رقم البحث(15)

EVALUATION OF ANTIFUNGAL ACTIVITY OF CLOVE OIL ON DERMATOPHYTES AND OTHER ASSOCIATED FUNGI IN VIVO AND IN VITRO

BY

***Eman abdeen, E. and ** El-Diasty, E.M.**

**Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, University of Sadat City*

*** Mycology Department, Animal Health Research Institute, Dokki, Egypt*

ABSTRACT

The incidence of fungal infections has been observed as well as a rise in the resistance of some species of fungus to different fungicides besides high treatment costs, all have stimulated the research for alternative natural drugs, such as essential oils. This study was carried out to evaluate the antifungal activity of Clove oil derived from Eugenia Caryophyllata at different dilutions (0, 10, 20, 50, and 100%) on *Trichophyton mantagrophytes*, *Microsporum canis*, *Aspergillus flavus* and *Candida albicans*. The results of this study concluded that Clove oil had successful antifungal activity against different fungal species in vitro and the applications of Clove oil as topical treatment in some cows suffering from dermatophytosis have useful and significant results.

Key word: Clove oil, *T. mentagrophytes, M. canis, A. flavus*, *C. albicans*

INTRODUCTION

Dermatophytes are the most common agents of fungal infections worldwide (**Yuanwu et al., 2009**). Dermatophytes infections have been considered to be a major health problem in many parts of the world. Dermatophytes infections are common in the developing countries and are particular concern in the tropics (**Guest and Sam**, **1998**). These infections are caused by 40 species of fungi which are grouped into three genera; Trichophyton, Microsporum and Epidermophyton (**David et al., 1997**). The mode of spread is

either by direct contact or indirect contact with an infected particles which usually a fragment of keratin containing viable fungus. Dermatophytes infections are rarely fetal but mostly debilitating disease that can give rise to permanent deformations if untreated (**Yuanwu et al., 2009**).

Essential oils have a long history of use as natural microbial agents and have recently been used in a number of pharmaceutical, food and cosmetic products since these oils effectively inhibit the growth of a wide range of microorganisms, with fewer side effects than synthetic antimicrobial agents in humans. Despite the widespread use of essential oils, details about the exact mechanism of their antimicrobial action are yet to be explored. Thus, many researchers have recently attempted to identify the antimicrobial properties of essential oils. Clove oil has been widely investigated due to its popularity, availability, and high essential oil content (eugenol) (**Park et al., 2007**). The increasing resistance to antifungal compound and the reduced number of available drugs led us to search for the new alternative among aromatic plants and their essential oils, used for their antifungal properties. The antifungal activity can be attributed to the presence of some components such as Eugenol (a phenylpropanoid, is an allyl chain- substituted guaiacol). (**Zuzarte et al., 2011**). We aimed to evaluate the effect of clove oil as antifungal agents.

MATERIALS AND METHODS

Samples collection:

Sixty sterile swab samples of skin scrapings (35) and hair (25) were collected from farm animals (cows and sheep) suffering apparently from skin lesions at minufiya governorate. The collected samples were brought to the laboratory in clean sterile Petri-dishes for mycological examination.

Table (1): Number and sources of examined samples.

Animals	**Type of samples**	
	Hairs	**Skin scrapings**
Cows	15	23
sheep	10	12

A- Mycological examination:

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 fugal elements (hyphea and spores around (ectothrix) or within the hairs (endothrix) using low and high power of microscopical examination (**Ellis et al., 2007**).

2- Isolation and identification of dermatophytes:

The collected specimens from different animals were isolated by inoculation in Sabouraud dextrose agar (SDA) with antibiotics (Chloramphenicol 50 mg/L and actidion (sigma) 0.5 g/L. The inoculated media were incubated at 30^0C 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):**

3-Isolation and identification of other associated fungi:

The suspected samples were inoculated into SDA with Chloramphenicol 50 mg/L. Aspergillus spp identified according to **(Samson, 1979).** While yeast (*Candida albicans*) was identified by culturing on corn meal agar medim **(Kriger Van Rij 1984)** and demonstration of germ tube on rabbit serum **(Koneman et al., 1992):**

B- Antifungal activity of clove oil:

Essential oil

Clove oil was obtained from pharmacognosy department, National Research Center, Doki, Giza, which dissolving in Tween 80.

