



The Antimicrobial Potential of Selenium Nanoparticles Singly and in Combination with Cinnamon Oil Against Fungal and Bacterial Causes of Diarrhea in Buffaloes

ATEF A. HASSAN*, MAREIM H. YOUSIF, HANAA M. M. ABD-ELKHALIQ, AHLAM K. A. WAHBA, AHMED M. A. EL-HAMAKY

Microbiology-Animal Health Research Institute (AHRI), Agriculture Research Center(ARC), Dokki, Giza Egypt.

Abstract | The antimicrobial potential of selenium nanoparticle (SeNPs) singly or in combination with cinnamon oil were evaluated against causes of diarrhea in buffaloes. The pathogens of *E. coli* and *C. albicans* were recovered from animal feeds, drinking water and feces of diarrheic animals at the top of all other iso-lates. The green synthesized selenium nanoparticles and cinnamon oil were had significant antimicrobial potential than traditional antibiotics against *E. coli* and *C. albicans*. The inhibitory concentrations of Se-NPs against *C. albicans* and *E. coli* were (0.4 and 0.3 mg/ml) and for Cinnamon oil were (0.5 %), respectively. The combination of SeNPs and Cinnamon oil completely inhibited the growth of *C. albicans* and *E. coli* sp. at (0.2 mg Se NPs / 0.2 % Cinnamon oil). It is concluded that the synergistic activity of metals NPs Se NPs / cinnamon oil were urgently required to overcome the microbial resistance against the traditional antibiotics and decrease the concentrations used of nanoparticles to avoid its toxicity for animals.

Keywords | Selenium nanoparticles, Synergy, Nano-emulsion, Cinnamon oil, Antimicrobial, Nanotechnology

Received | February 06, 2021; **Accepted** | March 17, 2021; **Published** | July 15, 2021

***Correspondence** | Atef Hassan, Microbiology-Animal Health Research Institute (AHRI), Agriculture Research Center(ARC), Dokki, Giza Egypt; **Email:** atefhassan2000@yahoo.com

Citation | Hassan A, Yousif MH, Abd-Elkhalik HMM, Wahba AKA, El-Hamaky AMA (2021). The antimicrobial potential of selenium nanoparticles singly and in combination with cinnamon oil against fungal and bacterial causes of diarrhea in buffaloes. *Adv. Anim. Vet. Sci.* 9(8): 1238-1248.

DOI | <http://dx.doi.org/10.17582/journal.aavs/2021/9.8.1238.1248>

ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

Copyright © 2021 Hassan et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Nowadays, there is a huge worldwide interest in applications of nanotechnology in different fields of biomedicine related to human and animal health (Hassanen et al., 2019; Khalaf et al., 2019). Where, the animal health and their production represent the major role in food security for human consumption and of huge economic importance in developing countries (Patel et al., 2010; Hassan et al., 2020b). The microbial infections resulted from opportunistic bacteria and fungi have been common especially in human and animals which being affected by special conditions likes' immune weakness. However, the fungal infections particularly by *C. albicans* and mycotoxigenic mold represent the widest spread causes of mycosis diseases of man and animals (Refai et al., 2014; Hassan et al., 2015, 2016, 2019a, 2020c). Moreover, several fungal and bac-

terial diseases adversely affect animal as mastitis, diarrhea and respiratory tract infections that resulted in decrease in their production and industry. The most important effects are related to economic losses due to decrease in milk yield (McDowell et al., 1995), diminished meat production due to diarrhea (Fagiolo et al., 2005) and respiratory disorders which are stress factors resulted in a bad production of animal (Quinn et al., 2002). The main recovered causes of animal diarrhea were *Staphylococcus* sp. *Streptococcus* sp., *E. coli*, *C. albicans*, *Aspergillus* sp. and *Penicillium* sp. (Yuan et al., 2012; Hassan et al., 2019a). However, in cases of calve diarrhea, enterotoxigenic *E. coli* (ETEC) are predominantly isolated which are producing toxin, *Salmonellae* sp. and *Y. enterocolitica* (Milnes et al., 2008). While, in water buffalo, *S. typhimurium* can induce a variety of clinical syndromes with different pathological lesions (Fagiolo et al., 2005). Some mold as members of *Aspergillus* sp., *Penicillium* sp.

and *Fusarium* sp. caused diseases disorder in buffalo (Hassan et al., 2019a, 2020b) and aflatoxicosis in cattle (Hassan et al., 2016). The problems in control of these microbial infections are attributed to drug resistance, which occurred because of prolonged wrong use of antibiotics (Williams, 2000; Jeykumar et al., 2013). Hence, the microbial pathogens maintain multidrug resistant genes that can be transferred to other pathogens (Goffeau, 2008; Daka and Yihdego, 2012). Therefore, novel antimicrobial agents are needed to overcome resistance to traditional antibiotics (Nabawy et al., 2014; Singh et al., 2018).

Recently, nanotechnology enable the production of effective antimicrobial agents from Nano sized materials particularly metals (Tran and Webster, 2011; Hassan et al., 2020 a, b). In addition, several studies confirmed the antioxidant, antibacterial, and antifungal activities of several metal and metal oxide nanoparticles. Examples for these nanoparticles are zinc oxide nanoparticles (ZnO-NPs) (Hassan et al., 2020a), copper nanoparticles (CuO-NPs) (Hassan et al., 2017), silver nanoparticles (Ag-NPs) (Fouda et al., 2019), and gold nanoparticles (GNPs) (Hassanen et al., 2020). Metals particularly selenium is the fundamental component or a micronutrient that used in disease treatment and could neutralize malignancy and decrease disease frequencies (Zonaro et al., 2015; Geofrion et al. (2020). The selenium nanoparticles can be produced by seed of *Mucuna pruriens* gave NPs of nearly (100–120 nm) and had IC50 (60 µg/mL) for inhibition the cell viability at 48 h. (Menon and Shanmugam, 2019). They detected that the preparation of SeNPs by green methods are cost-effective and environmental friendly and can be utilized further for future biomedical applications. This micronutrient is the primary part of glutathione peroxidase (GSH-Px), a cancer-inhibiting catalyst, which is associated with the protection of cells from oxidative stress (Ramamurthy et al., 2013; Kumari et al., 2018). The SeNPs reported to be more efficient than bulk selenium in activating selenoenzymes and have reduced toxicity (Mary et al., 2016; Jay and Shafkat, 2018). There are other several uses of SeNPs as antioxidant, anticancer and antimicrobial agents and protection from heart diseases (Ramamurthy et al., 2013, Nazıroğlu et al., 2017; Zhao et al., 2018). Recently, Meena et al. (2018) reported that the significant advantages of Nano-emulsions of oils are the simplicity, inexpensiveness, stability, versatility and the solubility of lipophilic substances that will protect them from degradation. Furthermore, the supplementation of oil with nanomaterials were successfully used in veterinary medicine as drug delivery agent and antimicrobial agents (Hassan et al., 2020a). Similarly, Abd-Elsalam and Khokhlov 2015, Hassan et al., 2020b) detected the antimicrobial potential of eugenol-Zinc Nano-emulsions against *Fusarium* sp. and *E. coli*. In addition, the contact of oil Nano-drops to the

microbial membranes cause its adhesion, destruction and final death of pathogens (Meena et al., 2018). Therefore, the objectives of present article were to detect the prevalence of fungal and bacterial causes of diarrhea in buffaloes. The most prevalent microbial agents that recovered from the present study were used for evaluation the effects of SeNPs singly and in-combination with cinnamon oil in inhibition their activities. The minimum inhibitory concentrations were measured during all tests in comparison with traditional antibiotics. Moreover, the activities mechanisms of SeNPs and oils and their benefits were fully discussed.

