



Original article

Antimicrobial activity of chemically and biologically synthesized silver nanoparticles against some fish pathogens



Hanan A. Ghetas^a, Nashwa Abdel-Razek^b, Medhat S. Shakweer^c, Mahmoud M. Abotaleb^d, Bilal Ahamad Paray^e, Sajad Ali^f, Elsayed A. Eldessouki^g, Mahmoud A.O. Dawood^{h,*}, Riad H. Khalilⁱ

^a Aquatic Animal Medicine and Management Department, Faculty of Veterinary Medicine, Sadat City University, Egypt

^b Fish Health and Management Department, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Agriculture Research Center, Egypt

^c Internal Medicine, Infectious and Fish Diseases Department, Faculty of Veterinary Medicine, Mansoura University, Egypt

^d Central Laboratory for Evaluation of Veterinary Biologics, Egypt

^e Department of Zoology, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Kingdom of Saudi Arabia

^f Department of Biotechnology, Yeungnam University, Gyeongsan 38541, South Korea

^g Department of Fish Health and Diseases, Faculty of Fish Resources, Suez University, Egypt

^h Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt

ⁱ Poultry and Fish Diseases Department, Faculty of Veterinary Medicine, Alexandria University, Egypt

ARTICLE INFO

Article history:

Received 7 August 2021

Revised 24 August 2021

Accepted 9 November 2021

Available online 15 November 2021

Keywords:

Silver Nanoparticles

Fish Pathogens

Antimicrobial Activity

Hemolysis

Transmission Electron Microscopy

ABSTRACT

Pathogens isolated from fish appear to possess considerable antimicrobial resistance and represent a problem for the economy and public health. Natural antimicrobial substitutes to traditional antibiotics represent an essential tool in the fight against antibiotic resistance. Nanotechnology has shown considerable potential in different research fields, and the antimicrobial properties of silver nanoparticles are known. Silver has been used for medical purposes since ancient times because of its bactericidal properties, and the highly reactive surfaces of silver nanoparticles (AgNPs) indicate that they might have a function in antimicrobial applications. This work aimed to study the antimicrobial properties of biologically produced AgNPs from *Origanum vulgare* leaves compared to chemically produced AgNPs. Both types were characterized by UV–vis spectrophotometry, TEM, and dynamic light scattering and tested against three bacterial strains (*Streptococcus agalactiae*, and *Aeromonas hydrophila*, both isolated from Nile tilapia and *Vibrio alginolyticus*, isolated from sea bass) and three fungal strains (*Aspergillus flavus*, *Fusarium moniliforme*, and *Candida albicans*, all isolated from Nile tilapia). Disk diffusion test and evaluation of ultrastructure changes of tested microorganisms treated with AgNPs by transmission electron microscopy were performed. Moreover, the hemolytic properties of AgNPs were studied on chicken and goat red blood cells. The results obtained declare that the green biological production of silver nanoparticles is safer and more effective than the chemical one; moreover, AgNPs have interesting dose-dependent antimicrobial properties, with better results for biologically produced ones; their effectiveness against tested bacterial and fungal strains opens the way to their use to limit fish diseases, increase economy and improve human health.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author.

E-mail addresses: bparay@ksu.edu.sa (B. Ahamad Paray), mahmoud.dawood@agr.kfs.edu.eg (M.A.O. Dawood).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<https://doi.org/10.1016/j.sjbs.2021.11.015>

1319-562X/© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Streptococcosis is considered a significant microbial disease in numerous fish species, and infections in marine and freshwater fish, both wild and aquaculture, are documented (Al-Sahzly et al., 2016; Mian et al., 2009; Van Doan et al., 2019). Salmon, mullet, golden shiner, pinfish, eel, sea trout, tilapia, sturgeon, and a variety of other fish species have been vulnerable to streptococcal infection (Inglis et al., 1993). Analogously, *Aeromonas hydrophila* is a harmful microbial pathogen for numerous fish and seafood (Janda, Abbott, 2010; Sarkar, Rashid, 2012). It is a causative agent

of bacterial hemorrhage in fish (Kumar et al., 2016) and is associated with economic losses in fish aquaculture worldwide (Illanchezian et al., 2010; Mohammadi et al., 2020b; Rashidian et al., 2021a). *Vibrio* species are well-known pathogenic bacteria for fish (Abdel-Tawwab et al., 2020; Ina-Salwany et al., 2019). *Aspergillus flavus* and associated aflatoxins represent a significant hazard to Nile tilapia aquaculture and fish health, leading to numerous clinical findings affecting animal and human health (Mohamed et al., 2017). *Fusarium moniliforme*, as mentioned by Magculia, Cumagun (2011), was found in freshwater fish in reservoirs, leading to mycosis and high mortality (Bisht et al., 2000). *Candida albicans* and other yeasts are more predominant in diseased fish than in apparently healthy fish (Tartor et al., 2018). Isolation of *C. albicans* from internal organs was also recorded by Zayed et al. (2016).

The growing phenomenon of antibiotic resistance in microorganisms isolated from fish is a highly topical concern and is documented almost everywhere globally, creating problems both from a health and economic point of view (Dawood, 2021; Mohammadi et al., 2020a). Serrano et al. (2015) demonstrated how bacteria attachment is altered in the presence of nanoscale topographies and described the best designs for preventing it while preserving biocompatibility. Fu et al. (2016) proposed an environmentally friendly method to synthesis silver nanoparticles (AgNPs) which had good cytocompatibility to cells and were highly poisonous to pathogenic microorganisms (*Escherichia coli*).

The utilization of herbal extracts for nanoparticle synthesis can be beneficial due to the simplicity of scaling up with fewer biohazards (Kumar et al., 2015; Vijay Kumar et al., 2014). *Origanum vulgare* L belongs to the family of Lamiaceae and grows wild in the Mediterranean and Eurasia (Alwafa et al., 2021). They contain vital essential oils and active derivatives, making them valuable phytochemicals for people and animals (Alagawany et al., 2020b). *O. vulgare* L contains many volatile compounds, including ketones, aldehydes, ethers, phenols, hydrocarbons, alcohols, and esters of phenolic and terpenic origins (de Torre et al., 2020; Veenstra, Johnson, 2019). The application of *O. vulgare* in aquaculture resulted in marked improvements in the production, feed utilization, antioxidative, and immune responses attributed to its potential role as an antibacterial agent (Alagawany et al., 2020a). Many investigators have reported green production of metal nanoparticles by resources from aqueous extracts in plants aimed at multidisciplinary usages (Kumar et al., 2013). Huang et al. (2017) investigated thorough knowledge of AgNPs' antibacterial characteristics and suggestions for improving AgNPs production for bacterial infection prevention. Sankar et al. (2013) reported that AgNPs from the *O. vulgare* plant leaves extract showed superior antimicrobial effects than manufactured antibiotics. The produced AgNPs reduced the counts of Gram-negative and Gram-positive bacterial infections such as *Escherichia coli* enteropathogenic strain, *Aeromonas hydrophila*, *Salmonella* spp *Salmonella dysenteriae*, *Salmonella paratyphi*, and *Shigella sonnei*. The desirability of AgNPs is mainly for its request in therapeutics, biomolecular recognition, catalysis, and antimicrobial mediators (Christopher et al., 2011; DeiviArunachalam et al., 2021; Rashidian et al., 2021b; Sadhasivam et al., 2010; Vali et al., 2020).

