

Antimicrobial Potential of Iron Oxide Nanoparticles in Control of Some Causes of Microbial Skin Affection in Cattle

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Abstract: Biosynthesis and characterization of iron oxide nanoparticles and evaluation of its antimicrobial potential against isolated bacterial and fungal causes of skin affection in cattle were investigated. Out of 120 cases of cattle suffering from obvious skin lesions, samples of skin scrapings and hairs from animals were collected. The recovered fungal species from samples included *Trichophyton verrucosum* and *T. mentagrophytes* and bacterial species of *Dermatophilus congolensis*. The biological synthesis of Fe₂O₃ NPs was done using *Candida albicans* and the particles were identified and characterized by UV –visible spectrophotometer and scanning by electron microscopy (SEM) for detection of their particle size and the purity of the prepared powder. The antimicrobial effect of prepared Fe₂O₃ NPs against isolated *Dermatophytes* and *Dermatophilus* species that recovered from skin affection of cattle was studied. The Fe₂O₃ NPs had an inhibitory effect against the growth of *T. verrucosum* at concentrations of 3 mg/ml and 4 mg/ml which yielded inhibitory zone diameter of (10 ±0.5mm and 14±0.7mm), respectively (using well diffusion test). Whereas, the concentration of 5 mg/ml of Fe₂O₃ NPs showed an inhibitory zone diameter of (10±0.1 mm and 20±0.5 mm) using disc and well diffusion tests, respectively. On the other hand, in case of *T.mentagrophytes*, iron oxide NPs revealed an inhibitory effect at concentration of 1, 2, 3, 4 and 5 mg/ml by well diffusion test and at concentration of 3, 4 and 5 mg/ml by disc diffusion test. The treatment by Fe₂O₃ NPs had no effect on the growth of *Dermatophilus sp.* at the concentration ranged from 1- 3 mg/ml using disc diffusion test. While, the treatment by 4 mg/ml or more resulted in inhibition of bacterial growth. But in case of well diffusion test, lower concentrations of Fe₂O₃ NPs were required (2 mg/ml or more) for inhibition of *Dermatophilus sp.* growth. When the treated fungi or bacteria were subjected to SEM, the damage and rupture of their cell wall were detected in the area surrounding growth leading to leakage of the cell contents and finally cell death. Further studies are needed to investigate the efficacy of preparations of Fe₂O₃ NPs as ointments, skin lotions and synergistic effects of nanoparticles in combination with other antibiotics in the treatment of animal diseases.

Keyword: *Dermatophytosis*, Cattle, *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, Ringworm, *Dermatophilus congolensis*, Iron oxide nanoparticles, Biosynthesis.

1. Introduction

The environmental bacterial and fungal pollution contributes a major problem to human and animal health. The most significant problems to date are the skin affections in animal which are responsible for high economical losses in cattle due to skin damages and decrease in milk and meat production and may be transmitted to contact human workers in farms of infected animals. The skin diseases are of worldwide distribution and considered as a public health problem all over the world [1]. The ringworm is one of the most common infectious fungal skin diseases in cattle, which affects mainly the keratin layer in the skin and hair resulted

in their damage and the disease is caused mainly by *Trichophyton* and *Microsporum spp.*[2]. Moreover, *Trichophyton verrucosum* is the usual zoophilic dermatophyte involved in cattle ringworm throughout the temperate regions of the world [3,4 and 5] and it also affects, but with lower prevalence, sheep, goat and other animals [6, 7, 8]. Whereas, the presence of *T. verrucosum* in the hair coat of free-ranging animals, especially non-ruminants, is uncommon [2].The lesions are described as papillomatous raised areas with alopecia , thickening and wrinkling of affected skin localized mainly on the face ,

