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Application of some inorganic metal oxide nanoparticles to control *E. coli* in raw milk

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Abstract

Background: Nanoparticles are regarded as magical bullets because of their exclusive features. Recently, the usage of nanoparticles has progressed in almost all aspects of science and technology due to its ability to revolutionize certain fields. In the field of food science and technology, the application of nanoparticles is being researched in many various areas thus provides the dairy industry with a variety of new attitudes for developing the quality, prolong shelf life, ensure the safety and healthiness of foods.

Aim: This study aimed to focus on the application of some inorganic metal oxide nanoparticles (zinc oxide (ZnO), magnesium oxide (MgO), and calcium oxide (CaO)) to control *E. coli* in raw milk and ensure its safety.

Methods: The antibacterial action of certain nanoparticles (ZnO, MgO, and CaO) with multiple concentrations (0.1, 0.05, 0.025, 0.0125, 0.006, and 0.003 mg/ml) was evaluated against *E. coli* strains in ultra heat treated (UHT) milk samples. Also, storage temperature and storage period effects were studied.

Results: The findings of the current research revealed that inorganic metal oxide nanoparticles had a significant antibacterial role against *E. coli*, in the following order; ZnO, MgO, and CaO, respectively. The antibacterial effect of inorganic metal oxide nanoparticles is more noticeable at lower temperatures.

Conclusion: Inorganic metal nanoparticles can be used in the food industry for the purpose of the control of *E. coli*, and extension of the shelf life of dairy products.

Keywords: Calcium oxide, *E. coli*, Magnesium oxide, Nanoparticles, Zinc oxide.

Introduction

Because it contains vital components including protein, carbohydrates, fat, vitamins, minerals, and water, milk is a perfect food source. However, bacteria may grow and multiply with the aid of these nutrients (Yohannes, 2018). Bacterial contamination found in milk has significant health issues that have raised concerns among the general public worldwide due to their potential to harm human health and cause social and economic difficulties.

One member of the facultative anaerobic, oxidase-negative, Gram-negative Enterobacteriaceae family of bacteria that ferments glucose to produce acid is *Escherichia coli* (*E. coli*). The latter can induce meningitis, sepsis, diarrhea, and urinary tract infections. Based on their virulence agents, different *E. coli* strains were named and classified. One of the most important food-borne infections that can be spread by milk and dairy products is *E. coli* (Hasan *et al.*, 2018). According to Zeinhom and Abdel-Latef (2014), *E. coli* is linked to potentially fatal conditions such as thrombotic thrombocytopenic purpura, hemolytic

uremic syndrome, and hemorrhagic colitis (HC). In addition, *E. coli* is one of the most important organisms that might result in difficulties and potentially fatal issues like bloodstream infections (Ibrahim *et al.*, 2022).

More affordable methods for creating nanoparticles with unique physical and chemical properties and restricted resistance have emerged as a result of recent advancements in microbial resistance to numerous antibiotics (Nazoori and Kariminik, 2018). The use of materials smaller than 100 nm is the focus of nanotechnology. In reality, they are used in many fields, such as material science, farming, the dairy sector, cosmetics, medicine, and diagnostics (Siddiqi *et al.*, 2018). Nanoparticles are referred to as "a wonder of modern medicine" these days. Because of their unique chemical and physical characteristics, nanostructures are now used more frequently (Abbas *et al.*, 2020). The most recent advancements in the field of nanoparticle components have a significant impact on the food industry, and nanotechnology has completely changed

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our social structure and is used extensively in daily life (Siddiqui and Alrumman, 2021).

The majority of the many types of nanoparticles found in food are made of inorganic substances including iron oxide, zinc oxide, calcium oxide (CaO), and magnesium oxide (MgO), all of which are safe for ingestion by humans (Pietrojusti *et al.*, 2016).

It is yet unknown how exactly nanoparticles work to inhibit bacteria. The antibacterial mechanism of nanoparticles has been explained by a number of mechanisms, including the generation of reactive oxygen species (ROS), which encourage lipid peroxidation in bacteria, the interaction of nanoparticles with bacteria that ultimately results in the destruction of the bacterial cell, and an alkaline impact. According to Tang and Lv (2014), the specific mechanism of action of metal oxide nanoparticles against bacteria is unclear and complex.

