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Toxicity of Zinc Oxide Nanoparticles from *Myristica fragrans* on Striped Panchax *(Aplocheilus lineatus)*

Nandini, N.J.[1*], Renjusha, S.[2], Siny G. Benjamin[3], Sajeeb Khan, A.[4], Ayana Gayathri, R.V.[5] and Shruthi, P.[6]

[1]Associate Professor, Department of Zoology, University College,*
University of Kerala, Thiruvananthapuram (Kerala), India.
[2]Assistant Professor, Department of Chemistry, N S S College, Pandalam,
University of Kerala, Thiruvananthapuram (Kerala), India.
[3]Assistant Professor, Department of Zoology, All Saint's College,
University of Kerala, Thiruvananthapuram (Kerala), India.
[4] Associate Professor, Department of Zoology, Government College for Women,
University of Kerala, Thiruvananthapuram (Kerala), India.
[5] Associate Professor, Department of Zoology, University College,
University of Kerala, Thiruvananthapuram (Kerala), India.
[6]Research Scholar, Department of Zoology, University College,
University of Kerala, Thiruvananthapuram, Kerala, India.

(Corresponding author: Nandini N.J.[*])
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ABSTRACT: The toxicity of Zinc Oxide nanoparticles from *Myristica fragrans* on striped panchax (*Aplocheilus lineatus*) was investigated in the present study. Usually, nanoparticles are synthesized chemically but safer synthesis methods are used to prevent risk for living organisms and the environment. Extracts of a diverse range of plant species have been successfully used in making nanoparticles. In the present study, zinc oxide nanoparticles are manufactured in the green synthesis method. The extracted powder was characterized and the synthesis of the nanoparticles was confirmed with UV-visible spectroscopy, IR spectroscopy, Scanning Electron Microscopy (SEM), and X-Ray Diffraction Technique (XRD). UV–visible absorption spectrum of synthesized nanoparticles is centred around 350 nm for ZNPs. In IR spectroscopy, the peaks were observed at 1600 and 500 cm^{-1} which correspond to Zn–O stretching and deformation vibration, respectively. SEM images clearly showed the existence of zinc oxide nanoparticles in spherical and crystalline form. XRD analysis showed a particle size of 25.26 nm. Toxicity evaluation was conducted on striped panchax (*Aplocheilus lineatus*) for 72 h. Three groups of the experiment fish were exposed to different concentrations (10, 50 and 100 mg/L) of ZnO nanoparticles. All the exposed fishes were daily observed and dead fishes were immediately removed and recorded. The obtained data were statistically analyzed using probit regression method. The LC_{50} value in the present study was found to be 47.86 mg/L for striped panchax, which makes this nanoparticle as a low toxicity substance in terms of toxicity classification. Based on the obtained LC_{50} it was revealed that the green nanoparticles synthesis is much less hazardous and a good alternative technique.

Keywords: Nanoparticles, Zinc oxide nanoparticles, Acute toxicity, Nanotechnology, LC_{50}.

INTRODUCTION

Nanotechnology has emerged as an interdisciplinary field of modern science and technology. Nanomaterials have found a wide range of different applications in many areas of human life. Nanoparticles have a large fraction of their atoms on the surface, which is among the reasons for their unique physicochemical characteristics, high surface energy and tendency for aggregation. The list of consumer goods in the nanofield is constantly increasing, involving many different products containing nanomaterials, such as sunscreens, toothbrushes, dental bonding, paints, textiles, plastic wrap, waterless car wash, corrosion resistance, golf clubs, tennis rackets, solar batteries, catalysts, and microelectronic devices (Gajewicz *et al.*, 2012; Savolainen *et al.*, 2010). According to Matinise *et al.* (2017), one of the groups of II–IV inorganic semiconductors for analytical measurement is zinc oxide nanoparticles. Additionally, zinc oxide nanoparticles have electrical, optical, and photocatalytic characteristics. Only recently has there been an increased focus on environment, human health and safety issues within the production, use and release of nanomaterials (Royal Society and Royal Academy of Engineering, 2004; Maynard, 2006; Moore, 2006).

It is crucial to acknowledge and address the hazards posed by these nanomaterials, particularly their toxicity. Zinc oxide (ZnO) nanoparticle is one of the most significant nanoparticles and is widely utilized in industry. The environment and human health are both harmed by these chemically produced nanomaterials (Roy and Barik 2010). Because of their potential for a wide range of applications, zinc oxide nanoparticles are among the most researched one (Mohan and Renjanadevi, 2016). The most widely used metal oxide after iron is zinc oxide nanoparticle, which is inexpensive, safe, and simple to create. The need for safer ways to produce nanoparticles, like biological methods, also referred to as the green method is growing (Song and Kim, 2009). These techniques typically involve the synthesis of nanoparticles using plants, microorganisms, or materials that are already found in nature. In the present study, zinc oxide nanoparticles (ZnO-NP) were synthesized using leaf extracts of *Myristica fragrans*. *M. fragrans*, commonly known as nutmeg, has numerous pharmacological activities. Its extracts and essential oils are valuable in drug development in South Africa, India, and other tropical countries. Using spectroscopy techniques such as UV-visible, IR, scanning electron microscopy (SEM), and X-ray diffraction (XRD), the synthesis of the nanoparticles can be verified.

