

**TOXICITY STUDY OF NANOSELENIUM ON SEED GERMINATION, BACTERIAL GROWTH AND FISH SURVIVABILITY**

**Abstract**

*Aim* : The use of nanoparticles in agriculture has recently increased to sustain crop productivity. Therefore, the effect of nanoparticles on the different ecosystems must be quantified before their use. This study aimed to examine the toxicity potential of nanoselenium on the germination of sorghum seed, growth of soil microorganisms viz., *Bacillus subtilis* and *Rhizobium* species, and survival of zebrafish. We hypothesize that nanoselenium could be non-toxic to sorghum seed germination, bacillus and rhizobium growth, and zebrafish survival up to 20 mg L<sup>-1</sup>.

*Study design* : Completely randomized design with four or five replications with respect to the experiments.

*Place and duration of the study* : Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore

*Methodology* : Sorghum seeds BTx 623 were soaked in different concentrations (0, 5, 10, 15 and 20 mg L<sup>-1</sup>) of nanoselenium for 24 h and then sown in Petri-dish to quantify germination potential and seedling growth. The growth medium of microorganisms was challenged from 0 to 20 mg L<sup>-1</sup> of nanoselenium, and the growth of microorganisms was assessed. Similarly, the survival of zebrafish from 0 to 20 mg L<sup>-1</sup> of nanoselenium was recorded.

*Result* : The results showed that nanoselenium up to 20 mg L<sup>-1</sup> did not cause toxic effects on all organisms representing soil, water and terrestrial ecosystems.

*Conclusion* : Hence, this study concluded that nanoselenium up to 20 mg L<sup>-1</sup> is not harmful to organisms representing soil, aquatic and terrestrial ecosystems.

*Keywords*

Bacterial growth, ecotoxicity, nanoselenium, seed germination, zebrafish

## Introduction

Currently, the practice of sustainable agriculture is followed to meet the food requirement of the global population. It is projected that the world population will be around 9.8 billion by 2050. To feed this ever-increasing population, global food production must be doubled to meet food, feed and fuel demand. However, crop production is highly impacted by the occurrence of various abiotic stress during the life cycle of the crop [1]. The year-to-year variability in crop yield is largely associated with rainfall and temperature during critical stages of crop development [2]. IPCC [3] as predicted that climate extremes are expected to increase with changes in climate variables, which can significantly limit crop production. Historical observations and model simulations suggested a high risk of drought across the globe [4]. Estimates indicated that across the globe, the yield of cereals, legumes, and oilseeds will be decreased by 10, 50, and 30%, respectively due to drought stress [5]. Future prediction of rainfall indicates a high chance of below-average precipitation in India which can exacerbate the drought stress [6]. Therefore, it is critical to develop crop management technologies to mitigate the drought stress effect to sustain the crop yield.

Studies have indicated that crop management practices like soil management, cultural practices, irrigation management, crop residues and mulching, use of stress-tolerant varieties, and application of nutrients and plant growth regulators can be used to alleviate the drought stress effects in plants [7]. Apart from this, recent studies have indicated that the application of nanomaterials to mitigate abiotic stress is one of the novel approaches [8]. Nanotechnology is a branch of science, that deals with a material having a dimension of 1-100 nm on any one side, which is referred to as a nanoparticle. Through nanomaterials, it is possible to reduce the input cost of agriculture by increasing nutrient use efficiency, controlling pests and diseases with new formulations, and detecting contaminants using nanosensor [9].

Nanoparticles have both positive and negative influences on crops, which are associated with their altered size, shape, and unique physio-chemical and biological properties [10]. Nanomaterials have a significant difference from bulk materials for their surface morphology and large surface area to volume ratio, which makes them more reactive compared to their bulk form [11, 12]. The efficiency of nanoparticles is dependent on their chemical composition, shape, surface morphology, and aggregation [13]. Nanomaterials with the same chemical

composition and different sizes and shapes and surface properties can create variations in their toxicity potential [14].

Recently studies on assessing the ecotoxicity of nanomaterials are increasing because it is mandatory to quantify the safety of nanomaterials at all trophic levels before their commercial use because there is a direct link between the negative effects of nanoparticles and the survival of organisms [15, 16]. Nanoparticles enter the plant system and accumulate in various plant parts and get biomagnified at various trophic levels by consumption [17]. Thus, the use of different types of nanomaterials in agriculture should be regularly examined for their toxicity [18].

