



# Antimicrobial Activity of Zeolite/Zinc Oxide Nanocomposite Containing *Aloe Vera* Gel Against *Shigella* spp. and Its Durability Effects on Strawberries

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## Abstract

Nanomaterials are novel applicable tools to improve fruit quality by using antimicrobial agents to prolong food storage as well as contamination reduction. The objective of this study was to evaluate the cytotoxicity and antibacterial effect of zeolite/zinc oxide nanocomposite (Zeo/ZnONC) alone and with *aloe vera* gel (Zeo/ZnONC-AG) on *Shigella sonnei* and *Shigella flexneri* and its effect on the durability of strawberries. First, ZnO materials were characterized with X-ray fluorescence (XRF), and Zeo/ZnONC was evaluated with field emission scanning electron microscopy (FE-SEM) analyses to confirm ZnO nanoparticle formation. Subsequently, the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of Zeo/ZnONC and Zeo/ZnONC-AG against *S. sonnei* and *S. flexneri* were assessed. The preservative application of hydrogel nanocomposite on the quality of strawberries was evaluated through the appearance of the fruits and the growth of mildew colonies. The MIC and MBC values of Zeo/ZnONC-AG against *S. sonnei* and *S. flexneri* were lower than the Zeo/ZnONC in equal concentrations of ZnO (1 mg/ml up to 8 mg/ml). The growth of mold on the surface of strawberries treated with Zeo/ZnONC-AG has shown a delay with the increase of ZnO concentration at refrigerator temperature. In conclusion, this study exhibited that Zeo/ZnONC-AG preservative has noticeable benefits in control of contamination and durability enhancement of the fruits like strawberries. The potential application of nanomaterials in industrial food suggests more evaluation on this compound by further studies.

**Keywords** Zinc oxide · Nanocomposites · *Aloe vera* · *Shigella sonnei* · *Shigella flexneri* · Natural food preservative

## 1 Introduction

Consumption of foods contaminated with infectious microorganisms causes serious diseases in humans. Preservation of fresh fruits is a challenging problem because they cannot be treated with heat or very harsh methods and still maintain their organoleptic quality. Disinfectants have been approved for fruits and vegetables, and chlorine has been widely used as a disinfectant [1, 2]. A comprehensive review of methods to kill pathogens in fresh fruits found that chlorine was not effective at concentrations commonly used (< 200 ppm) [3]. Therefore, safe and edible compounds such as lavender, chicory, and mentha pulegium coating oils are receiving recently a great deal with shelf life improvement and antimicrobial activity in the food industry [4–6].

Strawberry (*Vaccinium corymbosum*) is widely available among different fruits with nutritional features worldwide. However, this fruit is much more sensitive to

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nutritional, biochemical, and structural changes. These changes can mainly cause the loss of moisture and the activity of microorganisms, especially fungi [7]. *Shigella* is easily transmitted through human contact due to its low infectious dose of 10–200 cells [8]. *Shigella* has been isolated from almost all types of food, fruits, and vegetables [9]. It seems that the prevalence of antibiotic-resistant *Shigella* strains in foods is also increasing [10]. The significant number of occurrences of *Shigella* spp. each year in produce indicates the need to develop new non-antibiotic approaches for the disinfection of nutrients [11].

Zinc oxide nanoparticles (ZnONPs) have obtained abundant attention thanks to their distinctive physicochemical characteristics. ZnONPs are easily available at low production costs with high biocompatibility and have efficient catalytic activity and excellent bioactivity [12, 13]. More importantly, ZnO has been recognized as “generally recognized as safe” by the Food and Drug Administration (FDA) [14]. Zeolite (Zeo) is a microporous crystalline material of aluminosilicate, which can use for fruit preservation. NPs loaded Zeo are the subject of ongoing research due to their ethylene adsorption properties, hydrothermal stability of the cationic sites, durability, and safety which are particularly important toward industrial application and food marketing [15, 16]. The study’s results by Partoazar et al. indicated that ZnO/ZeoNC significantly inhibited the antibiofilm activity of *S. aureus* strains and eliminated the bacteria biofilm from the abiotic surface during 24-h cultivation. Those data suggest ZnO/ZeoNC can be safe and non-toxic for human cells while acting against infectious due to staphylococcal colonization [12]. In the study by Afrasiabi et al. [17], it was found that the growth of pathogenic bacteria such as *Streptococcus mutans* can be inhibited by using ZnONPs. These findings correspond to a Liu et al. [18], who achieved ZnONPs can potentially be used as an effective antibacterial agent to protect agricultural and food security.

