Acute Toxicity of Zinc Oxide Nanoparticles from Satureja hortensis on Rainbow Trout (Oncorhynchus mykiss)

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Article History
Received 09 April 2019
Accepted 04 December 2019
First Online 16 December 2019

Abstract
Nanoparticles are commonly synthesized chemically and because of their risk for living organisms and environment, safer synthesis methods are highly considered. This work was carried out to evaluate the acute toxicity of zinc oxide nanoparticles synthesized from Satureja hortensis on rainbow trout (Oncorhynchus mykiss) by static bioassay test. The average weight and length of trout fish used in the study were 20±3 g and 15±2 cm respectively. Three groups of the experimented fish were exposed to different concentrations (1, 10 and 100 mg/L) of ZnO nanoparticles. All the exposed fish were daily observed and dead fishes were immediately removed and recorded. The obtained data were statistically analyzed using probit regression method. The LC₅₀ value at 96 h was found to be 25.50 mg/L for trout fish which makes this nanoparticle as a low toxicity substance in terms of toxicity classification. According to the results lifetime reduced with increasing the concentration of ZnO. Based on the obtained LC₅₀ for the synthesized ZnO from Satureja plant in this study and comparison with that of the chemically fabricated in others work, it was revealed that the green nanoparticles synthesis is much less hazardous and a good alternative technique.

Introduction
In last decade, nanotechnology has been an attractive subject in nearly all fields from research to production and application. But the aspect of nanomaterials impact on human and animals has not been widely considered in many part of the world (Krysanov, Pavlov, Demidova, & Dgebuadze, 2010).

One of the most important nanoparticle is zinc oxide (ZnO) that is widely used in industries as an additive in various products (Fakhari, Jamzad, & Kabiri Fard, 2019). The increased production of nano-products results in an increase in their release to environment, particularly aquatic environments (Keller, McFerran, Lazareva, & Suh, 2013; Skjolding et al., 2016). Hence, assessment the risk of these nano materials and especially their toxicity is of great importance and necessity (Skjolding et al., 2016). Inadequate or lack of data on the fate of nanoparticles in aquatic environments and also their interaction with aquatic life have raised serious concerns (Ahmed, Chaudhry, & Ikram, 2017). In recent years, the production of nanoparticles has become an attractive topic in science and industry (Fakhari et al., 2019). In most cases, chemical methods are used to synthesize nanoparticles. These chemically synthesized nanomaterials are adversely harmful for human health and the environment (Ahmed et al., 2017; Roy & Barik, 2010).
Given this issue, the demand for the production of nanoparticles with safer methods, such as biological methods (also known as green method), is increasing (Agarwal, Kumar, & Rajeshkumar, 2017; Song & Kim, 2009). In these methods, plants or microorganisms or substances already exist in nature are commonly applied for nanoparticles synthesis (Agarwal et al., 2017). Iran is very diverse in terms of the native flora and more studies are needed in this regard (Baharara J & T., 2014). One of Iran’s most abundant herbs is savory (Satureja) plant, which is widely used in food and pharmaceutical industries and plays a special role in traditional medicine. It mainly contains carvacrol, thymol, parsimine and terpinene compounds (nooshkam. A, majonhoseini. N, hadian. J, jahansos.M, & khavazi, 2015). Although the savory is considered to be among the most important medicinal plants, but very few studies on nanoparticle synthesis from this plant, the toxicity and safety in the environment has been reported before (Afshar & Sedaghat, 2016).

Since the routine physical and chemical analysis cannot completely reveal the adverse effects of environmental pollutants, bioassay tests are commonly used to determine their toxicity (Clesceria, Greenberg, & and Eaton, 1998). These tests are generally used to find out the concentrations that can cause mortality in half of the examined organisms over a specific period of time (Prinsloo et al., 2017). These tests indicate that which pollutants have the greatest potential to impact on the environment and whether the pollutants exceed the standard limits or not (Azimi. Z, Sadeghpor, Porgholami, & Rofchai, 2015). Nowadays, for a variety of reasons, sea products play a significant role in supplying human food. As the nutritional value of sea foods are recognized, they receive more attention and are more consumed (Ardebili, 2017). Among sea products, fish is of great importance because of its high economic value and sensitivity to pollutants. Rainbow trout is among the most important cold-water fish belonging to the Salmonidae family and is one of the main species of fish cultivated in Iran (Kamani, Mortazavi, Safari, & Mehraban, 2016). Apart from its food value, this fish is also economically important. Studies have shown that salmon and trout farming projects in different provinces of Iran are considered as one of the most important production sectors contributing to job creation (Hoseinpour, Souri, Ghaemi, & 1, 2017).

