Animal Health Research Journal Vol. 7, No. 1, March 2019 pp. 76-94

ISSN: 2356-7767

Influence of selenium nanoparticles on some bacterial and fungal causes of mastitis in buffaloes

[*]Hassan, A.A.; [**]Mogda, K. Mansour; [***]Nahed, M.A. Shawky and [*]Rasha, M.H. Sayed El Ahl

[*]Department of Mycology and Mycotoxins, Animal Health Research Institute, Agriculture Research Center, Dokki, Giza, Egypt
[**] Department Biochemistry, Animal Health Research Institute, Agriculture Research Center, Dokki, Giza, Egypt
[***]Buffalo Diseases Research Department, Animal Health Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.

Received in 31/3/2019
Accepted in 16/4/2019

Abstract

Chemical synthesis of selenium nanoparticles (Se-NPs) and evaluation of their antimicrobial potential against some bacterial and fungal causes of diseases in buffaloes were investigated. Out of 80 samples were collected from dairy buffalo's farm which suffered from mastitis in Giza (60 mastitic milk samples and 20 samples of ration). The bacteriological and mycological examination of samples revealed that, the presence of *Staphylococcus* species and *Escherichia coli* as the most predominant isolates from different samples. While, *Aspergillus flavus* and *Candida albicans* were recovered in high percentages. On the other hand, the antimicrobial potential of selenium nanoparticles yielded a wide range against *S. aureus*, *E. coli*, *A. flavus* and *C. albicans*. As the concentration of Se-NPs increased from(100µg/ml to 600µg/ml), the diameters of inhibitory zone increased. When the treated fungal and bacterial cells were subjected to Scanning Electron Micrograph (SEM), the damage and rupture of their cell wall was detected or membrane damage and some pits and adhered to respiratory sequence of cytoplasm that had been caused leakage in inter cellular components and finally cell death. Hence, nano-selenium can be the alternative to the antibiotics and help to overcome the antibiotic resistance. It is concluded that, further studies are required for investigation the effect of combination of antioxidant Se-NPs with other traditional chemical drugs to obtain dual synergistic actions to decrease the amount of used chemicals and nanoparticles in the feeds manufacture.

Key words: *Selenium nanoparticles (Se-NPs), Antimicrobial potential, mastitis, buffaloes and Traditional antibiotic.*

Introduction

Bacteria, fungi and their toxins are natural contaminants of environment particularly foods and feeds even when the best condition of culture, harvest, storage and handling were used. The dairy buffalo is making a very significant contribution to the total food supply as milk, meat and leathers in many developing countries **(Patel *et al*., 2010)**. There are a number of diseases which adversely affect the health of this dairy animal, of these, mastitis which is conceder in both clinical and a subclinical form is a frustrating, costly and extremely complex disease that results in a marked reduction in the quality and quantity of milk **(Harmon, 2001 and Aamir *et al*., 2009).**

Whereas**, Manal *et al*. (2010)** reported that**,** the causes of mastitis involved a complex relationship of three major factors included host re-

sistance, causative agents and the environmental factors**.** It is mainly caused by microorganisms' usually Gram-negative and Gram-positive bacteria, mycoplasmas, yeasts and algae **(Zadoks *et al.*, 2011** and **Bhatt *et al.*, 2012)**. The main causative bacteria include: *S. aureus*, *S. agalactiae* (both of which are contagious), coli forms, streptococci and enterococci. All of these pathogens are found in the environment of the animals (water, feed, bedding, manure and soil), several other pathogens have been isolated from infected mammary glands which include *Trueperella pyogenes*, *Clostridiumperfringens* and other pathogens, such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Mannheimia haemolytica*, among others **(Conington *et al.*, 2005;Bhatt *et al.*, 2012** and **Emtenam *et al.*, 2018)**. On the other hand **Farid *et al.* (1975)** and **Mosherf (2005)** reported that, various *Candida* species particularly, *C. albicans* were the most common yeasts recovered from clinically and sub-clinically mastitic milk. Whereas, many mould isolates as *A. flavus* were recovered from mastitic milk of sheep and cattle by **Hassan *et al.*(2010).** The multiplicity of the cause and emergence of resistance due to indiscriminate and prolonged use of antibiotics in absence of antibiogram is a major hurdle in the control of mastitis **(Jeykumar *et al.*, 2013)**.

Inappropriate usage of antimicrobials such as wrong dose, drug or duration may be contributed to the increase in antimicrobial resistance without improving the outcome of treatment **(Williams, 2000)**.The usage of antibiotics correlates with the emergence and maintenance of antibiotic resistant traits within pathogenic strains. These traits are coded by particular genes that may be carried on the bacterial chromosome, plasmids and transposons or on gene cassettes that are incorporated into integrons **(Daka *et al.*, 2012),** thus are easily transferred among isolates **(Goffeau, 2008;** and **Hassan *et al.*, 2016**).

Thus, the development of more effective antimicrobial therapies understanding the mechanisms and decisions of cell death is of great importance **(Daka *et al.*, 2012; Nabawy *et al.*, 2014** and **Singh *et al.*, 2018)**.

In recent years, interest in nanoparticles and nanomaterials, with sizes ranging from 0.1 nm to 1,000 nm, has emerged due to their novel and enhanced applicability in various areas such as electronics, chemistry, energy, and the development of medicines **(Gajjar *et al.*, 2009** and **Tran and Webster, 2011)**. On the other hand, the selenium (Se) is an essential micronutrient for animals and humans that affect the chemotaxis, phagocytosis, and the respiratory burst of the neutrophils' activities under physiological and pathological conditions. The best-known biological roles of selenium are linked to its presence as the functional component in seleno-enzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) **(Ramamurthy *et al.*, 2013)**. Selenium incorporates into proteins such as seleno-cysteine and prevents oxidative damage to body tissues **(Rotruck *et al.*, 1973).** It plays an important role in antioxidant defensive system, regulation of thyroid hormones metabolism and cell growth **(Patching and Gardiner, 1999)**.

However, selenium has unique properties and great potential in the field of medicine, physics, biology and chemistry. The selenium nanoparticles are also used as antioxidant, antimicrobial, enzyme inhibition, anticancer agent but it is highly toxic.So, preparation of selenium nanoparticles and biomedical application is still a challenge **(Ramamurthy *et al.*, 2013)**. In addition, the selenium deficiency can lead to heart disease, hypothyroidism and a weakened immune system **(Dick and Agrawal, 2003)**. Whereas, the adequate levels of selenium in the feed prevent the accumulation of lipid hydroperoxides in organs and tissues and protect them from damage by oxygen radical species **(Hodgson *et al.*, 2006)**. Despite of the various advantages of selenium, the high doses can cause adverse effects. Recently, some studies are now focusing on how to overcome the drawbacks of high doses of selenium by using

its nanoparticles which have various biological effects such as the anti-cancer activity **(Ramamurthy *et al.*, 2013)**. In addition, selenium nanoparticles possess increased biological activity with reduced risk of selenium toxicity **(Ingole *et al*., 2010** and **Jay and Shafkat, 2018)**.

Therefore, the present study was undertaken to investigate the bacterial and fungal causes of some buffalo's mastitis. The recovered bacteria and fungi were subjected to treatment by Se-NPs in comparison with some traditional antibacterial and antifungal drugs. In addition, the efficacy of treatment by Se-NPs was evaluated by scanning electron microscope of the treated bacterial cells and fungal spores.

Materials and Methods

Collection of Samples: A total of 60 samples of milk from mastitic animals and 20 samples of ration were collected from a private dairy farm at Giza governorate before the buffalo treated with either intramammary or systemic antimicrobial agent according to **National Mastitis Council (NMC, 1999)**. Udder, teat orifice and hands of the milkers were perfectly cleaned with water and soap and disinfected with 70% ethyl alcohol before collection of milk samples. The first streams of milk were discarded and about 20 ml of milk were collected in clean sterile capped bottles, each bottle was given a serial number. Whereas, the rations samples collected under aseptic condition. All samples kept in ice box and sent directly to the laboratory for bacteriological and mycological examination as described by **Koneman *et al*.(1992)**.

Control of microbial agents: Using antifungal as fluconazol and itraconazole. While, the control antibacterial as enrofloxacin (fluoroquinolones) and curam (amoxycillin & claviolinic acid). All the used antimicrobial were purchased from Sigma Chemical Company (USA) and used as a comparable control.

Bacteriological Examination of samples: Following the standard microbiological technique and procedures for the diagnosis of bovine mastitis infection, the bacteriological culture was formed based on **Quinn *et al.* (2002)**.