Moreover, *Aloe vera* (*Aloe barbadensis* Miller), a member of the *Liliaceae* family, is one of the most biologically active natural plants. Due to its phenolic compounds, it acts as an antimicrobial and antioxidant. It is extensively employed in the food industry and pharmaceutical applications. A main characteristic of *aloe vera* is its high water content (above 90%) [19]. The antimicrobial activity of *Aloe vera* has been displayed against a wide variety of bacteria [20]. *Aloe vera* gel (AG) has been applied to maintain quality parameters and delay the microbial decay in fruit such as strawberry [7].

Therefore, in this study, we aimed to investigate the antibacterial activity of Zeo/ZnO nanocomposite (Zeo/ZnONC) and Zeo/ZnONC-AG against *S. sonnei* and *S. flexneri* as well as their effects on the shelf-life improvement of strawberry at refrigerator temperature.

## 2 Materials and Methods

### 2.1 ZnO/Zeo Composite Coating

Firstly, compounds of ZnO/Zeo nanocomposite, ZnO/Zeo, and alone zeolite were prepared according to the procedure that we developed in our previous studies [21, 22]. Composite materials were suspended in concentrations of 1.6 %, 0.8%, 0.4%, and 0.2% of (w/v) in distilled water using a stirrer tool. In addition, the hydrogel forms of the composites were mixed with *aloe vera* powder at 2% w/v under 7000 rpm stirring at 37 °C. The dipping technique was used coating the fruits that were immersed in the solution and then withdrawing and draining excessive solution. Zeolite elementals were analyzed to determine ZnO percentage using *X-ray fluorescence* (XRF, PW2404, Philips) system. The morphology of composite nanomaterials was examined by a field emission scanning electron microscopy (FE-SEM, MIRA3 TESCAN) system.

### 2.2 Preparation of the Aloe Vera Gel (AG)

*Aloe vera* leaves were collected. The leaves were rinsed with distilled water and then extracted using a method previously described by Arsene et al. to prepare hydrogel composition [23]. The extract was cooled at ambient temperature and stored at 4 °C.

### 2.3 Bacterial Strains and Culture Conditions

The strains used in the current study, i.e., *S. sonnei* ATCC 9290 and *S. flexneri* ATCC 12022, come from The American Type Culture Collection (ATCC). The strains were inoculated in tryptic soy broth (TSB, Merck, Darmstadt, Germany) for 24 h in an aerobic condition at 37 °C.

### 2.4 Antibacterial Assessment of Zeo/ZnONC and Zeo/ZnONC-AG

To evaluate the antibacterial properties of the Zeo/ZnONC and Zeo/ZnONC-AG, the MIC and MBC test was conducted using *S. sonnei* and *S. flexneri* strains. MICs were determined by microdilution methodology in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [24]. Twofold serial dilutions of Zeo/ZnONC and Zeo/ZnONC-AG in concentrations ranging from 1, 2, 4, and 8 mg/ml with an adjusted inoculum of  $10^8$  CFU/ml were tested to determine MIC value in Mueller–Hinton broth (MHB). The control contained only bacterial suspension. The plate was incubated for 24 h at 37 °C. The MIC is defined as the lowest concentration of products where no visible bacterial growth is observed.

After the MIC determination, aliquots of 10  $\mu\text{l}$  from all the wells with no visible growth were spread on Mueller–Hinton agar (MHA, Merck) plates and incubated for 24 h at 37  $^{\circ}\text{C}$ . When 99.9% of the final inoculum is killed at the lowest concentration of each agent, it is recorded as MBC endpoint.

