Development and use of nanoparticles from Lantana plant (*Lantana camara*) and Toad plant (*Tabernaemontana elegans*) in the management of southern root-knot nematode (*Meloidogyne incognita*)

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DECLARATION

I, Nicholus Mxolisi Mnyambo, hereby declare that this is my original research, it has never been submitted before by anyone for any degree or examination at any university other than the current submission at the University of Mpumalanga. The use of information and materials from any other sources has been fully acknowledged.

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DEDICATION

In loving memory of my late Mom **(01/09/2019)** my guardian angel.

To the experiences we never expected,

and the paths that were redirected.

To the friends and family who became part of this adventure.

To the nematode family that gave their lives for science,

And the plants that contributed their essence.

To the University of Mpumalanga community,

And to our Vice Chancellor, whose support opened new doors.

To my mentor and my dedicated core supervisors.

To Dr. Z.P. Dube *"Three years from now, when one reads this, you must be a professor."*

"When it looks impossible, look deeper. And then fight like you can win."
— Rost, *Horizon Forbidden West*

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PUBLICATIONS RELATED TO THE THESIS RESEARCH

1. Mnyambo, N.M., Rantho, L.P., Dube, Z.P. and Timana, M. (2024). Timing of Plant Extracts Application in the Management of *Meloidogyne incognita* on Tomato Plants. *International Journal of Plant Biology*, 15(4): 1108-1117.
2. Mnyambo, M.N., Dube, Z.P., Khanyile, N., Mdluli R.T. and De Waele, D. (2024). Development and efficacy of green-synthesised nanoparticles from medicinal plant extracts for sustainable management of root-knot nematodes (*Meloidogyne incognita*). One Health International Student Conference. 04-06 December 2024 (online). University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania. 4

ABSTRACT

Recent research on innovative nematicides has gained significant interest in addressing global crop losses caused by plant-parasitic nematodes. While chemical nematicides remain the primary control method, they pose risks to human health and the environment due to their toxicity, pollution potential, and residual effects. As a result, there is a growing demand for safer, more sustainable alternatives. Nanotechnology offers a promising solution by enhancing the effectiveness and targeted delivery of agrochemicals. Plant-based nanoparticle synthesis presents an eco-friendly approach by utilising natural bioactive compounds as reducing and stabilizing agents. The present study was carried out to explore the synthesis of green nanoparticles (NPs) derived from *Lantana camara* (NPlc) and *Tabernaemontana elegans* (NPte) using *Aloe vera* as reducing and stabilising agent (*A. vera*) and the sustainable management of plant-parasitic nematodes. This was achieved through four objectives: (1) to develop nanoparticles from nematicidal plants (*T. elegans* and *L. camara*) and determine their phytochemical composition, (2) to evaluate the suitability of *A. vera* gel as a reducing and stabilizing agent in the formulation of nanoparticles from *T. elegans* and *L. camara*, (3) to examine the effects of the prepared nanoparticles on hatch and mortality of *M. incognita* second-stage juveniles (J2) under *in vitro* conditions and (4) to explore the potential of the prepared nanoparticles in mitigating the impact of *M. incognita* and enhancing plant growth variables under greenhouse conditions. To achieve Objective 1 and 2, nanoparticles were synthesised by mixing four rations (25:75, 50:50,75:25, and 0:100 v/v) of *A. vera* gel with each plant extract. The experiment was a randomised complete design with five replications. The mixture was stirred using a magnetic stirrer for 12 h at room temperature. Nanoparticles and their mother plant extracts were screened for glycosides, flavonoids, alkaloids, tannins, phenols, saponins, reducing sugars and terpenoids phytochemicals using previously described qualitative methods, with energy-dispersive X-ray spectroscopy used to determine the chemical purity and elemental composition. Physical characterisation of the synthesised nanoparticles was done using UV-Vis spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM) and selected area electron diffraction (SAED) patterns. Plant-based nanoparticles were successfully synthesised from *T. elegans* and *L. camara* plant extracts with *Aloe vera* as reducing and stabilising agent. Generally, all pure plant extracts exhibited a high abundance of phytochemicals measured, with synthesised nanoparticles having an improved levels of phytochemicals when compared to the pure forms. Adding *A. vera* increased the levels of phytochemicals such as saponins, terpenoids and reducing sugars on both *L. camara* and *T. elegans* nanoparticles. Synthesised nanoparticle concentrations of 50:50 and 25:75 (extract : *A. vera*) had more phytochemicals at a higher levels. Energy-dispersive X-ray spectroscopy (EDS) spectrum also indicated presence of key elements such as potassium (K), phosphorus (P), magnesium (Mg), chlorine (Cl), and copper (Cu), in the synthesised nanoparticles. The UV-Vis spectra peak intensity and wavelength observed in the two plant extracts, *L. camara* and *T. elegans* nanoparticles in the presence of *A. vera* extracts were slightly shifted and broader, suggesting an interaction between the biomolecules from both extracts during the nanoparticle formation process. New peaks in the nanoparticle spectrum indicate the formation of distinct molecular species or structural changes due to nanoparticle synthesis. Electron microscopy provided detailed structural and morphological properties, which were a variety of shapes such as cubic, triangular, platelet, and irregular forms. The nanoparticle sizes varied from 5 to 21 nm, far smaller than the pure extract sizes that were greater than 450 nm. The Selected Area Electron Diffraction (SAED) patterns of nanoparticles where generally bright with distinct spots for 50:50 and 25:75 ratio concentrations, indicative of well-developed polycrystalline structures. In Objective 3, the *M. incognita* J2 hatch inhibition test was conducted under *in-vitro* conditions. Mortality J2 hatch rates differed significantly ($P \leq 0.05$) across these time intervals, with the highest rates recorded at 72 h and the lowest at 24 h. A