Milk samples were centrifuged at 3000 rpm for 20 minutes and discarded the supernatant fluid. Ration samples was inoculated into nutrient broth and incubated over night at 37ºC, then a loopfuls from all samples were streaked on 7% sheep blood agar plates and checked for bacterial growth after 24, 48 and up to 72 hours to rule out slow growing microorganisms such as *Corynebacterium* species, sample was considered negative if there is no growth after 72 hours. For primary identification, colony size, shape color hemolyticcharacteristic, gram reactions were considered. These colonies were subculture to get pure colonies to nutrient agar, MacConkey agar, Edwards's medium, Eothinemethyline blue (EMB) medium and mannitol salt agar plates. Characterizations of isolated mastitis causing bacteria was done by different biochemical tests such as catalase, oxidase, coagulase by using Staphylect plus reagent, sugar fermentation test, oxidation fermentation test and in dole test.

Mycological examination of samples: The collected milk and animals ration samples were prepared and examined for isolation of fungi according to the technique recommended by **Refai *et al*. (2012)**. In sterile Petri dishes or test tubes containing Sabouraud Dextrose Agar (SDA) and specimen was scattered over the surface of the medium and gently pressed down into the agar. The inoculated plates were incubated at 25-28 ºC for 3-5 days. The identification of molds was based on the morphology of the colony, the rate of growth and microscopic morphology of the isolates in a direct culture mount and micro-slide culture technique **(Koneman *et al*., 1992** and **Refai *et al*., 2014a)**. While, yeast isolates were identified according to **Kreger van Rij (1984)** and **Refai *et al*. (2014 b)**.

Synthesis and Characterization of selenium nanoparticles (Se-NPs): According to **Chudobova *et al*. (2014)** Se-NPs was prepared by dissolving sodium selenite pent hydrate $Na_2SeO_35H_2O$ in bi-distled water, and 3-mercaptopropionic acid was then slowly added to the solution under stirring. Afterwards, pH was adjusted to eight using 1 M NaOH. The reaction mixture was stirred for 2 h. The color of the solution changes from colorless to yellow after refluxing immediately and become orange after 30 minutes. Se-NPs were stored in darkness at 4°C. One millilitre of the solution contains 158 µg of Se nanoparticles (50–100 nm in diameter). Chemicals used in this study were purchased from Sigma-Aldrich in ACS purity unless noted otherwise. The UV-visible spectra of each solution were measured in a SHIMADZU UV-1800 double beam digital spectrophotometer. XRD patterns were obtained on a Philips X'pert MPD X-ray diffractomer using Cu Kα (1.54059 Å) radiation with the X-ray generator operating at 45 kV and 40 mA. TEM images were obtained on JEOL 2010 microscopes. The TEM sample was prepared by dropping a sample suspension in ethanol on a Cu grid coated with a carbon film. Selenium nanoparticles were prepared by the reduction of aqueous sodium seleno-sulphate solution with freshly prepared glucose solution.

Preparation of bacterial and fungal spore suspension of isolates (Koneman *et al*., 1992 and Gupta and Kohli, 2003): Suspension of tested bacterial and fungal isolates cultivated on trypticasesoy agar (TSA) for 24h at 37°C for bacteria and Sabouraud dextrose agar (SDA) for 1-3 days at 25°C for fungi, respectively. The bacterial colony and fungal mycelia / spore mat were washed off with a 6 ml of sterile distilled water and using sterile loop, the outer most layer of growth were scraped. This suspension was counted in haemocytometer slide and adjusted to 10^5/ml colony forming unit considering the dilution factor.

Antimicrobial activity of Se-NPs against some bacteria and fungi isolates by well diffusion method (Jin *et al*., 2009): The synthesized Se-NPs was tested for their antimicrobial activity by well diffusion method against pathogenic organisms like of *E.coli*, *S. aureus*, *A. flavus* and *C. albicans* that recovered from the present samples. The used media in this test were TSA for bacteria and SDA for fungi which poured into plates containing one ml of cell suspension of bacterial cell or spores suspension of fungus (10^5cells/ml), shaken over the table in rotary manner and remained till solidification. After solidification a hole with diameter of 6 to 8 mm is punched aseptically with sterile cork borer or a tip of pasture pipette. Using micropipette 50 µl of Se-NPs concentrations (100, 200, 300, 400, 500 and 600 µg/ml) in separate plates for bacteria or fungi were added. Whereas, in a separate welled plate the traditional antibacterial agent (curam & enrofloxacin) and antifungal agent (fluconazole &itraconazol) as control were poured at the concentrations of (50, 100, 150 and 200 mg) (each in separate wells). After incubation under suitable conditions depending upon the tested microorganism (for 24-48 h at 37^o C for bacteria and for 48h –5 days at 28-30^o C for fungi), the different diameters of zone of inhibition were measured.

Scanning Electron Microscopy (SEM) of the treated microbial cells (Gong *et al*., 2007): The morphological changes of treated bacterial and fungal cells by Se-NPs were observed with a scanning electron microscope (SEM). Strains were prepared by cutting the agar, fixed for a minimum of 3h in 2.5% glutaraldehyde (100 mM phosphate buffer solution, pH 7.2) and then fixed in1% osmium tetra oxide for 1h. 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 were dried with a critical-point drier using liquid CO_2 and coated with gold-coater for 5 min. The coated samples were observed under JSM-5600LVwith accelerating voltage of 10 kV.

Results and Discussion

The occurrence of microorganisms, such as bacteria, molds, yeasts and viruses in the living environment are often pathogenic and cause severe infections in both human beings and animals **(Reddy *et al.*, 2007)**. Bovine mastitis, the most important deadly disease of dairy animals is responsible for heavy economic losses due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%) **(Bhikane and Kawitkar, 2000 and Singh *et al.*, 2018)**. Also, **Kennedy and Miller (1993**) and **Bhatt *et al.* (2012)** reported that, mastitis is a complicated problem associated with almost every conceivable factor of management and the environment.

The continuing presence of the disease may be attributed to deficient management, improper milking procedures, faulty milking equipment, inadequate housing, and breeding for ever-increasing milk yield. All of these factors are probably involved, although herd investigations often fail to incriminate specific factors. It is important to recognize that mastitis is an infectious disease and that all methods of commercial milk production may provide suitable conditions for spreading mastitis organisms from animal to other **(Bachaya *et al.*, 2011)**.

Etiological agents of disease may vary from place to place depending on climate, animal species and animal husbandry and include wide variety of Gram positive and Gram negative bacteria and fungi **(Deb *et al.*, 2013).** About 137 microbial species, subspecies and serovars are isolated from the bovine mammary gland **(Watts, 1988)**. Among all the pathogens of bovine mastitis, *S.aureus* is the predominant organism **(Allore, 1993 and Kapur *et al.*, 1992).**

In the present study, a total of 80 samples (60 milk and 20 rations) were collected from a private dairy farm at Giza governorate, in which, the animals suffered from signs of mastitis (including enlarged udders, decreased feeds utilization and milk production). The total bacteria that recovered from mastic milk and animals' rations were 100%.The prevalence and incidence of bacterial infection alone and the mixed bacterial and fungal infection in milk samples were 25.01 and 73.33 respectively (Table, 1&2).Currently**,** the tabulated results illustrated that, *S. aureus, E. coli, S. agalactiae* spp. and *S. dysgalactiae* which considered the main causes of mastitis in bovine were isolated in a single form from mastitic buffalo milk only in a percentage of 10, 5, 1.67 and 1.67 respectively.

Also the Table (3) showed that, *S. aureus* was the predominant bacteria isolated from milk and ration samples (46.67%& 35% respectively), eitherin a single form (10%),mixed with other bacteria (5%) ormixed with all fungal pathogen recovered in the study (31.67%) from tested milk samples, and also mixed with all fungal in ration samples (35%).In addition Table (3) showed that, *E. coli, Coagulase–ve staphylococci, S. agalactiae* and *S. dysgalactiae*were isolated in an incidence of (43.33% &25%), (21.67% &25%),(11.67%&20%) and (8.33%&15%) from milk and rations amples respectively. These results come in accordance with the findings of **Kumar *et al.* (2017)** and **Allore (1993)** who said that**,** the most common mastitis pathogens are found either in the udder as contagious pathogens or in the animal surroundings such as bedding and manure soil or environmental pathogens. The most common contagious pathogens were *S. aureus* and *S. agalactiae*. These spread from infected to clean udders during the milking process through contaminated milker's hand and cloth towels used to wash or dry udder of more than one animal and may be by flies.