## 2.5 Experimental Design of Plant Material

Strawberries were collected from greenhouses in the center of Iran and transferred to the laboratory by maintaining cold chain conditions. Fruits without signs of mechanical damage, spots and disease were selected. Treatment of the strawberry was performed at refrigeration temperature (4 $^{\circ}\text{C}$ ) by immersing the fruits in a solution of either hydrogel NC (2, 4, 8, and 16 mg/ml) or distilled water as control fruits. Their quality was investigated during 7 days.

## 3 Results

### 3.1 Composite Analysis

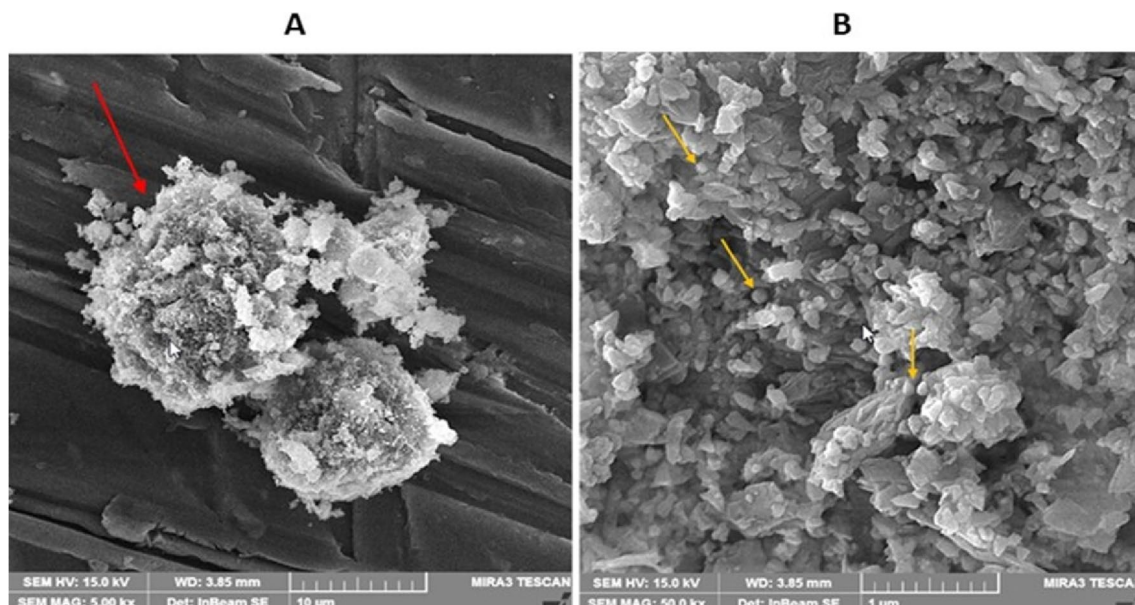
In this study, XRF technique was used for the determination of ZnO percentage for experimental compounds. Table 1 shows that the sample of Zeo/ZnONC has 18.39% ZnO among other elements while its quantities are 8.34% and 0.29% for Zeo/ZnO and raw Zeo, respectively. As shown in Fig. 1, FE-SEM imaging in lower magnification (Fig. 1A) determined the crystalline structure of the zeolite materials, and higher magnification (Fig. 1B) indicated that zinc oxide nanoparticles were formed on the zeolite surfaces.

### 3.2 Assessment of MIC Values Against *S. sonnei* and *S. flexneri*

The MIC values of Zeo/ZnONC and Zeo/ZnONC-AG against *S. sonnei* were 2 and 1 mg/ml, respectively,

**Table 1** Elemental analysis using XRF determined ZnO percentage in each compound [25]

Composition	XRF analysis (wt. percentage)						
	ZnO	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	CaO	MgO	Fe <sub>2</sub> O <sub>3</sub>	P <sub>2</sub> O <sub>5</sub>
ZnO/Zeo nanocomposite	18.39	55.52	8.77	3.14	0.87	0.96	0.147
ZnO/Zeo	8.34	65.81	8.91	3.62	0.56	1.29	0.031
Zeolite	0.29	66.32	12.14	4.43	0.31	0.33	0.003



