



IRAQI
Academic Scientific Journals



العراقية
المجلات الأكاديمية العلمية

TJAS

Tikrit Journal for
Agricultural
Sciences

ISSN:1813-1646 (Print); 2664-0597 (Online)

Tikrit Journal for Agricultural Sciences

Journal Homepage: <http://www.tjas.org>

E-mail: tjas@tu.edu.iq

Inhibitory Susceptibility of Synthetic Selenium Nanoparticles and some Conjugate Nutritional Compounds in Inhibition of some Bacterial Isolates Causing Food Poisoning

Saif E. AbdAlAziz¹, Karkaz M. Thalij¹, and Mohamed Gh. Zakari³

¹Food Science Department, College of Agriculture, Tikrit University, Tikrit, Iraq

²College of Medicine, Tikrit University, Tikrit, Iraq

* Corresponding author: E-mail: saifalkasab@gmail.com

ABSTRACT

KEY WORDS:

Se-NPs, Zn, Vit-D3, Cysteine, *E.coli*, *Staph. aureus*

Received: 20/05/2023

Accepted: 03/07/2023

Available online: 31/12/2023

© 2023 This is an open access article under the CC by licenses <http://creativecommons.org/licenses/by/4.0>



The research aimed to prepare the nanoparticles of zinc, vitamin D3 and cysteine with selenium nanoparticles (Se-NPs), and to determine the effect of each of them in inhibiting both the isolated *Escherichia coli* and *Staphylococcus aureus* isolated from food by estimating the minimum inhibitory concentration (MIC) with Kirby Bauer disk diffusion. The results showed that the conjugations of zinc, vitamin D3 and cysteine with se-NPs were more effective in bacterial inhibition compared to inhibition of Se-NPs alone. The MIC of Se-NPs alone or in combination with Zn, D3 or Cysteine against both species of bacteria was appeared at 1% and above. The inhibitory activity increased against the bacterial species *E.coli* and *S. aureus* as the concentrations used in the treatments were increased, while the diameter of the inhibition zone was 25% against the two types of bacteria *E.coli* and *Staph. aureus* was the highest effect when treated with Se Nps + D3, which was at 30 mm, and 22 mm, respectively.

القابلية التثبيطية للسيلينيوم النانوية المخلقة وبعض المركبات التغذوية المقترنة معه في تثبيط بعض العزلات البكتيرية المسببة للتسمم الغذائي

سيف عماد الدين عبد العزيز¹ و كركز محمد ثلج¹ و محمد غالب زكريا²
¹ا قسم علوم الاغذية ، كلية الزراعة ، جامعة تكريت ، العراق
²كلية الطب ، جامعة تكريت ، العراق

الخلاصة

هدف البحث الى تحضير المقترنات النانوية لكل من عنصر الزنك وفيتامين D3 والحامض الاميني Cysteine مع جسيمات السيلينيوم النانوي Se-NPs، وتحديد تأثير كل منها في تثبيط كل من عزلة *Escherichia coli* و *Staphylococcus aureus* المعزولة من الأغذية من خلال تقدير التركيز المثبط الأدنى وطريقة الحفر. بينت النتائج ان المقترنات لكل من عنصر الزنك وفيتامين D3 والحامض الاميني Cysteine مع جسيمات السيلينيوم النانوي Se-NPs كانت الأكثر تأثيراً في التثبيط البكتيري مقارنة مع التثبيط من جسيمات Se-NPs. كان التركيز المثبط الأدنى لكل من Se-NPs لوحده او مقترنا مع كل من Zn أو D3 او حامض Cysteine ضد نوعي البكتريا عند 1% فما فوق، وكانت الفعالية التثبيطية تزداد تجاه الانواع البكتيرية *S. aureus* و *E. coli* كلما زادت التراكيز المستخدمة في المعاملات، اما قطر منطقة التثبيط لتركيز 25% ضد نوعي البكتريا *Staph. aureus* و *E. coli* كانت اعلاها تأثيراً عند المعاملة بـ Se Nps + D3 التي كانت عند 30 ملم، و 22 ملم على التوالي.