MATERIALS AND METHODS

SAMPLES

Ninety samples (30 of each of animal feeds, water, and feces) were obtained from a private animal farm suffering from diarrhea at Giza governorates aseptically in sterile McCartney bottles. The samples were transferred, as soon as possible, to the laboratory and kept in fridge until examination.

COMMERCIAL ANTIBACTERIAL, ANTIFUNGALS AND OTHER CHEMICALS

A known commercial antibacterial, antifungal and reagents were purchased from Sigma Chemical Company (USA).

SELENIUM NANOPARTICLES AND CINNAMON OIL

The used SeNPs were synthesized by green method and were characterized by the laboratory of ALDRIK Sigma chemical company, USA. It was in amorphous powder form with 60 nm particle size. While cinnamon oil was purchased in crud form from Al Gomhorya chemical company, Egypt.

BACTERIOLOGICAL AND SEROLOGICAL EXAMINATION

Samples were cultured onto MacConkey agar medium for 24 hrs. at 37°C, then a peptone water cultures were prepared from appeared colonies to inoculate biochemical tests (Quinn et al., 2002). While, serological identification for *E. coli* and *Salmonella* sp. was undertaken according to (Edwards and Ewing 1972; Neville and Bryant, 1986).

MYCOLOGICAL EXAMINATION

The samples were prepared and examined for isolation of fungi as method as Refai et al. (2012). The samples were inoculated into Petri-dish plates contained Sabouraud's dextrose agar (SDA) and incubated 3-5 days at 25-28°C and identification of appeared mold and yeast colonies were identified according to (Pitt and Hocking, 2009; Refai and Hassan, 2013).

Green synthesis and characterization of selenium nanoparticles (Inregole et al., 2010).

One 100 ml (10⁻¹ M) sodium selenosulphate was treated with 10 ml 4% glucose solution and mixture was refluxed. The color of the solution changes from colorless to yellow after refluxing immediately and become orange after 30 minutes. The orange color solutions remained stable for months. The prepared nanoparticles were characterized via 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 diffractometer using Cu K α (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.

Measurement of MIC of prepared SeNPs and cinnamon oil against *C. albicans* and *E. coli* that isolated from diarrhea in buffaloes (CLSI 2008):

- Preparation of bacterial and fungal spore suspension of isolates (Koneman et al., 1992; Gupta and Kohli, 2003): Suspension of tested bacterial and fungal isolates were prepared from their cultures on MacConkey agar medium after incubation for 24h at 37°C for bacteria and on Sabouraud's 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 slid and adjusted to 10⁵/ml colony forming unit considering the dilution factor.
- The minimum inhibitory concentration (MIC) of SeNPs and Cinnamon oil for the tested isolates were determined by a broth micro-dilution method based on the National Committee for Clinical Laboratory Standards (NCCLS) for bacteria (Balachandran et al., 2015) and for yeasts (NCCLS, M27-A2 2002). In sterile 12- x 75-mm plastic test tubes , 900 µl of RPMI 1640 broth medium or SD broth medium (for fungi) or nutrient broth (for bacteria) were added. Then, 100 µl of spore suspension added separately to adjust the inoculum of *E. coli* and *C. albicans* to (1 x 10⁵ cells/ml). 100 µl of SeNPs concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5 mg/L) or Cinnamon oil at levels of (0.0, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%), were added. Similar tests applied the traditional antibacterial and antifungal agents in the separate assays.
- Combination effects of Se-NPs and Cinnamon oil was performed as above-mentioned tests but 100ul was added from each. All the test tubes were incubated for 48 hrs.-5days at 28-30° C (for fungi) and for 24-48 h at 37° C (for bacteria) and the experiment was

repeated twice. After end of incubation period, 5 µL of tested broth were inoculated on the sterile nutrient agar plates for bacteria and SDA plate for fungi and incubated at 37° C for 24 hr- 2 weeks. The turbidity of the growth in tubes was observed every 24 hrs. The growth was assayed by measurement of optical density and transmittance percentage of each tubes content at 405 nm using spectrophotometer. The MIC was the concentrations that remove the turbidity and decreased optical density (OD) and increased transmittance percentage (T %).

STATISTICAL ANALYSIS

The obtained data were computerized and analyzed for significance. Calculation of standard error and variance was according to SPSS 14, (2006).

RESULTS AND DISCUSSION

A total of ninety samples from animal feeds, drinking water and animal feces (30 of each) were examined for isolation and identification of bacterial and fungal pathogens of diarrhea in buffalo. The tabulated results in (Table 1) illustrated that the incidence rates of bacterial species were (50 %, 56.7 % and 83.3 %) for *E. coli* in animal feeds, drinking water and feces, respectively. While, it were (23.3 %, 16.7 % and 33.3 %) for *S. typhi* in samples, respectively. Meanwhile, *E. coli* and *S. typhi* were recovered from (63.3% and 24.4%) of total samples. However, isolates of *E. coli*, *Salmonellae* sp. and *Y. enterocolitica* were recovered from cases of calve diarrhea (Milnes et al., 2008). While, in water buffalo, *S. typhimurium* can induce a variety of clinical syndromes with different pathological lesions (Fagiolo et al., 2005). Another study reported that *S. typhi* causing bacterial gastro enteritis and some *Salmonella* sp. have the multidrug-resistant (Yan et al., 2004, Scallan et al., 2011). On the other hand, the environmental pollution by fungi affect upon the growth rate and health of human and animals and cause several diseases as thrush, candidiasis, aspergillosis, dermatophytosis and mastitis, anemia, carcinogenic, tremor-genic, hemorrhagic, pulmonary edema, immunosuppressive and hormonal effects (Hassan et al., 2016; 2019a; b, 2020b; Asfour et al., 2009). Currently, as observed in (Table 2), the incidence rates of molds and yeast species in animal feeds, drinking water and feces were (83.3 % , 56.7 % and 30 %), for *A. flavus*, (30 % , 83.3 % and 33.3 %) for *A. ocraceus* and (80 %, 80 % and 86.7 %) for *C. albicans*, respectively. *C. albicans* were recovered from (68%, 40% and 16%) of diseased cases in buffaloes with mastitis, diarrhea and respiratory disorders, respectively Hassan et al. (2014). Similar results were obtained by (Hassan et al., 2016, 2017, 2019a; 2020b) who recovered these fungi from cases of diarrhea and respiratory affections of buffaloes. In the present study, the most prevalent bacterial isolates in cases of diar

Table 1: Prevalence rates of bacterial species recovered from the examined samples

Bacterial isolates	Types of samples (30 for each)							
	Animals feeds (30)		Drinking water (30)		Feces (30)		Total (90)	
	No	%	No	%	No	%	No.	%
<i>E. Coli</i>	15	50	17	56.7	25	83.3	57	63.3
<i>S. typhi</i>	7	23.3	5	16.7	10	33.3	22	24.4

% calculated according to the number of samples examined (30)

Table 2: Prevalence rates of molds and yeast species isolated from the examined samples

Molds and yeast species	Types of samples (30 for each)							
	Animals feeds		Drinking water		Feces		Total (90)	
	No	%	No	%	No	%	No	%
<i>A.flavus</i>	25	83.3	17	56.7	9	30	51	56.6
<i>A.ochraceus</i>	9	30	25	83.3	10	33.3	44	48.8
<i>C .albicans</i>	24	80	27	80	26	86.7	77	85.5

% calculated according to the number of samples examined (30)

rhea was *E. coli* that recovered from (63.3%) and fungi of *C. albicans* from (85.5%) of all examined samples.