According to Golubeva et al. (2010), Hemolysis is the degradation of erythrocytes with the escape of hemoglobin. The interaction of certain peptide antibiotics with the erythrocyte membrane will harm it, allowing hemoglobin to escape the cell. The degree of damage caused by a specific antibiotic concentration is so great that the lipid layer's structural integrity is compromised. AgNPs are becoming increasingly popular in biomedical fields. Nevertheless, toxicological data are scarce on their impact on red blood cells (RBCs) and the mechanisms at operation.

The study evaluated the chemical and biological synthesis of silver-nanoparticles from *O. vulgare* leaves extract and evaluated antimicrobial properties on some bacterial and fungal fish pathogens.

2. Materials and methods

2.1. Microbial strains

All pathogenic strains were obtained from the Department of Poultry and Fish Diseases, Faculty of Veterinary Medicine, Alexandria University, Egypt. In Table 1, details about the tested strains are shown.

2.2. Production and characterization of AgNPs

2.2.1. Chemical production of AgNPs

In a three-necked round-bottomed flask, 20 mL of trisodium citrate (1%) and 75 mL of distilled water were blended for 15 min at 70 °C. After that, 1.5 mL from 1% silver nitrate solution (AgNO_3 , 99.99%, Roth, Karlsruhe Rheinshafen, Germany) were added; then 1% sodium borohydride (NaBH_4) was added, followed by quick mixing. The obtained mixed solution was heated for 60 min, cooled to room temperature, and water was added to a volume of 100 mL (Gunalan et al., 2012).

2.2.2. Biological production of AgNPs

AgNPs were synthesized using *Origanum vulgare* leaves extract as described by Shaik et al. (2018). One gram of *O. vulgare* tender leaves was cut into minor pieces; then, a grinder was used to obtain a powder which was dissolved in 10 mL of distilled water and then heated at 60 °C for 10 min. After cooling and filtering (Whatman No.1 filter paper), the solution was mixed with 90 mL of 1 mM silver nitrate (AgNO_3) solution and heated gradually at 60 °C for 10 min. The solution color transition to reddish-brown evidenced the production of AgNPs.

2.2.3. Silver nanoparticles characterization

The reddish, brown-colored reaction mixture was centrifuged at 12,000 rpm for 10 min. After that, the pellet was obtained and washed three times with deionized water, then lastly with acetone. The resulting dried pellet was stored for additional tests. Obtained AgNPs' optical properties were characterized by ultraviolet–visible (UV–vis) spectrophotometer (Thermo scientific SpectraScan) in the wavelength range of 340–900 nm. Production of nanoparticles was confirmed by measuring the absorption spectra of the solution at 420 nm by UV–vis spectrophotometer and by TEM and Differential Light Scattering (DLS) analysis (Mukherjee et al., 2008; Narayanan, Sakthivel, 2010; Wei et al., 2012).

Table 1
Types and sources of the tested strains.

Strains	Microbial species	Source of isolation
Bacterial strains	<i>Streptococcus agalactiae</i>	Nile tilapia (<i>Oreochromis niloticus</i>)
	<i>Aeromonas hydrophila</i>	Nile tilapia (<i>Oreochromis niloticus</i>)
Fungal strains	<i>Vibrio alginolyticus</i>	Sea bass (<i>Dicentrarchus labrax</i>)
	<i>Aspergillus flavus</i>	Nile tilapia (<i>Oreochromis niloticus</i>)
	<i>Fusarium moniliforme</i>	Nile tilapia (<i>Oreochromis niloticus</i>)
	<i>Candida albicans</i>	Nile tilapia (<i>Oreochromis niloticus</i>)

2.3. Studies of antimicrobial properties of AgNPs on the tested microorganisms

The antimicrobial activity of tested microorganisms was examined by the disc diffusion method (Bauer, 1966) using 6 mm discs with 20 μL of the test sample from both chemically and biologically produced AgNPs from *O. vulgare* leaves extract. Control (erythromycin for bacteria and fluconazole for fungi) and sterile discs were added. Standardized inoculation (0.5 Mc Farland standard) of the tested pathogens were seeded onto the surface of sterile Mueller Hinton agar and Sabouraud Dextrose Agar plates. After incubation at 37 °C for 24 hrs for bacteria and at 30 °C for 48hrs for fungi, the width of the zone of inhibition was measured with a caliper.

Three replicates of experiments were carried out. The diameter of the zone of inhibition was expressed as mean \pm standard error (SE).

2.4. Studies on hemolytic properties of AgNPs

Chemically and biologically produced AgNPs from *O. vulgare* leaves extract was examined for their hemolytic activity, for each concentration. 5 μL and 10 μL , on chicken and goat red blood cells (RBCs) (Paniprasad, 1997). The chicken and goat blood were obtained from the slaughterhouse in El-Behera Governorate, Egypt. A 5% EDTA solution was used as an anticoagulant; then, blood was centrifuged at 5000 rpm for 5 min. After discarding the supernatant with plasma and repeated (thrice) washing of the pellet, 1% erythrocytes suspension was prepared with 99 mL phosphate buffer and 1 mL of packaged erythrocytes. Serial two-fold dilutions of both chemically and biologically produced AgNPs were made in 100 μL of saline solution in a 96-well 'V' bottom microtitre plate. Then 100 μL of 1% erythrocytes suspension were. One hundred μL of saline solution was used as a positive control. The plate was slightly shaken and allowed to stand for 2 hrs at room temperature. The appearance of a uniform red colored suspension in wells was considered indicative of hemolysis, and a button development in the bottom of wells showed a lack of hemolysis. The reciprocal of the highest dilution showing hemolysis was considered as 1 hemolytic unit divided by protein content to obtain specific HU (Abirami et al., 2014).

2.5. TEM evaluation of ultrastructure changes of *S. agalactiae*, *A. hydrophila*, and *V. alginolyticus* treated with AgNPs

Cellular damage of tested bacteria by AgNPs was investigated by TEM. *S. agalactiae*, *A. hydrophila*, and *V. alginolyticus* 24hrs broth cultures were centrifuged at 10,000 rpm for 10 min, and pellets were obtained. Cells were washed thrice with PBS (pH 7.4) for 15 min; then, pellets were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer for 2 hrs. Samples were then rinsed (thrice/10 min each) with 0.05 M phosphate buffer (pH 7.2). Subsequently, a second fixation was made, submerging samples with 1% osmium tetroxide solution in 0.1 M phosphate buffer (pH 7.2) for 2 hrs at room temperature. Samples were thoroughly rinsed with phosphate buffer and serially dehydrated by ethanol (35, 70, 95, and 100%) for 15 min, and finally infiltrated by Epon Araldite Resin. Ultrathin sections were cut using a microtome with a diamond knife, placed on mesh copper grids, and counterstained as follows: 2% aqueous uranyl acetate for 10 min, distilled water rinse for 2–5 min, lead citrate for 4 min, a quick rinse with distilled water, then air-dried. Samples were observed by TEM (JSM-1400 plus-JEOL) at 120 kV in the Electron Microscope Unit, Alexandria University, Egypt (Kamonwannasit et al., 2013).