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breast and at the root of the animal tail [9,10]. On the other hand, the bacterial affections of cattle skin particularly with the *dermatophilus* species, can cause skin disease of animal, as reported by [11] and man [12]. This disease in cattle caused by a gram-positive actinomycetes, *Dermatophilus congolensis* which is characterized by acute or chronic, local or progressive and sometimes fatal exudative dermatitis; it starts as an erythema, progressing through serous exudation and drying to form characteristic matting of the hair [13,14,15]. Both fungal *dermatophytosis* and bacterial *dermatophilosis* which cause skin affections, have a worldwide distribution [2, 16] and had been reported by the Food and Agricultural Organization (FAO) to be major diseases, which affect cattle and other animals in the tropical and subtropical regions. The disease was reported in 1915 in cattle in the Belgian Congo [17]. The prevalence of the disease occurs during and immediately after the first rain [18]. The resistance to many of the traditional antifungal and antibacterial agents now in use has emerged. Although, antifungal drug resistance does not seem to be as much of a problem as resistance to antibacterial agents in bacteria, one long-term concern is that the number of fundamentally different types of antimicrobial agents that are available for treatment remains extremely limited. Hence, there is an inevitable and urgent medical need for antibiotics with novel antimicrobial mechanisms [19, 20]. In recent years, nanoparticle (NP) materials have received increasing attention due to their unique physical and chemical properties, which differ significantly from their conventional counterparts [21]. Recent studies have demonstrated antimicrobial activities of various NP materials, including silver [22, 23], copper [24], titanium dioxide [25], zinc oxide [26, 27, 28] and iron oxide nanoparticles [29, 30]. Therefore, the present study was undertaken to investigate the prevalence of fungal and bacterial skin affection in cattle, biosynthesis and characterization of Fe₂O₃ NPs and evaluation of its antimicrobial potential against isolated bacterial and fungal causes of skin affection in cattle. The traditional antimicrobials were used as a comparable agents to iron oxide nanoparticles.

2. Materials and Methods

2.1. Samples:

Samples were collected from 120 cases of cattle (of 6 months – 3 years' ages) in private farms at El- Minufiya governorate. The animals were housed indoors at the time of sampling. The obvious skin lesion that observed in animals was cleaned with cotton swabs soaked in 70% ethanol and then scrapped at the peripheral area with a sterile scalpel. The skin scrapings were collected in sterile petri-dishes labeled with the main animal characteristics and transferred to the laboratory for examination with standard methods [31].

2.2. Antifungal and antibacterial agents: Antifungal as (fluconazol 20 ug and griseofulvin 10 ug) and antibacterial as (gentamycin 10 µg and levofloxacin 5µg) were purchased from Sigma Chemical Company (USA).

2.3. Isolation and identification of fungi particularly dermatophytes species:

2.3.1. Direct microscopic examination for detection of dermatophytes species: After collection of samples, a piece of skin scraping or hairs were placed on a clean slide with few drops of KOH (10-25%) solution and/or Lacto-phenol cotton blue stain, covered with cover slip, and examined for

the presence of septet hyphae and characteristic arthroconidia of dermatophytes as recommended by [2].

2.3.2. Cultivation of specimens for isolation of dermatophytes species: All the collected skin scales and hair samples were divided into 2 parts the first was embedded into tubes containing Sabouraud dextrose agar with chloramphenicol or cyclohexamide. Each sample was inoculated into several tubes and all were incubated at 30-35 °C for 1-4 weeks. The cultures were examined periodically for the appearance of the characteristic growth features of dermatophytes species and were classified according to the keys of [2, 32].

2.4. Isolation and identification of the bacteria of dermatophilus species:

2.4.1. Direct microscopic examination for detection of dermatophilus species. The second part of samples of crusts and scabs collected from suspected skin lesions of cattle were subjected for direct impression smears and stained with Gram and Gimsa stains for direct microscopic examination according to the method described by [33].

2.4.2. Isolation and identification of the bacteria: The second part of skin scales and hair samples was inoculated into the test tubes containing nutrient broth and were incubated at 37 °C for 24 hrs. The subcultures were also made on nutrient agar, blood agar, EMB agar and MacConkey agar and incubated at 37 °C for overnight. Based on morphological and staining characteristics, hemolytic activities on blood agar, biochemical characteristic and antibiotic sensitivity test, the bacteria were isolated and identified as described by [33, 34, 35].

2.5. Biosynthesis and characterization of Iron Oxide nanoparticles; according to [36, 37, 38]: Biological synthesis of iron oxide nanoparticles was prepared using *Candida albicans* species. It was cultured and purified as described by [39] and incubated at 37°C for 24 hours and the fungal mycelia was harvested and washed thoroughly under sterile conditions. 20 g (wet weight) of the fungal biomasses were then re-suspended in 100 ml aqueous solutions of ferric chloride (FeCl₃, 0.074 g) and ferrous chloride (FeCl₂, 0.190 g) at a ratio of 2 to 1 molar ratio (pH 3.1) dissolved in 500 ml Erlenmeyer flasks and shaken (200 rpm) at 27 °C for a period of 24h. The bio-transformed products were collected by separating the fungal mycelia from the aqueous extract by filtration under sterile conditions. The nanoparticles were then removed from solution by magnetic separation and characterized as described by [40]. The prepared Iron Oxide NPs were subjected to optical measurements which were carried out by using a UV-Vis spectrophotometer (Lamda-25; PerkinElmer; Waltham, Massachusetts) and the size and morphology of nanoparticles were measured by scanning electron microscopy (SEM).

