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Presented By: MERBAI Yousra ; REDJEM Nouha et SOUKI
Safa

Jury Member:

Mme. Zaidi Nedjoua (Prof)	Président	Univ. August 20, 1955 – Skikda
Mme. Boucetta Sabrine (MCA)	Supervisor	Univ. August 20, 1955 – Skikda
Melle Boucetta Radja NADA(Doct)	Co- Supervisor	Univ. August 20, 1955 – Skikda
Mme. Laib Imen (MCA)	Examiner	Univ. August 20, 1955 – Skikda

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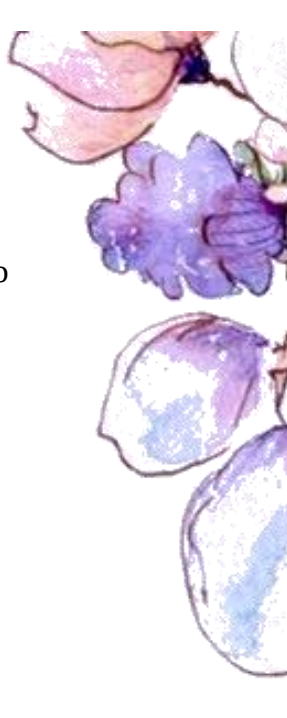
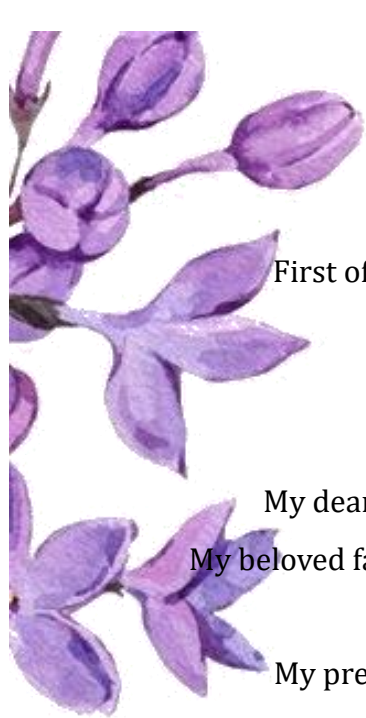
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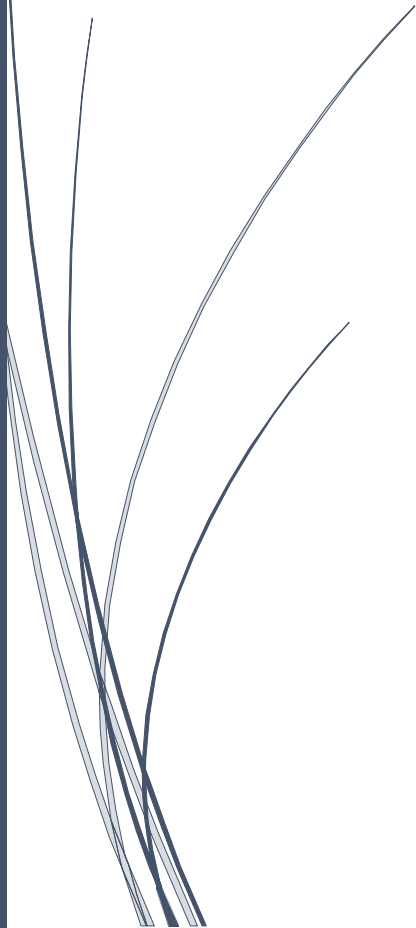
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Abstracts

Abstracts : French, English and Arabic



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Spécialité : Ecotoxicologie Animale

Auteurs : MERBAAI Yousra ; REDJEM Nouha et SOUKI Safa

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Résumé

L'utilisation de nanoparticules biosynthétisées à partir d'extraits végétaux représente une alternative prometteuse aux antibiotiques en aquaculture. Cette étude évalue l'effet de trois formulations naturelles administrées par voie alimentaire : les nanoparticules de zinc issues de *Moringa oleifera* (ZnO-MOR), et les nanoparticules d'argent synthétisées à partir de *Posidonia oceanica*, déposée (AgNPs-POD) et fraîche (AgNPs-POV).