Worldwide antibacterial agents, zinc oxide nanostructures are thought to be nontoxic and biosafe (Navale *et al.*, 2015). It has been discovered that ZnO nanoparticles selectively poison bacteria while having no negative effects on human health. These findings support the potential uses of ZnO nanoparticles in the dairy industry (Stankic *et al.*, 2016). The Food and Drug Administration (FDA) in the United States has approved zinc oxide as a food additive that extends shelf life and is "generally recognized as safe" (GRAS) (Siddiqui and Alrumman, 2021).

Moreover, milk-borne pathogenic bacteria are inhibited by nanoscale MgO (Jin and He, 2011). An antibacterial agent based on light metals that the body can process and completely resorb is the nano-MgO particle. Because MgO nanoparticles can be broken down and metabolized effectively in the body and can actually be removed from the body as long as kidney function is normal, MgO nanoparticles are an attractive alternative to heavy metal-based nanomaterials like silver nanoparticles. This helps to prevent excessive metal accumulation in the body (Nguyen *et al.*, 2018). The US FDA presently lists MgO nanoparticles as safe GRAS and as an addition to human feed. According to Anić *et al.* (2018), MgO nanoparticles are important inorganic metal oxide nanoparticles with a typical broad band-gap.

The most significant aspect of CaO nanoparticles is their environmentally friendly character, which makes them a unique agent for future industrial prospects. They are inexpensive, non-corrosive, and have an antibacterial role and potential heterogeneous catalytic impact (Boey *et al.*, 2021). When used at allowed concentrations, CaO nanoparticles are generally not harmful and remain stable in harsh environments. Because CaO nanoparticles have the ability to generate ROS, which ultimately causes microbial mortality, they may offer a therapeutic approach for inhibiting microbial growth (Kumari *et al.*, 2023).

According to Bertu *et al.* (2010), milk that is meant for human consumption cannot include any harmful bacteria. In order to assess the antibacterial activity of several nanoparticles (ZnO, MgO, and CaO) against *E. coli* strains *in vitro* on Muller Hinton Agar media and *in vivo* in ultra heat treated (UHT) milk samples contaminated with *E. coli*, the current study was conducted. Furthermore, the impact of storage temperature and duration on the antimicrobial activity of nanoparticles was investigated.

Materials and Methods

Preparation of the bacterial strain

The tested *E. coli* strain was isolated from examined raw milk samples obtained from small holders and biochemically identified by VITEK B Compact (BioMérieux, USA) at Mbarret El Asafra Labs, Alexandria. The molecular identification was carried out in the Reference Laboratory for Veterinary Quality Control in Poultry Production, Animal Health Research Institute, Giza, Egypt.

Preparation of nanoparticles (Zn oxide, mg oxide, and ca oxide)

For use in photo-electronic applications, ZnO nanoparticles with the NT-ZONP brand and an analytical certificate were purchased from NanoTech Egypt. The nanoparticles were 99.98% pure and had an average size of 30 nm. To achieve a homogenous solution of nanoparticles at different concentrations, distilled water was added to the nanoparticles in glass containers. The particles were then sonicated for 30 minutes to create a colloidal suspension and uniform dispersion.

NanoTech Egypt purchased MgO nanoparticles for Photo-Electronics under the NT-MgONP brand, which has an average size of 10 nm and a purity of 99.98%, along with an analytical certificate. To achieve a homogenous solution of nanoparticles at different concentrations, distilled water was added to the nanoparticles in glass containers. The particles were then sonicated for 30 minutes to establish a colloidal suspension and uniform dispersion (Ali *et al.*, 2018).

NanoTech Egypt purchased CaO nanoparticles with an average size of 80 nm and a purity of 99.98% for use in photo-electronics, as shown by the NT-CaO brand and an analytical certificate. To achieve a homogenous solution of nanoparticles at different concentrations, distilled water was added to the nanoparticles in glass containers. The particles were then sonicated for 30 minutes to establish a colloidal suspension and uniform dispersion (Ali *et al.*, 2018).