These results also agree with the findings of **Wyder *et al.* (2011)** and **El-Jakee *et al.* (2013)** who reported that, *Staphylococcus*, *E. coli* and *Streptococcus* species are recovered as the most common pathogens causes bovine mastitis. Also similar results to our findings were detected by **Kumar *et al.* (2017)** and **Emtenan**

et al. **(2018)** who said that, among environmental pathogens, the most common bacteria are *S. uberis, S. dysgalactiae,* coliforms such as *E. coli* and *Klebsiella*. Transmission of the pathogens may occur during milking but primarily between milking**.**

Table (1). Incidence of single and mixed bacterial pathogens isolated from mastitic milk samples:

Pathogens	**Milk (60)**	
	No.	**%**
Single isolated bacterial:		
S. aureus	6	10.00
E. coli	3	5.00
S. agalactiae.	1	1.67
S. dysgalactiae.	1	1.67
Mixed bacterial isolates:		
S. aureus +E. coli	2	3.33
S. aureus +S .agalactiae	1	1.67
E. coli +S. dysgalactiae+Coagulase–vestaphylococci	1	1.67
Total	15	25.01

N.B:% was calculated according to the total number of examined samples No: Number of positive samples

Table (2). Incidence of mixed bacterial, fungal Aspergillus and yeast pathogens inexamined samples:

Pathogens	**Milk (60)**		**Ration (20)**	
	No.	**%**	**No.**	**%**
Mixed bacterial and fungal isolates				
A.flavus+Coagulase–vestaphylococci	6	10.00	3	15.00
C. albicans +S. agalactiae+ A. fumigatus	4	6.67	3	15.00
C. albicans+S. dysgalactiae+A. flavus+A. fumigatus	3	5.00	3	15.00
C. albicans + E. coli spp+*A. flavus+ S. aureus*	14	23.33	3	15.00
A. niger+E. coli+ A. flavus+C. albicans	5	8.33	1	5.00
A.fumigatus+Coagulase–veStaphylococci+C .albicans	4	6.67	1	5.00
A. fumigatus+ Coagulase–veStaphylococci+A. flavus	2	3.33	1	5.00
C.albicans+ S. aureus.+A.niger	4	6.67	3	15.00
S. aureus+ S .agalactiae+ A.fumigatus	1	1.67	1	5.00
E. coli +A. niger+ C.albicans	1	1.67	1	5.00
Total	44	73.33	20	100

N.B: % was calculated according to the total number of examined samples No: Number of positive samples

Table (3). Incidence of bacterial pathogens in rations and mastitis milk samples:

Bacterial Species	Milk(60)		Ration(20)	
	No.	%	No.	%
S. aureus	28	46.67	7	35
E. coli	26	43.33	5	25
Coagulase–ve staphylococci	13	21.67	5	25
S.agalactiae	7	11.67	4	20
S.dysgalactiae	5	8.33	3	15

N.B: % was calculated according to the Total examined samples. No: number of positive sample

Mycotic mastitis are split into two big groups according to the moment of appearance, primary mycotic mastitis (bacterial preliminary mastitis) and secondary mycotic mastitis which appear often straight away without antibiotic treatment **(Akodouch *et al*., 2014)**. In addition, yeasts are single celled organisms that are ubiquitous in the environment and they are considered opportunistic pathogens of the mammary gland that produce disease when natural defense mechanism is lowered **(Pengova, 2002** and **Scaccabarozzi *et al*., 2011)**.

Outbreaks caused by yeast have particularly been reported in intensively managed herds in which there were failures in environmental hygiene or in association with repetitive intramammary treatment **(Geraldo Costa *et al.*, 2012*)***. Treatment with contaminated antibiotic solution as well as syringes, other materials brought in contact with the mammary gland may favor yeast colonization of cow's udder **(Zaragoza *et al*., 2011)**. In the present study, the same samples of mastitic milk and rations which investigated for bacteriological examination were subjected also for the fungal examination (Table, 2&4).

Table (4). Incidence of fungal Aspergillus and yeast pathogens in examined mastitic milk and rations samples:

Fungalspecies	Milk(60)		Ration(20)	
	No	%	No	%
A.flavus	30	50.00	11	55.00
A.fumigatus	14	23.33	9	45.00
A.niger	10	16.67	5	25.00
C.albicans	35	58.33	15	75.00

%: Was calculated according to the total number of examined sample. No: number of positive sample

The results were recorded in Tables 2 and 4whichillustrated that, the total fungi that recovered from mastic milk and animals' rations were 73.33 % and 100%, respectively. The most common isolates were *A. flavus* which isolated from 50% and 55%, followed by *C. albicans* from 58.3 % and 75% and *A. niger*16.67 % and 25%, while, the *A. fumigatus* were recovered from 23.33% and 45% of mastitis milk and rations of diseased animal respectively. Similar results to our findings were detected by several studies, where, yeasts as *C. albicans* were recovered from clinically affected buffaloes with mastitis in Egypt **(Farid *et al.*, 1975** and **Abdel-Halim *et al.*, 1990)**. While, **Mosherf (2005)** resulted that, various *Candida* species particularly *C. albicans* were the most common yeasts recovered from milk of clinically and subclinically mastitis milk. Also there are similarity between our result and the result of **AL-Ameed (2013)** in Iraq who registered 80 % positive fungal infection of

which 78.75% were yeast and 21.25 % were moulds. Our results are concordant with the result of **Pachauri *et al.* (2013)** who found, 64 samples were positive for fungal isolation, out of 100 samples. They recorded the most common isolates were *C.albicans, A. fumigatus* and *A. niger*. Whereas, **Hassan *et al.* (2010)** isolated *A. flavus* from animal feeds, mastitis milk and vaginal swabs at the rates of 80%, 50 % and 50% respectively.In addition several studies recovered that *Staphylococcus species, Streptococcus* species*, E. coli* species*, C. albicans, Aspergillus* spp. and *Penicillium* spp. is the dominant microbial isolates in case of mastitis **(Yuan *et al*., 2012; Hassan *et al.,* 2015** and **Kumar *et al.,* 2017)**.

Various studies reported that the selenium element is an essential trace element for animals and humans with a variety of biological functions **(Surai, 2006)**. It plays an important role in the regulation of thyroid hormone metabolism, cell growth, antioxidant defense systems and functions as a redox centre. For instance, the family of selenium-dependent glutathione peroxides could reduce hydrogen peroxide, lipid and phospholipid hydroperoxides to harmless products **(Rayman, 2005** and **Pappas and Zoidis, 2012)**. Due to the advantages of size effect and high surface reactivity, nanoparticles have been already used in pharmaceutical applications to increase the bioavailability of drugs and targeting therapeutic agents to particular organs **(Davda and Labhasetwar, 2002)**.

Recently, nano-elemental selenium has attracted wide spread attention due to its high bioavailability and relatively low toxicity. The nanometer particles exhibit novel characteristics, such as great specific surface area, high surface activity and lot of surface active centers, high catalytic efficiency, and the character of low toxicity of elemental selenium **(Zhang and Spallholz, 2011)**. In the present work, the synthesis by chemical method of Se-NPs was undertaken and characterized for their optical and nano structural properties by Scanning Electron Microscope and FT-IR spectra (Fourier Transform Infrared Spectrometer). The particles morphology of Se-NPs were spherical and granular in shape and they were well crystallized in the nano size of 60 nanometer scale as observed by scanning electron micrograph (SEM) (Fig.1).Whereas, the UV-Vis absorption spectrum of Se-NPs sample in the range 200–550 nm. It was reported that, the characterized absorption peak with absorbance intensity very close to a wave length of 400 nm (Fig, 2).

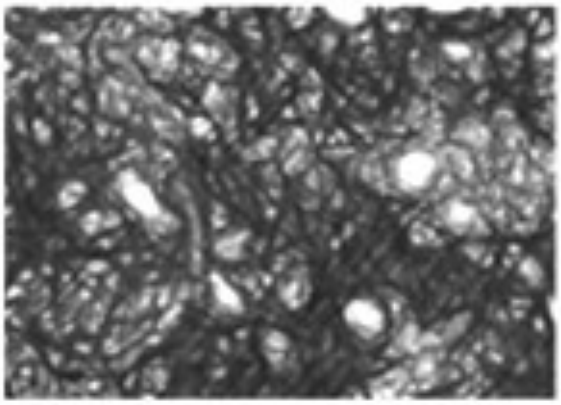


Fig. (1): SEM picture of Se–NPs showed the size and morphology (60 nm).