2.6. Evaluation of the effect of iron oxide nanoparticles on the growth of isolated strains from skin affection of cattle using diffusion tests [41,42]: The prepared iron oxide -NPs was evaluated for its antimicrobial activity against the fungal isolates of *T.mentagrophytes* and *T.verrucosum* and bacterial isolates of *Dermatophilus congelesis* which recovered from the cases of skin affection of cattle. The minimum inhibitory concentration (MIC) for each isolate was determined by a disc and well diffusion methods.

2.6.1. Well diffusion test: One ml of 10⁵ spore suspensions of tested fungus and bacteria were poured into sterile plate and over layered with the SDA medium or nutrient agar medium, respectively. After solidification of agar, wells of 5

mm in Φ were made on agar surface and filled by 100 μ l of the gradual concentrations of (0, 1, 2, 3, 4 and 5 mg /ml of Fe_2O_3 -NPs) and fluconazol 20 ug and griseofulvin 10 ug (control antifungal) or gentamycin 10 μ g and levofloxacin 5 μ g (control antibacterial) were used at the recommended units by producer company.

2.6.2. Disc diffusion technique: Filter paper discs Whatman No.1 of 5 mm Φ were impregnated for 10 minutes in 100 μ l of each concentration separately (0, 1, 2, 3, 4 and 5 mg /ml of Fe_2O_3 -NPs), fluconazole 20 ug and griseofulvin 10 ug (control antifungal) or gentamycin 10 μ g and levofloxacin 5 μ g (control antibacterial) were used at the recommended units by producer company. The prepared discs were dried by heating at 40- 50°C for one hour. Then, one ml of 10^5 spore suspensions was added to sterile plates and over layered with SDA (for dermatophytes) or nutrient agar medium (for bacteria). The plates were gently rotated to mix the content and allowed to solidify at room temperature.

On the surface of plates the paper discs of the drugs were pressed firmly for complete contact with the agar.

2.6.3. Incubation and reporting of the results: The plates were incubated at 37°C for 24 hrs (for bacteria) and for 1-3 weeks (for dermatophytes). After the end of incubation period, the sensitivity of fungi or bacteria to each tested drug was determined by measuring the diameter of the growth inhibition zone in mm around wells or discs.

2.7. Scanning Electron Microscopy (SEM) [43].

The morphological changes of *dermatophytes* species of *T.mentagrophyte* and *T.verrucosum* and *dermatophilus congelesis species* which treated by iron oxide -NPs were observed with a scanning electron microscope (SEM). From the petri-dishes of treatment, a selected treated and untreated part of agar were cut. The agar blocks were dehydrated through a graded series of ethanol (30, 50, 60, 70, 80, 90, 95, and 100%), each level was applied twice for 15 min each time) and ethanol : isoamyl acetate (3:1, 1:1, 1:3, and 100%) isoamyl acetate twice for 30 min). The agar blocks on grid were dried with a critical-point drier using liquid CO₂ and

coated with gold-coater for 5 min. The coated samples were observed under SEM, JSM-5600LV with accelerating voltage of 10 kV.

Statistical Analysis:

The obtained data were computerized and analyzed for Calculation of standard error and variance according to [44].

3.Results and Discussion

The fungal *dermatophytosis* infection in cattle (ringworm) and bacterial *dermatophilosis* are important skin infections which have received major consideration not only for economical losses in the animal breeding industry but also in regards to their zoonotic transmission to humans which represent the widest spread and most prevalent diseases of man and animal [45, 46].The occurrence of these serious skin affections have public health problem. Despite aggressive treatment with new or more established licensed antimicrobial agents, these infections are important causes of morbidity and mortality in animals [47]. The prevalence of dermatophytes infection in cattle was investigated by [46] who recovered *T.verrucosum* and *T.mentagrophytes* from cases of ringworm in cattle at incidence rates of 70% and

30% in skin scraping samples, respectively. Whereas, [48], reported that the most frequent dermatophyte isolated from cases of cattle ringworm was *Trichophyton verrucosum* (99% of total isolates) which was obtained from all culture positive cases except five cases (1.0%) infected with *Trichophyton mentagrophytes*.