Toxicity evaluation was conducted on striped panchax (*Aplocheilus lineatus*) for 72 h. As zinc oxide nanoparticles (ZnO-NPs) are involved in many industrial applications, researchers pay more attention to their toxic effects on living organisms. The objectives of the studies included to create zinc oxide nanoparticles from Myristica leaves and to assess its toxicity on striped panchax (*Aplocheilus lineatus*).

MATERIALS AND METHODS

Biosynthesis and Characterization of ZnO Nanoparticles

Fresh *Myristica fragrans* (jathi) leaves (Fig. 1) were collected from nearby localities. The leaves were washed several times with water to remove the dust particles and then sun-dried to remove the residual moisture. The extract was prepared by placing 50 g of washed dried fine cut leaves of *Myristica fragrans* in a 250 ml glass beaker along with 100 ml of sterile distilled water. The mixture was then boiled for 60 min. until the colour of the aqueous solution changed to light yellow. The leaf extract was cooled to room temperature and filtered. For the synthesis of ZnO nanoparticles, 50 ml of the leaf extract was taken and boiled at 60°C using a stirrer-heater. 5 g of Zinc nitrate was added to the solution at (60 °C). This mixture was boiled until it reduced to a deep yellow coloured paste (Fig. 2). This paste was then collected in a ceramic crucible and heated in the air-heated furnace at 400 °C for 2 hours and a light yellow coloured powder was obtained. The extracted powder was characterized and the synthesis of the nanoparticles was confirmed with UV–visible spectroscopy, IR spectroscopy, Scanning Electron microscopy (SEM), and X-Ray Diffraction Technique (XRD).

Experimental Fish, Acclimatization and Toxicity Test

Striped panchax (*Aplocheilus lineatus*) (Fig. 2) was caught from the nearby streams in Thiruvananthapuram district Kerala, India. The mean weight of the fishes was 5.73 g and their mean length was 8.13 cm. The healthy juvenile fishes were collected and transferred immediately to the laboratory for further experiment.

In the laboratory, the fishes were kept for three days to adapt to the new lab conditions. The temperature of water was controlled at 29-30 °C during the study period. The concentration of nanoparticle was constant in each container and the water was not replaced during the experiment. The fish were randomly divided into four groups of twenty, and placed in 500 ml beakers. One group was kept as a control and three other groups were exposed to different concentrations of zinc oxide nanoparticles (10, 50, 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 duration of the experiment was 72 h and the mortality rates were recorded at 24, 48, and 72 hours (Helfrich, *et.al*, 2009). During the experiment, all apparent symptoms and behaviours of the fish were carefully monitored and the observations were recorded.

Data Analysis. The probit regression method was used to analyze the data, and MS Excel to display the graphs.

RESULTS AND DISCUSSION

A. Characterization of ZnO NP

The synthesized zinc oxide nanoparticles were found to be light yellow coloured in the present study (Fig. 3). The characteristics of the obtained zinc oxide nanoparticles from the *Myristica fragrans* are highly consistent with other works. Devasenan *et al.* (2016) reported that the colour of the zinc oxide nanoparticle powder was brownish yellow. While, Joel and Badhusha (2016) reported that chemically fabricated zinc oxide nanoparticles were commonly white and the green-synthesized nanoparticles were brown. The extracted powder was characterized and the synthesis of the nanoparticles was confirmed with UV–visible spectroscopy, IR spectroscopy, Scanning Electron microscopy (SEM), and X-Ray Diffraction Technique (XRD). In the UV–visible spectroscopy the distinct peak centered around 350 nm and it is specific for ZnO NP, which is due to their large excitation binding energy at room temperature (Fig. 4A). The results of existing studies indicate that zinc oxide nanoparticles often have adsorption peaks between 305-375 nm (Dhanemozhi, *et al*., 2017; Senthilkumar and Sivakumar 2014; Yedurkar, *et al.*, 2016). Infrared studies were carried out in order to ascertain the purity and nature of the nanoparticles and also the presence of phytochemicals in the extract. The phytochemicals such as alcohols, phenols, amines and carboxylic acids can interact with the zinc surface and aid in the stabilization of ZnO NP. The peaks that were observed at 1600 and 500 cm^{-1} correspond to Zn–O stretching and deformation vibration, respectively (Fig. 4B). SEM images clearly showed the existence of zinc oxide nanoparticles in spherical and crystalline form (Fig. 4C), which confirmed the presence of zinc oxide nanoparticles in the sample. Other studies have also reported the same shapes especially spherical for fabricated zinc oxide nanoparticles (Anvekar *et al.*, 2017; Jamdagni *et al.*, 2018). XRD analysis showed a particle size of 25.26 nm, and the particle size of the synthesized zinc oxide nanoparticles was in close agreement with the previous findings (Fig. 4D). 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 and Badhusha 2016).

