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RESEARCH ARTICLE

Antifungal Activity of Clove Oil On Dermatophytes and Other Fungi

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Abstract

The incidence of fungal infections especially dermatophytosis has been observed increasing worldwide. The resistance of some species of fungi to antifungal drugs, high treatment costs and toxic effect of current drugs, all have encouraged the members of research for alternative natural compounds such as essential oils. The present study was carried out to evaluate the antifungal activity of Clove oil in vitro by agar disk diffusion method at different dilutions (0, 10, 20, 50, and 100%) on the isolated fungi (*Trichophyton mentagrophytes*, *Microsporum canis*, *Aspergillus flavus* and *Candida albicans)*, and confirmed the results through application of Clove oil on cows affected with dermatophytosis. The results concluded that, *T. mentagrophytes* and *M. canis* were the most prevalent isolates and Clove oil had a strong antifungal activity against tested isolated fungi especially against Dermatophytes spp in vitro and effectively as a topical treatment in cows with dermatophytosis .

INTRODUCTION

Dermatophytes are fungi that cause infection of skin, hair and nails of both humans and animals. (Chermette and Ferreiro,2008) Dermatophytes infections are caused by 40 species of fungi which are grouped into three genera; Trichophyton, Microsporum and Epidermophyton (David et al., 1997). The infections are rarely fetal but cause significant morbidity and economic costs because of their resistance to therapy (Yuan et al., 2009) the recent antifungal drugs used for these infections are toxic, expensive and need long term, so, the discovery of alternative new anti dermatophytic agent is critical (Soares et al.,2014)**.** Essential oils widely used as natural antimicrobial agents which effectively inhibit the growth of a wide range of microorganism and have low side effects than synthetic antimicrobial drugs(Rana et al., 2011). One of these oil is Clove oil which has been widely used due to its lower side effect and high essential oil content (eugenol) (Park et al., 2007). Antimicrobial effect of clove oil have been reported to inhibit the growth of molds, yeasts and bacteria (Matan et al., 2006). both eugenol and clove essential oil Changing permeability of cell membrane phospholipids therefore, inhibiting bacteria and different types of yeast. Moreover, have been described as useful antiseptic, analgesic and anesthetic effects (Chaieb et al., 2007a) . Therefore, the current study was focused to evaluate the effect of clove oil as antifungal agents.

Materials and methods

1-Samples collection:

Sixty (60) samples of skin scrapings (35) and hairs (25) were collected from farm animals cattle suffering apparently from skin lesions at minufia governorate. The collected samples were brought to the laboratory in clean sterile Petri-dishes for mycological examination.

2-- Mycological examination:

2.1-Direct Microscopical examination of collected samples:
broken hairs and some of skin scrapings of collected samples were placed in a drop of 20% KOH in a clean glass slide and covered with cover slide, heated gently and left for 1 hour, then examined for fungal elements (hyphea and spores around (ectothrix) or within the hairs (endothrix) using low and high power of microscopical examination (Ellis et al., 2007)**.**

2.2- Isolation and identification of dermatophytes:
The collected specimens from different animals were inoculated in Sabouraud's dextrose agar (SDA) with antibiotics(Chloramphenicol 50 mg/L and actidion (sigma) 0.5 g/L. the inoculated media were incubated at 30^{o}C for up to 21 days and examined daily. The isolated dermatophytes were identified by macroscopical examination which involved rate of growth, color, texture of the colony or consistency (Cottony, fluffy, suede-like and wiry), its surface topography (flat, folded, plicate, and rugose) and reverse side of colony (pigmentation of the medium), margins, elevation and detachability from the agar surface (Rippon, 1988 and Cheesbrough, 2003). While Microscopical morphology of the isolates was done by using wet mount preparation **(**Collee et al., 1996**).**

2.3- Isolation and identification of other associated fungi:
The suspected samples were inoculated into SDA with Chloramphenicol 50 mg/L. *Aspergillus flavus* identified according to (Samson, 1979**)**. While yeast (*Candida albicans*) was identified by culturing on corn meal agar medim (Kreger-van Rij, 1984) and demonstration of germ tube on rabbit serum **(**Konemanet al., 1992).

