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ANTIFUNGAL EFFECT OF SILVER NANOPARTICLES ON DERMATOPHYTES ISOLATED FROM CLINICAL SPECIMENS

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Abstract

Different isolates of eight species of dermatophytes: *M. canis*, *T. interdigetale*, *T. rubrum*, *T. mentagrophytes*, *T. schoenleinii*, *T. simii*, *T. verrucosum* and *Epidermophyton floccosum*, as well as *Candida* spp. Four species of dermatophytes were presented for different concentrations (25,50,100 ppm) of silver ions with plant in a mixture and under laboratory conditions. The colony diameter of the fungus was measured. The results showed that the inhibitory effect of silver ions was greater on the isolation of *M. canis., T. interdigetale* and *T. mentagrophtes* while the least affected was the *T. rubrum* cooperatively on the growth rate of the fungus. The concentration of 100 ppm silver nanoparticles gave the highest effect on fungus growth, The effect of nanoparticles depends on their concentrations and exposure time.

Key words : clinical specimens, nanoparticles, silver, antifungal.

Introduction

Dermatophytes are keratinophilic and ikeratinolytic fungi. They are characterized by high affinity to keratin-containing tissues, what make them responsible for superficial mycoses of skin (tinea faciei, tinea barbae, tinea corporis, tinea cruris, tinea manuum or tinea pedis), nails (onychomycosis, tinea unguium) and hair (tinea capitis) (Kalinowska *et al.*, 2009). Infections caused by dermatophyte fungi are very serious problem, not only clinical, but also epidemiological and therapeutic. The incidence of skin, hair and nail diseases does not depend on sex, age or social status. There are many species of dermatophytes causing mycoses, and geophilic dermatophytes are found in soils (Adamski and Batura Gabryel, 2007). In laboratory practice dermatophyte fungi belonging to three genera (*Trichophyton, Microsporum, Epidermophyton*), are known. *Trichophyton* and *Microsporum* genera are the most numerous and diverse, there are over 40 species belonging to these two taxonomic groups.

The field of nanotechnology is one among the foremost important and active areas of research in modern science. Nanotechnology deals with the formulation of experimental processes for the synthesis of nanoparticles with different sizes and shapes (Mahasneh, 2013). The application of nanoparticles, usually ranging from 1 to 100 nm, is a developing and interesting area of nanotechnology (Dahl *et al.,* 2007). Nanoparticles synthesized using metals have received extensive attention in recent years because of their remarkable properties and wide range of applications in catalysis (Paul *et al.*, 2014), plasmonics (Khlebtsov and Dykman, 2010), optoelectronics (Muruganandam *et al.*, 2014) biological sensor (Venkatesan and Santhanalakshmi.,2014) water treatment (Con and Loan, 2011) and pharmaceutical applications (Parashar *et al.,* 2009). To date, metallic nanoparticles are mostly prepared from noble metals, i.e. silver (Vankar and Shukla, 2012), gold (Dash *et al.* 2014), copper, zinc and titanium (Schabes-Retchki- man *et al.* 2006) as well from cadmium (Suresh, 2014), and alginate (Asadi, 2014). Among the noble metals, silver (Ag) is the metal of choice in the field. Green synthesis of nanoparticles is an emerging branch of nanotechnology (Roy and Barik, 2010).

The use of environmentally benign materials like plant extract (Imran, *et al.,* 2018), bacteria (Seshadri *et al.,* 2012), fungi (Muhsin and Hachim, 2014) and marine algae (Kannan *et al.,* 2013) for the synthesis of silver

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nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Green synthesis are found to be superior over physical and chemical method as it is economically feasible, environmental friendly, scaled up for mass-scale production without any complexity (Goodsell, 2004). Using green plants on biological nanoparticles is an amazing one, and it is somehow not well known (Korbekandi *et al.*, 2009). It should be mentioned that Nanoparticles produced by plants are more stable, and the rate of synthesis is faster than that in the case of other organisms (Iravani, 2011).