الكلمات المفتاحية: Se-NPs, Zn, Vit-D3, Cysteine, *E. coli*, *Staph. Aureus*

INTRODUCTION

The current era is considered to be the era of antibiotics resistant of microorganisms especially pathogenic and food poisoning microorganisms and the ability of bacteria has increased in the development of antagonistic mechanisms with traditional antibiotics, and it has become a life-threatening situation, the excessive use of antibiotics to the development of the ability of most species bacterial resistance to antibiotics (Vahdati and Tohidi, 2020). Earlier in this picture, the previous number of resistant bacterial strains, the latest of these discoveries was in the use of nanoparticles in microbial inhibition as well as their use in the fields of biology and medicine (Haddadian et al., 2022).

Selenium nanoparticles (Se-NPs) have been drawn in special attention for their outstanding properties such as stability in environmental conditions and synthesis at low temperatures, especially when synthesized by bioplates (Salem et al., 2021). Which distinguishes the product from nanoparticles, so that it is a non-toxic and low-risk particle (Hashem et al., 2022). The use of nanotechnology in biology has opened up opportunities in fields including tissue engineering, drug ticketing, imaging, and antibacterial alkalization with the need for novel antimicrobial agents, suggesting nanoparticles to treat inflammation have different ways for killing bacteria compared to conventional antibiotics, conventional nature human cells, as a result, consider nanomaterials as a promising alternative to antibiotics to control bacterial alleles (Salem and Fouda, 2021). As well as their anti-biofilm and anti-biofilm properties (Subhan and Muzibur, 2022). This study was aimed to conjugate selenium nanoparticles with zinc, vitamin D3 and the amino acid cysteine, and their inhibitory determination against isolates of *S. aureus* and *E. coli*.

MATERIAL AND METHODS

Collection of samples

Samples of both raw bovine milk and cake were prepared, from which it is intended to isolate the bacteria that cause food poisoning. Five samples of milk were collected and left in the laboratory for two days, and five samples of cake were left in the laboratory at temperature for four days.

Isolation and Diagnoses of bacterial Species from food samples

Staphylococcus aureus and *E.coli* were isolated according to the method of (Thongaram, 2016), *S.aureus* was isolated by mixing 10g of each cake sample left at room temperature for 4 days in 90 ml of physiological solution. While the *E.coli* was isolated by mixing 10 ml of milk samples presented in the laboratory for two days in 90 ml of physiological solution, then 100 microliters of the diluted of each cake solution and milk samples were taken to the surface of Mannitol salt agar and MacConkey agar media respectively and they were distributed homogeneously using a glass spreader in the shape of the letter (L), then incubated at 37 ° C for 48 hours. The single-growing bacterial colonies were taken and re-cultivated on the same medium above by the streaking method to obtaining of pure bacterial isolates. The process was repeated till to ensure complete purification, the isolates were kept on slanted cultures in the refrigerator until the diagnosis of the species was made.

Preparation of the serial solutions of Se-NPs and they're conjugated with each L-cysteine, Zinc and D3.

The serious dilutions were prepared by dilution of the stock solution of Se-NPs using the distilled water to become at 0.5, 1.0, 2.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0 and 100%.

Evaluation of the effectiveness of Se-NPs and their conjugation with each L-cysteine, Zinc and D3 as antimicrobials *in vitro*:

The effectiveness of Se-NPs, and their conjugated with each L-cysteine, Zinc and D3 against each species of *S. aureus* and *E. coli* that cause food poisoning and isolated from food samples were evaluated (CLSI, 2020) which included the following:

A bacterial suspension was prepared from each bacterial isolate to be treated with each Se-NPs, and it's conjugated with each L-cysteine, Zinc and D3.

The resulting solutions were compared with McFarland's standard tube solution at a concentration of 0.5 to stabilize the numbers at 1.5×10^8 CFU /mL of each suspension solution was withdrawn from it to the surface of the culture medium, Muller Hinton Agar, and spread on the surface of the medium and left for 15 minutes, then 50 microliters were transferred from each treatment at a concentration of 10, 15, 20, and 25% each of (Se-NPs and L-cysteine). + Se-NPs, Se-NPs + Zinc, and Se-NPs + D3) to holes with a diameter of 4 mm were made on the surface of the culture medium, after which the plates were incubated at a temperature of 37 ° C for 24 hours. Then, the effectiveness of each treatment against each bacterial species was determined by measuring the diameter of the inhibition zone in millimetres (mm).