The toxic effect of nanoparticles can be evidenced by genotoxicity, an increase in reactive oxygen species (ROS) production and decreased antioxidant enzyme activity [19]. It is observed that nanoparticles first interact with the cell walls, after entering the cells, the nanoparticle aggravates the alterations in the structure and functions of membranes, molecules, and cell organelles [20]. Initially, the metal radicals produced during oxidative stress, act as a signalling molecule to initiate the antioxidative defence system. If the oxidative damage is higher than antioxidative damage, which will cause toxicity of nanoparticles to the organism [19, 20].

Our earlier study has shown that selenium nanoparticles (Se-NPs) possess an antioxidative effect and can improve the yield of sorghum under high-temperature stress [21]. The higher beneficial effect of nanoselenium than bulk selenium is due to their higher surface area, higher bioavailability, size and shape morphology [22]. Bulk selenium particles reduced the stress effect by inducing the accumulation of secondary metabolites and increasing the activity of the antioxidant enzymes [23, 24]. Se-NPs mitigate the abiotic stress effects in plants, especially drought, salinity, and heavy metal stress by enhancing the antioxidant enzyme system and reducing the damages caused by ROS [25]. However, the toxicity potential of nanoselenium on different organisms was not studied in detail. Hence, this study aimed to examine the toxicity potential of nanoselenium on the germination of sorghum seed, growth of soil microorganisms viz., *Bacillus subtilis* and *Rhizobium* species, and survival of zebrafish. We hypothesize that nanoselenium could be non-toxic to sorghum seed germination, bacillus and rhizobium growth, and zebrafish survival up to 20 mg L<sup>-1</sup>.

## Materials and methods

### *Synthesis of nanoselenium*

Nanoselenium was synthesised by the chemical reduction method as described in Bisht et al. [26]. Selenious acid was used as the precursor and hydrazine was the reducing agent for the synthesis of nanoselenium. Briefly, 6.4 g of selenious acid was dissolved in water and transferred to a Teflon-lined autoclave to which 5 mL of hydrazine was added. Followed by this the content was transferred to a hot air oven maintained at 120 °C for 2 h. After the reaction time, the contents were filtered, and the particles were retained and dried in a hot air oven for 2 h. The contents were washed with water and ethanol five times and used for characterization and toxicity analysis. **The synthesised selenium nanoparticle was subjected to particle size analysis and its zeta potential using particle size analyser (Horiba, Nano particle analyser SZ-100).**

### *Evaluation of toxicity of Se-NPs*

#### Sorghum seed germination test

The experiment was designed in a completely randomized design with five replications. Sorghum variety BTx 623 was used as seed material. First, the seeds were surface sterilized with 0.1% mercuric chloride for 5 min, followed by washing in deionized water for five times. Sterilized seeds were soaked in different concentrations of nanoselenium (0, 5, 10, 15, and 20 mg L<sup>-1</sup>) **for 12 h as described by Acharya et al. [27].** After the expiry of time, the seeds were collected from the soaking solution and sown in a Petri plate. In each Petri plate, 20 seeds were sown. On 14<sup>th</sup> day after sowing, the number of seeds germinated per Petri plate was counted. Along with this, the shoot and root length of 10 seedlings per Petri plate was measured and **expressed in cm.** The emergence of the radicle was considered a criterion for germination. The vigour index is the product of germination percentage and seedling length.

$$\text{Vigour index} = \text{Germination \%} \times \text{Seedling length}$$

#### Growth of soil microorganism

The experiment was designed in a completely randomized design with five replications. *Bacillus* (*Bacillus subtilis*) is a gram-positive rod-shaped bacterium commonly found in the soil. It is a non-pathogenic bacterium. *Rhizobium* is a gram-negative bacterium that lives in the soil or root