consistent trend of increasing *M. incognita* juvenile hatch over time was observed. In addition, NPlc and NPte at the 50:50 (extract: *A. vera*) concentration demonstrated the lowest *M. incognita* juvenile hatch rates, followed by 25:75 (extract: *A. vera*). The mortality assay was conducted under *in-vitro* conditions as in the juvenile hatch inhibition, except that 100 *M. incognita* J2 were added into the Petri dish. *Meloidogyne incognita* J2 nematodes were considered dead if they were not mobile after transferring them into distilled water for 30 s even if they were probed with needles. The percentage of juvenile mortality was calculated. The highest mortality rate was recorded at 72 hours, while the lowest occurred at 24 hours, indicating a clear trend of increased juvenile mortality with extended exposure durations. The highest mortality rates were recorded at the 50:50 and 25:75 concentrations (extract: *A. vera*) of both NPlc and NPte, with no significant differences between these two concentrations. In Objective 4, plastic pots of 30-cm-diameter, were filled with a soil mixture of steam-pasteurized loamy and sandy soil at a ratio of 1:3 (v/v). Each pot was transplanted with one "Star 9009" tomato seedling. Two weeks after transplanting, seedlings were inoculated with 5000 *M. incognita* eggs and J2 using a 20 mL plastic syringe. One-week post-inoculation, nanoparticle treatments were applied to the pots. The experiment followed a 2 x 6 x 3 factorial arrangement in a randomized complete block design (RCBD) with five replications. The first factor consisted of two plant extracts (*L. camara* and *T. elegans*). The second factor consisted of five previously developed nanoparticle formulations; a positive control (Nemacur® 10GR at 5 g/plant); 100 % *A. vera*; and a negative control (nematode-inoculated plants without treatment) were also included. The third factor comprised of three nanoparticle application rates of 5 ml, 10 ml, and 15 ml. At 56 days, plant and nematodes variables were measured. Nematode management results highlighted a trend where increasing concentrations of *A. vera* in nanoparticle formation contributed to reductions in the number of *M. incognita* nematode J2 within the roots. The 50:50 and 25:75 nanoparticle concentration were particularly effective

in this regard, achieving the greatest reductions compared to other treatments. The application of nanoparticles directly influenced key plant growth parameters. This study represents the first documented use of *A. vera* gel in the green synthesis of nanoparticles from *T. elegans* and *L. camara*. The findings of this study conclusively highlight the capacity of green-synthesised nanoparticles to not only mitigate nematode infestations but also to enhance overall crop productivity, making them a valuable tool for sustainable agriculture. The study also paves the way for future research into optimising nanoparticle synthesis, conducting large-scale field trials, and evaluating the long-term environmental and economic impacts of their application.