L'expérimentation a été menée in vivo sur 120 individus de *Oreochromis* sp. répartis en trois régimes alimentaires pendant 8 semaines. Le groupe ZnO-MOR a montré la meilleure performance de croissance, avec un gain pondéral moyen de $38,9 \pm 3,8$ g et un SGR de $1,62 \pm 0,11$ %/jour. Le groupe POV a suivi avec un WG de $33,8 \pm 3,6$ g et un SGR de $1,41 \pm 0,15$ %/jour, contre $27,4 \pm 2,9$ g pour le témoin. Le taux de survie a été élevé dans tous les groupes traités, atteignant 100 % pour ZnO-MOR et 93 % pour AgNPs-POV.

Sur le plan nutritionnel, les concentrations en protéines musculaires ont été les plus élevées chez ZnO-MOR ($15,02$ µg/mg), suivies par POV ($12,01$ µg/mg) et POD ($10,6$ µg/mg). Des teneurs intestinales très élevées en glucides (1066 µg/mg) et en lipides (1066 µg/mg) ont été observées chez ZnO-MOR, traduisant une assimilation énergétique optimale. POD a favorisé un stockage hépatique des lipides (835 µg/mg), tandis que POV a montré un profil homogène et équilibré.

L'analyse hématologique a révélé une augmentation significative du taux d'hémoglobine (ZnO-MOR : $10,2$ g/dL), des globules rouges ($5,6 \times 10^6/\text{mm}^3$) et de l'IgM sérique, confirmant une stimulation immunitaire sans signe de stress oxydatif.

Sur le plan toxicologique, les DL_{50} déterminées via le modèle Probit sont proches pour ZnO-MOR et AgNPs-POV ($0,377$ mg/g), et légèrement plus élevées pour AgNPs-POD ($0,405$ mg/g). Les modèles statistiques montrent des courbes sigmoïdes classiques avec des pentes significatives, suggérant une toxicité modérée mais maîtrisable pour un usage nutraceutique contrôlé.

La posologie optimale déterminée dans cette étude se situe autour de $0,17$ à $0,31$ mg/g de poids corporel pour les nanoparticules administrées. À ces doses, les effets bénéfiques sur la croissance, la digestion et l'immunité sont maximisés, tout en restant en dessous des seuils toxiques identifiés. Ces données permettent d'établir un cadre posologique de référence pour une application rationnelle en élevage intensif.

En conclusion, les nanoparticules ZnO-MOR apparaissent comme les plus efficaces pour soutenir la croissance et l'immunité. Les AgNPs-POD et POV offrent quant à elles des effets ciblés intéressants sur la digestion et l'homéostasie métabolique. Ces résultats suggèrent que l'utilisation raisonnée de nanoparticules végétales peut constituer une alternative sûre, efficace et durable aux antibiotiques classiques en aquaculture.

Mots clés : Nanoparticules végétales, synthèse verte, *Moringa oleifera*, *Posidonia oceanica*, croissance, DL_{50} , posologie, hématologie, nutrition, aquaculture, santé animale.

Summary

The use of plant-based green-synthesized nanoparticles is a promising alternative to antibiotics in aquaculture. This doctoral research evaluates the pharmaco-nutritional and toxicological effects of three natural nanoparticle formulations administered via the diet: zinc oxide nanoparticles from *Moringa oleifera* (ZnO-MOR), and silver nanoparticles from deposited (AgNPs-POD) and fresh (AgNPs-POV) leaves of *Posidonia oceanica*.

The in vivo experiment involved 120 *Oreochromis* sp. individuals fed over 8 weeks with three experimental diets. The ZnO-MOR group showed the highest growth performance, with an average weight gain of 38.9 ± 3.8 g and a specific growth rate (SGR) of $1.62 \pm 0.11\%$ /day. POV followed with 33.8 ± 3.6 g (SGR = $1.41 \pm 0.15\%$ /day), compared to 27.4 ± 2.9 g for the control group. Survival rates reached 100% with ZnO-MOR and 93% with AgNPs-POV.

