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Research Article

EVALUATION OF THE SYNERGISTIC ANTIMICROBIAL ACTIVITIES OF SELENIUM NANOPARTICLES AND ROSEMARY OIL AGAINST *ASPERGILLUS FUMIGATU* AND *KLEBSIELLA PNEUMONIAE* RECOVERED FROM RESPIRATORY INFECTION IN CATTLE IN GIZA GOVERNORATE, EGYPT

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ABSTRACT: Synergistic and single antimicrobial activities of green synthesized selenium nanoparticles (SeNPs) and rosemary oil were investigated against predominant causes of respiratory diseases in cattle as *Aspergillus fumigatus* and *Klebsiella pneumoniae*. The prevalence rates of *A. fumigatus* were 14.28%, 12%, and 32% in the nasal swab, drinking water, and animal ration, respectively. While, *Klebsiella pneumoniae* was isolated from examined nasal swabs, water, and rations at the rates of 17.4%, 0%, and 8%, respectively. The minimal inhibitory concentration (MIC) of Se-NPs was 0.4 mg/ml and 0.5 mg/ml against *A. fumigatus* and *Kl. pneumoniae*, respectively. On the other hand, the inhibitory concentration of Rosemary against *A. fumigatus* and *Kl. pneumoniae* was 0.75 mg/ml and 1.0 mg/ml, respectively. The synergistic therapy of SeNPs dispersed with Rosemary oil reduced the MIC of SeNPs against *A. fumigatus* and *Kl. pneumoniae* was 0.1mg/ml and hence can be used as alternatives to their single forms in successful disease therapy. Moreover, these synergisms are essential to overcome the microbial resistance against the traditional antibiotics and decrease the concentrations used of nanoparticles to avoid their toxicity for animals.

Key words: Antimicrobial, *Aspergillus fumigatus*, *Klebsiella pneumoniae*, Rosemary oil, Selenium nanoparticles, Synergism.

INTRODUCTION

Nowadays, the worldwide problem of progressive raise in human populations resulted in the increased requirement of animal products, hence urgent significant attention for animal health and their productivity occurred. Hence, the health of large dairy animals as cattle gained intensive studies to improve all health factors related to their successful production (Barkema *et al*. 2015) The disease condition is considered to be the essential factor affecting their health. The respiratory diseases caused by some fungi in cattle as *Aspergillus* sp., particularly *Aspergillus fumigatus*, resulted in mycotic pneumonia, gastroenteritis, and mastitis (Seyedmousavi *et al*. 2015). As well as, the bacterium of *Klebsiella pneumoniae* that causes several infections in human and cattle nasal swabs (Cheng *et al*. 2018). The microbial drug resistance that occurred due to the prolonged wrong use of traditional antibiotics is considered as the most important problem in the control of these infections (Zhang *et al*. 2021). Therefore, novel antimicrobial agents are required to overcome microbial resistance to conventional antibiotics (Singh *et al*. 2018). Recently, there is progressive advancement in nanotechnology which enables the synthesis of novel nanosized materials that inhibit microbial growth and suppress their potentials in the occurrence of diseases among veterinary animals (Hassan *et al*. 2020). In addition, several studies confirmed the antioxidant, antibacterial, and antifungal activities of metals and metal oxide nanoparticles (Hassan *et al*. 2020, Fouda *et al*. 2020). In this respect, metal nanoparticles (NPs) particularly selenium NPs have significant and low toxicity (Zheng and Chen 2012).

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However, Geoffrion *et al*. (2020) used green biosynthesized selenium nanoparticles (SeNPs) and it has antibacterial potential against antibiotic-resistant bacteria *E. coli* and methicillin-resistant *S. aureus*. Also, Menon and Shanmugam (2019) detected that selenium nanoparticles are that produced by the seed of *Mucuna pruriens* gave NPs of nearly 100 -120 nm and had half inhibitory concentration (IC50)(60 µg/mL) for inhibition of the cell viability of bacteria at 48h. The green synthesis of SeNPs is cost-effective and environmental friendly that can be utilized in further biomedical applications.

