Nanoparticles and their potential application as antimicrobials

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Emerging infectious diseases and the increase in incidence of drug resistance among pathogenic bacteria have made the search for new antimicrobials inevitable. In the current situation, one of the most promising and novel therapeutic agents are the nanoparticles. The unique physiochemical properties of the nanoparticles combined with the growth inhibitory capacity against microbes has led to the upsurge in the research on nanoparticles and their potential application as antimicrobials. From centuries metals such as silver have been used for treating burns and chronic wounds, and copper has been used to make water potable. It is quite evident that some of the metallic compounds possess antimicrobial property. Recently, the confluence of nanotechnology and biology has brought to fore metals in the form of nanoparticles as potential antimicrobial agents. Nanoparticles have unique and well defined physical and chemical properties which can be manipulated suitably for desired applications. Moreover, their potent antimicrobial efficacy due to the large surface area to volume ratio has provided them an edge over their chemical counterparts which are facing the problems of drug resistance. This review focuses on the properties of different types of metallic nanoparticles such as copper, aluminium, gold, silver, magnesium, zinc and titanium nanoparticles. The mechanism of action of nanoparticles as bactericidal, antifungal and antiviral agents will be highlighted in this study. The potential application of nanoparticles will be also reviewed. The application of nanoparticles as antimicrobials is gaining relevance in prophylaxis and therapeutics, in medical devices, food industry and textile fabrics. The problems related to toxicity of nanoparticles will be addressed in brief.

Keywords: Nanoparticles, antimicrobials agents, therapeutics, drug resistance.

1. Introduction

The emerging infectious diseases and the development of drug resistance in the pathogenic bacteria and fungi at an alarming rate is a matter of serious concern. Despite the increased knowledge of microbial pathogenesis and application of modern therapeutics, the morbidity and mortality associated with the microbial infections still remains high [1]. Therefore, there is a pressing demand to discover novel strategies and identify new antimicrobial agents from natural and inorganic substances to develop the next generation of drugs or agents to control microbial infections.

Prior to the extensive use of chemotherapeutics in modern health care system, inorganic antimicrobials such as silver and copper were used since ancient times to treat microbial infections [2]. In the recent times, the advances in the field of nanosciences and nanotechnology has brought to fore the nanosized inorganic and organic particles which are finding increasing applications as amendments in industrial, medicine and therapeutics, synthetic textiles and food packaging products [3].

Nanoparticles usually ranging in dimension from 1-100 nanometers (nm) have properties unique from their bulk equivalent. With the decrease in the dimensions of the materials to the atomic level, their properties change. The nanoparticles possess unique physico-chemical, optical and biological properties which can be manipulated suitably for desired applications [4]. Moreover, as the biological processes also occur at the nanoscale and due to their amenability to biological functionalization, the nanoparticles are finding important applications in the field of medicine [5]. The nanoparticles are broadly grouped into organic and inorganic nanoparticles. The latter have gained significant importance due to their ability to withstand adverse processing conditions [6]. Currently, the metallic nanoparticles are thoroughly being explored and extensively investigated as potential antimicrobials. The antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms. The small size and the high surface to volume ratio *i.e.,* large surface area of the nanoparticles enhances their interaction with the microbes to carry out a broad range of probable antimicrobial activities. Metal nanoparticles with antimicrobial activity when embedded and coated on to surfaces can find immense applications in water treatment, synthetic textiles, biomedical and surgical devices, food processing and packaging [7]. Moreover, the composites prepared using metal nanoparticles and polymers can find better utilization due to the enhanced antimicrobial activity.

In this review, we focus on the role of various metallic nanoparticles as potential antimicrobials and the possible mechanism of their inhibitory actions. The increasing application of nanoparticles as antimicrobials in industries, medicine, cosmetics, textiles and food packaging which requires the assessment of the toxicity and risks associated with these particles will also be reviewed.

used in waste water treatment. It is considered non-toxic and has been approved by the American Food and Drug Administration (FDA) for use in human food, drugs, cosmetics and food contact materials [134]. Nowadays titanium dioxide nanoparticles are finding wide application as a self-cleaning and self-disinfecting material for surface coatings in many applications and in food industries for disinfecting equipments [135].

Zinc oxide (ZnO) and copper oxide nanomaterials due to their antimicrobial property are being incorporated into a variety of medical and skin coatings. ZnO nanoparticles are used in the wallpapers in hospitals as antimicrobials. ZnO powder is an active ingredient for dermatological applications in creams, lotions and ointments on account of its antibacterial properties [90].

5. Toxicity of nanoparticles

It is evident from the above studies that metal nanoparticles due to their unique physico-chemical and biological properties have far reaching industrial and medical applications. But there is a dearth of knowledge about the effect of the prolonged exposures to nanoparticle on human health and environment. The implication of nanoparticles on health and environment needs to be assessed completely before their large-scale production and application in various fields [136-138]. Studies conducted on the NP-induced toxicity have revealed that the metal-based nanoparticles can affect the biological behavior at the organ, tissue, cellular, subcellular, and protein levels. The size of the nanoparticles is small and these can easily access the skin, lungs, and brain and cause adverse affects [139-142]. For example, exposure of metal based nanoparticles to human lung epithelical cells leads to the generation of reactive oxygen species and result in oxidative stress and cellular damage. The toxicity of nanoparticles can be assessed by a number of *in vitro* and *in vivo* studies. For example, the toxic effects of nanoparticles can be carried out using zebrafish as a model due to its fast development and transparent body structure. Cell culture based assays are used as a pre-screening tool to understand the biological effects of nanoparticles. However, along with the *in vitro* assays it is necessary to confirm the *in vivo* biological activities of nanoparticles in animal models to study the suitability of their application [143-144]. There is an increasing use of microarray and real-time reverse transcription polymerase chain reaction for gene expression analyses as these are very sensitive and reliable methods to assess the changes in the expression levels of thousands of genes simultaneously under a wide variety of experimental conditions [145].

It is evident that metal based nanoparticles due to their biological and physiochemical properties are promising as antimicrobials and therapeutic agents. They can be used to address a number of challenges in the field of nanomedicine. But it must be remembered that they can also possibly cause adverse biological effects at the cellular and subcellular levels. Therefore, after the cytotoxicity and clinical studies the nanoparticles can find immense application as antimicrobials in the consumer and industrial products.

Table 1 Antimicrobial activity of metal based nanoparticles.

Properties	Mechanism of action	Examples of nanoparticles
Antibacterial	Interaction with phosphorus moieties in DNA, resulting in inactivation of DNA replication. Reacts with sulfur-containing proteins, leading to the inhibition of enzyme functions.	Silver nanoparticles have inhibitory activity against *E. coli, B. subtilis*, *S. aureus*, methicillin-resistant coagulase-negative staphylococci, vancomycin-resistant *Enterococcus faecium*, ESBL-positive *K. pneumonia*, *S. typhi, Vibri cholera* [29, 30, 32, 45, 52]. Gold nanoparticles have antibacterial activity against MRSA, VRE, *E. coli, Pseudomonas aeruginosa* [65-66, 72]. MgO nanoparticles have excellent against *E. coli, B.subtilis, B.megaterium.*[80,81] CuO strongly inhibits *B.subtilis* [86-88]. Aluminium oxide nanoparticles have growth inhibitory effect on *E. coli* [91]. TiO_2 nanoparticls are effective in killing *E. coli*, *S.aureus, Listeria monocytogenes* [99-102, 107,108]. ZnO nanoparticles inhibit food-borne bacteria *E. coli* 0157:H7,*B.subtilis, Pseudomonas fluorescens, L. monocytogenes, Salmonella enteritidis, S. aureus, S. typhimurium* [113-115].
Antiviral	Blocking of viral attachment to cell surface.	Gold nanoparticles have anti-HIV activity and inhibit several strains of influenza virus [119-120]. Silver nanoparticles inhibit HIV-1, Influenza virus, Herpes Simplex virus, Respiratory syncytial virus, Monkey pox virus [118,120, 125-128].
Antifungal	Disruption of cell membrane.	Silver nanoparticles have fungicidal and fungistatic effects on the dermatophytes *Trichophyton mentagrophytes* and *Candida species* [130-133].

References

[1] Kolar M, Urbanek K, Latal T. Antibiotic selective pressure and development of bacterial resistance. *Int J Antimicrob Ag*. 2001;17:357–363.
[2] Moghimi SM. Nanomedicine: prospective diagnostic and therapeutic potential. *Asia Pacific Biotech News*. 2005;9:1072-1077.
[3] Gajjar P, Pettee B, Britt DW, Huang W, Johnson WP, Anderson J. Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, *Pseudomonas putida* KT2440. *Journal of Biological Engineering*. 2009;3:9-22.
[4] Feynman R. There's plenty of room at the bottom. *Science*. 1991;254:1300-1301.
[5] Parak WJ, Gerion D, Pellegrino T, Zanchet D, Micheel C, Williams CS, Boudreau R, Le Gros MA, Larabell CA, Alivisatos AP: Biological applications of colloidal nanocrystals. *Nanotechnology*. 2003;14:15-27.
[6] Whitesides GM. The 'right' size in Nanobiotechnology. *Nature Biotechnology*. 2003; 21:1161-1165.
[7] Gutierrez FM, Olive PL, Banuelos A, Orrantia E, Nino N, Sanchez EM, Ruiz F, Bach H, Gay YA. Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. *Nanomedicine*. 2010;6:681-688.
[8] Eustis S, El-Sayed MA. Why gold nanoparticles are more precious than pretty gold: Noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. *Chemical Society Reviews*. 2006; 35:209-217.
[9] Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials*. 2005; 26:3995-4021.
[10] Jamieson T, Bakhshi R, Petrova D, Pocock R, Imani M, Seifalian AM. Biological applications of quantum dots. *Biomaterials*.2007; 28: 4717-4732.
[11] Samia ACS, Dayal S, Burda C. Quantum dot-based energy transfer: Perspectives and potential for applications in photodynamic therapy. *Photochemistry and Photobiology*. 2006; 82:617-625.
[12] Dunn K, Edwards-Jones V. The role of Acticoat with nanocrystalline silver in the management of burns. *Burns*. 2004; 30:1–9.
[13] Russell AD, Hugo WB. Antimicrobial activity and action of silver. *Prog. Med. Chem*. 1994;31:351-370.
[14] Ip M, Lui SL, Poon VK, Lung I, Burd A. Antimicrobial activities of silver dressings: an in vitro comparison. *J Med Microbiol*. 2006; 55:59–63.
[15] Petica A, Gavriliu S, Lungu M, Buruntea N, Panzaru C. Colloidal silver solutions with antimicrobial properties. *Mater Sci Eng*. 2008;152:22–27.
[16] Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv*. 2009; 27:76–83.
[17] Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH. Antimicrobial effects of silver nanoparticles. *Nanomedicine*. 2007;3:95–101.
[18] Li P, Li J, Wu C, Wu Q, Li J. Synergistic antibacterial effects of *β*-lactam antibiotic combined with silver nanoparticles. *Nanotechnology*. 2005;16:1912–1917.
[19] Ruparelia JP, Duttagupta SP, Chatterjee AK, Mukherji SM. A comparative study on disinfection potential of nanosilver and nanonickel. Technical poster, Proceedings of the 9th Annual Conference of the Indian Environmental Association (Envirovision-2006), entitled ‘‘Advances in Environmental Management and Technology”, Goa, India, September 21–23, 2006.
[20] Gupta A, Silver S. Silver as a biocide: will resistance become a problem? *Nat Biotechnol*. 1998;16:888-890.
[21] Matsumura Y, Yoshikata K, Kunisaki S, Tsuchido T. Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Appl Environ Microbiol*. 2003; 69:4278-4281.
[22] Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Yacaman MJ. The bactericidal effect of silver nanoparticles. *Nanotechnology*. 2005;16:2346–2353.
[23] Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology*. 2007;18:1–9.
[24] Bard AJ, Holt KB. Interaction of Silver(I) Ions with the Respiratory Chain of *Escherichia coli*: An Electrochemical and Scanning Electrochemical Microscopy Study of the Antimicrobial Mechanism of Micromolar Ag^+. *Biochemistry*. 2005; 44:13214-13223.
[25] Schreurs WJ, Rosenberg H. Effect of silver ions on transport and retention of phosphate by *Escherichia coli*. *J. Bacteriol.* 1982, 152:7-13.
[26] Bragg PD, Rainnie DJ. The effect of silver ions on the respiratory chain of *Escherichia coli*. *Can. J. Microbiol.* 1974; 228: 883-889.
[27] Semeykina AL, Skulachev VP. Submicromolar Ag^+ increases passive Na^+ permeability and inhibits the respiration-supported formation of Na+ gradient in *Bacillus* FTU vesicles. *FEBS Lett.* 1990; 269: 69-72.
[28] Dibrov P, Dzioba J, Gosink KK, Hase CC. Chemiosmotic mechanism of antimicrobial activity of Ag^+ in *Vibrio cholerae*. *Antimicrob. Agents Chemother.* 2002; 46: 2668-2670.
[29] Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mater Res*. 2000;52:662-668.
[30] Jung WK, Koo HC, Kim KW, Shin S, Kim SH, Park YH. Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. *Appl. Environ. Microbiol.* 2008; 74:2171-2178.
[31] Gogoi SK, Gopinath P, Paul A, Ramesh A, Ghosh SS, Chattopadhyay A. Green fluorescent protein-expressing *Escherichia coli* as a model system for investigating the antimicrobial activities of silver nanoparticles. *Langmuir* 2006;22:9322–9328.
[32] Alexander S, Klabunde K J, George MR, Christopher MS. Biocidal activity of nanocrystalline silver powders and particles. *Langmuir* 2008;24:7457–7464.
[33] Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. *Free Radic Biol Med*. 1995;18:321–36.