Nutritionally, the highest muscle protein content was recorded in the ZnO-MOR group ($15.02 \mu\text{g}/\text{mg}$), followed by POV ($12.01 \mu\text{g}/\text{mg}$) and POD ($10.6 \mu\text{g}/\text{mg}$). Glucose and lipid concentrations in the intestine were also highest in the ZnO-MOR group ($1066 \mu\text{g}/\text{mg}$ each), indicating optimal energy assimilation. AgNPs-POD promoted hepatic lipid storage ($835 \mu\text{g}/\text{mg}$), while AgNPs-POV induced a balanced and consistent biochemical profile.

Hematological analysis revealed increased hemoglobin (ZnO-MOR: $10.2 \text{ g}/\text{dL}$), red blood cell count ($5.6 \times 10^6/\text{mm}^3$), and IgM levels, supporting an improved immune response without signs of oxidative stress.

Toxicological evaluation (DL_{50}) via Probit modeling indicated moderate and comparable acute toxicity for ZnO-MOR and AgNPs-POV ($DL_{50} = 0.377 \text{ mg}/\text{g}$), slightly higher for AgNPs-POD ($0.405 \text{ mg}/\text{g}$). These values confirm a safe therapeutic window for dietary use.

The optimal dosage range was established between 0.17 and $0.31 \text{ mg}/\text{g}$ of body weight, balancing growth performance, immune stimulation, and safety margins. This dosage guidance may support rational application in intensive aquaculture settings.

In conclusion, ZnO-MOR nanoparticles demonstrated the most effective overall impact on growth and immunity. AgNPs-POD and POV showed targeted benefits in hepatic metabolism and immune regulation. These findings suggest that dietary administration of plant-based nanoparticles represents a safe, multifunctional, and sustainable alternative to synthetic antibiotics in aquaculture.

Keywords: Green nanotechnology, plant-based nanoparticles, *Moringa oleifera*, *Posidonia oceanica*, DL_{50} , hematology, nutrition, growth, dosage, sustainable aquaculture, animal health.

ملخص

تُعدُّ التقنيات النانوية الحيوية المستخلصة من النباتات خيارًا واعدًا لتعويض استخدام المضادات الحيوية في أنظمة الاستزراع المائي. تهدف هذه الأطروحة إلى تقييم التأثيرات التغذوية، المناعية، والسمية الحادة لثلاثة أنواع من الجسيمات النانوية النباتية: أكسيد الزنك المستخرج من *Moringa oleifera* (ZnO-MOR)، والفضة النانوية المستخلصة من أوراق *Posidonia oceanica* المترسبة (AgNPs-POD) والطاقجة (AgNPs-POV)، والمقدمة عن طريق العلف لأسمك البلطي (*Oreochromis sp.*).

تم تنفيذ التجربة الحيوية على 120 سمكة موزعة على ثلاث وجبات غذائية تجريبية لمدة 8 أسابيع. حقق ZnO-MOR أفضل أداء للنمو بزيادة وزنية بلغت 38.9 ± 3.8 غرام، ومعدل نمو نوعي 1.62 ± 0.11 %/يوم. تلاه POV بزيادة 33.8 ± 3.6 غرام (SGR = 1.41 ± 0.15 %/يوم)، مقابل 27.4 ± 2.9 غرام للمجموعة الشاهدة. بلغت نسبة البقاء على قيد الحياة 100% في ZnO-MOR و93% في POV.

من الناحية التغذوية، سجلت أعلى تركيزات البروتين العضلي في مجموعة ZnO-MOR (15.02 ميكروغرام/ملغ)، تلتها POV (12.01) ثم POD (10.6). كما ظهرت تراكمات مرتفعة من السكريات والدهون في أمعاء ZnO-MOR (1066 ميكروغرام/ملغ)، مما يدل على امتصاص طاقي ممتاز. بينما حقّز POD تخزين الدهون في الكبد (835 ميكروغرام/ملغ)، وحقق POV توازنًا كيميائيًا جيدًا.

بينت التحاليل الدموية ارتفاعات معتبرة في تركيز الهيموغلوبين (ZnO-MOR: 10.2 غ/دل)، وعدد الكريات الحمراء (5.6×10^6 /مم³)، مما يدل على تنشيط مناعي فعال دون مؤشرات على الإجهاد التأكسدي.