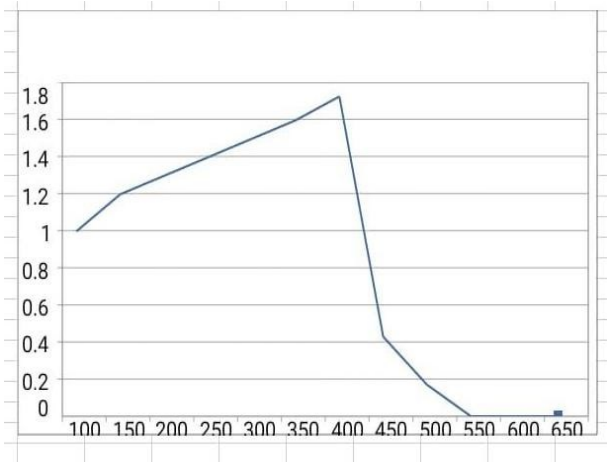


Fig. (2): The UV-VIS absorbance spectra of Se-NPS (60nm) at 400nmwave length

Similarly, **Atul *et al.* (2010)** synthesized Se-NPs by chemical method used glucose from an aqueous sodium selenosulphate precursor. They evaluated and characterized of produced Se-NPs by UV-visible optical absorption spectroscopy, X-ray diffraction and transmission electron microscopy techniques. The particle size of produced Se-NPs by this method was ranged from (20-80 nm). Recently, **Jas and Shafkat (2018)** biosynthesized of Se-NPs using plant extract from Allium Sativum and sodium selenite solution. Also, the prepared Se-NPs were characterized by using UV-Visible (UV-VIS) spectrophotometer, Transmission electron microscopy (TEM), Fourier transform spectroscopy (FTIR) and Energy dispersive X-Ray spectroscopy (EDAX). The selenium nanoparticles were observed as hollow and spherical particles in size of (60 nm) which are found more stable than two months.

Over the past decades, the bacterium of *S. aureus* was represent the major causative organism of mastitis, its emergence as multi drug resistant has become a deep concern for dairy industry worldwide. Because of the propensity of the organisms to acquire antimicrobial resistance, whereas most infection can be treated or prophylactic with antibiotic, antimicrobial resistance of *S. aureus* especially methicillin resistant *S. aureus* (MRSA) continues to be a problem for clinicians worldwide **(Shittu and Lin, 2006)**. Several reports had been published about the presence of multidrug resistant (MDR) *S. aureus* in subclinical mastitis which was also highly virulent that could be a major obstacle in the treatment of mastitis in China **(Memon *et al.,* 2013)**. MDR may be found in *S. aureus* and CNS isolates from mastitic milk **(Kumar *et al.,* 2010)**.

Nanotechnology hold promise advances for medication and nutrition because materials at the nanometer dimension exhibit novel properties different from those of both the isolated atom and bulk material **(Albrech *et al.,* 2006)**. Nano elemental selenium was studied in male mice, and at 20 to 60 nm, the bioavailability of Nano-Se was shown to be similar in increasing the activities of GSH-Px and thioredoxin reductase, although Nano-Se had a much lower toxicity compared with seleno-methionine **(Wang *et al.,* 2007)**.

The progressive increase in microbial infections that are resistant to conventional antibiotics has been observed, especially, the frequency of infections occurred by opportunistic bacterial and fungal strains has increased dramatically **(Milnes *et al*., 2008** and**Nabawy *et al.,* 2014)**. Furthermore, the outbreaks of food borne pathogens continue to draw public attention to food safety. So, there is a need to develop new antimicrobials to ensure food safety and extend shelf life. The use of antimicrobial agents directly added to foods or through antimicrobial packaging is one effective approach **(Wilczynski, 2000)**.

As other nanoparticles, selenium nanoparticles would have some unique mechanical, optical, electrical, biologic and chemical properties as compared with bulk materials. It has been reported that, the redness selenium nanoparticles has high biological activities and low toxicity **(Gao *et al.,* 2000)** and nano wires of trigonal selenium have novel photoconductivity **(Gates *et al.,* 2002)**. Recently, **Eleonora *et al.* (2016)** detected Se-NPs were active at low minimum inhibitory concentrations against a number of clinical isolates of *P. aeruginosa* but did not inhibit clinical isolates of the yeast species *C. albicans* and *C. parapsilosis*. They added that the biogenic Se-NPs appear to be reliable candidates for safe medical applications, alone or in association with traditional antibiotics, to inhibit the growth of clinical isolates of *P. aeruginosa* or to facilitate the penetration of *P. aeruginosa* and *Candida* spp. biofilms by antimicrobial agents.

Whereas, prolonged and intensive antibiotic therapy is an important predisposing factor in farm animals especially in Aspergillosis **(Krukowaski *et al.,* 2006)**. The multiplication of fungi during antibiotic therapy is stimulated

by the elimination of antagonistic bacterial flora decreased amounts of vitamin A in the glandular tissue, epithelium and irritating factors of antibiotic **(Wladyslaw *et al.*, 2010)**. Several studies evaluated the antimicrobial activity of nanoparticles of metals against bacteria and fungi in culture media. Metal oxides nanoparticles are characterized by very high surface area to volume particularly ZnO-NPs which is of the least hazards to the environment **(Hassan *et al.*, 2015** and **Nabawy, 2015)**.

In the present work, the prepared Se-NPs were evaluated for its antimicrobial activities against the most predominantly isolated bacteria (*E. coli* and *S. aureus*) and fungi (*A. flavus* and *C. albicans*) from mastitic buffalo's milk and their ration. Currently, the antibacterial activity of Se-NPs against *E.coli* and *S.aureus* and the minimum inhibitory concentration (MIC) was measured using well diffusion test. The inhibitory zone of Se-NPs against *E. coli* was 25mm at the concentration of 100µg/ml. However, the diameter zone of inhibition for *E. co*li was 27mmat the concentration of 200 µg/ml which reached to 40 mm when the used concentration of Se-NPs increased to 600 µg/ml (Table, 5&Fig.5). Similarly, in case of *S. aureus* as the concentration of Se-NPs increased, the inhibitory zone was also increased. Where, the inhibitory zone was 17mm at the concentration of 100µg /ml of Se-NPs and the diameter of inhibitory zone reached to 31 mm at the concentration of 600 µg/ml (Table, 5&Fig.3).

On the other hand, the control standard traditional antibiotic had comparatively higher effects. Where, the inhibitory zone of Curam antibiotic against *S. aureus* was increased from 52-60 mm as the concentration of curam antibiotic was increased from 250 mg/ ml to 1000 mg/ ml (Table, 5&Fig.4a), similar findings were yielded in case of use of curam against *E. coli*. As the concentration of antibiotic increased from 250 mg/ml to 1000 mg/ml, the diameters of inhibition zone was increased from 43 mm to 51 mm (Table, 5&Fig.6a). Currently, other traditional antibiotic enorofloxacin, yielded similar results as curamand relatively higher potential than Se-NPs(Table, 5&Fig.4b, 6b). Therefore, our results indicated that, the chemical traditional antibacterial showed higher efficiency than Se-NPs. Whereas, the chemical drugs caused many serious adverse effects which avoided by use of natural antibacterial as Se-NPs , Ag-NPS , Fe-NPS and Zn-NPs by their addition to animal feeds **(Hassan *et al.*, 2019)**.

Several studies evaluated the antimicrobial activities of nanoparticles of metal oxide which characterized by very high surface area to volume particularly ZnO powder sagainst *S. aureus*, *E. coli*or fungi in culture media. ZnO is 1 of 5 zinc compounds that are currently listed as generally recognized as safe (GRAS) by the United state food and drug administration (USFDA)**(Lopes de Romana, 2002)**. While **Khiralla and El- Deeb (2015),** detected the antimicrobial potential of Se-NPs on some food born pathogens as *B. cereus*, *E. faecalis*, *S. aureus* , *E. coli*, and *S.* Typhimurium and *S.* Enteritidis. The MIC90 (25 µg/ml) showed antimicrobial effects against the mentioned 6 bacteria. While, when the concentration of Se-NPs reached (75 µg /ml), acomplete inhibition of bacterial cells growth occurred. Recently, **Jay and Shafkat (2018)** synthesized Se-NPs by Allium sativum extract and evaluated antimicrobial activity by well diffusion method against pathogenic bacteria of *S. aureus* and *B. subtilis*. They found that, the MIC of Se-NPs (25 µl) and the highest antimicrobial activity against *S. aureus* (32mm) and *B. subtilis* (28mm) at concentration of 100µl Se-NPs. On therwise, as the concentration of Se-NPs increased, the inhibitory zones were also increased.