Moreover, SeNPs can be used as drug delivery, additives in food and feed, and detection and treatment of livestock diseases (Husen and Siddiqi 2014). On the other hand, the nano-oil emulsions were successfully used in veterinary medicine as drug delivery and antimicrobial agents (Hassan *et al*. 2020). Meena *et al*. (2018) illustrated that the advantages of using the oils. Nanoemulsions have the simplicity, inexpensiveness, stability, versatility and the solubility of lipophilic substances and the ability to protect them from degradation. One of the most useful oils is rosemary oil. It can be used in the processing of food as antioxidants and prevent microbial growth (Barreto *et al*. 2014). Therefore, the current research articles were undertaken to detect the fungal and bacterial causes of respiratory manifestations in cattle. The most prevalent microbial agents that recovered from the present samples were used for evaluating the effects of SeNPs singly and in combination with Rosemary oil in inhibiting the activities of pathogens. Moreover, the minimum inhibitory concentrations of these agents were measured during all tests in comparison with traditional antibiotics. Additionally, the activities mechanisms of SeNPs and oils and their benefits were fully discussed.

MATERIALS AND METHODS

Samples

A total of 120 samples (70 nasal swabs and 25 each of water and ration samples) were collected from private cattle farms at Giza Governorate. Approximately 100 gram of each ration sample was aseptically collected, properly seal the sample container to ensure that leakage will not occur during transport. One hundred (100) ml of each water samples was collected from water troughs in sterilized screw capped bottle. Nasal swab was gently inserted the entire soft tip of the swab into one nostril until a bit of resistance was felt and rubed in a circle around the walls atleast 4 times. One swab was used for both the nostrils and two nasal swabs were taken from each animal. The swab was put into the provided tube and screwed the red cap on tightly. Each sample was divided into two parts; one was subjected to mycological examination, while the second part was subjected to bacteriological examination.

Antibacterial, antifungal, and other chemicals

Antibacterial, antifungal, and reagents were obtained from Sigma Chemical Company (USA).

Selenium nanoparticles and Rosemary oil

The used Se-NPs were synthesized by green method as per Rai *et al* (2017) and characterized by the laboratory of ALDRIK Sigma chemical company, USA and it was