من حيث السمية الحادة، أظهرت نماذج Probit أن قيم DL₅₀ كانت متقاربة في ZnO-MOR و POV (0.377 ملغ/غ)، وأعلى قليلاً في POD (0.405 ملغ/غ)، ما يؤكد سلامة استخدامها بجرعات مدروسة.

تم تحديد الجرعة المثالية ما بين 0.17 إلى 0.31 ملغ/غ من وزن الجسم، وهي الجرعة التي تحقق التوازن بين النمو، المناعة، والأمان. تشير هذه النتائج إلى أن الاستخدام الغذائي المنظم للجسيمات النانوية النباتية يشكل بديلاً فعالاً، آمناً ومستداماً عن المضادات الحيوية في تربية الأسماك.

الكلمات المفتاحية: الجسيمات النانوية النباتية، التقنيات النانوية الخضراء، *Moringa oleifera*، *Posidonia oceanica*، النمو، DL₅₀، التغذية، الدم، صحة الحيوان، الاستزراع المائي المستدام.



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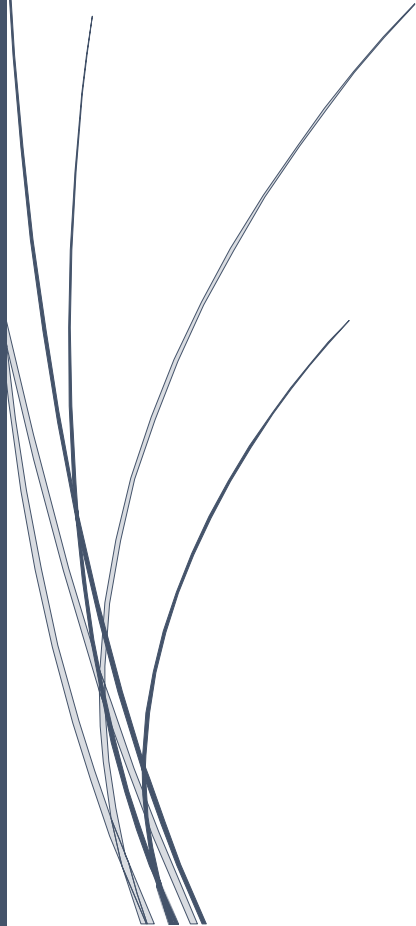


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Général Introduction

1. General context: Nanoparticles, a scientific revolution

Nanotechnologies now occupy a central position in contemporary scientific research, due to their potential for innovation in various fields such as electronics, medicine, catalysis, cosmetics, water purification and environmental protection [1]. At the heart of this revolution are nanoparticles (NPs), materials whose physicochemical properties are radically modified at the nanoscale. Their small size (<100 nm), large specific surface area and increased reactivity give them unique characteristics, which closely depend on their size, morphology, crystallinity and composition.

Among the most studied nanomaterials, zinc oxide nanoparticles (ZnO-NPs) and silver nanoparticles (AgNPs) occupy a prominent place.

ZnO-NPs, wide bandgap (3.37 eV) and high excitation energy (60 meV) semiconductors, exhibit high thermal and chemical stability, as well as remarkable optical and photocatalytic properties, which make them prime candidates for applications ranging from sensors to antimicrobial materials [5,9].

AgNPs, on the other hand, are distinguished by their powerful antimicrobial properties, high electrical conductivity, plasmonic activity, and ability to interact effectively with a wide range of microorganisms, even at very low concentrations. Their effectiveness against antibiotic-resistant bacteria makes AgNPs promising agents in the medical, pharmaceutical, and environmental fields [10,12].

2. Towards a green synthesis: sustainability and innovations

In the interest of sustainability and reducing environmental impacts, the green synthesis of nanoparticles, using plant extracts, is emerging as an innovative alternative to conventional chemical methods, which are often costly and polluting. These processes, carried out at room temperature and without toxic solvents, make it possible to obtain stable, functional NPs compatible with biomedical or environmental uses [3,8].