Rainbow trout is one of the most sensitive species to presence of contaminants in water even at low concentrations. Therefore, it can be considered as an appropriate indicator for pollutants assessment (Khodabakhshi, Porbagher, & Hosseini, 2017). Given the mentioned issues regarding to the importance of nanoparticles in industries, as well as the economic and nutritional importance of salmon and trout in our lives, this study aimed to synthesize green zinc oxide nanoparticles from the savory plant and assess its toxicity.

### Materials and Methods

In this work, zinc oxide nanoparticle was prepared biologically from the *Satureja* plant as follows. At first the savory plant was purchased from farms around Khorraramabad, Iran. After confirmation the characteristics by the University Herbarium, the leaves of the plant were removed and dried in shade and then crushed.

#### Biosynthesis of ZnO Nanoparticles and Characterization

Biosynthesis of ZnO nanoparticles of *S. hortensis* was mainly prepared according to the method presented Arinjoy Datta (Datta et al., 2017). To prepare the plant extract, 100 mL of double- distilled water and 10 g of dried savory plant were put into a beaker and then the beaker was put in an electric stirrer (speed of 300 rpm) at 150°C and a for 1 h. After cooling down, the extract was filtered using a Whatman paper filter (No 40) at room temperature and finally a uniform red solution was obtained. To synthesize zinc oxide nanoparticles, 100 mL of the savory extract was mixed with 7 g of zinc nitrate and 10 mL of sodium hydroxide, then the mixture was placed in an electric stirrer with a speed of 400 rpm at 150°C. Subsequently, the samples were centrifuged at 5,000 rpm for 5 min. The obtained precipitate was dried at 85°C for 90 min. The dried samples were then put into a porcelain mortar and completely crushed. The obtained powder was passed through a sieve (with a pore size of .015 mm) to complete the nanoparticle synthesis (Figure 1). The powder samples were analyzed and characterized using UV-vis (T80-UV-vis, England), Scanning electron microscope (SEM) (FE-SEM, tescan company, Czech Republic), energy-dispersive X-ray analysis (EDX) (mira3-tescan device, Czech Republic), and X-ray diffraction (XRD) (Stoe company, Germany).

#### Experimental Fish and Acclimatization

Rainbow trout (*Oncorhynchus mykiss*) was purchased from a fish cultivation filed around Khorrarambad city, Iran. The weight of the fish was 20±3 g and their mean length was 15±2 cm. For proper transferring the fish to the laboratory, a 60-L plastic container equipped with an air pump, and an oxygen cylinder was used. Furthermore, to control the water temperature during the transferring, ice pieces were used.

#### Water Analysis and Quality

The water used in this study was from public drinking water of Khorrarambad city. Water was aerated for three days (72 h) in order to complete removed of residual chlorine (Jahanbakshi, Hedayati, Pirbeigi, & Javadimoosavi, 2015). Before the experiments, water
quality was analyzed for common quality parameters using standard methods (Clesceria et al., 1998) as shown in Table 1.

**Data Analysis**

Data analysis was carried out using, probit regression method SPSS -19 (trial version) and the graphs were presented by SPSS and MS-Excel.

**Acute Toxicity Test**

In laboratory, the fish were kept for three days for adaptation to new lab condition. Experiments were planned and carried out based on the standard methodology of the Organization for Economic Cooperation and Development (OECD, 2009). Accordingly, the experiment was carried out statically, i.e. the concentration of nanoparticle was constant in each container and the water was not replaced during the experiment. Additionally, the feeding of the fish was stopped 24 h before the start of the experiment. The fish were randomly divided into four groups of nine, placed in 40-L plastic containers. An air pump and a water pump were placed in each container to circulate and aerate the water. One group was kept as control and three other groups were exposed to different concentrations of zinc oxide nanoparticle, (1, 10 and 100 mg/L). To reduce the stress of the fish and also to reduce the effect of their excrements on the results of the experiments, they were not fed during the experiment period. The water temperature was controlled between 18 and 20 °C., by placing plastic ice bags into the containers. To add the nanoparticles, a uniform suspensions were prepared based on the certain amount of each concentration and consequently added to the containers. The duration of the experiment was 96 h and the mortality rates were recorded at 24, 48, 72 and 96 hours (Helfrich, Weigmann, Hipkins, & Stinson, 2009). During the experiment, all apparent symptoms and behaviors of the fish were carefully monitored and the observations were recorded.