The aim of our study is isolate and identify the dermatophytes isolated from clinical specimens of tinea, and evaluate the different concentration dose of silver nanoparticles green synthesis on several species of dermatophytes.

Materials and Methods

Fungal samples

A total of 70 specimens were collected from patients with dermatophytoses. There are three types of specimens as follow: 7 (10%) nails clips, 16 (22.8%) hair fragment and 47 (67.1%) skin scraps. The characteristics of the patient such as age, gender, the site of lesion, residency, existence of domestic animals and presence of chronic disease were collected in advance forms.

Fungal identification

For identification of dermatophytes were cultured on Sabouraud‘s Dextrose agar (SDA) with chloramphenicol and cycloheximide and cultured at 26°C for up to 4 weeks. The identification of fungal agents were based on macro- and micromorphological characteristics. In addition, fungal identification was confirmed by the *In vitro* hair perforation test, urease production in Christensen’s medium and vitamin requirements in Trichophyton agar media (Kannan *et al.*, 2006).

Plant material and preparation of the extract

Aloe vera leaves were used to make the aqueous extract leaves. *Aloe vera* leaves is dried in a 60°C oven. Weigh 10 grams of dried plant, add 100 ml of water and leave it on fire for 5 minutes. Then we use Centrifuge for the plant extract and then filter it with the filter paper.

Synthesis of silver nanoparticles

10mM aqueous solution of Silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Aloe vera* leaf extract was added into 100 ml of silver nitrate at a concentration of 10 mM. Note 10 mM moler of silver nitrates Prepare 0.168 g of silver nitrate with 100 ml of water.

After the preparation of the plant extract with the silver nitrate, the solvent prepared with Centrifuges is separated by 10000 cycles for 10 minutes. The residue is then washed with distilled water and separated by Centrifuge at 10000 cycles for 10 minutes. The washing process is repeated with distilled water 3 times and after washing The precipitate obtained in the oven is obtained at 50 ° C and the minutes of the plant extract are obtained with silver nitrate (Dash *et al.*, 2014).

Antifungal assay

New plates have 15 ml of culture Intermediate feet with one of the concentrations (25, 50 and 100 ppm) of silver nanoparticles were cultures incubated under 37°C for 10 days. Mycelium growth measurement (diameter) was carried out every 2 d. Three replicates per concentration were conducted.

Statistical analysis

Statistical analysis was performed by using the chi-square test to find the significant correlation at ($p< 0.05$) level.

Results and Discussion

The result of in this study, showed Tinea corporis is the most common clinical form of dermatophytoses (42.8%) followed by T. capitis (22.8%), T. unguium (10%), T. cruris (10%), T. pedis (8.5%) and T. manuum (5.7%) respectively (Table 1). These results were consistent with the other studies (Mathur *et al.*, 2012; Prasad *et al.*, 2013) which showed that tinea corporis often have occupied the first place of dermatophytoses.

The effect of risk factors on clinlcal types of dermatophytosis was found that statistically significant effect ($P< 0.05$) table 1, the gender relationship were that Tinea capitis (11/16) male and (5/16) female. Patients with tinea corporis recorded the highest no. (16/30) males and (14/30) females while the lowest no. in male was of Tinea cruris (0/7) and in female was of Tinea pedis (3/6), usually males infected with Tinea capitis more than females because of hormones that are play a role in increase, or may be the reason due to attributed to the easy implantation of spores because of short hair and frequency of sharing comb, brushes, and cups (Ilkit and Demirhindi, 2008; Younes *et al*., 2012).

The influence of residence on distribution of dermatophytosis showed that 54.2% patients was from rural area while 45.7% patients was from urban area, the reason for elevation rural area infection may be its poor socioeconomic environmental that due to increase of infectious diseases especially among children (Hasan

Table 1: Distribution of dermatophytosis according to the risk factors.