3- Evaluate Antifungal activity of Clove oil.
Essential oil
Clove oil was obtained from pharmacognosy department, National Research Center, Doki, Giza, which dissolving in Tween80.
3.1- Evaluate antifungal activity of Clove oil in vitro.
Fungal spores were harvested after 14 days old (*T. mentagrophytes*, *Microsprum canis*) and 5 days old (*A. flavus* and *C. albicans*) on SDA slants. Culture was washed with 10 ml normal saline in 2% Tween 80 with aid of glass beads to help in dispersing of the spores. The spore suspensions were standardized to 10^5 spores/ml. 0.1 ml of each standardized spore suspension (10^5 spores/ml) was evenly spread on the surface of SDA plates by sterile glass rod. Filter paper disk (whatman No. 4mm diameter) impregnated with different dilutions (0, 10, 20, 50 and 100%) of Clove oil then placed on the surface of Petri dishes inoculated with spores by agar disk diffusion method according to (Bauer et al., 1966**)**. The plates were sealed with parafilm immediately after adding oil and incubated for 21 days at 25^{o}C in case of dermatophytes, while in case of *A. flavus* and *C. albicans* incubated for 5 days. The diameter (mm) of clear zone of growth inhibition was measured (Aggarwal et al., 2001).

3.2- Evaluate antifungal activity of Clove oil on field cases of ring worm.
After the clove oil exhibited successful antidermatophytic activity, trails for treatment of some infected farm animals were done in this research through treated group of cows at different places suffering from dermatophytosis. Pure clove oil used as a topical application 2-3 times daily for 7-10 days with daily examined animals.

Result and Discussion

1-Prevalence of Dermatophytes and other fungi

The prevalence of Dermatophytosis vary according to several factors as geographic distribution, environmental and culture factors (Havlickova et al.,2008) . The result obtained in table (1), out of 60 samples(35 skin scraping and 25 hair) from infected cattle, Forty samples (66.6%) were positive for dermatophytosis by KOH examination . but in culturing on SDA 12 (20%) were positive for dermatophytes. While culture positive for non dermatophytes were 10% and 16.66% in hair and skin scraping respectively. A lower prevalence rate (13.04%) recorded by (Akbarmehr, 2011). While (Shams-Ghahfarokhi et al., 2009) recorded a higher rate (71.6%). The result showed In table (2) two genera of dermatophytes were the most prevalent isolates (42.85%) *Trichophyton mentagrophytes*

and *Microsporum canis* (10.71%) and (32.14) respectively. followed by aspergillus spp (32.1%), *Candida albicans* (17.85%), other previous report (Shams-Ghahfarokhi *et al*., 2009) identified 4 species of dermatophytes (*T. rubrum* , *T. verrucosum* , *T. mentagropgytes* and *M. canis)* from cows and buffaloes.

2-Evaluation of antifungal activity of Clove oil in vitro

The Clove oil was respected under several studies to investigate its therapeutic uses. Previous studies have reported antifungal activity of clove oil and eugenol against yeast and filamentous fungi (Lopez et al., 2005**)** and human pathogenic fungi (Chaieb et al., 2007b). In the present study, pure Clove oil (100%) showed strong antifungal effect against all tested fungi, while the concentration 50% and 20% effectively against *Candida albicans*. and the most susceptible fungi were *T. mentagrophytes*, *M. canis*, *C. albicans* respectively which showed the largest inhibition zone (mm) as shown in table (3) and fig (1,3). This nearly agree with (Pinto et al., 2009) The EO and eugenol showed inhibitory activity against Candida , Aspergillus and Dermatopytes spp. Moreover the present study clarified that the largest inhibition zones was 50 and 45 mm in case of *T. mentagrophytes* and *M. canis* respectively as shown in fig (1). These results supported by (Chee and Lee, 2007) and (Pinto et al. 2009) Clove oil exhibited wide–spectrum antifungal activity against five different species of dermatophytes.