Evaluation of antibacterial activity of various metal oxide nanoparticles against the E. coli isolates

Many of the antibacterial activities can be assessed using the agar-well diffusion method (Magaldi *et al.*, 2004; Valgas *et al.*, 2007; Balouiri *et al.*, 2016). Inorganic metal oxide nanoparticle dilutions were made at concentrations of 0.1, 0.05, 0.025, 0.0125,

0.006, and 0.003 mg/ml. The tested isolate's colony suspension was calibrated to meet the 0.5 McFarland criteria. To put it succinctly, the bacterial isolate was cultivated on Plate Count Agar (HiMedia, India) and incubated for twenty-four hours at 37°C. A 0.5 McFarland standard solution was used to compare the four to five isolated colonies of the tested strain that were picked up and injected at a concentration of 1.5×10^8 CFU/mL in tubes containing sterile saline (5 ml in each) (Saeed and Tariq, 2005). To ensure that the isolated bacteria were evenly distributed throughout the surface of a Muller-Hinton agar plate, 100 µl of the bacterial solution was carefully transferred using a micropipette. Subsequently, a sterile cork borer or tip was used to punch an aseptic hole measuring 6 to 8 mm in diameter. A volume of 20–100 µl of the inorganic metal oxide nanoparticle suspensions at varying concentrations was then added to the wells. Agar plates were then incubated for 24 hours at $37^\circ\text{C} \pm 2^\circ\text{C}$. The studied microbial strain's growth is inhibited by the antimicrobial agent as it diffuses throughout the agar media. The zones of inhibition were measured and the results were interpreted.

Evaluation of antibacterial activity of different metal oxide nanoparticles against the *E. coli* isolates in UHT milk model

The study conducted by Mirhosseini and Afzali (2016) involved the application of varying concentrations of different inorganic metal oxide nanoparticle suspensions, such as zinc oxide, MgO, and calcium oxide nanoparticles, to UHT milk model samples that had already been inoculated with a known quantity of *E. coli*. The aim was to evaluate the effect of inorganic nanoparticles on *E. coli*. The inoculated milk was then stored at cold storage, while a similar group of inoculated milk was kept at room temperature to ascertain the impact of temperature and storage conditions on the effectiveness of nanoparticles for inhibiting *E. coli* over a three-day period.

Statistical analysis

The data were presented as means \pm SD and subjected to analysis of variance and the Tukey's

Kramer HSD test, where a significance level of $p < 0.05$ was applied.

Ethical approval

Not needed for this study.

Results

Results in Table 1 exhibited that the highest concentration (0.1 mg/ml) of ZnO nanoparticles had the widest zone of inhibition (ZOI) against *E. coli* on Muller Hinton agar media with a ZOI (mm) of 5.93 ± 0.21 . ZnO nanoparticles at a concentration of 0.012 mg/ml also exhibited a high inhibition zone 2.23 ± 0.15 . A concentration (0.1 mg/ml) of MgO nanoparticles resulted in the widest ZOI against *E. coli* with 5.27 ± 0.25 , additionally a concentration (0.1 mg/ml) of CaO nanoparticles had a ZOI of 1.33 ± 0.21 .

Data given in Table 2 summarize the effect of different concentrations of various nanoparticles on *E. coli* count inoculated into UHT milk stored at cold storage ($4^\circ\text{C} \pm 1^\circ\text{C}$) at the 1st day. Data revealed that the most effective concentration of zinc oxide, MgO, and CaO was 0.1 mg/ml with a mean inhibition zone value of 1.85 ± 0.01 , 1.33 ± 0.01 , 2.31 ± 0.01 (\log_{10} cfu/ml), respectively. Summarized data pointed out the impact of different concentrations of nanoparticles on *E. coli* inoculated in UHT milk stored at ambient temperature ($25^\circ\text{C} \pm 1^\circ\text{C}$) on the 1st day. Results showed that ZnO, MgO, and CaO nanoparticles with a concentration of 0.1 mg/ml significantly reduced *E. coli* counts to 2.10 ± 0.01 , 1.80 ± 0.03 , and 1.94 ± 0.02 .

