); *Trichophyton schonleinii* (4.5%); *Trichophyton verrucosum* (3%); *Microsporum canis* (3%) and *Trichophyton equineum* (1.5%). *Tinea capitis*; affected mostly the children *Tinea pedis*; affected house wives. Students and military personnel's were affected with *Tinea versicolor*. Al Ali reported, *Tinea pedis* and *Tinea versicolor* to account for the higher rates of infection, she attributed this high rate of their incidence to certain factors, such as occupation and high temperature.

This study aims to achieve the following objectives:

- 1- A thorough survey on the incidence of *Tinea pedis* and *Tinea versicolor* in Jordan.
- 2- Finding any relation between the disease incidence and age, sex, living standard, occupation and some behaviour of the patients in relation to

bathing, type of internal wear, contact with animal, type of shoes and underlying disease.

3- Determining the etiological agents of *Tinea pedis*.

For this purpose, it was possible to study cases from the dermatology clinics of the royal medical services, King Hussein Medical Center, Queen Alia Hospital, Gafgafa, Jwaydeh and Beerein prisoners.

LITERATURE

REVIEW



## 2.1 Introduction

Fungi are known to cause diseases of animals and human, causing mycoses Willard (1974) grouped mycoses on the basis of tissues affected into:

### 1- Superficial mycoses:

Involves the upper layers of the stratum corneum. The main superficial mycoses are the superficial *Candidiasis* and *Pityriasis versicolor*.

### 2- Cutaneous Mycoses:

Involves keratinized tissues, hairs, nails and stratum corneum of the skin. Such tissues are attacked by specialized dermatophytes or “ring worm” fungi. In addition to the dermatophytes, other fungi are some times involved in cutaneous infection. These include a wide variety of soil inhabiting yeasts and opportunistic fungi, and the disease caused by them is called dermatomycosis.

### 3- Subcutaneous mycoses:

Caused by opportunistic fungi that enter through wounds or punctures of the skin.

### 4- Systemic Mycoses:

Caused by opportunistic fungi that infect the major internal organs.

## 2.2. Dermatophytes

The Dermatophytes or ring worm infection (dermatophytosis) are acute or chronic superficial infections of the epidermis or keratinised structures such as nail or hair. The appearance of individual lesions varies with the identity of the causative organism, the site of infection and in some

instances the over all condition of the patients (Hay, 1983) Infections with dermatophytes in man originate from one of the three sources. Animals (Zoophilic); soil (geophilic) or other humans (anthrophilic). Zoophilic infections in man are frequently highly inflammatory, in contrast, anthrophilic infections are often minimally inflamed (Radentz , 1991; Grossman *et al.*, 1995 and Weitzman, 1995).

Dermatophyte infections are usually described by using the latin term *tinea*, followed by appropriate latin description of the site of infection as follows:

- Tinea corporis* is Dermatophytosis of the glabrous skin.
- *Tinea pedis* is Dermatophytosis of the foot (athletes foot).
- *Tinea unguium* is Dermatophytosis of the nails
- *Tinea barbae* is Dermatophytosis of the beard.
- *Tinea manum* is Dermatophytosis of the hands.
- *Tinea cruris* is Dermatophytosis of the groin.
- *Tinea capitis* is Dermatophytosis of the scalp

There are 41 recognized species of dermatophytes, classified within three genera:

- 1- *Trichophyton* (26) species
- 2- *Microsporum* (14) species
- 3- *Epidermophyton floccosum* (1) species

The dermatophytes were considered members of the class *Deuteromycotina* (Fungi imperfecti). Until the discovery of perfect stage in *Trichophyton ajelloi* and *Microsporum gypseum* and others which are make them in the class *Euascomyetes*. Among these, only 23 species are

known to produce infection in man and animals. 15 spp. of *Trichophyton*; 7 spp. of *Microsporum* and one species of *Epidermophyton floccosum*

Dermatophytes as Group are Characterized by :

- 1- Ability to grow on culture media, with alkaline pH and to resist cycloheximide up to 500 mg/ml.
- 2- Sensitivity to 25 mg/ml or less griseofulvin.
- 3- Production of two types of conidia, microconidia ( conidia with one cell) and macroconidia ( conidia with more than one cell).
  - genus *Microsporum* = fusiform macroconidia
  - genus *Trichophyton* = cylindrical macroconidia
  - genus *Epidermophyton* = clavate macroconidia.

### 2.2.1 *Trichophyton* species

The genus *Trichophyton* consists of a large number, of species which attack hair, nails and skin resulting in a wide variety of symptoms depending upon the species and the location of infection. This genus is the most likely cause of *Tinea pedis* and *Tinea unguium* in humans. Usually, no fluorescence occurs in hairs infected by *Trichophyton* species.

### 2.2.2 *Trichophyton rubrum*

#### A- Pathogenicity:

This has a world wide distribution causing *Tinea corporis*, *Tinea pedis*, *Tinea cruris* and *Tinea unguium*, cases are rare in animals, and reported in dogs, cats, rabbits, monkey and sheep (Al doory, 1980 and Larone, 1987) .

**B- Rate of Growth:**

Slow, matures within 14 days.

**C- Colony Morphology:**

Morphology is variable white and downy to fluffy with deep blood red pigment on the reverse side of the colony, some strains of this fungus with powdery surface, other strains may have heaped colonies which has relatively glabrous.

**D- Microscopic Morphology:**

Septate hyphae, with lateral tear drop shaped microconidia ( $2-3 \times 3-5 \mu\text{m}$ ) borne along the sides of hyphae and not in clusters. Macroconidia ( $4-6 \times 15-30 \mu\text{m}$ ) may be abundant, rare or absent. When present, they are long, thin walled, with thick attachment to the vegetative hyphae. Produce recquet hyphae, chlamydospores and nodular bodies. It dose not perforate Human hair in vitro and has a negative urease test.

**2.2.3 *Epidermophyton floccosum***

Consist of single species, and it infects skin and nails only.

**A- Pothogenicity:**

Has a world wide distribution common causes infections of human groin and foot and occasionally it infects body and nails. It dose not infect hairs (Al-dorry, 1980 and Larone, 1987).

**B- Rate of Growth:**

Moderate growth, mature within 10 days.

**C- Colony Morphology:**

A flat culture with surface color olive gray or khaki, radiating grooved developed with age. Aerial hyphae may developed as white tufts on the colony surface, the reverse side is burnt orange.

#### D- Microscopic Morphology:

Septate hyphae, microconidia are never formed, macroconidia are numerous club-shaped ( $7-12 \times 20-40 \mu\text{m}$ ) with rounded ends. Contain two to six cells; smooth, thin walled and are found singly or in characteristic clusters; macroconidia often transform into chlamydospores.

### 2.3 The Opportunistic Fungi

Opportunistic fungi are those, which under normal conditions are not pathogenic, but prove to be pathogenic under the influence of some hosts changes. However, some opportunistic fungi become parasitic without requiring any host modifications while others become parasitic only when host resistance is substantially impaired (Braathen *et al.*, 1980; Chaiclemenos *et al.*, 1995 and Rossmann *et al.*, 1996).

These fungi are considered to be keratinophilic and are isolated from the soil (Shtayeh, 1989) and sheep hairs. The potential pathogenicity of these keratinophilic fungi is considered to be as a natural evolution from keratin utilization to invasion of stratum corneum in animals (Shtayeh *et al.*, 1989).

#### 2.3.1 *Alternaria* sp.

##### A- Pathogenicity:

Commonly considered as a contaminant but occasionally reported as the opportunistic causative agent of allergic pulmonary disease, skin infection and nasal infection in compromised patients (Chaiclemenos *et al.*, 1995)

##### B- Rate of Growth:

Colonies of *Alternaria* grow rapidly and mature within 5 day.

**C- Colony Morphology:**

At first they appear gray with wooly surface then become greenish-black olive green or black, the reverse side of the colony is black.

**D- Microscopic Morphology:**

Hyphae appear septate and dark, conidiophores are septate of variable length and some times branched. Conidia are muriform, have both longitudinal and transverse septation ( 7-10 × 23-34 μm), brown and are produced in succession, forming chains of conidia which are characterisitc to this genus; conidia may be formed singly, also conidia some times produce germ tubes which are ovate to elongate round at the end nearest the conidiophore and narrow at the far end.

**2.3.2 *Cladosporium* sp****A- Pathogenicity:**

There are several pathogenic species in this genus which may cause chromomycosis, cladosporiosis and other mycoses. The saprobic species are characterized by forming poor to no growth at 36°C, or on media containing cycloheximide antibiotic.

**B- Rate of Growth:**

Moderately rapid, mature within 7 days.

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**C-Colony Morphology:**

The surface is greenish brown or black with grayish, aging deepens the color of the colonies toward black, colony is powdery, velvety and becomes heaped and slightly folded, the reverse of the colonies is always black.

**D- Microscopic Morphology:**

Hyphae septate, dark, conidiophores are dark, branched, vary in length, the tip of each branch produces two or more conidial chains by

budding at the distal poles. The cell bearing the conidial chains are large, some times septate, the conidia (3-6 × 4-12 μm) are brown oval, usually smooth form branching tree like chains and easily dislodged.

### **2.3.3 *Penicillium* sp**

#### **A- Pathogenicity:**

*Penicillium* spp. are reported as opportunistic pathogens for human beings, they can form flattened fronds of hyphae along lines of weakness within the substratum by means of perforating organs. They can penetrate deep into a hard resistant substratum (Decan, 1984)

#### **B- Rate of Growth:**

Rapid mature within 4 days.

#### **C- Colony Morphology:**

Surface at first white and flat then velvety and later on it become powdery, bluish green with white border. This genus include a large number of species with variation in macroscopic and microscopic appearances. Some species exhibit a yellow red or brown color, caused by an exudate from the fungus and not from pigments in fungal mycelium or conidial heads; reverse side is usually white but may be red or brown.

#### **D- Microscopic Morphology:**

Hyphae appear hyaline, branching and septate, with branched or unbranched conidiophores that have secondary branches known as metula. Flask shaped phialides (strigmata) are arranged in whorls on the metula that bear unbranched chain of smooth or rough round conidia giving the appearance of brush (broom).

### 2.3.4 Yeasts

Yeasts provoking mycoses of the feet are usually *Candida* species *Candida albicans* and *Candida krusei* (Rieth, 1978 and Larone, 1987)

## 2.4 *Tinea pedis*

*Tinea pedis* is disease caused by dermatophytes, yeasts and moulds which infect the interdigital spaces, subdigital folds and the soles. (Rieth, 1978 and Leyden, 1978).

The disease is also known as [athlete's foot, hongkong foot, epidermophytosis and ring worm of the foot].

### 2.4.1 Pathogenesis

The development of the infection is encouraged by a number of factors which stimulate infection creating the conditions for fungal growth. The most important factors are the moisture and warmth of the toe clefts which are induced by shoes and socks providing a humid tropical environment which encourages the growth of fungi, (Willard, 1974 and Rieth 1978). Infection is more pronounced in the space between the fourth and the fifth toes and it is the site which is most frequently involved. The disease is of a world wide distribution and is generally occurring in adult life.

### 2.4.2 Clinical Description.

Willard (1974); Ashton and Leppard (1993) recognized four different patterns of tinea on the foot.



### 1- The Chronic Intertriginous

This is the commonest, characterized by peeling, maceration and fissuring of the area between the third and fourth toes, which are the most often involved. The webs, subdigital and interdigital surfaces are the favoured sites. The area is covered with dead, white macerated epidermis and with often a foul odor and erythematous edge; the major symptom is itching.

The infection begins on one foot only and later it may spread to the other foot and or toe nails. The infection may be intensified by hot, humid summer weather and becomes severely pruritic.

### 2- The Chronic Hyperkeratotic

Is very persistent and difficult to treat. It is characterized by the presence of areas of pink skin, covered with fine silvery white scales, and is commonly bilateral. The lesions may involve the whole foot; in such a case the disease is termed "moccasin foot", the etiologic agent is usually *T. rubrum*.

### 3- The Vesicular or Subacute

In which the lesion is characterized by the appearance of vesicles, vesiclopustules and some times bullae. The involved area may extend from the intertriginous areas to include the dorsal surface of the foot; the eruptions vary in size up to 7-9 mm and are either Isolated or occur in patches.

### 4- The Acute Ulcerative

This is characterized by ulceration of the epidermis and formation of purulent vesicles. This form of the disease is complicated by secondary bacterial infection and the "ide" reaction is common.

### 2.4.3 Dermatophytosis Complex

Dermatophytosis complex present as white maceration and soggy interdigital scaling accompanied by bad odor; is caused by the proliferation of web space flora (Roth and James, 1988).

Simple scaling type of infection is caused by dermatophytes invasion of the stratum corneum, maceration and over growth is caused by bacteria. Particularly *Brevibacterium epidermidis*, *Micrococcus sedantarius* and various gram negative flora, such as *Proteus* sp. and *Pseudomonas* sp. (Noble, 1984 and Lyden, 1994).

### 2.4.4 Etiology

The majority of cases of foot ring worm throughout the world is caused by *Trichophyton rubrum*, *Trichophyton interdigitale* and *Epidermophyton floccosum* (Rook *et al.*, 1987).

Double infection with any two of these species may occur and for precision, especially in clinical surveys, it is useful to adopt the following terminology (Rook *et al.*, 1987), to describe them:

1. **Combined Infections:-** are those in which different causal organisms are present in the same lesion.
2. **Concurrent Infections:-** are those in which different causal organisms are found in different lesions at the same time.
3. **Consecutive Infections:-** are those in which the same patient has different causal organism at a single site at different occasions.

Obasi *et al.*, (1988) found that men suffering from *Tinea pedis* (21 of 65 patients) were mycologically positive. However the etiologic agents were isolated from only 10 cases; *Hendersonula trouloidea* (2), *Trichophyton*

*interdigitale* (1) *Trichophyton soudanense* (1), *Epidermophyton floccosum* (1) *Candida albicans* (1) and *Fusarium* species (4).

Kombila *et al.*, (1990), isolated pathogenic fungi from 149 Gabonese patients, who exhibited clinical signs of *Tinea pedis*. The isolated fungi were *Hendersonula toruloidea*, isolated from 51 cases (34.2%) which often was accompanied with *Candida* species.

In another study made by Nolting *et al.*, (1993) on 382 patients, a total of 381 fungi were isolated. Such fungi were *Trichophyton rubrum* (196) *T. mentagrophytes* (73), other dermatophytes (17) *Candida albicans* (65), other yeasts (23), *Aspergillus* sp.; *Penicillium* sp. and *Mucor* (7).

Hope *et al.* (1984) found that out of 514 coal miners, 1/3 of coal miners populations were infected with, *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum*. Gram negative bacilli accounted for most of the remaining cases. However, he reported the bacteria (*Actinobacter* sp.) to be non-pathogenic, while (*Aeromonas*, *Pseudomonas* and *Protus* sp.) are potentially pathogenic.

Al- Ali (1995), studying 33 patients with *Tinea pedis*, isolated. *Trichophyton mentagrophytes* from 14 cases; *Trichophyton rubrum* from 6 cases; *Epidermophyton floccosum* from 8 cases and *Candida* sp. from 5 cases.

#### 2.4.5 Differential Diagnosis of *Tinea Pedis*.

Infections with *Tinea pedis* are largely restricted to the toe cleft. On the other hand, *erythrasma* which is usually asymptomatic but often resembles *Tinea pedis*. However, woods lamp using, *erythrasma* may show pink fluorescence. *Candidiasis*, also resembles *Tinea pedis* by being characterized in forming a rather more white macerated skin with

erythematous base. Other bacterial infections with G-ve bacteria including *Actinobacter* can produce infection accompanied by inflammation and often a foul odor (Leyden, 1994) in addition to this bacterial over growth particularly that of *Pseudomonas* sp, show under woods lamp a greenish fluorescence. Clinically diagnosed *Tinea pedis* is further ascertained by microscopic examination and culturing of repeatedly taken scrapings.