3-Evaluation of antifungal activity of Clove oil on field cases with dermatophytosis.

Several previous studies focused on antifungal activity of Clove oil in vitro. In the current research, the antidermatophytic activity of pure Clove oil in cows with dermatophytosis was evaluated through used EO as a topical treatment on affected lesion 2-3 times daily for 7-10 days. We noticed that the healing of affect lesions and hair grown up as shown in fig (4). Theses findings supported by(Zuzarte et al., 2011) used Clove oil as a topical treatment in animal models with ringworm. Also Clove oil and eugenol have also tested as antifungal agents in animal models (Ahmad et al., 2005). Ointment formulations with oils were applied in guinea-pigs previously infected with dermatopytosis (lee et al, 2007). From previous we recommended that, essential oils as Clove oil can be used as a topical treatment for ringworm infection in animals and human as alternative natural drug to the conventional antifungal agents for treatment of dermatophytosis.

Table 1: prevalence of positive samples for dermatophytes by KOH 20% and culturing on SDA medium.

Samples	No. of examined Samples	+ve Microscopical sample with KOH (20%)		+ve Culture samples for Dermatophytes		+ve Culture samples for Non dermatophytes		-ve Cultue samples	
		No.	%	No.	%	No.	%	No	%
Hair	25	14	23.33	5	8.33	6	10	3	5
Skin scraping	35	26	43.33	7	11.66	10	16.66	9	15
Total	60	40	66.6	12	20	16	26.6	12	20

% was estimated according to total samples (60).

Table 2: Prevalence of dermatophytes and non dermatophytes spp (n=28).

Fungal isolates	Samples				
	Hair		Skin scraping		Total
	No	%	No	%	
Dermatophytes:					
Microsporum canis.	1	3.57	2	7.14	10.71
Trichophyton mentagrophytes	0	0	9	32.2	32.14
Non dermatophytes	No	%	No	%	total
A.flavus	1	3.57	3	10.71	14.28
A.versicolor	0	0	1	3.57	3.57
A.niger	1	3.57	1	3.57	7.14
A.fumigatus	1	3.57	1	3.57	7.14
Cladosporium spp	1	3.57	0	0	3.57
Penicillium spp	0	0	1	3.57	3.57
Candida albicans	1	3.57	4	14.28	17.85

% was estimated according to total number of isolates (28).

Table 3: Antifungal activity of Clove essential oil on dermatophytes spp. and some non dermatophytes isolates in vitro.

Fungal isolates	The mean values of the inhibition zones in mm				
	0%	10%	20%	50%	100%
Trichophyton Mentagrophytes	-ve	-ve	1	2	50
Microsporum canis	-ve	-ve	1	1	45
A. flavus	-ve	-ve	2	6	30
C. albicans	-ve	-ve	15	20	26