Results mentioned in Table 3 revealed the impact of different concentrations of various nanoparticles on *E. coli* count inoculated in UHT milk stored at cold storage ($4^\circ\text{C} + 1^\circ\text{C}$) on the 2nd day. ZnO, MgO, and CaO nanoparticles at 0.1 mg/ml significantly reduced *E. coli* counts to 2.75 ± 0.001 , 2.52 ± 0.001 , 2.76 ± 0.004 (\log_{10} cfu/ml), respectively. The impact of different concentrations of various nanoparticles on *E. coli* inoculated into UHT milk stored at ambient temperature ($25^\circ\text{C} \pm 1^\circ\text{C}$) on the 2nd day revealed that 0.1 mg/ml was the most effective concentration that achieved a significant reduction in *E. coli* counts

Table 1. Impact of different concentrations of various nanoparticles on viability of *E. coli* isolated from the examined milk sample using agar well diffusion method on Muller Hinton agar.

Ca oxide nanoparticles	Mg oxide nanoparticles	Zn oxide nanoparticles	Concentration of nanoparticles
Inhibition zone diameter (mm) (Mean \pm SD)			
$1.33 \pm 0.21^{\text{Ac}}$	$5.27 \pm 0.25^{\text{Ab}}$	$5.93 \pm 0.21^{\text{Aa}}$	0.1 mg/ml
$1.20 \pm 0.17^{\text{Bc}}$	$4.30 \pm 0.26^{\text{Bb}}$	$5.60 \pm 0.44^{\text{Ba}}$	0.05 mg/ml
$1.00 \pm 0.17^{\text{Cc}}$	$4.13 \pm 0.32^{\text{Ca}}$	$2.30 \pm 0.27^{\text{Cb}}$	0.025 mg/ml
0.00 ± 0.000	$3.03 \pm 0.15^{\text{Da}}$	$2.23 \pm 0.15^{\text{Db}}$	0.012 mg/ml
0.00 ± 0.000	$2.13 \pm 0.15^{\text{Eb}}$	$2.17 \pm 0.21^{\text{Ea}}$	0.006 mg/ml
0.00 ± 0.000	$2.03 \pm 0.15^{\text{Fa}}$	$2.10 \pm 0.27^{\text{Fa}}$	0.003 mg/ml

SD: Standard deviation. Means carrying different superscript capital letter on the same column are significantly different ($p < 0.05$). Means carrying different superscript small letter on the same row are significantly different ($p < 0.05$).

Table 2. Impact of different concentrations of various nanoparticles on viability of *E. coli* inoculated in UHT milk stored at (4°C ± 1°C) and (25°C ± 1°C) for 1st day.

Ca oxide nanoparticles		Mg oxide nanoparticles		Zn oxide nanoparticles		Concentration of nanoparticles
Room	Cold	Room	Cold	Room	Cold	
Mean ± SD (log ₁₀ cfu/ml)						
3.03 ± 0.003 ^{Aa}	2.65 ± 0.01 ^{Ab}	3.03 ± 0.003 ^{Aa}	2.65 ± 0.01 ^{Cb}	3.03 ± 0.003 ^{Aa}	2.65 ± 0.01 ^{Ab}	Control
1.94 ± 0.02 ^{Db}	2.31 ± 0.006 ^{Cb}	1.80 ± 0.03 ^{Fb}	1.33 ± 0.01 ^{Ea}	2.10 ± 0.01 ^{Fb}	1.85 ± 0.01 ^{Fb}	0.1 mg/ml
2.61 ± 0.003 ^{Ca}	2.61 ± 0.002 ^{Bb}	1.96 ± 0.01 ^{Eb}	2.50 ± 0.002 ^{Da}	2.38 ± 0.01 ^{Ea}	2.33 ± 0.004 ^{Eb}	0.05 mg/ml
2.63 ± 0.002 ^{Ba}	2.65 ± 0.004 ^{Aa}	2.56 ± 0.01 ^{Da}	2.55 ± 0.002 ^{Ca}	2.52 ± 0.01 ^{Db}	2.53 ± 0.004 ^{Db}	0.025 mg/ml
0.00 ± 0.000	0.00 ± 0.000	2.58 ± 0.002 ^{Cb}	2.58 ± 0.001 ^{Bb}	2.52 ± 0.001 ^{Db}	2.52 ± 0.003 ^{Db}	0.012 mg/ml
0.00 ± 0.000	0.00 ± 0.000	2.58 ± 0.003 ^{Cb}	2.58 ± 0.002 ^{Bb}	2.59 ± 0.002 ^{Ca}	2.59 ± 0.004 ^{Ca}	0.006 mg/ml
0.00 ± 0.000	0.00 ± 0.000	2.98 ± 0.002 ^{Ba}	2.69 ± 0.001 ^{Ab}	2.80 ± 0.003 ^{Ba}	2.62 ± 0.003 ^{Ba}	0.003 mg/ml