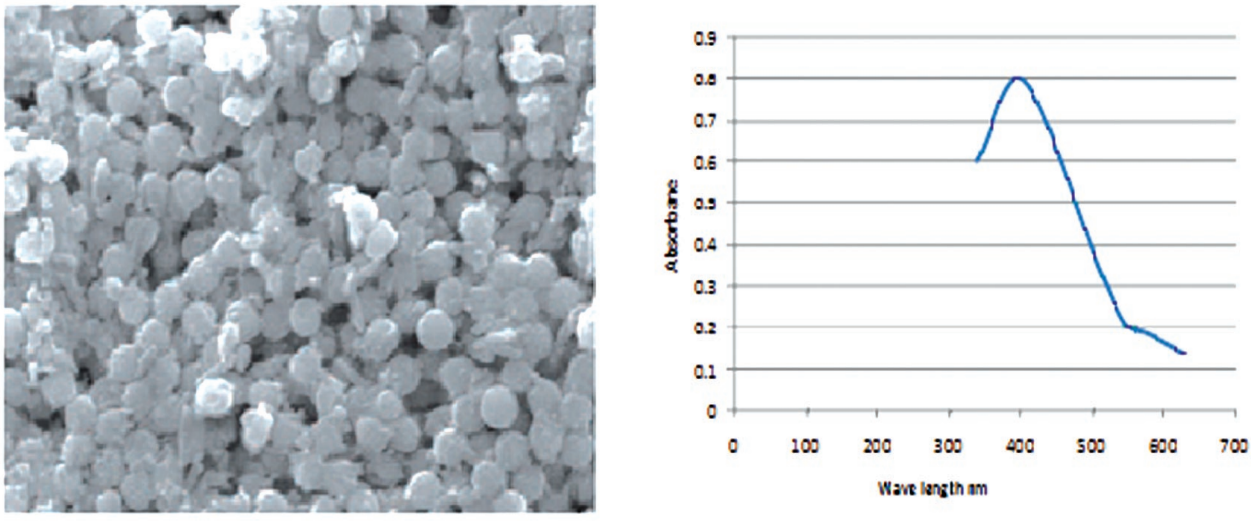

Fig. 1. SEM pictures of Se–NPs showed the size and morphology (60 nm) and the UV-VIS absorbance spectra of Se-NPS (60 nm) at 405 nm wavelength.

in amorphous powder form of 60 nm particles size. While, Rosemary oil was purchased in crud form from Al Gomhorya chemical company, Egypt.

Mycological examination

The collected samples were prepared, enriched by incubation in Sabouraud dextrose broth at 28°C for 24 hrs and examined for isolation of fungi as a method as (Refai *et al*. 2012). The samples were cultured on Sabouraud dextrose agar (SDA) and incubated 3-5 days at 25-28 °C. After incubation, the plates were examined macroscopically and microscopically. Identification based on the morphology of the colony, the rate of growth, and microscopic morphology of the isolates according to the description in textbooks dealing with molds (Pitt and Hocking 2009). The fungal isolates were sub-cultured by single spore isolation technique on SDA, CZ, CYA and MEA media. The fungal cultures were separated into groups based on their morphological characteristics including colony size (diameter), texture and surface. The fungal cultures were examined periodically during the incubation period. The culture characteristics and sporulation on different culture media were recorded after 7 days of the incubation period at 28°C. The morphological characteristics of each fungal isolate were determined using the light microscope. The microscopic examination of fungal isolates was described after the fungal colonies were sporulated on the different culture media. For this purpose, small mycelia part from the centre and edge of the growing colony was mounted onto microscope slide using distilled water and covered by a cover slip. The characteristics of vegetative and reproductive structures such as hyphal colour and structures, spore shape, as well as spore size were determined.

Bacteriological and Serological Examination

The samples were collected in swabs containing nutrient broth and kept at 37°C for 24 hrs. A loopful from each broth was streaked onto the following media: blood agar, MacConkey's agar, Edwards agar and incubated aerobically at 37°C for 24-48 hrs. The growing surface colonies (pink, mucoid colonies with 3-4 mm in diameter) were picked up, purified, and re-inoculated into a nutrient broth for further identification which is based on cultural, morphological, and biochemical characteristics (Quinn *et al.* 2011). Different bacteria were identified routinely using morphological and biochemical tests, followed by different specialized tests, serotyping and antibiotic resistance patterns etc. Water samples were examined according to Oblinger and Koburger (1975).

Antibiotic sensitivity test

It was done by disc diffusion method according to (Cruichshank *et al.* 1975). The results were interpreted according to (NCCLS 2004).

Green synthesis and Characterization of selenium nanoparticles (Inregole *et al.* 2010)

One 100 ml (10-1M) sodium selenosulphate was

Table 1. Prevalence rates of mould, yeast and bacteria species recovered from the examined samples collected from cattle.

Fungal species	Type of examined samples					
	Nasal swabs (n=70)		Water (n=25)		Ration (n=25)	
	No.	%	No.	%	No.	%
Aspergillus fumigatus	10	14.28	3	12	8	32
Aspergillus flavus	9	12.8	19	76	1	4
Pencillium sp.	2	2.8	1	4	0	0
Fusariium sp.	5	7.1	1	4	1	4
Mucor sp.	4	5	1	4	1	4
Candida albicans	5	7.1	1	4	0	0
Geotrichum sp.	2	2.8	-	0	0	0
Trichosporum sp.	1	1.4	-	0	0	0
Klebsiella pneumoniae	12	17.14	0	0	2	8
Klebsiella oxytocha	5	7.14	0	0	1	4
Pseudomonas aeroginosa	3	4.28	4	16	0	0
Staph. aureus	2	2.85	0	0	1	4
Strept. pyogens	2	2.85	0	0	0	0

Table 2. Optical density and transmittance of treated *A. fumigatus* and *Kl. pneumoniae by* SeNPs and Rosemary oil.