## **2.5 Pityriasis Versicolor.**

symptoms are : bran-like and the term is used for scaly rash; of different colors (Ashton and Leppard, 1993). The disease is a superficial, chronic, mild fungal disease of the stratum corneum, usually asymptomatic. It is also known as *Tinea versicolor*, *Chromophytosis*, *Tinea flava* and *Liver spots*.

### **2.5.1 Clinical Description**

The lesions are characterized by branny consistency and always scaly when scratched. Small scales are less than 1cm in diameter, discrete and some may join together to form larger plaques, coloured white orange to brown or dark brown, according to the normal pigmentation of the skin, exposure of the area to sunlight and the severity of the disease. The areas usually involved are the chest, neck, abdomen, upper arms and the back. Lesions may also be found elsewhere on the skin, with the exception of soles and palms, (Willard, 1974; Ashton and Leppard, 1993; Faergemann, 1993 and Roberts, 1969).

## 2.5.2 Etiology and Pathogenesis

Infection is caused by the organism *Malassezi furfur*, the two commensal form of this fungus *Pityrosporum orbiculare* and *Pityrosporum ovale* are both epiphytes, exist as resident members of the normal skin flora. On the basis of microscopic morphology *P. orbiculare* is with round yeast cells and buds from a narrow neck; *P. ovale* is with ovale cells and buds from a wide neck. Both are lipophilic yeast (Ashbee *et al*, 1993) *P. orbiculare* and *P. ovale* can be cultured not only from patients with pityriasis versicolor, but also from normal looking skin. In (1913) Castellani and Chalmers isolated aliphilic ovale budding yeast from normal skin and seborrheic dermatitis. They named the organism *Pityrosporm ovale* and their description of the organism was based on its appearance, both in skin scales and in the laboratory setting. Faergemann (1993) in a culture study from normal looking skin in healthy children, could not culture *P. orbiculare* from those less than 5 years of age, but in 15 year old children the fungus was found with the same incidence as in adults. Other investigators have found great variations in the type of the causal organism (*P. ovale* or *P. orbiculare*) but *P. orbiculare* was mos prevalent (Faergemann, 1985, 1993). Colonization starts during puberty and in elderly persons; number of yeasts diminish due to a decrease in skin lipid.

Under the influence of predisposing factors; such as high temperatur, high relative humidity, occlusion by clothes, occupation and immunodifficiency, *P. orbiculare* or *P. ovale* in *pityriasis versicolor* increase in numbers and undergo changes from the round budding blastospore form to mycelial form. The name given to the mycelial form is *Malassezia furfur* which is a dimorphic yeasts lipophilic, the yeast is normally resides on human skin in the sebum-rich areas of the upper trunk and head. It is found on skin where sebaceous glands are present in mature ages when the

sebaceous gland are most active. the predisposing factors can be either endogenous to the individual or exogenous (Roth and James, 1988; Faergemann, 1993).

*Malassezia furfur* in its yeast phase based on serological differences in cell surface antigens can be classified into three serovars A, B and C. Microscopically serovars A and B have round blastospores, whereas serovars C possesses oval blastospores. Historically, these may have been described as *P.orbicular*e and *P.ovale* (Cunningham *et al.*, 1992).

There is relatively little information concerning the carriage and distribution of the three serovars of *Malassezia furfur* on the skin of normal individuals at various ages. It has recently been shown that *P.ovale* was the dominant species on the scalp, *P.orbicular*e was the more numerous on the trunk and is present in more than 90% of subjects (Roberts, 1969).

The chief complaint of the patients is with cosmetic problems, but pruritus may some times be troublesome and it is more pronounced when the patients are sweating. The earliest lesions are frequently red but then become depigmented.

Modification of Pigmentation and damage of melanocytes are characteristic features of skin colonisation with *Pityrosporum* hyphae. In pityriasis versicolor, the yeast is lipophylic and lipid dependent, capable of oxidising unsaturated lipid components of skin surface lipid. Skin surface lipid consist, of a mixture of saturated and Un-Saturated fats of sebaceous and epidermal origin (Nicollier,1986). *Pityrosporum* is capable of oxygenation of Un-saturated free fatty acids containing a 1,4 -cis pentadien system, catalyzed by lipoxygenases enzymes. The oxidation of un-saturated free fatty acids give rise to dicarboxylic acid which is thought to cause damage to melanocytes as well as to inhibit tyrosinase which results in the dys pigmentation ( Deluca *et al.*, 1996 and Nazzaro, 1986).

The explanation for the hyper pigmentation remains obscure, although electronmicroscopy reveals abnormally large melanosomes in hyperpigmented lesions and smaller than normal melanosomes in hypopigmented ones.

*Pityriasis versicolor* is not a contagious disease. Twenty volunteers were studied, ten were normal individuals and the other ten were patients who had a history of infection with *pityriasis versicolor*. Three adhesive plaster tapes were placed on the upper back of each volunteer. The first tape contained *pityrosporum* species, the second tape *Malassezia furfur* and the third tape was empty and used as control. Two weeks later all tapes were removed and the skin was re-examined both clinically and mycologically. Among patients with a history of *pityriasis versicolor*, lesions appeared under the first tape in 8 patients (80%) and among the second and third tapes in 6 patients (60%). Among the control group, *pityriasis versicolor* lesions appeared under the first tape in 2 volunteers (20%) and under both the second and third tapes in one patient each (10%). These findings indicated that these organisms yield clinical *Pityriasis* lesions only in the susceptible individuals, and contamination by fungal spores doesnot automatically give rise to lesion. Under normal conditions, healthy skin has a high resistance to the development of mycoses. In order to develop infections, the fungus require of a predisposing factors (Galadari, 1990).

### 2.5.3 Differential diagnosis of *Tinea versicolor*

The pigmentary changes may be very distressing to patients who may believe that they herald the onset of other forms of skin disease such as *vitiligo* and *chloasma* which are distinguishable by complete absence of scaling in them.

*Erythrasma* may closely mimic *Pityriasis versicolor* with pigmentary change and scaling, but satellite lesions are less common fluorescence under the woods Lamp is pink. *Pityriasis versicolor*, on the other hand has yellow fluorescence and it easily diagnosed under the light microscope by using a potassium hydroxide (KOH) preparation of skin scraping, and observing the hyphae which are numerous, short, straight or angular, may surround the clusters of thick walled round cells (Beneke and Rogers 1980; Hay, 1983).



**MATERIALS AND  
METHODS**

Fungal skin diseases of the feet (*Tinea pedis*) and *Pityriasis versicolor* (*Tinea versicolor*) in Amman area were surveyed between the period of June (1996) and may (1997). Among patients of Royal Medical center, Queen Ali'a hospital and Gafgafa, Beerein, Jwaeydeh and military prisoners.

During this period specimens were taken from 169 patients, suffering from fungal infection of the toe webs (*Tinea pedis*) and smooth skin (*Tinea versicolor*), which were clinically diagnosed at the dermatology clinic. Besides of this 64, specimens were taken from prisoners at Gafgafa, Beerein, Jwaydeh and military preson suffering from *Tinea pedis* and *Tinea Versicolor*.

### **3.1 Collection of Specimens for Mycological Examination**

#### **3.1.1 *Tinea pedis***

The specimens of skin (toe webs) were taken from an area previously cleansed with 70% alcohol to reduce the amount of contamination and to remove any topical ointement.

Specimens were taken from the web between the fourth and fifth toes, where infection usually starts. Specimens were taken by removing as much dead white macerated epidermis from the active edge of the lesion with sterile blade (Martin, Germany), placed on a microscopic slide or in container for examination (Al-Doory, 1980).

#### **3.1.2 *Tinea versicolor***

Specimens of diseased skin were obtained following two methods :

- (i)- by stretching the skin lesion in both directions (longitudinal and transverse), so that a bran-like scales would appear. Then scales were

scraped with a blade and placed on a slide or in a container for examination (Beneke and Rogers, 1980).

- (ii)- Pressing a piece of clear scotch tape on the lesion then removing the tape containing infected fragments of the horny layer of the skin, held with a forcep and is dipped into Giemsa stain solution for staining (Benek and Rogers, 1980).

### **3.2 Examination of the Specimens.**

Enough amount of skin scraping was collected for both microscopic examination and culturing.

#### **3.2.1 Direct Microscopic Examination**

Small parts of scraped specimens were examined microscopically, 10-20% solution of potassium hydroxide following Larone (1987) methods as follows:

1. A drop of KOH solution 20% was first placed on a clean glass slide.
2. Small pieces of specimens was transferred to the drop of KOH.
3. A cover slip was placed over the specimen.
4. Heating gently over flame for few seconds, avoid boiling to prevent crystal formation by KOH.
5. The slide were examined with a light compound microscope (Olympus) using 10X and 40X.

#### **3.2.2 Direct Stain on Plastic Tape**

This method of Beneke and Rogers (1980) is recommended for use in a staining for fungal infection of the horny epidermis, as follows:

1. Placing a tape with adhesive material, side up on a slide.
2. Staining for one minute with Giemsa stain solution.
3. Rinsing with alcohol for a moment
4. Drying and mounting on a slide.

**Giemsa Solution Stock.** (Al- Dorry, 1980).

- i. Giemsa stain powder.....4. g
- ii. Glycerine..... 250mL
- iii. Methanol..... 250mL

**Giemsa Working Solution.**

- i- Giemsa stock solution..... 4mL
- ii- Distilled water (buffered PH. 6.8)..... 96mL.

### 3.3 Culturing of the Specimens.

Specimens of *Tinea pedis* were cultured on sabouraud dextrose agar, dermatophyte test medium and Chrom agar media. However routine culture for the *Tinea versicolor* is not recommended for diagnosis, yet the causal organism can be cultured at 37°C on sabouraud dextrose agar, containing cycloheximide, streptomycin and penicillin, with a layer of sterile olive oil over the surface (Beneke and Rogers, 1980; Larone, 1987; Leeming and Notman, 1987).

**A- Sabouraud Dextrose Agar With Antibiotic (SDA).**

This medium is useful for isolation of pathogenic fungi from clinical materials heavily contaminated with bacteria and saprophytic fungi.

**Formula for One Liter:-**

- i- Dextrose ..... 20. g
- ii- Mycological peptone..... 10.g
- iii- Agar.....17.g
- iv- Distilled water.....1000.mL.
- v- 0.5 g of cycloheximide dissolved in 10mL acetone.
- vi- 0.05g of chloramphenicol dissolved in 10 mL of 95% ethanol.

The antibiotics are added to the medium before autoclaving at 15 psi for 10 min, Final pH 6.9.

**B. Dermatophyte Test Medium (D.T.M).**

This medium is used for presumptive identification of dermatophytes. Most dermatophytes, produce a red color in the medium within 14 days, while most saprophytic fungi, yeasts and bacteria do not modify the yellow color of the medium.

**Formula for One Liter:-**

- i- phyton.....10.g
- ii- Dextrose.....10.g
- iii- Agar.....20.g
- iv- Phenol red solution..... 40 mL (0.5 g of phenol red dissolved in 15mL of 0.5 N (NaOH) made up to 100 mL with distilled water).
- v- 0.8 M Hcl..... 6mL
- vi- Cycloheximide..... 0.5g dissolved in 2 mL of distilled water.
- vii- Gentamicin sulfate ..... 0.1g dissolved in 2 mL of acetone.

Autoclave at 15 psi for 10 minutes and cool to  $\approx 47^{\circ}\text{C}$ , pH  $5.5 \pm 0.1$ .

viii- Hydrochlorated Chlorotetracycline.....0.1 g. dissolved in 25 mL of sterile distilled water and add to the medium.

### C. Chrom Agar

Chrom agar is a selective and differential medium that allows selective isolation of yeasts and simultaneously identifies (by color reaction and colony morphology) colonies of *Candida albicans*, *Candida tropicalis* and *Candida krusei*, with a high degree of accuracy and can provide results within 24- 48h (Pfaller *et al.*, 1996). This medium was purchased as powdered prepared according to the manufactures instructions.

### 3.3.1 Culture Methods.

- Sabouraud dextrose agar medium, with antibiotic or dermatophyte test medium, were dispensed into screw cap bottles or tubes, autoclaved, slanted and cooled; culturing was also made in petri dishes.
- Inoculations were made by placing fragments of infected skin on the agar surface using inoculating sterile needles.
- Cultures were incubated at temperature, 25-30°C.
- Examination of cultures, were done twice weekly. Dermatophytes take 5-25 days, to grow and yeasts require 24-48. hours; saprophytic fungi grows within, 2-4 days. Cultures which did not yield any growth were discarded after 4 weeks (Larone, 1987).

## 3.4 Identification of Fungi

### 3.4.1 Macroscopic Examination

The visual examination of a fungal colony was made considering the following characteristics:

- i- The rate of growth. Slow, moderate, rapid.
- ii- Texture of the colony e.g. fluffy, fuzzy, floccose, glabrous, silky, powdery, velvety, cottony... etc.
- iii- The topography of the colony e.g. flat, folded, grooved, .... etc.
- iv- The color of the colony e.g. olive - green, cream, light gray, brown,....etc.
- v- The reverse pigmentation e.g., black, deep red, brownish, ... etc.

### **3.4.2 Microscopic Examination.**

Microscopic examination, was performed to observe the following fungal characteristic:

- i- Mycelium septation, branching, spiral hyphae, racquet hyphae, faveic chandeliers, formation of pectinate bodies, nodular organs. Reflexive hyphae, ....et.
- ii- Macroconidia. formation their shape, size, presence of septa and the degree of abundance.
- iii- Microconidia formation their shape, size, special arrangement in relation to the mycelium and their abundance or absence.

### **3.4.3 Slide Preparation for Microscopic Examination**

#### **A. Tease Mount Technique**

- i- A drop of lactophenol cotton blue stain was placed on the slide.
- ii- A bent needle sterilized by flaming was used to remove a small portion of the colony to be studied. The sample was taken at a point intermediate between the center and the periphery of the colony, then teased apart with a dissecting needle.

- iii- A cover slip was placed over the fungal specimens and was gently pressed to ensure that the fungal fragments were flat against the slide and to avoid air bubbles.
- iv- The slide was left for a minutes, to allow the stain to penetrate the fungal specimen.

### **B. Scotch Tape Technique**

- i- The tape strip is looped back on itself with the adhesive side out.
- ii- The adhesive side was then pressed firmly to the surface of a fungal colony, so that aerial hyphae will cling to the sticky surface.
- iii- The tape strip was then opened up and placed on a small drop of lactophenol-cotton blue.
- iv- A cover slip was placed and left for one minute to allow stain to penetrate the fungal specimen.

Lactophenol-cotton blue, was used as a mounting fluid and a stain

### **Lactophenol-Cotton Blue Formula**

- i- Lactic acid..... 20. mL
- ii- Phenol crystals.....20.g
- iii- Glycerol.....40 mL
- iv- Distilled water..... 20 mL.
- v- Cotton blue.....0.05g

Dissolve phenol in the lactic acid glycerol and water by gently heating, then add cotton blue and mix well (Larone, 1987).



## Identification

The following references were used for identification of the isolated fungal genera and species:

(Willard, 1974; Al. Doory, 1980; Beneke and Rogers, 1980 and Larone, 1987)

### 3.4.4 Hair culture technique.

This technique was used to differentiate between *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

*Trichophyton mentagrophytes* perforates hair in vitro, but *Trichophyton rubrum* does not. This test is done only when the causal organism is one of these two fungi (Larone, 1987).

#### Procedure:

- i- Place about 10 small pieces of sterilized human hair into a glass or a plastic petridish.
- ii- Add 0.1 mL of 10% sterile yeast extract and 20 mL of sterile distilled water to the plate .
- iii- With sterile needle place several fragments of un-known fungal culture into the petri dish and mix well.
- iv- Incubate the culture at 30°C for 4 week.
- v- At weekly intervals, examine hairs by placing one or two fragments into adrop of lactophenol-cotton blue on a slide with cover slip.
- vi- Observe for growth of the fungus; if there is growth around the hair, the organism is *T.rubrum*, if hairs are perforated by the fungus, it is *T.mentagrophytes*.