Ten mL of Muller Hinton broth, AgNPs, and bacteria were added to a 20 mL test tube to get a final concentration of 10 mg/L of

AgNPs and 10⁶ CFU/mL of bacterial cells. A control group without AgNPs was considered. The test tubes were incubated at 37 °C \pm 2 °C and shaken at 150 rpm for 12hrs, after which the cultures were centrifuged and pellets harvested to study morphology and structure by TEM according to Li et al. (2010).

Antibacterial properties of AgNPs at different concentrations were investigated on *Proteus* and *Klebsiella* by Ouda (2014) and on different bacterial fish pathogens by Shaalan et al. (2017).

2.6. Statistical analysis

The results were subjected to one analysis of variance (ANOVA) to test the effect of treatments on fish performance. Differences between means were compared using Duncan multiple range tests to test the significance level among means of treatments using SAS (2011) at $P < 0.05$. Significant differences between treatment and within the same strain as shown by the t -test ($P < 0.05$).

3. Results

3.1. UV–visible spectrophotometric profile of synthesized AgNPs

Manufactured AgNPs revealed a color shift from yellow to brown (Fig. 1) owing to the reduction of silver salt by reducing compounds in leaves extract of *O. vulgare*. Additionally, AgNPs formation was demonstrated by UV-absorption spectra which occupied a range from 200 to 800 nm, with an absorption spectrum of nanoparticles at 416 nm (Fig. 2), moreover characterization of nanoparticles by TEM confirmed the size of AgNPs were 21 and 100 nm, as well as by DLS size were 26.4 and 96 nm, for chemical and biological synthesis, respectively.

3.2. Antimicrobial activity of AgNPs

Data in Table 2 show the antibacterial activity of chemically and biologically produced AgNPs from *O. vulgare* extract on *Streptococcus agalactiae*, *Aeromonas hydrophila*, and *Vibrio alginolyticus* with different concentrations of 5 $\mu\text{L}/\text{disc}$ and 10 $\mu\text{L}/\text{disc}$ for the zone of inhibition (mm). The most significant inhibition zones within the three mentioned bacterial strains were obtained by 10 $\mu\text{L}/\text{disc}$ of biological AgNPs as 23.7 \pm 1.5, 31.3 \pm 1.5, and 26.1 \pm 1.0, while the smallest one was reported by 5 $\mu\text{L}/\text{disc}$ of chemical AgNPs as followed: 14.0 \pm 1.0, 16.3 \pm 1.5, and 9.0 \pm 1.0, respectively. Within the same treatment, by increasing the concentration of AgNPs, the antibacterial effect increased ($P < 0.05$), and overall treatment, the biological one was more potent antibacterial than the corresponding chemical one ($P < 0.05$). Also, Gram-negative bacteria were

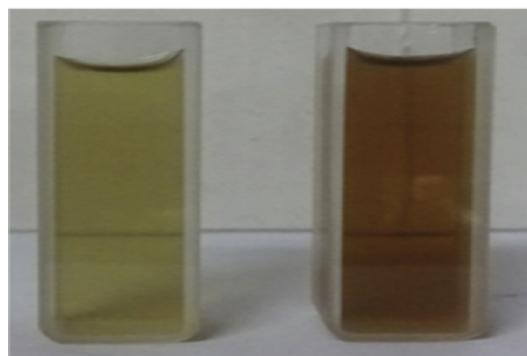


Fig. 1. Color shift from yellow to brown due to the synthesis of silver nanoparticles after adding the silver nitrate solution in the leaves extract of *Origanum vulgare*.

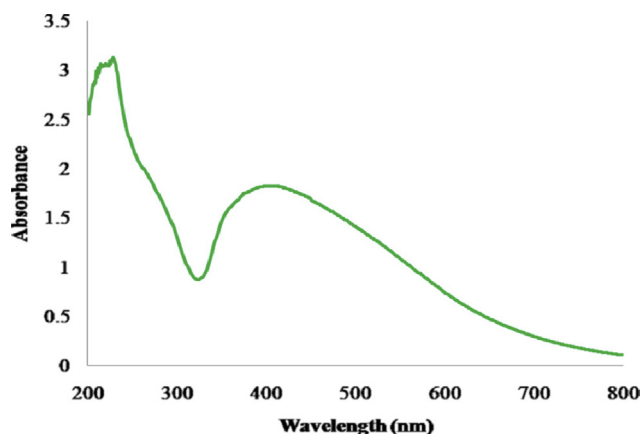


Fig. 2. UV-vis absorption spectrum of AgNPs produced from *O. vulgare* leaves extract.

more affected than Gram-positive ones by both chemically and biologically produced AgNPs.

As for antifungal activity, data in Table 3 show that antifungal activity of chemically and biologically produced AgNPs from *O. vulgare* extract on *Aspergillus flavus*, *Fusarium moniliform*, and *Candida albicans* with different concentrations of 5 $\mu\text{L}/\text{disc}$ and 10 $\mu\text{L}/\text{disc}$ for the zone of inhibition (mm). The most significant inhibition zones within the three mentioned fungal strains were obtained by 10 $\mu\text{L}/\text{disc}$ of biological AgNPs as 11.0 ± 1.0 , 13.0 ± 1.0 , and 18.0 ± 1.0 , while the smallest one was reported by 5 $\mu\text{L}/\text{disc}$ of chemical AgNPs as followed: 5.0 ± 1.0 , 5.0 ± 1.0 and 8.0 ± 1.0 , respectively. The antifungal property increased by increasing concentration for both treatments ($P < 0.05$), and overall treatment, the biological one was more potent antifungal than the corresponding chemical one ($P < 0.05$). Also, yeast was more affected than molds by both chemically and biologically produced AgNPs.

3.3. Haemolytic activity of AgNPs

Data in Table 4 showed that chemical AgNPs in most cases are more hemolytic of biological AgNPs, and that chicken is more sensitive than red goat cells ($P < 0.05$).

3.4. Transmission electron microscopy microphotograph

Figs. 3–5 showed interface among AgNPs and *S. agalactiae*, *Aeromonas hydrophila*, and *Vibrio alginolyticus* exposed to the chemical (A and B) and biological (C and D) production of AgNPs at 5 and 10 $\mu\text{L}/\text{mL}$, respectively), bad effect ranged from involvement into the external membrane, broadening of periplasmic space, the occurrence of AgNPs within periplasm and noticeable intra-cytoplasmic edema.

Table 2

Antibacterial activity (zone of inhibition in mm) of different concentrations of chemically (Treatment A) and biologically (Treatment B) produced AgNPs.

Bacterial Strain	Treatment A		Treatment B		Overall treatment	
	5 $\mu\text{L}/\text{disc}$	10 $\mu\text{L}/\text{disc}$	5 $\mu\text{L}/\text{disc}$	10 $\mu\text{L}/\text{disc}$	A	B
<i>S. agalactiae</i>	14.0 ± 1.0^b	21.0 ± 1.0^{ci}	17.0 ± 1.0^a	23.7 ± 1.5^{ci}	17.5 ± 3.9	$20.3 \pm 3.8^*$
<i>A. hydrophila</i>	16.3 ± 1.5^a	$25.7 \pm 2.1^{a†}$	19.0 ± 1.0^a	$31.3 \pm 1.5^{a†}$	21.0 ± 5.4	$25.2 \pm 6.9^*$
<i>V. alginolyticus</i>	9.0 ± 1.0^c	23.3 ± 1.5^{bi}	12.0 ± 1.0^b	26.0 ± 1.0^{bi}	16.2 ± 7.9	$19.0 \pm 7.7^*$

Values are means \pm standard deviation. Means within a column without a common letter differ significantly ($P < 0.05$). $†$ Within treatment, means of the two concentrations differ significantly ($P < 0.05$). $*$ Within strain, the overall means of the two treatments differ significantly ($P < 0.05$).