B-1- in vitro antifungal efficacy of clove oil

Fungal spores were harvested after 14 days old (*T. mentagrophyte*, *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 discs (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 disc diffusion method according to **(Bauer et al., 1966)** The plates were sealed with parafilm immediately after adding oil and incubated for 21 days at 25^0C 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**).

B-2- in vivo antifungal efficacy of clove oil:

After the clove oil exhibited successful antidermatophytic activity, trails for treatment of some infected farm animals were done in this study through treated group of cows at different places suffering from ringworm in head and neck. Pure clove oil used as a topical application 3 times daily for 10 days with daily examined animals.

RESULTS

Table (2): Correlation between positive KOH 20% and positive culture of examined samples (n = 60)**:**

Type of samples	No. of examined samples	+ve microscopical Sample with KOH (20%)		+ve culture samples for Dermatophytes		+ve culture samples for Non-dermatophytes		-ve culture samples	
		No.	%	No.	%	No.	%	No.	%
Hair	25	14	56	5	20	6	24	3	5%
Skin scraping	35	26	74.3	7	20	10	28.6	9	15
Total	60	40	66.6	12	20	16	26.6	12	20

Table (3): Identification of dermatophytes and nondermatophytes isolated from samples (n=28).

Fungal isolates	Number of isolates				Total Percentage%
	Hair		Skin scraping		
	No.	%	No.	%	
Dermatophytes:					
Microsporum canis.	1	3.6	2	7.1	10.7
Trichophyton mentagrophyte	0	0	9	32.2	32.2
Non dermatophytes:					
A. flavus	1	3.6	1	3.6	7.2
A. versicolor	0	0	1	3.6	3.6
A.niger	1	3.6	3	10.7	14.3
A. fumigatus	1	3.6	1	3.6	7.1
Cladosporium spp.	1	3.6	2	7.1	10.7
Penicillium spp.	0	0	2	7.1	7.1
Candida albicans	1	3.6	1	3.6	7.2

Table (4): Antifungal activity of clove essential oil on dermatophytes spp. and some nondermatophytes isolated from examined samples.

Isolated fungi	The mean values of the inhibition zones in mm				
	0%	10%	20%	50%	100%
Trichophyton Mentagrophyte	-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