SD: Standard deviation. Means carrying different superscript capital letter on the same column are significantly different ($p < 0.05$). Means carrying different superscript small letter on the same row are significantly different ($p < 0.05$).

Table 3. Impact of different concentrations of various nanoparticles on viability of *E. coli* inoculated in UHT milk stored at (4°C ± 1°C) and (25°C ± 1°C) on the 2nd day.

Ca oxide nanoparticles		Mg oxide nanoparticles		Zn oxide nanoparticles		Concentration of nanoparticles
Room	Cold	Room	Cold	Room	Cold	
Mean ± SD (log ₁₀ cfu/ml)						
3.11 ± 0.001 ^{Aa}	2.85 ± 0.000 ^{Ab}	3.11 ± 0.001 ^{Ba}	2.85 ± 0.000 ^{Ab}	3.10 ± 0.001 ^{Bb}	2.85 ± 0.000 ^{Aa}	Control
2.60 ± 0.001 ^{Db}	2.76 ± 0.004 ^{Cb}	2.69 ± 0.002 ^{Fa}	2.52 ± 0.001 ^{Eb}	2.78 ± 0.001 ^{Fa}	2.75 ± 0.001 ^{Fb}	0.1 mg/ml
2.85 ± 0.002 ^{Ca}	2.84 ± 0.003 ^{Bb}	2.89 ± 0.001 ^{Ea}	2.64 ± 0.003 ^{Db}	2.83 ± 0.001 ^{Eb}	2.77 ± 0.002 ^{Eb}	0.05 mg/ml
2.89 ± 0.003 ^{Ba}	2.85 ± 0.001 ^{Ab}	2.96 ± 0.002 ^{Da}	2.64 ± 0.003 ^{Db}	2.92 ± 0.001 ^{Da}	2.82 ± 0.001 ^{Db}	0.025 mg/ml
0.00 ± 0.000	0.00 ± 0.000	3.00 ± 0.001 ^{Ca}	2.70 ± 0.002 ^{Cb}	2.97 ± 0.001 ^{Ca}	2.83 ± 0.002 ^{Cb}	0.012 mg/ml
0.00 ± 0.000	0.00 ± 0.000	3.00 ± 0.001 ^{Ca}	2.79 ± 0.003 ^{Bb}	3.18 ± 0.001 ^{Aa}	2.83 ± 0.001 ^{Cb}	0.006 mg/ml
0.00 ± 0.000	0.00 ± 0.000	3.12 ± 0.001 ^{Aa}	2.85 ± 0.001 ^{Ab}	3.18 ± 0.000 ^{Aa}	2.84 ± 0.001 ^{Bb}	0.003 mg/ml

SD: Standard deviation. Means carrying different superscript capital letter on the same column are significantly different ($p < 0.05$) within the same nanoparticle. Means carrying different superscript small letter on the same row are significantly different ($p < 0.05$) within the same nanoparticle.

to 2.78 ± 0.001, 2.69 ± 0.002, 2.60 ± 0.001 log₁₀ cfu/ml, respectively. Also, data in Table 3 illustrated a comparison among different concentrations of various nanoparticles on *E. coli* inoculated into UHT milk at both cold storage (4°C ± 1°C) and ambient temperature (25°C ± 1°C) on the 2nd day.