Concentration of SeNPs/R. mg/ml	*A. fumigatus*		*Kl. pneumoniae*	
	OD	T%	OD	T%
0.0	2.00	0.80	0.63	23.4
0.1 SeNPs/0.75 R	0.0	100	0.0	100
0.1 SeNPs/ 1.0 R	0.0	100	0.0	100
0.2 SeNPs/ 0.75 R	0.0	100	0.0	100
0.2 SeNPs/1.0 R	0.0	100	0.0	100

* R = Rosemary oil, T= Transmittance.

treated with 10ml 4% glucose solution and the mixture was refluxed. The color of the solution changes from colorless to yellow after refluxing immediately and becomes orange after 30 minutes. The orange color sols 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∝ (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 Se-NPs and rosemary oil for *Aspergillus fumigatus* and *Klebsiella pneumonia*e (CLSI 2008)

Preparation of bacterial and fungal spore suspension of isolates (Koneman *et al.* 1992, Gupta and Kohli 2003).

The fungal mycelia and bacterial colony were washed off with 6 ml of sterile distilled water per test tube, the outer layer of growth was scraped by a sterile loop. These spores suspensions were counted in a hemocytometer and adjusted dilution factor and the spores count was adjusted to10^5 spores /ml.

The inhibitory levels of Se-NPs and rosemary oil were estimated determined by a broth microdilution method for bacteria (Balachandran *et al.* 2016) and mold (NCCLS M27-A2, 2002). Briefly, in test tubes, 900 µl of SD broth medium (for fungi) or nutrient broth (for bacteria) were added. 100µl of spore suspension added separately of the inoculum of *A. fumigatus* and *Kl. pneumoni*ae and to 1 X 10^5 cells/ml. Then, 100 µl of SeNPs concentrations 0, 0.1, 0.2, 0.3, 0.4, 0.5 mg/ml or rosemary at levels of 0.0, 0.5, 0.75, 1.0, 1.5; 2.0 mg/ml, were added. Similar tests were applied using the traditional antibacterial and antifungal agents in the separate assays.

Combination effects of Se-NPs and rosemary oil was performed as above mentioned tests with some modification in addition as follow 0.1 SeNPs/0.75 R, 0.1 SeNPs/1.0 R, 0.2SeNPs/0.75R ; 0.2SeNPs/ 1.0R mg/ml (Shakibaie *et al.* 2015, Menon *et al.* 2020, Abozahra *et al.* 2020). All the test tubes were incubated for 48 hrs – 5 days at 28-30°C (for fungi) and 24-48 hrs at 37°C (for bacteria). The experiment was repeated twice and the MIC for fungi and bacteria was defined as the lowest Se-NPs concentration that showing no visible fungal or bacterial growth after incubation time. Also, 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 hrs - 2 weeks. The lowest levels of Se-NPs and rosemary oil that inhibiting the visual growth of the test cultures on the agar plate were reported as MIC. The turbidity of the growth in tubes was observed every 24 hrs. The growth was assayed by measurement of optical density and transmittance % of each tube's content at 405 nm using a spectrophotometer (NCCL - M27-A2, 2002).

Application of SeNPs singly and in combination with Rosemary for control of *A. fumigatus* and *Kl. pneumoniae* growth on sterilized yellow corn (Gupta and Kohli 2003)

The same procedures were repeated using sterilized commercial yellow corn contaminated with a spore suspension of *A. fumigatus* or *Kl. pneumoniae* instead of synthetic media. The total colony count of *A. fumigatus* or *Kl. pneumoniae* was evaluated before and after treatment.