Table (5). Antibacterial activity of Se-NPs by well diffusion method (Zone of inhibition of mm) in comparison with traditional antibiotics:

Tested Bacterial Pathogen (10^5/ml)	Zones of bacterial growth inhibition(mm) at gradual concentrations of :													
	Se-NPs (μg/ml)						Controlantibiotic (Curam)(mg/ml)				Control antibiotic (Enorfloxacin)(mg/ml)			
	100	200	300	400	500	600	250	500	750	1000	250	500	750	1000
S. aureus	17	18	20	21	22	31	52	53	57	60	37	39	43	46
E.coli	25	27	28	31	32	40	43	46	47	51	38	40	44	45

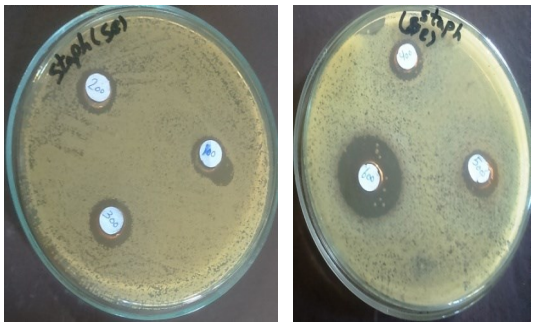
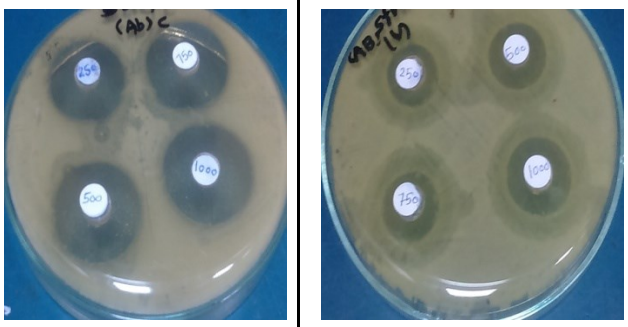


Fig. (3): Antibacterial activity of Se-NPs by well diffusion method. Zone of inhibition shows in plates of *S. aureus.*



(a) **(b)**

Fig. (4): Antibacterial activity of curam (a) and enorfloxacin (b) by well diffusion method. Zone of inhibition shows inplates of *S. aureus*

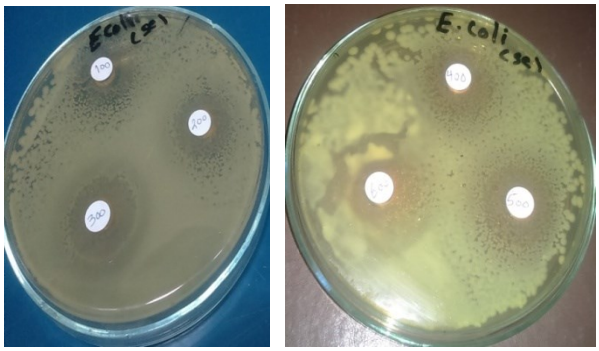
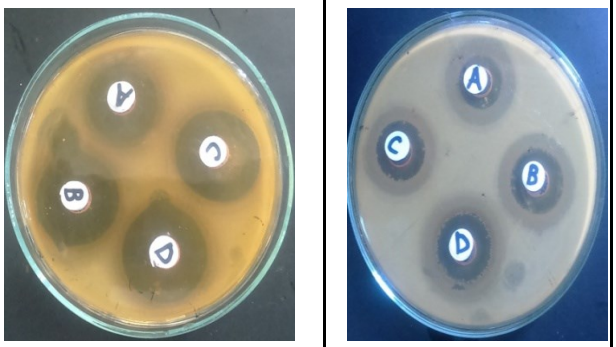


Fig. (5): Antifungal activity of Se-NPs by well diffusion method. Zone of inhibition shows in plates of*E. coli*



(a) (b)

Fig. (6): Antibacterial activities of (a) curam and enorfloxacin (b) by well diffusion method. Zone of inhibition shows inplates of*E. coli*

Regarding, the antifungal potential of Se-NPs against *A.flavus* and *C.albican*, the minimum inhibitory concentration (MIC) was measured using well diffusion test. The inhibitory zone of Se-NPs against *A. flavus* was increased from zero mm to 30 mm as the concentration of Se-NPds increased from 100µg/ ml to 600 µg/ ml. However, the diameter zone of inhibition for *C. albican* was increased from 10 mm to 40 mm as the concentration of Se-NP was increased from 100 µg/ ml to 600 µg/ ml **(**Table, 6&Fig.7, 9). On the other hand, the control standard traditional antifungal had comparatively similar effects to Se-NPs. Where, the inhibitory zone of fluoconazol against *A. flavus* and *C. albicans* were increased from 30 mm to 55 mm as the concentration of fluconazole increased from 50 mg/ ml to 200 mg/ ml (Table, 6&Fig.8a, 10a).

Currently, nearly similar findings were yielded in case of use antifungal itraconazole against both *A. flavus* and *C.albicans*. As the concentration of antibiotic increased from 50 mg/ ml to 200 mg/ ml, the diameters of inhibition zone was increased from 20 mm to 35 mm (Table, 6&Fig. 8b, 10b). Further more, different studies conducted in different laboratories showed that, the antimicrobial activity is influenced by not only nanoparticles concentration but also the size of the metal nanoparticles **(Violeta *et al.*, 2011)**.

Table (6). Antifungal activity of Se-NPs by well diffusion method (Zone of inhibition of mm) in comparison with traditional antibiotics:

Tested fungal Pathogen (10^5/ml)	Zones of fungal growth inhibition(mm) at gradual concentrations of:													
	Se-NPs (µg/ml))						Controlantifungal (Fluconazol)(mg/ml)				Control antifungal (Itraconazol)(mg/ml)			
	100	200	300	400	500	600	50	100	150	200	50	100	150	200
A.flavus	00	20	25	27	30	30	30	35	40	55	20	25	30	35
C.albicans	10	20	30	35	37	40	30	40	45	55	25	25	30	30

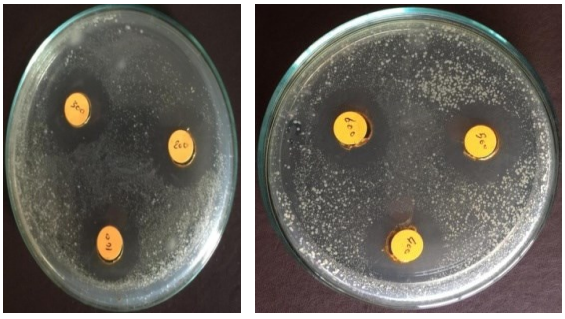
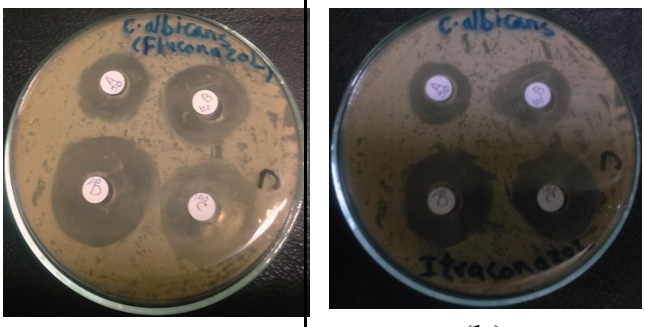


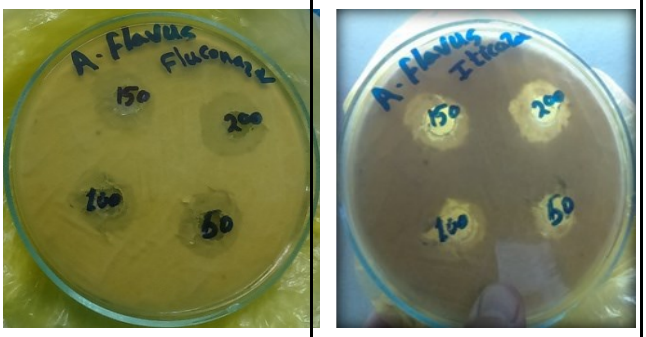
Fig. (7): Antifungal activity of Se-NPs by well diffusion method. Zone of inhibition shows in plates of *C. albicans.*



(a) (b)

Fig. (8): Antifungal activity of fluconazole (a) and itraconazole (b) by well diffusion method. Zone of inhibition shows in plates of *C.albicans*

Fig. (9): Antifungal activity of Se-NPs by well diffusion method. Zone of inhibition shows in plates of*A. flavus*

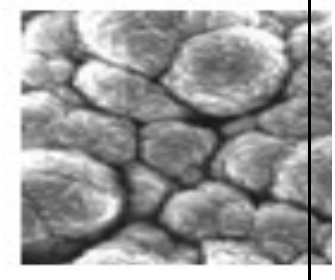


(a) (b)

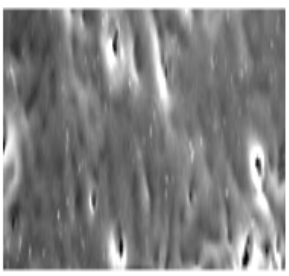
Fig. (10): Antifungal activity of fluconazole (a) and itraconazole (b) by well diffusion method. Zone of inhibition shows in plates of *A. flavus*

Furthermore, different studies conducted in different laboratories showed that, the antimicrobial activity is influenced by not only nanoparticles concentration but also by the size of the nanoparticles **(Violeta *et al.*, 2011**). Several studies propose that selenium may attach to the surface of the cell membrane disturbing permeability and respiratory function of the cell. It is also possible that selenium nanoparticles not only interact with the surface of membrane but can also penetrate inside the bacteria **(jay and Shafkat, 2018)**. While, **Moraru *et al.* (2003)** reported that, the antimicrobial effect of metal nanoparticles occurs by 2 ways. The first is the formation of H2O 2 on the surface of NPs due to the possible formation of hydrogen bond between hydroxyl group of cellulose molecules of fungi with oxygen atom of NPs leading to inhibition of the microbial growth, while the second is the release of metal ions + that causes damages of cell membrane and interacts with intraocular contents.