**Fig. 1** Representative SEM imaging of zinc oxide nanocomposite materials. **A** Crystalline form of zinc oxide/ zeolite nanocomposite is determined by red arrows. **B** Nanoparticles of zinc oxide doped on

the surface of zeolite composite are seen mostly as spherical shaped which are marked by yellow arrows

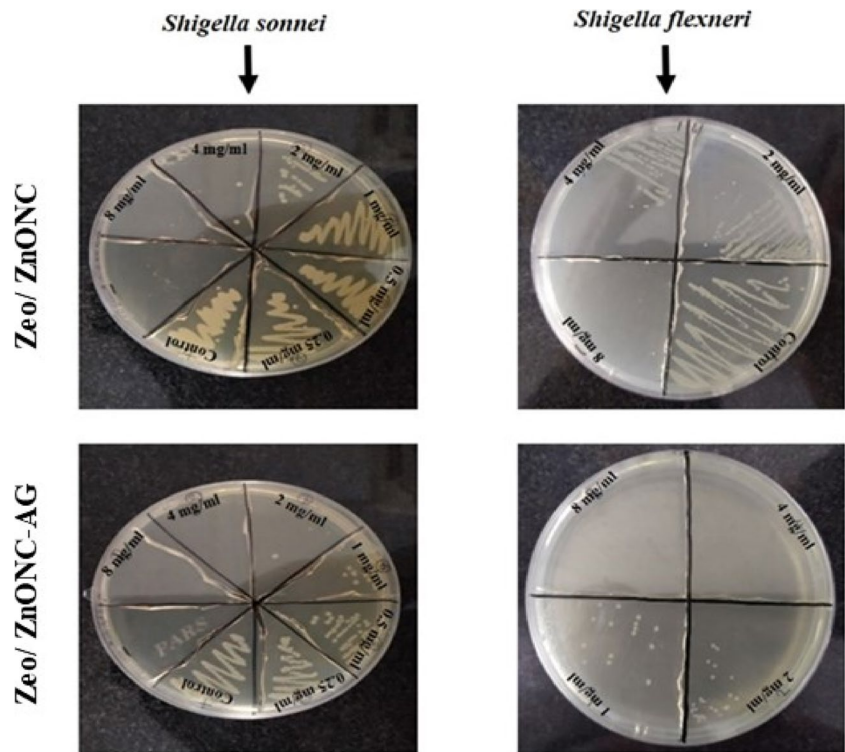


whereas the MBC values were 4 and 2 mg/ml for Zeo/ZnONC and Zeo/ZnONC-AG, respectively. The MIC values of Zeo/ZnONC and Zeo/ZnONC-AG against *S. flexneri* were 4 and 2 mg/ml, respectively, whereas the MBC values were 8 and 4 mg/ml for Zeo/ZnONC and Zeo/ZnONC-AG, respectively (Fig. 2).