[66] Perni S. The antimicrobial properties of light-activated polymers containing methylene blue and gold nanoparticles. *Biomaterials*. 2009;30:89–93.
[67] Zharov VP. Photothermal nanotherapeutics and nanodiagnostics for selective killing of bacteria targeted with gold nanoparticles. *Biophys. J*. 2006;90:619–627.
[68] Norman RS. Targeted photothermal lysis of the pathogenic bacteria, *Pseudomonas aeruginosa*, with gold nanorods. *Nano Lett*. 2008;8:302–306.
[69] Simon-Deckers A. Impact of gold nanoparticles combined to X-Ray irradiation on bacteria. *Gold Bull.* 2008;41:187–193.
[70] Huang WC. Functional gold nanoparticles as photothermal agents for selective-killing of pathogenic bacteria. *Nanomedicine*. 2007;2:777–787.
[71] Burygin, G.L. On the enhanced antibacterial activity of antibiotics mixed with gold nanoparticles. *Nanoscale Res. Lett.* 2009;4:794–801.
[72] Gu, H, Ho P L , Edmond Tong, Ling Wang, Bing Xu. Presenting vancomycin on nanoparticles to enhance antimicrobial activities. *Nano Lett*. 2003;3:1261–1263.
[73] Grace NA, Pandian K. Antibacterial efficacy of aminoglycosidic antibiotics protected gold nanoparticles—A brief study. *Colloids Surf. A Physicochem. Eng. Asp*. 2007; 297: 63–70.
[74] Saha, B. In vitro structural and functional evaluation of gold nanoparticles conjugated antibiotics. *Nanoscale Res. Lett.* 2007; 2:614–622.
[75] Rai A, Prabhune A, Perry CC. Antibiotic mediated synthesis of gold nanoparticles with potent antimicrobial activity and their application in antimicrobial coatings. *J. Mater. Chem*. 2010;20:6789-6798.
[76] Das SK. Gold nanoparticles: microbial synthesis and application in water hygiene management. *Langmuir*. 2009; 25: 8192–8199.
[77] Stoimenov PK. Metal oxide nanoparticles as bactericidal agents. *Langmuir*. 2002;18:6679-86.
[78] Klabunde KJ, Stark J, Koper O, Mohs C, Park D, Decker S, Jiang Y, Lagadic I, Zhang D. Nanocrystals as Stoichiometric Reagents with Unique Surface Chemistry *J. Phys. Chem.* 1996;100:12142-12153.
[79] Huang L. Controllable preparation of Nano-MgO and investigation of its bactericidal properties. *J Inorg Biochem*. 2005; 99:986-993.
[80] Richards R, Li W, Decker S, Davidson C, Koper O, Zaikovski V, Volodin A, Rieker T, Klabunde K. Consolidation of Metal Oxide Nanocrystals. Reactive Pellets with Controllable Pore Structure That Represent a New Family of Porous, Inorganic Materials *J. Am. Chem. Soc*. 2000;122:4921-4925.
[81] Koper O, Klabunde J, Marchin G, Klabunde KJ, Stoimenov P, Bohra L. Nanoscale Powders and Formulations with Biocidal Activity Toward Spores and Vegetative Cells of *Bacillus* Species, Viruses, and Toxins. *Current Microbiology*. 2002;44:49-55.
[82] Cava RJ. Structural chemistry and the local charge picture of copper oxide superconductors. *Science*. 1990;247:656–62.
[83] Tranquada JM, Sternlieb BJ, Axe JD, Nakamura Y, Uchida S. Evidence for stripe correlations of spins and holes in copper oxide superconductors. *Nature*. 1995;375:561-565.
[84] Kwak K, Kim C. Viscosity and thermal conductivity of copper oxide nanofluid dispersed in ethylene glycol. *Korea–Australia Rheol J*. 2005;17:35–40.
[85] Xu JF, JiW, Shen ZX, Tang SH, Ye XR, Jia DZ, Xin X Q Preparation and characterization of CuO nanocrystals. *J Solid State Chem*. 1999;147:516–519.
[86] Ren G, Hu D, Cheng EWC, Vargas-Reus MA, Reip P, Allaker RP. Characterisation of copper oxide nanoparticles for antimicrobial applications. *International Journal of Antimicrobial Agents*. 2009;33:587–590.
[87] Cioffi N, Torsi L, Ditaranto N, Tantillo G, Ghibelli L, Sabbatini L. Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. *Chem Mater*. 2005;17:5255–5262.
[88] Rupareli JP, Chatterjee AK, Duttagupta SP, Mukherji S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomaterialia*. 2008;4:707-771.
[89] Sadiq M, Chowdhury B, Chandrasekaran N, Mukherjee A. Antimicrobial sensitivity of *Escherichia coli* to alumina nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 2009;5:282–286.
[90] Martínez Flores E, Negrete J, Torres Villaseñor G. Structure and properties of Zn-Al-Cu alloy reinforced with alumina particles. *Mater Des*. 2003;24:281-286.
[91] Li B, Logan BE. Bacterial adhesion to glass and metal oxide surfaces. *Colloids Surf B*. 2004;36:81-90.
[92] Mohammad G, Mishra VK, Pandey HP. Antioxidant properties of some nanoparticles may enhance wound healing in T2DM patient. *Digest J Nanomater Biostruct*. 2008;3:159-62.
[93] Chorianopoulos NG, Tsoukleris DS, Panagou EZ, Falaras P, Nychas G-JE. Use of titanium dioxide (TiO_2) photocatalysts as alternative means for *Listeria monocytogenes* biofilm disinfection in food processing. *Food Microbiology*. 2011;28:164-170.
[94] Fujishima A, Honda K. Electrochemical photocatalysis of water at semiconductor electrode. *Nature*. 1972;238:27-38.
[95] Fujishima A, Hashimoto K, Watanabe T. *TiO_2 Photocatalysis*: *Fundamentals and Applications*. Japan. BKC Inc., 1992.
[96] Wei C, Lin WY, Zainal Z, Williams NE, Zhu K, Kruzic AP, Smith RL, Rajeshwar K. Bactericidal activity of TiO_2 photocatalyst in aqueous media: toward a solar-assisted water disinfection system. *Environ. Sci. Technol.* 1994;28:934-938.
[97] Pham HN, McDowell T, Wikins E. Photocatalytically-mediated disinfection of water using TiO_2 as a catalyst and spore-forming *Bacillus pumilus* as a model. *J. Environ. Sci. Health A*. 1995;30:627-636.
[98] Ireland JC, Klostermann P, Rice EW, Clark RM. Inactivation of *Escherichia coli* by titanium dioxide photocatalytic oxidation. *Appl. Environ. Microbiol*. 1993;59:1668-1670.
[99] Matsunaga T, Tomada R, Nakajima T, Wake H. Photochemical sterilization of microbial cells by semiconductor powders. *FEMS Microbiol. Lett.* 1998;29:211-214.
[100] Matsunga T, Tomoda R, Nakajima T, Nakamura N, Kmine T. Continuous-sterilization system that uses photosemiconductor powders. *Appl.Environ.Microbiol.* 1988;54:1330-1333.

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Antifungal activity of ZnO nanoparticles—the role of ROS mediated cell injury

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Abstract

Metal oxide nanoparticles have marked antibacterial activity. The toxic effect of these nanoparticles, such as those comprised of ZnO, has been found to occur due to an interaction of the nanoparticle surface with water, and to increase with a decrease in particle size. In the present study, we tested the ability of ZnO nanoparticles to affect the viability of the pathogenic yeast, *Candida albicans* (*C. albicans*). A concentration-dependent effect of ZnO on the viability of *C. albicans* was observed. The minimal fungicidal concentration of ZnO was found to be 0.1 mg ml^{-1} ZnO; this concentration caused an inhibition of over 95% in the growth of *C. albicans*. ZnO nanoparticles also inhibited the growth of *C. albicans* when it was added at the logarithmic phase of growth. Addition of histidine (a quencher of hydroxyl radicals and singlet oxygen) caused reduction in the effect of ZnO on *C. albicans* depending on its concentration. An almost complete elimination of the antimycotic effect was achieved following addition of 5 mM of histidine. Exciting the ZnO by visible light increased the yeast cell death. The effects of histidine suggest the involvement of reactive oxygen species, including hydroxyl radicals and singlet oxygen, in cell death. In light of the above results it appears that metal oxide nanoparticles may provide a novel family of fungicidal compounds.

1. Introduction

Nanosized metal oxide particles, are receiving increasing attention for a large variety of applications. Titanium dioxide (TiO_2) and zinc oxide (ZnO) nanoparticles (NPs) are included in toothpaste, beauty products, sunscreens, and textiles. Ceramic nano-powders of metal oxides such as ZnO have been found to exhibit marked antibacterial activity [1–5]. The use of metal oxides as antimicrobial agents has the advantage of improved safety and stability, as compared to organic antimicrobial agents [6]. Although there are numerous studies regarding the antibacterial effect of ZnO, little is known about the mechanism underlying their bactericidal effect. Sawai *et al* [7–9] proposed that hydrogen peroxide generation is responsible for the bacterial destruction, whereas Stoimenov *et al* [10] suggested that the binding of ZnO particles to the bacteria's surface due to electrostatic forces kills the pathogens.

Although metal oxide NPs have been widely studied for their antibacterial properties, there are almost no studies regarding their antifungal activities. For example, Sawai *et al* found that ZnO powder (at the microscale) exhibits a very weak antifungal activity against *C. albicans*. Though growth inhibition was observed, conductivity change was observed only at concentration above 100 mg ml^{-1}. Consequently ZnO powder was found to be unsuitable for the control of fungi [11]. Gomes *et al* [12] tested ZnO with calcium hydroxide plus 2% chlorhexidine to treat microbial infections at the external tooth root surface but had no success. More promising results were shown by Fang *et al* [13] where ZnO in a 'whisker' form was found to inhibit the growth of *C. albicans*. There are relatively few agents that can be used to treat fungal

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Full Length Research Paper

The prevalence of cattle ringworm in native dairy farms of Sarab city (East Azarbayjan province), Iran

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Ringworm is a fungal and zoonotic infectious disease, caused by different species of dermatophytes. Lesions of ringworm are usually found on the head, muzzle, ears, neck, and particularly, around the eyes of the infected animals. This study was conducted to determine the prevalence of cattle ringworm in native farms of Sarab city in Iran. A total number of 1150 cattle in native dairy farms of Sarab city were examined in this study from July 2007 till June 2008. The animals were classified into two age groups of less than 2 years and more than 2 years. Each animal was thoroughly examined for skin ringworm lesions, then, microscopic and culture examinations were carried out on samples obtained from ringworm lesions of infected animals. According to the results of this study among 1150 cattle, 188 of them (16.34%) were clinically positive for skin ringworm lesions. After microscopic and culture examinations, 150 animals (13.04%) were confirmed for dermatophyte infections. *Trichophyton verrucosum* was isolated in 100% of positive samples. The prevalence of ringworm among cattle less than 2 years and more than 2 years were 15 and 9% respectively, which showed significant difference between them. This study is the first research on the prevalence of cattle ringworm in Sarab city which indicated the relatively high prevalence of the disease and revealed the dominant role of *T. verrucosom* in cattle ringworm.

Key words: Ringworm, dermatophyte, cattle, Sarab.

INTRODUCTION

Ringworm is a fungal and zoonotic infectious disease caused by different species of dermatophytes. These microorganisms are a group of closely related fungi which utilize keratin and tend to be confined to the superficial integument, including skin, nails, claws and hair of both animals and humans (Aala et al., 2010). Dermatophytes can be divided into three groups of anthropophilic, zoophilic and geophilic, depending on their natural habits and host preferences (Dehghan et al., 2009). The geophilic dermatophytes inhabit the soil, and can exist there as free-living saprophytes. *Microsporum gypseum* and *M. nanum* are examples of geophilic dermatophytes. The zoophilic dermatophytes are primarily parasites of animals, although they can cause infection in humans. Zoophilict species of dermatophytes including, *M. canis*, *Trichophyton Verrucosum*, and *T. mentagrophytes* are associated with dermatophytosis in wild and domestic animals. These species are the most common dermatophytes as causative agents of tinea in rural areas in Iran (Mahmoudabadi, 2010). Humans are the main host for anthropophilic dermatophytes. Most of the dermatophytes causing lesion in animals, are also capable of producing ringworm in humans (Quinn et al., 1994). Cattle ringworm has worldwide distribution and has been considered a major public and veterinary health problem in the world (Al-ani et al., 2002). The disease is responsible for great economic losses due to skin injuries and many casualties in animal products (wool, meat, etc.). Cattle of all ages may be affected but the disease mainly occurs in young animals (Shams et al., 2009). Ringworm is more commonly seen during the winter months in stabled animals but may occur at any time. *T. verrucosum* is the most frequent cause of ringworm in cattle however, *T. mentagrophytes* is occasionally isolated. The fungus is resistant and may survive for a long time in dry scales shed by the infected animals (Quinn et al., 1994). Infection is transmitted readily from animal to animal and from animal to man by direct or indirect contact. The human infection with zoophilic dermatophytes has been reported from different

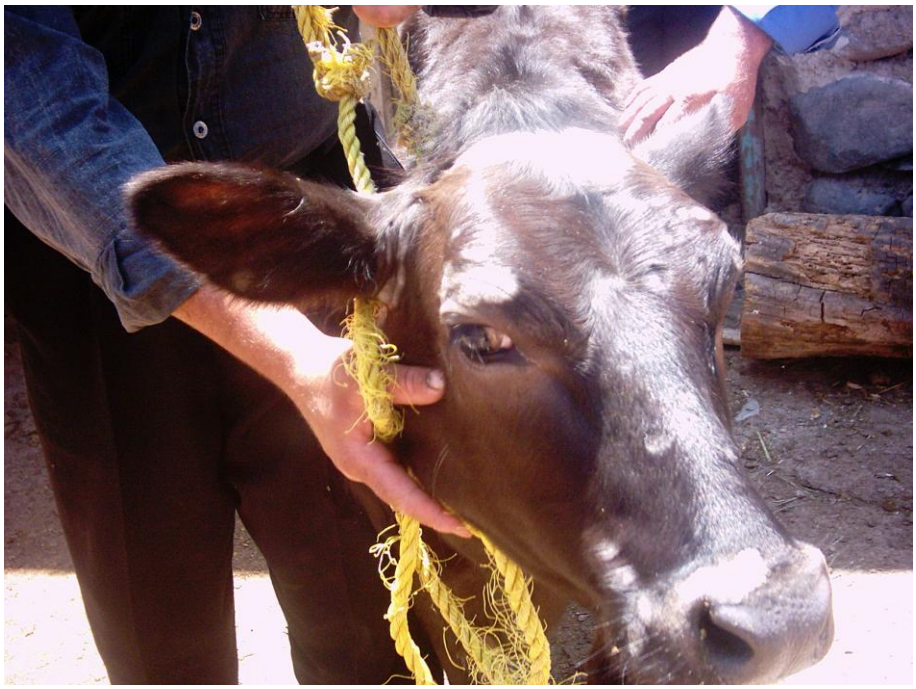

Figure 1. 15-months-old calf from dairy farm of Sarab city with lesions of ringworm around the eyes and muzzle (infected by *T. verrucosum*).

provinces in Iran (Khosravi et al., 1994; Mahmoudabad, 2010). However, very limited studies on cattle ringworm have been published in Iran and the disease is considered to be common in most dairy farms of this country (Shams et al., 2009; Aghamirian et al., 2009). In the present study, the prevalence of cattle ringworm in native dairy farms of Sarab city, which is located in East Azarbayjan province in Iran, were determined by microscopic and culture examinations. With this research, it may be possible to reach effective program for preventing the disease and eliminating the causative fungi.

MATERIALS AND METHODS

A total number of 1150 cattle from 50 different dairy farms which were selected randomly in Sarab city were examined in this study from July 2007 till June 2008. The animals were classified into two age groups (less than 2 years and more than 2 years) and subjected for clinical and laboratory examinations. The skin of each animal was thoroughly examined for evidence of ringworm lesions including hair loss and crusting. Clinical lesions in affected animals were first rubbed with a cotton swab impregnated with 70% ethyl alcohol to remove surface adhering microorganisms and then, skin scales were collected by scraping of the lesion using a sterile scalpel. The skin scrapings were collected into sterile Petri dish and transferred to laboratory for culture and microscopic examinations. For each sample, the age of animal, date of sampling and the name of dairy farm were also recorded. Microscopic examination was performed using KOH wet mount method described by Queen et al. (1994). According to this method, 1 to 2 drops of 20% KOH (potassium hydroxide) were placed on a microscopic slide and a small amount of the specimen was added and then, the slide was gently passed through a low flame and covered by a cover slip. After 2 h, the specimen was examined for the presence of arthrospores and hyphae under a light microscope. For isolation and identification of pathogenic fungi, a portion of each sample was inoculated on sabouraud dextrose agar (Merck), supplied with cyclohexamide and chloramphenicol, and then, incubated at, 28°C for, 2 to 6 weeks. The ringworm dermotophytes were identified by considerations of the rate of growth, texture and pigmentation of the obverse and reverse side of the colony and microscopic features of macroconidium which are pencil shaped and divided by septa into 3 to 8 cells in *Trichophyton* spp. Moreover, *T. verrucosum* produce chains, forming chlamydospores, which are characteristic of this species.

RESULTS

Out of 1150 animals examined from 50 dairy farms of Sarab city, 188 of them (16.34%) showed clinical signs of ringworm (circumscribed, circular, grayish and crusty lesions) as demonstrated in Figure 1. Most of the lesions were found on the neck, head and around the eye of the affected animals. According to Table 1, out of 750 cattle examined in age group of less than 2 years, 145 (19.33%) of them were clinically positive for ringworm lesions. While in the age group of more than 2 years, out of 400 animals, 43 (10.75%) of them showed clinical signs of the disease. The results of direct microscopic examinations showed that 15.2% of cattle in age group of less than 2 years and 9% of those in age group of more than 2 years were positive for dermatophytosis. In

Table 1. The prevalence of cattle ringworm in 2 age groups of cattle in native dairy farms of Sarab city-Iran.

Age group	No. of examined animals	No. of positive case (clinical signs)		No. of positive case (DME)	
		Number	%	Number	%
Less than 2 years	750	145	19.33	114	15.2
More than 2 years	400	43	10.75	36	9
Total	1150	188	16.34	150	13.04

DME= direct microscopic examination.

overall, out of 1150 animals examined in this study, 150 (13.04%) animals had positive results in direct microscopic examinations. All of the KOH positive samples were culture positive on sabouraud dextrose agar. According to the results of microscopic examinations, after growing the pathogenic fungi on agar media, *T. verrucosum* was isolated from all culture positive samples (100%).

DISCUSSION

Ringworm is a contagious fungal infection of the skin and superficial integument, which is worldwide in distribution. In this study, 13.04% of examined cattle were positive for dermatophyte in direct microscopic examinations. Based on culture examination, *T. verrucosum* was found in all of the positive DME cases. The results of this research showed that, *T. verrucosum* was the most dominant dermatophyte caused cattle ringworm in native dairy farms of Sarab city. As Table 1 shows, there was significant difference between the prevalence of ringworm among cattle less than 2 years (15.2%) and the age group of more than 2 years (9%). The lower prevalence rate of ringworm infection in adults may be due to development of immunity system by increasing age. Several researchers have reported the occurrence of dermatophytosis in different areas in Iran. According to the research of Shams et al. (2009) which was carried out in dairy farms of Mashhad city, *T. verrucosum* and T*. mentagrophytes* were isolated from the skin lesions of affected animals where *T. verrucosum* with frequency of 99% was the most frequent dermatophyte. In the present study, no *T. mentagrophytes* was isolated, which showed that the prevalence of this dermatophyte may differ in different geographic area.

Another study which was carried out by Aghamirian et al. (2009), *T. verrucosum* was reported as an exclusive fungus isolated from cattle in Iran. Authors in different countries have reported different prevalence rate of *T. verrucosum* among cattle herds. According to the research of Papini et al. (2009) which was done in 20 farms in Italy, *T. verrucosum* was the most dominant fungi species which isolated. Al-ani (2002) isolated *Trichophyton* spp. from 69.01% of infected animals in Jordan. He also reported that, *T. verrucosum* was the most frequent fungi species which identified. All of the above studies agree with our results in the present study and revealed the dominant role of *T. verrucosum* in cattle ringworm. Zoophilic dermatophytosis is a major public health problem. *T. verrucosum* is able to survive in skin lesions of the infected animals for several months and can be easily transmitted to human. Many researches have reported the transmission of dermatophytes among animals and humans (Aghamirian et al., 2009; Ming et al., 2006; Ameh et al., 2004).