In the present work, when the treated bacteria or fungi by Se-NP were subjected to SEM, the damage and rupture of their cell wall were detected. The normal cell of bacteria, yeast or mold has aspherical shape and smooth cell wall and intact cell membrane. The effect of high concentration of Se-NPs on the treated bacteria or fungi at was observed as membrane damage of cells and some pits that have been caused in intercellular components, leading to leakage and finally cell death (Fig. 11:14).

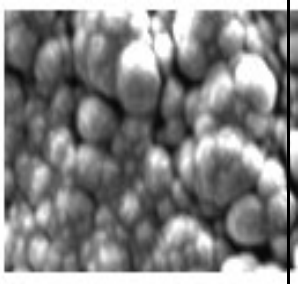


(a)

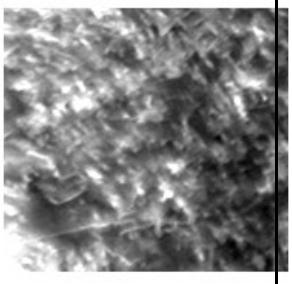


(b)

Fig. (11): SEM of *E.coli* before (a) and after (b) treatment by Se-NPs.*(X3000)*



(a)



(b)

Fig. (12): SEM of *S. aureus* befor and after treatment by Se-NPs.*(X3000)*

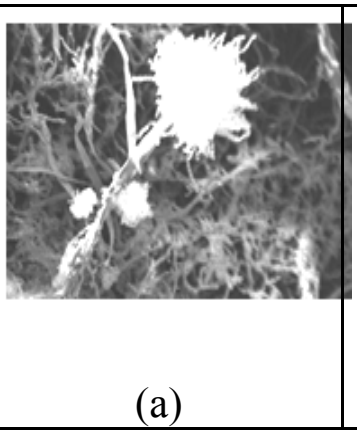


Fig. (13): SEM of*A. flavus* before (a) and after (b) treatment by Se-NPs*(X3000)*.

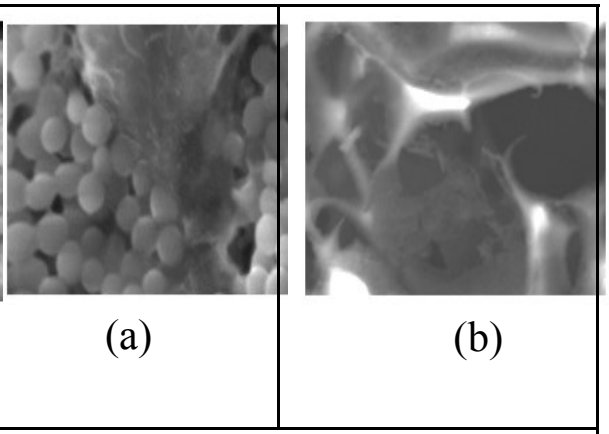


Fig. (14): SEM of *C.albicans* before (a) and after (b) treatment by Se-NPs. *(X3000)*

Similarly, several natural and engineered nanomaterials have demonstrated strong antimicrobial properties through diverse mechanisms including photocatalytic production of reactive oxygen species that damage cell components and viruses, compromising the cell envelope (e.g. peptides, chitosan carboxy fullerene, carbon nanotubes, ZnO and interruption of energy transduction **(Matei *et al.,* 2010** and **Violeta *et al.,* 2011)**. In addition, selenium is an essential trace element that has a large number of biological functions in human and poultry organisms. The most important and known action is its antioxidant effect because it forms selenocysteine, part of the active center of glutathione peroxidase (GSH-Px; **(Navarro-Alarcón and López-Martínez, 2000)**. In chicken health, **Zhouand Wang (2011)** concluded that, Nano-Se supplementation of chicken diets with 0.30 mg/kg was effective in increasing the growth performance of chicken and improving the selenium content in tissues and the quality of meat. Furthermore, dietary supplementation of Nano-Se was effective in enhancing the serum and hepatic GSH-Px activities of chicken.

Conclusions: The present study was undertaken to evaluated cases of mastitis in buffaloes by microbiological examination of milk and rations. Whereas, chemical synthesis and characterization of Se-NPs were carried out and the particles observed as hollow and spherical particles in size of 60 nm which is found more stable more than two months. On the other hand, the most predominant isolates recovered from mastitis milk and feed were subjected for evaluation the antimicrobial efficacy of Se-NPs in comparison of traditional chemical antimicrobial. It is reported that Se-NPs may attach to the surface of the cell membrane disturbing permeability and respiratory function of the cell, interact with the surface of membrane and penetrate inside the bacteria or fungi. Hence, nano-selenium can be the alternative to the antibiotics and help to overcome the antibiotic resistance. Further studies are required for investigating the effect of combination of antioxidant Se-NPs with other chemical traditional drugs to obtain dual synergistic actions to decrease the amount of used chemicals and nanoparticles in the feeds manufacture. Advanced field application of the present findings in direct treatment of diseased animal and as food additive should be of interest in near future for improvement the animal and human health.

Acknowledgments
The authors are gratefully acknowledged to Dr. Hazem Hassan Mahmoud, Chairman of Laboratory of Elemental and Isotopic Analysis, Nuclear Research Centre, Atomic Energy Authority, Egypt, for his kind assistance and fund in identification and characterization of the prepared and used selenium nanoparticles and scanning the treated cultures of fungal and bacterial cells by electron microscopy for evaluation the efficacy of treatments.

References

Aamir, S.; Ghulam, M.; and Muhammad, A. (2009). Mastitis in Buffaloes Pakistan J. Zool. Suppl. Se, (9):479-490.

Abd El-Halim, M.M.; Abu Zaid A.A.; Waffa, W.R. and Rifai M.K. (1990). Occurrence of Microsporumgypseum in some Egyptian soil inhabited by animals. Vet. Med. J., 38: 173-181.

Akodouch, L.; Aissi, M.; Zenia, S. and Saadi, A. (2014). Importance of Yeasts in the Mammary Infection of the Cattle in the Region of Sidi M'Hamed Ben Ali, Wilaya of Relizane, Algeria. J. Vet. Sci. Technol. 5: 2-7.

Al-Ameed, A.I. (2013). Isolation and identification of fungi from infected milk samples obtained from cattle with mastitis and studying the antifungal activity of Rosemary Ethanolic extract agaimst the main strains. Diyala Agricultural Sci. J. 5 (2) 1–13.

Albrecht, M.A.; Evans, C.W. and Raston, C.L. (2006). Green chemistry and the health implications of nanoparticles. Green Chem. 8: 417–432.

Allore, H.G. (1993). A review of the incidence of the mastitis in buffaloes and cows. *Pakistan Vet. J.,* 13: 1-7.

Atul, R.I.; Sanjay, R.T.; Khati, N.T.; Atul, V.W.; and Burghate, D.K. (2010). Green synthesis of selenium nanoparticles under ambient condition. 7 (7): 485 – 489.

Bachaya, H.A.; M.A. Raza; S. Murtaza and Akbar, I.U.R. (2011). Sub-clinical bovine mastitis in Muzaffar Garh District of Punjab (Pakistan). The J. Anim. Plant Sci., 21(1): 16 -19.

Bhatt, V.D.; Ahir, V.B.; Koringa, P.G.; Jakhesara, S.J.; Rank, D. N.; Nauriyal, D.S.; Kunjadia, A.P. and Joshi, C.G. (2012). Milk microbiome signatures of subclinical mastitis affected cattle analysed by shotgun sequencing. J Appl Microbiol., 112(4): 639-50.

Bhikane, A.V. and Kawitkar, S.B. (2000). Hand book for Veterinary Clincian. Venkatesh Books. Udgir, India.

Chudobova, D.; Cihalova, K.; Dostalova, S.; Ruttkay-Nedecky, B.; Rodrigo, M.;Tmejova, K.; Kopel, P.; Nejdl, L.; Kud, J.; Gumulec, J.; SonaKrizkova, S.; Kynicky, J.; Kizek, R. and Vojtech Adam, V. (2014). Comparison of the effects of silver phosphate and selenium nanoparticles on *Staphylococcus aureus* growth reveals potential for selenium particles to prevent infection. FEMS Microbiol Lett 351: 195–201.

Conington, J.; Cao, G.; Stott, A. and Bünger, L. (2005). Towards a better understanding of using breeding to control mastitis in sheep and cattle. Scottish Agricultural College (SAC) Technical Note. Sustainable Livestock Systems Group, SAC, Edinburgh.