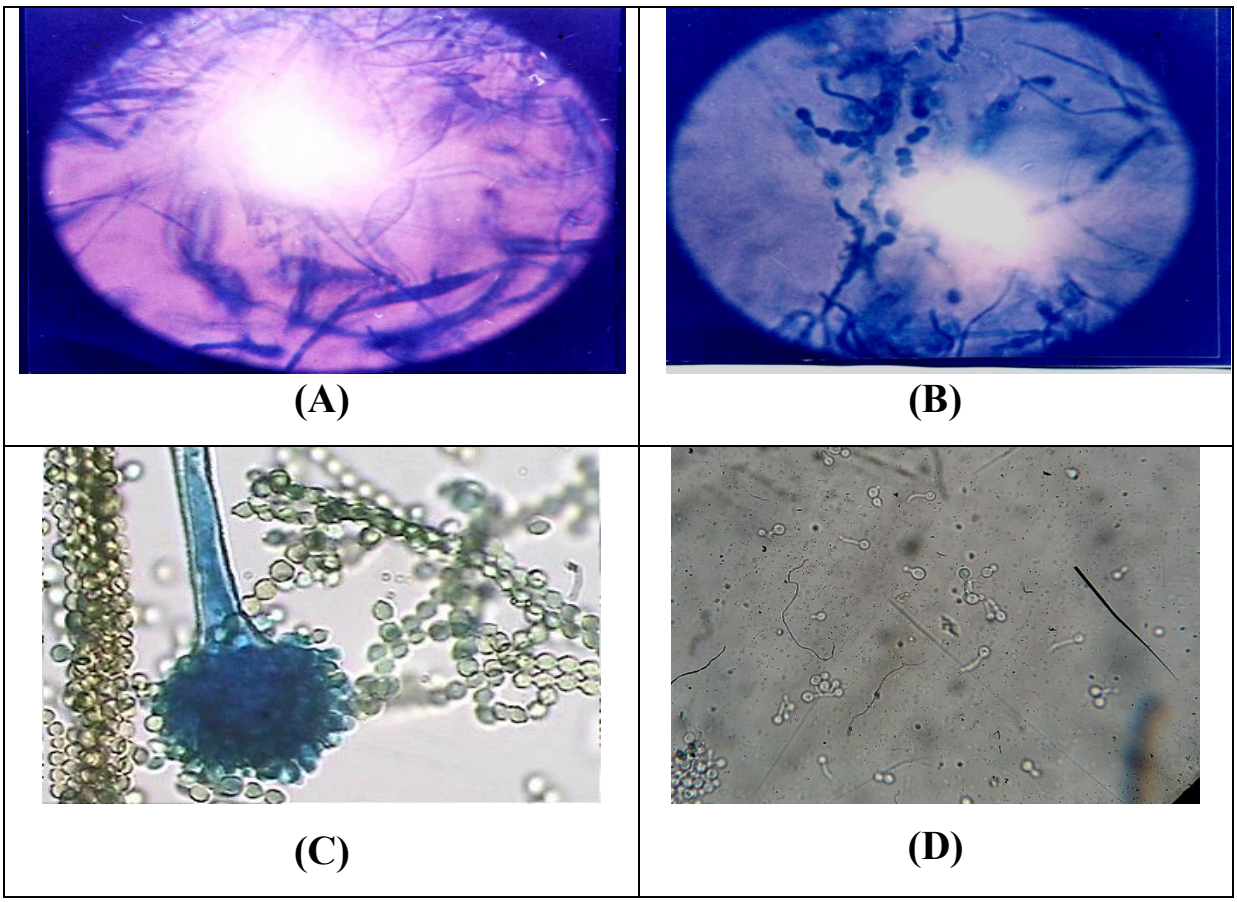


Photo (1): Showing microscope examination of different isolated fungi.
(A): *Microsporum canis* **(B):** *Trichophyton mentagrophytes,* **(C):** *Aspergillus flavus* and **(D):** Germ tube formation of *C. albicans.*

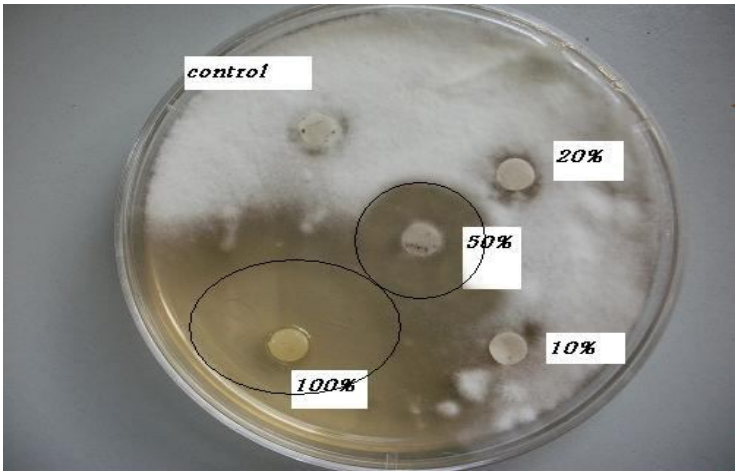
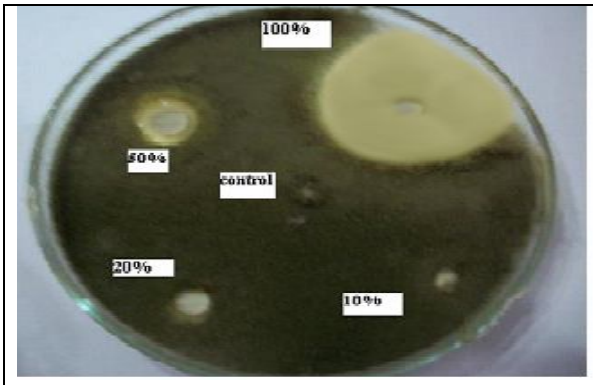
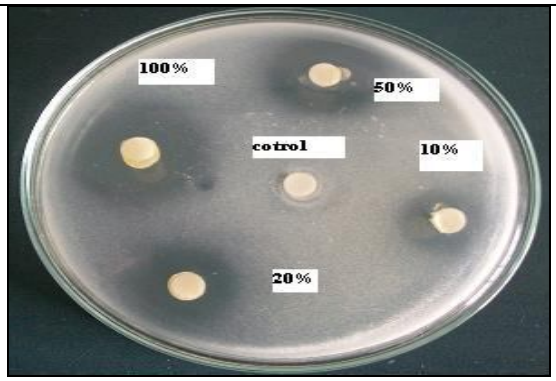