**Table 1.** Chemical parameters of the water used for the experiments

| Parameter              | Unit       | Min - max | Mean  | Method of analysis  |
|------------------------|------------|-----------|-------|---------------------|
| pH                     | -          | 7.1-7.9   | 7.5   | pH meter            |
| Total Hardness         | mg/L (as CaCO₃) | 152-336  | 244   | Titration with EDTA |
| Electrical conductivity | µSiemens/cm | 330-803  | 566.5 | Conductivity meter  |
| Total dissolved solids | mg/L       | 204-488  | 346   | TDS meter           |
| Chloride               | mg/L       | 8-48.5   | 285   | Argentometry        |
| Nitrate                | mg/L       | 1.3-28.3 | 14.8  | Spectrophotometry   |
| Nitrite                | mg/L       | 0.03-2.2 | 2.1   | Spectrophotometry   |
| Dissolved oxygen       | mg/L       | 6-7      | 6.5   | DO meter            |
| Ammonium               | mg/L       | 0        | 0     | Titrimetric method  |

**Figure 1.** Preparing zinc oxide nanoparticles produced from Satureja plant.
Results

Characterization of ZnO NP

The synthesized zinc oxide nanoparticle powder, was brown in color. This powder was characterized and the synthesis of the nanoparticles was confirmed with the available instruments. UV spectroscopy revealed that the absorption peak of the powder was at the 330 nm. SEM images clearly showed the existence of zinc oxide nanoparticles in spherical and crystalline form, that confirmed the presence of zinc oxide nanoparticles in the sample. EDX analysis also demonstrated the presence of zinc and oxygen elements as the main elements in the sample. XRD analysis showed a particle size of 35.88 nm, and the obtained peaks showed that the most frequent synthesis phases assigned to zinc, confirming the synthesis of zinc oxide nanoparticles from the savory plant (Figure 2).

Toxicity

In the control group and at a concentration of 1 mg/L, no mortalities were observed. The first mortality in this study occurred at concentration of 10 mg/L, with one death in the first 24 h. At concentration of 100 mg/L, a high mortality rate was observed in the fish population, so that in the first 24 h of the experiment, and in the first 48 h, 3 and 8 fish died, respectively. The mortality rates of the tested fish at the pre-determined times and the concentrations are presented in Table 2. The results of LC50, LC10, LC50 and LC10 are presented in Table 3. Based on the results (Table 3), the four-day LC50 (96h) was calculated to be 25.50 mg/L. The probability deaths of the fish at different concentrations of nanoparticles for 24, 48, 72 and 96 h are shown in Figure 3. LC50 values at the above periods were obtained 2127.99, 19.03, 25.50, and 25.50, respectively. Figure 4 shows the logarithm of the concentration of ZnO nanoparticles synthesized from the Satureja plant on trout. As it is evident, nanoparticle concentration decreases with the elapsing the time. Given the results of the lethal concentration at different periods, the maximum allowable concentration of zinc oxide nanoparticles can be determined. According to the OECD standard (OECD, 2009), the allowable lethal concentration, which is also called the maximum ineffective concentration, is 10% of LC50 96h, and since LC50 96h is 25.50 mg/L, the maximum allowable concentration is 2.55 mg/L. Based on the same standard, the minimum effective concentration is equal to LC50 10h which is 4.69 mg/L. The results of no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) and their comparisons with LC50 96h, are presented in Figure 5. As noted before, the LC50 96h value in this study was obtained 25.50 mg/L. Therefore, based on the toxicity levels as determined by the Globally Harmonized System of Classification and Labelling of Chemicals (Table 4), zinc oxide nanoparticles biologically prepared from the savory plant belong to the low toxicity category (Helfrich et al., 2009).

Behavioral Effects

Observation of the behavior of the fish during the experiment indicated that they showed abnormal movements and behaviors during the first hours of exposure to the nanoparticles. These behaviors included rising to the surface of the water, fast swimming, restlessness in moving round and round the container, gathering in one part of the aquarium, and the quick opening and closing of the gill slits. At concentrations of 1 and 10 mg/L, the fish quickly adapted to the nanoparticles in the water, and their abnormal behaviors subsided after 24 h and they began slow swimming in the water. At high concentrations, the fish that remained alive, even on the fourth day, were restless when responding to external stimuli. As soon as the researcher approached the container, they began to swim swiftly. The fish went towards air bubbles and opened and closed their gills. Mucus secretion was observed in small amounts and backward swimming was also observed.