Daka, D.; Silassie, S. and Yihdego, D. (2012). Antibiotic resistanc *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. Annals of Clinical Microbiology and Antimicrobials, 11: 26.

Davda, J. and Labhasetwar, V. (2002). Characterization of nanoparticle uptake by endothelial cells. Int. J. Pharm., 233: 51-59**.**

Deb, R.; Kumar, A., Chakraborty, S., Verma, A.; Tiwari, R.; Dhama, K.; Singh, U. and Kumar, S. (2013). Trends in diagnosis and control of bovine mastitis: a review. Pak. J. Biol. Sci. 16 (1) 1653-1661.

Dick, D.M. and Agrawal, A. (2003). The genetics of alcohol and other drug dependence, Alcohol Research and Health; 31:111-118.

Eleonora, C.; Emanuele, Z.; Marta, D.; Silvia, L. and Marzia, B. (2016). Biogenic selenium nanoparticles: characterization, antimicrobial activity and effects on human dendritic cells and fibroblasts. MicrobBiotechnol. 9(6): 758–771.

El-Jakee, J.; H.S. Hableel; M. Kandil; O.F. Hassan; E.A. Khairy and M. Marouf (2013). Antibiotic resistance patterns of *Streptococcus agalactiae* isolated from mastitic cows and ewes in Egypt. GlobalVeterinaria.10 (3):264-270.

Emtenan, M.H.; Wahid, M.A.; Magdy, M.Z. and Hesham, H. (2018). Updates in Bovine

Mastitis Middle-East Journal of Scientific Research 26 (1): 01-09.

Farid, A.; M. Abdel- Ghani and Oof,F. (1975). Bacterial and mycotic flora of apparently normal and mastitic buffalo milk samples. Egypt. Vet. Med. J. 23 (23): 265.

Gajjar, P.; Pettee, B.; Britt, D.; Huang, W.; Johnson, W. and Anderson, J. (2009). Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, *Pseudomonas putida* KT2440. Journal of Biological Engineering, 3: 9-22.

Gao, X.Y.; J.S. Zhang; L.D. Zhang and M.X. Zhu (2000). China public Health, 16,421.

Gates, B.; Mayers, B.; Cattle, B. and Xia, Y. N. (2002). Adv. Funct. Mater.12, 219

Geraldo, M.C.; Ulisses, de P.; Maria, Aparecida, G. and Nivaldo, da S. (2012). Yeast mastitis outbreak in a Brazilian dairy herd Braz. J. Vet. Res. Anim. Sci., São Paulo, 49 (3): 239-243.

Goffeau, A. (2008). Drug resistance: the fight against fungi. Nature, 452(7187): 541 -542.

Gong, P.; Li, H.; HeX, S.; Wang, K.; Hu, J.; Zhang, S. and Yang, X. (2007). Preparation and antibacterial activity of Fe3O4@Ag nanoparticles. Nanotechnology, 18, No. 28.

Gupta, A.k. and Kohli, Y. (2003). In-vitro susceptibility testing of ciclopirox, terbinafine, Ketoconazole and Itraconazolee against dermatophytes and nondermatophytes, and in vitro evaluation of combination antifungal activity. Br. J.Dermatol., 149: 296-306.

Harmon, R.J. (2001). Somatic cell count: A primer. In: Annual Meeting National Mastitis Council, 40. Reno. Proceedings. Madison: National Mastitis Council. 3- 9.

Hassan, A.A.; Hanan, K.M.; Taha, H.; Rasha, M.H. and Mahmoud, H.H. (2015). c.Herbalbiosynthesis of zinc Nanoparticles and evaluation of their antifungal and antibacterial effect for buffaloes skin affections. International Journal of Current Research, Vol. 7, Issue, 12, pp. 24338-24349.

Hassan, A.A.; Howayda M. El Shafei and Rania M. Azab (2010). Influence of solar simulator, gamma irradiation and laser rayes on the growth and aflatoxin production of *A. flavus* and *A. parasiticus*. *4th Sci. Congr.*of Egypt.

Hassan, A.A.; Mogda, K.M; Ibrahim, E.M.; Naglaa, M.A.; Flourage, A.M.; Rady, M. and Darwish, A.S. (2016). Aflatoxicosis in rabbits with particular reference to their control by N. Acetyl Cysteine and Probiotic. International Journal of Current Research, Vol. 8, Issue, 1, pp.

Hassan, A.A.; Rasha, M.H.; Noha, H.Or.; and El Hamak, A.M. (2019). Metal Nanoparticles for Controlling Mycotoxigenic Fungi and Mycotoxicosis Diseases of Animals and Poultry. Chapter in Nanomycotoxicology book, Elsevier publishing company (under press).

Hodgson, J.C.; Watkins, C.A. and Bayne, C.W. (2006). Contribution of respiratory burst activity to innate immune function and the effect of disease status and agent on chemiluminescence responses by ruminant phagocytes in vitro. Vet Immunopathol. 112: 12–23.

Ingole, A.R.; Thakare, S.R.; Khati, N.T.; Wankhade, A.V. and Burghate, D.K. (2010). Green synthesis of selenium nanoparticles under ambient condition. ChalcogenideLett.7: 485–489.

Jay, V. and Shafkat, R. (2018). Synthesis of selenium nanoparticles using *Allium sativum* extract and analysis of their antimicrobial property against gram positive bacteria. The Pharma Innovation Journal; **7**(9): 262-26.

Jeykumar, M.; Vinodkumar, G.; Bashir, B. and Krovvidi, S. (2013). Antibiogram of mastitis pathogens in the milk of crossbred cows in Namakkal district, Tamil Nadu. Veterinary World, 6(6): 354-356.

Jin, T.; Sun, D.; Su, J.Y.; Zhang, H. and Sue, H.J. (2009). Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogenes*, *Salmonella* Enteritidis and *Escherichia coli* O157:H7. J. Food Sci. 74**:** M46-M52.

Kapur, M.P.; Anshu, S. and Bahardwal, R. M. (1992). Bacteriology of clinical mastitis in buffaloes. Buffalo Bull., 11: 32-35.

Kennedy, P.C. and Miller, R.B. (1993). The mammary gland. In Pathology of Domestic Animals. Vol. 3.4th edn.KVF Jubb, P.C. Kennedy and N. Palmer (Editor). Academic press Inc. pp. 454-469.

Khiralla, M. and El-Deeb, B. (2015). Antintimicrobial and antibiofilm effects of selenium nanoparticles on some food borne pathogens. Food sienceabd Technology, 63(2): 1001-1007**.**

Koneman, E.W.; Allen, S.D.; Janda, W.M.; Scheckenberger, P.C. and Winn, W.C. (1992). Color Atlas and text book of Diagnostic Microbiology. 4thEdn. J.B. Lippincott Company. Philadelphia.

Kreger-van Rij, N.J.W. (1984). Systems of classification of the yeasts. In: Kreger-van Rij, N. J. W. (ed.). The Yeasts: A Taxonomic Study Third revised and enlarged edition Elsevier. Amsterdam K.

Krukowski, H.; Lisowski, A.; Rózański, P. and Skórka, A. (2006). Yeasts and algae isolated from cows with mastitis in the southeastern part of Poland. Pol. J. Vet Sci. 9 (3): 181-184.

Kumar, N.; Manimaran1, A.; Manimaran1, A.; Kumaresan, S.; Jeyakumar, L.; Sreela, P.; Mooventhan and M. Sivaram (2017). Mastitis effects on eproductive performance in dairy cattle: a review Trop Anim Health Prod DOI 10.1007/s11250-017-1253.

Kumar, R.; B.R. Yadav and Singh, R.S. (2010). Genetic determinants ofantibiotic resistance in *Staphylococcus aureus* isolates from milk of mastitic cross bred cattle. Curr. Microbial, 60: 379–386.

Lopes de Romana, D.; Brown, K.H. and Guinard, J.X. (2002). Sensory trial to assess the accept-ability of zinc fortificants added to iron-fortified wheat products. J. Food Sci.67: 461– 5.

Manal M.; Hesham, Y.; and Hussin, A. (2010). Environmental Organisms as Risk Factors in the Occurrence of Mastitis in Dairy Buffaloes with Suggested Methods of Control. A Field Study. Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt Cairo University, Giza, Egypt. E-mail: drmanalmoustafa2008@yahoo.com.

Matei, E.; Enculescu, I.; Vasilache, V. and Teodorescu, C.M. (2010). Cobalt doped ZnO prepared by electrochemistry: chemistry, orphologyand magnetism. Physica Status Solidi, 207(11): 2517-2522.