Photo (3): Effect of clove oil on *Trichophyton mentagrophytes.*

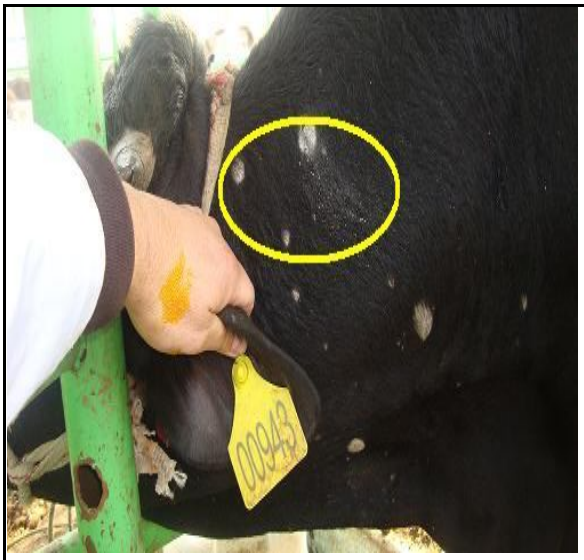


(A)

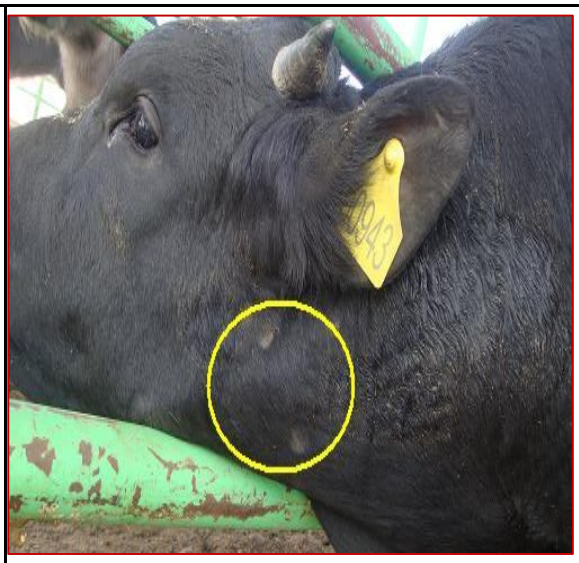


(B)

Photo (4): (A) showing Effect of clove oil on *A. flavus* (B): Effect of clove oil on *C. albicans*.



Before treatment



After treatment

Photo (5): Treatment with essential clove oil 100% dilution.

DISCUSSION

Dermatophytosis is common and clinically multifaceted fungal skin disease affecting both humans and animals. Dermatophytosis causes more economic losses among livestock not only the skin suffer, but causes metabolic disorders, rapid weight loss and a slowdown in growth.

Direct microscopical examination of the skin scraping using 20% KOH revealed fungal mycelia and arthrospores in the macerated debris and infected hair which characterized by large-spore ectothrix arrangement of arthrospores. The obtained results in table (2) showed that total number of 60 skin scraping samples and hair which collected from infected cattle and sheep give 40 cases positive by using KOH 20% in which 56% and 74.3% were positive in hair and skin scraping samples, respectively. Not all of KOH positive samples were culture positive. On SDA 5 (20%), 7 (20%) for hair and skin scrapes, respectively. While, culture positive for non dermatophytes were 6 (24%) and 10 (28.6%) for hair and skin scrapes, respectively. These results in agreement with (**Nada 2000), (Hanaa 2003**), (**Akbarmehr 2011**), **and (EL-Diasty *et al*. 2013**).