### 3.4.5 Urea Agar.

This medium is used for the differentiation of the yeast like fungi and also for the identification of some trichophyton spp. and aerobic Actinomycetes urease positive organisms produce an alkaline reaction indicated by a pink-red color within seven days. It is available from commercial sources (Oxoid).

#### Content :

I- Peptone.....	1.g
ii- Dextrose.....	1.g
iii- Sodium chloride.....	5.g
iv- Disodium phosphate.....	1.2g
v- Mono potassium phosphate .....	0.8g
vi- Phenol Red .....	0.012g
vii- Agar .....	15g.

---

Formula : added 24 g of dehydrated powder to 950 ml of distilled water

Autoclave at 15 psi for 15 min then, cool the medium to  $\approx 50^\circ\text{C}$  and added the 50 ml of sterilized Urea broth, final pH  $6.8 \pm 0.2$ .

### 3.4.6. Animal inoculation

This technique is usefull for laboratory study of the nature of the lesions developed by the organisms and for study of immunity. Guinea pigs and some times mice, are the preferred animals for incoulation (Beneke and Rogers, 1980; Hahn, 1988 and Hernadoze *et al.*, 1986) .

**Procedure:**

- i- A shaved area on the back of the mice and Guinea pigs about 2cm<sup>2</sup>.
- ii- Prepare a heavy suspension of the fungus (*Penicillium*, *Alternaria*, *Cladosporium*, *Trichophyton rubrum* and *Epidermophyton floccosum*), by grinding with water to make a paste.
- iii- Apply the paste to the area and rub in well by using surgical tape.
- iv- Examine for the presence of erythema, scaling and pustules, after 7-10 days.
- v- Reisolate the fungus from the lesions.

### 3.5 Epidemiological studies

The patients were asked to fill the following questionnaire for collecting data.

<b>Questionnaire Dermatophytosis</b>	
1. Name.....	
2. Age.....	
3. Sex	<input type="checkbox"/> F <input type="checkbox"/> m
4. Place of living .....	
5. Date of residence.....	
6. Occupation .....	
7. Contact with Animal	<input type="checkbox"/> yes      Type of animal.....
	<input type="checkbox"/> No
<b>Habits</b>	
1. Bathing	<input type="checkbox"/> Daily <input type="checkbox"/> Weekly <input type="checkbox"/> Monthly
2. Clothes: Type of internal wear	<input type="checkbox"/> Cotton <input type="checkbox"/> Wool <input type="checkbox"/> Silk <input type="checkbox"/> Polyester
3. Shoes: Type.....	
<b>Underlying Disease</b>	
	<input type="checkbox"/> Diabetic <input type="checkbox"/> high blood pressure <input type="checkbox"/> diabetic and high blood pressure
<b>Type of Infection</b>	
<i>Tinea versicolor</i>	
a- Location of lesions.....	
b- Itching	<input type="checkbox"/> yes <input type="checkbox"/> No
<i>Tinea pedis</i>	
Type :	
a) Intertriginous	
b) Hyperkeratotic type	
c) Vesicular	
d) Acute Ulcerative	
Symptoms.....	
* Results	- Direct Examination.....
	- Culture .....
* Code Number	<input type="text"/>

### **3.6. Statistical Analysis**

A descriptive statistics was used applying SPSS-package for descriptive analysis. Through the descriptive analysis, the frequencies and percentages were used to describe the sample of the study. On the other hand, Chi-square test was used to predict the statistical differences of the different hypothesis of the study.

### **3.7 Microscopy**

Plates were photographed using colored film with the help Nikon camera. Slides were examined and photomicrographs were taken using a Zeiss photomicroscope I.

# RESULTS

The study which was made on 233 cases of various hospitals and prisons included 99 cases (42.5%) of *Tinea versicolor* and 70 cases (30%) of *Tinea pedis* were collected from the dermatological clinics in King Hussein medical center and Queen Alia Hospital; 53 cases (22.7%) of *Tinea versicolor* and 11 cases (4.7%) were *Tinea pedis* on the prisoners in Gafgafa, Jwaydeh, Beerein and military prisoners.

### **- Incidence of, *Tinea pedis* and *Tinea versicolor* according to age**

The study showed that, *Tinea versicolor* to be the most common among age group of 21-30 years old (60.5%); followed by the age group 13-20 years old (21.1%); very low cases (1.2%) were reported among elderly persons and no cases were reported among individuals of less than 12 years old. In contrast, *Tinea pedis* was found to be most common among ages of more than 40 years old (40.7%); followed by the age group 31-40 years (32.1%) and no cases were reported among individuals of less than 12 years old. Distribution of *Tinea versicolor* and *Tinea pedis* according to age was evaluated by statistical analysis using the chi-square test which indicated significant relationship between diseases incidence and age at a level  $P < 0.05$  (Table 1).

Table (1): Distribution of *Tinea pedis* and *Tinea versicolor* in relation to age.

Age level	<i>Tinea versicolor</i>		<i>Tinea pedis</i>		Total	
	Count	%	Count	%	Count	%
<=12	2	1.3%	1	1.2%	3	1.3%
13-20	32	21.1%	7	8.6%	39	16.7%
21-30	92	60.5%	14	17.3%	106	45.5%
31-40	19	12.5%	26	32.1%	45	19.3%
>40	7	4.6%	33	40.7%	40	17.2%
Total	152	100%	81	100%	233	100%

### - Incidence of *Tinea pedis* and *Tinea versicolor* according to sex

Out of 152 cases of *Tinea versicolor*, 124 cases (81.6%) were reported among males and the rest of the cases (18.4%) were reported among females, indicating males to be much more susceptible to *Tinea versicolor* infection. Almost similar results were obtained on the incidence of *Tinea pedis*. Out of 81 cases, 53 cases of this disease (65.4%) were reported among males and the rest (34.6%) were reported among females (Fig. 1).

### - Incidence of, *Tinea pedis* and *Tinea versicolor* according to occupation

The result revealed that *Tinea versicolor* to be more prevalent among the military men (53.5%), who seem to be more susceptible followed by the workers (10.1%) and still fewer cases occurred among teachers and nurses (3.0%). In contrast, *Tinea pedis* was more common among House wives (32.9%); followed by the workers (21.4%) and to less extent among teachers (1.4%) Chi-square analysis indicated strong relationship between the occurrences of tineas and occupation, at a level of  $P < 0.05$  (Fig. 2).



strong relationship between the occurrences of tineas and occupation, at a level of  $P < 0.05$  (Fig.2).

### **- Relationship of *Tinea pedis* and *Tinea versicolor* occurrences and underlying diseases**

Out of 152 cases of *Tinea versicolor* infection, only 2 cases (1.3%) were reported to occur among patients with high blood pressure and 1 case (0.7%) was reported to occur among patients with diabetes and high blood pressure. The rest of cases (98.0%) were reported in normal individuals. On the other hand, out of 81 cases of *Tinea pedis* infection, 8 cases (9.9%) were reported among diabetes patients, 4 cases (4.9%), were reported to occur among patients with high blood pressure and 2 cases (2.5%) were reported among individuals with high blood pressure and diabetic (Table. 4).

### **- Relationship between incidence of *Tinea pedis* and *Tinea versicolor* with animal contact.**

Among the 233 cases of *Tinea pedis* and *Tinea versicolor* only 17 cases were found on individuals who have contact with animals, 5 cases of *Tinea versicolor* were recorded on individual who contacted regularly with sheep, 6 cases on individuals who have contacts with goats and two cases on individuals who have contacts with chicken. In case of *Tinea pedis*, only two cases were found on individuals who have contact with sheep and one case on an individuals who have contacts with goats and another 5 cases on an individual who contacted chicken. (Fig. 3)

**- The temporal incidence of *Tinea pedis* and *Tinea versicolor***

The study indicated that, high incidence of *Tinea versicolor* occurs mostly in summer during of July,96 (26.3%), and no cases of the disease were reported during Feb 97. In contrast, high incidence of *Tinea pedis* Occurred mostly during May, 97 (30.9%) and no cases were reported during Feb, 97 Fig (4.A-4.B).

**- Incidenc of *Tinea pedis* and *Tinea versicolor* in relation to place of living**

Amman area was found to harbour the highest rate of *Tinea versicolor* infection (25.3%); followd by Wadi-Al Sir (14.1%). As for other cities Zarqa accounted for (22.2%); followed by Irbid and Salt each of which accounted for (7.1%). The other cities had the lowest infection rates. *Tinea pedis* was also the highest in Amman area (38.6%); followd by Wadi-Al Sir (4.3%). As for other cities, Zarqa accounted for (34.3%) of the disease incidence, followed by Salt which accounted for (12.9%). Other Jordan cities had much lower rates of the disease incidence (Fig. 5).

**- The incidence of *Tinea pedis* and *Tinea Versicolr* among prisoners**

The study revealed that out of 233 cases of *Tinea pedis* and *Tinea versicolor*, sixty four cases (27.5%) were found to occur among prisoners in GafGafa, Jwyedh, Beerein and military prisoners. Among these, 53 cases of *Tinea versicolor*, (82.2%) and *Tinea pedis* (17.2%). The highest incidence of *Tinea versicolor* occurred among GafGafa and Beerein prisoners, each of which accounting for (28.3%) of all cases. In contrast, *Tinea pedis*

*Tinea pedis* occurred among Jwayedh and Gafgafa prisoners, each of which accounted for 3 cases (27.3%) Fig(6).

### **- Incidence of *Tinea versicolor* according to body location**

Out of 152 cases of *Tinea versicolor* infections the highest incidence of infection occurred on the neck, chest and back. This may be mostly due to the existence of higher numbers of sebaceous glands in these locations. Infections on other body sites were much less and no cases were reported on the palm of the hands or the feet (Fig. 7).

### **- Incidence of *Tinea pedis* according to its type**

Out of 70 cases of *Tinea pedis*, house wives accounted for 23 cases (32.9%) and workers on the other hand accounted for 14 cases (20.0%) all cases were of the intertriginous type of *Tinea pedis*. Except for four cases which were of the hyperkeratotic type, two cases (2.9%) of the hyperkeratotic type of infection were found among retired male individuals, one case among each of the prisoners and military men. Statistical analysis using chi-square test revealed significant relationship between the type of *Tinea pedis* and occupation at a level of  $P < 0.05$  (Fig. 8 ,9).

### **- Incidence of *Tinea versicolor* in relation to type of internal wear**

Out of 152 cases of *Tinea versicolor* 141 cases (92.8%) occurred among individuals with cotton internal wear; followed by 8 cases (5.2%) among individuals with silky internal wear and 3 cases (2.0%) on individuals with wool internal wear (Table 2). The highest infection rate with the disease incidence among such individuals occurred during July (23.7%) which were associated with cotton internal wear. Yet, such results may not indicate certain relationship with frequency of the disease incidence and cotton internal wear (Fig. 10).

### **- Incidence of *Tinea versicolor* in relation to bathing**

The results, indicated that (94.1%) of 152 cases of *Tinea versicolor* occurred among individuals who bathed on daily basis and (5.1%) was found to occur among individuals who bathed on weekly basis, such results may indicate certain relationship with frequency of the disease incidence and bathing ; however this phenomena is not explainable as yet (Fig. 11)

### **- Incidence of *Tinea pedis* in relation to shoe type**

Out of 81 cases of *Tinea pedis*, 64 cases (79%) occurred among individuals with leather type of shoes; followed by 17 cases (21%) among individuals with plastic type of shoes (Table 3). The highest infection rate with the disease incidence among such individuals occurred during May (22.2%), which were associated with leather type of shoes. Yet, such results may not indicate certain relationship with the frequency of the disease incidence and leather type of shoes (Fig. 12).

**Table (2):** Incidence of *Tinea versicolor* in relation to type of internal wear and month of year.

Type of clothes	DATE												Total	%	
	Jun. 96	Jul.96	Aug.96	Sep. 96	Oct. 96	Nov. 96	Dec. 96	Jan. 97	Feb.97	Mar. 97	Apr.97	May.97			
Cotton	Total	14	36	11	11	12	12	17	8	0	2	1	17	141	92.8
	% of Total	9.2%	23.7%	7.2%	7.2%	7.9%	7.9%	11.2%	5.3%	0	1.3%	0.7%	11.2%		
Wool	Total	0	0	0	0	0	0	0	0	0	0	0	3	3	2.0
	% of Total	0	0	0	0	0	0	0	0	0	0	0	2%		
Silky	Total	0	4	2	0	0	0	0	0	0	0	0	0	8	5.2
	% of Total	0	2.6%	1.3%	1.3%	0	0	0	0	0	0	0	0	152	100.0%

**Table. (3) : Incidence of *Tinea pedis* in relation to type of shoes and month of year.**

		Jun. 96	July 96	Aug.96	Sep. 96	Oct. 96	Nov.96	Dec. 96	Jan.97	Feb.97	Mar. 97	Apr. 97	May. 97	Total	%
		leather	count	6	12	5	3	0	1	6	2	0	5	6	18
	%	7.4%	14.8%	6.2%	3.7%	0	1.2%	7.4%	2.5%	0	6.2%	7.4%	22.2%		
Plastic	count	0	5	1	0	0	0	0	0	0	0	4	7	17	21%
	%	0	6.2%	1.2%	0	0	0	0	0	0	0	4.9%	8.6%		
Total	count													81	100%

## - Isolation of etiologic agents

out of 81 clinically positive cases of *Tinea pedis* it was possible to microscopically examine only 35 cases. Twenty three of these cases yielded fungal growth on cultures. Organisms isolated from these cases were: *Candida albicans* from 13 cases; *Trichophyton rubrum* from 5 cases; other *Candida* species from 3 cases and *Epidermophyton floccosum* from two cases. The rest of the cases (46 cases) were not examined microscopically.

Upon culturing 38 cases yielded cultures of *Candida albicans* (13 cases); *Candida krusei* (6 cases), other *Candida* species (8 cases); *Hansenula* sp. (1 case); *Epidermophyton floccosum* (1 case); *Penicillium* sp. (4 cases); *Cladosporium* sp. (3 cases) and *Alternaria* sp. (2 cases). In addition to this, *Trichophyton rubrum* (5 cases) was isolated only from the hyperkeratotic type of infection. On the other hand, *Epidermophyton floccosum* (3 cases); moulds (9 cases) and yeasts (41 cases) were isolated from the intertriginous cases. Statistical analysis using chi-square test at a level of  $P < 0.05$  indicated positive relationship between the type of *Tinea pedis* and the fungal isolates (Table 4).

Yeast species associated with diabetic patients and patients with high blood pressure, accounted for 11 cases with *Candida albicans* being the most frequently isolated among the yeasts, it accounted for 8 cases. Among dermatophytes, *Trichophyton rubrum* accounted for 1 case and *Alternaria* sp. accounted for another case. Statistical analysis using Chi-square test at a level of  $P > 0.05$  indicated no significant relationship between the isolated species and underlying disease. (Table 5).

**Table: (4):** Fungal isolates obtained from specimens of *Tinea pedis* on sabouraud dextrose agar

Direct exam.	Type of <i>Tinea pedis</i>	-ve		<i>candida albicans</i>	<i>candida krusei</i>	other <i>Candida</i> species	<i>Hansenula</i> sp.	<i>Trichophyton rubrum</i>	<i>Epidermophyton floccosum</i>	<i>penicillium</i> sp.	<i>Cladosporium</i> sp.	<i>Alternaria</i> sp.	Total
		count	%										
-Ve. exam	intertriginous	count	8	13	0	3	0	0	2	0	0	0	26
		%	30.8%	50.0%	0	11.5%	0	0	7.7%	0	0	0	100.0%
	hyperkeratotic	count	0	0	0	0	0	5	0	0	0	0	5
		%	0	0	0	0	100.0%	0	0	0	0	0	100.0%
+Ve. exam	intertriginous	count	4	0	0	0	0	0	0	0	0	0	4
		%	100%	0	0	0	0	0	0	0	0	0	100%
No direc exam	intertriginous	count	8	13	6	8	1	0	1	4	3	2	46
		%	17.4%	28.3%	13.0%	17.4%	2.2%	0	2.2%	8.7%	6.5%	4.3%	100.0%
Total count			20	26	6	11	1	5	3	4	3	2	81
%			24.7%	32.1%	7.4%	13.6%	1.2%	6.2%	3.7%	4.9%	3.7%	2.5%	100.0%



**Table (5):** Distribution of causative agent of *Tinea pedis* among patients suffering from diabetes and high blood pressure.