nodules of leguminous plants. Bacteria were subcultured in the Luria-Bertani broth in an Erlenmeyer flask under aseptic conditions and incubated for 24 h in a rotary shaker at 30 °C and 150 rpm. Luria-Bertani broth consists of 10 g L<sup>-1</sup> of tryptone, 5 g L<sup>-1</sup> of yeast extract, and 1 g L<sup>-1</sup> of sodium chloride. The LB mediums with different nanoselenium concentrations (0, 5, 10, 15, and 20 mg L<sup>-1</sup>) were prepared by sonication and autoclaved at 121 °C for 20 min and 15 lbs sq inch<sup>-1</sup> pressure. After sterilization, the LB medium was poured into a sterile Petri plate under an aseptic condition and kept undisturbed. The subculture of bacillus and rhizobium having 0.1 optical density was used for inoculation. The subculture was inoculated into the plates by spot inoculation method. After inoculation, the plates were wrapped with kiln film and incubated for overnight. After the incubation period, the plates were taken, and the growth of microorganism colonies were measured and expressed in cm.

#### Zebrafish survivability

The experiment was designed in a completely randomized design with three replications. Zebrafish (*Danio rerio*) is one of the small tropical freshwater fish, and it is the best model to study the toxicity of nanomaterial on aquatic ecosystems because of its small size, very high reproducibility, and easy handling. Different concentrations of nanoselenium solution (0, 5, 10, 15, and 20 mg L<sup>-1</sup>) were prepared and sonicated and to which seven adult zebrafish were left in the tank to test their survivability. Fishes were given their natural environment other than nanoselenium treatment. On the next day the survival percentage of fish in the tank of each concentration of nanoselenium was examined.

#### Statistical analyses

The data from each experiment were analyzed using SAS program. Observations were analyzed using the PROC GLM procedure of SAS. The standard error was shown as an estimate of variability, and means of various variables were separated for significance by the LSD test at a probability level of 0.05.

#### Result and discussion

It is mandatory to increase food production to feed the ever-growing population by adopting new technologies like nanotechnology. However, the safety of the nanomaterial to the ecosystem is of high priority. In the present study, the toxicity of nanoselenium was studied. The major finding

of this study was seed germination, microbial growth, and the survival percentage of zebrafish were not affected at 5, 10, 15, and 20 mg L<sup>-1</sup> of nanoselenium.

### **Nanoparticle characterization**

The analysis of synthesized nanoselenium in the particle size analyzer (Horiba, Nano particle analyser SZ-100) indicated that the particle size of the synthesized nanoselenium was 580.2 nm and the zeta potential of the synthesized nanoselenium falls between -40 mV to 0 mV (Fig. 1a-b).

### **Impact of nanoselenium on sorghum seed germination**

Uniform seed germination is important for better crop establishment [28]. The seed germination process involves the activation of enzymes namely amylases, proteases, and lipases, and these enzymes are involved in the breaking down of macromolecules for the growth and development of the embryo [29]. The present study indicated that seed treatment with nanoselenium from 5 to 20 mg L<sup>-1</sup> did not affect the sorghum seed germination percentage (Fig. 2a). This study showed that hydrolytic enzymes were not affected by treatment with nanoselenium. However, studies have indicated seed germination was positively influenced by chitosan or zinc oxide nanoparticles treatment [30]. In contrast, silver nanoparticle influences seed germination in both positive and negative way [31]. Application of high concentration of copper nanoparticle of 800 mg L<sup>-1</sup> in cowpea reduced the germination index, chlorophyll content, carotenoids and increased the lipid peroxidation and total sugars [32]. Similar to seed germination, the effect of nanoselenium on root length, shoot length and vigour index was not obvious compared to control (Fig. 2b-e).

### **Impact of nanoselenium on the growth of soil microbes**

Nanoparticles can be classified into four types based on toxicity, namely (i) carbon-based nanomaterials, (ii) metal and metal-based nanomaterials, (iii) nanomaterials based on metallodendrimers and (iv) metal composites [33]. The microbial interactions of nanoparticles would lead to the persistent entry of nanoparticles into the food chain. Studies indicated that bacteria are more sensitive to nanoparticles compared to human fibroblast [34, 35]. The present study showed that the growth of bacillus and rhizobium was not affected by the presence of nanoselenium up to 20 mg L<sup>-1</sup> (Fig. 3a-d). Like the present study, the growth of *E. coli* was not affected by the application of iron composite nanoparticles and there was no toxicity [36].