Clinical samples	Gender		Residency		Domestic animals		Chronic disease		Age		Total no. (%)
	Female	Male	Urban	Rural	+	-	+	-	Young	Adult	
Tinea capitis	5	11	3	13	11	5	1	15	4	12	16 (22.8)
Tinea corporis	14	16	14	16	14	16	7	23	20	10	30 (42.8)
Tinea manuum	1	3	2	2	2	2	0	4	3	1	4 (5.7)
Tinea unguium	6	1	4	3	3	4	1	6	7	0	7 (10)
Tinea cruris	7	0	5	2	2	5	2	5	6	1	7 (10)
Tinea pedis	3	3	4	2	2	4	2	4	3	3	6 (8.5)
Total no (%)	36 (51.4)	34 (48.5)	32 (45.7)	38 (54.2)	34 (48.5)	36 (51.4)	13 (18.5)	57 (81.4)	43 (61.4)	27 (38.5)	70 (100)

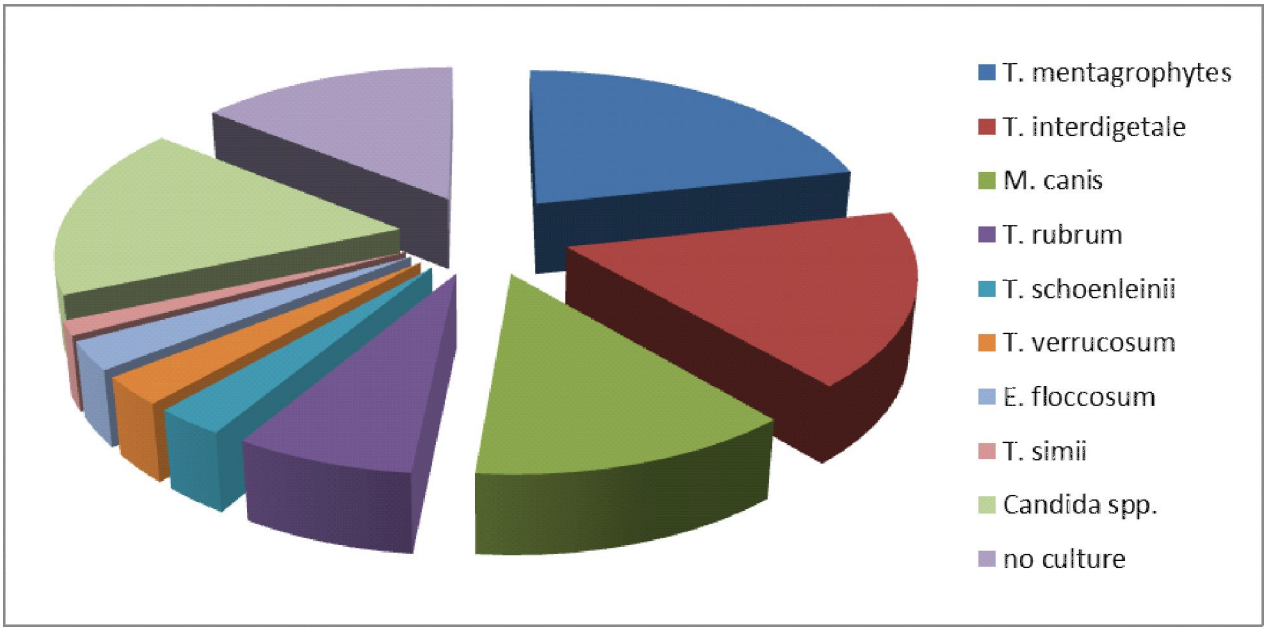

Fig. 1: Percentage of fungal species from dermatophytosis.