4. Discussion

Due to its unique functionality and wide range of applications, nanotechnology is a rapidly growing field. Nanomedicine investigates the potential for using nanotechnology's expertise and techniques to prevent, treat, diagnose, and control diseases (Almatroudi, 2020). A green, direct, and cost-effective fabrication method is proposed for Eco-environmentally AgNPs (Javan bakht Dalir et al., 2020). The study showed the synthesis of AgNPs by a color change from yellow to brown, which agreed with Hambarzumyan et al. (2020), who reported the exact color change from yellow to brown. Moreover, its detection through UV- absorption spectra occupied a range from 200 to 800 nm, with an absorption spectrum of nanoparticles at 416 nm. Similarly, Mukherjee et al. (2008), who reported at ~ 410 nm in the UV-vis spectrum, clearly reveals the formation of AgNPs, while de Aragão et al. (2019) confirmed that absorption peaks of AgNPs ranged from 400 to 450 nm. Throughout the procedure of nanoparticle production, incapability for silver salt to become reduced to silver ions via adding *O. vulgare* leaves extract was observed. Similar results were obtained by Hamouda et al. (2019), who found that bio fabrication of AgNPs could be ascertained using cyanobacterium *Oscillatoria limnetica*. The color transition from green to brown using cyanobacterium implies the biotransformation of Ag ions, indicating the synthesis of AgNPs. Further, *O. vulgare* L was used to synthesize AgNPs at ~ 430 nm (Shaik et al., 2018) and 480 nm (Landeros-Páramo, Rosas, 2019).

The sizes of chemically and biologically synthesized AgNPs in this study were 21 and 100 nm, respectively. The obtained sizes are slightly larger than those obtained by Mukherjee et al. (2008), who used TEM to characterize AgNPs, which showed the range of 13–18 nm in green synthesis, and DLS size was 26.4 and 96 nm, respectively. Indeed, the small size of AgNPs (1–100 nm) is unique to shape complex nanostructures, an extraordinary range of applications wound recovery, bactericidal and anticancer properties, and other therapeutic capacities, as well as their development cost-effectiveness addition (Almatroudi, 2020).

Biologically produced AgNPs showed more antimicrobial activity than the chemically produced AgNPs in this study. The zone of inhibition of biologically synthesized AgNPs is 25.2 ± 6.9 $\mu\text{L}/\text{disc}$ while in the case of chemically synthesized AgNPs, the zone of inhibition is 16.2 ± 7.9 $\mu\text{L}/\text{disc}$. Shaik et al. (2018) reported that the antifungal inhibition zone is 6.8 ± 2.1 and 14.0 ± 4.5 by chemically and biologically produced AgNPs, respectively. Both antimicrobial treatment effects increased by increasing the dose of AgNPs, and Gram-negative bacteria were more susceptible than Gram-positive bacteria. Yeast is more affected than mold; this may be attributed to the structure of pathogens. This agreed with Shrivastava et al. (2007), who found that antibacterial activity of the AgNPs was dose-dependent and greater against Gram-negative bacteria than against Gram-positive bacteria. Shaik et al. (2018) stated that AgNPs have contradictory biological effects with Gram-negative and Gram-positive bacterial and fungal strains.

Table 3

Antifungal activity (zone of inhibition in mm) of different concentrations from chemically (Treatment A) and biologically (Treatment B) produced AgNPs from *O. vulgare* leaves extract.

Strain	Treatment A		Treatment B		Overall treatment	
	5 μ L/disc	10 μ L/disc	5 μ L/disc	10 μ L/disc	A	B
<i>Aspergillus flavus</i>	5.0 \pm 1.0 ^b	8.7 \pm 0.6 ^{bi}	7.7 \pm 0.6 ^b	11.0 \pm 1.0 ^{ci}	6.8 \pm 2.1	9.3 \pm 2.0*
<i>Fusarium moniliforme</i>	5.0 \pm 1.0 ^b	10.0 \pm 1.0 ^{bi}	7.0 \pm 1.0 ^b	13.0 \pm 1.0 ^{bi}	7.5 \pm 2.9	10.0 \pm 3.4*
<i>Candida albicans</i>	8.0 \pm 1.0 ^a	14.3 \pm 1.5 ^{ai}	10.0 \pm 1.0 ^a	18.0 \pm 1.0 ^{ai}	11.2 \pm 3.7	14.0 \pm 4.5*

Values are means \pm standard deviation. Means within a column without a common letter differ significantly ($P < 0.05$). †Within treatment, means of the two concentrations differ significantly ($P < 0.05$). *Within strain, the overall means of the two treatments differ significantly ($P < 0.05$).

Table 4

Specific hemolytic unit (HT/mg) hemolytic activity of different concentrations of chemically (Treatment A) and biologically (Treatment B) produced AgNPs against chicken and goat blood.

Blood type	Treatment A		Treatment B		Overall treatment	
	5 μ L	10 μ L	5 μ L	10 μ L	A	B
Chicken	0.034 \pm 0.003 ^a	0.047 \pm 0.005 ^{ai}	0.032 \pm 0.004 ^a	0.026 \pm 0.004 ^a	0.040 \pm 0.008	0.029 \pm 0.005*
Goat	0.017 \pm 0.001 ^b	0.027 \pm 0.007 ^{bi}	0.019 \pm 0.004 ^b	0.014 \pm 0.002 ^b	0.022 \pm 0.007	0.016 \pm 0.004*

Values are means \pm standard deviation. Means within a column without a common letter differ significantly ($P < 0.05$). †Within treatment, means of the two concentrations differ significantly ($P < 0.05$). *Within blood type, the overall means of the two treatments differ significantly ($P < 0.05$).