Key words: *Lantana camara*, *Tabernaemontana elegans*, nanoparticles, plant extracts, *Aloe vera.*

CHAPTER 1

GENERAL INTRODUCTION

1.1.Background

Agriculture faces an array of challenges, including poor soil health, pest and disease infestations, climate change, and limited access to quality water supplies. These issues collectively threaten agricultural productivity and food security, particularly as the global population continues to increase (Pradhan *et al*., 2018). To address these challenges and ensure sustainable food production, innovative and eco-friendly agricultural practices are necessary (Mnyambo *et al*., 2023). Among the key threats to agriculture are root-knot nematodes (*Meloidogyne* spp.), which are responsible for approximately 90% of the total crop damage caused by plant-parasitic nematodes (Kalaiselvi *et al*., 2017). These plant-parasitic nematodes, affecting over 3,000 host plant species, inflict serious damage on various crops worldwide, making them a major concern in agricultural systems (Ghareeb *et al*., 2020).

Plant-pathogenic nematodes, including root-knot nematodes, lead to significant crop yield losses estimated at 8.8% in developed countries and up to 14.6% in tropical and subtropical regions where agriculture is most vulnerable (Ghareeb *et al*., 2022). Farmers in these regions often have limited access to effective management tools such as resistant crop varieties, chemical nematicides, and advanced diagnostic technologies. Agricultural practices in these areas, which are frequently dominated by smallholder or subsistence farming, may lack proper crop rotation and soil health management, contributing to the persistence and spread of nematodes. While, developed countries often benefit from stronger agricultural infrastructure, better access to control methods, and more resilient food systems, which help reduce the overall impact of nematode infestations. The reliance on old-generation pesticides to address these biotic stressors has introduced severe environmental and health concerns. Excessive application of especially old-generation chemical pesticides often results in soil degradation, water pollution through chemical runoff, and reduced soil fertility due to nutrient depletion (Mali *et al*., 2020; Sharma and Singhvi, 2017). These issues are compounded by the lack of effective and sustainable nematode management strategies, leaving farmers with limited options to protect their crops.

To meet increasing food demands and tackle agricultural challenges such as declining land productivity, low input efficiency, and pest and disease outbreaks, sustainable solutions are essential. Nanotechnology offers a promising approach to address these issues. The application of nanomaterial substances with dimensions between 1-100 nm has shown potential in managing plant diseases, including plan-parasitic nematodes, by enhancing the delivery and efficacy of agrochemicals (Prasad *et al*., 2021). Nanoparticles synthesized using biological materials, such as plant extracts, fungi, bacteria, and seaweed, have gained attention for their eco-friendliness, reduced toxicity, targeted/direct effect, and compatibility with agricultural and biomedical applications (Saratale *et al*., 2018).

1.2 Rationale

Root-knot nematodes (*Meloidogyne* spp.) are among the most economically significant plant-parasitic nematodes, causing extensive damage to a wide range of crops worldwide (Khosa *et al*., 2020). Global economic losses due to plant-pathogenic nematodes are estimated at $85 billion annually, highlighting the critical need for effective and sustainable management strategies (Gamalero and Glick, 2020). While old-generation nematicides are commonly used to mitigate these pests, their benefits are often short-lived, offering only temporary suppression of plant-parasitic nematode populations. Additionally, the widespread reliance on nematicides raises significant concerns regarding environmental contamination, human health risks, and the growing resistance of plant-parasitic nematodes to chemical treatments (Ghareeb *et al*., 2022; Dube *et al*., 2018).

Nanotechnology has emerged as a promising alternative for pest and disease management due to the unique properties of nanoparticles. The use of nanoparticles in agriculture particularly in the form of nanopesticides allows for targeted delivery, controlled release, and enhanced effectiveness of active ingredients, thereby reducing the quantity needed and minimizing environmental contamination. Globally, there is a clear upward trend in the adoption of agricultural nanotechnologies, driven by the need for more sustainable and efficient farming practices. The global nanopesticide market is expected to grow significantly, particularly in regions like Asia-Pacific, where high agricultural productivity is essential. However, this growth is tempered by concerns over the environmental fate of nanoparticles, high development costs, and a lack of clear regulatory guidelines. Despite these challenges, the integration of nanotechnology into precision agriculture and integrated pest management (IPM) strategies reflects a broader movement toward smarter and greener agricultural solutions (Kah et al., 2018; Servin et al., 2015; Chen & Yada, 2011).