Summarized results in Table 4 showed the effects of different concentrations of various nanoparticles on *E. coli* inoculated into UHT milk stored at cold storage (4°C ± 1°C) on the 3rd day. Data revealed that the most effective concentration of zinc oxide, MgO, and CaO nanoparticles was 0.1 mg/ml with mean counts of 3.06 ± 0.000, 2.82 ± 0.002, and 2.74 ± 0.002 (log₁₀ cfu/ml). Results in Table 5 point out the impact of the storage temperature and zinc oxide nanoparticles concentrations on *E. coli* inoculated into UHT milk stored over 3 days.

Discussion

Inorganic metal oxide nanoparticles such as ZnO, CaO, and MgO gain great attention as they are not

only constant under strict process, but also their antimicrobial role gains clear importance for their probable application in the dairy branch (Vasilache et al., 2011; Husen, 2017).

The obtained results of the current study revealed that the highest concentration of ZnO evolved the best results. ZnO nanoparticles with concentration (0.012 mg/ml) also exhibited a high inhibition zone 2.23 ± 0.153 which is in line with other reports (Vasilache et al., 2011; Bai et al., 2015). They found that 15 µg/ml zinc oxide nanoparticles had a significant effect against *E. coli*. Zinc oxide nanostructures are global antimicrobial compounds and are regarded bio-safe and nontoxic. The antibacterial efficacy was previously examined using agar well diffusion assay at higher concentrations (compared to our work) (256, 128, 64, 32 mg/ml) of ZnO NPs. The findings of the study revealed a higher reduction in *E. coli* counts (12.66 ± 0.66 mm) (Navale et al., 2015). In contrast, higher concentrations of ZnO (0.5 and 2 mM) did not exhibit

Table 4. Impact of different concentrations of various nanoparticles on viability of *E. coli* inoculated in UHT milk stored at (4°C ± 1°C) and (25°C ± 1°C) on the 3rd day.

Ca oxide nanoparticles		Mg oxide nanoparticles		Zn oxide nanoparticles		Concentration of nanoparticles
Room	Cold	Room	Cold	Room	Cold	
Mean ± SD (log ₁₀ cfu/ml)						
**	3.15 ± 0.001 ^A	**	3.15 ± 0.001 ^A	**	3.15 ± 0.001 ^A	Control
**	2.74 ± 0.002 ^C	**	2.82 ± 0.002 ^F	**	3.06 ± 0.000 ^E	0.1 mg/ml
**	3.01 ± 0.001 ^B	**	2.94 ± 0.001 ^E	**	3.07 ± 0.001 ^D	0.05 mg/ml
**	3.15 ± 0.000 ^A	**	2.95 ± 0.001 ^D	**	3.13 ± 0.000 ^C	0.025 mg/ml
**	0.00 ± 0.000	**	3.00 ± 0.001 ^C	**	3.13 ± 0.001 ^C	0.012 mg/ml
**	0.00 ± 0.000	**	3.09 ± 0.001 ^B	**	3.14 ± 0.001 ^B	0.006 mg/ml
**	0.00 ± 0.000	**	3.15 ± 0.000 ^A	**	3.15 ± 0.001 ^A	0.003 mg/ml

SD: Standard deviation. Means carrying different superscript capital letter on the same column are significantly different ($p < 0.05$) within the same nanoparticle. **Milk subjected to spoilage at 3rd day of room storage.

Table 5. Impact of storage temperature and zinc oxide nanoparticles concentrations on viability of *E. coli* inoculated in UHT milk stored over 3 days.