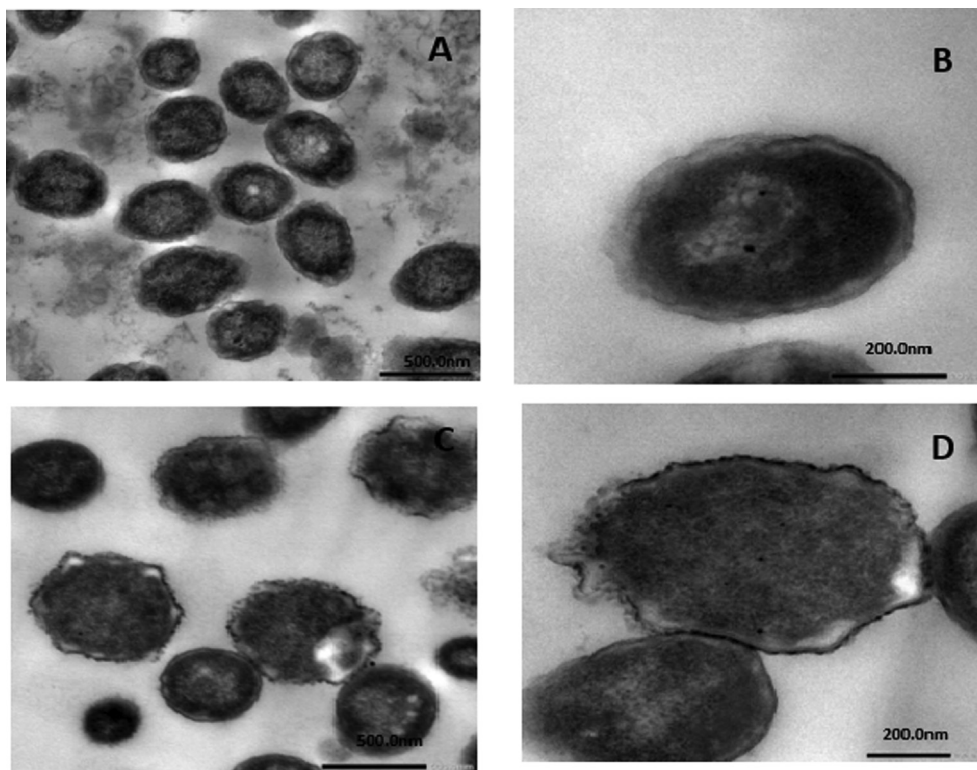


Fig. 3. The action of chemically (A and B) and biologically (C and D) produced AgNPs on *Streptococcus agalactiae* observed by TEM at 5 and 10 μ L/mL, respectively).

Data illustrated in Table 4 highlighted the hemolytic activity of chemically and biologically produced AgNPs, with different concentrations of 5 and 10 μ L; the direct association of the nanoparticle with RBCs causes oxidative stress, membrane damage and hemolysis, which leads to cytotoxicity. Overall, the findings indicate that concentration has a significant impact on AgNPs-RBC interaction. Lower values of hemolysis were observed with biologically produced AgNPs than chemically produced AgNPs. The results indicated that the biological AgNPs are safer for cell cytotoxicity and more potent as antimicrobial efficacy than chemically synthesized AgNPs. This agreed with Meléndrez et al. (2010), who said the fact that increasing concentrations of AgNPs resulted in a

high degree of hemolytic activity suggests that AgNPs toxicity is concentration-dependent, and Choi et al. (2011) and Sopjani et al. (2009) who reported that RBCs died when silver ions were released, even at low concentrations, according to previous toxicity studies. Also similar to Chen et al. (2015), who mentioned that hemolysis, membrane damage, lipid peroxidation, and antioxidant enzyme activity were all size and dose-dependent toxic effects. Moreover, disagree with Huang et al. (2016) who mentioned that although several studies on the biological effects of AgNPs in blood have been conducted, the materials employed in each study differed in size and coating, making comparisons impossible. Furthermore, there are insufficient controls to determine the actual cause

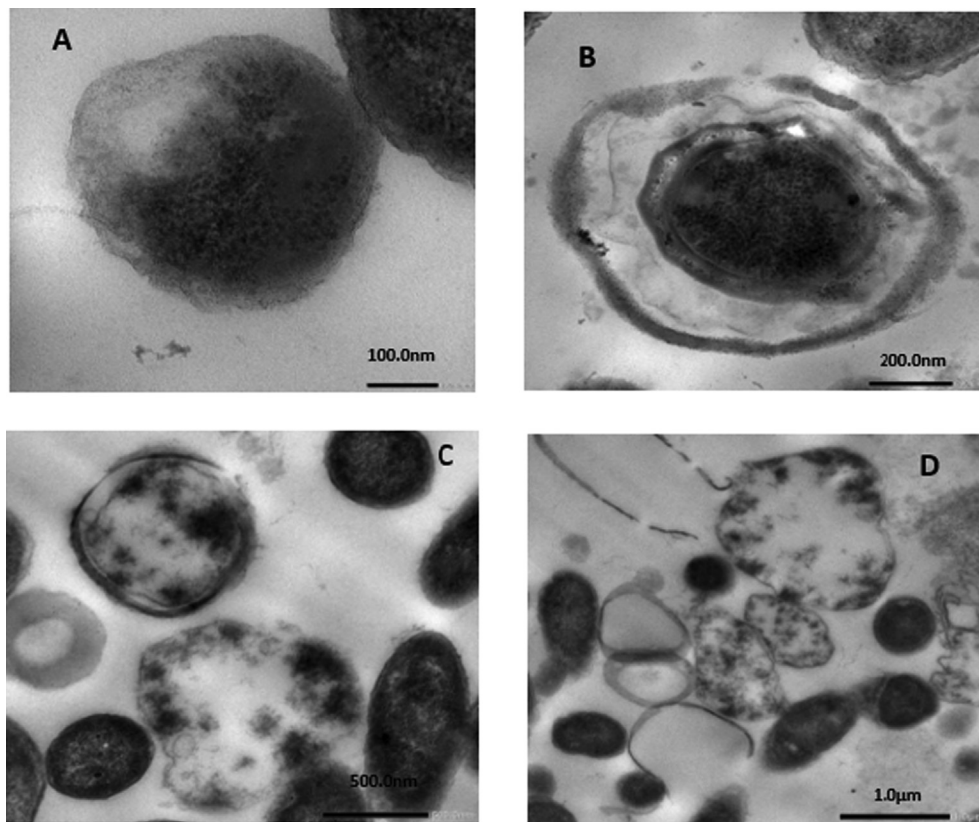


Fig. 4. The action of chemically (A and B) and biologically (C and D) produced AgNPs on *Aeromonas hydrophila* observed by TEM at 5 and 10 µL/mL, respectively).

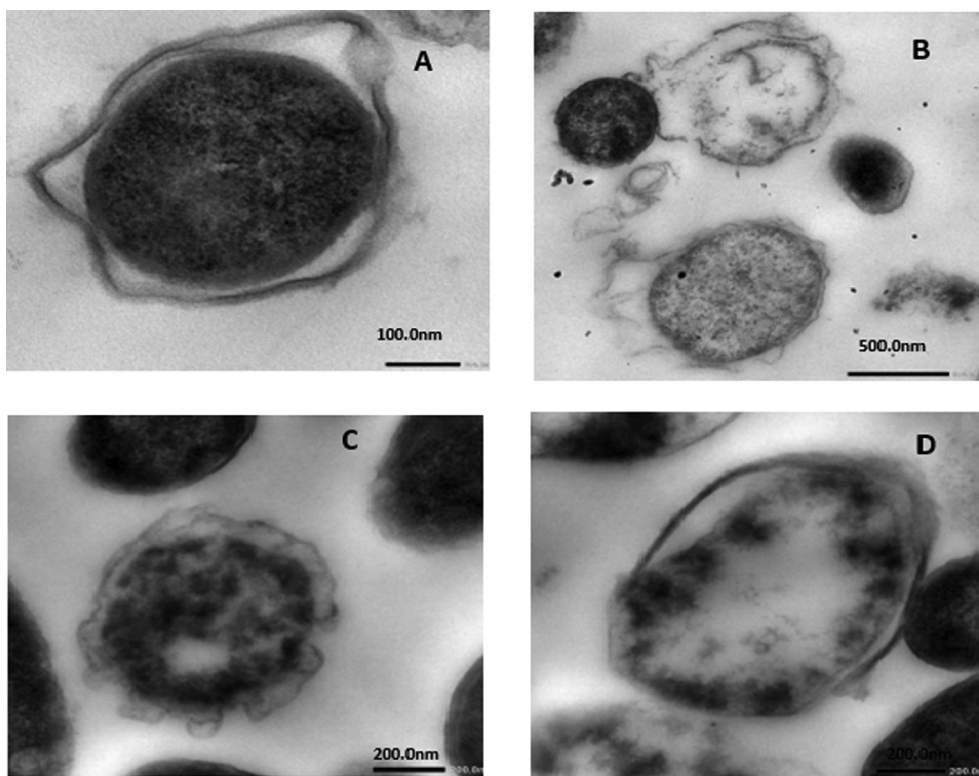


Fig. 5. The action of chemically (A and B) and biologically (C and D) produced AgNPs on *Vibrio alginolyticus* observed by TEM at 5 and 10 µL/mL, respectively).