Plant extracts are now widely used in aquaculture as natural feed supplements to stimulate growth, enhance immunity, and mitigate the toxic effects of nanoparticles (NPs) [20]. In particular, nanoparticles biosynthesized from plant extracts are distinguished by their

porosity, stability, and ability to effectively penetrate target cells, thus contributing to enhancing the antioxidant and immune status of fish [21,22].

Among these medicinal plants, *Moringa oleifera* (MO), belonging to the *Moringaceae* family, is native to India and is now widely distributed in Africa and Asia [23,24]. Also called horseradish tree or miracle tree, MO is an exceptional source of micronutrients and bioactive compounds. Its leaves are rich in potassium, iron, phosphorus, vitamins A, C, E and D, flavonoids, ascorbic acid, β -carotene, oxidase and catalase [25–27]. They contain up to 17 times more calcium than milk, and six spoons of powder can cover the daily iron and calcium needs of a pregnant woman [27]. The pods are also useful for digestive health and colon cancer prevention [28].

Due to their high antioxidant content, MO leaves protect against hyperglycemia, cerebral oxidative stress and dementia, while acting as an anticancer and anti-anemic agent [29–32]. In traditional medicine, MO is used to treat more than 300 pathologies, including hypertension, bacterial infections, hypercholesterolemia and inflammation [33,34]. Studies have demonstrated its hepatoprotective effects and potent immuno-antioxidant properties against toxicity induced in *Oreochromis niloticus* [26,35,36]. However, little research has explored the effect of MO extracts on stresses caused by ZnO-NPs, making this study a pioneering contribution in this field [22,37].

Similarly, *Posidonia oceanica* (PO), a marine plant endemic to the Mediterranean, represents a valuable resource that is still underexploited in aquaculture. Its leaves, whether detached green (POV) or deposited brown (POD), constitute a reservoir of phenolic compounds, flavonoids, tannins, and secondary pigments with antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective properties [38–41]. In particular, PO extracts have shown their ability to scavenge free radicals, inhibit oxidative stress, and protect liver cells against toxic damage [42,43]. Studies have also highlighted their immunomodulatory potential and their ability to improve zootechnical performance in fish, particularly in the context of environmental or chemical stress [44,45]. The richness of PO in bioactive compounds, coupled with its bioavailability in the marine environment, makes it an ideal candidate for the green biosynthesis of nanoparticles, such as AgNPs, as a substitute for synthetic antibiotics.

Thus, this study aims to comparatively evaluate the protective effects of nanoparticles biosynthesized from *Moringa oleifera* (ZnO-NPs) and *Posidonia oceanica* (AgNPs) against NP-induced toxicity in *Oreochromis niloticus*. It focuses in particular on their influence on growth, physiology, hepatorenal histology, immunity and antioxidant status of fish, with the aim of proposing ecological and functional alternatives for sustainable aquaculture.

In the framework of this study, two types of biomaterials are mobilized for green biosynthesis:

- *Moringa oleifera* (MOR) leaves are used as a reducing and stabilizing agent for the synthesis of ZnO-NPs, allowing to obtain homogeneous, bioactive particles with notable photocatalytic properties.
- The leaves of *Posidonia oceanica*, a marine plant endemic to the Mediterranean, are used for the synthesis of AgNPs. Two types of leaves are distinguished: POV: detached green leaves, still rich in phenolic compounds and POD: brown leaves deposited on the marine substrate, subject to natural degradation.

This choice of plant resources reflects an ecological, local and enhancing approach to available biomass, contributing to nanotechnology that respects ecosystems.

3. Benefits of nanoparticles in fighting infections

The growing emergence of bacterial resistance constitutes a major public health issue, compromising the effectiveness of conventional antibiotics and promoting the persistence of serious nosocomial infections [14,16]. In this context, metallic nanoparticles, notably ZnO-NPs and AgNPs, appear as innovative alternatives.

ZnO-NPs act through the generation of reactive oxygen species (ROS), the release of Zn²⁺ ions, and the disruption of bacterial cell membranes. They have shown antibacterial efficacy against a broad spectrum of pathogens, including multidrug-resistant strains, and further exhibit the ability to inhibit biofilm formation [2].