Determination of the minimum inhibitory concentration (MIC) of Se-NPs, and it's conjugated with each L-cysteine, Zinc and D3.

The method of the tube dilution is used to prepare serial dilution of Se-NPs, and it's conjugated with each L-cysteine, Zinc and D3 from Stock solution at 0.5, 1.0, 2.0, 5, 10 and 20%. The tubes were

inoculated with each bacterial suspension of *S. aureus* and *E.coli* and compared with McFarland 0.5 tube. The two tubes of control are prepared. The first one as a negative control contained each of the Se-NPs, and it's conjugated with each L-cysteine, Zinc and D3 without bacterial suspension, while the second (Positive control) tubes were containing the bacterial suspension only. The tubes were incubated at 37°C for 24hrs then the MIC results were obtained according to the tubes that appeared to be innate to inhibit the bacterial growth after checking with a spectrophotometer at 575 nm (Baron et al., 1998).

Statistical analysis:

The biological experiment was implemented according to the randomized complete design CRD, and the analysis of variance was carried out using the General Linear Model within the SAS (SAS, 2012) program. When there are significant differences between the means, Duncan's test (Duncan, 1955) was used to determine the significance of the differences between the different means at a probability level of 0.05.

RESULTS AND DISSCUSION

Isolation and identification of food poisoning bacteria

cake samples were used as sources of isolation for the bacterial type *S. aureus*, as the method of planning was used on the culture medium, Mannitol salt agar, after making the appropriate dilutions of the samples (Roberts & Greenwood, 2003). This bacterial species was distinguished from the rest of the other species of the same genus in its ability to decompose mannitol and turn the colour of the food medium to yellow resulting from acid production. The colonies resulting from the development of these bacteria appeared to be circular, convex, shiny, smooth, and golden in colour, with diameters ranging between 2-3 mm, arranged in short chains or the form of grape clusters. When tested under the microscope, the cells were shown to be spherical, clustered nonmotile clusters, positive for the Gram stain, and the biochemical tests showed that they were positive for the catalase test, which was shown through the production of gas bubbles from CO₂ resulting when the fresh culture was added to 3% hydrogen peroxide. They do not produce the oxidase enzyme, which was indicated by the absence of a violet colour after 10-15 seconds of adding the reagent. It was also found that these isolates were unable to produce H₂S after growing them on the medium. All isolates were capable of fermenting mannitol by turning the colour of the medium. Mannitol salt agar turned to a pale colour, indicating a positive fermentation, and it was also positive for the blood clotting enzyme (Coagulase test). This is one of the important diagnostic tests to distinguish *S. aureus* from other species, as this enzyme has an important relationship in the pathogenic events of these bacteria, as it works to convert fibrinogen into fibrin in red blood cells, which causes thrombus formation (Jawetz et al., 2004) The above mentioned diagnostic characteristics indicate that the bacterial species is *S. aureus* (Table 1).

While the bacterial type *E.coli* they were obtained from raw bovine milk samples after culturing the samples on MacConkey agar medium, and the diagnosis was completed after development at 37 °C for 24 hours (Roberts & Greenwood, 2003). The bacterial colonies were diagnosed by comparing the results obtained with the tests used in Table (1). The colonies appeared in a pink colour, and they were small and dry on the medium of the MacConkey agar. A gram dye was used to determine the shape of the cells. The result was gram-negative short bacilli, and not capable of

lysis. It was positive for the catalase test when hydrogen peroxide was added to the young colonies. It does not produce H₂S gas and is negative for the oxidase test when the reagent is added and the violet colour does not appear. When conducting the mobility test, it showed its ability to move by peripheral flagella (Atlas *et al.*, 1995) and showed its ability to ferment .. mannitol (Msalya, 2017).