The human infection with *T. verrucosum* has been reported in different regions in Iran. According to the research of Chadeganipour et al. (19970 which was carried out in Isfahan city in Iran, T. *verrucosum* was reported as the most frequent dermatophyte isolated from patients. They also found a relationship between the spread of dermatophytosis and livestock infected with dermatophytes. The same results have been reported by several authors in different provinces in Iran (Dehghan et al., 2009; Khosravi et al., 1994; Sepahvand et al., 2009; Bassiri-Jahromi et al., 2009; Falahati et al., 2003; Pakshir et al., 2006). Finally, this study showed the importance of cattle ringworm in Sarab city and revealed the dominant role of *T. verrucosum* in cattle dermatophytosis. Therefore, routine and regular inspection of animals, isolation of infected animals, disinfection of contaminated stables and other effective control program should be highly recommended.

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REFERENCES

Aala F, Yusuf UK, Khodavandi A, Jamal F (2010). In vitro antifungal activity of allicin alone and in combination with two medication against six dermatophytic fungi. Afr. J. Microbiol. Res., 4(5): 380-385.

Aghamirian MR, Ghiasian SA (2009). Dermatophytes as cause of epizoonoses in dairy cattle and humans in Iran: epidemiological and clinical aspects. *Mycoses*, no. doi: 10.1111/j.1439-0507.2009.011832.x.

Inorganic nano mental oxides used as anti-microorganism agents for pathogen control

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Abstract: Recently, inorganic antimicrobial agents are being increasingly for control of microorganism in various areas, especially in dentistry. Particle size of mental oxides had an impact on their anti-microorganism activity. There is growing interest in nanoscale particles since materials exhibit unique properties which offer considerably from those of macroscopic materials. Inorganic nano mental oxides including MgO, ZnO and CaO have been shown anti-microorganism activity. This chapter highlighted inorganic nano mental oxides used as anti-microorganism agents for pathogen control. Preparation of MgO, ZnO and CaO nanoparticles, effect of nanoparticles on anti-microorganism activity and antimicrobial mechanism of nanoparticles were discussed. Study of MgO, ZnO and CaO nanoparticles as anti-microorganism agents in our group was also introduced.

Keywords nano metal oxdies; anti-microorganism; pathogen control

Introduction

Fruit juices have a low pH that inhibits most bacteria, which are mainly acid tolerant bacteria such as Gram positive lactobacilli and leuconostocs. The lactobacilli are important in the spoilage of fruit juices. Some strains are quite acid tolerant and can metabolize citric and malic acids. This reduces the acidity, resulting in a bland flavour and loss in astringency. The production of diacetyl, hydroxyl butanone and dihydroxybutane by these organisms results in buttermilk like flavour in fruit juice. *Leuconostoc mesenteroides* produces dextranes, conferring an unpleasant slimy texture to juices [1]. *Alicyclobacillus* spp. is increasing being acknowledged as causative agents of spoilage of fruit juices. Major characteristics of *Alicycobacillus* spp. are their ability to survive commercial pasteurization processes, and produce off-flavors in juices [2]. Yeasts and fungi also can contaminate and ferment fruit juices. The most frequently occurring yeasts are: *Saccharomyces cerevisiae*, *Candida stellata* and *Zygosaccharomyces rouxii* [3]. If the sugar concentration is high, the osmophilic yeasts, *Saccharomyces rouxii* et al *S. mellis* can ferment the sugars to alcohol, and *Acetobacter* can convert the alcohol to acetic acid giving the fruit juice the vinegar flavour. Species of fungi identified in the spoilage of fruit juices include *Penicillium*, *Aspergillus*, *Paecilomyces* et al *Fusarium*. It has been shown that some anamorphic fungi (*Paecylomyces variotii* et al *Fusarium sp*.) could cause spoilage of pasteurized fruit juices [4]. Fungal biomass suspensions of *P. variotii* strains were able to survive higher temperatures for a longer time than spores.

Although potential spoilage organisms in fruit juices are heat sensitive and can be eliminated by pasteurization process, the juice industry faces potential economic losses caused by microbial production of off-flavors in pasteurized fruit juices. Heat resistance of thermophilic and acidophilic charcteristics of *Alicyclobacillus* species and anamorphic fungi, *Paecilomyces variotti* and *Fusarium* sp., enable them to survive current pasteurization process. In addition, post contamination of pasteurized juices can limit their shelf life. To guarantee longer shelf life and reduce losses, pasteurized juices must be aseptically packaged, or stored and distributed under refrigeration conditions. Because of high costs associated with aseptic processing as well as refrigeration, processors use chemical preservatives such as benzoate and sorbate to control spoilage organisms in pasteurized juices. Addition of chemical agents is an effective low-cost solution, which enables the processor to distribute price competitive fruit juices and drinks under ambient conditions. Since consumers are reticent towards chemical additives, there is great interest in the use of nature products as antimicrobial compounds in fruit juices. A variety of substances have been investigated in an effort to replace benzoate and sorbate: bacteriocins, lysozyme, propolis, chitosan, polylysine, isothiocyanates isolated from white mustard seeds, limoid glucosides, flavonoids, vanillin et calcium lactate [5-10]. Although these substances are effective antimicrobial agents, their use in juices is limited because they either alter the sensory attributes of fruit juices or they are costly. Juice processors need alternative substances that are functionally effective without altering organolepic quality of the juices, and which also price competitive. Heath beneficial agents would be all the more attractive for both consumers and the processors.

In recent years, inorganic antimicrobial agents are being increasingly for control of microorganisms in various areas, especially in dentistry. The key advantages of inorganic agents are improved safety and stability compared with organic antimicrobial agents [11]. The antibacterial activity of metal oxides including MgO and ZnO was shown by a Japanese group [12]. There is growing interest in nanoscale particles since materials exhibit unique properties which differ considerably from those of macroscopic materials. The finding that nanosized silver exhibits a strong antimicrobial activity has inspired investigations on the antimicrobial activity of nanoscale metal oxides, in particular MgO. From the

The alkali metal oxides CaO and MgO exhibited somewhat similar trends in their lethal effects against lactic bacteria. *L. helveticus* was more susceptible and *L. plantarum* as well as *L. mesenteroides* were more resistant when exposed to them for 6 h. However, the latter were resistant to MgO even after 24 h of exposure. Both alkali metal oxides contribute to the alkalinity of the medium and they are also relatively more soluble compared to ZnO. Given the differences between *L. helveticus* and *L. plantarum* as well as *L. mesenteroides*, the susceptibility of *L. helveticus* to the alkali metal oxides can be understood from pH effect, lipid oxidation provoked by free radicals and by the effect of free ions.

The effects of calcium oxide, magnesium oxide and zinc oxide on spores of *Alicyclobacillus acidoterrestris* were also examined. The results shown no antibacterial effect against spore of *A. acidoterrestris* after 96 h of exposure. Spore cells are much more robust with their thick proteinous spore coat than vegetative cells. The CaO and MgO slurries were able to kill the spores of *B. subtilis* in physiological saline at a higher concentration of the metal oxide used in this study. This fact suggested that the concentrations used were not enough to promote the spore killing of *A. acidoterrestris*. The target of the oxidizing agent, such as peroxide, could be lipids or the proteins of the inner membrane. In the case of *Alicyclobacillus*, due to its unique fatty acid composition (essentially composed of saturated branched and cyclic fatty acids of this membrane[71], proteins could be the major target for antibacterial agents [72]. Yamazaki *et al.*[73] reported that under low pH condition, spores of *A. acidoterrestris* exhibited strong binding character of Ca^{2+} and they were not affected by Mg^{2+}. These two facts may explain the strong resistance ability of spores against metal oxide tested.

Conventional and nano-assembled metal oxides (CaO, MgO and ZnO) were evaluated for their lethal effect on yeasts (*S. cerevisiae* and *C. tropicalis*) and fungal spores (*A. niger*, *P. variotii* and *Byssochlamys spp.*) and growth inhibitory effect on yeasts and fungi, which are implicated in the spoilage of fruit juices and drinks. The effect of exposure time, pH, concentration and particle size of metal oxides were examined. The lethal effect was determined by exposing yeast cells or fungal spores to specified concentrations of metal oxides. The viable cells were counted on culture media.

Alkalinity effect is considered as a major factor in the antimicrobial action of CaO and MgO. The bactericidal actions of CaO and MgO were compared with alkaline (NaOH) solution and were found to be higher than those of NaOH solution at identical pH. The pH values of isotonic solution containing different metal oxide powder showed a very high alkalinity for CaO (10.7 to 11.2 at 100 ppm to 12.3 at 1000 ppm). At 100 ppm, both yeasts were killed by the high pH (pH control) of CaO. *S. cerevisiae* was more sensible to the pH variation than *C. tropicalis*. This suggests that the fungicide action of CaO is due to its surface alkalinity, which could lead to solubilisation of cell surface proteins and cell wall alkali-soluble polysaccharides [74]. The change in the pH of MgO with increase of its concentration in isotonic solution was small and stabilized around 10.5, as noted by Makhluf *et al.* [47] for MgO nanoparticles. At this pH, the viabilities of yeasts were reduced by about 1 log. Compared to this value, MgO nanoparticles had a higher killing effect at 1000 ppm against *S. cerevisiae*. In the growth medium, change of pH tends to reduce considerably the growth of both yeasts tested, because no growth was observed in the control pH 8.5. However, control pH corresponding to ZnO slurries pH in isotonic solution did not disturb the viability of yeasts and molds, whereas in nutrient broth, their growth was inhibited by change of pH. Praphailong and Fleet [75] showed that the effect of pH on the growth of *S. cerevisiae* and that its growth was inhibited at pH 8.0. CaO and MgO are also relatively soluble in water compared with ZnO, which are alkali metal oxides with high solubility constants (CaO, 5.02×10^{-6}; MgO, 5.61×10^{-12}; and ZnO, 3.0×10^{-17}). It suggests that CaO and MgO nanoparticles may kill or inhibit micro-organisms by other properties than their high alkalinity alone.

Sawai and Yoshikawa [76] evaluated antifungal activity of metallic oxides (MgO, CaO and ZnO) by an indirect conductimetric assay. Their results indicated that CaO and MgO had antifungal activity above 1600 ppm against *S. cerevisiae* and other fungi. However, ZnO exhibited only a weak antifungal activity against *S. cerevisiae*, but some growth inhibition was observed at 100 ppm. Our results shown that there was a little or no effect of particle size on either the lethality or growth inhibition of metal oxides.

Our work shown for the first time a fungicidal effect of ZnO against yeasts and also a fungiostatic action against molds. Unlike CaO and MgO, pH of ZnO dispersed in physiological solution was between 7.7 and 8.6, irrespective of its particle size or concentration. It did not affect microbial survival or growth [59, 75]. The difference between yeasts and molds could be attributable to the difference in their cell wall and membrane structure. Chitin makes up to 45% of the cell wall of *A. niger*, however, it is present only 3% in *S. cerevisiae* (Roller and Covill, 1999). For almost all fungi, the central core of the cell wall is a branched β-1,3, 1,6 glucan that is linked to chitin via a β-1,4 linkage [74]. This prove that spore of molds are much more resistant to environment perturbation than yeast. However, S. *cerevisiae* is known to possess the enzymes catalase that catalyses the breakdown of H_2O_2 to O_2 and H_2O [77]. It suggests that metal oxides may inhibit fungi by other characteristics than their generation of superoxides at their surface. The binding of the oxides particles on the fungal cell surface through electrostatic interactions could be a possible mechanism [46]. Concerning ascospores of *Byssochlamys spp.*, the results shown no effect against them. This was expected because ascospores are more resistant to environmental stress than spore itself. Also, younger ascospores might succumb to stress more rapidly than older one because of their relatively 'weak' cell wall, whereas mature ascospores have a denser cell wall which may protect them from perturbation [78].

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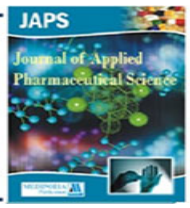

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A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi

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ABSTRACT

The antifungal activity of zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles prepared by bio-safe method was evaluated for *Alternaria alternate*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus*. It was observed from the study that all the nanoparticles at different concentrations brought about significant inhibition in the germination of spores of *Alternaria alternata, Fusarium oxysporum, Rhizopus stolonifer and Mucor plumbeus*. However, the highest inhibition in the germination of all the test fungi was observed at higher concentrations followed by lower concentrations of nanoparticles. The nanoparticles of MgO at highest concentration was found most effective in reducing the spore germination followed by nanoparticles of ZnO at the same concentration.

Keywords: Nanoparticles; anti fungal activity; spore germination

INTRODUCTION

Nanosciences has reached within the last decade the status of a leading science with fundamental and applied research prospects in all basic cognitive sciences such as physical, life and earth sciences: from physics and chemistry, biology and medicines, to engineering and agriculture. Nanotechnology is the next industrial revolution and all most all industries will be radically transformed by it in few years and this technology would directly benefit a common man when it comes to commercial use. The emerging field of nanosciences and nanotechnology is leading to a technological revolution in the world (Shah and Towkeer, 2010). Nanotechnology has the potential to revolutionize the agriculture and food industry too with new tools for molecular treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients (Patolsky *et al*., 2006). Nanoscale devices could be used to identify plant health issues before these become visible to the former. Smart devices could be used to deliver chemicals in a controlled and targeted manner in the same way as nano-medicine has implications for drug delivery in humans. Technologies such as encapsulation and controlled release methods have revolutionized the use of pesticides and herbicides (Scrins and Lyons, 2006). Besides, plants and/or their extracts provide a biological synthesis route of several metallic nanoparticles which is more eco-friendly and allows a controlled synthesis with well defined size and shape (Bar *et al*., 2009).

Plants are often attacked by various pathogens such as fungi, bacteria and viruses which results in great loss to farmers (Esfahani, 2006). Several conventional methods have been used for the control of these pathogens and each of these methods has one or other limitations. Some of these methods such as use of pesticides cause hazardous effect on the environment and human health. Thus, use of nanoparticles has been considered an alternate and effective approach which is eco-friendly and cost effective for the control of pathogenic microbes (Kumar and Yadav, 2009). These nanoparticles have a great potential in the management of plant diseases as compared to synthetic fungicides (Park *et al*., 2006). Zinc oxide (ZnO) and magnesium oxide (MgO) nanoparticles are an effective antibacterial and anti-odor agent Shah and Towkeer, 2010). The increased ease in dispensability, optical transparence and smoothness make ZnO and MgO nanostructures an attractive antibacterial ingredient in many products. Both have also been proposed as an anti-microbial preservative for wood or food products (Aruoja *et al*., 2009; Huang *et al*., 2006; Sharma *et al*., 2009). Properly functionalized nanocapsules provide better penetration through cuticle and allow slow and controlled release of active ingredients on reaching the target weed. The use of such nano-biopesticide is more acceptable since they are safe for plants and cause less environmental pollution in comparison to conventional chemical pesticides (Bark *et al*., 2008). In this communication, the antimycotic effects of ZnO and MgO nanoparticles having average size of ~30 ± 10nm and ~50 ±10nms respectively are tested on some pathogenic fungi such as *Alternaria alternate, Fusarium oxysporum, Rhizopus stolonifer and Mucor plumbeus***.** The nanoparticles of MgO at highest concentration were found most effective in reducing the spore germination followed by nanoparticles of ZnO at the same concentration. To the best of our knowledge and belief, the use of nanostructures prepared without organics or toxic solvents has not been used for in-vitro studies so far.

MATERIALS AND METHODS

Preparation of nanoparticles and Fungal culture

The method employed for preparation is same as described earlier (Al-Harbi *et al*., 2011; Shah and Quarshi, 2009). In a typical synthesis of ZnO and MgO nanoparticles, 3 mg of zinc and magnesium powder was taken in a vial containing 30 ml of de-ionized water separately and was well sonicated for 10 minutes each before placing at desired temperature in a Teflon bomb. The reaction mixture was transferred to teflon-lined stainless steel chamber and has been kept at 110^{o}C in an oven for 6h. After the desired time, the system was naturally cooled to room temperature. The reaction mixture was centrifuged to reclaim the precipitated sample and was washed with distilled water. After drying in air, synthesized nanoparticles were characterized by various techniques. The average particle size of ZnO and MgO nanoparticles is ~ 30 ± 10nm and 50 ±10nms respectively (Figure 1 and 2). The Fungal culture was prepared in Plant Pathology Laboratory, Department of Botany, University of Kashmir on Potato dextrose Agar medium PDA. Fungal species of *Alternaria alternate, Fusarium oxysporum* and *Rhizopus stolonifer.and Mucor plumbeus* were isolated from rotten samples of tomato and bringal, cultured on PDA and identified following Koch's postulates.