Table (3) showed that 2 genera of dermatophytes (Trichophyton and Microsporum) and 4 genera of non-dermatophytes were identified from the examined samples. The frequencies of isolated dermatophytes genera were *Microsporum canis* 3 (10.7%) and *Trichophyton mentagrophyte* 9 (32.2%). While, the frequencies of isolated non-dermatophytes genera were *A.flavus* 2 (7.1%), *A.versicolor* 1 (3.7%), *A.niger* 4 (14.3%), *A.fumigatus* 2 (7.1%), Cladosporum spp. 3(10.7%), Penicillium spp 2 (7.1%) and *Candida albicans* 2 (7.1%). These results come in agreement with (**El-Diasty *et al*., 2013)** who identified 2 genera of dermatophytes from cows and buffaloes, the identified isolates were *Trichophyton mentagrophytes* (33.33%) and *Microsporum canis* (26.67%). On the other hand (**Shams-Ghahfarokhii *et al*., 2009**) who revealed that 4 species of dermatophytes were identified from 35 positive cultures of cows ringworm suspected lesions, these species were (*T. rubrum* (19), *T. verrucosum* (10), *T. mentagropgytes* (5), and *M. canis* (1) from cows and buffaloes. Also (**Al-Ani et al., 2002**) reported that *T. verrucosum* was the most isolated fungi (47.88%) and *T. mentagrophytes* was the second frequent isolated fungi from ringworm infection in calves. While **(Sudad and Mohammed, 2011)** identified 3 species of dermatophytes which were T. rubrum, T. verrucosum and T. mentagrophytes from sheep in Iraq.

The clove oil from Syzygium aromaticum and eugenol have been described as useful antiseptic, analgesic and anesthetic effects (**Chaieb et al., 2007a**) and are largely used in dental medicine. Previous studies have reported antifungal activity for clove oil and eugenol against yeast and filamentous fungi, such as several food-borne fungal species (**Lopez et al., 2005**) and human pathogenic fungi (**Chaieb et al., 2007b**). Clove oil and eugenol have also tested as antifungal agents in animal models (**Ahmad et al., 2005**).

Table (4) showed the results of the disc diffusion method which indicated that *T. mentagrophytes, M. canis, A. flavus* and *C. albicans* were more sensitive to clove essential oil. The results showed that the largest inhibition zones 50 and 45 mm in case of *T. mentagrophytes* and *M. canis* respectively as shown in photo (3). These result agreed with **(Chee and Lee, 2007) and (Eugenia et al., 2009**) who's reported that the clove oil exhibited wide-spectrum antifungal activity and the highest level of activity was observed against five different species of dermatophytes. Also had antifungal effect on Aspergillus and Candida strains as shown in photo (4) that *Candida albicans* and *Aspergillus flavus* were sensitive and inhibited by pure clove oil and showing wide zone of inhibition. Moreover **(Ahlam and Hanaa 2013)** revealed that clove oil inhibit the growth of dermatophytes as *M. canis*, *T. mentagrophytes* and *T. verrucoscum*. (**Guy et al., 2012**) evaluated the effect of eugenol (active principle of clove oil) against 38 Candida species. It was found that eugenol exhibited highly antifungal activity.

photo(5): showing cow suffering from area of alopecia and crust formation and the other part of photo showing healing the lesions and hair grown up after application of pure clove oil on lesion 2-3 times daily for 7-10 days . The similar results obtained by (**Zuzarte et al., 2011**) who used clove oil as a topical treatment in animal models with dermatophytosis and revealed that the most active antifungal compounds of essential oils (EO) proved to be able to attack cell walls and membranes, affecting the permeability and release of intracellular constituents as well as several invasive targets, allowing together inhibition of fungal infection.

REFERENCES

Aggarwal, K. K.; Khanuja, S.P.S.; Ahmad, A.; Kumar, T.R.S.; Gupta, V.K. and Kumar, S. (2001): Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of Menthaspicata and Anethumsowa. Flav.Fragr. J., 17: 59–63.