Statistical analysis

The statistical evaluation was done by SPSS version 21 software package (SPSS, Inc, USA) through one-way ANOVA followed by Dunnett tests for control negative group (G1) comparison and p value ≤ 0.05 was considered statistically significant. All data were tabulated as Means±SD. according to SPSS 14 (2006).

RESULTS AND DISCUSSION

In the present study, a total of 120 samples were collected from private cattle farms with respiratory manifestation (70 nasal swabs, 25 drinking water, and 25 animal rations). The presented samples examined for isolation and identification of bacterial and fungal pathogens causing respiratory manifestations in dairy cattle with special reference to *A. fumigatus* and *Kl. pneumoniae*. The obtained tabulated results in Table 1 showed that the prevalence rates of *A. fumigatus* were

14.28%, 12%, and 32% in the nasal swab, drinking water, and animal ration, respectively. Other species were identified as *A. flavus, penicillium, fusarium,* and *Mucor sp.* in different comparatively low frequencies. Moreover, yeast species were recovered from the nasal swabs as, *Candida albicans* 7.1%, *Geotrichum* sp. 2.8%, and *Trichosporum* sp. 1.4%. Similar results have already been reported by Al-Khalidi *et al.* (2012). On the other hand, *Kl. pneumoniae* is a facultatively anaerobic, Gram-negative bacterium of the *Enterobacteriaceae* family, and a reported opportunistic pathogen. Several studies recorded it as the main cause of pneumonia in mammals (Newire *et al.* 2013). The invasion of *Kl. pneumoniae* in the domestic animal has a potential threat to public health since these animals can act as a reservoir of multidrug-resistant *Kl. pneumoniae* strains (Cheng *et al.* 2018). Regarding results reported in Table 1, it is evident that the prevalence of *Kl. pneumoniae* isolated from examined nasal swab, water and rations were 17.14%, 0%, 8%, respectively. While, *Kl. oxytocha* recorded in 7.14%, 0%, 4% of examined nasal swab, water and rations samples, respectively. On the other hand, *Pseudomonas aeroginosa, Staph. aureus* and *Strept. pyogenes* were isolated from the examined nasal swabs in a percentage of 4.28%, 2.85%, and 2.85% respectively.

It is common to detect pulmonary mixed infection as in the bovine respiratory tract which acts as reservoirs for pathogenic microorganisms that cause pneumonia (Moustafa 2004). Approx. 2.8% of examined nasal swab samples showed mixed infection with *A. fumigatus* and *Kl. pneumoniae*, while, there is no mixed infection recorded in ration and water samples. These results may be attributed to the antagonistic interactions between *Kl. pneumoniae* and several *Aspergillus* species including *A. fumigatus*, where, *Kl. pneumoniae* can prevent *Aspergillus sp*. spores germination and hyphae development (Nogueira *et al.* 2019).

In the present study, *A. fumigatus* was resistant to Fluconazole and Amphotericin B. However, it was sensitive to Voriconazole and Itraconazole at the rate of 70% and 80% respectively. Moreover, *Klebsiella* spp. were 100% resistant for each of E.Mox Clav (AMC30), Rifampicin (RD5) and Erythromycin (10µg). On the other hand, the isolates were sensitive to Ampicillin Sulbactom (SAM20), Nitrofurantion (F300), Amikacin (30µg), Oxytetracyclin (Ot30), and Oxacillin (OX1) in a percentage of 41.6%, 66.6%, 75%, 83.3%, 83, and 3% respectively. Today, the frequent use of traditional antibiotics that are resisted by some pathogens resulted in drug resistance and failure of disease treatments and this becomes a major world health concern (Hassan *et al.* 2020).

Herein, the used SeNPs were 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 (Transmission electron microscopy). The formation of selenium nanoparticles in presence of glucose is primarily authenticated from UV-Vis spectrophotometry (Fig.1). They are safe methods and environmentally friendly and available for large-scale production. The organisms may cause changes in the toxic metals by decreasing the toxic effects (Inregole *et al.* 2010).