of AgNPs toxicity, although they said hemolysis also was dose dependent. As well as similar to [Raja et al. \(2016\)](#) who published that AgNPs were shown to be safe at maximum concentrations of

1–5 µg/mL and harmful at concentrations of 15–50 µg/mL in an in vitro hemolytic test. Furthermore, these biologically produced nanoparticles were less hazardous and more effective against some

bacterial strains. Data also illustrated the effect of antibacterial effect of AgNPs on *S. agalactiae*, *Aeromonas hydrophila*, and *Vibrio alginolyticus* exposed to chemical and biological production of AgNPs at 5 and 10 µL/mL, respectively. Vazquez-Muñoz et al. (2019) obtained similar results, assuming that the effect of AgNPs, particularly with multi-drug resistant microbes, can be exploited to curb the current problem of antibiotic resistance due to membrane damage on Gram-negative bacteria resulting in a bactericidal effect.

TEM exploration revealed that AgNPs meaningfully mark the cell membrane integrity in Gram-negative bacteria; nevertheless, no cell wall disruption was detected in treatments on Gram-positive bacteria. This agreed with Shrivastava et al. (2007), who said bacterial cell lysis might be one of the causes of the antibacterial effect; nanoparticles have changed the phosphatase levels. Further, Huang et al. (2017) concluded that untreated bacterial cells with AgNPs displayed intact cell membrane and wall. Cells with uniform electron density recommended that the cells were in a normal state. AgNPs showed advanced harmfulness against Gram-negative bacteria than against Gram-positive ones due to structural differences. Gram-positive bacteria are secure via a denser cell wall, whereas Gram-negative stand extra effortlessly destroyed because of interface among distinct external membrane and nanoparticles. In contrast with Hambardzumyan et al. (2020), who recorded that when compared to Gram-negative stains, Gram-positive bacteria were more sensitive to AgNPs and attributed that may be related to species differences.

The biosynthesis process was very beneficial above additional chemical methods of production AgNPs; this was as same as that mentioned by Chand et al. (2020), who concluded that green synthesis of AgNPs is more effective than chemical one moreover safe for the environment. Also, the use of plants and plant extracts to synthesize silver AgNPs (green synthesis) has become a breakthrough in biotechnology, especially for the control of pathogenic bacteria (Mashwani et al., 2015).

5. Conclusions

Silver nanoparticles (AgNPs), mainly green ones, possess potent antimicrobial characters in competing for many fish microorganisms; Gram-positive and Gram-negative bacteria also molds, and yeasts affect fish. More specifically, green biological production of AgNPs is more safe and powerful than chemical ones principally by increasing their concentrations. The obtained results help overcome antimicrobial resistance to limit fish diseases, increase economy and, in consequence, improve human health.

Funding

This research was funded by the Researchers Supporting Project Number (RSP-2021/144), King Saud University, Riyadh, Saudi Arabia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP-2021/144), King Saud University, Riyadh, Saudi Arabia.

References

- Abdel-Tawwab, M., Khalifa, E., Diab, A.M., Khallaf, M.A., Abdel-Razek, N., Khalil, R.H., 2020. Dietary garlic and chitosan alleviated zearalenone toxic effects on performance, immunity, and challenge of European sea bass, *Dicentrarchus labrax*, to *Vibrio alginolyticus* infection. *Aquaculture International*. 28 (2), 493–510.
- Abirami, P., Arumugam, M., Giji, S., Nagarajan, S., 2014. Bio-prospecting of catfish sting venom *Arius maculatus* available along South East coast of India. *Int J Pharm Pharm Sci*. 6, 110–115.
- Al-Sahzly, M., Khalil, R., Saad, T., 2016. Studies on Streptococcus Infection in *O. niloticus* Fish and Its Vaccine Protection 11, 191–198.
- Alagawany, M., Farag, M.R., Salah, A.S., Mahmoud, M.A., 2020a. The role of oregano herb and its derivatives as immunomodulators in fish. *Reviews in Aquaculture*. 12 (4), 2481–2492.
- Alagawany, M., Abd El-Hack, M.E., Farag, M.R., Shaheen, H.M., Abdel-Latif, M.A., Noreldin, A.E., Khafaga, A.F., 2020b. The applications of *Origanum vulgare* and its derivatives in human, ruminant and fish nutrition—a review. *Annals of Animal Science*. 20, 389–407.
- Almatroudi, A., 2020. Silver nanoparticles: synthesis, characterisation and biomedical applications. *Open Life Sciences*. 15, 819–839.
- Alwafa, R.A., Mudalal, S., Mauriello, G., 2021. *Origanum syriacum* L. (Za'atar), from Raw to Go. *A Review*. *Plants*. 10 (5), 1001. <https://doi.org/10.3390/plants10051001>.
- Bauer, A.W., 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol*. 45, 149–158.
- Bisht, D., Bisht, G., Khulbe, R., 2000. Fusarium—a new threat to fish population in reservoirs of Kumaun. *India. Current Science*, 1241–1245.
- Chand, K., Cao, D., Eldin Fouad, D., Hussain Shah, A., Qadeer Dayo, A., Zhu, K., Nazim Lakhani, M., Mehdi, G., Dong, S., 2020. Green synthesis, characterization, and photocatalytic application of silver nanoparticles synthesized by various plant extracts. *Arabian Journal of Chemistry*. 13 (11), 8248–8261.
- Chen, L.Q., Fang, L.I., Ling, J., Ding, C.Z., Kang, B., Huang, C.Z., 2015. Nanotoxicity of silver nanoparticles to red blood cells: size-dependent adsorption, uptake, and hemolytic activity. *Chemical Research in Toxicology*. 28 (3), 501–509.
- Choi, J., Reipa, V., Hitchins, V.M., Goering, P.L., Malinauskas, R.A., 2011. Physicochemical characterization and in vitro hemolysis evaluation of silver nanoparticles. *Toxicological Sciences*. 123, 133–143.
- Christopher, P., Xin, H., Linic, S., 2011. Visible-light-enhanced catalytic oxidation reactions on plasmonic silver nanostructures. *Nature Chemistry*. 3 (6), 467–472.
- Dawood, M.A.O., 2021. Nutritional immunity of fish intestines: important insights for sustainable aquaculture. *Reviews in Aquaculture*. 13 (1), 642–663.
- de Aragão, A.P., de Oliveira, T.M., Quelemes, P.V., Perfeito, M.L.G., Araújo, M.C., Santiago, J.d.A.S., Cardoso, V.S., Quaresma, P., de Souza de Almeida Leite, J.R., da Silva, D.A., 2019. Green synthesis of silver nanoparticles using the seaweed *Gracilaria birdiae* and their antibacterial activity. *Arabian Journal of Chemistry*. 12 (8), 4182–4188.
- de Torre, M.P., Vizmanos, J.L., Caverio, R.Y., Calvo, M.I., 2020. Improvement of antioxidant activity of oregano (*Origanum vulgare* L.) with an oral pharmaceutical form. *Biomedicine & Pharmacotherapy*. 129, 110424. <https://doi.org/10.1016/j.biopha.2020.110424>.
- DeiviArunachalam, K., Kuruva, J.K., Pradhoshini, K.P., Musthafa, M.S., Faggio, C., 2021. Antioxidant and antigenotoxic potential of *Morinda tinctoria* Roxb. leaf extract succeeding cadmium exposure in Asian catfish, *Pangasius sutchi*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 249, 109149. <https://doi.org/10.1016/j.cbpc.2021.109149>.
- Fu, L.-H., Deng, F.u., Ma, M.-G., Yang, J., 2016. Green synthesis of silver nanoparticles with enhanced antibacterial activity using holocellulose as a substrate and reducing agent. *RSC advances*. 6 (34), 28140–28148.
- Golubeva, O.Y., Shamova, O.V., Orlov, D.S., Pazina, T.Y., Boldina, A.S., Kokryakov, V.N., 2010. Study of antimicrobial and hemolytic activities of silver nanoparticles prepared by chemical reduction. *Glass Physics and Chemistry*. 36 (5), 628–634.
- Gunalan, S., Sivaraj, R., Rajendran, V., 2012. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International*. 22 (6), 693–700.
- Hambardzumyan, S., Sahakyan, N., Petrosyan, M., Nasim, M.J., Jacob, C., Trchounian, A., 2020. *Origanum vulgare* L. extract-mediated synthesis of silver nanoparticles, their characterization and antibacterial activities. *AMB Express*. 10, 162.
- Hamouda, R.A., Hussein, M.H., Abo-elmagd, R.A., Bawazir, S.S., 2019. Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica*. *Scientific Reports*. 9, 13071.
- Huang, H., Lai, W., Cui, M., Liang, L., Lin, Y., Fang, Q., Liu, Y., Xie, L., 2016. An evaluation of blood compatibility of silver nanoparticles. *Scientific reports* 6 (1), 1–15.
- Huang, X., Bao, X., Liu, Y., Wang, Z., Hu, Q., 2017. Catechol-Functional Chitosan/Silver Nanoparticle Composite as a Highly Effective Antibacterial Agent with Species-Specific Mechanisms. *Scientific Reports*. 7, 1860.
- Illanchezian, S., Jayaraman, SathishKumar, Manoharan, M.S., Valsalam, S., 2010. Virulence and cytotoxicity of seafood borne *Aeromonas hydrophila*. *Brazilian Journal of Microbiology*. 41 (4), 978–983.
- Ina-Salwany, M.Y., Al-saari, N., Mohamad, A., Mursidi, F.-A., Mohd-Aris, A., Amal, M. N.A., Kasai, H., Mino, S., Sawabe, T., Zamri-Saad, M., 2019. Vibriosis in Fish: A Review on Disease Development and Prevention. *Journal of Aquatic Animal*