Moreover, the mixed infection by *E .coli* with *C. albicans* in diarrheic buffaloes was investigated. The results revealed that out of 30 examined of feces 10 were showed mixed infection and 5 out of 30 feed samples had mixed infection. But, no mixed infection by *E .coli* with *C. albicans* was detected in water samples (Table 3). Okela, (2010) and Blanchard P.C. (2012) who detected mixed infection of *E.coli* and yeast in cases of bovine Diarrhea reported similar findings.

Herein, Susceptibility of *E. coli* to commercial antimicrobial agents were illustrated in (Table 4) which showed that *E. coli* isolates were resistant for each of (Ampicillin, Kanamycin, Tetracycline, Trimethoprim sulfate) in a percentages of (100 %, 80 %, 95 % and 100%), respectively. While the isolates were sensitive for Amikacin, Colestin, Ofloxacin (80%, 90 %, 100 %), respectively. Currently, the results in (Table 5) detected the sensitivity to commercial antifungals against *C. albicans* which was resistant for (Fluconazole) at the rate of (100 %). While, it was sensitive for (Itraconazole and Nystatin) (100 % for each).

Table 3: Prevalence of mixed infection with *E .coli* & *C. albicans* recovered from diarrhoeic buffaloes

Types of samples (30 for each)	Prevalence of <i>E .coli</i> & <i>C. albicans</i>	
	No.	%
Animals feeds (30)	5	16.7%
Drinking water (30)	0	0%
Feces (30)	10	33.3%

Since microbes have progressively eroded the effectiveness of previously successful antibiotics by developing resistance, the emergence of resistant and more virulent strains

Table 4: Antibiotic sensitivity test of representative *E.coli* isolated from diarrhoeic buffaloes

Antibacterial agents	Bacterial isolates			
	<i>E .coli</i> (20)			
	R		S	
	No	%	No	%
Ofloxacin (10 µg)	0	0	20	100
Amikacin (10µg)	4	20	16	80
Colestin (100 µg)	2	10	18	90
Ampicillin (10 µg)	20	100	0	0
Kanamycin (10 µg)	16	80	4	20
Tetracyclin (30 µg)	19	95	1	5
Trimethoprim (25 µg)	20	100	0	0

No = Number % = Percent . R = Resistant S = Sensitive.

of bacteria and fungi has outpaced the development of new antibiotics. Therefore, there is an inevitable and urgent medical need for antibiotics with novel antimicrobial mechanisms (Whitesides, 2003). Several recent studies revealed that the metals nanomaterials present in different forms as ZnNPs, AgNPs, Sins, CuNPs, CS-CuNPs, core/shell (CS) NPs; polymer-coated NPs and others have significant antimicrobial potential (Hassan et al., 2019a, 2020a). They have prominent biomedical activity than their bulk material (El-Sayed and Kamel, 2020). It is being applied not only in the treatment and the prophylaxis of infectious diseases but also used as diagnostics tools of infections (Hassan et al., 2019a; 2020a). Whereas, Hassanen and Ragab (2021) evaluated the antibacterial effect of low doses (5 mg/kg bwt) nanoparticles of chitosan (Ch-NPs), silver (Ag-NPs), and chitosan-silver nanocomposites (Ch-Ag NCs) against experimentally chronic infection induced by methicillin-resistant *S. aureus* in rats. They resulted that mixing between chitosan and silver nanoparticles in one

Table 5: Antibiotic sensitivity test of representative *C. albicans* isolated from diarrhoeic buffaloes

Fungal Isolates (Numbers)	Antifungal Agent									
	Fluconazole (10 g)		Voriconazole (1g)		Itraconazole (10g)		Nystatin (100g)		AmphotericinB (100µg)	
	R	S	R	S	R	S	R	S	R	S
<i>C. albicans</i> (20)	20	0	12	8	0	20	0	20	10	10
%	100%	0	60%	40%	0	100%	0	100%	50%	50%

S = Sensitive R = Resistant

Table 6: Optical density and Transmittance of treated *C. albicans* and *E. coli* by Se NPs .

Concentration of Se NPs (mg/l)	<i>C. albicans</i>		<i>E.coli</i>	
	OD	T%	OD	T%
0.0	2.06	0.87	2.06	0.87
0.1	0.15	10.1%	0.20	63.1
0.2	0.05	70.8%	0.03	93.3
0.3	0.02	95.5%	0.0	100
0.4	0.00	100%	0.0	100
0.5	0.00	100%	0.0	100

OD = Optical Density . T = transmittance

nanocomposite (Ch-Ag NCs) had a noticeable effect on bacterial count as well as the MIC value of this conjugate *in vitro*. In addition, they can be added in drinking water of broiler as in use of Gold NPs (0.5 mg/ kg of b.w.) which increased the growth performance and immune defense of broilers (Hassanen et al., 2020).

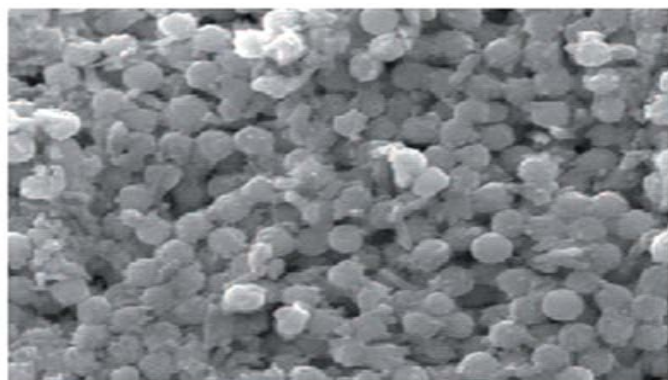


Figure 1: SEM pictures of Se-NPs showed the particle size and morphology (60 nm).

Herein, the used SeNPs was synthesized by green method to form glucose stabilized SeNPs from an aqueous sodium selenosulphate precursor under ambient conditions and the characterized NPs have amorphous powder form and the particles size was (60 nm) detected using TEM (Figure 1). The formation of selenium nanoparticles in presence of glucose is primarily authenticated from UV-Vis spectrophotometry shown in (Figure 2). They are safe methods and environmentally friendly and available for large-scale production. The organisms may cause changes in the toxic metals by decreasing their toxic effects (Inregole et al., 2010).