Table 1: Results of phenotypic and biochemical tests for *Staphylococcus aureus* and *E. coli* isolate

+ test Positive - test Negative.

Test type	Test name	Test result	
		<i>Staphylococcus aureus</i>	<i>E. coli</i>
Phenotypic tests	colonies	Smooth round golden color	Colonies are small, round, sticky, with a smooth edge
	the movement	-	+
	Gram staining	+	-
	Figure cells under a light microscope	cocci	Bacilli
Biochemical tests	catalase	+	+
	oxidase	-	-
	H ₂ S	-	-
	Coagulation	+	-
	Manthol fermentation	+	+

Inhibitory activity of Se-NPs, Se-Nps+ L-cysteine, Se-Nps+ Zinc and Se-Nps+ D3 against test bacteria species

Determination of the minimum inhibitory concentration (MIC) of the nanoparticles:

Determination of the use of Se NPs, Se Nps + L-cysteine, Se Nps + Zinc and Se Nps + D3 at serial concentrations of 0.5, 1.0, 2.0, 5.0, 10 and 20% each against two types of bacteria, *S. aureus* and *E. coli* results are shown in Table (2) Results The minimum inhibitory concentration (MIC) for both types of bacteria, *S. aureus* and *E. coli*, of selenium nanoparticles was 1.0 and 1.0%, respectively. Increased concentration of nanoparticles. The results agreed with what was mentioned by (Boroumand *et al.*, 2019) and (Al Jahdaly *et al.*, 2021) who indicated that the minimum inhibitory concentration of Se-NPs against *E.coli* and *S.aureus* was 1.3%, while sensitive bacteria were 2.0 and 5.0%, respectively.

As a conclusion of nanoparticles role in the inhibition of microorganism, the electron transfer process and its plan, and the process of osmosis regulation (Medina & Webster, 2018), the positively charged ions can be liberated by nanoparticles inside the cell that can be classified into ribosomes, protein synthesis or inhibit the process of multiplying the genetic material of bacteria in those nanoparticles in the substance producing free radicals causing In cellular formation in bacterial cell formation (Al Jahdaly *et al.*, 2021). It is known that Gram-negative bacteria possess the outer envelope consisting of the outer membrane LPS and related proteins in their general life such as toxins, drugs, detergents, broken enzymes and degraded enzymes from the cell membrane, which has the role of the hypothalamus of the bacterial cell. Causing gradual release of LPS and

proteins, causing the gradual release of LPS and proteins, linking binding to cytosolic membranes (Boroumand *et al.*, 2019).

Also, the size of the nanoparticle plays a major role in the antibacterial activity, through the fact that the cell membranes of bacteria have holes with a diameter of nanometers, while the nanoparticles have a size less than the size of the holes, so they have distinctive properties to cross through the cell membrane without any barrier, and this in turn explains the effectiveness of the Se-NPs used in the study, which had a particle size of 1-57 nm.

Table 2. The minimum inhibitory concentration of each of the Se-NPs, Se-Nps+ L-cysteine, Se-Nps+ Zinc and Se-Nps+ D3 against the two types of test bacteria.

The type of bacteria tested	Type of Nanoparticles	The inhibitory ability of the test materials according to the concentration used(%)					
		0.5	1	2	5	10	20
<i>E. coli</i>	Se NPs	-	+	++	++	+++	++++
	Se Nps+ L-cysteine	-	-	+	+	++	+++
	Se Nps+ Zinc	-	+	+	++	+++	++++
	Se Nps+ D3	-	-	+	+	++	+++
<i>S. aureus</i>	Se NPs	-	+	+	++	+++	++++
	Se Nps+ L-cysteine	-	-	+	++	+++	++++
	Se Nps+ Zinc	-	+	+	++	+++	++++
	Se Nps+ D3	-	-	+	++	+++	++++

• (-) insensitive, (+) low sensitivity, (++) medium sensitivity, (+++) high sensitivity, (++++) fully sensitive. • The concentrations of Se NPs, Se Nps+ L-cysteine, Se Nps + Zinc and Se Nps + D3 were at 0.5, 1.0, 2.0, 5, 10 and 20%. • Se-NPs = selenium nanoparticles, Se Nps+ L-cysteine = selenium nanoparticles loaded with the amino acid cysteine, Se Nps + Zinc = selenium nanoparticles loaded with zinc, • Se Nps+ D3 = selenium nanoparticles loaded with D3