In addition, AgNPs exert a very powerful bactericidal action, thanks to their ability to penetrate the cell wall, disrupt cellular respiration and interact directly with DNA. Their antimicrobial spectrum is even broader, including bacteria, fungi and viruses. AgNPs also demonstrate excellent synergy with certain antibiotics, reinforcing their effect in cases of multi-resistance [19].

Thus, these two types of nanoparticles represent promising tools for antimicrobial control, with distinct but complementary modes of action, opening the way to new therapeutic strategies.

4. Emerging risks and ecotoxicological concerns

Despite their potential benefits, the increasing use of nanoparticles in the environment raises concerns about their toxicity to aquatic organisms and their impact on ecosystems.

ZnO-NPs, due to their partial solubility and their potential to generate ROS, can cause pathophysiological disorders in freshwater fish such as *Oreochromis niloticus* or *Cyprinus carpio*: oxidative stress, hepatic alterations, inflammation, disruption of enzymatic activity, and bioaccumulation in tissues [2].

AgNPs, although highly effective as antimicrobial agents, also exhibit acute and chronic toxicity to many aquatic species. Their small size facilitates their cellular penetration, and the released Ag^+ ion can disrupt essential cellular functions. Studies have reported negative effects on the growth, reproduction, and survival of fish, mollusks, and aquatic invertebrates [5].

It therefore appears crucial to compare the ecotoxicological effects of these two types of nanoparticles, in order to choose the least harmful formulations in aquaculture application contexts.

5. Focus on aquaculture: health challenges and prospects for integrating nanotechnologies

Aquaculture, a rapidly expanding industry worldwide, faces increasing pressure from infectious diseases, chemical pollution, and the need to maintain sustainable productivity. Exposure of fish to nanoparticles, whether intentional (for therapeutic purposes) or involuntary (via product release), requires a rigorous assessment of their benefits and risks.

ZnO-NPs, incorporated into food formulations, have shown improved growth, immunity, and resistance to bacterial infections. However, side effects at high doses, such as oxidative imbalances, have also been observed.

AgNPs, on the other hand, can be used at very low concentrations for potent antimicrobial effects, particularly against *Aeromonas hydrophila*, a major pathogen in aquaculture. However, their accumulation in tissues and their long-term effects on fish health and the food chain raise unresolved questions.

It therefore becomes essential to adopt an integrated approach, based on green synthesis, environmental safety and therapeutic efficacy, to integrate nanotechnologies into sustainable aquaculture.

The objective of the study:

This study is part of the context of the eco-responsible valorization of Mediterranean and tropical plant resources, through the green synthesis of metallic nanoparticles (AgNPs and ZnO-NPs). Carried out for the first time in Algeria, this research aims to explore the effectiveness of these bio-sourced nanoproducts as alternative antimicrobial food supplements to chemical antibiotics, in the diet of Nile tilapia (*Oreochromis niloticus*), an aquaculture species of great economic importance.

The specific objectives of this manuscript are as follows:

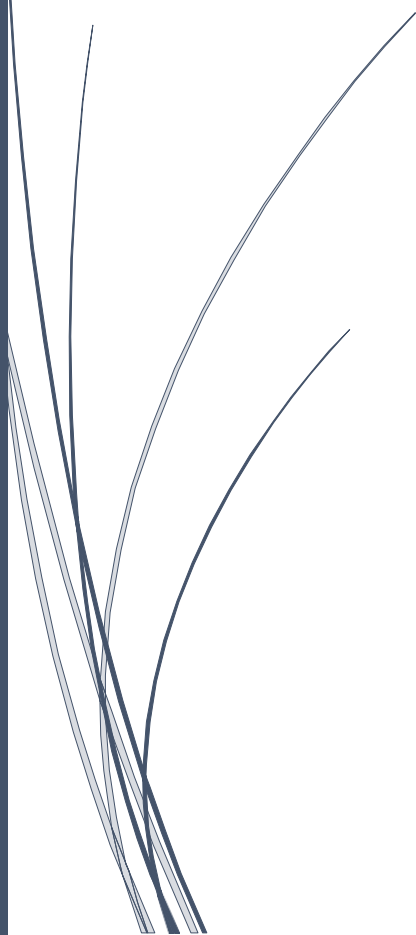
1. Synthesize and characterize silver nanoparticles (AgNPs) from *Posidonia oceanica* leaves (POV: detached green leaves; POD: deposited dead leaves) and zinc oxide nanoparticles (ZnO-NPs) MOR) from *Moringa oleifera* leaves, evaluating their yield, morphology, stability and physicochemical properties.