Culture		Undrlying disease				Total
		diabetic	High blood pressure	Diabetic & High Blood Pressure	Normal	
00-ve	Count	0	1	0	19	20
	%	0	1.2%	0	23.5%	24.7%
<i>Candida albicans</i>	Count	5	2	1	18	26
	%	6.2%	2.5%	1.2%	22.2%	32.1%
<i>Candida Krusei</i>	Count	0	0	0	6	6
	%	0	0	0	7.4%	7.4%
Other <i>Candida</i> Species	Count	2	1	0	8	11
	%	2.5%	1.2%	0	9.9%	13.6%
<i>T. rubrum</i>	Count	0	0	1	4	5
	%	0	0	1.2%	4.9%	6.2%
<i>E. Floccosum</i>	Count	0	0	0	3	3
	%	0	0	0	3.7%	3.7%
<i>Penicillium sp.</i>	Count	0	0	0	4	4
	%	0	0	0	4.9%	4.9%
<i>Cladosporium sp.</i>	Count	0	0	0	3	3
	%	0	0	0	3.7%	3.7%
<i>Alternaria sp.</i>	Count	1	0	0	1	2
	%	1.2%	0	0	1.2%	2.5%
<i>Hensenula sp.</i>	Count	0	0	0	1	1
	%	0	0	0	1.2%	1.2%
Total	Count	8	4	2	67	81
	%	9.9%	4.9%	2.5%	82.7%	100.0%

*Malassezia furfur*, the causative organism of *Tinea versicolor*, this etiologic agent was identified by direct microscopic examination in 152 cases (124 males and 28 females). Attempts to culture this agent on sabauround dextrose agar medium failed.

Attempts on the pathogenecity tests for *Penicillium* sp; *Cladosporium* sp. and *Alternaria* sp. on mice failed to cause any infection on the test animals. The hair penetration test and urease test indicated the fungus to be *Trichophyton rubrum* and not *Trichophyton mentagrophytes*.

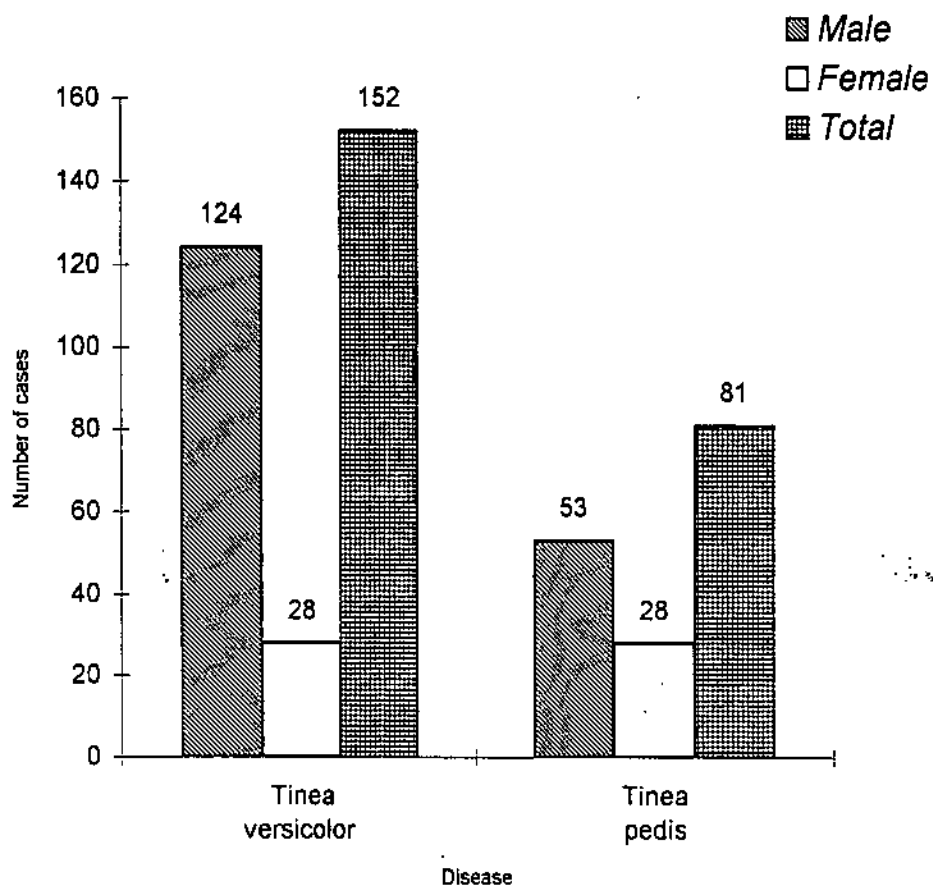


Fig (1) : Distribution of *Tinea pedis* and *Tinea versicolor* in relation to sex .

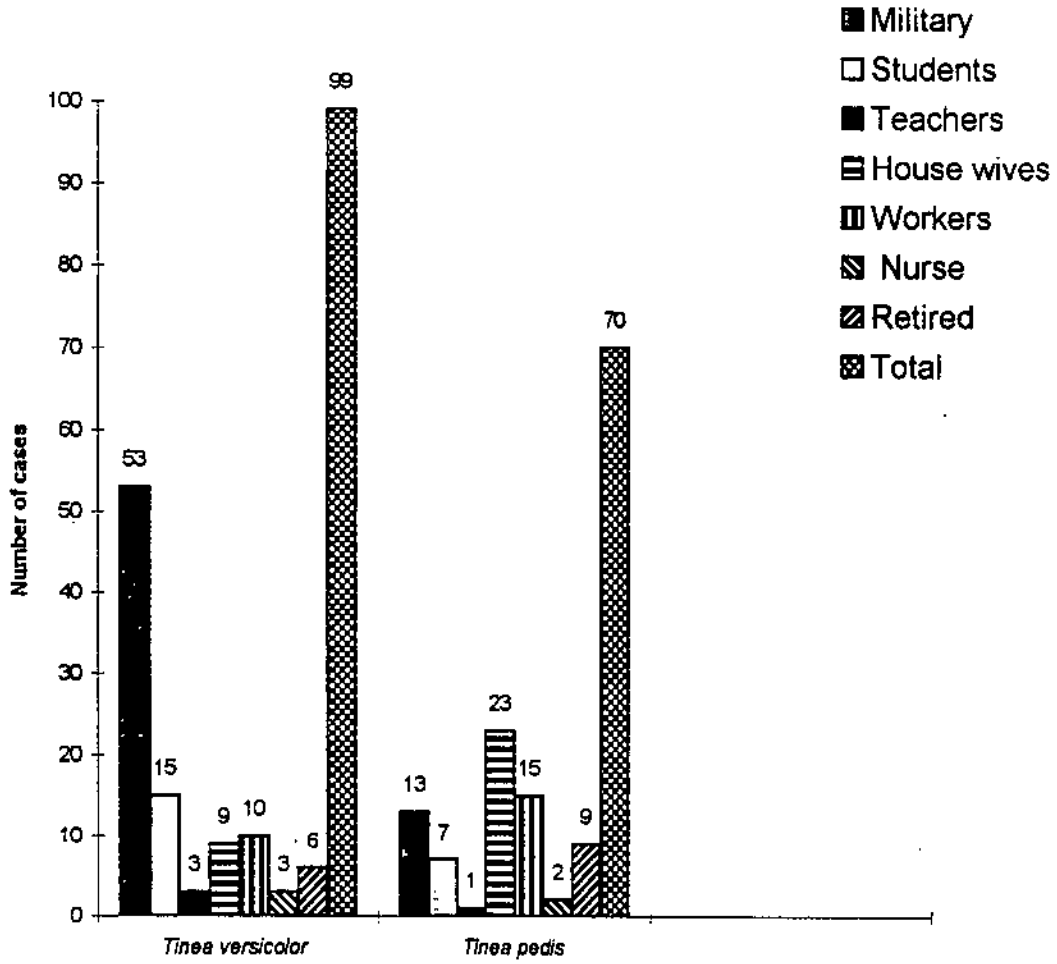


Fig (2): Distribution of *Tinea pedis* and *Tinea versicolor* in relation to occupation .

**Table 6:** distribution of *Tinea pedis* and *Tinea versicolor* in relation to underlying disease.

Underlying disease		Type of Disease		Total
		<i>Tinea versicolor</i>	<i>Tinea pedis</i>	
diabetic	Count	0	8	8
	%	0	9.9%	
high blood pressure	Count	2	4	6
	%	1.3%	4.9%	
diabetic and high blood pressure	Count	1	2	3
	%	0.7%	2.5%	
Normal	Count	149	67	216
	%	98%	82.7%	233

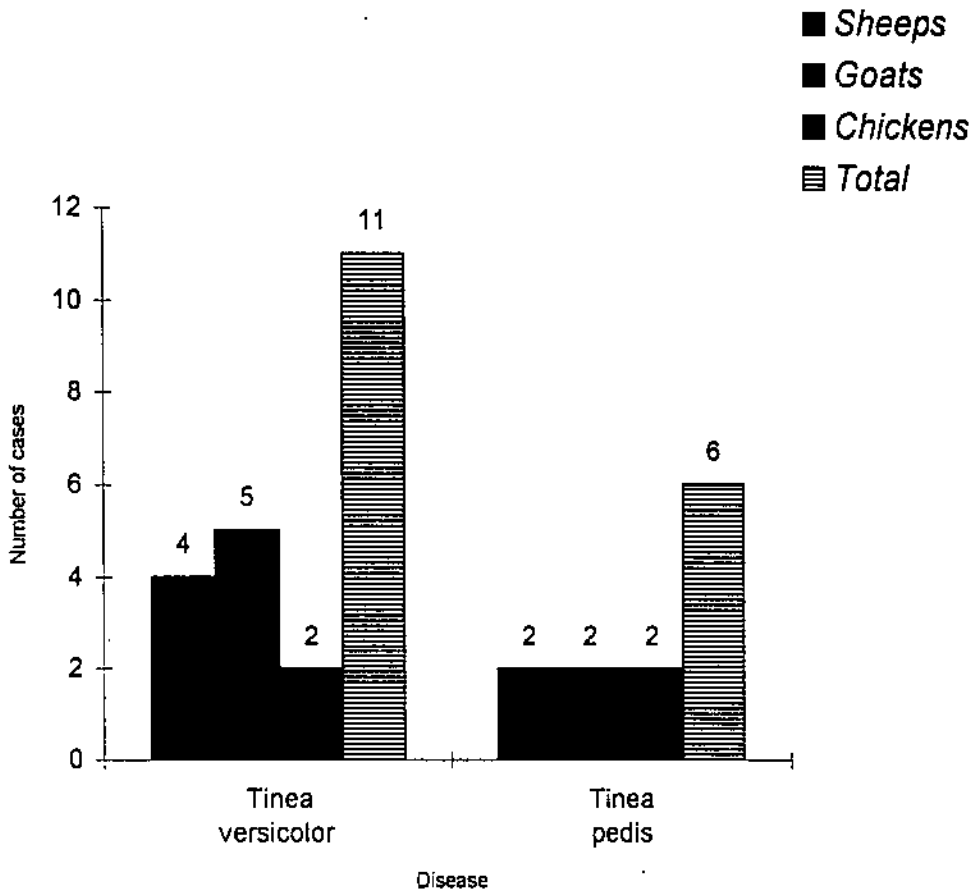


Fig (3) : Distribution of *Tinea pedis* and *Tinea versicolor* in relation to animals contact.

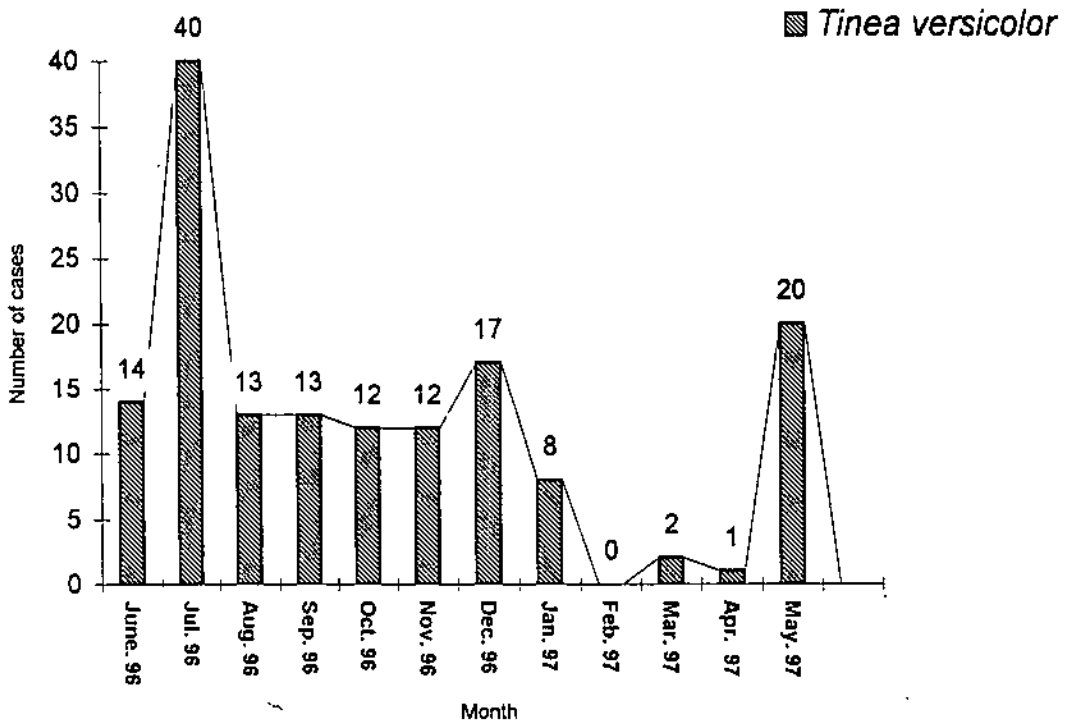


Fig (4.A): Temporal distribution of *Tinea versicolor* during June 96 until May 97.

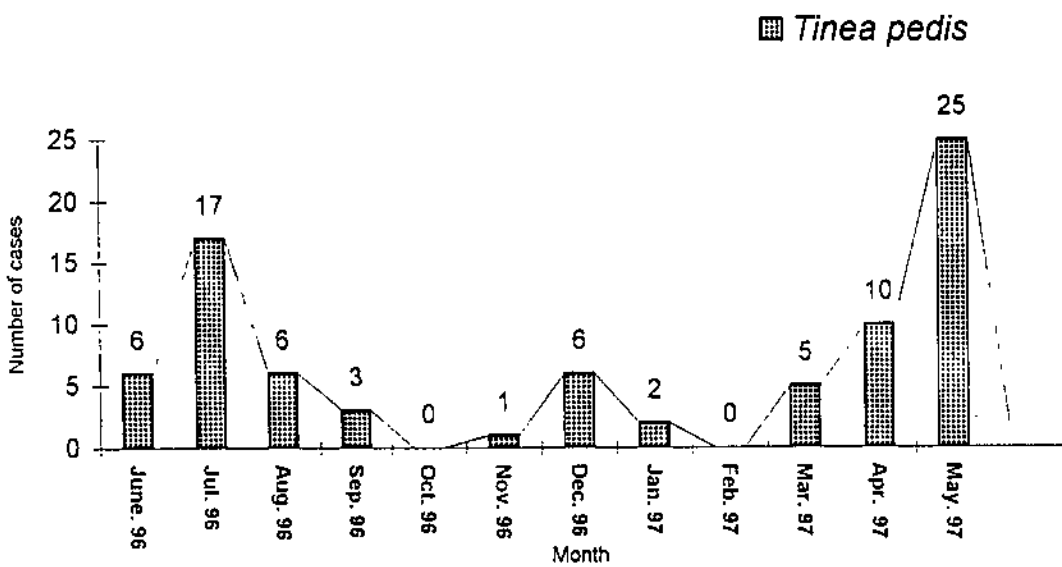


Fig (4.B): Temporal distribution of *Tinea pedis* during June 96 until May 97.