In the present study, the Se-NPs were evaluated for inhibition of the growth of *A. fumigatus* and *Kl. pneumoniae* that *isolated* from nasal swabs, drinking water, and ration. The recorded results were shown in (Table 2) illustrated that the MIC of Se-NPs against *A. fumigatus* was (0.4 mg/ml) and it was (0.5 mg/ml) for *Kl. pneumoniae*. The optical density of treated spore suspension was decreased till reach zero and transmittance 100%.

Table 3. MIC of SeNPs combined with rosemary aganist *A. fumigatus and Kl. Pneumonia.*

Concentration of Se NPs (mg/ml)	*A.fumigatus*		*Kl. pneumoniae*		Concentration of Rosemary (mg/ml)	*A.fumigatus*		*Kl. pneumoniae*	
	OD	T%	OD	T%		OD	T%	OD	T%
0.0	2.13	0.79	0.63	23.4	0.0	2.13	0.79	6.30	23.4
0.1	0.27	53.1	0.15	63.1	0.5	0.44	36.3	0.34	45.1
0.2	0.10	79.9	0.08	93.3	0.75	0.00	100	0.29	63.1
0.3	0.00	100	0.06	87.1	1.0	0.00	100	0.00	100
0.4	0.00	100	0.02	95.5	1.5	0.00	100	0.00	100
0.5	0.00	100%	0.0	100	2.0	0.00	100	0.0	100

These results were confirmed by re-cultivation of inoculums from treated tubes onto the specific medium SDA for fungi and nutrient agar for bacteria. Shahverdi *et al.* (2010) investigated that the complete inhibition of *A. fumigatus* was seen in the presence of 80 µg/ml of biogenic selenium nanoparticles. Eswarapriya and Jegatheesan (2015) detected the antifungal potential of Se-NPs against *Aspergillus sp.* at a level of 12.5 µg/ml. Furthermore, Shakibaie *et al.* (2015) found that the MIC Selenium Nanoparticles (Se NPs) against *A. fumigatus* was 100 µg/ml. While the antimicrobial of Se-NPs against gram-negative bacteria was detected by many authors (Hariharan *et al.* 2012). Menon *et al.* (2020) found that the inhibition zone of Se-NPs against *Kl. pneumoniae* measured 10.5 ± 02.8 mm at a concentration of 100 µg/ml.

On the other hand, the antimicrobial potential of Rosemary oil against *A. fumigatus* and *Kl. pneumoniae* (Table 2) indicated that the Optical density and transmittance were concentration-dependent. When the concentrations of Rosemary increased up to 0.75 mg/ml, the optical density of treated *A. fumigatus* was decreased till reach 100% transmittance and clear medium. On the other hand, the inhibitory concentration of Rosemary oil that inhibited the growth of *Kl. pneumoniae* was 1.0 mg/ml. Herein, the antifungal potential of Rosemary oil against *Aspergillus* sp. was confirmed by Mihai and Popa (2015). Abozahra *et al.* (2020) determined the antimicrobial effect of rosemary against *Kl. pneumoniae*. The mechanism antimicrobial mechanisms of action of plant essential oils are suggested to be due to their contents of hydrophobic bioactive compounds which destruction of microbial cell walls and cell functions (Souza *et al.* 2013). They added that the contents of the oil affect ATP production, prevent cell protein synthesis, induce cytoplasmic changes and interfere with quorum sensing.

In the present study, results in (Table 3) showed the combined antimicrobial potentials of SeNPs with Rosemary against bacteria and fungi. It is obvious that the MIC of SeNPs against *A. fumigatus* and *Kl. pneumoniae* was decreased to 0.1 mg/ml when combined with Rosemary 0.75 mg/ml. The synergistic and combination therapy of SeNPs with the essential oil additives decreases the used concentration of nanoparticles. There have been numerous studies that have reported the potential of both essential oils and metal/metal oxide nanocomposites with broad spectra of bioactivities including antioxidant, anticancer, and antimicrobial activities (Hassan *et al.* 2020). Combination therapy represents an important field that needs greater attention and future investigations (Basavegowda *et al.* 2020).