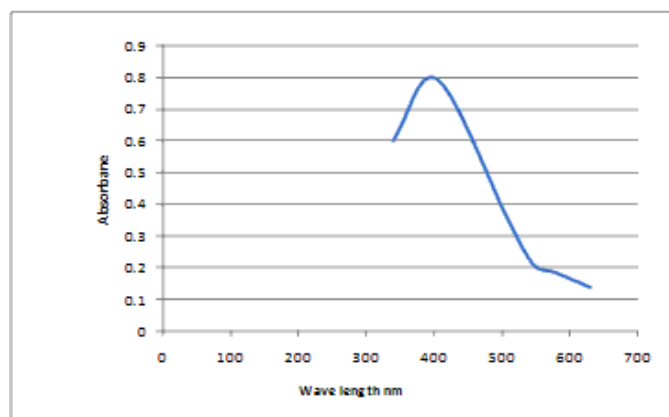


Figure 2: The UV-VIS absorbance spectra of Se-NPS (60nm) at 405 nm wave length

In the present study, SeNPs was evaluated for antimicrobial activities against the most predominantly isolated bacteria *E. coli* and fungi *C. albicans* from buffalo’s feed, drinking water and feces. The tabulated results in (Table, 6, Figure 3, 4), illustrated that the antimicrobial potential of Se-NPs against *C. albicans*, *E. coli* was concentration dependent. When the concentrations of SeNPs increased up to (0.5 mg/l), the OD of treated spore suspension were decreased until reach zero and T% increased to 100%. The inhibitory concentration of SeNPs against *C. albicans* was (0.4 mg/l) and it was (0.3 mg/l) for *E.coli* sp. Khiralla and El-Deeb (2015), detected the antimicrobial potential of Se-NPs against some bacterial food borne pathogens included *E. coli*, and *S. Typhimurium* and *S. Enteritidis* and MIC90 was (25 µg/ml for all). While, when the concentration of SeNPs reached (75 µg /ml), a complete inhibition of bacterial cells

Table 7: Optical density and transmittance of treated *C. albicans* and *E. coli* by cinnamon oil.

Concentration of cinnamon oil	<i>C. albicans</i>		<i>E.coli</i>	
	OD	T%	OD	T%
0.0	2.06	0.87	2.10	0.79
0.1%	0.40	3.98	0.37	42.6
0.2%	0.150	10.8	0.22	60.2
0.3%	0.06	87.1	0.04	91.2
0.4%	0.02	95.5	0.02	95.5
0.5%	0.0	100	0.0	100

OD = Optical Density . T = Transmittance

Table 8: Optical density and transmittance of treated *C. albicans* and *E. coli* by Combination of Se NPs / cinnamon oil

Concentration of SeNPs/CO	<i>C. albicans</i>		<i>E. coli</i>	
	OD	T%	OD	T%
0.0	2.06	0.87	2.10	0.79
0.1mg Se NPs/0.1% CO	0.40	3.98	0.37	42.6
0.1 mg Se NPs/ 0.2% CO	0.150	10.8	0.22	60.2
0.2 mg Se NPs/ 0.1% CO	0.06	87.1	0.04	91.2
0.2 mg Se NPs/0.2% CO	0.00	100	0.00	100

OD = Optical Density . T = Transmittance

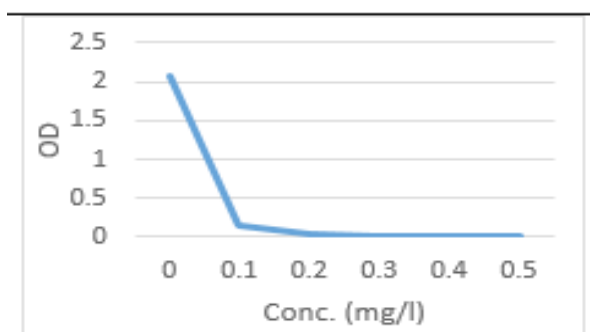


Figure 3: O.D of treated *C. albicans* with gradual concentration of Se NPs

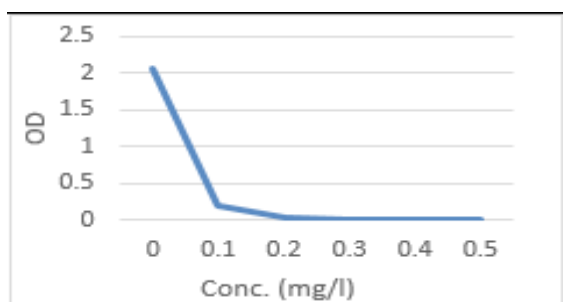


Figure 4: O.D of treated *E. coli* with gradual concentration of Se NPs.

growth occurred. Jay and Shafkat (2018) biosynthesized SeNPs and detected their anti-microbial activity against *S. aureus* and *B. subtilis*. They found that the MIC detected at (25 µl) and the highest zone of inhibition observed in *S. aureus* (32mm) and lower in *B. subtilis* (28mm) at concentration of 100µl SeNPs. While, Menon et al. (2020) detected antibacterial activity of SeNPs for wide range of

bacterial strains. However, their antimicrobial activity have been detected against pathogenic bacteria, fungi and yeasts (Shahverdi et al., 2010, Hariharan et al., 2012, Beheshti et al., 2013; Hassan et al., 2019a b).

Regarding, the antimicrobial potentials of essential oil, they have antibacterial and antifungal activities (Vitoratos et al., 2013). They are able to control microorganisms related to skin and food spoilage, including Gram-negative and Gram-positive bacteria. Clove, cinnamon, mandarin, lime, and basil oils are the best examples that are commonly used as natural antibacterial and antifungal agents, attracting the growing interest of scientists for use as food preservatives (Ghosh et al., 2013).

Currently, the present results of the antimicrobial potential of Cinnamon oil against *C. albicans* and *E. coli* sp. (Table, 7 and Figure 5, 6), yielded that the Optical density and transmittance were also concentration dependent. When the concentration of Cinnamon oil increased up to (0.5 %), the OD of treated spore suspension was decreased till reach 100 % T. The inhibitory concentration of Cinnamon oil that inhibited the growth of *C. albicans* and *E. coli* sp. was 0.5 % .Similar findings were detected by Eugénia et al. (2009) as he detected the significant fungicidal effect of eugenol, against *Candida*, *Aspergillus* and *dermatophytes* including fluconazole-resistant strains. In other study, Wahba and Abd-khaliq, (2013) recorded that the clove oil in a pure state has a bactericidal and fungicidal effect where it inhibit growth of bacteria such as *St. aureus*, *St. lentus* , *dermatophytes* as *Microsporium canis*, *Trichophyton mentagro-*

phyte and *T. verrucosum* at concentrations of (25, 20, 15, 10, 5 μ l), respectively.