- Health. 31 (1), 3–22.
- Inglis, V., Roberts, R.J., Bromage, N.R., 1993. Chapter 12: Streptococcal infections". In: Bacterial Diseases of Fish, Halsted Press, John Wiley & Sons Inc, NY, pp. 196–197.
- Janda, J.M., Abbott, S.L., 2010. The Genus *Aeromonas*: Taxonomy, Pathogenicity, and Infection. *Clinical Microbiology Reviews*. 23 (1), 35–73.
- Javan bakht Dalir, S., Djahaniani, H., Nabati, F., Hekmati, M., 2020. Characterization and the evaluation of antimicrobial activities of silver nanoparticles biosynthesized from *Carya illinoensis* leaf extract. *Heliyon*. 6 (3), e03624. <https://doi.org/10.1016/j.heliyon.2020.e03624>.
- Kamonwannasit, S., Nantapong, N., Kumkrai, P., Luecha, P., Kupittayanant, S., Chudapongse, N., 2013. Antibacterial activity of *Aquilaria crassna* leaf extract against *Staphylococcus epidermidis* by disruption of cell wall. *Annals of Clinical Microbiology and Antimicrobials*. 12, 1–7.
- Kumar, P., Govindaraju, M., Senthamilselvi, S., Premkumar, K., 2013. Photocatalytic degradation of methyl orange dye using silver (Ag) nanoparticles synthesized from *Ulva lactuca*. *Colloids and Surfaces B: Biointerfaces*. 103, 658–661.
- Kumar, P.P.N.V., Shameem, U., Kollu, P., Kalyani, R.L., Pammi, S.V.N., 2015. Green synthesis of copper oxide nanoparticles using *Aloe vera* leaf extract and its antibacterial activity against fish bacterial pathogens. *BioNanoScience*. 5 (3), 135–139.
- Kumar, R., Pande, V., Singh, L., Sharma, L., Saxena, N., Thakuria, D., Singh, A., Sahoo, P., 2016. Pathological findings of experimental *Aeromonas hydrophila* infection in golden mahseer (*Tor putitora*). *Fish Aquac J.* 7, 2.
- Landeros-Páramo, L., Rosas, G., 2019. Green Synthesis of Silver Nanoparticles Using the Mixture of Aqueous Extracts of *Sedum praecaltum* and *Origanum vulgare*. *Microscopy and Microanalysis*. 25 (S2), 2372–2373.
- Li, W.-R., Xie, X.-B., Shi, Q.-S., Zeng, H.-Y., OU-Yang, Y.-S., Chen, Y.-B., 2010. Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*. *Applied Microbiology and Biotechnology*. 85 (4), 1115–1122.
- Magculia, N.J.F., Cumagun, C.J.R., 2011. Genetic diversity and PCR-based identification of potential fumonisin-producing *Fusarium verticillioides* isolates infecting corn in the Philippines. *Tropical Plant Pathology*. 36, 225–232.
- Mashwani, Z.-u.-R., Khan, T., Khan, M.A., Nadhman, A., 2015. Synthesis in plants and plant extracts of silver nanoparticles with potent antimicrobial properties: current status and future prospects. *Applied Microbiology and Biotechnology*. 99 (23), 9923–9934.
- Meléndrez, M.F., Cárdenas, G., Arbiol, J., 2010. Synthesis and characterization of gallium colloidal nanoparticles. *Journal of Colloid and Interface Science*. 346 (2), 279–287.
- Mian, G.F., Godoy, D.T., Leal, C.A.G., Yuhara, T.Y., Costa, G.M., Figueiredo, H.C.P., 2009. Aspects of the natural history and virulence of *S. agalactiae* infection in Nile tilapia. *Veterinary Microbiology*. 136 (1–2), 180–183.
- Mohamed, H.M.A., Emeish, W.F.A., Braeuning, A., Hammad, S., 2017. Detection of aflatoxin-producing fungi isolated from Nile tilapia and fish feed. *EXCLI J.* 16, 1308–1318.
- Mohammadi, G., Rashidian, G., Hoseinifar, S.H., Naserabad, S.S., Doan, H.V., 2020a. Ginger (*Zingiber officinale*) extract affects growth performance, body composition, haematology, serum and mucosal immune parameters in common carp (*Cyprinus carpio*). *Fish & Shellfish Immunology*. 99, 267–273.
- Mohammadi, G., Rafiee, G., El Basuini, M.F., Abdel-Latif, H.M.R., Dawood, M.A.O., 2020b. The growth performance, antioxidant capacity, immunological responses, and the resistance against *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*) fed Pistacia vera hulls derived polysaccharide. *Fish & Shellfish Immunology*. 106, 36–43.
- Mukherjee, P., Roy, M., Mandal, B.P., Dey, G.K., Mukherjee, P.K., Ghatak, J., Tyagi, A.K., Kale, S.P., 2008. Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*. *Nanotechnology*. 19 (7), 075103. <https://doi.org/10.1088/0957-4484/19/7/075103>.
- Narayanan, K.B., Sakhivel, N., 2010. Biological synthesis of metal nanoparticles by microbes. *Advances in Colloid and Interface Science*. 156 (1–2), 1–13.
- Ouda, S.M., 2014. Some nanoparticles effects on *Proteus* sp. and *Klebsiella* sp. isolated from water. *Am. J. Infect. Dis. Microbiol.* 2, 4–10.
- Paniprasad, K., 1997. Microhaemolytic Assay: In: training manual on advance techniques marine Biotoxinology, Venkateshvaran K, Prasad KP. Cent Ins Fish Edu, India. 41.
- Raja, A., Salique, S.M., Gajalakshmi, P., James, A., 2016. Antibacterial and hemolytic activity of green silver nanoparticles from *Catharanthus roseus*. *Int. J. Pharm. Sci. Nanotechnol.* 9.