Inhibitory activity of Se-NPs, Se-Nps+ L-cysteine, Se-Nps+ Zinc and Se-Nps+ D3 against test bacteria species:

The results showed in Table (3) that the nanoparticles at a concentration of 10, 15, 20 and 25% each of Se NPs, Se Nps + L-cysteine, Se Nps + Zinc and Se Nps + D3 were effective in their ability to inhibit *E. coli* and *S. aureus*, as the addition of selenium nanoparticles Se NPs to bacterial cultures of type *E. coli* showed that the area of diameter of inhibition was in the range of 12, 16, 20 and 28 mm, while the Se Nps + L-cysteine particles showed an area of diameter of inhibition on the same bacterial isolate of the range of 13, 15, 19 and 30 mm, also the Se Nps + Zinc particles showed that the area of diameter of inhibition was within the limits of 12, 17, 21 and 26 mm, while the Se Nps + D3 particles showed an area of diameter of inhibition on the same bacterial isolate within the limits of 10, 14, 20 and 30 mm, while the results of using selenium nanoparticles (Se NPs) indicated bacterial cultures of the type *S. aureus*, showed that the area of inhibition diameter was in the range of 7, 12, 16 and 21 mm, while the Se Nps + L-cysteine particles sh ability to inhibit owed at the same area of inhibition diameter. Bacterial isolates within 8, 11, 15 and 17 mm, also the Se Nps + Zinc particles showed that the area of inhibition diameter was within the range of 10, 14, 18 and 20 mm,

while the Se Nps + D3 particles showed an area of inhibition diameter on the same bacterial isolate of the range 8, 12, 17 and 22 mm, which indicates that the sensitivity of *E. coli* to selenium nanoparticles and materials loaded with it was higher than that of *S. aureus*. The results agreed with what was found by (Medina & Webster, 2018) and (Al Jahdaly et al., 2021), as they stated that selenium nanoparticles have the ability to inhibit *E.coli*, *S. aureus*, and other different types of bacteria due to the formation of Se-NPs nanoparticles with an increase in the concentration of Reactive Oxidative Species (ROS). This leads to a failure in the internal metabolism of the bacteria, which leads to its death.

Table 3. Diameter of inhibition (mm) of nanoparticles of selenium and its loaded materials against the test bacteria species.

The type of bacteria tested	Type of Nanoparticles	The diameter of the zone of inhibition (mm) against the test bacteria species according to the concentration used(%)			
		%10	%15	%20	%25
		(mm)			
<i>E. coli</i>	Se NPs	12	16	20	28
	Se Nps+ L-cysteine	13	15	19	30
	Se Nps+ Zinc	12	17	21	26
	Se Nps+ D3	10	14	20	30
<i>S. aureus</i>	Se NPs	7	12	16	21
	Se Nps+ L-cysteine	8	11	15	17
	Se Nps+ Zinc	10	14	18	20
	Se Nps+ D3	8	12	17	22

• The concentrations of Se NPs, Se Nps + L-cysteine, Se Nps + Zinc and Se Nps + D3 were at 10, 15, 20 and 25%.

• Se-NPs = selenium nanoparticles, Se Nps+ L-cysteine = selenium nanoparticles loaded with cysteine, Se Nps+ Zinc = selenium nanoparticles loaded with zinc, Se Nps+ D3 = selenium nanoparticles loaded with D3

CONCLUSION

The results show the effective effect of selenium nanoparticles alone or in conjunction With zinc, vitamin D3 and the amino acid cysteine, it effectively inhibits bacteria *S. aureus* and *E. coli*, The treatment with Se-NPs + D3 was the best treatment to inhibit the bacterial species that cause food poisoning under study

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the staff of the [Department, College or University] for their technical and general support.