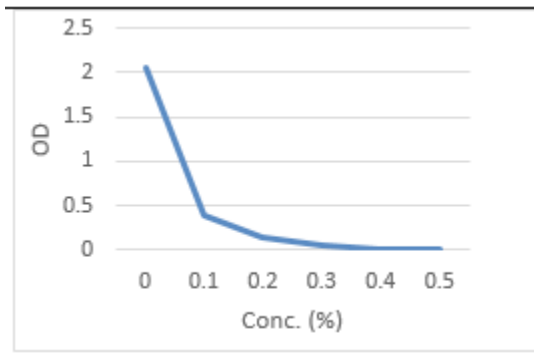


Figure 5: O.D of treated *C. albicans* with gradual concentration of Cinnamon oil

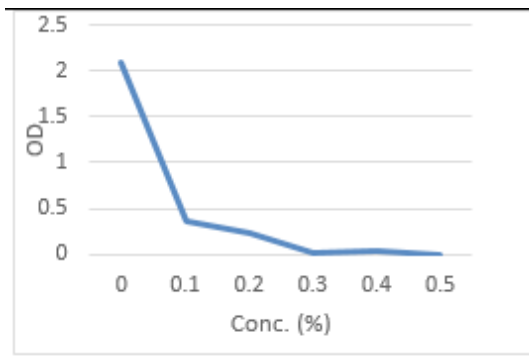
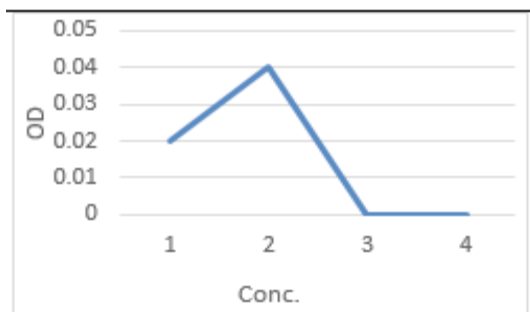


Figure 6: O.D of treated *E. coli* with gradual concentration of Cinnamon oil

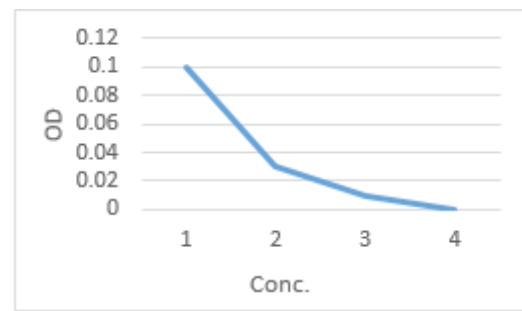


Conc. (1)	(Se 0.1mg/Oil 0.1%)
Conc. (2)	(Se 0.1mg/Oil 0.2%)
Conc. (3)	(Se 0.2mg/Oil 0.1%)
Conc. (4)	(Se 0.2mg/Oil 0.2%)

Figure 7: O.D of treated *C. albicans* with combination of Se NPs and Cinnamon oil

Moreover, herein the antimicrobial potential of synergistic activity of SeNPs with Cinnamon oil was evaluated. The findings in (Table, 8 and Figure 7, 8) indicated that the required concentrations for growth inhibition of *C. albicans* and *E. coli* sp. in combination of Se NPs and Cinnamon oil were at rates of (0.2 mg Se NPs / 0.2 % Cinnamon oil) which was lower than if each used separately (Table, 6 -8

and Fig. 1-6). The significance for using low concentrations of Se NPs by conjugation with cinnamon oil was to ensure their nontoxicity for human and animal cells.



Conc. (1)	(Se 0.1mg/Oil 0.1%)
Conc. (2)	(Se 0.1mg/Oil 0.2%)
Conc. (3)	(Se 0.2mg/Oil 0.1%)
Conc. (4)	(Se 0.2mg/Oil 0.2%)

Figure 8: O.D of treated *E. coli* with combination of Se NPs and Cinnamon oil

Hassan et al. (2017, 2019 b and 2020b), detected the more antimicrobial potentials of conjugation of ZnNPs with cinnamon oil or ozone than their single activities against bacteria and fungi. In addition, the essential oil additives loaded into mesoporous silica nanoparticles (MSNs) have the ability to suppress the growth of *A. niger* (Bernardos et al., 2015). The combination of Nano-emulsions and matrix of certain nanostructures such as lipids and polysaccharides were more effective in inactivation of *E. coli* than the traditional and classical emulsions and used lower doses (Salvia-Trujillo et al., 2017).

On the other hand, there are several mechanisms of antimicrobial activity of NPs included contact of NPs and penetration of the cell walls, destroying a microbial cell generating ROS release of metals ions and caused oxidative stress (Rudramurthy et al., 2016). The release of metallic ions resulted in depolarization of cell membranes, lipid peroxidation, protein oxidation, and DNA damage (Huang et al., 2020). Based on oxidative stress, Chang et al. (2012) found that NPs may enter the microbial cells via endocytosis process this related to induction ROS and the ions released by the nanostructures. While, Jay and Shafkat (2018); Hassan, et al. (2019b) reported that Se-NPs caused destruction of cell wall, leakage of cytoplasm contents and loss of treated fungal and bacterial cell functions as detected when they subjected to SEM. Zhao et al. (2018), found that a high stress due to accumulation of SeNPs on surface of cells stimulated the production of ROS which help in inhibition of bacterial cells. In general, the antimicrobial effect of nanoparticles occurs by two ways (Moraru et al., 2003). The first is the formation of H₂O₂ on the surface of NPs due to the possible formation of hydrogen bond between hydroxyl group of cellulose molecules of fungi and

bacteria. The synergistic and combination therapy of metals NPs as SeNPs with oils was urgently required to decrease the used concentration of nanoparticles, overcome the microbial resistant to traditional antibiotics, and resulted in more efficient antimicrobial activity of metal nanoparticles for the treatment of human and animal diseases.

Moreover, there will be several benefits of metallic nanomaterials to be used in improvement the biomedical applications. Although, data related to their harmful effects are not sufficient and special attention is required for known their toxicity risk before to biomedical applications. Hence, several toxicological studies are needed before nanotechnology applications in biomedicine and animal health.

CONCLUSION

The buffaloes' diarrhea results in significant losses in animal health and causes important burdens to the country's economy regarding to meat, milk, wool and leather industries. The frequent testing program of the animal feeds and other environmental factors for fungal and bacterial contamination is a critical demand. The metals nanomaterials are used as antimicrobial agents beside to other benefits strategies as diseases detection, diagnosis and therapy, additives to food, feeds and their products, and finally food safety. Our results detected that Se-NPs and cinnamon oil administration have significant antimicrobial potential against fungal and bacterial causes of diarrhea and their combination showed the requirement of lower concentrations from both to obtain the antimicrobial effects than their single form. Therefore, the synergistic therapies are needed to reduce the used doses of nanoparticles and hence overcome its toxicity and more efficient antimicrobial activities. Furthermore, the production of the production of SeNPs. Using glucose as a reducing agents and stabilizer avoid the aggregations of particles under ambient condition which can be used in large scale production and are safe method and environmentally friendly. The mechanisms of their activity are due to its penetration of cells membrane, damage of cytoplasmic contents, loss of function and cell kill. Hence, advanced and further investigations are required for direct treatment of farm animals by SeNPs in combination with other safe herbs and biological compound to avoid toxic effects of nanomaterials, which may result from misusing doses of nanoparticles. Moreover, the effects of green synthesized nanomaterial have long-term use on health need to concern and toxicity in surrounding environment and must be resolved in future. Therefore, the toxicity risk of nanomaterials must be determined before applications of green synthesized metallic nanomaterials in biomedicine for safe human and livestock health and their activities and production.