Toxicity. The mortality rates of the experiment fish and the corresponding concentrations of zinc oxide nanoparticles are presented in Table 1. In the control and at concentration of 10 mg/L, no mortalities were observed during the whole experiment (Table 1). Similarly, no mortalities were recorded in the experiment fishes during the 24h exposure to zinc nanoparticles in all the treated concentrations. Mortalities were only recorded during the 48h and 72 h exposure in the concentration of 50 mg/L and 100 mg/L. At the concentration of 50 mg/L, 12 and 24 fishes were dead at 48 h and 72 h, respectively. The first mortality was recorded in the 48 h of exposure at a concentration of 50 mg/L, with 12 deaths (Table 1) and during 72 h the total number of dead fishes increased to 36 (Table 1). In the concentration of 100 mg/L, at 48 h of exposure, it recorded mortality of 24 fishes. During 72 h of exposure complete mortality of experiment fishes were observed (Table 1). Taherian *et al*. (2020) also reported the acute toxicity effects of zinc oxide nanoparticles in *Oncorhynchus mykiss*. The probability of stripped panchax, (*Aplocheilus lineatus*) mortality at different concentrations of ZnO nanoparticles from Myristica leaves during different exposure time is given in Fig. 5.

The results of LC_{10}, LC_{30}, LC_{50} $LC_{70,}$ and LC_{90} are presented in Table 2. Based on the results (Table 2), the LC_{50}value calculated was 47.86 mg/L. Taherian *et al*., (2020) also reported lower LC_{50} values and confirmed that the green synthesis of nanoparticles is much less hazardous and a good alternative technique. The $LC_{10,}$ LC_{30}, $LC_{70,}$ and LC_{90} values obtained in the present study were 31.62 mg/L, 40.74 mg/L, 56.23 mg/L and 70.79 mg/L, respectively (Table 2). The obtained results indicated that a lower concentration of zinc oxide nanoparticles is needed to kill 50% of the fish population and it belongs to the low toxicity category (Helfrich *et al*., 2009). Bita *et al*. (2017) reported the superiority of biological methods over chemical methods. Based on the present study, it is confirmed that the biological synthesis of nanoparticles is safe, clean, biocompatible, and eco-friendly (Ghobadi *et al*., 2014; Akl *et al*., 2020; El-Saadony *et al*., 2021).

Behavioural Effects. Different behaviour changes were observed in experiment fishes when treated to different exposure doses of zinc oxide nanoparticles. After the first exposure, its overall behaviour recorded a significant improvement. Both the exposure doses of zinc oxide nanoparticles (10 mg/L and 50 mg/L) recorded reduced mortality rate. Exposure dose of 100 mg/L showed nearly 50% mortality during 48 h and complete mortality during 72 h. Behaviour of experiment fishes in all the three exposure doses (10 mg/L, 50 mg/L and 100 mg/l) showed odd movements during the first few hours of exposure. The abnormal behaviours included buoyancy issues, gasping its mouth at surface, rapid gill movement and strange swimming behaviours. The strange swimming behaviours included fishes crashing the bottom of the container, hitting the sides of the container and flashing behaviour. The abnormal behaviours are signs of stress experienced by the experiment fishes (Taherian *et al*., 2020; Ghobadi *et al*., 2014).

Table 1: Mortality rate in stripped panchax exposed to different concentrations of ZnO nanoparticles from *Myristica fragrans* at different exposure times (24-72 h).

	Concentration of Zinc oxide	24 h		48 h		72 h	
		Live	Mortality	Live	Mortality	Live	Mortality
Control	0 mg	60	0	60	0	60	0
Group 1	10 mg	60	0	60	0	60	0
Group 2	50 mg	60	0	48	12	36	24
Group 3	100 mg	60	0	36	24	0	60

Table 2: Lethal concentration of ZnO nanoparticle from *Myristica fragrans* for stripped panchax.

Lethal concentration	LC_{10}	LC_{30}	LC_{50}	LC_{70}	LC_{90}
Value (mg/L)	31.62	40.74	47.86	56.23	70.79

Fig. 1. Leaves of *Myristica fragrans.*

Fig. 2. Biosynthesis of ZnO nanoparticles from Myristica leaves.