3rd day		2nd day		1st day		Concentration of nanoparticles
Room	Cold	Room	Cold	Room	Cold	
Mean ± SD (log ₁₀ cfu/ml)						
**	3.15 ± 0.001 ^{Aa}	3.10 ± 0.001 ^{Bb}	2.85 ± 0.000 ^{Ad}	3.03 ± 0.00 ^{Ac}	2.65 ± 0.008 ^{Ac}	Control
**	3.06 ± 0.000 ^{Ea}	2.78 ± 0.001 ^{Fb}	2.75 ± 0.001 ^{Fc}	2.10 ± 0.006 ^{Fe}	1.85 ± 0.011 ^{Fe}	0.1 mg/ml
**	3.07 ± 0.001 ^{Da}	2.83 ± 0.001 ^{Eb}	2.77 ± 0.002 ^{Ec}	2.38 ± 0.006 ^{Ec}	2.33 ± 0.004 ^{Ed}	0.05 mg/ml
**	3.13 ± 0.000 ^{Ca}	2.92 ± 0.001 ^{Db}	2.82 ± 0.001 ^{De}	2.52 ± 0.001 ^{Dd}	2.53 ± 0.004 ^{De}	0.025 mg/ml
**	3.13 ± 0.001 ^{Ca}	2.97 ± 0.001 ^{Cb}	2.83 ± 0.002 ^{Cc}	2.52 ± 0.006 ^{De}	2.52 ± 0.003 ^{Dd}	0.012 mg/ml
**	3.14 ± 0.001 ^{Bb}	3.18 ± 0.001 ^{Aa}	2.83 ± 0.001 ^{Cb}	2.59 ± 0.002 ^{Cd}	2.59 ± 0.004 ^{Cc}	0.006 mg/ml
**	3.15 ± 0.001 ^{Ab}	3.18 ± 0.000 ^{Aa}	2.84 ± 0.001 ^{Bc}	2.80 ± 0.003 ^{Bd}	2.62 ± 0.003 ^{Bd}	0.003 mg/ml

SD: Standard deviation. Means carrying different superscript capital letter on the same column are significantly different ($p < 0.05$). Means carrying different superscript small letter on the same row are significantly different ($p < 0.05$). ** Milk subjected to spoilage at 3rd day of room storage.

an inhibitory zone on *E. coli*, according to Mirhosseini and Firouzabadi (2013). In contrast, the inhibition zone against *E. coli* for 5 and 10 mM of ZnO was 12 mm. The outcomes of our experiment matched those of Abdulrahman and Nssaif (2016).

Because of their stability, surface characteristics, and structure, MgO nanoparticles play a significant role as antibacterial materials in dairy applications (He et al., 2016). MIC of 500 µg/ml was the antibacterial activity of MgO nanoparticles against *E. coli*. Because of the existence of oxygen vacancy defects at the surface of the nanoparticles, it was hypothesized that the mechanism of MgO nanoparticles' antibacterial effect would include lipid peroxidation and ROS (Krishnamoorthy et al., 2012). Our results contradicted those of Kumari et al. (2023), who reported that calcium oxide nanoparticles exhibited the maximum inhibition zone against *E. coli*.

To assess the antibacterial activity of CaO NPs against a variety of bacterial strains, including *E. coli*, the Agar well diffusion method was utilized. A 16.6 mm zone of inhibition was observed for *E. coli* at doses of CaO NPs (1 mg/ml) (Anantharaman et al., 2016). As the nanoparticle concentration rose, the zone of inhibition progressively widened. It is likely that Sawai (2003) stated that as the quantity of MgO nanoparticles increased, so did their activity against *E. coli*.

It has been demonstrated that inorganic metal oxide nanoparticles, like ZnO, are extremely hazardous to *E. coli* (Hu et al., 2009). When used at low concentrations, ZnO nanoparticles dramatically prevent *E. coli* from growing (Hirota et al., 2010). Researchers looked at the mechanism underlying ZnO nanoparticles' antibacterial effectiveness against *E. coli*. The chemical interactions between hydrogen peroxide and membrane proteins, as well as the chemical interactions between other unknown chemical species produced as a result of ZnO

nanoparticles existing within the lipid bi-layer, are found to be the primary mechanisms of such antibacterial efficacy. Under the circumstances of this investigation, it is discovered that the impact of direct physical interactions between nanoparticles and biological cells is comparatively minimal (Zhang *et al.*, 2010). Both the size and concentration of the nanoparticles affect how effective they are against germs. It has been observed that nanoparticle concentration matters more than particle size (Vasilache *et al.*, 2011).