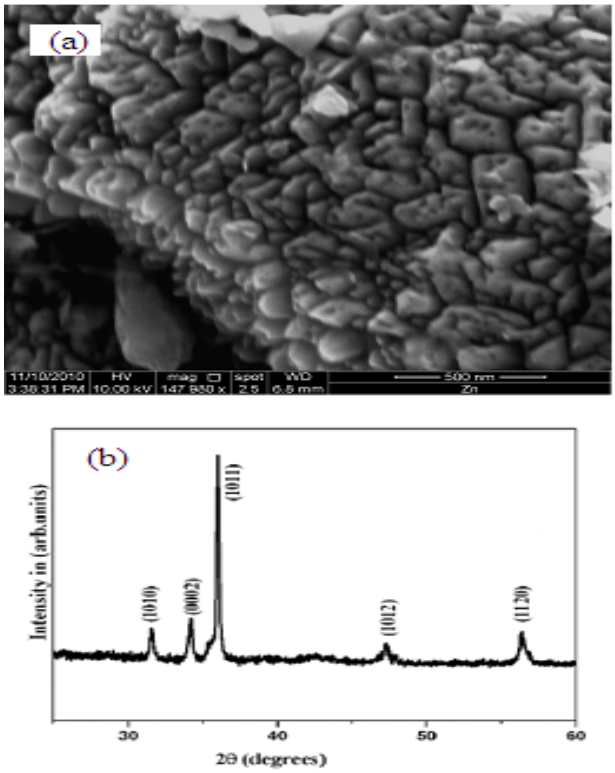

Fig. 1: (a) FESEM image of ZnO nanoparticles (b) XRD of the samples.

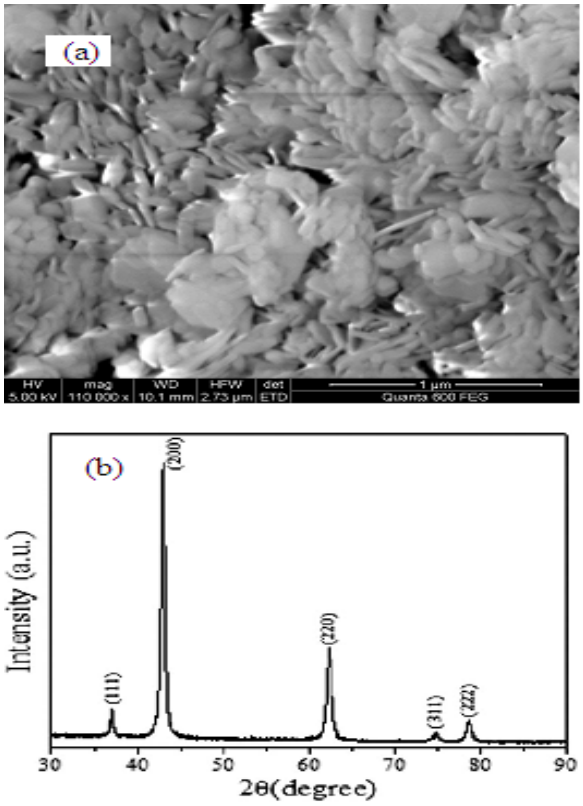

Fig. 2: (a) FESEM image of MgO nanoparticles (b) XRD of the samples.

Patolsky F, Zheng G & Lieber CM. Nanowire sensors for medicine and life sciences, *Nanomedicine*. 2006; 1. 51–65

Perez-de-Luque & Diego AR. Nanotechnology for parasitic plant control, *Pest Management Science* 2009; 65. 540–545

Scrinis G & Lyons K. The emerging nano-corporate paradigm: nanotechnology 653and the transformation of nature, food and agri-food systems. *Int. J. Sociol. Food Agric.* 2006; 15. 22–44..

Shah MA & Towkeer A. Principles of Nanosciences and Nanotechnology, Naroosa Publishing House, New Delhi, (2010).

Shah MA & Quarshi A. Novel surfactant free synthesis of MgO nanoflakes. *J. of alloys and Comp.* 2009; 252. 583

Sharma V K, Yngard RA & Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. *Adv. Colloid Interface Sciience* 2009; 145. 83–96

Vahabi K, Mansoori GA & Karimi S. Biosynthesis of silver nanoparticles by fungus Trichoderma Reesei . *Inscience J*. 2011; (1): 65-79.

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An epidemiological survey on cattle ringworm in major dairy farms of Mashhad city, Eastern Iran

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ABSTRACT

Background and Objectives: Cattle dermatophytosis (syn. cattle ringworm), an important skin infection, has received major consideration not only for economical losses in the animal breeding industry but also in regards to its zoonotic transmission to humans. For effective control measures, it is important to determine the disease prevalence in cattle herds.

Materials and Methods: To determine ringworm prevalence, a total number of 3,540 cattle in different age groups at three major farms of Mashhad including Kenebist (KB) in the east, Mazraeh Nemooneh (MN) in the south, and Moghoofat Malek (MM) in the north-east were examined. Skin scrapings were prepared from all animals clinically suspected to have dermatophytosis. The samples were examined microscopically for fungal elements (hyphae and/or arthrospores) by adding potassium hydroxide (KOH) to samples. To isolate the etiologic dermatophytes, all samples were cultured on selective agar for pathogenic fungi medium for 4 weeks at room temperature.

Results: Among 684 suspected cases (19.3%) selected from a total number of 3,540 cattle based on clinical signs, 604 cases (88.3%) were KOH positive in direct microscopy, while 490 cases (71.6%) were culture positive on selective agar for pathogenic fungi (SAPF) medium. The most frequent dermatophyte isolated was *Trichophyton verrucosum* (495 isolates accounting for 99% of total isolates) which was obtained from all culture positive cases except five cases (1.0%) infected with another dermatophyte named *Trichophyton mentagrophytes.*

Conclusion: This work is the first comprehensive study on cattle ringworm in Iran. With respect to the high prevalence of cattle ringworm, particularly in young animals reported in the present study, effective management programs such as vaccination and improved hygiene are necessary for disease control in the herds.

Keywords: Dermatophytosis, Cattle, *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, Iran.

INTRODUCTION

Dermatophytosis (syn. ringworm, tinea) is a zoonotic skin infection of keratinized tissues caused by a specialized group of fungi named dermatophytes. The disease has worldwide distribution and it has been considered as a public health problem all over the world (1). Animal dermatophytosis is responsible for high economical losses especially in cattle farming due to skin damages and decrease in milk and meat production.

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Dermatophytes include geophilic, anthropophilic and zoophilic species living in soil, human beings and animals respectively (2). For each animal species, the dermatophytes involved depend on the host studied and on the geographical and environmental conditions. *Trichophyton verrucosum* is the usual zoophilic dermatophyte involved in cattle ringworm throughout the temperate regions of the world. It also affects, but with lower prevalence, sheep, goat and other ruminants (3,4). The presence of *T. verrucosum* in the hair coat of free-ranging animals, especially non-ruminants, is uncommon. The animal age and trauma are important predisposing factors of disease (5).

Cattle ringworm mainly occurs in young animals (calves) and is rapidly spread in the herd via infected propagules, i.e. hyphae, and specialized fungal spores

Table 1. The prevalence and distribution of cattle ringworm in major dairy farms of Mashhad city in East Iran with special reference to direct examination and culture results.

Farm	No. of animals with clinical sings (%)	Age in month (No.)	No. of KOH positive cases	No. of culture positive cases	Fungal isolates (No.)		Total number of animals in each farm
Kenebist (KB)	207 (16.9)	3-6 (137)	125	105	*T. mentagrophytes* (2) *T. verrucosum* (103)	535	1223
		7-12 (65)	52	36	*T. verrucosum* (36)	275	
		>12 (5)	5	3	*T. verrucosum* (3)	413	
Mazraeye Nemooneh (MN)	295 (19.7)	3-6 (236)	224	178	*T. verrucosum* (178)	703	1500
		7-12 (52)	29	29	*T. verrucosum* (29)	294	
		>12 (7)	6	4	*T. verrucosum* (4)	503	
Moghoofate Malek (MM)	182 (22.3)	3-6 (153)	138	117	*T. mentagrophytes* (3) *T. verrucosum* (114)	306	817
		7-12 (25)	21	14	*T. verrucosum* (14)	72	
		>12 (4)	4	4	*T. verrucosum* (4)	439	

named arthrospores. The disease occurs worldwide and *T. verrucosum* is the almost exclusive etiologic agent (6,7). Besides cattle, it has been reported as the major agent of dermatophytosis in ruminants such as goat, sheep and camel (3,4,8,9). Aside from animal involvement, several human outbreaks of *T. verrucosum* infection have been reported so far by direct contact with infected animals or indirect contact with infectious propagules in the environment (10-12). Although various parts of human body may be involve by the fungus, the face and the body are mainly affected during the fungal infection. Human cases of *T. verrucosum* infection have been successfully treated by different antifungal agents such as azoles, but therapy for cattle is more difficult (12). Some reports support the efficacy of vaccination and improved hygiene for management of cattle ringworm in affected herds (7, 13, 14).

Despite the existing data about different clinical features of human dermatophytosis in Iran (15-18), little has been documented about animal involvement to date. In the present study, the prevalence and distribution of cattle ringworm in three major farms of cattle breeding in Mashhad were evaluated with special reference to etiologic dermatophytes.

MATERIALS AND METHODS

Animals and sample preparation. Three major dairy farms of Mashhad located in different geographic areas including Mazraeh Nemooneh (MN) in the south, Kenebist (KB) in the east and Moghoofat Malek (MM) in the north-east were involved in this study during the Summer of 2004. All animals (calves and cattle ; Holstein breed) in different age groups (3-6 months (1,544 cases), 7-12 months (641 cases) and >12 months (1,355 cases)) were examined . The animals were housed indoors at the time of sampling. The entire body of each animal was thoroughly examined for evidences of scaling, crusting, hair loss and erythema. Clinical lesions consistent with dermatophytosis observed in 684 of 3,540 animals were cleansed with cotton swabs soaked in 70% ethanol and then scrapped in the peripheral area with a sterile scalpel. Some of the animals which had no obvious lesions were also randomly sampled. The skin scrapings were collected in sterile petri-dishes labelled with the main animal characteristics and transferred to the laboratory for examination with standard methods.

Direct microscopic examination (DME). For

common agents of disease. In this study, from a total number of 490 dermatophyte isolates obtained from infected animals on examination at mentioned farms, 485 isolates were identified as *T. verrucosum*, while only 5 isolates were diagnosed as *T. mentagrophytes*. Kakepis *et al*. (25) reported the cattle ringworm with the sole etiology of *T. mentagrophytes* with a frequency of about 35% in seven cattle barns during 1976-1977. The zoophilic fungus, *T. mentagrophytes* isolated in this study may be associated with frequent contacts of the calves with rats and pet animals like dogs and cats as it was reported for cattle from traditional-type farms (26). A recent report indicated that the relative frequency of the isolation of *T. verrucosum* (cattle ringworm) has decreased by 90% during a 25 years period from 1980 to 2005 in United Kingdom (27). This may be a reflection of improving hygienic condition of people and use of effective programs for controlling the ringworm in cattle herds.

In regards to the correlation between the prevalence of ringworm and economic losses or public health hazards observed in the present study, different aspects of disease were noticed. Some of the workers who had direct contact with the infected animals showed involvement of the face area mainly as tinea barba (data not shown). These individuals were treated with common antifungal agents. On visual inspection, an obvious reduction in weight gain was seen in some affected calves compared with unaffected animals of similar age. As indicated by the farmers, milk production and the quality of leather was also affected in heavily infected dairy cattle in all three farms examined. Despite the above-mentioned economical losses in cattle breeding farms, real losses to the cattle breeding industry in Iran is not possible to calculate due to lack of informative and documented data from different parts of the country.

In conclusion, results of the present study clearly highlight the importance of cattle ringworm in Iran. The high prevalence of disease seems to be associated with high density and poor hygienic conditions of animals in the herds. Routine evaluation of all cattle and calves accompanied with suitable control strategies, i.e. vaccination and improved hygiene, may be useful for managing ringworm as an economically important zoonotic infection.

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REFERENCES

1. Kane J, Summerbell R, Sigler L, Krajden S, Land G (1997). Laboratory Handbook of Dermatophytes. Star Publishing Company, Belmont, CA ,USA.
2. Weitzman I, Summerbell RC. The dermatophytes. *Clin Microbiol Rev* 1995; 8: 240-259.
3. Pier AC, Smith JMB, Alexiou H, Ellis DH, Lund A, Pritchard RC. Animal ringworm – its aetiology, public health significance and control. *J Med Vet Mycol* 1994; 32: 133–150.
4. 4. Stenwig H. Isolation of dermatophytes from domestic animals in Norway. *Nord Vet Med* 1985: 37: 161–169.
5. Oborilova E, Rybnikar A. Experimental dermatophytosis in calves caused by *Trichophyton verrucosum* culture. *Mycoses* 2005; 48: 187–191.
6. Wabacha JK, Gitau GK, Bebora LC, Bwanga CO, Wamuri ZN, Mbithi PM. Occurrence of dermatophytosis (ringworm) due to *Trichophyton verrucosum* in dairy calves and its spread to animal attendants. *JS Afr Vet Assoc* 1998; 69: 172–173.
7. Weber A. Mycozoonoses with special regard to ringworm of cattle. *Mycoses* 2000; 43: 20–22.
8. Pepin GA, Austwick PKC. II.–Skin disease, mycological origin. *Vet Rec* 1968; 82: 208–221.
9. Fadlelmula A, Agab H, Le Horgne JM, Abbas B, Abdalla AE. First isolation of *Trichophyton verrucosum* as the aetiology of ringworm in the Sudanese camels (*Camelus dromedarius*). *Rev d'élev Méd Vét Pays Trop* 1994; 47: 184-187.
10. Roman C, Massai L, Gianni C, Crosti C. Case reports. Six cases of infection due to *Trichophyton verrucosum*. *Mycoses* 2001; 44: 334–337.
11. Maslen MM. Human cases of cattle ringworm due to *Trichophyton verrucosum* in Victoria, Australia. *Australian J Dermatol* 2000; 42: 1-4.
12. Ming PX, Ti YL, Bulmer GS. Outbreak of *Trichophyton verrucosum* in China transmitted from cows to humans. *Mycopathologia* 2006; 161: 225-228.
13. Bredahl L, Gyllensvaan C. Incidence and control of cattle ringworm in Scandinavia. *Mycoses* 2000; 43: 8–10.
14. Rybnikar A, Vrzal V, Chumela L. Protection efficacy of vaccines against bovine dermatophytosis after double and single vaccination. *Mycoses* 1998; 41: 83–86.
15. Mahmoudabadi AZ. A study of dermatophytosis in South West of Iran. *Mycopathologia* 2005; 160: 21-24.
16. Chadeganipour M, Shadzi S, Dehghan P, Movahed M. Prevalence and etiology of dermatophytoses in Isfahan, Iran. *Mycoses* 1997; 40: 321-324.
17. Kazemi A. Tinea unguium in the north-west of Iran. *Rev*

Mycopathologia **137:** 107–113, 1997.

Dermatophytes isolated from domestic animals in Barcelona, Spain

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Abstract

This retrospective study deals with the main samples studied at the Mycology Diagnostic Service of the Faculty of Veterinary Science of Barcelona: animals with suspected dermatophytosis. Over a ten-year period from 1986 to 1995, 136 dermatophytes were identified from dog and cat cultures submitted for identification and from specimens submitted for mycological examination from a variety of other domestic animals. The most frequent dermatophytes isolated were *Microsporum canis* (55.9%), *Trichophyton mentagrophytes* var. *mentagrophytes* (27.2%), *Microsporum gypseum* (7.4%) and *Trichophyton verrucosum* (7.4%). The identity of the dermatophytes from dog and cat cultures submitted for identification was *M. canis* (77.8%), *T. mentagrophytes* (13.3%) and *M. gypseum* (8.9%). Dermatophytes were cultured from 15 of 105 (14.3%) canine specimens and 19 of 56 (33.9%) feline specimens submitted for mycological examination during this period. *Microsporum canis* was the most common species isolated (73.3% and 94.7% respectively). The percentage of positive microscopic examinations of the specimens of hair in culture positive submissions from dogs and cats was 58.8%. There was a high proportion of positive cultures from both dogs and cats less than 1 year of age, and in some breeds of dogs, but there was no significant difference between the sexes. Although dermatophytes were more frequently isolated in autumn and winter months, no significant difference was detected in the seasonal distribution of the canine and feline dermatophytosis. *Trichophyton mentagrophytes* was the most prevalent dermatophyte in rabbits and *T. verrucosum* in ruminants. Other isolated species were *T. equinum* and *M. equinum* from horses.

Key words: dermatophytes, dermatophytosis, domestic animals

Introduction

Animals serve as reservoirs of the zoophilic dermatophytes. The incidence of dermatophytosis varies according to climate and with the natural reservoirs. However the pattern of the species of dermatophytes involved in dermatophytosis may be different in similar geographical conditions, both in humans and animals, and it has been related, among other factors, to the decline in the incidence of animal ringworm in these areas or the degree and closeness of animal to human contact [14]. In other countries, epidemiological studies on the isolation of dermatophytes from domestic animals with suspected lesions of dermatophytosis have been reported [1, 4, 8, 13, 17, 18].