Ahlam K. A. Wahba and Hanaa, M. M. Abd-El khaliq (2013): The effect of clove oil on Staphylococcus aureus and dermatophytes isolated from skin affections in buffaloes. 12th Sci. Cong., Egyptian Society for Cattle Diseases, 3-6 Dec. 2013, Hurgada, Egypt.

Ahmad, N.; Alam, M. K.; Shehbaz, A.; Khan, A.; Mannan, A.; Rashid Hakim, S.; Bisht, D. and Owais, M. (2005): Antimicrobial activity of clove oil and its potential in the treatment of vaginal candidiasis. J. Drug Target 13: 555–561.

Akbarmehr, J. (2011): The prevalence of cattle ringworm in native dairy farms of Sarab city (East Azerbaijan province), Iran. African Journal of Microbiology Research, 5(11): 1268-1271.

Al- Ani, F. K.; Younes, F. A. and Al-Rawashdeh, O. F. (2002): Ringworm infection in cattle and horses in Jordan, Acta Vet. Brno, 71: 55–60.

Bauer, A.W.; Kirbay, W.A.; Sherris, J.S. and Turk, M.(1966): Antibiotic susceptibility testing by a standardized single disc method. Am. J.Clin. Pathol, 45:493-496.

Chee, H. Y. and Lee, M. H. (2007): Antifungal Activity of Clove Essential Oil and its Volatile Vapour against Dermatophytic Fungi. Mycobiology 35(4): 241-243.

Cheesbrough, M. (2003): Distinct laboratory practical in tropical countries, part 2 Cambridge University press, and UK pp. 136.

Chaieb, K.; Hajlaoui, H.; Zmantar, T.; Kahla-Nakbi, A. B.; Mahmoud, R.; Mahdouani, K. and Bakhrouf, A. (2007a): The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzigium aromaticum L. Myrtaceae): a short review. Phytother Res., 21: 501–506.

Chaieb, K.; Zmantar, T.; Ksouri, R.; Hajlaoui, H.; Mahdouani, K.; Abdelly, C. and Bakhrouf, A. (2007b): Antioxidant properties of the essential oil of Eugenia caryophyllata and its antifungal activity against a large number of clinical Candida species. Mycoses, 50: 403–406.

Collee, J. G.; Duguid, J. P.; Fraser, A. G.; Marmion, B. P. and Simmons, A. (1996): Mackie and McCartney Practical Medical Microbiology. 14thEd. The English Language Book Society and Churchill Living Stone Edinburgh and New York.

David, G.; Richard, C.B.S. and John, F.P. (1997): Medical microbiology. A guide to microbial infections palliogenesis, immunity, laboratory diagnosis and control Ed. 15th EL ST Publishers. 558-564.

El-Diasty, E.M.; Ahmed, M.A.; Nagwa, O.; Salwa .F. M.; Samaa, I.E.; Hanaa, M. A. E. and Mariam h. Y. (2013): Antifungal activity of zinc oxide non particles against dermatophytic lesions of cattle. Romanian J. Biophys., 23(3): 191–202.

Ellis, D.; Davis, S.; Alexiou, H.; Handke, R. and Bartley, R. (2007): Description of medical fungi.2ndEdition the National Library of Australia.

Eugénia, P.; Vale-Silva, L.; Cavaleiro, C. and Salgueiro, L. (2009): Antifungal activity of the clove essential oil from Syzygiumaromati-cum on Candida, Aspergillus and dermatophyte species, J Med Microbiol . 58(11): 1454-1462.

Guest, P.J. and Sam, W.M. (1998): Dermatophyte and superficial fungi In: Sam www jrlynch P. J. Principle and practice Dermatology. New York pp. 3-4.

Guy, P. K.; Ilze, V. and Alvaro, M. V. (2012): Eugenol—From the Remote Maluku Islands to the International Market Place: A Review of a Remarkable and Versatile Molecule. Molecules, 17:6953-6981.

Hanaa, M.A. (2003): Studies on ringworm in animals with special reference to methods of direct microscopy. M.D. Thesis, Faculty of Veterinary Medicine, Zagazig University.