Discussion

In this study the characteristics of the obtained zinc oxide nanoparticles from the Satureja plant, are highly consistent with other works. The synthesized zinc oxide nanoparticles are often brown like as the present work found out. Devasenan et. al. reported that the color of the zinc oxide nanoparticle powder was brownish green (Devasenan,S, Beevi,N, & Jayanthi,S, 2016). While, Joel reported that chemically fabricated zinc oxide nanoparticles were commonly white and the green-synthesized nanoparticles were brown in color (Joel.C & Badhusha, 2016). The results of existing studies indicate that zinc oxide nanoparticles often have adsorption peaks between 305-375 nm (Dhanemozhi, Rajeswari, & Sathyajothi, 2017; Senthilkumar & Sivakumar, 2014; Surtradhar & Saha, 2016; Yedurkar, Maurya, & Mahanwar, 2016). The 330 nm absorption peak obtained in this study is in complete agreement with the previously published works. The nanoparticles synthesized in the present study are predominantly spherical and crystalline in shape. Other studies have also reported the same shapes especially spherical, for fabricated zinc oxide nanoparticles (Anvekar, Chari, & Kadam, 2017; Hoseinpour et al., 2017; Jamdagni, Khatri, & Rana, 2018). Furthermore, XRD spectrum showed that the zinc oxide nanoparticles have hexagonal structures and their average diameter is 35.88 nm. Various studies have confirmed the hexagonal structure of the zinc oxide nanoparticle, with nearly the same diameters, depending on the experimental conditions, plant type, region type, and genetic properties (Joel.C & Badhusha, 2016; Taghavi A & Entezari M, 2017). The results of
Figure 2. Characterisation of ZnO NP by Satureja plant, a: UV pectrum, b: XRD analysis, c: SEM image, d: EDX analysis.

Table 2. Mortality rate in trout fish exposed to different concentrations of ZnO nanoparticles by Satureja plant at different times (24-96h)

| Treatment   | Concentration Of ZnO (mg/L) | 24h Live fish mortality n(%) | 48h Live fish mortality n(%) | 72h Live fish mortality n(%) | 96h Live fish mortality n(%) |
|-------------|-----------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Control     | 0                           | 9                            | 0                            | 9                             | 0                             |
| Group1      | 1                           | 9                            | 0                            | 9                             | 0                             |
| Group2      | 10                          | 8                            | 1(11)                        | 8                             | 1(11)                        |
| Group3      | 100                         | 6                            | 3(33)                        | 1                             | 8(89)                        |

Table 3. Lethal concentration (LC<sub>1-99</sub>) of ZnO nanoparticle by Satureja plant depending on time (24-96h) for trout fish

| LC   | Concentration (mg/L) | 24h Log Con. | 48h Log Con. | 72h Log Con. | 96h Log Con. |
|------|----------------------|--------------|--------------|--------------|--------------|
| LC<sub>10</sub> | 4.69                | 0.672        | 0.92         | 0.32         | 1.28         |
| LC<sub>30</sub>  | 174.19               | 2.24         | 5.57         | 0.74         | 7.50         |
| LC<sub>50</sub>  | 2127.99              | 3.32         | 19.03        | 1.28         | 25.50        |
| LC<sub>70</sub>  | 25996                | 4.41         | 66.81        | 1.825        | 86.71        |
| LC<sub>90</sub>  | 964486               | 5.98         | 401.32       | 2.60         | 507.53       |
Figure 3. The probability of trout fish mortality at different concentrations of ZnO nanoparticles from Satureja, A- 24h, B- 48h, C- 72, D- 96h.

Figure 4. Effect of ZnO nanoparticles from Satureja on Trout fish.

Figure 5. Acute toxicity testing statistical endpoints of ZnONP.
bioassay studies are acceptable only when the maximum deaths in the control group are not more than 10% (Helfrich et al., 2009; OECD, 2009). In this study, no deaths were observed in the control group. Based on the results obtained in this study, the amount of LC$_{50}$ 96h of zinc oxide nanoparticles prepared from savory on trout, was 25.50 mg/L. In addition, the LC$_{50}$ 24h value of the zinc oxide nanoparticles was calculated as 2127.99 mg/L.