REFERENCES

- Al Jahdaly, B. A., Al-Radadi, N. S., Eldin, G. M., Almahri, A., Ahmed, M. K., Shoueir, K., and Janowska, I. (2021). Selenium nanoparticles synthesized using an eco-friendly method: dye decolorization from aqueous solutions, cell viability, antioxidant, and antibacterial effectiveness. *Journal of Materials Research and Technology*, 11:85-97.
- Atlas, R. M.; Brown, A. E. and Parks, L. C. (1995). *Laboratory manual experimental microbiology*. Mosby Company.
- Baron, E. J. and Fingold, S. E. (1994). *Diagnostic Microbiology* .9th . Ed. the C.V. Mosby Company. Baltimore.
- Boroumand, S., Safari, M., Shaabani, E., Shirzad, M., and Faridi-Majidi, R. (2019). Selenium nanoparticles: synthesis, characterization and study of their cytotoxicity, antioxidant and antibacterial activity. *Materials Research Express*, 6(8), 0850d8.
- CLSI, (2011). *Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement*. Vol. 30 (1): 145-157.
- Duncan, D. B. (1955) Multiple range and F. test. *Biometric* 11: 42.
- Haddadian, A., Robattorki, F. F., Dibah, H., Soheili, A., Ghanbarzadeh, E., Sartipnia, N., and Mirzaie, A. (2022). Niosomes-loaded selenium nanoparticles as a new approach for enhanced antibacterial, anti-biofilm, and anticancer activities. *Scientific Reports*, 12(1), 21938.
- Hashem, A. H., Selim, T. A., Alruhaili, M. H., Selim, S., Alkhalifah, D. H. M., Al Jaouni, S. K., and Salem, S. S. (2022). Unveiling antimicrobial and insecticidal activities of biosynthesized selenium nanoparticles using prickly pear peel waste. *Journal of Functional Biomaterials*, 13(3): 112.
- Jawetz, E.; Brooks, G. F.; Butel, J. S. and Morse, S. A. (2004). *Jawetz, Melnick and Adelberg, s Medical microbiology 23rd ed.* McGraw-Hill Com., Singapore.
- Medina Cruz, D., Mi, G., and Webster, T. J. (2018). Synthesis and characterization of biogenic selenium nanoparticles with antimicrobial properties made by *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Pseudomonas aeruginosa*. *Journal of Biomedical Materials Research Part A*, 106(5): 1400-1412.
- Msalya, G. (2017). Contamination Levels and Identification of Bacteria in Milk Sampled from Three Regions of Tanzania: Evidence from Literature and Laboratory Analyses. *Veterinary Medicine International*.
- Roberts, D. and Greenwood, M. (2003). *Practical food microbiology*. 3ed Edt., Blackwell publishing Inc., 350 Malden, Massachusetts 02148-5018, USA.
- Salem, S. S. and Fouda (2021) A. Green synthesis of metallic nanoparticles and their prospective biotechnological applications: An overview. *Biol. Trace Element Res.* 199, 344–370.

- Salem, S. S., Fouda, M. M., Fouda, A., Awad, M. A., Al-Olayan, E. M., Allam, A. A., and Shaheen, T. I. (2021). Antibacterial, cytotoxicity and larvicidal activity of green synthesized selenium nanoparticles using *Penicillium corylophilum*. *Journal of Cluster Science*, 32, 351-361.
- SAS, (2012). *Statistical Analysis System, User's Guide*. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
- Subhan, M. A., and Muzibur Rahman, M. (2022). Recent development in metallic nanoparticles for breast cancer therapy and diagnosis. *The Chemical Record*, 22(7), e202100331.
- Thongaram, T. (2016). In vitro evaluation of selected probiotic properties of lactic acid bacteria isolated from the traditional fermented vegetable. *Conference Proceedings*. Paper presented at International Scientific Conference on Probiotics and Prebiotics, Budapest. In *Kysucke Nove Mesto*, ISBN- 978-80-89589-14-2.
- Vahdati, M., and Tohidi Moghadam, T. (2020). Synthesis and characterization of selenium nanoparticles-lysozyme nanohybrid system with synergistic antibacterial properties. *Scientific reports*, 10(1): 510.