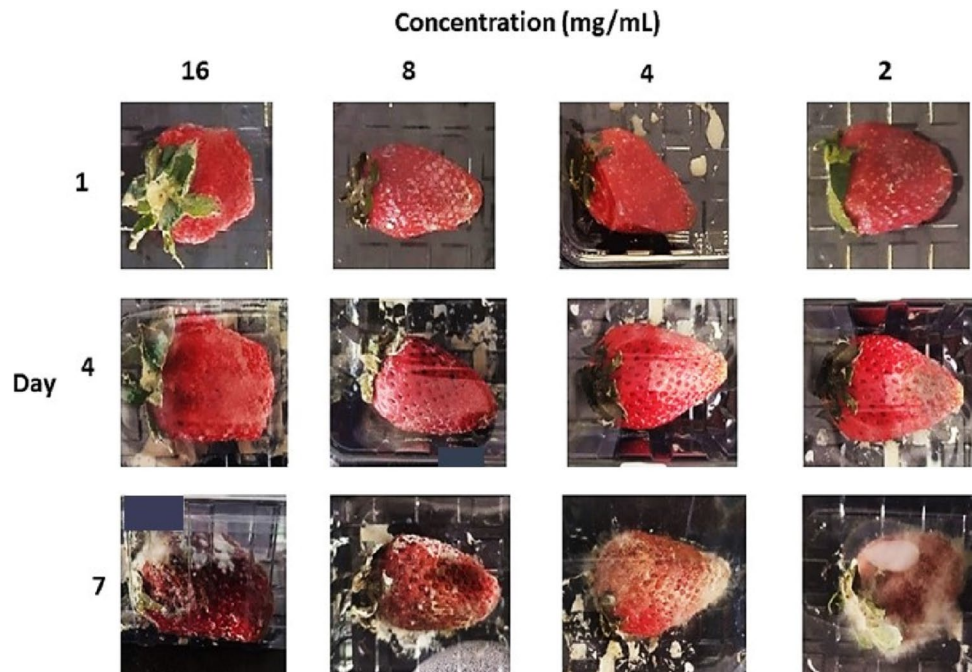
### 3.3 Strawberry Storage and Preservation

Figure 3 and Table 2 show the changes of strawberries after Zeo/ZnONC-AG application during 7 days experimentation. Throughout seven days storage of treated fruits, mildew colonies firstly developed in the control group. The moldy

**Fig. 2** The determination of minimum bactericidal concentration of zeolite/zinc oxide nanocomposite with and or without *aloe vera* gel against *Shigella sonnei* and *Shigella flexneri*



**Fig. 3** The effect of different concentrations of zeolite/zinc oxide nanocomposite (Zeo/ZnONC) with *aloe vera* gel on strawberries at refrigerator temperature, 4°C, condition during 7 days



**Table 2** The effect of different concentrations of Zeo/ZnONC-AG on strawberries in refrigerator temperature conditions

Concentrations	2 mg/ml	4 mg/ml	8 mg/ml	16 mg/ml	Control
Days					
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	+
4	+	-	-	-	+
5	+	+	-	-	+
6	+	+	+	-	+
7	+	+	+	+	+

Zeo/ZnONC-AG zeolite/zinc oxide nanocomposite with *aloe vera* gel, + mold growth, - no mold growth

strawberries appeared in the group that was exposed to 2 mg/ml of Zeo/ZnONC-AG after the strawberries were stored for 4 days, indicating that Zeo/ZnONC-AG has a positive preservative effect on strawberries. However, the group was soaked in 16 mg/ml of Zeo/ZnONC-AG can prolong the storage time of strawberries compared with the control.

## 4 Discussion

Fruits and vegetables are daily produced and consumed worldwide. However, there are concerns about their storage [26]. Hence, it is crucial to develop new and effective strategies to control fruit losses during the storage process [27]. In this study, 2 and 4 mg/ml of Zeo/ZnONC showed an inhibition effect against *S. sonnei* and *S. flexneri* in MIC test, respectively. These values reached 1 and 2 mg/ml for Zeo/ZnONC-AG, respectively. Therefore, Zeo/ZnONC-AG show more enhanced bactericidal activity against *S. sonnei* and *S. flexneri*. On the other hand, the lowest MIC and MBC were observed against *S. sonnei*.

Gunalan et al. investigated the antibacterial properties of ZnO NPs on various bacterial and fungal pathogens and showed that the antimicrobial activity of NPs depends on the particle dose, contact time, size, and synthesis method [28]. ZnO NPs can induce oxidative stress due to the reactive oxygen species (ROS) generation, which causes membrane destruction of reducing lipids, proteins, DNA, and also decreases cell viability [29]. The disorganization of cell membrane structure due to the accumulation of NPs in the bacterial membrane as well as their cellular internalization is also one of the causes of bacterial cell death. In addition, the release of Zn<sup>2+</sup> ions that attach to the cell membrane surface causes antimicrobial effects. Through close contact with the cell, NPs cause a change in the microenvironment of the bacteria, and by increasing the solubility of the metal or producing ROS, they eventually cause damage to the membrane [30].