Memon, J.; Y. Yang; J. Kashif; M. Yaqoob; R. Buriro; J. Soomro; W. Liping and Hongjie, F. (2013). Genotypes, virulence factors and antimicrobial resistance genes of *Staphylococcus aureus* isolated in bovine subclinical mastitis from Eastern China. Pak. Vet. J., 33(4): 486-491.

Milnes, A.S.; I. Stewart; Clifton-Hadley and F.A. Davies (2008). Intestinal carriage of verocytotoxigenic *Escherichia coli* O157, *Salmonella*, thermophilic *Campylobacter* and *Yersinia enterocolitica,* in cattle, sheep and pigs at slaughter in Great Britain during 2003, Epidemiology Infect 136: 739-751.

Moraru, C.I.; Panchapakesan, C.P.; Huang, Q.; Takhistove, P.; Liu, S. and Kokini, J.L. (2003). Nanotechnology: a new frontier in food science. Food Technol 57(12): 24–29.

Mosherf, B.S. (2005). Studies causes of inastitis in buffaloes. Ph.D. thesis of Fac. Vet.Med. Cairo univ. Egypt.

Nabawy, Gehan. A. (2015). Effect of metal element nanoparticles in the growth of aflatoxogenic *A. flavus* and aflatoxin production in feed, Master thesis in veterinary science (Microbiology), Faculty of Veterinary Medicine, Cairo University.

Nabawy, Gehan A.; Hassan, A.A.; El- Ahl, Rasha, H.S. and Refai, M.K. (2014). Effect of metal nanoparticles in comparison with Commercial antifungal feed additives on the growth of Aspergillus flavus and aflatoxin b1 production. Journal of Global Biosciences, 3(6): 954-971.

National Mastitis Council (NMC), (1999). Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Madison, WI, USA.

Navarro-Alarcón, M. and López-Martínez, M.C. (2000). Essentiality of selenium in the

human body: Relationship with difference diseases. Sci. Total Environ. 249:347–371.

Pappas, A.C. and Zoidis, E. (2012). The Role of Selenium in Chicken Physiology: New Insights. In: Chickens: Physiology, Diseases and Farming Practices, Kapur, I. and A. Mehra (Eds.). Nova Science Publishers, New York, USA. ISBN-13: 9781620810279, pp: 167.

Pachauri, S.; Varshney, P.; Dash, S. and Gupta, M.K. (2013). Involvement of fungal species in bovine mastitis in and around Mathura, India Vet. World 6(7): 393-395.

Patching, S.G. and Gardiner, P.H. (1999). Recent developments in Se metabolism and chemical speciation: a review. J Trace Elem Med Biol 13: 193–214.

Patel, D.V.; Singh, S.P.; Shukla, H.R.; Devanand, C.P. and Kasiraj, R. (2010). Superovulatory response to FSH and embryo recovery rate in Pandharpuribuffaloes (Bubalusbubalis) Buffalo Bulletin, 29 (4): 244-249.

Pengova (2002). A prevalence of mycotic mastitis in cows. Acta Vet. (Beograd), 52 (2-3): 133-136.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donelly, W.J. and Leonard, F.C. (2002). Veterinary Microbiology and Microbiological Diseases. 1st Iowa State University Press Blackwell Science.

Ramamurthy, C.H.; Sampath, K.S.; Arunkumar, P.; Suresh Kumar, M.; Sujatha, V.; Premkumar, K. and Thirunavukkarasu, C. (2013). Green synthesis and characterization of selenium nanoparticles and its augmented cytotoxicity with doxorubicin on cancer cells. Bioprocess Biosyst. Eng. 36: 1131–1139.

Rayman, M.P. (2005). Selenium in cancer prevention: a review of the evidence and mechanism of action. ProcNutr Soc. 2005; 64 (4): 527–542.

Reddy, K.M. (2007). Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Appl. Phys. Lett. 90: 2139021 -2139023.

Refai, M.; Heidy Abo El-Yazid and El-Hariri, M. (2014a). Monograph on Dermatophytes: A guide for isolation and identification of dermatophytes, diseases and treatment. Academia edu. egy.

Refai, Mohamed K.; Heidy Abo El Yazied and M. El Hariri (2012). Monograph of yeast (Updated). htt//Cairo, academic edu/ Mohamed Refai.

Refai, M.K.; Mona, El- Enbawy and Hassan, A.A. (2014b). Monograph on *Candida albicans*. https://www. academia.edu.eg./7688721. Atef Hassan https: // www. Research gate. net / publication/ Atef Hassan.

Rotruck, J.T.; Pope, A.L. and Ganther, H.E. (1973). Selenium: biochemical role as a component of glutathione peroxidase. Science 179: 585–590.

Sawai, J. and Yoshikawa, T. (2004). Quantitative evaluation of antifungal activity of metallic oxide powders (MgO, CaO and ZnO) by an indirect conduct metric assay. J Apple Microbial.96: 803–9.

Scaccabarozzi, L.; Locatelli, C.; Pisoni, G.; Manarolla, G.; Casula, A.; Bronzo, V. and Moroni, P. (2011). Short communication: Epidemiology and genotyping of Candida rugosa strains responsible for persistent intramammary infections in dairy cows. J Dairy Sci. 94 (9): 4574-4577.

Shittu, A.O. and J. Lin (2006). Antimicrobial susceptibility patterns and characterization of clinical isolates of *S. aureus* in Kwazulu-Natal province, South Africa. BMC Infect. Dis., 6(1): 125.

Singh, A.; Chhabra, R.; Sikrodia, S.; Shukla, S.; Sharda R. and Audarya, S. (2018). Isolation of *E. coli* from Bovine Mastitis and their Antibiotic Sensitivity Pattern. Int. J. Curr. Microbiol. App. Sci 7(10): 11-18.

Surai, P.F. (2006). Selenium in nutrition and health, Notting University Press, Nottingham.

Tran, P.A.; O'Brien-Simpson, N.; Reynolds, E.C.; Pantarat, N.; Biswas, D.P. and O'Connor, A.J. (2016). Low cytotoxic trace

element selenium nanoparticles and their differential antimicrobial properties against *S. aureus* and *E. coli*. *Nanotechnology*. 27(4): 045101.

Tran, P.A. and Webster, T.J. (2011). Selenium nanoparticles inhibit *Staphylococcus aureus* growth. Int. J. Nanomed. 6: 1553–1558.

Violeta, V.; Catalin, P.; Constantin, F.; Monica, A. and Marius, B. (2011). Nanoparticles applications for improving the food safety and food processing. 7thInternational Conference on Materials Science and Engineering, Bramat, Brasov, 24–77.

Wang, H.; Zhang, J. and Yu, H. (2007). Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice. Free Radic-Biol Med 42: 1524–1533.

Watts, J.L. (1988). Etiological agents of bovine mastitis. Vet. Microbiol., 16: 41-66.

Wilczynski, M. (2000). Anti-microbial porcelain enamels. Ceram EngSciProc 21: 813.

Williams, R. (2000). The impact of antimicrobial resistance. Acta Vet. Scand., Suppl., 93:17-20.

Władysław, W.; Mariola, B. and Tomasz, P. (2010). Yeast mastitis in dairy cows in the middleeastern part of Poland Bull Vet. Inst. Pulawy 54: 201-204.

Wyder, A.B.; R. Boss.; J. Naskova.; T. Kaufmann; A. Steiner and H.U. Graber (2011). *Streptococcus spp.*and related bacteria their identification and their pathogenic potential for chronic mastitis-amolecular approach. Res. Vet. Sci., 91(3): 349-357.

Yuan-Yuan, C.; Zheng-Tao, Y.; Wen-Bo, L.; Qiao-Cheng, C.; Li-Guo, W. and Nai-Sheng, Z. (2012). Prevalence and Major Pathogen Causes of Dairy Cows Subclinical Mastitis in Northeast China. Journal of Animaland Veterinary Advances, 11: 1278-1280

Zadoks, R.N.; Middleton, J.R.; McDougall, S.; Katholm, J. and Schukken, Y.H. (2011). Molecular epidemiology of mastitis pathogens of dairy cattle and comparative relevance to humans. J. Mammary gland Biol. Neoplasia, 16(4): 357-72

Zaragoza, C. S.; Olivares, R.A.C.; Watty, A.E.D.; Moctezuma, A.D.P. and Tanaca, L.V. (2011). Yeast isolation from bovine mammary glands under different mastitis status in the Mexican High Plateu. Rev Iberoam Micol, 28(2): 79-82.

Zhang, J. and Spallholz, J. (2011). Toxicity of selenium compounds and nano-selenium particles. In: Casciano D, Sahu SC, editors. *Handbook of Systems Toxicology*. West Sussex, UK: John Wiley and Sons.

Zhou, X. and Wang, Y. (2011). Influence of dietary nano elemental selenium on growth performance, tissue selenium distribution, meat quality, and glutathione peroxidase activity in Guangxi Yellow chicken. Poultry Science 90: 680–686.