Currently, obtained findings in (Table 4) showed that the inhibitory effect of SeNPs against *A. fumigatus* and *Kl. pneumoniae* in the ration which revealed that the MIC was 0.3 mg/ml and 0.4 mg/ml, respectively. Whereas, the combination of polluted rations with SeNPs and Rosemary oil caused a reduction in MIC of SeNPs to 0.1mg/ml for each.

Similarly, Hassan *et al.* (2017), detected the significantly stronger antimicrobial potentials of ZnNPs in conjugation with cinnamon oil or ozone than their single activities against bacteria and fungi. Also, the essential oil additives loaded into mesoporous silica nanoparticles can suppress the growth of fungi (Bernardos *et al.* 2015). The combination of nanoemulsions and matrix of certain nanostructures such as lipids and polysaccharides were more effective in the inactivation of bacteria 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

Table 4. Influence of treatments of SeNPs singly and in combination with oil in control of *A. fumigatus* and *Kl. pneumoniae* in infected ration.

Tested isolates	Mean log CFU/ml at gradual concentrations of SeNPs									
	Non treated (N.T.)	SeNPs (0.1mg /ml)	SeNPs (0.2mg /ml)	SeNPs (0.3mg /ml)	SeNPs (0.4mg /ml)	SeNPs (0.5mg /ml)	Non treated (N.T.)	*0.1 SeNPs/ 0.75 R*	*0.2 SeNPs/ 0.75 R*	*0.1 SeNPs/ 0.1 R*
A. fumigatus	3.3±0.18	1.9±0.04	0.7±0.054	00	00	00	3.3±0.18	00	00	00
Kl. pneumo.	3.1±0.11	2.17±0.22	1 ±0.036	0.6±0.73	00	00	3.1±0.11	0.3±0.085	00	00

microbial cells via the endocytosis process this related to induction ROS and the ions released by the nanostructures. While, Jay and Shafkat (2018) reported that Se-NPs destroyed the cell wall, leakage of cytoplasm contents, and loss of treated fungal and bacterial cell functions as detected when they subjected to SEM. While, Zhao *et al*. (2018), found that high stress due to the accumulation of SeNPs on the surface of cells stimulated the production of ROS which help in the inhibition of bacterial cells Also, Se-NPs destructed the cell wall, leakage of cytoplasm contents and loss of treated fungal and bacterial cell functions as detected when they subjected to SEM (Jay and Shafkat 2018). The synergistic and combination therapy of SeNPs with oils is of significant importance to reduce the used levels of metals NPs, avoid the microbial resistance to traditional antibiotics, and resulted in more efficient antimicrobial activity in the therapy of human and animal diseases. Moreover, there will be several benefits of metallic nanomaterials to be used in improving biomedical applications. Although, data related to their harmful not suf?cient and special attention is required for known their toxicity risk before to biomedical applications. Hence, future several toxicological studies are needed before nanotechnology applications in biomedicine and animal health.

CONCLUSION

Our forgoing results concluded that respiratory diseases are responsible for huge economic losses in livestock especially in large ruminants due to important burdens to the country's economy regarding meat, milk, wool, and leather industries. Hence, 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 besides other benefits strategies as disease detection, diagnosis and therapy, additives to food, feeds and their products, and finally food safety. Our results detected that Se-NPs and rosemary oil administration have significant antimicrobial potential against fungal and bacterial causes of respiratory infection and their combination showed the requirement of lower concentrations from both (0.1 mg/ml Se-NPs in combination with Rosemary 0.75mg/ml) to obtain significantly higher antimicrobial effects than their single form. This combination resulted in decreasing the concentrations from both to obtain the antimicrobial effects. Therefore, synergistic therapy is needed to reduce the used levels of metal nanoparticles and hence overcome their toxicity and more efficient antimicrobial activities occurred.

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