The antibacterial role of MgO nanoparticles was almost >3 mg/ml concentration required to observe the destruction effect on *E. coli* (Jin and He, 2011). These results are extensively higher than our experiment as we demonstrated that the application of 0.1 mg/ml of MgO nanoparticles gave the best significant effect against *E. coli* compared to control. From the analysis of the antibacterial activity of MgO nanoparticles, it is revealed that 7.5 µg/ml of a dose is sufficient for killing *E. coli* (Maji *et al.*, 2020) this is parallel to our findings which approved that usage of MgO nanoparticles with concentration (0.006 mg/ml) decreased the count of *E. coli* from 2.65 ± 0.008 to 2.58 ± 0.002 (\log_{10} cfu/ml) significantly. Likely, ZnO and MgO nanostructures cause a major reduction in *E. coli* number in the broth medium.

A wide group of researchers found that nano-sized ZnO reveals diverse morphologies and exhibits strong antibacterial activity over a broad spectrum of bacterial species (Raghupathi *et al.*, 2011). The highest *E. coli* bacterial count was seen at the lowest doses of zinc oxide and MgO nanoparticles, which was significantly different from the control. *E. coli* is susceptible to the antibacterial activity of MgO nanoparticles (Makhluf *et al.*, 2005). The antimicrobial activity of MgO and CaO nanoparticles is dependent on a variety of parameters, including nanoparticle size, shape, biodegradation, high-temperature stability, high surface charge, and surface adsorption ability, all of which can be extremely harmful to biological systems (Dizaj *et al.*, 2014).

Cold storage significantly plays a great antimicrobial role in *E. coli* count compared to room temperature storage. These results are in the same line with data reported before (Lo' Ay and Ameer, 2019) who said that most nanoparticles' effect was more pronounced at lower temperatures. In general, it has been found that the antibacterial activity of ZnO NPs is increased at lower temperatures. This is likely due to the fact that ROS are more stable at lower temperatures. Additionally, the cell membrane of bacteria is more susceptible to damage at lower temperatures (Qu *et al.*, 2010; Sirelkhatim *et al.*, 2015).

Cold storage affects *E. coli* microbial count compared to the room temperature. Also, most concentrations of ZnO nanoparticles affect *E. coli* count at cold storage significantly compared to the room temperature. This means that ZnO nanoparticles play a synergistic role

with cold storage on *E. coli* count on the 2nd day of storage. All concentrations of MgO nanoparticles have a great effect on *E. coli* count together with the aid of cold storage.

The storage period can have a vital role in the activity of zinc oxide nanoparticles and in the viability of *E. coli* due to agglomeration, nanoparticles tend to agglomerate or form clusters over time especially if they are not stored under proper conditions which can reduce their surface area and hinder their reactivity. Surface oxidation, some nanoparticles such as metal-based ones may undergo surface oxidation when exposed to air during storage for an extended period of time; this oxide layer can alter the nanoparticles properties and affect its activity. Contamination and storage conditions might lead to contaminants in the end products. The stability of certain types of nanoparticles is influenced by storage conditions such as temperature and humidity levels, if stored improperly; these particles may degrade or lose their desired properties over time. Particle size distribution changes over long periods of storage, there might be changes in particle size distribution due to various reasons including agglomeration leading to change in catalytic performance (Mukha *et al.*, 2013; Abdou *et al.*, 2012).

Conclusion

Nanoparticles became among the most commonly used preservatives due to their specific chemical and physical features, and their antibacterial properties against milk-containing pathogens as *E. coli*. According to our study, it can be concluded that ZnO, MgO, and CaO nanoparticles are effective antibacterial agents for *E. coli*.

Conflict of interest

The authors declare that there is no conflict of interest.

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Author's contributions

All authors participated in lab work, data analysis, and writing, revising, and approving the manuscript.

Data availability

Data of the current study are available upon request.

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