However, iron nanoparticle influences bacterial growth in both positive and negative way [37]. In contrast, microbe treated with 500 mg L<sup>-1</sup> SeNPs get destructed within 12 hours because SeNPs change the membrane permeability and it caused the leakage of proteins and polysaccharides [38]. The decreased effect of selenium nanoparticles on microbial growth may be associated with efflux systems, redox state of the metal ions, extracellular complexation of metals, and the changes in membrane composition [39, 40].

### **Impact of nanoselenium on the survival percentage of zebrafish**

In general, the toxicity of nanomaterial to aquatic ecosystems is very important because it is the main point of nanomaterial entry into the various ecosystem. The zebrafish is an established vertebrate model for studying the development and disease [41]. The zebrafish and human genomes share ~70% similarity [42, 43]. Therefore, the study was conducted with zebrafish to evaluate the toxicity potential of nanoselenium. The result indicated that nanoselenium did not affect the survivability of zebrafish (Fig. 4). Similar to the present study, gold, magnesium, copper and carbon nanotubes did not cause any toxicity to zebrafish as evidenced by 100% survivability [44]. In contrast, 100 mg L<sup>-1</sup> of TiO<sub>2</sub>NP caused oxidative damage in zebrafish [45].

### **Conclusions**

This study showed that nanoselenium from 5 to 20 mg L<sup>-1</sup> did not affect the seed germination process, bacillus and rhizobium growth and zebrafish survivability. Overall, nanoselenium, up to 20 mg L<sup>-1</sup> did not have any toxic effect on terrestrial, soil and aquatic ecosystem representing sorghum seed germination, soil microbes and zebrafish respectively.

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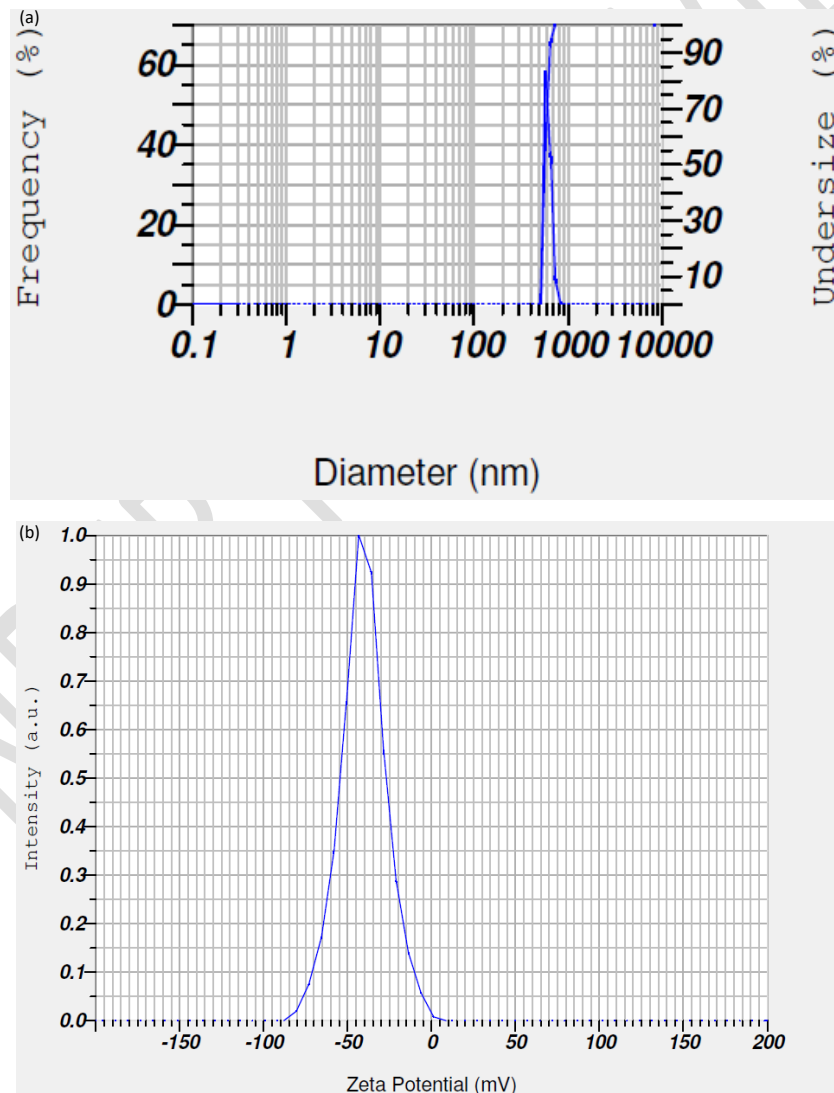
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Fig. 1. Showing the (a) Particle size distribution and (b) zeta potential of synthesized nanoselenium using particle size analyser (Horiba, Nano particle analyser SZ-100).