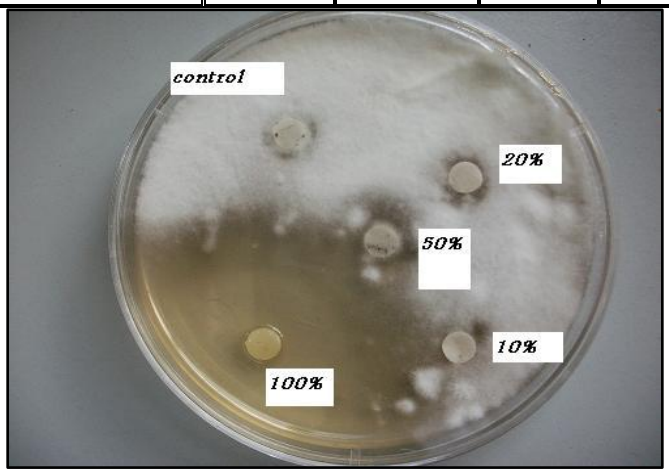


Fig (1): Effect of clove oil on *T. mentagrophytes*

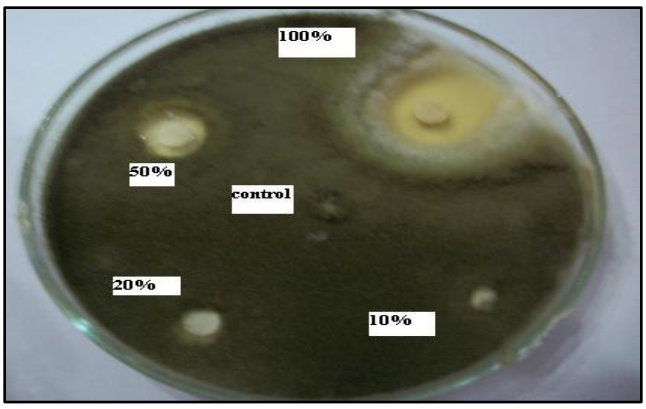


Fig (2): Effect of clove oil on *A. flavus*

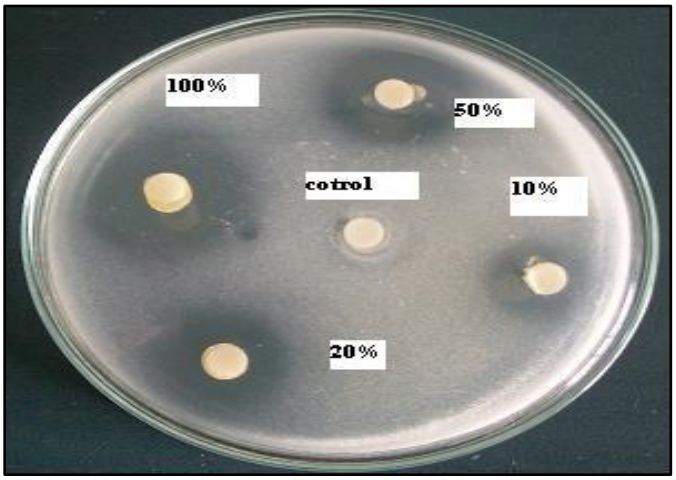
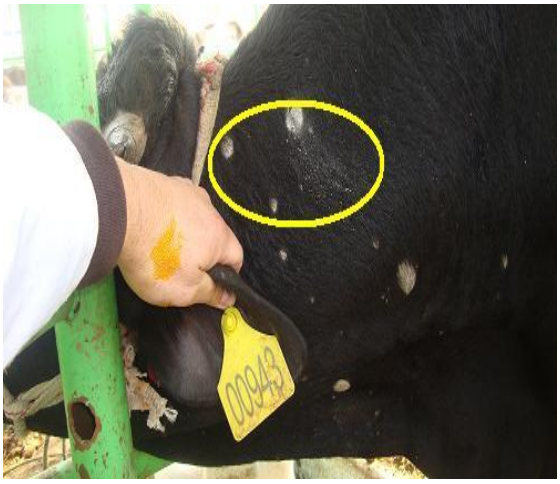
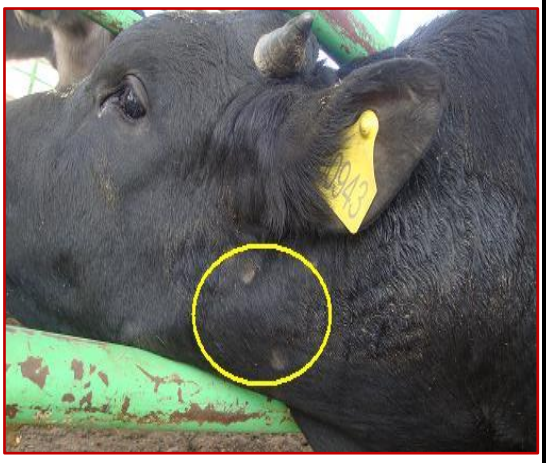


Fig (3): Effect of clove oil on *C. albicans*



Before treatment



After treatment

Fig (4):Topical Treatment of cows with Clove Oil

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