The obtained results indicated that as the exposure time elapsed, lower concentration of zinc oxide nanoparticles is needed to kill 50% of the fish population. It has been confirmed that one of the most important factors affecting mortality in fish is exposure time (Bazari Moghaddam et al., 2015; shamlofar, Jorjani, & ghelichi, 2015). When the fish are gradually exposed to a constant concentration of nanoparticles, their resistance to the pollutant decreases, and the nanoparticle gets enough time to affect the fish (shamlofar et al., 2015). On the other hand, the gradual accumulation of a toxin in fish body also increased the adverse effects within the 4 days of the experiments and led to a decrease in LC$_{50}$ value (Bazari Moghaddam et al., 2015). Khodabakhshi revealed that LC$_{50}$ 96h value of chemically synthesized zinc oxide nanoparticles on rainbow trout was 0.75 mg/L (Khodabakhshi et al., 2017). The value obtained in present study was 25.50 mg/L that indicates the low risk of biologically produced nanoparticle in comparison with chemically prepared one. The same finding has been reported for other nanomaterials. for instance, Gobadi et al. showed that chemically synthesized silver nanoparticles had a severe effect on salmon and trout at a very low concentration of 50 μg/L and destroyed all gonadal cell lines (Ghobadi, Farahmand, & Mirjalili, 2014). While Bita et al., reported that the LC$_{50}$ value of biologically produced silver nitrate nanoparticles from the Sargassum algae was 11.34 mg/L. However, this finding indicates the superiority of biological methods to chemical methods (S Bita, Mesbah, Shahryari, & Ghorbanpoor Najafabadi, 2017). Fish Abnormal behaviors resulting from pollutant exposure have also been reported in many works. For example, exposure to thyme, was reported to have similar behaviors on salmon, such as backward swimming, purposeless fast swimming, irritability, mucus secretion (especially in high concentrations), respiratory problems and death (Sharif Rohani, Haghighi, & Assaeian, 2011). The effects of silver nanoparticles on zebrafish, also showed the occurrence of abnormal behaviors, such as swimming at the surface of the water, gathering at a specific point in the aquarium, rapid gill movements and mucus secretion (Mazarei, Sajadi, Sarinejad, Johari, & Asadi, 2016). The above symptoms, as well as muscle contractions and abnormal curving of the spine have been reported in studies by Jegede (Jegede, 2007). These observations are consistent with the behavioral outcomes observed in the present study. In general, different fish show nearly similar symptoms in exposure to nanoparticles.

**Conclusion**

This study mainly aimed to synthesize zinc oxide nanoparticles using Satureja and evaluate its toxicity on rainbow trout. The characterization analysis confirmed the proper fabrication of the nanoparticle.

Considering the obtained LC$_{50}$ values, the findings of this study indicated that biologically synthesized nanoparticles are less hazardous for the environment and aquatic organisms than chemically prepared nanomaterials. Plants can be considered as the main option for the synthesis of nanoparticles due to their species diversity and they should be considered as an alternative to chemical methods.

**Acknowledgments**

The authors would like to express their heartfelt gratitude to the colleagues of the Faculty of Health and Nutrition of the Lorestan University of Medical Sciences, who liberally put the laboratory facilities of the faculty at the disposal of the researchers.

**References**

Afshar, P., & Sedaghat, S. (2016). Bio-synthesis of silver nanoparticles using water extract of satureja hortensis L and evaluation of the antibacterial properties. *Current Nanoscience*, 12(1), 90-93. http://dx.doi.org/10.2174/1573413711666150529022 38

Agarwal, H., Venkat Kumar, S., & Rajeshkumar, S. (2017). A review on green synthesis of zinc oxide nanoparticles—-an eco-friendly approach. *Resource-Efficient Technologies*, 3(4), 406-413. https://doi.org/10.1016/j.refit.2017.03.002

Ahmed, S., Chaudhry, S. A., & Ikram, S. (2017). A review on biogenic synthesis of ZnO nanoparticles using plant extracts and microbes: a prospect towards green
Ardebili, A. (2017). Iranian seafood consumers’ willingness to pay for fresh fish. Norwegian University of Life Sciences, Ås.

Azimi, Z., Sadeghpour, A., Porgholami, A., & Rofchaj, R. (2015). Determine LC50 of sodium nitrite and its effect on white liver kidney tissue. Journal of Applied Fisheries Research, 2(3), 59-72.