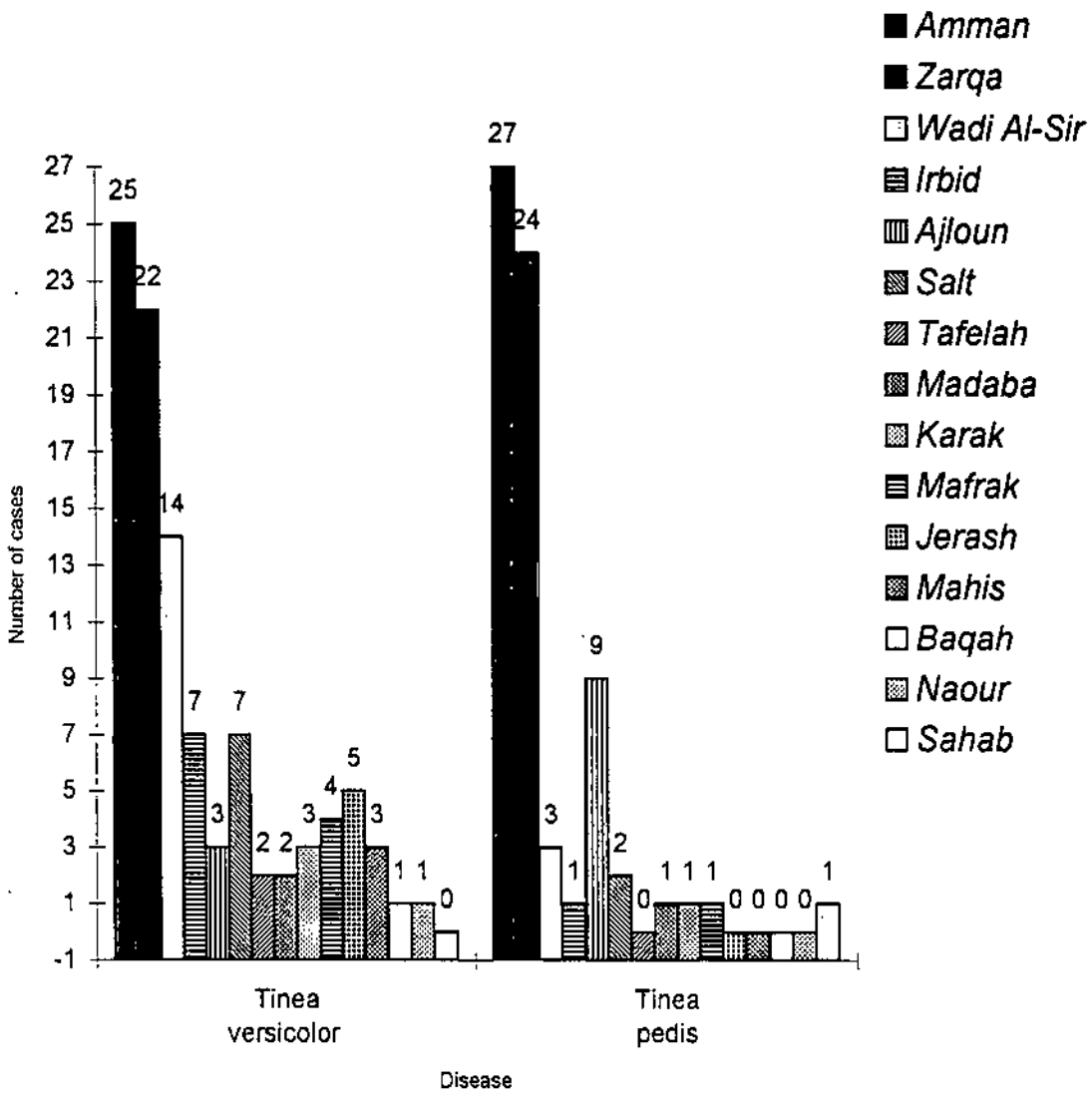


Fig (5) : Distribution of *Tinea pedis* and *Tinea versicolor* in various Jordan cities.



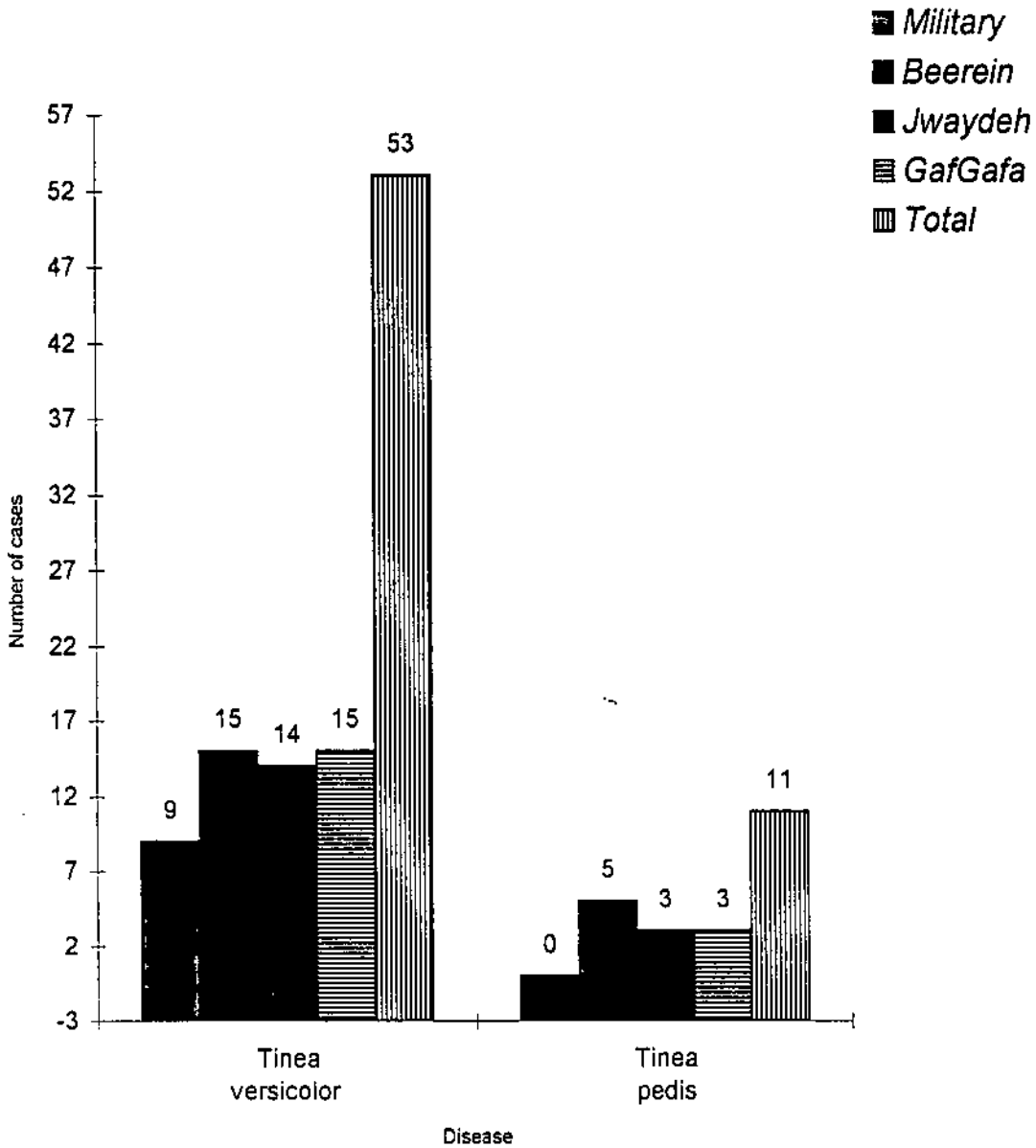


Fig (6) : Distribution of *Tinea pedis* and *Tinea versicolor* among prisoners .

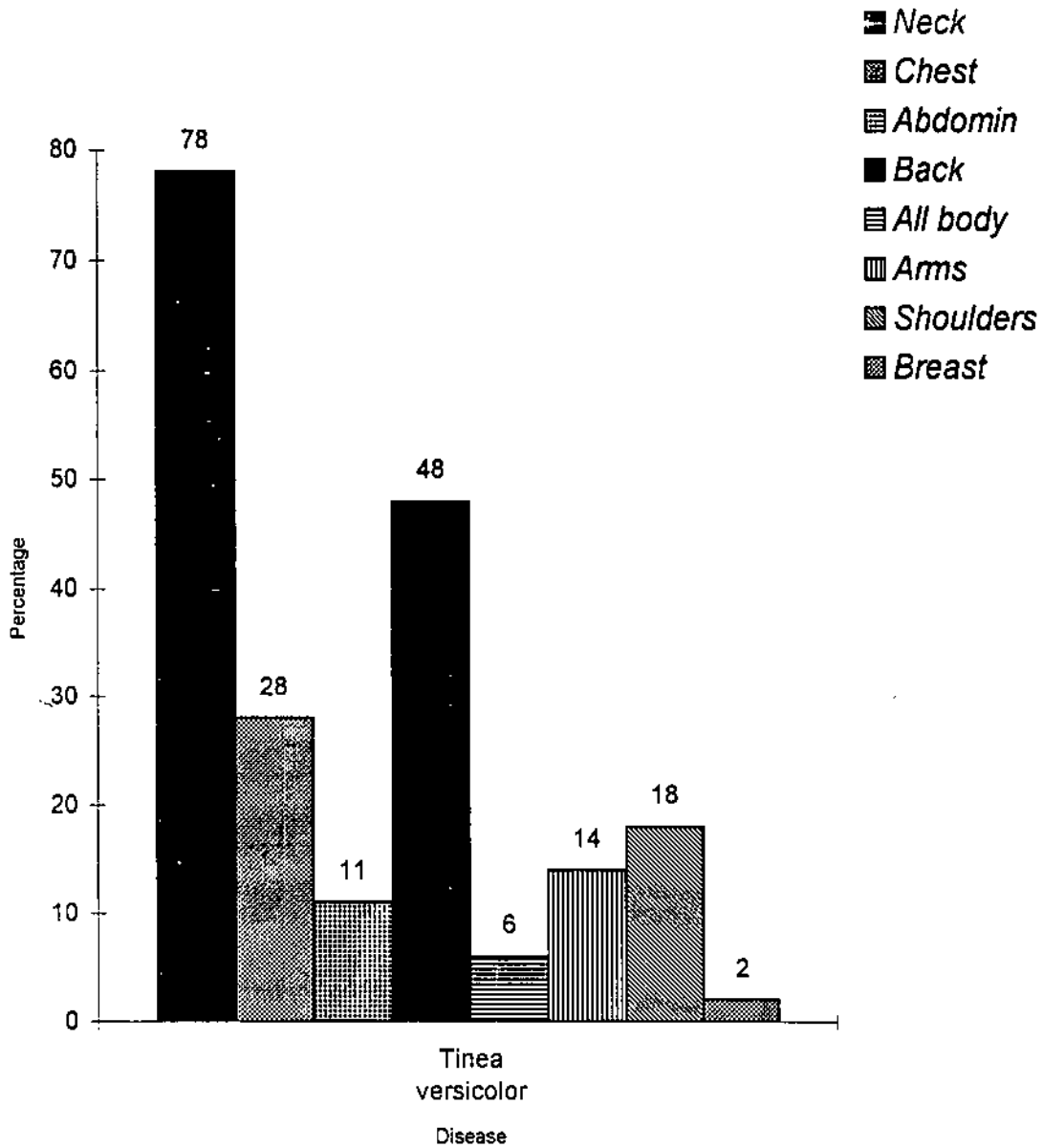


Fig (7) : Distribution of *Tinea versicolor* on various parts of the body.

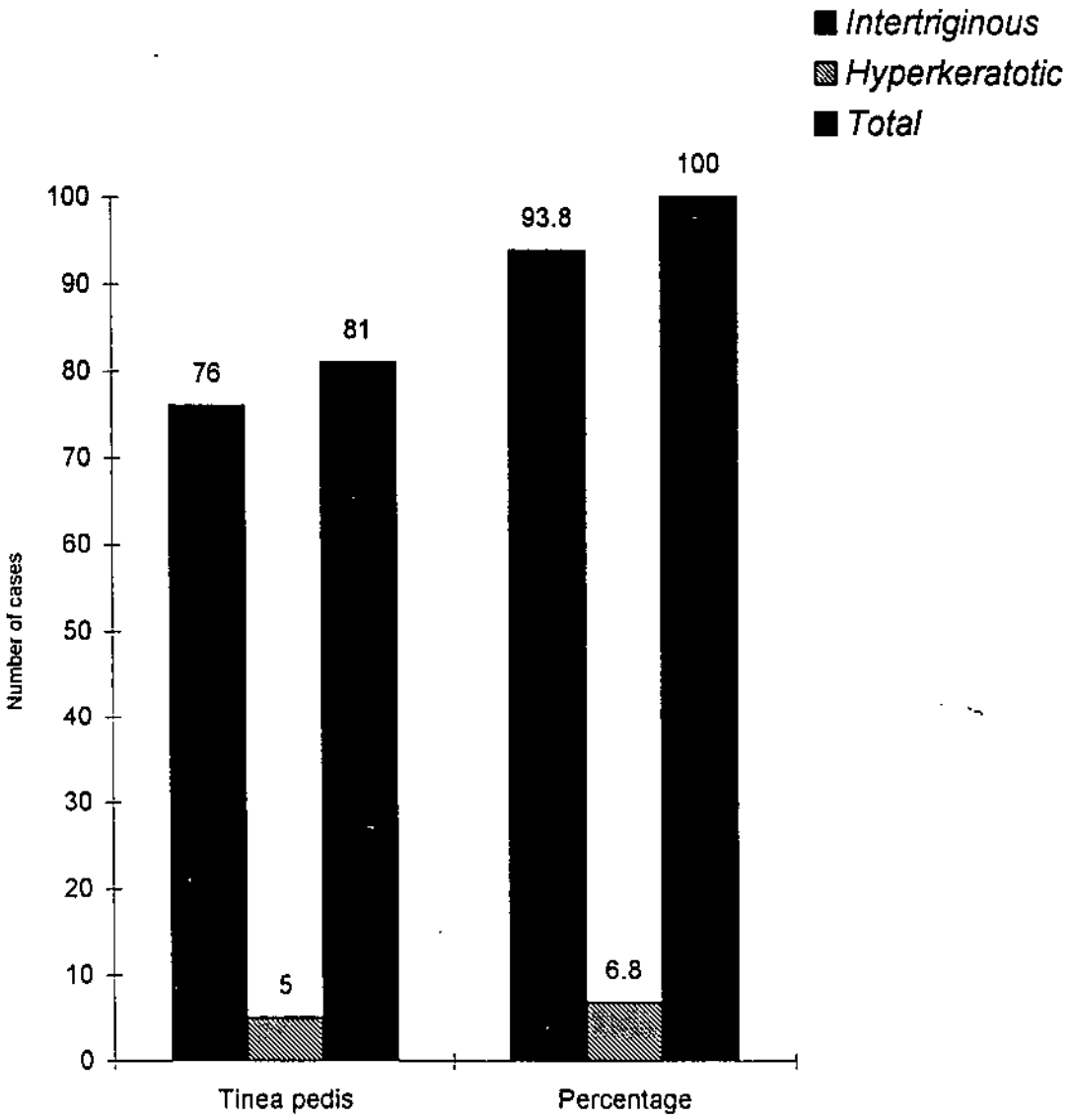


Fig (8) : Distribution of types of *Tinea pedis*.