Different forms of ZnO, such as powder, film, polyvinylpyrrolidone (PVP)-capped, and coating showed a superior antibacterial effect against the wide range of bacteria [31]. In a previous study, the authors demonstrated that the Zeo/ZnONC hamper the biofilm formation of *Enterococcus faecalis*. This antibacterial activity occurs via the reduction of *esp* gene expression promoted by ZnONPs. Spherical-shaped ZnO nanoparticles with an average size of 30 nm displayed a leakage of cationic zinc from ZnONP-doped natural zeolite as long-lasting [21]. Another relevant study reported the antimicrobial activity of Zeo/ZnONC against *Klebsiella pneumonia* [22]. Furthermore, ZnONP-AG exhibited antibacterial activities towards the Gram<sup>+</sup> and Gram<sup>-</sup> bacteria [32, 33].

In the current study, strawberries treated with Zeo/ZnONC-AG demonstrated fewer changes than the control. Generally, when strawberries are stored at ambient temperature for just one day, they begin to spoil and change in taste. Today, common preservation methods include chemical preservation and refrigeration condition in controlled atmosphere storage [34]. However, these expensive methods require equipment investment. Currently, natural compounds allowed for a simple, cost-effective preservation procedure of fruits [35]. According to previous investigations on the antibacterial effect of Zeo/ZnONC and AG, we applied Zeo/ZnONC-AG to the preservation of strawberries. The results showed that with the increase of Zeo/ZnONC-AG concentration, the appearance of mold on the surface of strawberries demonstrated a downward trend. Probably, due to the shielding effect of Zeo/ZnONC-AG on strawberry fruit, the risk of water loss and bacterial proliferation of fresh strawberry will be greatly reduced [35]. In terms of safety and biocompatibility, our recent results showed that Zeo/ZnONC-AG have the least cytotoxicity effect and good biocompatibility with the host cells. Interestingly, the percentage of Caco-2 cells exposed to ZnONPs containing AG indicated significantly higher cell viability compared to ZnONPs/Zeo group, in equal concentrations [25]. In the research, ZnONPs and *A. vera* were introduced an eco-friendly and the results of the microdilution test and bacterial count displayed antibacterial activity against *S. typhi* and *S. paratyphi A* using ZnONPs [25]. Furthermore, ZnONC containing chitosan hydrogel have shown significant biofilm formation and metabolic activity reduction through gene expression down-regulation of *Streptococcus mutans*. ZnONC containing chitosan exhibits a non-cytotoxic on human gingival fibroblast cells within 72-h investigation [17].

Therefore, Zeo/ZnONC-AG improves the quality of the strawberry. Overall, the findings of the current study demonstrated that the antibacterial activity of Zeo/ZnONC-AG was obviously better than Zeo/ZnONC. These results offer insight into the effects of Zeo/ZnONC-AG on other bacteria involved in food contamination. One of the limitations of the current study was that only strawberry was examined. Therefore, this study can be completed on other fruits and foods. Overall,

further investigations are required to confirm the antimicrobial and antifungal activity of Zeo/ZnONC-AG on fruits.

## 5 Conclusion

The data presented above suggests that Zeo/ZnONP-AG has an efficient antibacterial performance against *S. sonnei* and *S. flexneri*. Moreover, Zeo/ZnONP-AG slowed down the visible mold development on strawberries and also improved the durability of the fruit in cold conditions. ZnO nanocomposite can be a promising preservative in the control of foodborne contamination as well as the shelf life enhancement of sensitive fruits like strawberries. However, other aspects of the preservative criteria of Zeo/ZnONP-AG like risk assessments, stability, broad-spectrum activity, etc. need more investigations in future studies.

**Author Contribution** A.P.: methodology and writing and editing the manuscript. S. A.: writing and editing the manuscript. M. F. G.: experimental assessment. G-K.: data curation and advisor. MM. S.D.: supervisor.

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## Declarations

**Informed Consent** None.

**Research Involving Humans and Animals Statement** None.

**Competing Interests** The authors declare no competing interests.

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