Nanoparticles have a high surface area-to-volume ratio, which enhances their biological reactivity and enables the efficient delivery of active compounds to target organisms (Mali *et al.*, 2020). In nematode management, nanotechnology has demonstrated potential in improving the efficacy of agrochemicals, reducing the required dosages, and minimizing non-target effects. This improvement is largely attributed to the use of nanocarriers and nanoformulations that facilitate controlled and targeted delivery of nematicides, allowing for better root penetration and increased contact with soil-dwelling nematodes. Chhipa (2017) has emphasised how nanoparticles like silver (AgNPs), zinc oxide (ZnO), and chitosan possess not only delivery capabilities but also inherent antimicrobial and nematicidal properties, making them dual-function agents in crop protection. For example, AgNPs have been found effective in significantly reducing populations of *Meloidogyne incognita*, one of the most economically damaging root-knot nematodes, by disrupting their cellular metabolism and reproduction

(Gurunathan et al., 2014; Siddiqi and Husen, 2017). For instance, metal-based nanoparticles, such as silver and copper nanoparticles, have been widely studied for their antimicrobial and pesticidal properties. These nanoparticles are effective in disrupting nematode physiology and suppressing the populations of plant-parasitic nematodes (PPN). However, the use of metal-based nanoparticles is not without drawbacks. Metal nanoparticles can pose significant risks to the environment and non-target organisms. Studies have shown that their persistence in soil and water can disrupt microbial communities, reduce soil fertility, and potentially bioaccumulate in the food chain, raising concerns about long-term ecological and health impacts (Castillo-Henríquez *et al*., 2020). For example, silver nanoparticles have been reported to alter soil microbial biomass, while copper nanoparticles had been found to inhibit plant root elongation and negatively impacting soil enzyme activities (Punniyakotti *et al*., 2024). These risks highlight the need for safer and more sustainable approaches to nanoparticle synthesis.

Plant-based solutions offer an eco-friendly alternative for developing nanoparticles. Extracts from various plants are rich in natural bioactive compounds, which can serve as reducing and stabilizing agents in the synthesis of green nanoparticles. These plant-derived nanoparticles combine the nematicidal properties of plant extracts with the enhanced efficacy of nanotechnology, offering a dual advantage in pest management (Khosa *et al*., 2020). Moreover, green nanoparticles eliminate the need for toxic chemicals and heavy metals, significantly reducing environmental and health risks. This study focuses on synthesising nanoparticles from *Tabernaemontana elegans* Stapf and *Lantana camara* L. plant extracts using *Aloe vera* gel as a natural reducing and stabilizing agent. *Aloe vera* is rich in phytochemical composition and multifunctional properties. It contains a wide variety of bioactive compounds, including polysaccharides (like acemannan), phenolics, flavonoids, saponins, vitamins (e.g., vitamin C and E), enzymes, and amino acids. These compounds serve dual roles in green synthesis: they reduce metal ions (e.g., Ag^+ to Ag^0 in silver nanoparticle formation) and stabilise the nanoparticles by capping their surfaces, thus preventing aggregation. Furthermore, Aloe vera is widely available, non-toxic, eco-friendly, and cost-effective, making it an ideal alternative to hazardous chemical reagents commonly used in nanoparticle synthesis. Its antioxidant and antimicrobial properties also enhance the functional potential of the resulting nanoparticles, especially when used in applications like pest or disease management.

By leveraging the unique benefits of nanotechnology, the research aims to develop an innovative and sustainable solution for nematode control while improving plant growth. This approach not only addresses the limitations of old-generation chemical nematicides and metal nanoparticles but also aligns with global efforts to promote sustainable agriculture and environmental conservation.

1.3 Overall aim and objectives

1.3.1. Aim

Investigating the formulation and potential application of nanoparticles synthesised from *T. elegans* and *L. camara* extracts as an innovative, sustainable approach for controlling root-knot nematodes (*M. incognita*).

1.3.2. Objectives

i. To determine the phytochemical composition of the nematicidal plants (*T. elegans* and *L. camara*) and nanomaterials in relation to their effects on *M. incognita* nematode.

ii. To evaluate the suitability of *A. vera* gel as a reducing and stabilising agent in the formulation of nanoparticles from *T. elegans* and *L. camara*.

iii. To examine the effects of the prepared nanoparticles on the hatching, mobility, and mortality of *M. incognita* second stage juveniles under *in vivo* conditions.

iv. To explore the potential of the prepared nanoparticles in mitigating the adverse impact of *M. incognita* and enhancing plant growth variables under greenhouse conditions.