- Rashidian, G., Boldaji, J.T., Rainis, S., Prokić, M.D., Faggio, C., 2021a. Oregano (*Origanum vulgare*) Extract Enhances Zebrafish (*Danio rerio*) Growth Performance, Serum and Mucus Innate Immune Responses and Resistance against *Aeromonas hydrophila* Challenge. *Animals*. 11 (2), 299. <https://doi.org/10.3390/ani11020299>.
- Rashidian, G., Lazado, C.C., Mahboub, H.H., Mohammadi-Aloucheh, R., Prokić, M.D., Nada, H.S., Faggio, C., 2021b. Chemically and Green Synthesized ZnO Nanoparticles Alter Key Immunological Molecules in Common Carp (*Cyprinus carpio*) Skin Mucus. *International Journal of Molecular Sciences*. 22 (6), 3270. <https://doi.org/10.3390/ijms22063270>.
- Sadhasivam, S., Shanmugam, P., Yun, KyuSik, 2010. Biosynthesis of silver nanoparticles by *Streptomyces hygroscopicus* and antimicrobial activity against medically important pathogenic microorganisms. *Colloids and Surfaces B: Biointerfaces*. 81 (1), 358–362.
- Sankar, R., Karthik, A., Prabu, A., Karthik, S., Shivashangari, K.S., Ravikumar, V., 2013. *Origanum vulgare* mediated biosynthesis of silver nanoparticles for its antibacterial and anticancer activity. *Colloids and Surfaces B: Biointerfaces*. 108, 80–84.
- Sarkar, M.J.A., Rashid, M.M., 2012. Pathogenicity of the bacterial isolate *Aeromonas hydrophila* to catfishes, carps and perch. *Journal of the Bangladesh Agricultural University*. 10 (1), 157–161.
- Serrano, C., García-Fernández, L., Fernández-Blázquez, J.P., Barbeck, M., Ghanaati, S., Unger, R., Kirkpatrick, J., Arzt, E., Funk, L., Turón, P., del Campo, A., 2015. Nanostructured medical sutures with antibacterial properties. *Biomaterials*. 52, 291–300.
- Shaan, M.I., El-Mahdy, M.M., Theiner, S., El-Matbouli, M., Saleh, M., 2017. In vitro assessment of the antimicrobial activity of silver and zinc oxide nanoparticles against fish pathogens. *Acta Veterinaria Scandinavica*. 59, 1–11.
- Shaik, M.R., Khan, M., Kuniyil, M., Al-Warthan, A., Alkhatlan, H.Z., Siddiqui, M.R.H., Shaik, J.P., Ahamed, A., Mahmood, A., Khan, M., Adil, S.F., 2018. Plant-Extract-Assisted Green Synthesis of Silver Nanoparticles Using *Origanum vulgare* L. Extract and Their Microbicidal Activities. *Sustainability* 10.
- Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P., Dash, D., 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology*. 18 (22), 225103. <https://doi.org/10.1088/0957-4484/18/22/225103>.
- Sopjani, M., Föller, M., Haendeler, J., Götz, F., Lang, F., 2009. Silver ion-induced suicidal erythrocyte death. *Journal of Applied Toxicology*. 29 (6), 531–536.
- Tartor, Y., Taha, M., Mahboub, H., El Ghamery, M., 2018. Yeast species associated with diseased fish: Occurrence, identification, experimental challenges and antifungal susceptibility testing. *Aquaculture*. 488, 134–144.
- Vali, S., Mohammadi, G., Tavabe, K.R., Moghadas, F., Naserabad, S.S., 2020. The effects of silver nanoparticles (Ag-NPs) sublethal concentrations on common carp (*Cyprinus carpio*): Bioaccumulation, hematology, serum biochemistry and immunology, antioxidant enzymes, and skin mucosal responses. *Ecotoxicology and Environmental Safety*. 194, 110353. <https://doi.org/10.1016/j.ecoenv.2020.110353>.
- Van Doan, H., Hoseinifar, S.H., Sringarm, K., Jaturasitha, S., Yuangsoi, B., Dawood, M.A.O., Esteban, M.Á., Ringø, E., Faggio, C., 2019. Effects of Assam tea extract on growth, skin mucus, serum immunity and disease resistance of Nile tilapia (*Oreochromis niloticus*) against *Streptococcus agalactiae*. *Fish & Shellfish Immunology*. 93, 428–435.
- Vazquez-Muñoz, R., Meza-Villecas, A., Fournier, P.G.J., Soria-Castro, E., Juarez-Moreno, K., Gallego-Hernández, A.L., Bogdanchikova, N., Vazquez-Duhalt, R., Huerta-Saqueró, A., Mishra, Y.K., 2019. Enhancement of antibiotics antimicrobial activity due to the silver nanoparticles impact on the cell membrane. *PLoS one*. 14 (11), e0224904. <https://doi.org/10.1371/journal.pone.0224904>.
- Veenstra, J.P., Johnson, J.J., 2019. Oregano (*Origanum vulgare*) extract for food preservation and improvement in gastrointestinal health. *Int J Nutr.* 3, 43–52.
- Vijay Kumar, P.P.N., Pammi, S.V.N., Kollu, P., Satyanarayana, K.V.V., Shameem, U., 2014. Green synthesis and characterization of silver nanoparticles using *Boerhaavia diffusa* plant extract and their anti bacterial activity. *Industrial Crops and Products*. 52, 562–566.
- Wei, X., Luo, M., Li, W., Yang, L., Liang, X., Xu, L., Kong, P., Liu, H., 2012. Synthesis of silver nanoparticles by solar irradiation of cell-free *Bacillus amyloliquefaciens* extracts and AgNO₃. *Bioresource Technology*. 103 (1), 273–278.
- Zayed, M., Soliman, M., Kalil, R., Saad, T., 2016. Isolation of *Candida albicans* from naturally infected freshwater fish. *Kafrelsheikh Veterinary Medical Journal*. 14 (1), 21–45.