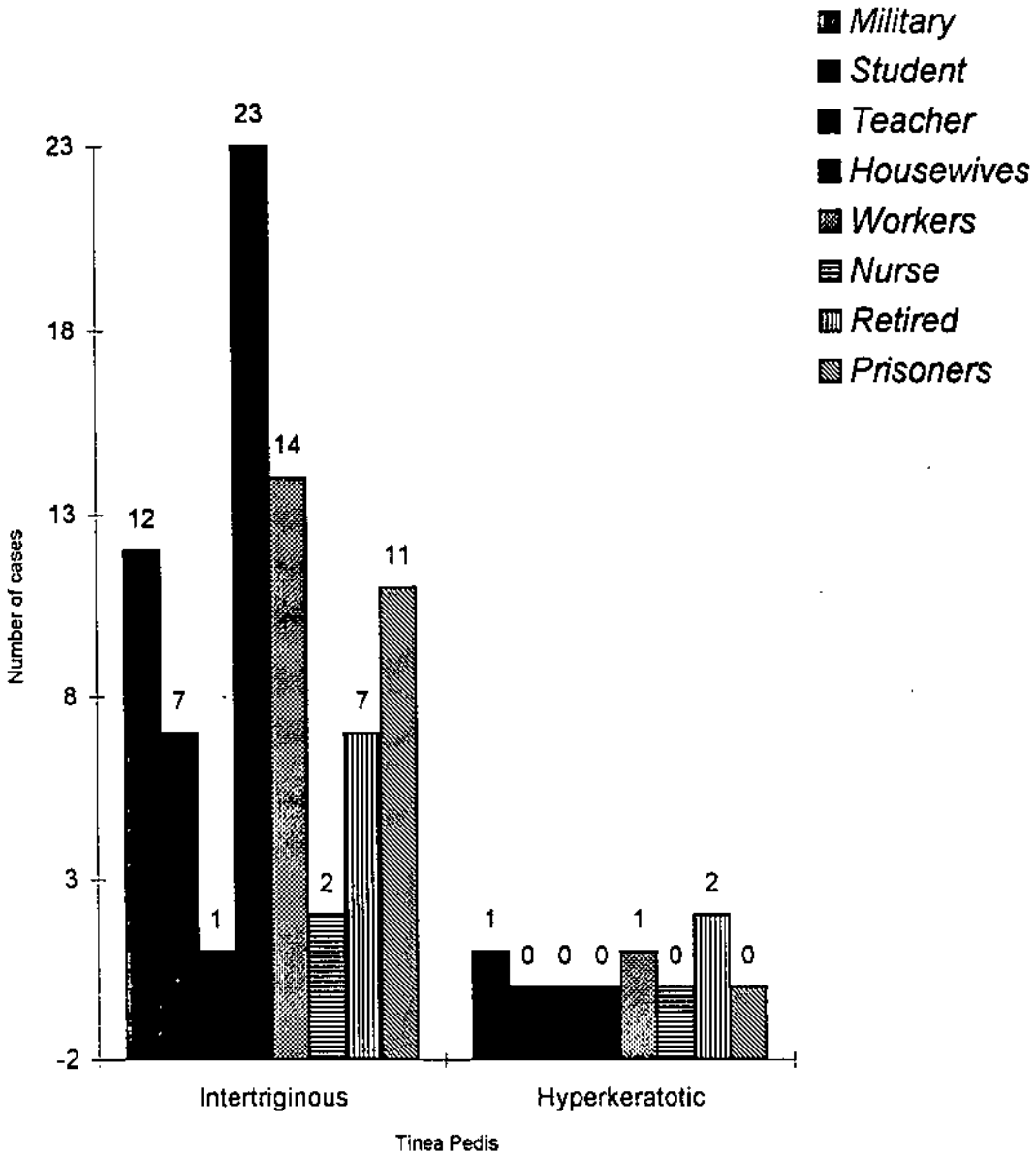


Fig (9) : Distribution of types of *Tinea pedis* among individuals of various occupations.

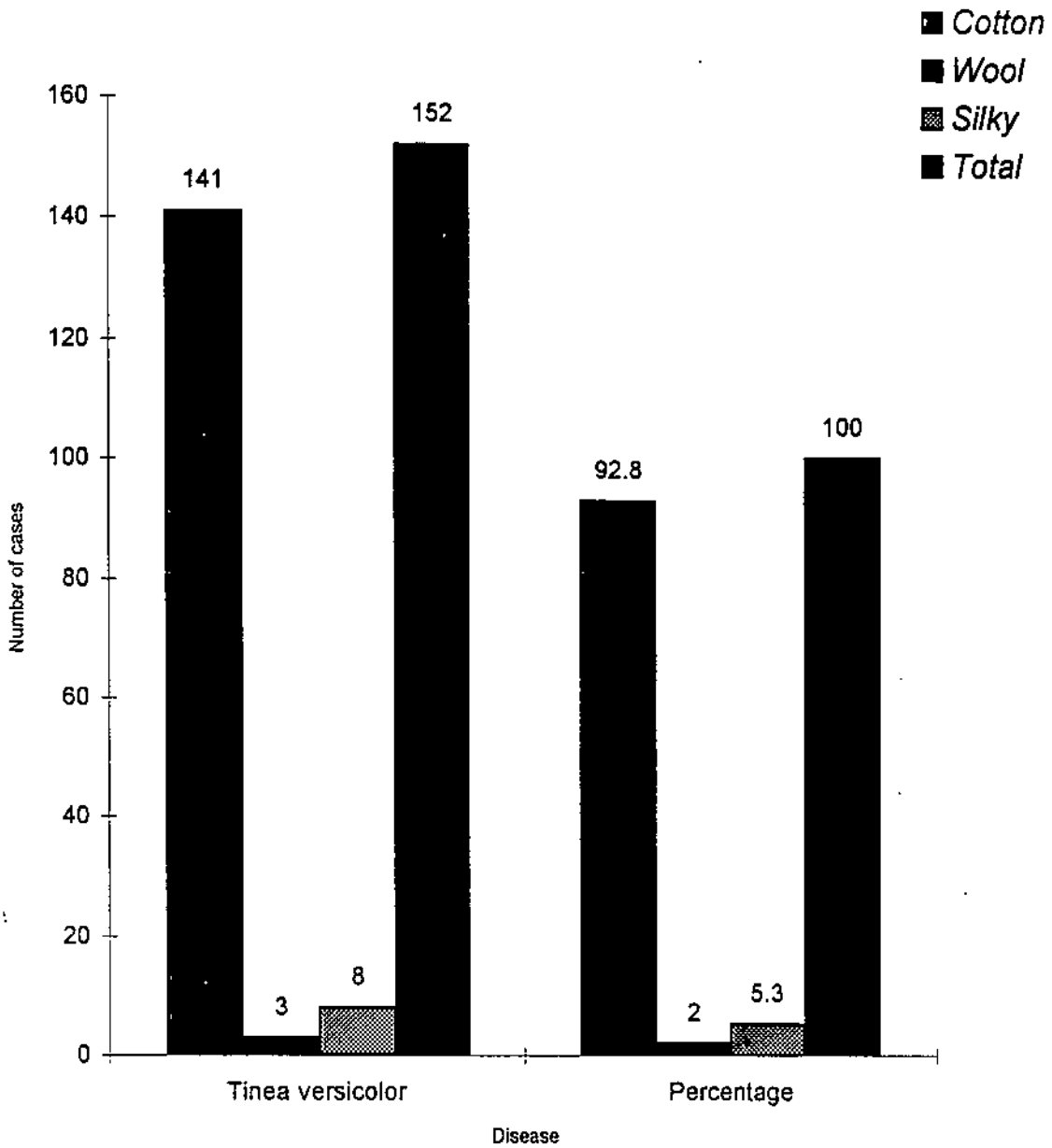


Fig (10) : Distribution of *Tinea versicolor* in relation to the type of internal wear.

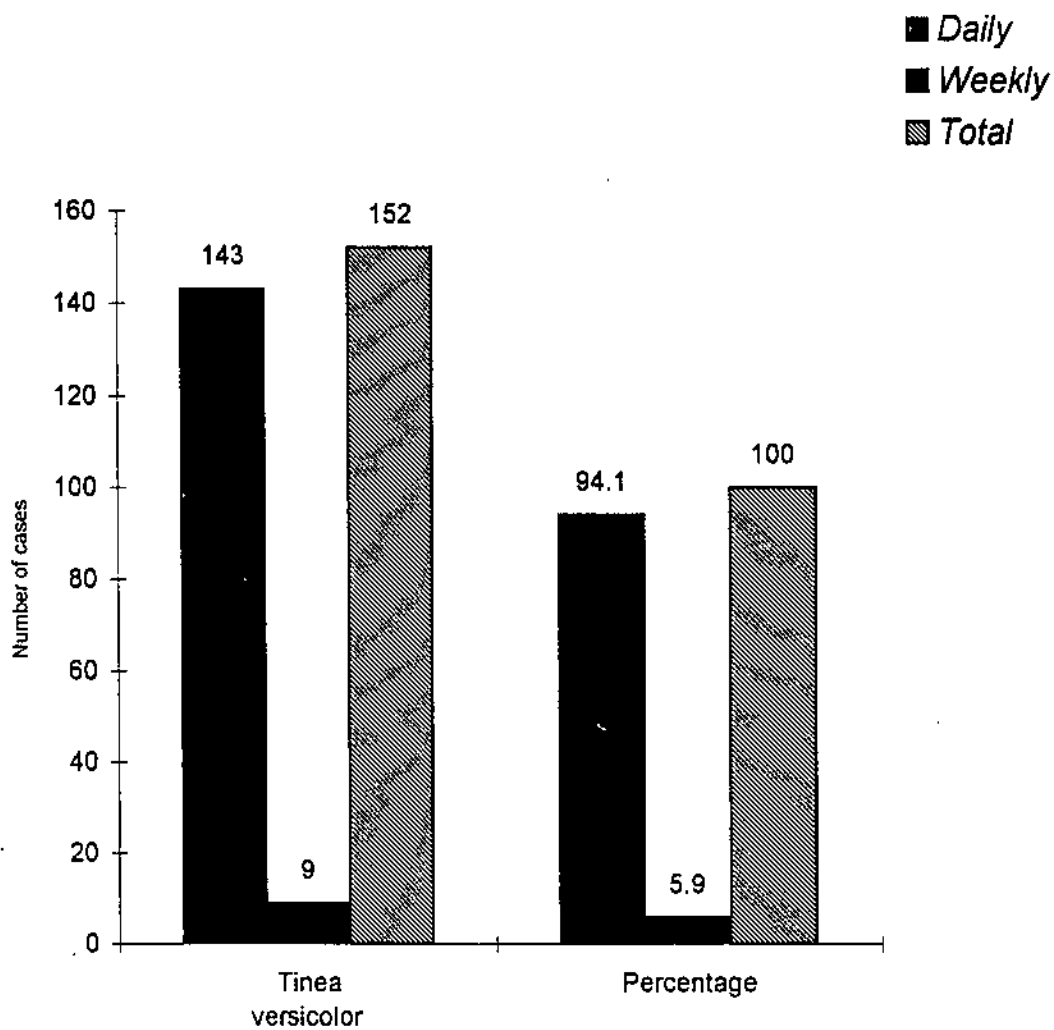


Fig (11) : Distribution of *Tinea versicolor* in relation to bathing frequency.

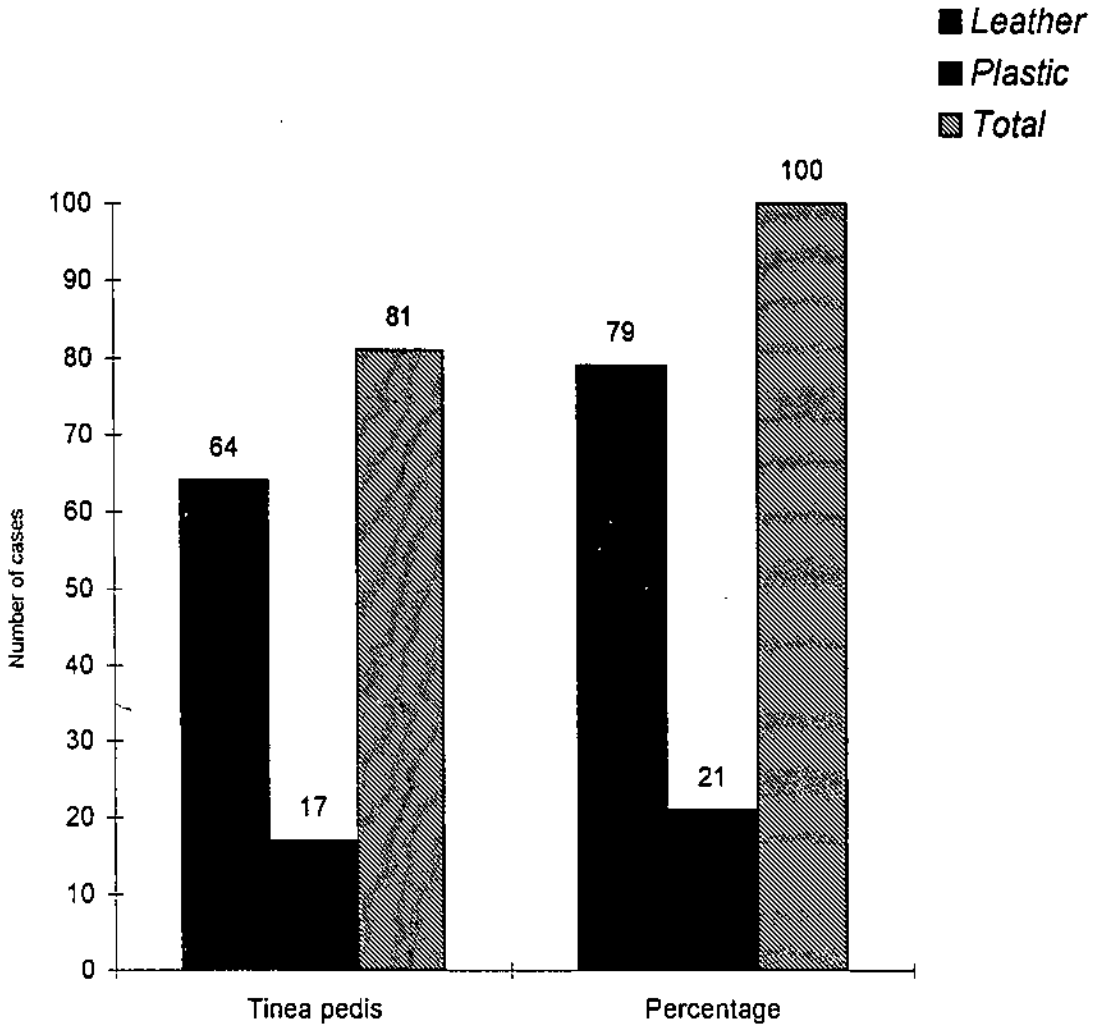
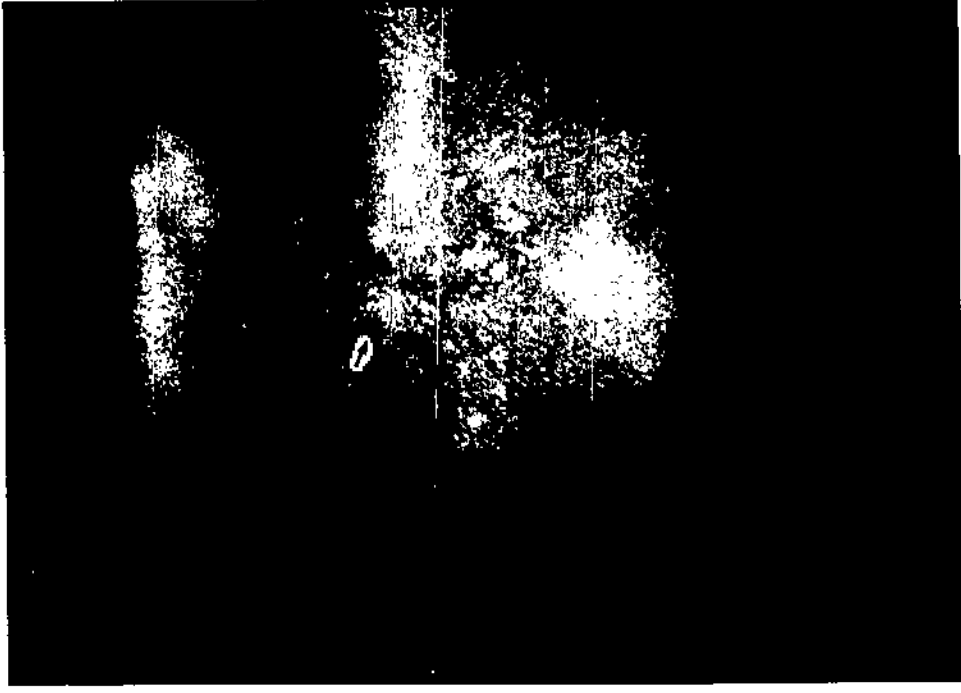
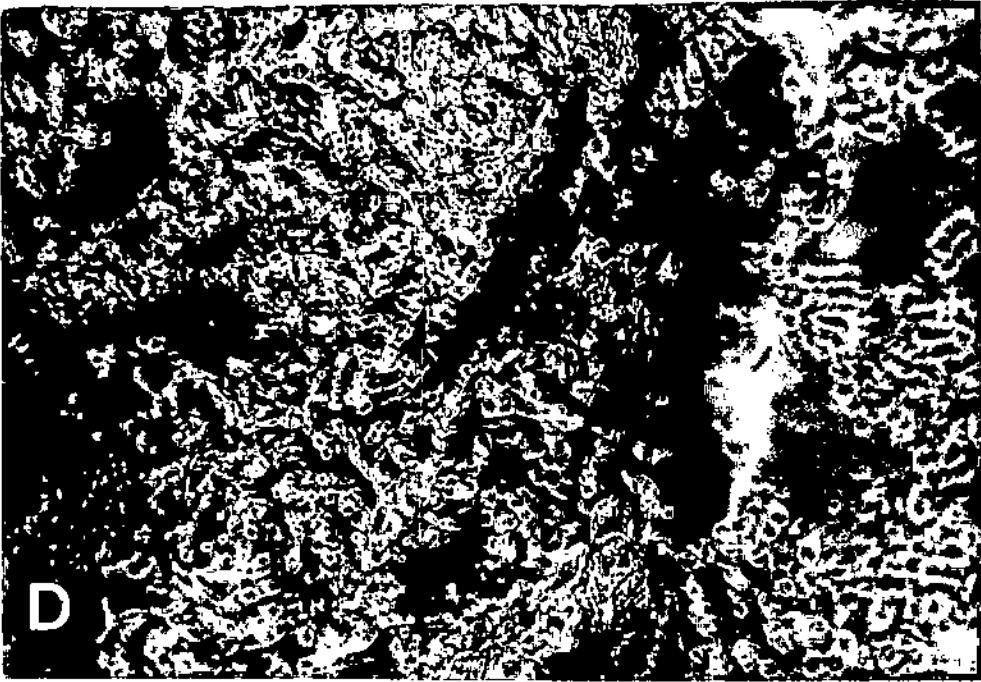