This first-ten-year retrospective study (1986–1995) deals with the main samples studied at the Mycology Diagnostic Service, which was stablished in 1986 at the Faculty of Veterinary Science of Barcelona. Animals with suspected dermatophytosis, including the identification of cultures submitted to us and specimens, were processed in our laboratory.

Material and methods

Cultures submitted for identification. During this ten-year period 47 cultures, 35 from dogs and 12 from cats (mainly on Dermatophyte Test Medium), were received for identification. These cultures were submitted by practitioners from different Veterinary Clinics to our laboratory, and were related to samples from dogs and cats suspected of having dermatophytosis.

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A Survey of Dermatophytes Isolated from Cows and Sheep in Iraq

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Summary

A total of 100 animals were examined during the period of beginning of September - 2010 till the end of March 2011 at dept of Microbiology college of Veterinary Medicine Baghdad University Baghdad Iraq. These animals include 50 cow and 50 sheep. Hairs and scales were submitted to direct KOH mount smear and culture on modified Sabouraud's Dextrose agar medium The direct smear was positive in 40 (80%) for both cows and sheep while the growth of dermatophyte was positive in 35 (70%) and 38 (76%) for cows and sheep respectively. Species identification revealed the presence of *Trichophyton rubrum* (19 isolates) *Trichophyton verrucosum* (10 isolates) *Trichophyton mentagrophytes* (5 isolates) and *Microsporum canis* (one isolate) in cow while *Trichophyton rubrum* (22 isolates) *Trichophyton verrucosum* (2 isolates) and *Trichophyton mentagrophytes* (14 isolates) was recorded in sheep.

Keyword: Dermatophytes Trichophyton verrucosum , Trichophyton mentagrophytes, Microsporum canis , Trichophyton rubrum.

دراسة بحثية عن الفطريات الجلدية المعزولة من الابقار والاغنام في العراق

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الخلاصة

تم فحص مائة حالة من الابقار والاغنام خلال الفترة من بداية شهر أيلول 2010 الى نهاية شهر أذار 2011 وفحصت النماذج المأخوذة من الجلد والشعر في كلية الطب البيطري / جامعة بغداد. خضعت النماذج المأخوذة للفحص المباشر بأستخدام الشريحة المرطبة بمحلول هيدروكسيد البوتاسيوم (10% KOH) مع الزرع على وسط السابرويد المطور وكانت نتجة الفحص المباشر 40 (80%) لكل من الابقار بينما كان نمو الفطريات الجلدية موجبة في 35 (70%) و 38 (76%) للابقار والاغنام على التوالي. أظهر فحص تشخيص الانواع ففي الابقار وجود 19 عزلة للنوع *Trichophyton rubrum* 10عزلات نوع *Trichophyto verrucosum* و 5 عزلات للنوع *Trichophyton mentagrophytes* و *عزلة* واحدة للنوع *Microsporum canis* بينما في الاغنام شخصت 22 عزلة من النوع *Trichophyton rubrum* و عزلتان من النوع *Trichophyton verrucosum* و 14 عزلة من النوع *Trichophyton mentagrophyte*.

Introduction

Dermatophytes are fungi that require keratin for growth. These fungi can cause superficial infections of the skin hair and nails They are spread by direct contact from other people (Anthropophilic organisms) animals (Zoophilic organisms) and soil (Geophilic organisms) as well as indirectly from fumiest (1). Dermatophytes infection usually refer to as Tinea are caused mostly by the genera Epidermophyton Microsporum and Trichophyton.

Variation in the distribution pattern of dermatophytes infection a many different countries of the world are evident in the studies (2 - 6). This distribution pattern of dermatophytes infections in different parts of the world has been attributed to factors of climate life - style and prevalence of immunodeficiency disease in the community and also the reluctance of patients to seek treatment because of embarrassment or minor nature of disease unless the condition becomes sufficiently serious to effect the quality of life (7).

Materials and Methods

A total of 100 animals were examined during the period of beginning of September 2010 till the end of March 2011 at dept of Microbiology college of Veterinary Medicine Baghdad University Baghdad Iraq These animals include 50 cows and 50 sheep hairs and scales were submitted to microscopic examinations after immersion in 10% potassium hydroxide solution with gentle heating Hairs and scales from active outer border of the lesions of all animals were inoculated on modified Sabouraud's Dextrose agar containing chloramphenical (005 mg/ml) and cyclohexmide (05 mg/ml) Culture were incubated at 28 c with daily observation for a period of 4 – 5 weeks before they were considered negative The identification of dermatophyte species was based on the gross and microscopic cultural characteristics produced on this standard medium according to Emmon *et al* (8) and Rippon (9).

Results

A total of 50 cows were examined. Twenty five (50%) cases were positive by both direct KOH mounts smear and culture Fifteen (30%) cases were culture negative after six weeks incubation at room temperature (28 c) although the direct KOH mount examination was positive. The remaining 10 (20%) cases gave a positive culture result although the direct KOH mount smear examinations were negative (table1).

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A Clinico-mycological Study of Onychomycosis

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Abstract

Background and Objectives:

Onychomycosis is a chronic fungal infection of nails. The clinical diagnosis of onychomycosis needs to be confirmed by direct microscopy and culture for identification of specific pathogen and proper selection of antifungal treatment. Our aim is to study the morphological pattern of onychomycosis and to analyze the mycological and cultural positivity of onychomycosis with respect to various etiological agents.

Methods:

A prospective study was conducted on 200 patients of onychomycosis. A detailed history and thorough clinical examination was done in all patients. The samples were taken from involved nails and skin and subjected to potassium hydroxide (KOH) examination and fungal culture on Dermatophyte test medium (DTM) and Sabouraud's dextrose agar (SDA) medium. The identification of isolate was done from the growth on Sabouraud's dextrose agar (SDA) medium.

Results:

Distal and lateral sub-ungual onychomycosis (DLSO) was the commonest type (67%) and proximal Trichophyton mentagrophyte

subungual onychomycosis (PSO) was the least common (1%). Out of 200 KOH positive cases, 72 were culture positive on Sabouraud's dextrose agar (SDA) medium (36%) and 54 cases (27%) were positive on Dermatophyte test medium (DTM). Trichophyton rubrum was the most common (45%) isolate followed by (25%) and Candida albicans (14%). Aspergillus niger was only non-dermatophyte mould isolated from 7 cases (3.5%) of toe nail onychomycosis.

Conclusion:

Dermatophytes were the most common group followed by yeasts and non-dermatophyte moulds in the etiology of onychomycosis. Trichophyton rubrum was the most common isolate. Dermatophyte test medium (DTM) is a rapid and easy method to confirm dermatophyte infection.

Introduction

Onychomycosis is defined as the fungal infection of nails caused by dermatophytes, yeasts and non-dermatophytic fungi. It is one of the commonest nail disorders and accounts for up to 30% of all superficial infections of the skin [1] Recently there has been a worldwide increase in the incidence of onychomycosis with social, cultural and economical factors contributing to it [2]. The disease per se is asymptomatic but it poses a serious concern to the clinician as it often becomes a chronic source of recurrent superficial skin infections. Besides this, the destruction and disfigurement of nail plate in onychomycosis can lead to self-consciousness and impairment in doing fine work [3].

The clinical diagnosis of onychomycosis can be confirmed by direct microscopy of potassium hydroxide (KOH) preparation. However a fungal culture is required to identify the specific genus and species of pathogens.

In the past most of the research work has been done on the superficial mycosis of the skin as compared to onychomycosis. In India relatively less work has been done on the onychomycosis as compared to western countries. The evolving role of non-dermatophytic moulds has added a new dimension to the clinical patterns of onychomycosis. There is a need for further studies on onychomycosis and other dermatophytosis in view of the introduction of several newer systemic antifungal drugs.

The present study was conducted to study the morphological pattern of onychomycosis and to analyze the mycological and cultural positivity in view of the paucity of literature on onychomycosis from this part of the country.

Methods

This was a prospective study carried out in the outpatient department

Dermatophytes: Their taxonomy, ecology and pathogenicity

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Summary

Current concepts of anamorphic and teleomorphic states of dermatophytes, sampling techniques and techniques for mating studies are discussed. Ecological groupings and sources of infection; pathogenicity with emphasis on proteolytic enzymes including its biochemical assays, characterization and molecular weight size are reviewed.

Key words

Dermatophytes, Taxonomy, Ecology, Pathogenicity

In culture dermatophyte morphology, for purposes of nomenclature, can be divided into two states on the basis of stages in the life cycle, the anamorphic and the teleomorphic states. The anamorph is the state where asexual or somatic reproduction occurs and has a distinct morphology. The teleomorph, on the other hand, is the sexually reproductive ("perfect") state, morphologically (and/or karyologically) differentiated from the anamorph [1]. Sexual reproduction has been demonstrated in a number of species which requires two compatible isolates ("+"and "-") on a suitable medium. A workable classification of dermatophytes is best based on the macroscopic and microscopic morphology of the asexual state in culture and the Emmons [2] classification system emphasizes these characteristics.

Definition

Dermatophytes are a group of morphologically and physiologically related molds some of which cause well-defined infections: dermatophytoses (tineas or ringworm) [3]. They possess two important properties: they are keratinophilic and keratinolytic. This means they have the ability to digest keratin *in vitro* in their saprophytic state and utilize it as a substrate and some may invade tissues *in vivo* and provoke tineas. However, their morphology in the parasitic growth phase is different from the morphology exhibited in culture or *in vitro.*

Anamorphic states

Dermatophytes as saprophytes reproduce asexually by simple sporulation of arthro-, micro- and macroconidia produced from specialised conidiogenous cells. Dermatophyte species also exhibit a range of vegetative structures with typical arrangement on hyphae, chlamydospores, spirals, antler-shaped hyphae (chandeliers), nodular organs, pectinate organs and racquet hyphae [2,4]. In addition, some physiological characteristics based on nutritional requirements [4-8]such as vitamin deficiency can be used to identify some dermatophytes.

Most dermatophyte colonies develop forms and pigmentation which can allow a presumptive identification of that dermatophyte species. The appearance of a fungus colony depends on the medium used, but for comparative purposes Sabouraud dextrose agar (SDA) medium is conventionally used to obtain colonies which can be compared to others reported in the literature [4]. Ajello [4] lists five important colony characteristics to look for in presumptive identification of a dermatophyte culture when it is one to three weeks old: (1) rate of growth (2) general topography (flat, heaped, regularly or irregularly folded) (3) texture (yeast-like, glabrous, powdery, granular, velvety or cottony) (4) surface pigmentation and (5) reverse pigmentation.

Based on the above criteria, particularly on differences in conidial morphology, dermatophyte species can be classified into three genera within the Fungi Imperfecti (or Deuteromycotina) namely: *Epidermophyton*, *Microsporum*, and *Trichophyton* [2]. The studies of Cole and Samson [9] have shown that the ontogeny of the holothallic conidia of *Microsporum* and *Trichophyton* is essentially the same. Their only difference is the macroconidial cell-wall thickness and presence of echinulations in *Microsporum* species which are absent in *Trichophyton* species [10-11].

However, there has been some controversy in the broad classification of some dermatophytes. Benedek [12] felt that the genus *Achorion* should have been retained [2] as was *Epidermophyton* simply because of its "established usage" and that Emmon's proposal cannot be considered a natural classification. The proposed system [13,14] distinguished the genera *Epidermophyton*, *Microsporum*, *Trichophyton*, *Microides* and *Keratinomyces*. Ajello [15] rejected Vanbreuseghem's genus *Microides* based on the similarity in morphology of *M. interdigitalis* to *T. mentagrophytes*. He proposed that *M. interdigitalis* should be considered a variety of *T. mentagrophytes*, to be known as *T. mentagrophytes var. interdigitale* while Emmons [2] considered *T. interdigitale* to be a synonym of *T. mentagrophytes*.

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A. fulva to be the least pathogenic, while no notable differences have been observed with *A. gypsea* and *A. incurvata* [75,76]. The distribution of the *M. gypseum-fulvum* complex is world-wide [47,77-79].

For pathogenic geophilic dermatophytes, infective propagules originate from saprobic sources, are transmitted either directly or indirectly, and are referred to as saprobic-parasitic (S-P) infections [3,70]. This mode of infection is common for *M. gypseum*, where the source of most infections in man and animals is the soil [4]. In children facial ringworm by *M. gypseum* can follow recreational exposure to soil-borne propagules of this fungus [3]. Occupational exposure is illustrated by reported cases in gardeners [3] and small epidemics observed in, for example, cucumber growers [80,81]. The macro- and microconidia, ascospores and other propagules are produced during the saprophytic growth of dermatophytes on keratin in soil or other biotopes (e.g. birds nests in the case of *M. ripariae*) [70] and it is these which form the potential inoculum.

The *T. terrestre* complex is considered to be non-pathogenic [33], although human infections by *T. terrestre* have been reported [82] and experimental animal infections have also been successfully induced [83]. Other geophilic dermatophytes include *M. cookei* and *T. ajelloi* which are non-pathogenic. *Microsporum cookei* is a geophile with a global distribution, often isolated from soil and also from rodents and other animals not showing any clinical symptoms of ringworm [84,139,159]. Human infections by *M. cookei* have rarely been reported [86,87]. *T. ajelloi* is commonly found in colder climates but is sporadic in hot climates [47], possibly because higher temperatures inhibit its growth. The fungus has been found to be more often associated with acid soils than with alkaline soils [47].

Zoophiles

Zoophilic species are basically animal pathogens, often with a single preferred animal host or very limited host range, outside which they are found only in exceptional circumstances [88]. Zoophilic dermatophytes rarely grow actively as saprophytes but survive in a dormant state on contaminated materials of animal origin.

M. canis, *T. verrucosum* and *T. mentagrophytes* are common agents of ringworm in animals but are also frequently associated with human infection. The amount of literature on human infections due to the three dermatophytes is enough evidence of their human affinity. Of the three, *M. canis* is the best documented [89-92]. This is mainly because it causes a lot of scalp ringworm in children [88]. *M. canis* commonly infects pet animals and especially cats and dogs which shed infective particles into the domestic environment and contact with this results in familial infections [3]. Like other types of ringworm, young children particularly in the age range 5-14 years are more susceptible to infection than adults. Similarly, kittens and puppies are more susceptible to ringworm than adult animals [93]. *M. canis* is also known to cause ringworm in horses, monkeys, apes and chinchillas [14].

Another dermatophyte species closely related to *M. canis* is *M. distortum*, known to cause ringworm infections in monkeys, dogs and cats. It has been reported to occur mainly in New Zealand [94,95], Australia and the United States [14]. It is now regarded as a variety of *M. canis*. *T. verrucosum*, on the other hand, is a common cause of *tinea* in cattle. It has also been reported in donkeys, dogs, goats, sheep and horses [48]. Close contact by man with infected animals and their fomites leads to contracting the fungus. It is also generally accepted that in countries with cold winters where housing of the animals is required, the incidence of *T. verrucosum* rises in both animals and humans at that time of the year [88]. Cattle breeders and veterinarians, occasionally suffer from tineas due to *T. verrucosum*, which is mainly an agent of inflammatory skin and scalp lesions (kerion). Members of the *T. mentagrophytes* complex (with the exception of *T. mentagrophytes var. interdigitale*) are transmitted from wild rodents and the prevalence of human infections due to this fungus is known to be higher in rural areas where there is a reservoir of rodents e.g. North America and Europe [96-98]. *T. mentagrophytes* has occasionally been isolated from the soil [45,99] where it can survive for several months.

Anthropophiles

Anthropophilic species are primarily adapted for parasitism of man, but some species occasionally cause ringworm in animals. For example, *T. rubrum* has been reported to have caused an infection in a dog [74,100]. Anthropophilic dermatophytes are mainly associated with community life. Since transmission is man to man, contracting the disease therefore requires human contact. The spread of anthropophiles is more common in communities like schools, barracks, prisons and the family [93,101]. In concentrated communities, the use of facilities such as shower-rooms, and common headgear leads to rapid spread of infection.

Four of the *Microsporum* species, according to Vanbreuseghem, can be distinguished from each other on clinical, epidemiological and mycological grounds: *M. audouinii*, *M. langeroni*, *M. rivalieri* and *M. ferrugineum*. *M. langeroni* [22,102,103] has been separated from the classic *M. audouinii* by its geographic region (restricted to Central Africa) and unlike *M. audouinii* can cause *tinea corporis* (ringworm of the glabrous skin) and can be inoculated to produce experimental lesions in guinea pigs. However, most mycologists consider *M. langeroni* and *M. rivalieri* as variaties of *M. audouinii*.