2. To quantify the bioactive compounds (total polyphenols) and antioxidant activity of plant extracts used in green synthesis, depending on the leaf type (fresh or decomposing for *P. oceanica*) and the extraction solvent, in order to correlate their richness in secondary metabolites with the reducing capacity of the extracts for the formation of NPs.
3. To evaluate the zootechnical performances (growth, survival rate, feed conversion ratio) and biochemical composition of Nile tilapia fry fed with feed formulations enriched with AgNPs (POV and POD) and ZnO-NPs (Moringa), under controlled experimental conditions.
4. To study the physiological and immune effects of these food nanoproducts on fry, through the analysis of hemato-biochemical parameters, oxidative stress markers, and specific immune responses.
5. To compare the antimicrobial efficacy and safety of use of AgNPs and ZnO-NPs, in relation to their potential to replace antibiotics in the aquaculture sector, while minimizing ecotoxicological impacts.



Chapter 01

Materials and Methods



1.2. Synthesis of zinc oxide (ZnO-NPs) and silver (AgNPs) nanoparticles

The nanoparticles were synthesized at the technological hall (University of August 20, 1955 Skikda) by green synthesis method under ultrasonic irradiation.

In this process, zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, $\geq 98\%$, Sigma-Aldrich) and silver nitrate (AgNO_3 , $\geq 99\%$, Sigma-Aldrich) were used as precursors. Sodium hydroxide (NaOH , $\geq 97\%$, Sigma-Aldrich), used as a precipitation and pH regulating agent [109].

0.1 M zinc ion solution was prepared by dissolving the appropriate amount of zinc acetate in 100 mL of deionized water. This solution was then subjected to ultrasonic irradiation using a Hielscher UP400S (400 W, 24 kHz, Berlin, Germany) at 79% amplitude and 0.76 cycle for 5 minutes at 40 °C [109].

Green synthesis of silver nanoparticles (AgNPs) was carried out from aqueous extracts of *P. oceanica*, and that of zinc oxide nanoparticles (ZnO-NPs) from aqueous extracts of *M. oleifera*, using an eco-compatible method [109].

The aqueous extracts of POD, POV, and MO were prepared separately by adding 5 g of dried plant extract in 100 mL of distilled water, heated at 60 °C for 30 minutes with constant stirring. After filtration, 90 mL of each extract was added dropwise to 10 mL of silver nitrate (0.1 M AgNO_3) for AgNPs, or zinc acetate dihydrate (0.1 M $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) for ZnO-NPs with moderate stirring at 75–80 °C [109].

The color change (dark brown) confirmed the formation of AgNPs and ZnO-NPs, which were then centrifuged at 10,000 rpm for 15 minutes, washed with distilled water, and then dried at 60 °C to obtain a dry powder. The obtained nanoparticles (POD-AgNPs, POV-AgNPs and ZnO-NPs) were stored at room temperature in a tightly closed sterile bottle until use [109].



Figure 03 : The Bioassay *Tilapia Oreochromis sp.*

The choice of *Oreochromis sp* as a model organism for manipulation studies in the natural environment is judicious because it satisfies three essential criteria:

- 1. Ecosystem representativeness:** *Oreochromis sp* is a freshwater fish species widespread in many aquatic ecosystems, making it representative of the environments studied.
- 2. Euryecia:** This species has a high tolerance to variations in environmental conditions, demonstrating its euryecious character.
- 3. Ecophysiological knowledge:** The ecophysiology of *Oreochromis sp* is extensively documented, allowing an in-depth understanding of its responses to experimental manipulations.

In addition, *Oreochromis sp* presents several additional advantages favoring its use as a model organism:

- Ease of handling and sampling
- Mastered and well-established captive breeding techniques
- Ability to feed on artificial foods
- Availability throughout the annual cycle