Fig (12) : Distribution of *Tinea pedis* in relation to type of shoes.

**Fig (13)**

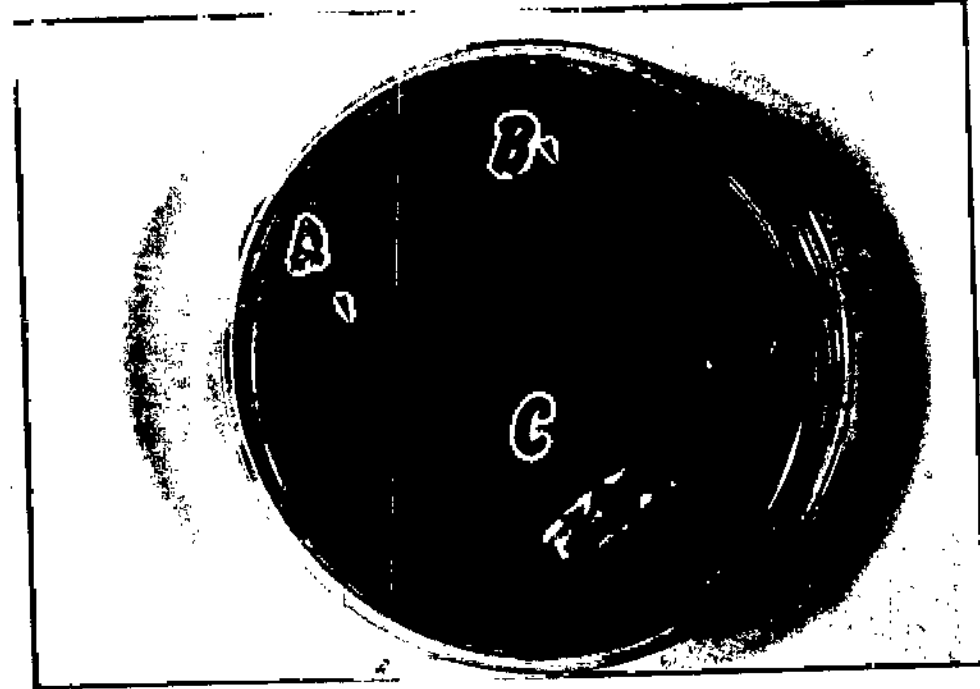
- A- Typical lesions of *Pityriasis versicolor* (arrow head)
- D- Photomicrograph showing round blastospore yeast cell within skin scales (arrow head , 360 x)





**Fig (14)**

- A- Typical lesions of intertriginous type of *Tinea pedis* (arrow head)
- D- Cultures of *Candida*, isolated from the intertriginous area on chrom agar (arrow head)
  - A- *Candida albicans* (Green)
  - B- *Candida krusei* (Pink)
  - C- Other *Candida* species (whitish)



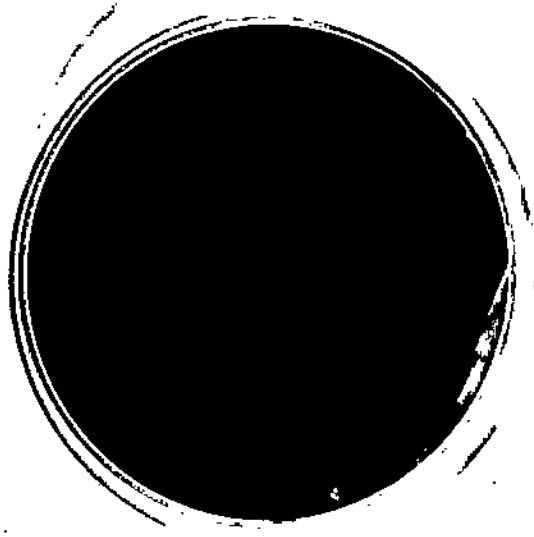
**Fig (15)**

Culture of *Penicillium*

B- Colony morphology

A- Reverse pigment production

C- Photomicrograph showing conidiophore with conidia (arrow head. 360 x)



**A**



**B**



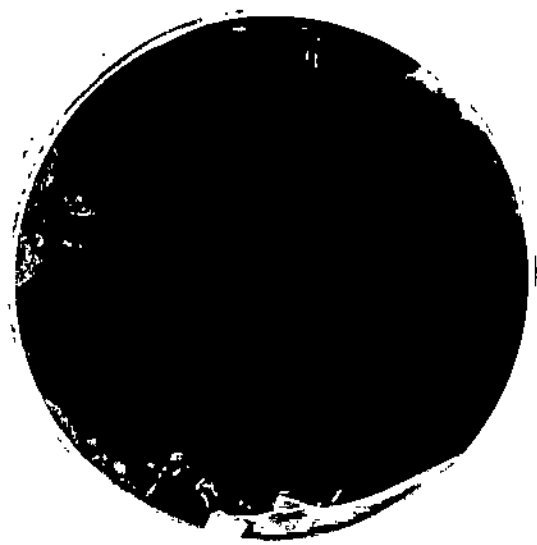
**Fig (16)**

Culture of *Alternaria*

B- Colony morphology

A- Reverse pigment production

C- Photomicrograph showing conidia (arrow head. 360 x)



A



B



C

**Fig (17)**

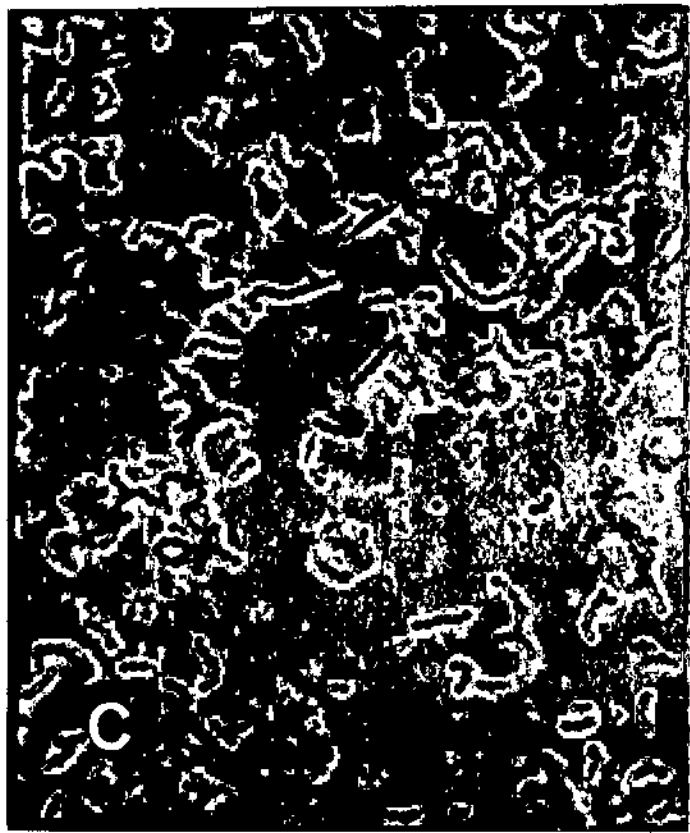
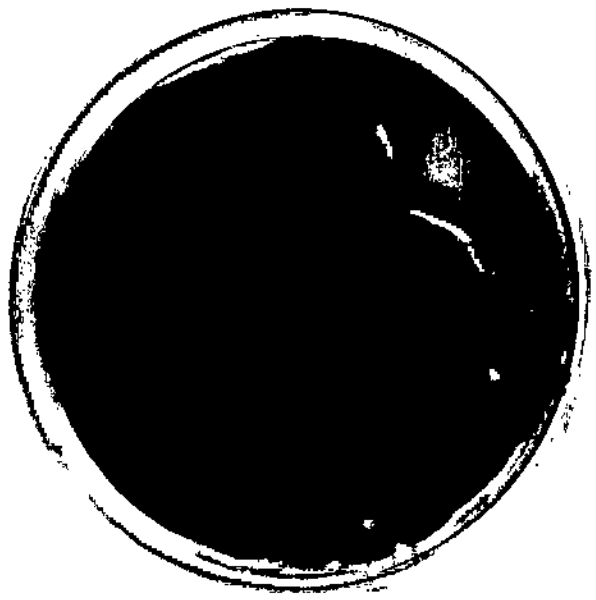
Culture of *Cladosporium*

B- Colony morphology

A- Reverse pigment production

C- Photomicrograph showing chain of conidia (arrow head. 360 x)





A

B

C

**Fig (18)**

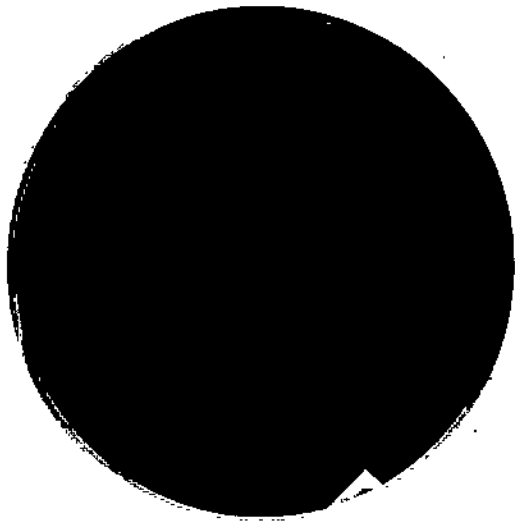
Culture of *Trichophyton rubrum*

B- Colony morphology

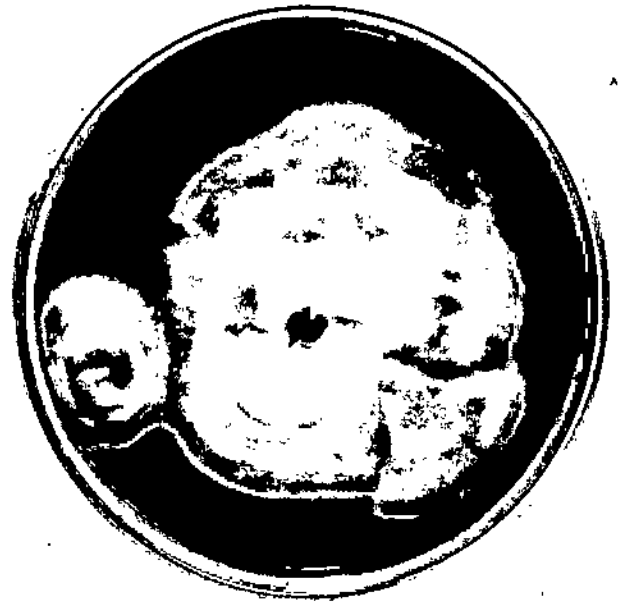
A- Reverse pigment production

C- Photomicrograph showing macroconidia (arrow head. 360 x)

D- Photomicrograph showing microconidia (arrow head. 360 x)



A



B



C



D

**Fig (19)**

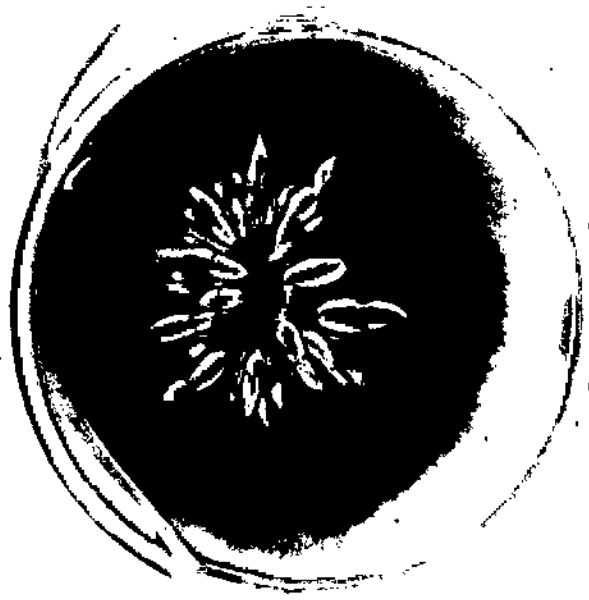
Culture of *Epidermophyton floccosum*

B- Colony morphology

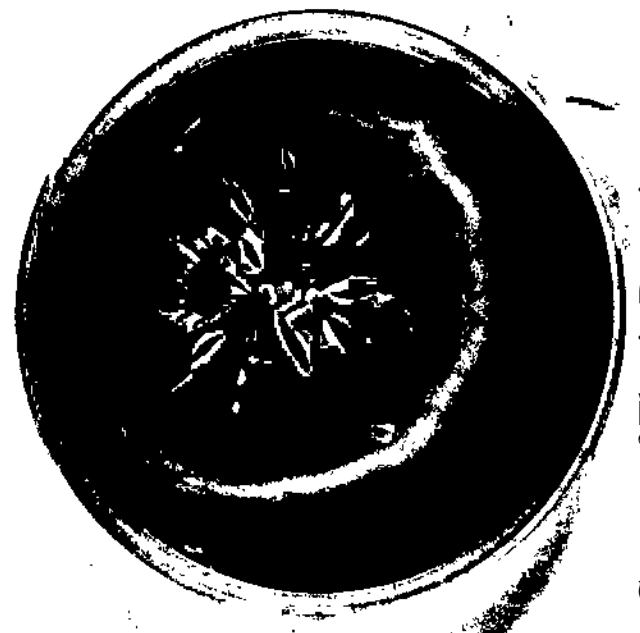
A- Reverse pigment production

C- Photomicrograph showing macroconidia (arrow head. 360 x)

D- Photomicrograph showing chlamydospore (arrow head. 360 x)



**A**



**B**



**C**



**D**

# DISCUSSION

### ***Tinea versicolor:***

The study revealed age group of 21-30 to have the highest rate of disease incidence (60.5%). Individuals, of less than 20 year old accounted for (22.4%) of the disease incidence, the rest of infection with the disease occurred among individuals of more than 30year old. Such results clearly indicate the disease to spread mostly among puberty age. This may be due to the fact that sebum production is most active among individuals of this age. *Pityrosporum* species, the most causative organism of the disease is a lipophilic requiring a higher skin lipid level. At age of puberty the sebaceous glands become most active and produce more sebum, which favours the growth of the fungus (Rook *et al.*, 1987).