2. Effect of different concentrations of nanoselenium on (a) sorghum seed germination percentage, (b) shoot length (cm plant<sup>-1</sup>), (c) root length (cm plant<sup>-1</sup>), (d) vigour index, and (e) an overview of the experimental setup. Results indicated that the seed germination, seedling growth, and vigour index were not affected at all concentrations of nanoselenium.

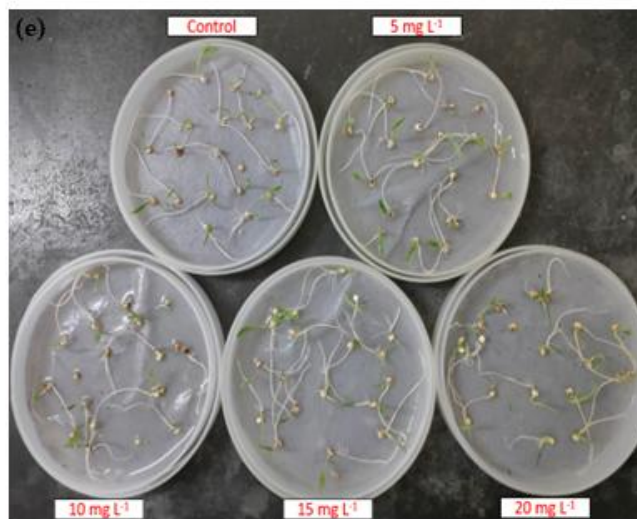
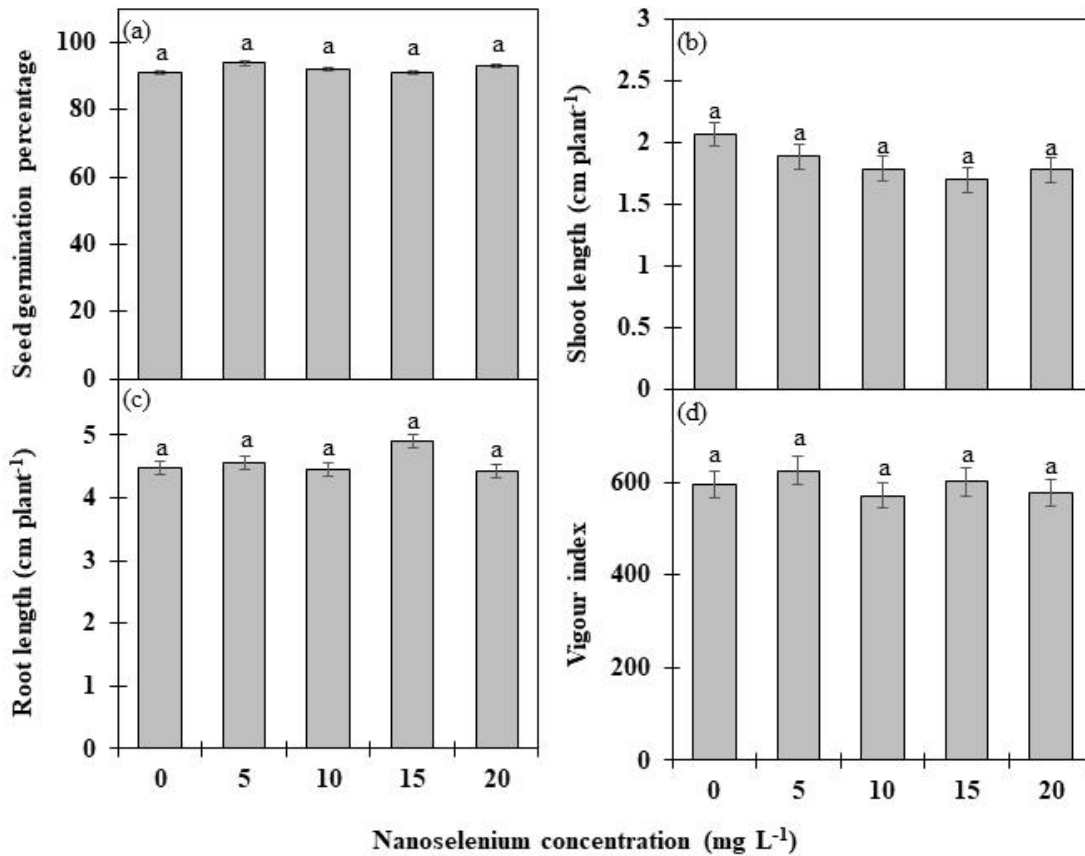
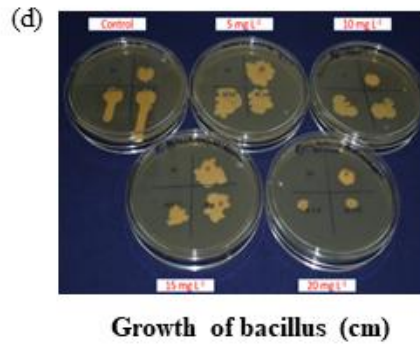
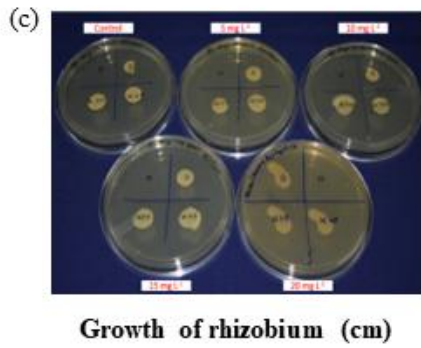
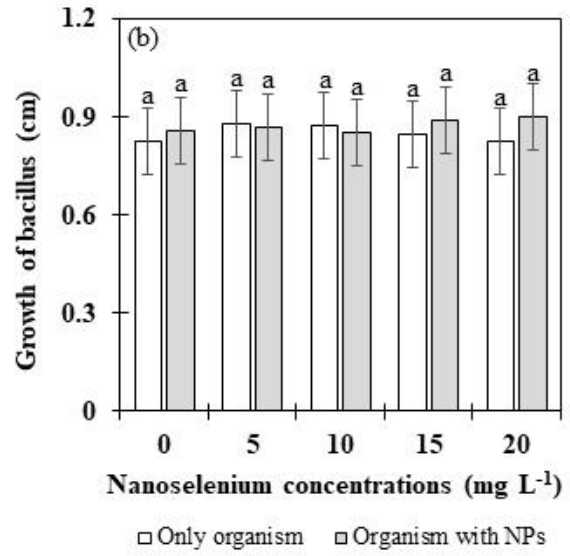