Baharara J., & Ramezani, T., (2014). Nanoparticle synthesis of gold from Shiraz plum breed. Genesis Biology Series, 7(2), 8.

Bazori Moghadam, S., Sharif Rohani, M., Sharifpour, I., Pajand, Z., Jallipour, J., & Masoumzadeh, M. (2015). Determination of lethal concentration (LC96h) of hydroalcoholic extract of garlic (Allium sativum L.) and its effects on gill tissue of Persian sturgeon (Acipenser persicus) fingerlings. Iranian Journal of Medicinal and Aromatic Plants, 30(6), 915-922. DOI: http://dx.doi.org/10.22092/jimnap.2015.11926

Clesceria, L., S., Greenberg, A.E., & and Eaton, A.D. (1998). Standard methods for the examination of water and wastewater (20th ed.). Washington, D.C.

Datta, A., Patra, C., Bharadwaj, H., Kaur, S., Dimri, N., & Khajuria, R. (2017). Green synthesis of zinc oxide nanoparticles using parthenium hysterophorus leaf extract and evaluation of their antibacterial properties. Journal of Biotechnology and Biomaterials, 7, 271-275. DOI: 10.4172/2155-952X.1000271

Devasen, S., Hajara Beevi, N., & Jayanthi, S. (2016). Green Synthesis and Characterization of Zinc Nanoparticle Using Andrographis paniculata Leaf Extract. 39(1), 5.

Dhanemozhi, A.C., Rajeswari, V., & Sathyajothi, S. (2017). Green synthesis of zinc oxide nanoparticle using green tea leaf extract for supercapacitor application. Materials Today: Proceedings, 4(2), 660-667. https://doi.org/10.1016/j.matpr.2017.01.070

Fakhari, S., Jamzad, M., & Kabiri Fard, H. (2019). Green synthesis of zinc oxide nanoparticles: a comparison. Green Chemistry Letters and Reviews, 12(1), 19-24. https://doi.org/10.1080/17518823.2018.1547925

Ghobadi, M., Farahmand, H., & Mirjalli, M. (2014). Study of damage induced by silver nanoparticles on rainbow trout gonadal cell lines in vitro conditions. Journal of Genetics, 8(3), 251-560.

Helfrich, L.A., Weigmann, D.L., Hipkins, P.A., & Stinson, E.R. (2009). Pesticides and aquatic animals: a guide to reducing impacts on aquatic systems.

Hoseinpour, V., Souri, M., Ghaemi, N., & Shakari, A. (2017). Optimization of green synthesis of ZnO nanoparticles by Dittrichia graveolens (L.) aqueous extract. Health Biotechnology and Biopharma, 1(2), 11. http://dx.doi.org/10.22034/HBB.2017.10

Jahanbakhsh, A., Hedayati, A., Pirbeigi, A., & Javadimoosavi, M. (2015). Determination of acute toxicity and the effects of sub-acute concentrations of CuO nanoparticles on blood parameters in Rutilus rutilus. Nanomedicine Journal, 23(1), 195-202. http://dx.doi.org/10.7508/nmj.2015.03.004

Jamdagni, P., Khatri, P., & Rana, J.S. (2018). Green synthesis of zinc oxide nanoparticles using flower extract of Nycanthes arbor-tristis and their antifungal activity. Journal of King Saud University-Science, 30(2), 168-175. doi: https://doi.org/10.1016/j.jsus.2016.10.002

Jegede, T. (2007). Acute-toxicity of sodium chloride (NaCl) on Oreochromis niloticus fingerlings. Journal of Fisheries International, 2(4), 292-294. http://medwelljournals.com/abstract?doi=jifish.2007.2

Joel, C., & Badhusha, S.M. (2016). Green synthesis of ZnO Nanoparticles using Phyllanthus emblica Stem extract and their Antibacterial activity. Der Pharmacia Lettre, 8(11), 6.

Kamani, M., Mortazavi, S.A., Safari, M., & Mehraban, M. (2016). Investigation of changes in the composition of rainbow trout (Oncorynchus mykiss) carcass during storage at refrigerator temperature. Journal of Food Science and Technology, 8(1), 83-89.

Keller, A.A., McFerran, S., Lazareva, A., & Suh, S. (2013). Global life cycle releases of engineered nanomaterials. Journal of nanoparticle research, 15(6), 1692.