Authors declare that there is conflict of interest.

AUTHORS' CONTRIBUTION

All authors contributed equally.

REFERENCES

- Abd-Elsalam KA, Khokhlov AR (2015). Eugenol oil nanoemulsion: antifungal activity against *Fusarium oxysporum* f. sp. *vasinfectum* and phytotoxicity on cottonseeds. *Appl. Nanosci.* 5(2): 255-265. <https://doi.org/10.1007/s13204-014-0398-y>
- Asfour HAE, El-Metwally AE, Kotb MH (2009). Yeast as a cause of bovine mastitis and their histopathological effect on the mammary gland tissues. *J. Egypt. Vet. Med. Assoc.*, 69(4): 41-72.
- Balachandran RS, Heighington CS, Starostina NG, Kipreos ET (2015). A novel pathway for the degradation of cyclin B presented in International Worm Meeting. Unpublished information; cite only with author permission.
- Beheshti N, Soflaei S, Shakibaie M, Yazdi MH, Ghaffarifar F, Dalimi A, Shahverdi AR (2013). Efficacy of biogenic selenium nanoparticles against *Leishmania major*: in vitro and in vivo studies. *J. Trace Element. Med. Biol.* 27(3): 203-207. <https://doi.org/10.1016/j.jtemb.2012.11.002>
- Bernardos A, Marina T, Žáček P, Pérez-Esteve É, Martínez-Mañez R, Lhotka M, Klouček P (2015). Antifungal effect of essential oil components against *Aspergillus niger* when loaded into silica mesoporous supports. *J. Sci. Food Agric.* 95(14): 2824-2831. <https://doi.org/10.1002/jsfa.7022>
- Blanchard PC (2012). Diagnostics of Dairy and Beef Cattle Diarrhea. *Vet Clin Food Anim* 28 (2012) 443-464. <http://dx.doi.org/10.1016/j.cvfa.2012.07.002> – 2012 Elsevier Inc. All rights reserved.
- CLSI (Clinical and Laboratory Standards Institute) (2008). Reference method for Broth dilution antifungal susceptibility testing of filamentous fungi; approved standard second—Edition CLSI document M38-A2 (ISBN1-56238-668-9) (2008) Clinical and Laboratory Standards Institute, 940, West valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
- Chang YN, Zhang M, Xia L, Zhang J, Xing G (2012). The toxic effects and mechanisms of CuO and ZnO nanoparticles. *Materials.* 5:2850-2871. <https://doi.org/10.3390/ma5122850>
- Daka D, Yihdego D (2012). Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. *Ann. Clin. Microbiol. Antimicrob.* 11(1): 26. <https://doi.org/10.1186/1476-0711-11-26>
- Edwards PR, WH Ewing (1972). Identification of Enterobacteriaceae, third ed. Burgess Publishing Co., Minneapolis. pp: 208-339.
- El-Sayed A, Kamel M (2020). Advanced applications of nanotechnology in veterinary medicine. *Env. Sci. Poll. Res.* 27(16): 19073-19086. <https://doi.org/10.1007/s11356-018-3913-y>
- Eugénia P, Vale-Silva L, Cavaleiro C, Salgueiro L (2009).