Similar results were reported in our work, out of 120 cases of cattle in private farms at El-Minufiya governorate suffered from obvious skin patches, samples of skin scrapings and hairs from all animals were collected. *Trichophyton verucosum* and *T. mentagrophytes* were recovered from (46.6% and 33.3%) of hair and from (33.3% and 26.6%) of skin scales that collected from animals showed obvious skin patches, respectively(Table,1).

Table (1): Prevalence of dermatophytes in hair and skin scraping collected from cattle:

| samples | Cattle had Infected skin (120 cases) | | | |
|-------------------------|--------------------------------------|------|-------------|------|
| | Hair | | Skin scales | |
| | No. | % | No. | % |
| <i>T. verrecosum</i> | 56 | 46.6 | 40 | 33.3 |
| <i>T. mentagrophyte</i> | 40 | 33.3 | 32 | 26.6 |

Several dermatophytes were isolated from skin scales of affected animals, from which *T. verrucosum* was reported as the exclusive agent of cattle ringworm [49]. It was interesting to report here that the isolation of *Trichophyton verucosum* and *T. mentagrophytes* from cases of cattle ringworm indicated that both fungi are the most common dermatophytes affecting animals in Egypt [2, 46].The primary dermatophyte isolation in this work was achieved by using a commercial Sabouraud-cycloheximide medium and direct microscopy for any given specimen type as recommended by [1]. It is worthy to mention that dermatophytes are able to survive in skin scales of infected animals for up to several months in moist and dark places where they can be easily transmitted to human and other animals [50]. The zoophilic fungus, *T. mentagrophytes* isolated in this study may be associated with frequent

contacts of the cattle with rats and pet animals like dogs and cats as it was reported in cattle cases from traditional type farms[51].

Regarding the bacterial skin affection of cattle, several factors are involved in the pathogenesis of *dermatophilosis*, among them are the mechanical injury to the skin, rainfall, tick infestation, concurrent diseases and or stresses that compromise the hosts immune system [52, 53]. It is suggested that the infection occurs when the integrity of the skin is impaired, as in case of long exposure to rain or traumatic injuries resulting from arthropod bites e.g. from ticks, flies and mosquitoes which serve as mechanical transmitters of *Dermatophilus congolensis* into epidermal layers, where germination zoospores takes places [54]. These germinating zoospores can penetrate into the epidermis to reach the basement membrane and they form

multi-dimensional branching filaments [55]. However, the high relative humidity has a significant influence on the maturation and motility of the infective zoospores and it has been claimed to be a major predisposing factor in the spread and epizootiology of *Dermatophilosis* [54] and latently infected animals may serve as the major reservoir of infection [16]. The organism persists in dry scabs and crusts and can survive in the environment for long periods (up to 42 months) [56, 57]. It may be transmitted by direct contact

Table(2): Incidence of *dermatophilus congolensis* in hair and skin scraping collected from cattle

| samples Bacterial species | Cattle had Infected skin (120 cases) | | | |
|----------------------------------|--------------------------------------|------|-------------|----|
| | Hair | | Skin scales | |
| | No. | % | No. | % |
| <i>Dermatophilus congolensis</i> | 16 | 13.3 | 18 | 15 |

The incidence of *Dermatophilus congolensis* in this study was nearly similar to the prevalence which reported by [63] which was 15.2% and so far from results reported by [64, 65] which was 1.04%. Many studies indicated that there was significant prevalence of *Dermatophilus* infection in cattle infested with ticks. This may be due to the fact that toxins present in saliva of ticks result in immunosuppression of the animals. It has been noticed that ticks and seasons of the year were the commonest risk factors associated with bovine *Dermatophilosis*. In cattle, it cause high economic losses as body weight loss, decreased milk yield and the acceptance of live animals in the market would also be reduced relative to *Dermatophilosis* free animals.