Of the anthropophilic *Trichophyton* species *T. rubrum* is a very common cause of *tinea unguium*, *cruris*, and *pedis* [3,34,101,104]. *T. rubrum* very rarely invades hair *in vivo*. The distribution of *T. rubrum* is global, cutting across all populations and ethnic groups [3,34]. It is a dermatophyte becoming more prevalent among urban populations, especially in developed countries, due mainly to the "modern" way of life such as the wearing of occlusive shoes, which maintain heat and humidity [93]. It is also able to adapt to its environment in a way other species can not emulate [105]. In India, *T. rubrum* causes *tinea corporis* in women and *tinea cruris* in men due to the sari (worn by women around the waist) and the dhobie (loin cloth) worn by men, both of which are tight-fitting [34,68]. *T. rubrum* is also known to cause chronic forms of infections and it has been suggested that the amino acid composition of perspiration may predispose individuals to chronic infection. Certain amino acids are considered "inducers" of *T. rubrum* infections [68]. Pushkarenko and Pushkarenko [106] in their investigations found patients with chronic *T. rubrum* had a higher than normal content of leucine, lysine, asparagine and histidine in their sweat. Rippon and Scherr [107] were able to induce arthroconidia formation in *T. rubrum* at 32°C and 37°C with a medium containing a high amino acid concentration.

T. mentagrophytes var. interdigitale, a member of the *T. mentagrophytes* complex, is essentially a cause of *tinea pedis* and *tinea cruris*, and does not invade hair *in vivo* [108]. The infection of the skin of the foot usually

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Full Length Research

Isolation of Dermatophytes and Screening of selected Medicinal Plants used in the treatment of Dermatophytoses

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Antidermatophytic activities of five selected medicinal plants (leaves) used traditionally in the treatment of dermatophytoses. The screened plants include; *Euphorbia balsamifera* Ait, *Mitracarpus scaber Zucc, Pergularia tomentosa* L*, Streospermum kunthianum* Cham and *Holarrhena floribunda* (g, Don) Dur. And Schiz. A total of two hundred and fifty samples were obtained from infected skin, hair and nails of individuals within Sokoto metropolis. Four dermatophytes were identified to specie level and one to genus level; they include *Trichophyton rubrum, Trichophyton mentagrophyte, Microsporum audouinii, Microsporum gypseum* and *Microsporum* specie. The percentage prevalence of isolated dermatophytes indicated *T. rubrum* had the highest percentage of 27.6 %, while other samples that could not be identified as dermatophytes had the least percentage of 8.4 %. The results of aqueous, hexane and chloroform extracts of *P. tomentosa* and *M. scaber* have exhibited promising antidermatophytic activities against *T. rubrum*, *T. mentagrophyte and M. gypseum* at 10 mg/ml respectively. Minimum Inhibitory Concentrations and Minimum Fungicidal Concentrations of the extracts produced inhibitory action on *T. rubrum*, *T. mentagrophyte* and *M. gypseum* at 10 mg/ml each. Findings from this research proved that *P. tomentosa* and *M. scaber* are more active than the conventional antifungal drug Grisiofulvin. Therefore, this reseach reinforce the use of *P. tomentosa* and *M. scaber* in Nigerian traditional medicine for treating skin infections caused by some dermatophytes.

Keywords: Isolation Dermatophytes, medicinal plants, dermatophytoses and screening

INTRODUCTION

Dermatophyes are the most common agents of fungal infections worldwide (Robert *et al.,* 2004; Yuanwu *et al*., 2009). Dermatophytic infections have been considered to be a major public health problem in many parts of the world. The infections are common in the developing countries, and are of particular concern in the tropics, especially in infants (Guest and Sam, 1998). The 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 or indirect contact with an infected particle which is usually a fragment of keratin containing viable fungus. Indirect transfer may occur via the floor of swimming pools, bath rooms or on brushes, combs, towels and animal grooming implements (Nweze, 2001; Ngwogu and Otokunefor, 2007). Dermatophytes infections are hardly fatal but mostly debilitating and disfiguring diseases that can give rise to permanent deformations if untreated (Elewski, 1996 and Yuanwu *et al*., 2009).

Dermatophytes are susceptible to common disinfectants, particularly those containing aerosol, iodine or chlorine. In some cases combine topical and systemic treatment are often used. However, in our society (Sokoto metropolis) today lack of education particularly in knowledge related to clinical mycology, such treatments are not administered at the beginning of such infections until when they have progressed to chronic level. More to this are the cost device and availability of such antifungal agents which are sometimes beyond the reach of the common man, as a result people revert as usual to the traditional means of treatment.

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The use of medicinal plants is very wide spread in many parts of the world, Nigeria inclusive (Zavala *et al.,* 1997). Traditional medicine is the oldest method of curing diseases and infections and various plants are used in different part of the world to treat human diseases and infections (Caceres *et al*., 1991; Venugopal and Venugopal 1995; Nweze *et al.,* 2004; Vineela and Elizabeth, 2005).

In Nigeria, many plants are used against infectious diseases, which today are frequent due to very poor hygienic conditions, cost and microbial resistance to the time- honoured antibiotics (Ngono Ngane *et al*., 2000). The continuing increase in the incidence of fungal infections together with the gradual rise in resistance of bacterial and fungal pathogens for antibiotics and antifungals highlights the need to find alternative sources from medicinal plants (Berkowitz, 1995, Jennifer and Paul, 2000). This work was designed to isolate and identify dermatophytes from infected individuals with physical skin lesions and to screen five medicinal plants for potential antidermatophytic activity. The selection of these plants for evaluation was based on ethanomedical information obtained from traditional healers in who used the plants for treatment of dermatophytic infection.

MATERIALS AND METHODS

Sample collection

Fresh leaves of *Euphorbia balsamifera* Ait, *Mitracarpus scaber* Zucc*, Pergularia tomentosa* L*, Streospermum kunthianum* Cham and *Holarrhena floribunda* (g, Don) Dur. And Schiz*,* were collected around Usmanu Danfodiyo University (permanent site) Sokoto. The plants were identified and confirmed at Usmanu Danfodiyo University, Sokoto Herbarium (Botany unit, Department of Biological Science). Voucher specimens were deposited in the Herbarium. The plant materials (fresh leaves) were air dried, pulverized into a fine powder.

A total of two hundred and fifty (250) samples of infectd skin, hair and nail were collected from infected patients with clinical manifestations of dermatophytosis within Sokoto metropolis (schools, barbing salons and hospitals).The sites of infections were first cleaned with surgical spirit, scales from the skin lesions were collected by scraping outwards with a blunt scalpel from the edge of the lesion. Specimens from the scalp were collected using forceps to pluck from the scalp. Samples of nail were collected by scraping materials from underneath the nail and from edge of the nail. All the samples were collected on a clean piece of paper (5cm square) the papers were folded to enclose the specimen, they were labelled and transferred to mycology laboratory of the Biological Sciences Department of Usmanu Danfodiyo University, Sokoto for the culture of associated dermatophytes.

Extraction and fractionation procedure

Extraction and fractionation of the plants extract was carried out by activity guided fractionation according to Moris and Aziz, 1976. The procedure was carried out using ethanol-water (1:1 v/v) and different organic solvents, (Hexane, Petroleum ether and Cloroform).

Forty grams of the powdered plant materials were extracted using percolation process in 250ml distilled water and 250ml ethanol at 35^{o}C overnight. The extract was filtered and the filtrate was partitioned with 250ml hexane. The extract was separated by filtration. The hexane filtrate was evaporated to dryness at 40^{o}C to obtain residue. The remaining water ethanol extract was further partitioned with petroleum ether and chloroform using the same procedure above. The last remaining water ethanol extract was also evaporated to dryness to yield residue. The dried extracts were reconstituted in water at different concentrations of 10, 20, 40, 80 and 160 mg/ml respectively. Another extraction was carried out using 40g of procured plant material with 500 ml distilled water at 35^{o}c overnight. The extract was filtered and evaporated to dryness, and residues were obtained in gram.

Isolation and identification of dermatophytes

Microscopic examination

Microscopic examination was carried out in accordance with Mackie and McCartney (1999). The hair, nail and skin scrapings were examined microscopically for the characteristic of macroconidia and microconidia, presence of hyphae and arthroconidia. Samples were treated with 20% potassium hydroxide (KOH) solution by flooding on slides. Cover slips were used with application of gentle heat. Microscopy was carried out under low power and subdued light. Infected hair and skin were seen encased in regular sheath of arthrospores that doubled their normal thickness; lactophenol cotton blue was used to improve visualization.

Inoculation and isolation of dermatophytes from samples

Scrapings, of skin and nail were reduced in size to pieces approximately 1 mm across and the hair roots were cut into similarly sized fragments. Both samples were planted on the surface of selected medium that is Sabourad Dextrose Agar containing chloramphenicol at 500 mg/L.The culture media were incubated at 30^{o}C for up to 21 days. After isolation the cultures were transferred to freshly prepared SDA media to obtain pure cultures. Pure cultures were also maintained in SDA slants at 5±1^{0}c. The test dermatophytes were identified by their cultural morphology and microscopic characteristics (Hartman and Rohde, 1980 and Cheesbrough, 2003).

Identification of dermatophytes

Identification of dermatophytes was done in accordance with Hartman and Rohde (1980). The identification was based on colonial appearance, pigment production and the micro morphology of the spore produced. Cultures were examined at 4 or 5 days intervals from the onset. Some characteristics were also noted the texture, colour and shape of the upper thallus and the production of pigment on the underside. Identified isolates were sent for confirmation at IITA Ibadan (Germ plasm unit). The confirmed isolates were: - *T. rubrum, T. mentagrophyte*, *M. gypseum*, *M. audouinii* and *Microsporum specie*.

Invitro Anti-Fungal Assays

Determination of the antidermatophytic activity using Agar incorporation method

The preliminary anti-dermatophytic activities of the aqueous and organic solvents extracts were evaluated using dermatophytes

this work. We would also like to acknowledge the efforts made by the laboratory assistants who assisted during laboratory analysis.

REFERENCES

Abere TA, Onwukaeme DN, Eboka CJ (2007). Pharmacognostic evaluation of the leaves of *mitracarpus scaber* Zucc (rubiaceae) *Trop.* J. Pharmaceut. Res. 6(4): 849-853.

Anonymous (2008). Fungal infectiosn of the skin and skin structure. Available at http://www.doctorfungus.org/mycoses/human/ other/skin-index.htm. p. 1-2.

Berkowitz FE (1995). Antibiotic resistance of bacteria. South *Africa med. J.* 88: 797-806.

Chakraborty A, Chowdhury BKI, Bhattacharya P (1995). Clausenol and Clausenine two carbazole alkaloids from clause nine two carbazole alkaloids form *Clausena anisata* phytochemistry; *60: 295-298.*

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

David G, Richard CBS, John FP (1997). Medical microbiology. Aguide to microbial infections palliogenesis, immunity, laboratory diagnosis and control Ed 15th EL ST publishers. p. 558-564.

Elewski BE (1996). The dermatophytoses. Semin Cutanmed Surg. 2: 1043-1044.

Guest PJ, Sam WM Jr (1998). Dermatophyte and superficial fungi In: Sam wwwjrlynch P. J. Principle and practice Dermatology. New York p. 3-4.

Gupta AK, Sibbalad RG, Lynde CW (1997). Onychomycosis in children prevalence and treatment strategies *JAM Acad Dermatol* 36(3): 395-402.

Gbodi TA, Irobi ON (1992). Antifungal properties of crude extracts of Aspergillus quardrilineatus. *Afr. J. pharm. Pharm. Sci.* 23(1): 32-33

Hartmann G, Rohde B (1980). *Introducing mycology* Dermatophytes pp. 8-14.

Hassan SW, Umar RA, Ladan MJ, Nyemike P, Wasagu RSU, Lawal M, Ebbo AA (2007). Nutritive value, phytrochemical and antifungal properties of *Pergularia tomentosa* L. (Asclepiadaceae). *Int'l J. Pharmacol.* 3(4): 334-340

Jennifer MF, Paul AL (2000). Emerging Novel anti fungal agents infectious Diseases *Reserch Pharmacia and up john. USA*. 5(1): 25-32.

Mackie, McCartney (1999). Practical medical microbiology edn 14th Harcourt brace and company limitd p. 695-696.

Moris KS, Azizi K (1976). Tumour inhibitors 114 Aloe Emodin: Anti leukemic principle isolated from Rhamnus frangular L. J. Nat prod. Lioydia. 39(4): 223-225.

Mukoma FS (2000). Dermatophytes: Their taxonomy, ecology andD epartment of biological sciences, University of Bots wana, Garborone, Bost wana pp. 4-6.

Ngono Ngane A, Biyif L, Amvamzolo PH, Bouchet PH (2000). Evaluation of antifungal activity of extracts of two camerounian Ruthceae zanthoxy lep rieumii Guill and *Zanthoxylum xanthory* loides waterm. *.J. Ethano Pharmacol* 70: 335-342.

Nweze ELO, Okafor JI, Njoku O (2004). Antimicrobial activities of methanolic extracts of *Trema guineensis* (Schumm and thorn) and Morinda lucida bench used in Nigeria Bo research 2: 39.

Padmaja V, Thankamany V, Hara N (1995). Biological activities of Amonaglabra. J. Ethanopharmacoli. 48: 21-24.

Rober T, Brodell MG, Giorgro Vescera MSn (2004). File://A: postgraduate medicine pearls indeamatology.

Simpanya MF, Baxter M (1996). Isolation of fungi from the pelage of cats and dogs using the hair brush technique, mycop athologia 134: 129-133.

Subramanian SD, Sathish K, Arulselvan P, Senthikumar GPO (2006). Invitro antibacterial and antifungal activities of ethanolic extrac of Aloe Vera Leaft Gel *J. Pant Sci.* 1: 348-355

Van-wyk B (1997). Medicinal plants of South Africa. Petoria: Briza publication p. 88-102.

Venugopal PV, Venugopal TV (1994). Antiderrmatophytic activity of neem (*Azdirachta indica*) leaves *Invito In. J. Pharmacol.* 26: 141-143.

Vineela CH, Elizabeth KM (2005). Antimicrobial activity of marine algae of visakha patnamcity. And hrapradesh. *Asian Jr. Microbial. Biotech Env.* Sc. 7: 209-212.

Wokoma EC, Essien IE, Agwa OK (2007). The invitro antifungal activity of garlic (*Allium sativum)* and onion (*Allium cepa)* extracts against dermatophytes and yeast. *The Nig. J. Microbiol.* 21: 21489

Yuanwu JY, Fanyang T, Wenchuan L, Yonglie C, Qijin (2009). Recent dermatophyte divergence revealed by comparative and phylogenic analysis of mitochondrial genomes. BMC genomics 10: p. 238, pp. 1471-2164.

Zacchino AS, Lopez NS, Pezzenati DG, Furlan LR, San Tecchia BC, Munoz L, Giannini MF, Rodriguez MA, Enriz DR (1999). Invitro Evaluation of antifungal properties of pheny propanoids and related compounds acting against dermatophytes *J. Nat. Prod.*, 62: 1353-1355.

Zavala MAS, Salaud, PG, Perez RMG (1997). Anti miorobial screening of some medicinal plants. Phytothe. Res. p. 11, pp. 368-371.

Dermatophyte Infections

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Dermatophytes are fungi that require keratin for growth. These fungi can cause superficial infections of the skin, hair, and nails. Dermatophytes are spread by direct contact from other people (anthropophilic organisms), animals (zoophilic organisms), and soil (geophilic organisms), as well as indirectly from fomites. Dermatophyte infections can be readily diagnosed based on the history, physical examination, and potassium hydroxide (KOH) microscopy. Diagnosis occasionally requires Wood's lamp examination and fungal culture or histologic examination. Topical therapy is used for most dermatophyte infections. Cure rates are higher and treatment courses are shorter with topical fungicidal allylamines than with fungistatic azoles. Oral therapy is preferred for tinea capitis, tinea barbae, and onychomycosis. Orally administered griseofulvin remains the standard treatment for tinea capitis. Topical treatment of onychomycosis with ciclopirox nail lacquer has a low cure rate. For onychomycosis, "pulse" oral therapy with the newer imidazoles (itraconazole or fluconazole) or allylamines (terbinafine) is considerably less expensive than continuous treatment but has a somewhat lower mycologic cure rate. The diagnosis of onychomycosis should be confirmed by KOH microscopy, culture, or histologic examination before therapy is initiated, because of the expense, duration, and potential adverse effects of treatment. (Am Fam Physician 2003;67:101-8. Copyright© 2003 American Academy of Family Physicians.)

Members of various family practice departments develop articles for "Practical Therapeutics." This article is one in a series coordinated by the Department of Family Medicine at the Medical University of South Carolina. Guest editor of the series is William J. Hueston, M.D.

See page 7 for definitions of strength-of-evidence levels.

The dryness of the skin's outer layer discourages colonization by microorganisms, and the shedding of epidermal cells keeps many microbes from establishing residence.[1] However, the skin's mechanisms of protection may fail because of trauma, irritation, or maceration. Furthermore, occlusion of the skin with nonporous materials can interfere with the skin's barrier function by increasing local temperature and hydration.[2] With inhibition or failure of the skin's protective mechanisms, cutaneous infection may occur.

Microsporum, Trichophyton, and Epidermophyton species are the most common pathogens in skin infections. Less frequently, superficial skin infections are caused by nondermatophyte fungi (e.g., *Malassezia furfur* in tinea [pityriasis] versicolor) and Candida species. This article reviews the diagnosis and treatment of common dermatophyte infections.