- Antifungal activity of the clove essential oil from *Syzygium aromati-cum* on *Candida*, *Aspergillus* and dermatophyte species. *J. Med. Microbiol.* 58(11): 1454-1462. <https://doi.org/10.1099/jmm.0.010538-0>
- Fagiolo A, Roncoroni C, Lai O, Borghese A (2005). Buffalo pathologies. In *Buffalo Production and Research*. Edited by Borghese A. Rome: FAO Regional Office for Europe Inter-Regional Cooperative Research Network on Buffalo; 2005:249-296.
 - Fouda A, Hassan SE, Abdo AM et al (2019). Antimicrobial, Antioxidant and larvicidal activities of spherical silver nanoparticles synthesized by endophytic *Streptomyces* spp. *Biol. Trace Elem. Res.* 1-18. <https://doi.org/10.1007/s12011-019-01883-4>.
 - Geoffrion LD, Hesabizadeh T, Medina-Cruz D, Kasper M, Taylor P, Vernet-Crua A, Chen J, Ajo A, Webster TJ, Guisbiers G (2020). Naked Selenium Nanoparticles for Antibacterial and Anticancer Treatments. *ACS Omega.* 5: 2660-2669. <https://doi.org/10.1021/acsomega.9b03172>
 - Ghosh V, Saranya S, Mukherjee A, Chandrasekaran N (2013). Cinnamon oil nano-emulsion formulation by ultrasonic emulsification: investigation of its bactericidal activity. *J. Nanosci. Nanotechnol.* 13(1): 114-122. <https://doi.org/10.1166/jnn.2013.6701>
 - Goffeau A (2008). Drug resistance: the fight against fungi. *Nature.* 452(7187): 541-542. <https://doi.org/10.1038/452541a>
 - Gupta AK, Kohli Y (2003). In vitro susceptibility testing of ciclopirox, terbinafine, ketoconazole and itraconazole against dermatophytes and nondermatophytes, and in vitro evaluation of combination antifungal activity. *Brit. J. Dermatol.* 149(2): 296-305. <https://doi.org/10.1046/j.1365-2133.2003.05418.x>
 - Hariharan H, Al-Harbi N, Karupiah P, Rajaram S (2012). Microbial synthesis of selenium nanocomposite using *Saccharomyces cerevisiae* and its antimicrobial activity against pathogens causing nosocomial infection. *Chalcogenide Lett.* 9(12): 509-515.
 - Hassan SED, Fouda A, Radwan AA, Salem SS, Barghoth MG, Awad MA, Abdo AM, El Gamal MS (2019). Endophytic actinomycetes *Streptomyces* spp mediated biosynthesis of copper oxide nanoparticles as a promising tool for biotechnological applications. *J. BIOL. INORG. CHEM.* 24:377-393. <https://doi.org/10.1007/s00775-019-01654-5>
 - Hassan AA, Abo-Zaid KF, Oraby NH (2020b). Molecular and conventional detection of antimicrobial activity of zinc oxide nanoparticles and cinnamon oil against *Escherichia coli* and *Aspergillus flavus*. *Adv. Anim. Vet. Sci.* 8(8): 839-847. <https://doi.org/10.17582/journal.aavs/2020/8.8.839.847>
 - Hassan AA, Howayda M, El-Shafei, Hanan KM (2017). Antimicrobial Potential of Ozone on Fungal and Bacterial Contamination of Animal Feed That Caused Diseases in Some Buffalo Farms. 1st International Conference, Animal Health Research Institute, ARC, Egypt. 9-13 Nov. 2017.
 - Hassan AA, Mansour MK, El Hamaky AM, El Ahl RMS, Oraby NH (2020a). Ch 24: Nanomaterials and nanocomposite applications in veterinary medicine. In *Multifunctional Hybrid Nanomaterials for Sustainable Agri-Food Ecosyst.* (pp. 583-638). Elsevier. <https://doi.org/10.1016/B978-0-12-821354-4.00024-8>
 - Hassan AA, Mogda KM, Noha HO, Aliaa AE Mohamed (2016). The efficiency of using silver nanoparticles singly and in combination with traditional antimicrobial agents in control of some fungal and bacterial affection of buffaloes. *Int. J. Curr. Res.* 8(4): 29758-29770.
 - Hassan AA, Oraby NA, Mohamed AA, Mahmoud HH (2014). The possibility of using Zinc Oxide nanoparticles in controlling some fungal and bacterial strains isolated from buffaloes. *Egypt. J. Appl. Sci.* 29(3): 58-83.
 - Hassan AA, Oraby NH, El-Dahshan EME, Ali MA (2015). Antimicrobial potential of iron oxide nanoparticles in control of some causes of microbial skin affection in cattle. *Euro. J. Acad. Essays.* 2(6): 20-31.
 - Hassan AA, Sayed-Elahl RM, Oraby NH, El-Hamaky AM (2020 c). Metal nanoparticles for management of mycotoxigenic fungi and mycotoxicosis diseases of animals and poultry. In *Nanomycotoxicology* (pp. 251-269). Academic Press. <https://doi.org/10.1016/B978-0-12-817998-7.00011-2>
 - Hassan AA, Mogda KM, Noha HO, Aliaa AE (2019a). The efficiency of using silver nanoparticles singly and in combination with Traditional antimicrobial agents in control of some fungal and bacterial affection of buffaloes. *Int. J. Curr. Res.* 8(4): 29758-29770. April, 2016.
 - Hassan AA, Mogda K Mansour, Nahed MA, Shawky, Rasha M.H. Sayed El Ahl (2019b). Influence of selenium nanoparticles on some bacterial and fungal causes of mastitis in buffaloes. *Anim. Health Res. J.* 7(1): 76-94.
 - Hassanen EI, Morsy EA, Hussien AM, Ibrahim MA, Farroh KY (2020). *Biosci. Rep.* (2020) 40 BSR20194296 <https://doi.org/10.1042/BSR20194296>.
 - Hassanen EI, Khalaf AA, Tohamy AF, Mohammed ER, Farroh KY (2019) Toxicopathological and immunological studies on different concentrations of chitosan-coated silver nanoparticles in rats. *Int. J. Nanomed.* 14: 4723-4739. <https://doi.org/10.2147/IJN.S207644>.
 - Hassanen EI, Ragab E (2021). In Vivo and In Vitro Assessments of the Antibacterial Potential of Chitosan-Silver Nanocomposite Against Methicillin-Resistant *Staphylococcus aureus*-Induced Infection in Rats. *Biolog. Trace Element Res.* 199:244-257. <https://doi.org/10.1007/s12011-020-02143-6>.
 - Huang W, Yan M, Duan H, Bi Y, Cheng X, Yu H (2020). Synergistic Antifungal Activity of Green Synthesized Silver Nanoparticles and Epoxiconazole against *Setosphaeria turcica*. *J. Nanoma.* Vol. 2020, Article ID 9535432, pp. 1-7. <https://doi.org/10.1155/2020/9535432>
 - Inregole A. R., Thakare S.R., Khati N.T, Wankhade A. V., Burghate D. K. (2010). Green synthesis of selenium nanoparticles under ambient condition. *Chalcogenide Letters* Vol. 7, No. 7, July 2010, p. 485 - 489.
 - Jay V, Shafkat R (2018). Synthesis of selenium nanoparticles using *Allium sativum* extract and analysis of their antimicrobial property against gram-positive bacteria. *Pharm. Innovat. J.* 7(9): 262-26.
 - Jeykumar M, Vinodkumar G, Bashir B, Krovvidi S (2013). Antibigram of mastitis pathogens in the milk of crossbred cows in Namakkal z, Tamil Nadu. *Vet. World.* 6(6):354-356. <https://doi.org/10.5455/vetworld.2013.354-356>
 - Khalaf AA, Hassanen EI, Azouz RA, Zaki AR, Ibrahim MA, Farroh KY (2019). Ameliorative Effect Of Zinc Oxide Nanoparticles Against Dermal Toxicity Induced By Lead Oxide In Rats. *Int. J. Nanomed.* 14: 7729-7741, <https://doi.org/10.2147/IJN.S220572>.
 - Khiralla GM, El-Deeb BA (2015). Antimicrobial and antibiofilm effects of selenium nanoparticles on some foodborne pathogens. *LWT-Food Sci. Technol.* 63(2):