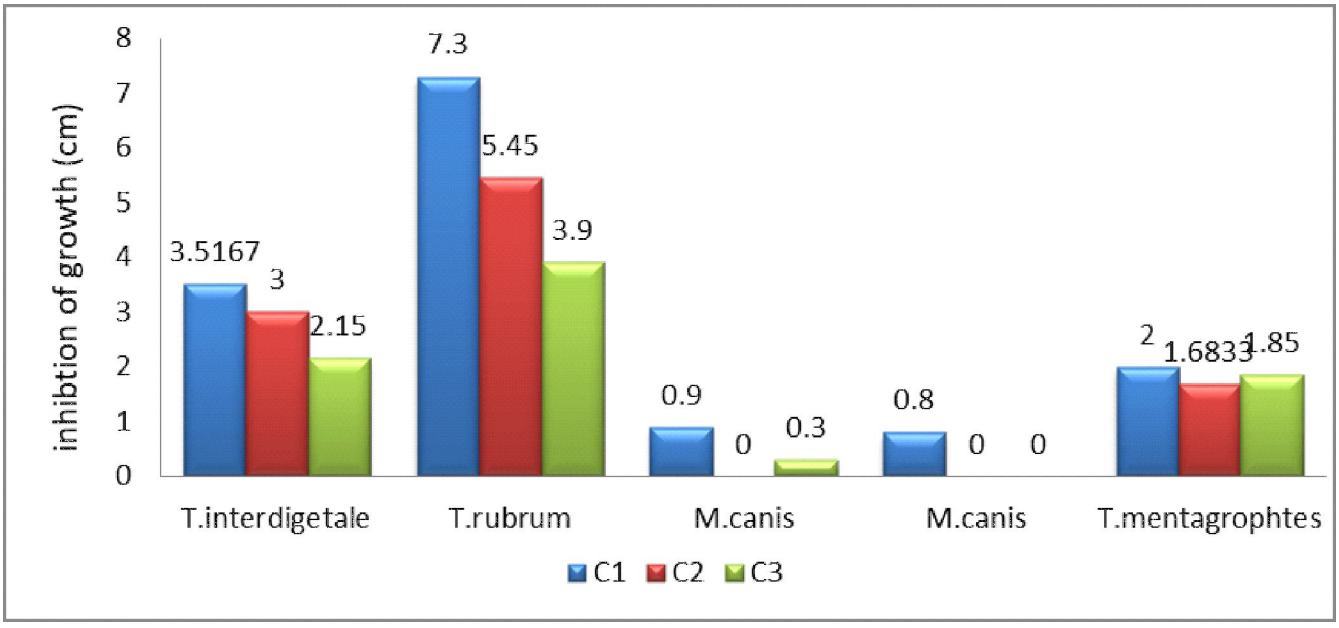

Fig. 2: The inhibition growth of dermatophytes strains against nanoparticles of *Aloe vera*.