A characteristic feature of dermatophyte infections is an inflammatory pattern at the edge of the skin lesion, noted by redness and scaling or, occasionally, blister formation.

Dermatophytoses

Because dermatophytes require keratin for growth, they are restricted to hair, nails, and superficial skin. Thus, these fungi do not infect mucosal surfaces. Dermatophytoses are referred to as "tinea" infections. They are also named for the body site involved.

Some dermatophytes are spread directly from one person to another (anthropophilic organisms). Others live in and are transmitted to humans from soil (geophilic organisms), and still others spread to humans from animal hosts (zoophilic organisms). Transmission of dermatophytes also can occur indirectly from fomites (e.g., upholstery, hairbrushes, hats).

Anthropophilic organisms are responsible for most fungal skin infections. Transmission can occur by direct contact or from exposure to desquamated cells. Direct inoculation through breaks in the skin occurs more often in persons with depressed cell-mediated immunity. Once fungi enter the skin, they germinate and invade the superficial skin layers.

In patients with dermatophytoses, physical examination may reveal a characteristic

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Epidemiology of dermatophytoses in an area south of Tehran, Iran

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Abstract

Dermatophyte infections have been considered to be a major public health problem in many parts of the world. The aim of this study was to identify the etiological and epidemiological factors of dermatophyte infections in an area south of Tehran. A total of 1254 patients suspected to have dermatophytic lesions were examined over a period of three years (1999-2001). Material collected from skin, hair, and nails was submitted to direct microscopic examination using KOH, cultured in Sabouraud dextrose agar and microscopically examined for colony morphology, in order to the identify the 169 dermatophytes isolated. The prevalence of dermatophytoses was 13.5% (95% CI: 11.7–15.5%). Their incidence was 10.6 per 100,000 person-years (95% CI: 8.5–13.2). *Epiderophyton floccosum* was the most frequent dermatophyte isolated (31.4%) followed by *Trichophyton rubrum* (18.3%), *T. mentegrophytes* (17.2%), *T. violaceum* (16.6%), *Microsporum canis* (6.5%), *T. verrucosum* (4.7%) and *M. gypseum* (4.1%). *Epidermophytes floccosum* was found to be the most common isolated dermatophyte in age groups 20–29 (30.2%). Tinea corporis (31.4%) was the most common type of infection, followed by tinea cruris (20.7%), tinea manuum (15.4%), tinea capitis (12.4%), tinea pedis (10.6%), tinea faciei (7.1%), and tinea unguium (2.4%). The frequency rate of all of the types of tinea was higher in males than in females. The anthrophilic species *E. floccosum* was the most common dermatophyte as a causative agent of tinea. The most prevalent fungal infection was tinea corporis caused by *E. floccosum*.

Key words: dermatophytoses, epidemiology, Iran, tinea

Introduction

Mycotic infections have been considered to be a major public health problem in many parts of the world [1–4]. They are most prevalent in economically underdeveloped and developing countries where they constitute a major public health problem [5–12]. The prevalence of superficial mycotic infections has risen to such a level that skin mycoses now affect more than 20–25% of the world's population, making them one of the most frequent forms of infection [13]. The cause for this increase in the prevalence of skin mycoses possibly may be found in the public's socioeconomic status as well as, poor hygiene and sanitary conditions.

The distribution of the dermatophytoses and their etiological agents varies with geographical location; some species are widely distributed whereas others are geographically restricted [9, 14–15]. The trend of living in communities, contact with animals, the use of -antibiotics, corticosteroids, and antineoplastic drugs are some of the factors that contribute to the increase in the risk of infection by fungi especially by dermatophytes [9–14].

The prevalence and distribution of the dermatophytoses have not been well defined in Iran and extensive epidemiological surveys of these infections have not been carried out. However, a few epidemiological studies on the dermatophytoses in Iran have been published [7, 16–19]. Identification of the dermatophyte species involved may be useful in directing surveys to the environmental and animal sources of infection. There is a need to educate the general population concerning the danger of acquiring infections from infected persons as well as from animals. For these

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Structural and optical characterization of metal tungstates (MWO_4; M=Ni, Ba, Bi) synthesized by a sucrose-templated method

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Abstract

Background: Metal tungstates have attracted much attention due to their interesting structural and photoluminescence properties. Depending on the size of the bivalent cation present, the metal tungstates will adopt structures with different phases. In this work, three different phases of metal tungstates MWO_4 (M= Ba, Ni and Bi) were synthesized via the sucrose templated method.

Results: The powders of $BaWO_4$ (tetragonal), $NiWO_4$ (monoclinic) and Bi_2WO_6 (orthorhombic) formed after calcination temperatures of 750, 650 and 600°C for 4 h respectively are found to be crystalline and exist in their pure phase. Based on Scherrer estimation, their crystallite size are of nanosized. BET results showed $NiWO_4$ has the highest surface area. $BaWO_4$ exhibited less Raman vibrations than the $NiWO_4$ because of the increased lattice symmetry but Bi_2WO_6 showed almost the same Raman vibrations as $BaWO_4$. From the UV-vis spectra, the band gap transition of the metal tungstates are of the order of $BaWO_4$ > Bi_2WO_6 > $NiWO_4$. Broad blue-green emission peaks were detected in photoluminescence spectra and the results showed the great dependence on morphology, crystallinity and size of the metal tungstates.

Conclusion: Three different phases of metal tungstates of $BaWO_4$ (scheelite), $NiWO_4$ (wolframite) and Bi_2WO_6 (perovskite layer) in their pure phase were successfully prepared by the simple and economical sucrose-templated method. The highest surface area is exhibited by $NiWO_4$ while largest band gap is shown by $BaWO_4$. These materials showed promising optical properties.

Keywords: Metal tungstates, Sucrose, Optical

Introduction

Metal tungstates with formula MWO_4 have attracted much attention due to their interesting structural and photoluminescence properties [1-5]. These materials have found applications in scintillation counters, lasers and optical fibers [6,7]. Some of the divalent transition metal tungstates have also gained commercial interest in lasers and fluorescent lamps, while some are of special importance due to their electrical conductivity and magnetic properties. In addition, these materials also find applications as catalysts and humidity sensors [8,9].

In the MWO_4 compounds, if M^{2+} has small ionic radius < 0.77 Å (Ni = 0.69), it will belong to the wolframite-type monoclinic structure where the tungsten atom adopts an overall six-fold coordination [10]. However, if larger bivalent cations with ionic radius > 0.99 Å (Ba=1.35), they exist in the so-called scheelite-type tetragonal structure where the tungsten atom adopts tetrahedral coordination. Bismuth tungsten oxide belongs to the orthorhombic system, space group $Pca2_1$, and crystallizes in a layered crystal structure including the corner-shared WO_6. The Bi atom layers are sandwiched between WO_6 octahedral layers [11]. It is the simplest member of the Aurivillius family from $Bi_2A_{n-1}B_nO_{3n+3}$ (A=Ca, Sr, Ba, Pb, Bi, Na, K and B=Ti, Nb, Ta, Mo, W, Fe) (when n=1) of layered perovskites, which structurally comprises of alternating perovskite-like slabs of WO_6 and $[Bi_2O_2]^{2+}$ layers. Recently, many studies have been reported on the preparation and characterization of metal tungstates using various

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preparation methods such as Czochralski [12], precipitation [13,14], hydrothermal [11,15], solid state [16], pulsed laser deposition [17]. Meanwhile the nanostructures of metal tungstates in different crystal structures including nanorods, nanoparticles, hollow clusters and others have been prepared by chemical and physical methods. For Bi_2WO_6, its nanometer sheet shaped was obtained through hydrothermal treatment at pH=11, heated at 200°C for 24 hours and finally thermally treated at 400, 600 and 800°C for 3 hours [18]. $BaWO_4$ in the rhombic shape was prepared by a molten flux reaction using alkali metal nitrates as the reaction media [19]. Nickel tungstate ($NiWO_4$) nanoparticles were successfully synthesized at low temperatures by a molten salt method at a temperature as low as 270°C, where the mixture of $NaNO_3$ and $LiNO_3$ was used as the molten salt medium with 6:1 mass ratio of the salt to the $NiWO_4$ precursor [20]. Generally, these methods require expensive and sophisticated equipment, high temperatures with long processing times, expensive precursors and high consumption of electric energy.

Prabhakaran et al. [21] had used a cheaper and simpler method of using sucrose in order to synthesize yttria-stabilized zirconia (YSZ) nanoparticles in both acidic and basic solutions. The analyses consistently reported to have fairly uniform nanoparticles with small size, containing both tetragonal and monoclinic phases with crystallite size between 10 and 30 nm. Due to its simplicity, the sucrose-template method has great potential for manufacturing high quality ultrafine ceramic oxides economically [22] and this creates a new approach for synthesis of the other ceramic materials. In this method, the -OH and -COOH groups of the decomposed sucrose products help in binding the metal ions in the homogeneous solution, which reduces the chances of precipitation. During the decomposition process, a voluminous, organic-based, black, fluffy mass of carbonaceous material is formed which upon heating will decompose further into carbon dioxide and water and a large amount of heat is generated. The outgoing gases prevent agglomeration, and form pores and fine particles with high surface area in the final products. The aim of this paper is to synthesize the different crystal structures of $BaWO_4$, $NiWO_4$ and Bi_2WO_6 by a sucrose templated method and to characterize the materials for their structural and optical property by X-ray Diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM), Brunaer-Emmet-Teller (BET) and Raman spectroscopy while optical properties were investigated using UV-vis and photoluminescence spectroscopy.

Experimental details

Preparation of powders

The desired metal nitrates [$Ba(NO_3)_2$, $Ni(NO_3)_2 \cdot 6H_2O$, $Bi(NO_3)_2$] of 2.6135, 2.9081 and 4.8511 g were individually dissolved in distilled water before being mixed into an aqueous solution of sucrose. This is followed by addition of an equal volume of 2.4633 g of ammonium metatungstate to maintain stoichiometric ratio (1:1) with continuous stirring. Sucrose acts as a template and the ratio of sucrose to metal used was 3:1. Towards the end of the evaporation, the precursor solution (after further heating) gave rise to a fluffy black organic mass. The carbon rich mass was easily crushed to form the precursor powders. Precursor powders are denoted as MW*p* (M= Ba, Ni, and Bi). Calcination treatment was applied in the next step because of the large amount of organic compounds present in the crunchy powders. The temperatures and durations for calcinations were derived from the results of the thermogravimetric analysis whereby processes such as dehydration and other volatilizations to go to completion before proceeding to higher temperatures.

The calcination treatment applied to the samples involved heating at the rate of 10°C/min and the temperature was held constant for 4 h for each thermal change as inferred from the thermal analysis to allow completion of each of the processes. The three powdered precursors, MW*p* (M= Ba, Ni, and Bi) were subsequently calcined at 750, 650 and 600°C respectively for 4 h and the samples were denoted as MWO_4 (M= Ba, Ni, and Bi).

Characterization

The formation of oxides was monitored by X-ray diffraction (XRD) measurements using Siemen D5000 with a copper K_α radiation tube and wavelength λ of 1.54 Å, operated at 40 kV and 40 mA. The X-ray powder diffraction patterns were obtained in the range 5-60°, with increments of 0.05°. The crystalline phases were identified by using the International Centre for Diffraction Data (ICDD). The full width at half maximum (FWHM) of the diffraction peaks obtained from the refinement have been used to calculate the crystallite size. Specific surface area (S_{BET}) measurements were made with a Quantachrome AUTOSORB-1 model by nitrogen adsorption at -196°C using the BET isotherm. Samples were degassed under flowing argon at 250°C for 9 h before being adsorbed by nitrogen. The surface morphology of the samples was e analyzed using the Field Emission Scanning Electron Microscope, FESEM JSM-7500F/7500FA (JEOL) at magnification of 20,000 ×. This morphological analysis can provide information on the prevalent surface features. FESEM images allowed us to estimate the average particle size distribution of all three samples through the counting of approximately 150 particles using Image tool software. Diffuse reflectance spectra were obtained using a UV-Visible Spectrophotometer (Shimadzu). Raman spectra was collected by InVia Raman Microscope Renishaw spectrometer using

Biology

STUDIES ON FUNGI ASSOCIATED WITH LABORATORY ANIMAL 'GOLDEN HAMSTER' AND ANTIBIOTIC EFFECTS OF ALOE SAP, GARLIC EXTRACT AND ONION OIL

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SUMMARY: Healthy hair samples from golden hamsters were examined for the presence of dermatophyte and non-dermatophyte using baiting technique and direct inoculation. Thirty four species and 2 varieties attributed to 17 genera were recovered. Paecilomyces variotii (isolated from 84.4% of the examined hair) and A. niger (81.3%) were more frequently on Sabouraud's dextrose agar (SDA) without cycloheximide. Our results have clearly demonstrated that the hair of hamster was free from true dermatophyte. Using the dilution plate method many different fungal species were isolated from cage material (7 genera and 10 species + 1 variety); from faeces (10 genera and 17 species); from standard chow (3 genera and 6 species) of hamster on SDA without cycloheximide. P. variotii which was the most frequent fungus in the preceding 3 substrates; was completely absent in the presence of cycloheximide in SDA. The present study has demonstrated for the first time the isolation of Trichophyton rubrum from hamster faeces. Also several saprophytic and cycloheximide resistant fungi were isolated. In the air of hamster cage Cladosporium cladosporioides, Penicillium chrysogenum, Alternaria alternata and Scopulariopsis brevicaulis were the most dominant species on SDA with or without cycloheximide.

Using the agar diffusion method; Aloe sap, onion oil, garlic bulb extract and aqueous leaf extracts of Andropogon citratus, Euphorbia sp. and Ruta graveolens were tested for their antifungal activity on 10 Fungal species. It was observed that onion oil exhibited a high inhibitory effect against most of the tested fungi.

Key Words: Saprophytic fungi, cycloheximide, hamster, hair, faeces, antifungal activity.

INTRODUCTION

The skin of animals is contaminated by numerous fungi, some of which are opportunistic pathogens or allergens. Several investigations have reported the occurrence of dermatophytes on the apparently healthy skin of domestic and wild animals (1-3). Also animal pens and animal faeces represent a good habitat for keratinophilic and saprophytic fungi (4,5).

The objective of this work, was to determine:

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Original Article

Use of Single-enzyme PCR-restriction Digestion Barcode Targeting the Internal Transcribed Spacers (ITS rDNA) to Identify Dermatophyte Species

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Abstract

Background: Dermatophytes are the most common causative agents of superficial mycoses. Species identification of these fungi is important from therapeutic and epidemiological point of wive. Traditional approaches for identification of dermatophytes at the species level, relying on macroscopic and microscopic features of the colonies, usually are time-consuming and unreliable in many circumstances. Recently a broad varieties of rapid and accurate DNA-based techniques were successfuly utilized for species delineation of dermatophytes.

Methods: The ITS1-5.8S-ITS2 region of rDNA from various reference strains of dermatophyte species were amplified using the universal fungal primers ITS1 and ITS4.The PCR products were digested by a single restriction enzyme, *Mva*I. The enzyme was evaluated in both *in silico* and practical PCR-RFLP assay to find the exact differentiating restriction profiles for each species. To validate the standardized PCR-RFLP system, all tested strains were subjected to sequencing and sequence analysis.

Results: The obtained RFLP patterns were specific for many species including *T. interdigitale*, *T. rubrum*, *T. violaceum*, *M. persicolor*, *M. audouinii*, *M. nanum* (*A. obtusum*) and *E. floccosum* but were similar for some closely related species such as *M. canis* / *M. ferrugineum*. Sequencing of the ITS1-5.8S-ITS2 fragment from all type strains affirmed the RFLP findings.

Conclusion: It was practically revealed that the ITS-PCR followed by *Mva*I-RFLP is a useful and reliable schema for identification and differentiation of several pathogenic species and can be used for rapid screening of even closely related species of dermatophytes in clinical and epidemiological settings.

Keywords: Dermatophytes, Identification, ITS, PCR-RFLP

Introduction

Dermatophytes are a group of specialized molds, affecting the superficial keratinized structures (skin, hair and nails) of human and animal hosts, producing dermatophytosis, commonly referred to as 'ringworm' or tinea. They are classified in three genera, *Epidermophyton*, *Microsporum*, and *Trichophyton* containing three ecological groups of anthropophilic, zoophilic and geophilic species (1, 2). Dermatophytes are the most common agents of cutaneous fungal infections worldwide(3, 4). Infections are contagious and represent a significant public health problem in many parts of the world. Dermatophytosis is not a reportable disease but

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SOME EPIDEMIOLOGICAL STUDIES ON RINGWORM IN CATTLE AT ASSIUT GOVERNORATE, EGYPT

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ABSTRACT

Ringworm is an infectious disease of animals caused by different species of dermatophytes. It is a major public and veterinary health problem reported in the different parts of the world including Egypt and causes great economic loss, through body weight loss, skin injuries, and long course of treatment. This study was carried out on 230 cattle infected with skin lesions from a total number of 1350 animals, in different ages, sex, breeds and in different seasons and localities at Assiut Governorate. These animals were subjected to clinical and laboratory examinations and the infected animals were subjected for treatment with different antifungal agents as (Mycostatin ointment, Trocid Lotion, Tincture iodine, Garlic Juice, Mange-cide ointment.). The obtained results indicated that animals housed in close proximity to each other for long periods in the fattening period in the presence of infected one lead to spreading spores of fungus between animals and so a greater infection rate was recorded in males compared with females. Also, the result reported a higher infection rate in winter than summer season. The percentage clinically diagnosed cattle with ringworm was 17% at Assiut Governorate with higher frequency of isolated *Trichophyton verrucosum* (98 %) from the examined animals infected with ringworm (98%). The most effective and cheap drug used for treatment of infected animals was Tincture iodine in concentration of 5 to 7% which used as paint or spray with 100 % recovery rate.