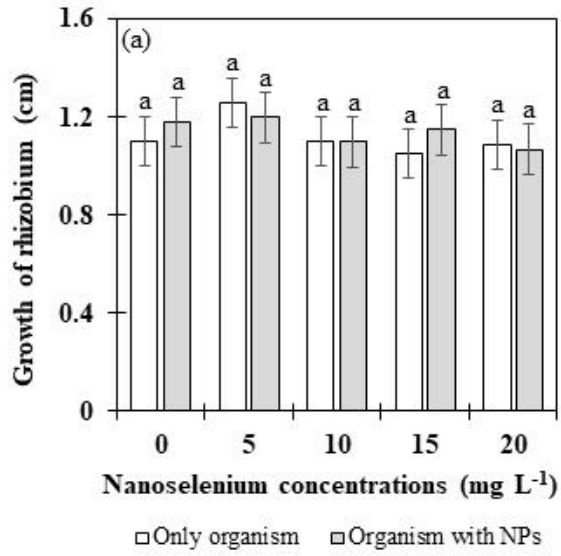


Fig. 3. Effect of different concentrations of nanoselenium on the growth of (a) rhizobium (cm), (b) bacillus (cm), (c) a view of rhizobium growth, and (d) a view of bacillus growth. The result indicated that the growth of rhizobium or bacillus were not affected at all concentrations of nanoselenium.



UNDER REVIEW

Fig. 4. Effect of different concentrations of nanoselenium on survival of zebrafish. In all the treatments, the fishes survived indicating no significant differences among the treatments.