Up to date it is difficult to control such microbial affections by traditional antibiotics due to the formed subsequent resistance in the successive generations of microbial causes and to overcome this resistance, it is important to explore novel antimicrobial agents, which may replace current control strategies [66]. Recently, nanoparticles (NPs) materials have received increasing attention due to their unique physical and chemical properties which differ significantly from their conventional counterparts [21]. The metallic nanoparticles are most promising as they contain remarkable antimicrobial properties due to their large surface area to volume ratio, which is of interest to researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains [28, 43, 67]. Among nano- material Fe_2O_3 has gained more attention due to their special properties and less hazard to environment, however, like most of nanoparticles, they are toxic to organisms, which can be used as antibacterial [68, 69] and antifungal agents [23, 29, 30, 70]. In addition, the magnetite (Fe_3O_4) had been widely studied for biomedical applications in drug delivery and targeting [71, 72] and cancer treatment and inhibition of microbial colonization [73]. The magnetic nanoparticles as delivery nano-systems

with a carrier host, by contaminated fomites or by biting and non biting flies and is also encountered in a wide range of hosts such as buffalo, rabbit and sheep and rarely in man [58,59, 60, 61].The current results in table (2) revealed that the *Dermatophilus congolensis* was recovered from 13.3% of hair samples and from 15% of skin scales scraping, these results agreed with that revealed by [62].

are considered effective new tools to tackle the current challenges in treating infectious diseases, by improving the therapeutic index of antimicrobial drugs, and diminishing the local and systemic side effects including cutaneous irritation, peeling, scaling and gut flora reduction [74]. The use of eukaryotic organisms such as fungi holds promise for large scale metal nanoparticles production as the enzymes secreted by fungi is an essential element for the biosynthesis of metal nanoparticles [75]. Different fungi such as *Fusarium oxysporum*, yeast and *Colletotrichum sp.* had been reported to synthesize metal nanoparticles [76, 77] and *C.albicans* [38].

In the present study, the biological synthesis of Fe_2O_3 nanoparticles by fungal strains of *C.albicans* was investigated. The conversion of bulk ferrous and ferric chlorides to iron oxide nanoparticles occurred by a reduction of metal ion and the formation of nanoparticles. Bio-reduction indicated the presences of reducing agent which served as electron shuttle in this reduction reaction and it was also reported that, fungus reduction was most probably either by reductase action or by electron shuttle quinones or both [78]. Moreover, this process can be easily scaled up, economically viable with the possibility of easily covering large surface areas by suitable growth of mycelia [79]. The prepared Fe_2O_3 -NPs were identified and characterized by visual inspection; in a UV-visible spectrophotometer and Scanning electron microscope (SEM) for detection of their particle size and the purity of the prepared powder. The UV-Vis absorption spectrum of sample observed at the range 200–550 nm of wavelength. It showed peaks absorbance intensity at wavelength of 400 nm (Fig.1, B).

Whereas, the size and morphology of nanoparticles were measured by Scanning electron microscope depending on charge oscillation principle in iron oxide nanoparticles that exhibit by light, which detected as black dots of 80 nm in size (Fig. 1, A).

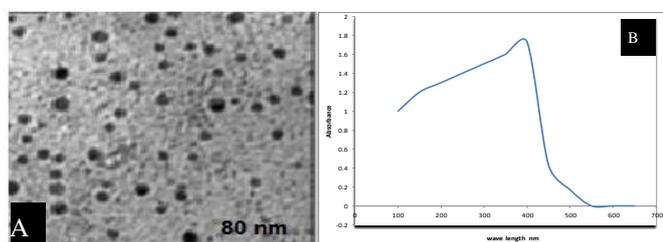


Fig. (1):(A)The micrograph of the particles size of iron oxide nano particles black (80nm) (black dots) under SEM. ($\times 20000$)(B) The UV-VIS absorbance of Fe_2O_3 -NPs.

The ultra-small size is comparable to naturally occurring proteins and bio-molecules in the cell and is notably smaller than the typical diameter ($\sim 7 \mu m$) of many biological cells.

The structural, morphological and chemical properties enabling them to interact in unique ways with cell biomolecules and enable their physical transport into the interior structures of cells and reduction of materials to the nanoscale which can frequently alter their electrical, magnetic properties [78]. In addition, iron oxide nanoparticles had been utilized to develop the present imaging techniques for *in vivo* diagnosis of biomedical disorders and it being used in patients for both diagnosis and therapy, leading to more effective medication with less unfavorable effects [80]. On the other hand, the antifungal potential of nanoparticles was investigated in many studies as [70] evaluated the inhibition of aflatoxigenic mold growth and aflatoxins production on yeast extract sucrose medium by zinc oxide NPs and they detected that a lower concentration of (8 ug/ml) was required to obtain effective inhibition. While, [29] illustrated the inhibitory effect of prepared iron oxide nanoparticles for *C.neoformance* using well and disc diffusion tests. The MIC of Fe₂O₃-NPs by the use of disc diffusion test was efficient than well diffusion test, where the MIC of Fe₂O₃-NPs for *C.neoformance* was 50 ppm but in case of well diffusion test the MIC was 100 ppm or more.