Due to dryness of the skin and decrease in skin lipid among elderly persons (over 40 years old) the disease incidence is far less among individuals of this age (Faergmann, 1993).

As to the disease occurrences on the body, most of its lesions were found to be restricted to the upper part of the trunk. This may be due to the fact that sebaceous glands are largest and most numerous in this part of the body (Rook *et al.*, 1987).

Most cases of infection with *pityrosporum* species . were found to occur during summer; during which a rather hot and humid condition prevail and individuals perspire more, which make them more susceptible to the disease. Such conditions favour *pityrosporum* growth and induce the mycelial form instead of plastospore form of the fungus (Noble, 1984).

Among the infected individuals, highest rate of the disease incidence was found to occur among military men, accounting for (34.8%) followed by the students (9.9%) and workers who accounted for (10.1%) of the disease incidence.

High rates of disease incidence among such groups of individuals may be accounted for by the fact that their occupation requires physical efforts, which induce sweating and make them more suitable to infection. Moreover such individuals, who are mostly of the puberty age, have a seborrheic skin which encourage the growth of the fungus on it (Faergemann, 1993).

Considering sex, males were found to be more susceptible than females, accounting for (81.6%) of the disease incidence. This may be mostly due to the nature of men occupations which often induce sweating, which increases body humidity and render them more susceptible to infection. Internal clothing cause occlusion of the sweat which seems to be inducive to the colonization of the *Pityrosporum* species (Noble, 1984).

*Tinea versicolor* seems to be a noncontagious disease. This study did not reveal any infections among sisters and brothers in the same family. Upon questioning the infected prisoners it was found that they caught the disease inside the prison after their imprisonment. Accordingly, it may be concluded that infection spreads among prisoners through their use of common facilities, particularly the baths.

Moreover most patients suffered from cosmetic problems. Aside of this, some patient suffered from itching but only when sweating. However, out of 152 cases of *Tinea versicolor* only 40 of them (26.3%) suffered from prurites.

### ***Tinea Pedis:***

The study revealed individuals of more than 40 year old, to have highest rate of disease incidence (40.7%). On the other hand, individuals of less than 20 year old accounted for (9.8%) of the disease incidence. The rest of infection with the disease, occurred among individuals of more than 30 year old. Such result clearly indicate that, the disease spreads mostly among



elderly people. This may be due to the fact that, individuals of such age are mostly religious and therefore have more contact with water due to their ablution and normally they don't wipe their webs in the proper manner and wear their socks and shoes directly after ablution. In such cases the stratum corneum is often modified in a way to become more susceptible to infection and growth of the etiologic agent (Rieth, 1978).

Considering sex, males were found to be more susceptible than females, accounting for (65.4%) of the disease incidence. This is perhaps due to the fact that men generally wear more occlusive and heavier foot wear, through the year than women. This in turn induce sweating and provoke maceration of the horny layer of the epidermis, making them more suseptable to infection (Rook *et al.*, 1987).

Considering the occupation of infected individuals, highest rate of the disease incidence was found to occur among house wives, who accounted for 32.9% of the cases. This may be explained by the fact that, house wives in our society are in more contact with water and for prolonged periods of time. This in turn modifies the natural cutaneous resistance and provokes maceration of the stratum corneum. In addition to this, certain alkaline soaps and detergents used in washing induce changes in the horny layer of the skin and make it more permeable for pathogenic fungi (Rieth, 1978).

Military men and workers accounted for the next higher incidence rates, (16%), (18.5%) respectively. This is most likely due to the fact, they wear heavier occlusive boots. Furthermore, the occupation of such individuals normaly require physical efforts which induce foot sweating, which provokes maceration of the horny layer. This in turn facilitates the penetration of the deeper layers of the skin by the etiologic agents (Rieth, 1978).

Most cases of infection with *Tinea pedis* were found to occur during summer, during which a rather hot and humid conditions prevail which are inducive to sweating and provoke maceration of the stratum corneum, rendering the individual more susceptible to the disease .

Most of *Tinea pedis* infection cases were found to occur among individuals with leather shoes accounting (79%). However leather type of shoes may not have any significant effect on the disease incidence. The highest infection rate among such individuals occurred during summer time, perhaps due to the fact that occlusion by wearing shoes during the hot summer for prolonged periods during the day, which normally induces more foot sweating and this provokes maceration and render them more susceptible to fungal infection.

Individuals infected with *Tinea pedis*, who have diabetes and or blood pressure, accounted for 14 cases (17.3%) where found to harbour 11 yeast spp in the site of their infections. The most frequently isolated yeast was *candida albicans* which accounted for (6.2%), followed by *Trichophyton rubrum* and *Alternaria* sp. which were isolated from only one case for each (1.2%).

Opportunistic yeast were isolated from such patients because such patients normally encourage the growth of opportunistic fungi (Buxton. *et al.*, 1995 ; Dahl, 1994; Leibovici, 1995; Litch Field *et al.*, 1996 and Sahnle *et al.*, 1983).

The most, common type of *Tinea pedis* is the intertriginous which develops mainly between the fourth and the fifth toes. This may be due to the fact that, the fourth and fifth toes are in contact with each other, causing accumulation of higher humidity rate between them. Such humidity along with sweating, cause maceration of the horny layer of the stratum corneum, leading to infection.

Due to almost similar social life in Jordan, the disease was found to have similar distribution among Jordan cities. Most of the isolated fungal species from infections were well known agent of foot mycoses. Among these, *Trichophyton rubrum* was isolated from 5 cases (6.2%); *Epidermophyton floccosum* was isolated from 3 cases (3.7%). However, *Candida* species were of the highest rate of distribution; among the isolates. *Candida albicans*, had the highest distribution ; it was isolated from 26 cases (32.1%); *Candida krusei* was isolated from 6 cases (7.4%); *Hansenula* sp. was isolated from 1 case only (1.2%). Other unknown *Candida* sp. were isolated from 11 cases (13.6%) *Penicillium* sp was isolated from 4 cases (4.9%) ; *Cladosporium* sp. was isolated from 3 cases (3.7%) and *Alternaria* sp. was isolated from 2 cases (2.5%).

*Trichophyton rubrum* was found to be the most common dermatophyte. It was isolated from *Tinea pedis* cases. Elder-Smith, 1984 and Nolting *et al.*, (1992) reported similar results.

In addition to this, *Trichophyton rubrum* was isolated only from the hyperkeratotic cases and was revealed to be the most common isolate of this type of *Tinea pedis*. Similar finding was reported by Willard (1974). In contrast, *Epidermophyton floccosum* was isolated only from intertriginous type of *Tinea Pedis*. Both species are considered to be anthropophilic species (Hay, 1983) *Trichophyton rubrum* was isolated from two military men; one worker and one prisoner and the a retired person. In contrast, *Epidermophyton Floccosum* was isolated from two House wives and one worker. Infection with these two fungi most likely occurs by direct contact between patients and healthy individuals; they are more liable to be transferred through shoes and socks.

The opportunistic fungi i.e. *Penicillium* sp.; *Alternaria* sp. and *cladosporium* sp. were isolated from intertriginous area, *penicillium* species

were most predominant. Similar results were reported by Nolting *et al.*, (1993). On the other hand, *Alternaria* sp. and *Cladosporium* sp. were reported to be potentially pathogenic (Benek and Roger, 1980 and Willard, 1974), yet their role in the mycotic foot infection is still not will know. *Candida albicans* was isolated from 26 cases of the positive cultures.

Binding between the yeast spores and target tissue cell membranes, occurs at specific surface active sites on the yeast spores. The adherent spores change the configuration of the host cell membrane (Cavitates it) stimulate or facilitate germ tube formation which in turn penetrates the host cell surface mainly with the aid of acid proteinase enzyme (Shepherd *et al.*, 1986).

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# ملخص انتشار ومسببات سُعف القدم و سُعف الجسم في الأردن

اعداد

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المشرف

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الفطريات الجلدية هي تلك الفطريات التي تصيب الأجزاء الخارجية من الجسم والتي تحتوي على الكيراتين كالشعر والجلد والأظافر. تتعلق هذه الدراسة بمرضى من هذه الأمراض الذين يصيبها القدم والجسم من حيث انتشار هذين المرضين ومدى ارتباطهما بكل من العمر، الجنس، مكان السكن، أمراض تؤثر في مناعة الجسم، الاحتكاك مع الحيوانات، بعض العادات مثل كثافة الاستحمام، نوع اللباس الداخلي، نوع الحذاء وكذلك معرفة الاجناس الفطرية المسببة في حالات فطريات القدم.

من خلال هذه الدراسة تم جمع (233) عينة مرضية من هذه الامراض الفطرية ما بين الفترة من ايار 1996 الى حزيران 1997 من ضمن هذه العينات (169) عينة من مرضى عيادات مدينة الحسين الطبيه ومستشفى الملكة علياء و (64) عينة من مرضى السجون في كل من بيرين، قفقفا، الجويده، والسجن العسكري، وقد تم فحص العينات بواسطة الميكروسكوب مبدئياً باستعمال (25%) هيدروكسيد البوتاسيوم (KOH) كما تم ايضاً زراعة العينات على ثلاث بيئات غذائية مختلفة.

وقد أمكن خلال هذه الدراسة الحصول على النتائج التالية:

سُجّلت ٨١ حالة اصابه بمرض فطريات القدم وكانت تشكل نسبة مقدارها (٣٤,٨%) حيث ان الاشخاص الذين تزيد اعمارهم عن ٤٠ عاماً هم الاكثر عرضه للاصابه حيث شكلوا ٣٣ حالة اصابة ونسبة مقدارها (٤٠,٧%). علاوة على ذلك تبين من خلال الدراسة ان الذكور هم الاكثر اصابة بذلك المرض حيث بلغت عدد الحالات المصابة ٥٣ حالة ونسبة مقدارها (٦٥,٤%). تبين كذلك من خلال الدراسة ان الاشخاص المصابين بداء السكري هم عرضه للاصابة حيث شكلوا ٨ حالات اصابة ونسبه مقدارها (٩,٩%) وتبين كذلك من خلال الدراسة انه لا توجد هناك علاقة بين الاصابه بهذا المرض والحيوانات المحيطة وكذلك وجد من خلال الدراسة ان اعلى نسبة انتشار سُجّلت في شهر أيار/٩٧ وكانت تشكل ٢٥ حالة اصابة ونسبه مقدارها (٣٠,٩%) وان منطقة عمان هي اكثر المناطق انتشاراً حيث سجلت ٢٧ حالة اصابة ونسبه مقدارها (٣٨,٦%) وانه لا توجد هناك علاقة مباشرة بين نوع الحذاء والاصابة بهذا المرض .

اما الفطريات المسببة لهذا المرض فقد تم عزل عدة اجناس منها وان اكثرها انتشاراً هي فطر *Candida sp* حيث تم عزلها من ٤٤ حالة اصابة من فطريات ما بين أصابع القدمين وكانت تشكل نسبة مقدارها (٥٤,٣%) ومن اهم انواعها *Candida albicans* حيث تم عزلها من ٢٦ حالة اصابة وكانت تشكل نسبة اصابة مقدارها (٣٢,١%) يليه *Candida Krusei* حيث تم عزلها من ٦ حالات اصابة وكانت تشكل نسبة مقدارها (٧,٤%) وانواع اخرى من *Candida sp* لم تعرف حيث تم عزلها من ١١ حالة اصابة وكانت تشكل نسبة مقدارها (١٣,٦%) يليه جنس *Hansenula sp.* حيث تم عزلها من حالة واحدة فقط (١,٢%) وكذلك وجد ان الفطريات الجلدية dermatophytes كانت لها نسبة انتشار قليلة حيث عزلت من ٨ حالات مصابة فقط (٩,٩%) وان اكثرها انتشاراً هو *Trichophyton rubrum* حيث تم عزلة من ٥ حالات فقط من فطريات جانب القدمين (٦,٢%) يليه *Epidermophyton floccosum* حيث تم عزلة من ٣ حالات (٣,٧%).

وكذلك تم عزل بعض انواع العفن (moulds) حيث تم عزلها من ٩ حالات مصابة وكانت نسبة انتشارها (١١,١%) وان اكثرها انتشاراً هي *Penicillium sp* حيث تم عزلها من ٤ حالات مصابة (٤,٩%) يليه *Cladosporium sp* حيث تم عزلها من ٣ حالات مصابة (٣,٧%) يليه *Alternaria sp* حيث تم عزلها من حالتين فقط (٢,٥%).

- سُجلت ١٥٢ حالة إصابة من مرض تَبَع الجلد وكانت تشكل نسبة مقدارها (٦٥,٢%) وان معظم الاصابات كانت محصورة في الفئة العمرية (٢١-٣٠) حيث سُجلت ٩٢ حالة مصابة وكانت تشكل نسبة مقدارها (٦٠,٥%) علاوة على ذلك وجد من خلال الدراسة ان الذكور هم الاكثر إصابة حيث سُجلت ٥٣ حالة إصابة ونسبة انتشار مقدارها (٥٣,٦%)، تبين كذلك من خلال الدراسة ان المرضى المصابون بداء ضغط الدم حيث شكلوا حالاتي إصابة فقط ونسبة مقدارها (١,٣%) كذلك أشارت الدراسة الى عدم وجود علاقة بين الإصابة بهذا المرض والحيوانات المحيطة . تبين كذلك من خلا، الدراسة ان منطقة عمان هي الاكثر انتشاراً حيث سُجلت ٢٥ حالة إصابة ونسبة مقدارها (٢٥,٣%)، كذلك وجد من خلال الدراسة ان اعلى نسبة انتشار سُجلت في شهر تموز/٩٦ وكانت تشكل ٤٠ حالة إصابة ونسبة مقدارها (٢٦,٣%)، وكذلك بينت الدراسة أن نوع الالبسة الداخلية وكثافة الاستحمام ليس لها علاقة مباشرة بحدوث الإصابة أما الدراسة المجهرية اشارة الى وجود فطر *Malassezia furfur* .

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