Koneman, E. W.; Allen, S. D.; Janda, W. M.; Schrecken Berger, P. C. and Winn, J. W.C. (1992): Color atlas and text book of diagnostic Microbiology. 4th Ed. J. B. Lippincott Company, Philadelphia.

Kriger Van Rij, N.J.W. (1984): The yeasts: A taxonomic study .3rd Ed. Amsterdam, Elsevier.

Lo´ pez, P.; Sa´ nchez, C.; Batlle, R. and Nerı´n, C. (2005): Solid and vapour phase antimicrobial activities of six essential oils: susceptibility of selected food borne bacterial and fungal strains. J Agric Food Chem., 53: 6939–6946.

Nada, H. S. (2000): Mycological studies on dermatophytes among goats in Egypt 22"Veterinary Medical Journal Giza, 48 (3): 335-340.

Park, M.J.; Gwak, K.S.; Yang, I.; Choi, W.S.; Chang, J.W.; Jeung, E.B. and Choi, I.G. (2007): Antifungal activities of the essential oils in Syzygium aromaticum (L.) Merr. Et Perry and Leptospermum petersonii Bailey and their Constituents against various dermatophytes. The J.Microbiol. 45: 460-465.

Rippon, J.W. (1988): Medical Mycology, the pathogenic fungi and the pathogenic Actinomycetes, 3rd ed., W.B. Saunders Co., Philadelphia.

Samson, R.A. (1979): Compilation of the Aspergillia described since 1965. Studies in Mycology 18: 1-38. Study 18.

Shams-Ghahfarokhii, M.; mosleh-Tehrani, F.; Ranjbar-Bahadori, S. and Razzaghi A. M. (2009): An epidemiological survey on cattle ringworm in major dairy farms of Mashhad city, Eastern Iran, Iran. J. Microbiol. , 1:31–36.

Sudad, J.M. and Mohammed, K.F. (2011): A survey of dermatophyes isolated from cows and sheep in Iraq. The Iraqi. J. Vet. Med. Vol 35(2):40-45.

Yuanwu, J.Y.; Fanyang, T.; Wenchuan, L. and Yonglie, C. Q. (2009): Recent dermatophyte divergence revealed by comparative and phylogenic analysis of mitochondrial genomes. BMC genomics 10: 1471-2164.

Zuzarte, M.; Gonçalves, M. J.; Canhoto, J. and Salgueiro, L. (2011): Antidermatophytic activity of essential oils. Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Ed.) FORMATEX.

الملخص العربي

تقييم النشاط الفطري المضاد من زيت القرنفل على الفطريات الجلدية والفطريات المرتبطة الأخرى معمليا و علي حيوانات المزرعة

*إيمان الشحات عابدين و**ايمان محمود الدياسطي

*مدرس ·قسم البكتيريا والفطريات والمناعة - كلية الطب البيطرى، جامعة مدينة السادات

** قسم بحوث الفطريات - معهد بحوث صحة الحيوان بالدقي

إن حدوث الإلتهابات الفطرية وكذلك الإرتفاع الملحوظ في مقاومة بعض الأنواع من الفطريات لمضادات الفطريات المختلفة إضافة الى إرتفاع تكاليف العلاج، كل هذه العوامل جميعا قد حفزت الأبحاث لإيجاد أدوية بديلة طبيعية، مثل الزيوت الأساسية .فقد أجريت هذه الدراسة لتقييم النشاط الفطري المضاد لزيت القرنفل المستمدة من يوجينيا كاريوفيلاتا عند التخفيفات مختلفة (٠ ، ١٠، ٢٠، ٥٠، و ١٠٠) على كل من ترايكوفيتون منتاجروفيت ، ميكروسبوريم كانز ، الأسبرجيليس فلافس والكانديدا البيكانز.و أظهرت نتائج هذه الدراسة أن زيت القرنفل له نشاط مضاد ناجح ضد الأنواع الفطرية المختلفة في المختبر .أيضا عند تطبيق زيت القرنفل كعلاج موضعي في بعض الأبقار يعانون من إصابات جلدية كانت لها نتائج مفيدة وهامة.