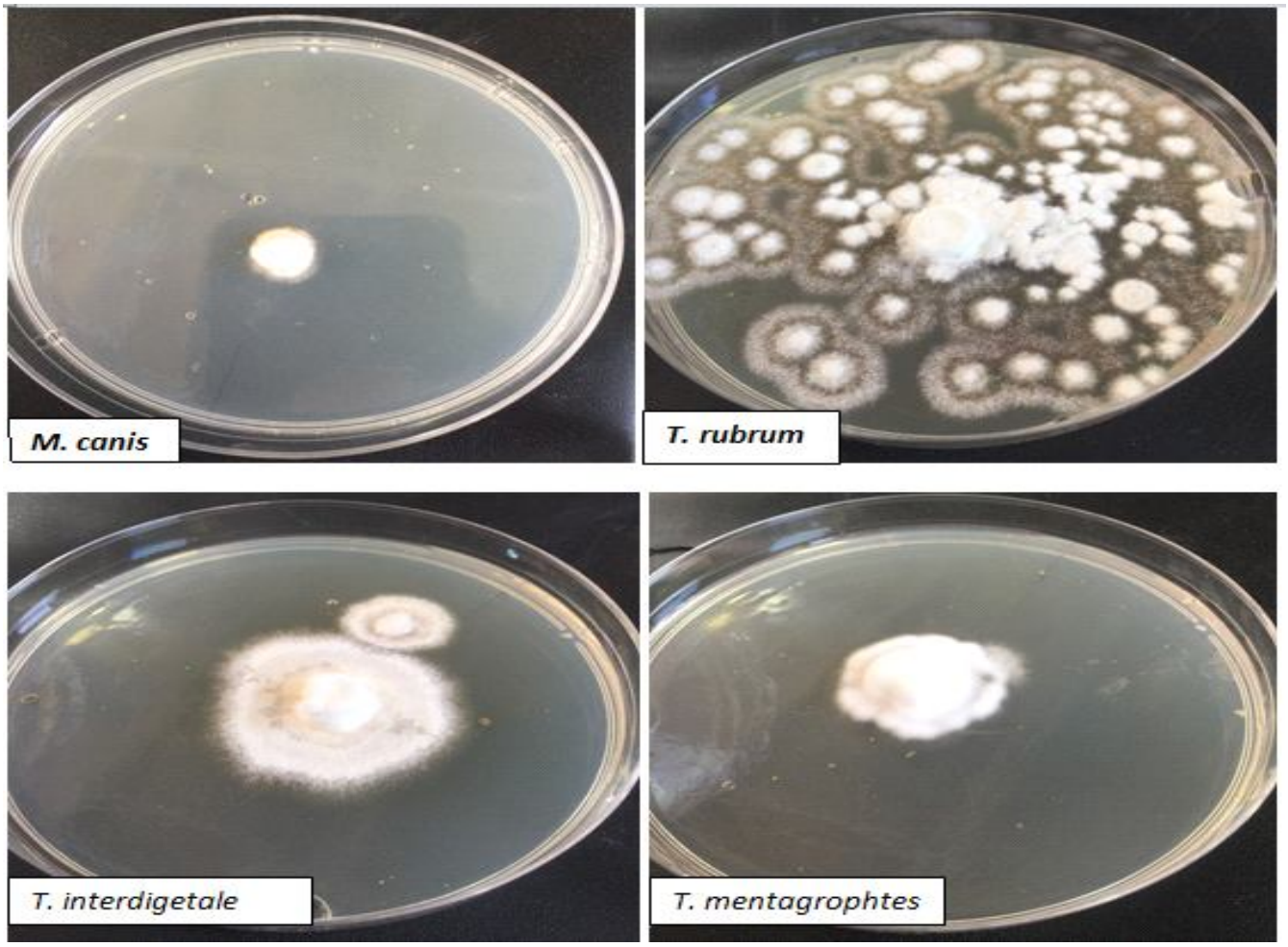

Plate 1: Concentrate effect 25 ppm AgNPs on the diameter of the colonies four species of dermatophytes (10) days of incubation.