However, [81], evaluated the antifungal potential of iron oxide Nano-particles against *Candida albicans* and reporting the dose- and time-dependent toxic potential. He added that 65% viability of *Candida albicans* detected at lower concentrations (5-30 µg/mL) of iron oxide NPs and up to 12 hours of exposure, whereas at higher concentrations (30-100 µg/mL) and prolonged (24-48 hours) exposure viability reduced to 80-95%. In other study [82] evaluated the antifungal activity of zinc oxide nanoparticles against species of *Trichophyton mentagrophyte*, *Microsporium canis*, *Candida albicans* and *Aspergillus fumigatus* that isolated from diseased cases. They detected that the largest inhibition of the germination of all the tested fungi was observed at largest ZnO nanoparticles concentration (40 mg/ml).

In general, the use of iron oxide nanoparticles in the biomedical applications require that these nanoparticles have

high magnetization values and size smaller than 100 nm with overall narrow particle size distribution [83].

Recently, [28] evaluated the antifungal potential of Fe₂O₃ nanoparticles in comparison with commercial antifungal feed additives. The authors revealed that the mean of growth inhibition zone diameters for non-aflatoxigenic strains ranged from (10.5±0.5 mm) to (21.0±0.9 mm) when the concentration of iron oxide nanoparticles elevated from 25-250 ug/ml using well diffusion test. Whereas, at the same concentrations of iron oxide nanoparticles, the mean growth inhibition zone diameters of aflatoxigenic strains were relatively smaller (ranged from 8.0±0.4 to 18.0±0.82 mm). They obtained similar patterns for the mean growth inhibition zone diameters in disc diffusion test and concluded that the antimycotoxin effects of nanoparticles were limited to their addition to food and feed during processing preparation to prevent fungal growth and mycotoxins production and even toxicities and it could be used in the field of veterinary medicine as fungicide in successful treatment of microbial diseases.

Similar results were obtained in our work, where the current results in table (3,5) (fig.,2,3), showed the inhibitory zone of iron oxide nanoparticles (mm) in comparison with some traditional antifungal against isolated dermatophytes species recovered from skin affection of cattle. From the obtained data, it is clear that iron oxide NPs had no inhibitory effect on *T.verrecosum* at concentration of 1 mg/ml and 2 mg /ml. While, the growth inhibition detected at concentrations of 3 mg/ml and 4 mg/ml (10±0.5 mm and 14±0.7 mm), respectively (using well diffusion test). Whereas, the concentration of 5 mg/ml of iron oxide NPs showed an inhibitory zone diameter of (10±0.1 mm and 20±0.5 mm) using disc and well diffusion test, respectively.

On the other hand, in case of *T.mentagrophyte*, iron oxide NPs revealed inhibitory effect at concentration of 1, 2, 3, 4 and 5 mg/ml by well diffusion test and at concentration of 3, 4 and 5 mg/ml by disc diffusion test.

Table (3): Diameters of inhibitory zones caused by iron oxide nanoparticles (mm) compared with some* traditional Antifungal against isolated dermatophytes species from skin affection of cattle.

| Fungi | Concentrations of iron oxide NPs mg/ml | | | | | | | | | |
|------------------------|--|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | 1 mg | | 2 mg | | 3mg | | 4 mg | | 5mg | |
| | ⁽¹⁾ D | ⁽²⁾ W | ⁽¹⁾ D | ⁽²⁾ W | ⁽¹⁾ D | ⁽¹⁾ W | ⁽¹⁾ D | ⁽²⁾ W | ⁽¹⁾ D | ⁽²⁾ W |
| <i>T.verrecosum</i> | - | - | - | - | - | 10±0.5 | - | 14±0.7 | 10±0.1 | 20±0.5 |
| <i>T.mentagrophyte</i> | - | 20±0.5 | - | 20 ±0.9 | 16±0.8 | 24 ±1.0 | 20±2.0 | 30±2.0 | 22±1.5 | 30±3.0 |

*Traditional antifungal used:

Grisofulven 10 ug: Yielded 15 mm zone diameter of inhibition for both *T.verrecosum* & *T.mentagrophyte*

Fluconazole 20 ug: Yielded 14mm zone diameter of inhibition for *T.verrecosum* & 16 mm. for *T.mentagrophyte*

(1): Dis diffusion test (2): Well diffusion test.