Khodabakhshli, L., Porbagher, H., & Hosseini, S.V. (2017). The effect of time and concentration of nanoparticles of zinc oxide on histological pathology of rainbow trout gill. Iranian Natural Resources Journal, 70(1), 84-95.

Krysanov, E.Yu., Pavlov, D.S., Demidova, T. B, & Dgebuadze, Yu.Yu. (2010). Effect of nanoparticles on aquatic organisms. Biology Bulletin, 37(4), 406-412. DOI: 10.1134/S1062359010040114

Mazarei, S., Sajadi, M., Sorinejad, A., Johari, S.A, & Asadi, M. (2016). Determination of lethal concentration of nano silver in common zebra (Aphanius dispar Ruppell, 1829). Aquatic Ecology Journal, 4(4), 110-115.

nooshkam, A., majoonhoseini, N., hadian, J., jahansos, M., & khavazi, K. (2015). Comparison of vegetative body performance and essential oil yield of two medicinal species of Khuzeastan and Souresh Rashingari in two regions of Khuzeastan and Lorestan in field. 38(2), 12.

OECD. (2009). OECD Guidelines for the Testing of Chemicals Test No. 203: Fish, Acute Toxicity Test. Section 2: Effects on Biotic Systems.

Prinsloo, G., Papadi, G., Hiben, M.G., de Haan, L., Louisse, J., Beekmann, K., . . . Rietjens, I.MCM. (2017). In vitro bioassays to evaluate beneficial and adverse health effects of botanicals: promises and pitfalls. Drug discovery today, 22(8), 1187-1200. ttp://dx.doi.org/10.1016/j.drudis.2017.05.002

Roy, N., & Barik, A. (2010). Green synthesis of silver nanoparticles from the unexploited weed resources. International Journal of Nanotechnology and Applications, 4(2), 95-101.

S Bita, S., Mesbah, M., Shahryari, A., & Ghorbanpoor Najafabadi, M. (2017). Effects of silver nanoparticles synthesized by bioreduction method on gill antioxidant defense system response of common carp, Cyprinus carpio. Iran. J. Health & Environ, 10(3), 339-348.

Senthilkumar, S.R., & Sivakumar, T. (2014). Green tea (Camellia sinensis) mediated synthesis of zinc oxide (ZnO) nanoparticles and studies on their antimicrobial activities. Int J Pharm Pharm Sci, 6(6), 461-465.

shamlofar, M., Jorjani, S., & ghelihi, A. (2015). Determination of LC50 and examination of swine venom tissue lesions in whitefish. Journal of Aquaculture Development, 9(1), 43-52.
Sharif Rohani, M., Haghighi, M., & Assaeian, H. (2011). The lethal concentration (LC50) of Zataria multiflora essential oil in fryies of rainbow trout (Oncorhynchus mykiss). Iranian Scientific Fisheries Journal, 20(2), 89-96. http://dx.doi.org/10.22092/isfj.2017.109994

Skjolding, L.M., Sørensen, S.N., Hartmann, N.B., Hjorth, R., Hansen, S.F., & Baun, A. (2016). Aquatic ecotoxicity testing of nanoparticles—the quest to disclose nanoparticle effects. Angewandte Chemie International Edition, 55(49), 15224-15239. https://doi.org/10.1002/anie.201604964

Song, J.Y., & Kim, B.S. (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess and biosystems engineering, 32(1), 79. http://dx.doi.org/10.4314/tjpr.v15i11.4

Sutradhar, P., & Saha, M. (2016). Green synthesis of zinc oxide nanoparticles using tomato (Lycopersicon esculentum) extract and its photovoltaic application. Journal of Experimental Nanoscience, 11(5), 314-327. http://dx.doi.org/10.1080/17458080.2015.1059504

Taghavi A., & Entezari M. (2017). Biosynthesis and characterization of the Silver Nanoparticles by Aqueous Extract of Saffron corm and Evaluation of their Antibacterial and Mutagenesis activity. Journal of police medicine, 1(6), 10.

Yedurkar, S., Maurya, Ch., & Mahanwar, P. (2016). Biosynthesis of Zinc Oxide Nanoparticles Using Ixora Coccinea Leaf Extract—A Green Approach Open Journal of Synthesis Theory and Applications, 5(1), 14. doi: 10.4236/ojsta.2016.51001. http://dx.doi.org/10.4236/ojsta.2016.51001