- 1001-1007. <https://doi.org/10.1016/j.lwt.2015.03.086>
- Koneman EW, Allen SD, Janda WM, Scheckenberger PC, Winn WC (1992). Color Atlas and textbook of Diagnostic Microbiology. 4thEdn. J.B. Lippincott Company. Philadelphia.
 - Kumari M, Purohit MP, Patnaik S, Shukla Y, Kumar P, Gupta KC (2018). Curcumin loaded selenium nanoparticles synergize the anticancer potential of doxorubicin contained in self-assembled, cell receptor-targeted nanoparticles. *European J. Pharmaceut. Biopharmaceut.* 130: 185–199. <https://doi.org/10.1016/j.ejpb.2018.06.030>
 - Mary TA, Shanthi K, Vimala K, Soundarapandian K (2016). PEG functionalized selenium nanoparticles as a carrier of crocin to achieve anticancer synergism. *RSC Adv.* 6: 22936–22949. <https://doi.org/10.1039/C5RA25109E>
 - McDowell RE, Wilk JC, Shah SK, Balain DS, Metry GH (1995). Potential for commercial dairying with buffaloes. North Carolina State University, USA.
 - Meena NS, Sahni YP, Thakur D, Singh RP (2018). Applications of nanotechnology in veterinary therapeutics. *J. Entom. Zool. Stud.* 6(2): 167–175.
 - Menon S, Agarwal H, Rajeshkumar S, Rosy JP, Shanmugam VK (2020). Investigating the Antimicrobial Activities of the Biosynthesized Selenium Nanoparticles and Its Statistical Analysis. *BioNano Sci.* 10:122–135. <https://doi.org/10.1007/s12668-019-00710-3>
 - Milnes AS, Stewart I, Clifton-Hadley FA, Davies RH, Newell DG, Sayers AR, Cheasty T, Cassar C, Ridley A, Cook AJ, Evans SJ, Teale CJ, Smith RP, McNally A, Toszeghy M, Futter R, Kay A, Paiba GA (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, *Epidemiol. Infect.* 136: 739–751. <https://doi.org/10.1017/S0950268807009223>
 - Moraru CI, Panchapakesan CP, Huang Q, Takhistove P, Liu S, Kokini JL (2003). Nanotechnology: a new frontier in food science. *Food Technol.* 57(12): 24–29.
 - Nabawy GA, Hassan AA, Sayed El-Ahl RH, Refai MK (2014). Effect of metal nanoparticles in comparison with commercial antifungal feed additives on the growth of *Aspergillus flavus* and aflatoxin b1 production. *J. Global Biosci.* 3(6): 954–971.
 - Naziroğlu M, Muhamad S, Pecze L (2017). Nanoparticles as potential clinical therapeutic agents in Alzheimer's disease: focus on selenium nanoparticles. *Expert Rev. Clin. Pharmacol.* 10(7): 773–782. <https://doi.org/10.1080/17512433.2017.1324781>
 - NCCLS, M27-A2 (2002). Reference method for broth dilution antifungal susceptibility testing of yeasts: Approved standard M27-A2, 2nd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 - Neville J, Bryant AF (1986). *Laboratory Immunology and Serology*. 2nd ED., Saunde Company Copyright, Toronto.
 - Okela MA, El-Sheik A, Khadr A, Bekheit AA, Badawy MA (2010). Aerobic bacteria and yeast associated with diarrhoea among calves. *ISSN 110-2047 Alex. J. Vet. Sci.* 30 (1): 57–70.
 - Patel DV, Singh SP, Shukla HR, Devanand CP, Kasiraj R (2010). Superovulatory response to FSH and embryo recovery rate in Pandharpuri buffaloes (*Bubalus bubalis*) *Buffalo Bulletin*, 29 (4):244–249.
 - Pitt JI, Hocking AD (2009). *Fungi and food spoilage* (Vol. 519). Ch 4: Methods for Isolation, Enumeration and Identification, pp. 1955. 3rd Ed. New York: Springer Science International Publishing.
 - Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC (2002). *Veterinary Microbiology and Microbiological Diseases*. 1st Iowa State University Press Blackwell Science.
 - Ramamurthy CH, Sampath KS, Arunkumar P, Kumar MS, Sujatha V, Premkumar K, Thirunavukkarasu C (2013). Green synthesis and characterization of selenium nanoparticles and its augmented cytotoxicity with doxorubicin on cancer cells. *Bioproc. Biosyst. Eng.* 36(8): 1131–1139. <https://doi.org/10.1007/s00449-012-0867-1>
 - Refai MK, Mona El- Enbawy, Hassan AA (2014). Monograph on Candida albicans. <http://cairo.academia.edu/MohamedRefai/Manuals>. Atef Hassan (<https://www.researchgate.net/publication/AtefHassan>).
 - Refai MK, Hassan AA (2013). Monograph on Mycotoxigenic Fungi and Mycotoxins in food and feeds with synopsis of the authors done on Mycotoxigenic Fungi and Mycotoxins in Foods and Feeds. <http://cairo.academia.edu/MohamedRefai/Manuals>.
 - Refai MK, Abo El Yazied H, El Hariri M (2012). Monograph of yeast (Updated). <http://Cairo.academic.edu/MohamedRefai>.
 - Rudramurthy G, Swamy M, Sinniah U, Ghasemzadeh A (2016). Nanoparticles: alternatives against drug-resistant pathogenic microbes. *Molecules.* 21: 836. <https://doi.org/10.3390/molecules21070836>
 - Salvia-Trujillo L, Soliva-Fortuny R, Rojas-Graü MA, McClements DJ, Martín-Belloso O (2017). Edible nanoemulsions as carriers of active ingredients: A review. *Ann. Rev. Food Sci. Technol.* 8: 439–466. <https://doi.org/10.1146/annurev-food-030216-025908>
 - Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Griffin PM (2011). Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17(1): 7–15. <https://doi.org/10.3201/eid1701.P11101>
 - Shahverdi AR, Fakhimi A, Mosavat G, Jafari-Fesharaki P, Rezaie S, Rezayat SM (2010). Antifungal activity of biogenic selenium nanoparticles. *World Appl. Sci. J.* 10(8): 918–922.
 - Singh A, Chhabra R, Sikrodia S, Shukla S, Sharda R, 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. <https://doi.org/10.20546/ijemas.2018.710.002>
 - SPSS 14 (2006). “Statistical Package for Social Science, SPSS for windows Release 14.0.0”, 12 June, 2006.” Standard Version, Copyright SPSS Inc., 19892006, All Rights Reserved, Copyright R SPSS Inc.
 - Tran PA, Webster TJ (2011). Selenium nanoparticles inhibit *Staphylococcus aureus* growth. *Int. J. Nanomed.* 6:1553–1558. <https://doi.org/10.2147/IJN.S21729>
 - Vitoratos A, Bilalis D, Karkanis A, Efthimiadou A (2013). Antifungal activity of plant essential oils against *Botrytis cinerea*, *Penicillium italicum* and *Penicillium digitatum*. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca.* 41(1): 86–92. <https://doi.org/10.15835/nbha4118931>
 - Wahba AK, Abd-ElKhalig HM (2013). The effect of clove oil on *Staphylococcus aureus* and dermatophytes isolated from skin affections in buffaloes. 12th Cong. Eyp. Society for cattle diseases. 3–6 dec. 2013.
 - Whitesides GM (2003). The right size in Nanobiotechnology.

- Nat. Biotechnol., 21(10): 1161-1165. <https://doi.org/10.1038/nbt872>
- Williams R (2000). The impact of antimicrobial resistance. Acta Vet. Scand., Suppl., 93:17-20.
 - Yan SS, Pendrak ML, Abela-Ridder B, Punderson JW, Fedorko DP, Foley SL (2004). An overview of Salmonella typing: public health perspectives. Clin. Appl. Immunol. Rev. 4(3): 189-204. [https://doi.org/10.1016/S1529-1049\(03\)00085-0](https://doi.org/10.1016/S1529-1049(03)00085-0)
 - Yuan-Yuan C, Zheng-TaoYang, Wen-Bo L, Qiao-Cheng C, Li-Guo W, Nai-ShengZhang (2012). Prevalence and Major Pathogen Causes of Dairy Cows Subclinical Mastitis in Northeast China. J. Anim. Vet. Adv. 11: 1278-1280.
 - Zhao G, Wu X, Chen P, Zhang L, Yang CS, Zhang J (2018). Selenium nanoparticles are more efficient than sodium selenite in producing reactive oxygen species and hyper-accumulation of selenium nanoparticles in cancer cells generates potent therapeutic effects. Free Rad. Biol. Med. 126: 55-66. <https://doi.org/10.1016/j.freeradbiomed.2018.07.017>.
 - Zhu X, Radovic-Moreno AF, Wu J, Langer R, Shi J (2014). Nanomedicine in the management of microbial infection—overview and perspectives. Nano Today. 9(4): 478-498. <https://doi.org/10.1016/j.nantod.2014.06.003>
 - Zonaro E, Lampis S, Turner RJ, Junaid S, Vallini G (2015). Biogenic selenium and tellurium nanoparticles synthesized by environmental microbial isolates efficaciously inhibit bacterial planktonic cultures and biofilms. Front. Microbiol. 6: 1-11. <https://doi.org/10.3389/fmicb.2015.00584>