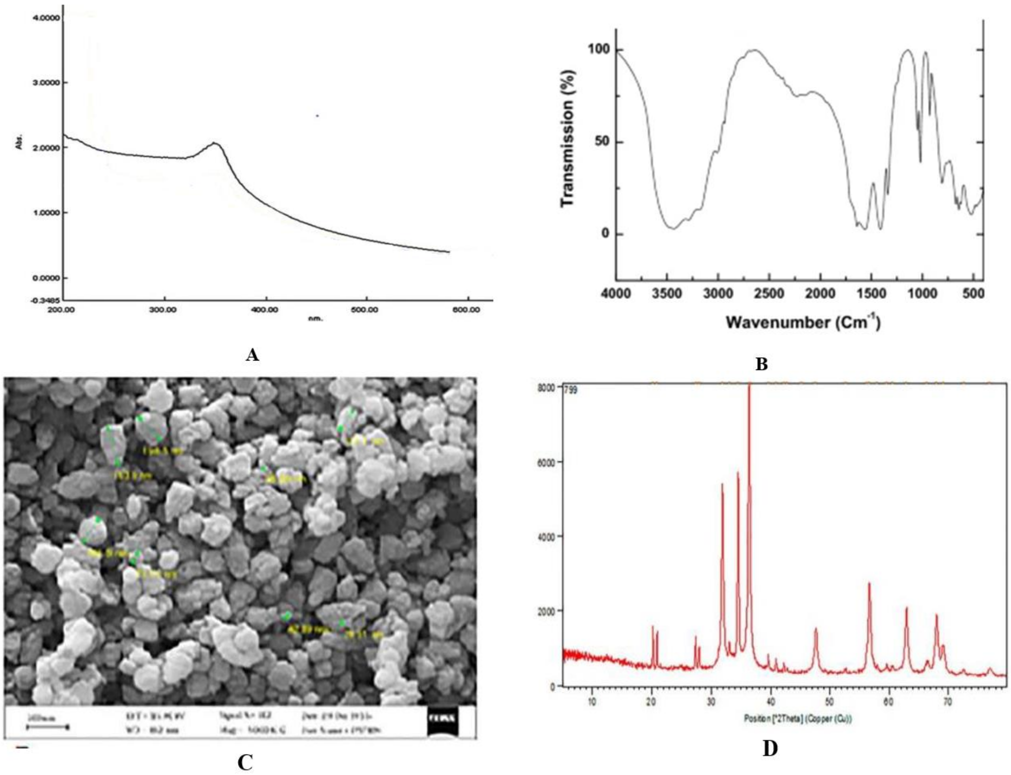

Fig. 3. Characterisation of ZnO NP by *Myristica fragrans* leaves. A: UV-vis spectrum, B: Infrared spectroscopy C: SEM image, D: XRD analysis.

Fig. 4. Striped panchax *(Aplocheilus lineatus*).

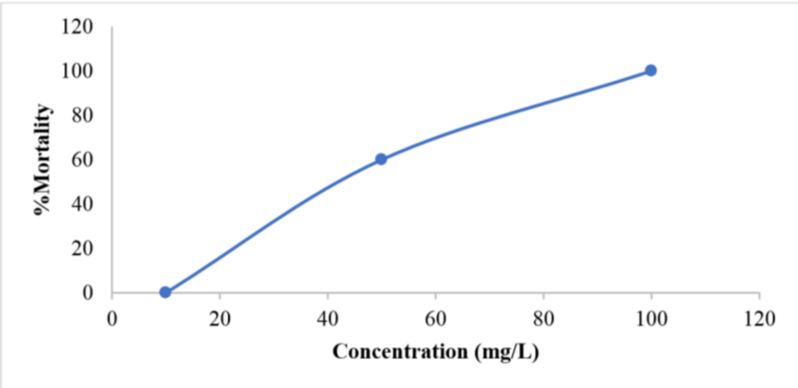

Fig. 5. The probability of stripped panchax mortality at different concentrations of ZnO nanoparticles from *Myristica fragrans.*

CONCLUSION

The primary objectives of this study were to synthesise zinc oxide nanoparticles using leaf extracts from Myristica leaf and to assess the detrimental effects of these particles on striped panchax. The ZnO NPs were successfully synthesized by the biological method and it was found to be simple, cheap, safer and eco-friendly than the chemical and physical methods.

The precise development of the nanoparticle was validated by the characterization analysis. The outcomes of this study suggest that biologically synthesized nanoparticles pose less threat to aquatic organisms compared to chemically prepared nanomaterials, based on the obtained LC_{50} values. Because of their species diversity, plants can be thought of as the primary option for the synthesis of nanoparticles and should be viewed as an alternative to chemical methods. Since green synthesis is less expensive, creates less pollution, and improves the safety of the environment and human health, it is preferable to traditional chemical synthesis. The development of green synthetic methods has been driven by the increasing demand for green chemistry and nanotechnology.

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