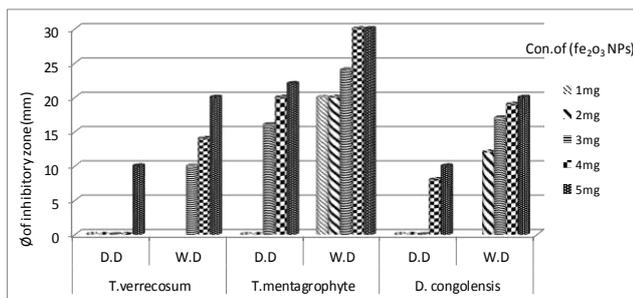


Fig (2): Diameters of inhibitory zones of different concentrations of iron oxide NPs (mm) against different isolated strains from skin affection of cattle.

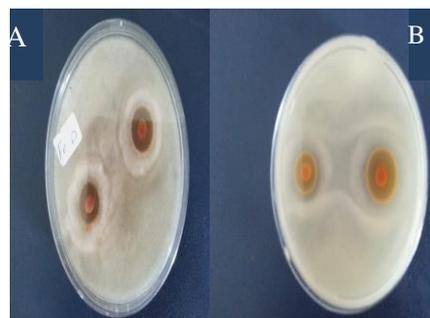


Fig. (3)(A): DD test for effect of iron oxide NPs against *T.verrucosum* **(B):** WD test for effect of iron oxide nanoparticles against *T.verrucosum*.

Regarding the antibacterial potential of iron oxide NPs against isolated *dermatophilus sp.*, the results illustrated that the treatment by iron oxide nanoparticles (mm) had no effect on the growth of *Dermatophilus sp.* at the concentration ranges from 1- 3 mg/ml using disc diffusion test. While, the treatment with 4 mg/ml or more resulted in inhibition of bacterial growth (Table (4,5), fig. (2, 4.).

On the other hand, the evaluation of antibacterial effects of iron oxide nanoparticles was more efficient using well diffusion test than disc diffusion test where the effect against bacterial growth was evident at concentration ranged from 2 mg or more. However in case of disc diffusion tests, the nanoparticles required more concentrations to be effective.

Table (4) Diameters of inhibitory zones caused by iron oxide nanoparticles (mm) compared with some*traditional Antibacterial against isolated bacteria of dermatophylas from skin affection of cattle

| Bacteria | Concentrations of iron oxide NPs mg/ml | | | | | | | | | |
|----------------------------------|--|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | 1 mg | | 2 mg | | 3mg | | 4 mg | | 5mg | |
| | ⁽¹⁾ D | ⁽²⁾ W | ⁽¹⁾ D | ⁽²⁾ W | ⁽¹⁾ D | ⁽¹⁾ W | ⁽¹⁾ D | ⁽²⁾ W | ⁽¹⁾ D | ⁽²⁾ W |
| <i>Dermatophilus congolensis</i> | - | - | - | 12±1.0 | - | 17±1.5 | 8±2.0 | 19±2.5 | 10±1.0 | 20±0.8 |

*Traditional antifungal as:

Gentamycin 10 ug/ml and Levofloxacin 20 ug/ml: Both Yielded 14 mm zones diameter of inhibition for *Dermatophilus congolensis* .

(1): Dis diffusion test (2): Well diffusion test



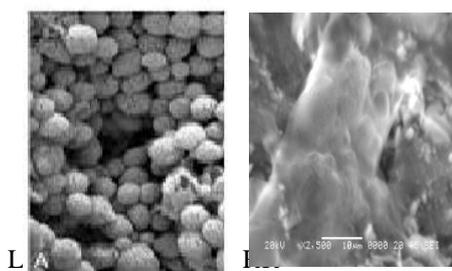
Fig (4): WD test for antifungal effect of iron oxide nanoparticles on the *D. congolensis*

Table (5): the mean diameters of inhibitory zones of iron oxide nanoparticles (mm) against isolated strains from skin of cattle:

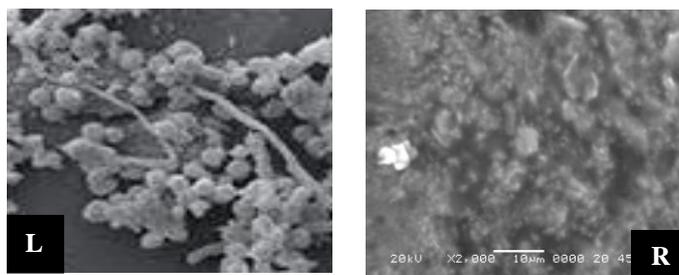
| Mean strains | DD (disc diffusion) | | | WD (well diffusion) | | |
|----------------------------------|---------------------|------|-------------|---------------------|-------|-------------|
| | Mi n | Max. | Mean±SE | Mi n | Ma x. | Mean±S |
| <i>T.verrucosum</i> | 0 | 10 | 2±0.2x10 | 0 | 20 | 8±0.6x10 |
| <i>T.mentagrophyte</i> | 0 | 22 | 11.6±0.9x10 | 0 | 30 | 24.8±1.3x10 |
| <i>Dermatophilus congolensis</i> | 0 | 10 | 2±1.7x10 | 0 | 20 | 13.6±1.9x10 |

However, the obtained efficient results in well diffusion tests suggested that it was essential for nanoparticles molecules to contact or penetrate into microbial cells to express their antibacterial activities. These data might also be interpreted as a requirement for interaction of nanoparticles with the fungal cell wall or membrane for expression of antifungal activity [84]. Several studies showed that nanoparticles as zinc homeostasis is regulated through a number of specific and nonspecific membrane-bound uptake and efflux pumps

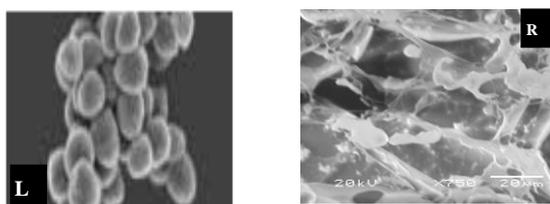
[85] and prevents sulfhydryl groups from oxidation [86]. In the present work, it was proved that the antifungal and antibacterial effects of metal nanoparticles was probably due to the damage of the cell wall of the microbial cells leading to leakage of the cell contents and finally cell death, as confirmed when the treated dermatophytes or dermatophilus species were subjected to SEM, the damage and rupture of their cell wall were detected in the area surrounding growth (Fig. 5, 6 and 7).



Fig, 5 :(L) Scanning Electron Microscopy (SEM) of normal *T. mentagrophyte* (R): Scanning Electron Microscopy (SEM) of *T. mentagrophyte* after treatment with Fe_2O_3 NPS.



Fig, 6 :(L) Scanning Electron Microscopy (SEM) of normal *T. verucosum*(R): Scanning Electron Microscopy (SEM) of *T. verucosum* after treatment with Fe_2O_3 NPS.



Fig, 7: (L) Scanning Electron Microscopy (SEM) of normal *D. congolensis* bacteria (R): Scanning Electron Microscopy (SEM) of *D. congolensis* after treatment with Fe_2O_3 NPS.

The normal conidial cell of dermatophytes fungi had a spherical shape singly or in clusters and intact cell membrane. The effect of high concentration of Fe_2O_3 nanoparticles on the treated fungi was observed as membrane damage of cells and some pits that have been caused in inter cellular components, leading to leakage and finally cell death. Similar findings were also reported by [27, 28, 87, 88]. As a first step in this direction, various nanoparticles as zinc oxide (ZnO) nanoparticles, gold nanoparticles, carbon nanotubes and iron oxide nanoparticles had been explored for improving bacteria and biofilm adhesion, penetration, generating reactive oxygen species and killing bacteria and fungi, potentially providing a novel method for fighting infections that is non drug related as reported by [89]. Also,

6. References

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[90] detected that adhesion of ZnO NPs with bacterial cells causing membrane disruption through direct interactions or through free radical production.

4. Conclusions

From the foregoing results it is suggested that iron oxide NPs could be used as an effective fungicide and bactericide in agricultural and food safety applications particularly treatment of animal skin diseases. The biosynthesis of nanoparticles in this study was environment friendly and commercially economic. Biological methods generous highlighting to biogenic synthesis is documented here. Further studies are needed to investigate the efficacy of pharmaceutical preparation of ointments, skin lotions and synergistic therapy with other traditional antibiotics in the treatment of animal diseases.

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