Key words: Epidemiology, Ringworm, Cattle, Treatment.

this study. Among *Trichophyton* species, *T. verrucosum* was the most commonly identified (98% of the total fungi isolated). The fungi grow slowly on mycobiotic agar producing slightly folded, heaped glabrous gray white colony. On slide culture slides stained with lactophenol cotton blue, a characteristic septet hyphae with chlamydocondias arranged in chains with single microconidia were identified. *Trichophyton mentagrophytes* was the second frequent isolated fungi from calves with ringworm (2 % of the total isolated fungi). The colony formed on mycobiotic agar after 2-4 weeks of culture at 28 °C appeared as a buff to tan thallus color and exhibited radial folds. On microscopic examination, the microconidia were pyriform in shape while the macroconidia were cigar-shaped with thin walls having a narrow attachment to the hyphae containing 3-5 cells.

The present study revealed that 17.03 % of examined animals in Assiut Governorate were infected by species of superficial dermatophytes. This finding less than that reported by ***Al Ani et al. (2002)*** who found the prevalence of ringworm in infected calves 30.6% and more than that recorded by ***Haab et al. (1994)*** who found the prevalence of ringworm in infected calves 7.7 %.

Moreover, higher prevalence of infection was found amongst calves under the age of 6month (18.7%) in comparison with age more than 6 month (14.5%). This result showed that infection decreased with increase animal age. This result agreed with that stated by ***Acha and Szyfres (2003)*** who reported that dermatophytosis is more common when animals are immunosuppressed, have poor nutrition or are kept in high density populations and infections can be more persistent or widespread in young or sick animals.

Whether an animal becomes infected, after contact with a dermatophyte, may depend on the animal's age, the condition of its exposed skin, and grooming behavior. Young animals are more likely to have symptomatic infections ***(Weirzman and summerbell, 1995).***

Among livestock, ringworm infection was common in cold season (28.0%) in comparison to hot season (10.6%) and this result may be attributed to that the animals were stabled for long periods of time during cold season as well as high humidity during winter season, which facilitate the growth of spores and increases the susceptibility of animals to infection ***(Nooruddin and Singh, 1987)***.

Sex distribution of ringworm infection among animals showed that more males (28.3%) than female (8.0%) were infected. This result may be attributed to animals housed in close proximity to each other for long periods in the fattening period and the presence of infected one leads to spreading

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An outbreak of a mixed infection of *Dermatophilus congolensis* and *Microsporum gypseum* in camels (*Camelus dromedarius*) in Saudi Arabia

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Summary

Although both *Dermatophilus congolensis* and *Microsporum gypseum* infections have been reported separately in camels, mixed infection involving both agents has not been reported to date. The authors describe a mixed infection of *D. congolensis* and *M. gypseum* in camels reared on a dairy farm in Saudi Arabia. A total of 131 out of 559 camels (23.4%) were affected. Forty-eight camels less than one year of age had discrete, circumscribed, crusty, hairless lesions, found in particular on the neck and forelegs. Eighty-three camels of varying ages had extensive hair matting with crusty, hairless lesions, especially on the flanks. Camel calves and young camels demonstrated a relatively greater amount of skin lesions. *D. congolensis* and *M. gypseum* were diagnosed by direct microscopy, isolation and histopathology.

Keywords

Camels – Dermatophilosis – Microsporum gypseum – Saudi Arabia.

Introduction

The Kingdom of Saudi Arabia has a surface area of 2,250,000 km^2, divided into three main geographical areas: the Asir-Hejaz region along the west coast; the Najd plateau sloping gently from the Asir highlands across the country to the Arabian Gulf; and the Rub'al Khali desert in the south.

Camel dermatophilosis, a skin disease characterised by hair matting and encrustation, has been reported in Kenya (5, 6, 7) and the Sudan (H. Agab, personal communication), both of which are tropical countries. This outbreak is the first to be reported from a country in the Middle East. In Africa, most camels are reared by pastoralists, for whom their milk provides sustenance, especially during the dry season when other animals fail to thrive, or die. In Saudi Arabia, however, camel rearing is one of many agricultural activities which is positively encouraged by the state as a commercial enterprise, where husbandry is of a higher quality. Therefore, it is important to examine the occurrence of camel dermatophilosis in the context of a different management system and ecoclimatic setting.

Different pathogenic dermatophytes have been reported in camels (2, 3, 4, 8, 9). The authors describe the first outbreak of camel dermatophilosis to occur concurrently with infection by a dermatophyte, *Microsporum gypseum*.

Materials and methods

Epidemiology

The Qaşīm region is a large farming area in Najd, 300 miles north of Riyadh, the capital of Saudi Arabia. The region is arid, with temperatures ranging from 2°C in the winter to 50°C in the summer. The annual rainfall in the region is 120 mm (1). The topography is that of a dry, rocky desert, with rain in both winter and spring. Most of the area is cultivated, with wheat, barley, dates and citrus fruit grown using underground irrigation. In one privately operated dairy farm, 559 camels

are reared intensively in pens with no shelter, exposing them to sunlight and rain. Camels were examined once a week for disease from February to May 1997. This was performed by giving each camel a thorough physical examination in a crush. Skin scabs were then examined from four infected adult camels, four infected young camels, three infected camel calves (less than one year of age) with discrete lesions, and three infected camel calves (one year of age) with generalised lesions.

Bacterial and fungal isolation

The fourteen skin scabs were emulsified with Ringer's solution and then inoculated on sheep blood agar (SBA) (6% sheep blood agar, nutrient agar and 0.4% sodium chloride), and Sabouraud's dextrose agar (SDA) plates. After incubation for 48 h, *Dermatophilus congolensis* colonies were obtained on the SBA plates, emulsified with Ringer's solution and inoculated into sugar fermentation tubes, gelatin and litmus milk. The catalase test was performed by combining one colony from an SBA plate with a drop of 30% hydrogen peroxide on a glass slide and observing the evolution of gas.

Plate disc antibiotic sensitivity was tested by inoculating the emulsified organism from the nutrient broth onto an SBA plate with a wire loop to obtain confluent growth. Sensitivity discs 6 mm in diameter were placed on each plate, and the zones of growth inhibition measured qualitatively after incubation at 37°C for 72 h. Another set of fourteen skin scabs from the same camels were mixed in 10% potassium hydroxide (KOH) solution for direct microscopic examination. A separate set of fourteen skin scrapings from the same camels were inoculated into SDA slants and incubated at room temperature (23°C-27°C) for fourteen days. Needle-mounts from the colonies were stained with lactophenol cotton blue and examined by oil-immersion microscopy.

Histopathology

The biopsy specimens were fixed in 10% formal saline, embedded in wax, cut at 5 µ and stained with haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) in order to conduct histopathological studies.

Results

An infection was found in 131 camels out of 559 (23.4%) (Table I). There were two types of infection, as shown by the two different types of lesions. The first type, found in forty-eight camels less than one year old, was characterised by discrete, well-circumscribed, crusty, hairless lesions 1-2 cm in diameter. The lesions were located primarily on the neck and forelegs, but there were also a few on the shoulders. In the 'Majaheem' breed, the lesions were especially prominent as their white colour contrasted with the black colour of the skin of the camel (Fig. 1). On removal of the crusts, the skin was almost normal, with no erythema, inflammation or bleeding.

The second type of infection was found in 83 camels of varying ages. The infections were diffuse, covering the flanks, legs and the ventral aspects. There was extensive matting of the hair; when the hair was removed, raw, hyperaemic areas became apparent. In many cases, large, brown crusts of variable sizes were present (Fig. 2). Young and growing calves showed a proportionally higher incidence of the disease, with lesions covering 50% or more of the skin.

Table I
The lesions in different age groups of camels

Camel groups	Camels affected			
	Adults (> 4 years)	Growing calves (1 to 4 years)	Young calves (< 1 year)	Total
Total examined	259	48	252	559
Number affected	14	19	98	131
Diffuse lesions	14	19	50	83
Discrete lesions	0	0	48	48
Percentage affected	5.4	39.6	38.9	23.4

One of the observations made during examination of the camels was that no tick had been found on any camel for the previous five months.

Direct 10% KOH examination of the skin scrapings revealed fungal mycelia and arthrospores in the macerated debris and infected hairs, characterised by large-spore (6 to 8 µ) ectothrix arrangement of arthrospores. Mycelia were seen within the hair shaft running parallel to the length of the hair (Fig. 3). A direct Gram's stain was carried out on smears of the scrapings, revealing dense forms of Gram-positive branching filaments with a diameter of approximately 1 µm.

Isolation processes

After incubation for 48 h, all the inoculated SBA plates revealed colonies typical of *D. congolensis*. The colonies were white, 1-2 mm in diameter, rough, convex, with a crater-like shape and a 1 mm zone of complete haemolysis. The colonies were firmly attached to the agar. Microscopy revealed a predominance of filamentous forms, but after several passages, coccoid forms and filaments featuring both longitudinal and transverse divisions were present (Fig. 4). The biochemical behaviour was similar to that described by Gitao *et al.* in 1990 (7). The *D. congolensis* isolate was sensitive to streptomycin (10 µg), ampicillin (25 µg), kanamycin (30 µg), gentamicin (10 µg), sulphamethoxazole (200 µg), chloramphenicol (30 µg). The *D. congolensis* isolate was resistant to co-trimoxazole (25 µg) and tetracycline (25 µg).

The culture on SDA revealed three saprophytes. One was brown, folded and leathery, and microscopy revealed no distinct morphological identification pattern. The second was velvety, green and folded, and was white on the reverse side. Microscopy revealed a large number of sporangiospores. The

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Full length Research Paper

Anti-fungal evaluation of *Diodia scandens SW* leaf extracts against some dermatophytes in Ukwuani Region of Delta State, Nigeria

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Diodia scandens **leaf is applied topically for the treatment of various superficial skin infections among the Ukwuani aborigines of Delta state, South-South Nigeria. To obtain a scientific base for this practice, its phytochemistry and antidermatophyte potentials against three dermatophytes, *Microsporium gypseum, Trichophyton mentagrophytes* and *Trichophyton rubrum* isolated from infected hairs, nails and skins (mostly children) in Obiaruku, Ukwuani Local Government Area of Delta State, Nigeria were evaluated. Methanol and aqueous extracts of the leaves were tested using the agar diffusion method at extract concentrations 100mg/ml, while griseofulvin was used as the standard drug. The phytochemical investigation revealed the presence of saponin, flavonoid and tannins and cardiac glycosides. The anti-fungal activity showed that all the isolates were susceptible to both the organic and aqueous extracts, though the methanolic extracts had wider diameter of inhibitions than the aqueous extract. The highest susceptibility was observed against *T. mentagrophytes* (22.2mm) followed by *T. rubrum* (21.0mm), while *M. gypseum* (18.5mm) was the least. Their minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) value was 3.13/6.25mg/ml for *T. mentagrophytes* and *T. rubrum*, and 6.25/12.5mg/ml for *M. gypseum.* The anti-fungal activity of the extract was not significantly affected by variations in temperature (30-100°C) and pH (2.0-10) for 1hour. This work, therefore may probably justify the traditional application of this plant in the management of skin diseases.**

Keywords: *Diodia scandens,* superficial skin infections, antimicrobial activity, and agar diffusion method

INTRODUCTION

Dermatophytes are a group of closely related fungi known to cause human superficial mycoses in many tropical countries where their prevalence still remain a public health problem. They are classified into 40 species of three genera; *Microsporium, Trichophyton, and Epidermophyton* based on the types of microconidia they produce (David et al*.,* 1997). *Microsporium* spp mostly affect hairs and skins, but not nails; *Trichophyton* spp infect hairs, skins and nails; while *Epidermophyton* spp infect skin and nails, but not hairs (David et al., 1997). The mode of spread is either by direct or indirect contact with an infected particle which is usually a fragment of keratin containing viable fungus. Indirect transfer may occur via the floor of swimming pools, bath rooms or on brushes, combs, towels and animal grooming implements (Shinkafi and Manga, 2011). The infections resulting from dermatophytes are hardly fatal but mostly debilitating and

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rubrum and Trichophyton mentagrophytes) used in this study were isolated from children with dermatophyte infections(infected skin, hairs and nails) in primary schools in Obiaruku, Ukwuani Local Government area of Delta state, Nigeria. The sites of the infection were first cleaned with surgical spirit, before using scalpel to scrap the scales, hairs and nails into clean white paper and all samples labelled appropriately for analysis. The collected samples were examined microscopically (with 10-20% KOH) for the characteristic macroconidia and microconidia, presence of hyphae and arthroconodia, and also plated on Sabouraud Dextrose agar (Lab M, India)(SDA) supplemented with 0.05% chloramphenicol and incubated at ambient temperature (28-30^{o}C) for 7-14 days. The fungal isolates were identified based on colonial appearance, pigment production on the underside and microscopic characteristics (Rebel and Taplin, 1970; Campbell, 1980; Hartman and Rohde, 1980). The pure culture was further confirmed by comparing them with stock cultures kept at the National Institute for Veterinary Research VOM, Nigeria.

Standardization of Inoculum

Fungal spores were harvested after 7 days old SDA slant culture was washed with 10ml normal saline in 2% Tween 80 with the aid of glass beads to help in dispersing the spores. The spore suspensions were standardized to 10^{5}spores /ml.

Antifungal Susceptibility Studies

Sabouraud Dextrose Agar (SDA) (Lab M, India) was prepared according to specifications, autoclaved (121^{o}C for 15minutes) supplemented with 0.05% chloramphenicol and dispensed into 11cm diameter Petri dishes. 1ml of each standardized spore suspension (10^{5}spores/ml) was evenly spread on the surface of the gelled SDA plates. Then, sterile cork a borer (6mm in diameter) was used to make well at the centre of each seeded plates. Thereafter, 0.2ml of the reconstituted aqueous and methanol extracts (100mg/ml) was applied into each labeled well. 0.2ml each of 20% DLMSO and standard drug griseofulvin, (100mg/ml) (Clarion Medicals Ltd. Lagos, Nigeria), served as negative and positive control respectively. The plates were incubated at ambient temperature for 1-7days and observed for growth. Anti-fungal activities of the extract as well as the controls were measured and recorded as means diameter of zones of inhibition around the three wells.

Determination of Minimum Inhibitory Concentration (MIC) of extracts

The MIC of the extracts was also carried out using broth dilution method as described in Ibekwe et al, 2001. Sabouraud dextrose broth prepared according to the manufacturer's instruction in separate test-tube sterilized at 121^{o}C for 15minutes and then allowed to cool. Two-fold serial dilutions of the aqueous and methanol extracts in the broth were made from the stock concentration of the extract to obtain 50, 25, 12.5, 6.25, 3.13 and 1.56mg. 0.1ml of the standardized inoculums (10^{5}spores/ml) was then inoculated into the different concentrations of the extracts in the broth. Controls were also set up along the test experiment. The test tubes of the broth were incubated at 30^{o}C for 1-7days and observed for turbidity of growth. The lowest concentration which showed no turbidity in the test tube was recorded as the MIC.

Determination of the Minimum Fungicidal Concentration (MFC) of extracts

Fresh Sabouraud Dextrose agar media were prepared, sterilized at 121^{o}C for 15mins and was poured into sterile Petri-dishes and left to cool and solidify. The contents of the MIC in the serial dilution were then sub-cultured onto the media and incubated at 30^{o}C for 1-7days and observed for colony growth. The MFC was the plate with the lowest concentration of extract and without colony growth.

Effect of temperature and pH on stability of extracts

The method of Doughari and Sunday, (2008) was followed in this analysis. 50mg/ml concentration of extracts was reconstituted in 20%DMSO, and 5ml of the suspension dispensed into five test tubes. The tubes were then treated at 4^{o}C in the refrigerator, 30^{o}C at room temperature, 60^{o}C, 70^{o}C and 100^{o}C using water bath for 1hours. They were tested for antifungal activities as described earlier. The effect of pH was determined by treating the extract at pH ranges of 2.0-10.0 using 1N HCL and 1N NaOH solutions respectively in a series of test tubes for 1hour. After 1hour treatment, each of the extract was neutralized (pH 7.0) once again using 1N HCL and 1N NaOH as the case may be. They were also