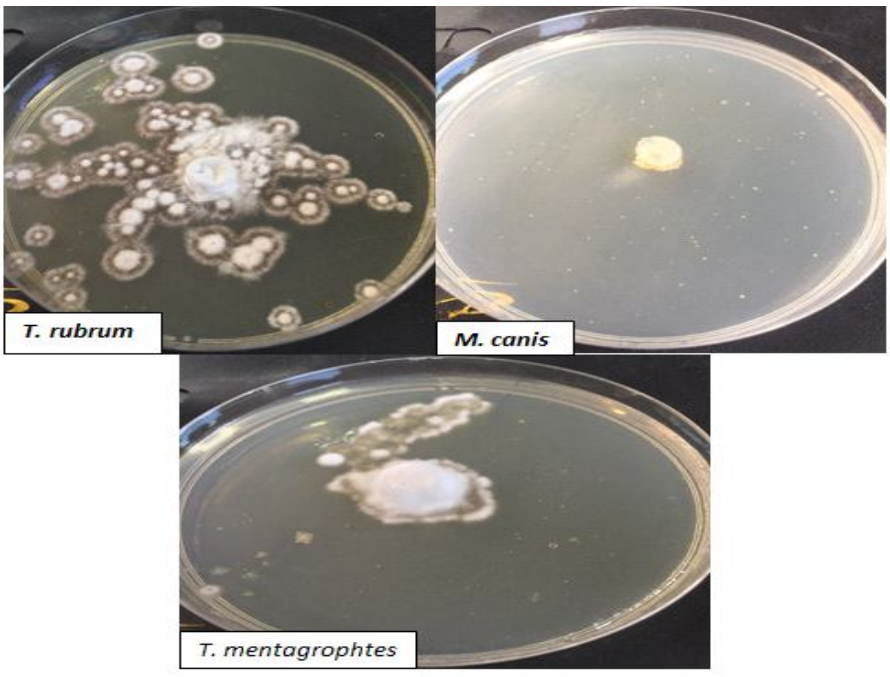

Plate 2: Concentrate effect 50 ppm AgNPs on the diameter of the colonies four species of dermatophytes (10) days of incubation.

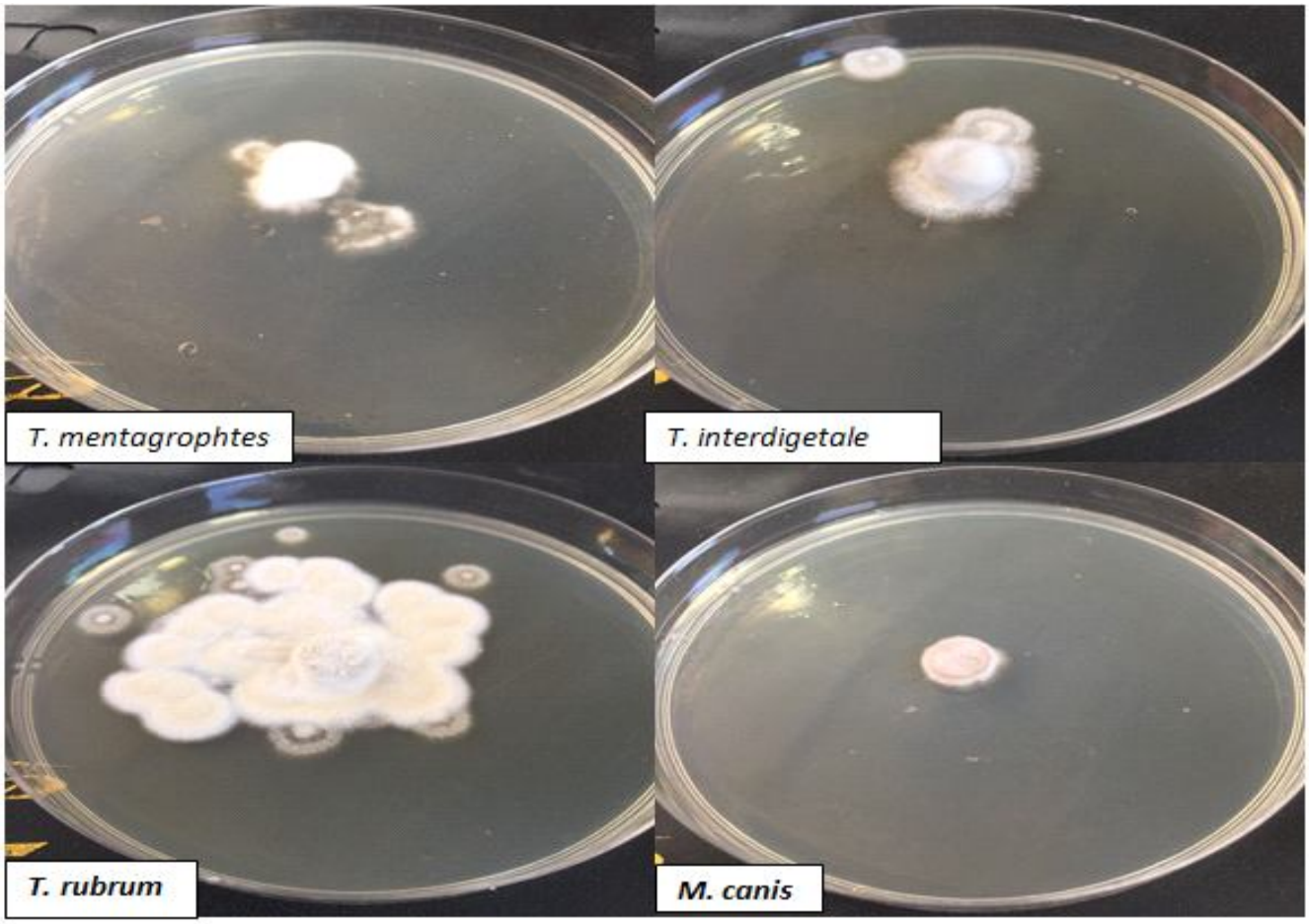

Plate 3: Concentrate effect 100 ppm AgNPs on the diameter of the colonies four species of dermatophytes (10) days of incubation.

and Al-Shibli, 2016).

Often of cases were not suffering from any chronic disease (81.4%) compared with cases that suffering from chronic disease (18.5%). Tinea corporis recorded the highest no. including (7/30) cases with chronic disease followed by both Tinea cruris and Tinea pedis. This results refer to the disease with dermatophytes infected both immunocompetent or immunocompromised patients. Almost affect 10-20% of the world's population and it is possible to infect immunocompetent hosts, in many cases, the treatment methods are very difficult, or may re-infection after recovery (Recurrent) (Grumbt *et al*., 2011). The patients in contact and no contact with domestic animals were convergent, Tinea corporis recorded the highest no. including (14/30) cases with existence of domestic. These results were consistent with Ayesh (2013) when he studied dermatophytosis in Gaza, Palestine.

According to the patients age it was found that statistically significant effect ($P< 0.05$) on the distribution of dermatophytoses, The results exhibited that the young patients had the highest frequency (61.4%), while the adult recorded (38.5%), the results was agreement with Hasan and Al-Shibli, (2016) they found the highest frequency of dermatophytoses in age 30-40 years while the lowest frequency in age more than 70 years. The higher incidence in young males could be due to greater physical activity and increased sweating (Al-Galibi and Imran, 2014).

Eight species of dermatophytes were identified in clinical specimens: *T. mentagrophytes* 15 (21.4%) showed the highest frequency followed by *T. interdigetale* 12 (17.1%), *M. canis* 9 (12.8%), *T. rubrum* 5 (7.1%), and all of *T. schoenleinii*, *T. verrucosum* and *Epidermophyton floccosum* 2 (2.8%) (Fig. 1), as well as *Candida* spp 12 (17.1%). These results were consistent with the other studies (Hasan and Al-Shibli, 2015; Prasad *et al.*, 2013).

In present study the inhibitory effects of nanoparticles of *Aloe vera* various concentrations were tested on the growth of *M. canis*, *T. interdigetale*, *T. rubrum* and *T. mentagrophtes*.The results of inhibition growth of dermatophaytes strains to nanoparticles of valo vera are

illustrated in (Fig. 2). *M. canis* had the highest inhibition, following *T. mentagrophytes* and *T. interdigetale*, while *T. rubrum* was low inhibition growth (Plate 1-3). The differences were considered significant at P value < 0.05.

Dermatophytosis is caused by the keratinophylic fungus called dermatophytes (Coelho *et al*., 2008). In some cases, treatment of the disease with the current therapeutic agents can result in the damage of host tissues due to the similarity between eukaryotic cells in human and fungi structure, emergence of drugs resistance to fungal strains, and treatment failures (Pakshir *et al*., 2009). Different research groups have investigated the efficacy of AgNPs on yeasts, molds, bacteria, and viruses (Mehrbod *et al*., 2009; Namasivayam *et al*., 2011). But, information about anti-dermatophyte activities of nano-silver particles is few (Noorbakhsh *et al*., 2011). The AgNPs had good antifungal and antimicrobial effects (Vaidyanathan *et al*., 2009; Wasif *et al*., 2009). Atef *et al*., (2013) reported the growth inhibition of the AgNPs on *T. mentagrophytes* and *C. albicans*.

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