1.3.3. Hypotheses

i. The phytochemical composition of *T. elegans*, *L. camara* and synthesised nanoparticles contains bioactive compounds with nematicidal properties.
ii. *Aloe vera* gel is a suitable reducing and stabilizing agent for the formulation of nanoparticles from *T. elegans* and *L. camara*.
iii. Nanoparticles synthesized from *T. elegans* and *L. camara* reduce the hatching, mobility, and motility mortality of *M. incognita* juveniles under in vivo conditions.
iv. Nanoparticles synthesized from *T. elegans* and *L. camara* reduce the adverse impact of *M. incognita* infestations and improve plant growth variables under greenhouse conditions.

1.4. Scientific contribution

This research represents a transformative step in advancing sustainable agricultural practices by introducing nanoparticles synthesized from *T. elegans* (NPte- nanoparticle synthesised from *T. elegans* and *A. vera*) and *L. camara* plant (NPlc- nanoparticle synthesised from *L. camara* and *A. vera*) extracts, with *A. vera* gel serving as a natural reducing and stabilizing agent. The development of these nanoparticles marks a significant improvement over traditional methods of nematode management by harnessing the power of nanotechnology to enhance the properties of bioactive plant compounds. At the nanoscale, these formulations exhibit improved physical and chemical properties, such as greater stability, increased bioavailability, and precise targeted delivery of active ingredients. These enhancements enable the nanoparticles to suppress plant parasitic nematode populations more efficiently, requiring lower dosages compared to some conventional plant extracts or some chemical nematicides.

One of the key advantages of nanoscale formulation is the dramatically increased surface area of the nanoparticles (Mondéjar-López *et al.*, 2024). This feature facilitates better interaction with plant parasitic nematodes, allowing the bioactive compounds to act more effectively on their target organisms. By concentrating the active components in smaller, more reactive particles, the nematicidal efficacy is significantly enhanced, ensuring rapid and consistent action against plant parasitic nematode populations. Moreover, the stability of these nanoparticles means they are less prone to degradation under adverse environmental conditions, such as fluctuating temperatures or prolonged exposure to sunlight, which can often limit the effectiveness of raw plant extracts. This prolonged bioactive lifespan makes them a reliable and practical option for use in diverse agricultural settings.

The research also underscores the importance of eco-friendly innovation in pest management. The green synthesis process employed here avoids the use of toxic, old-generation chemicals and heavy metals, ensuring that the final product poses minimal risks to the environment and non-target organisms. Traditional old-generation chemical or metal-based nanoparticles, while effective, often come with ecological trade-offs, including soil contamination, disruption of microbial communities, and potential accumulation in the food chain. By contrast, the plant-extract-based nanoparticles developed in this study align with global efforts to reduce agricultural pollution and promote biodiversity.

The practical benefits of this innovation are far-reaching, particularly for farmers in resource-limited settings. These nanoparticles offer an affordable and effective alternative to costly and potentially harmful old-generation chemical nematicides. Their enhanced efficiency means that smaller quantities are required, further reducing costs and environmental impact. Additionally, the ability of these nanoparticles to suppress nematode populations can directly lead to improved plant health, increasing crop yields and supporting food security. This is particularly critical for smallholder farmers in developing regions who often lack access to advanced agricultural inputs.

Beyond individual farmers, the broader agricultural sector stands to benefit from the development and adoption of such green technologies. Policymakers and environmental advocates can leverage these innovations to promote sustainable farming practices, aligning with international goals for reducing greenhouse gas emissions, preserving and optimising soil health, and safeguarding water quality. These nanoparticles not only reduce the ecological footprint of agriculture but also contribute to building more resilient and productive farming systems.

Ultimately, this research demonstrates the potential for science and technology to address critical challenges in agriculture, offering solutions that are both effective and environmentally responsible. By combining the natural bioactivity of plant extracts with the advanced capabilities of nanotechnology, this study lays the groundwork for future innovations in pest management, crop enhancement, and sustainable farming practices.

1.5. Thesis Structure

Chapter 1: Provides an overview of the research topic, its significance, objectives, and scope.

Chapter 2: Reviews existing research and identifies gaps that the study aims to address.

Chapter 3: Focuses on the chemical analysis and synthesis of nanoparticles using plant extracts and *A. vera* gel.

Chapter 4: Investigates the bioactivity of synthesised nanoparticles on nematode hatch and mortality.

Chapter 5: Evaluates the effectiveness of nanoparticles in reducing nematode infestation and improving plant health under greenhouse conditions.

Chapter 6: Summarizes the research, highlights key findings, discusses their implications, and offers recommendations for future studies.

1.6. References

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CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Agriculture is the backbone of livelihoods for many communities in South Africa, providing essential food and income. However, traditional agricultural practices, which rely heavily on chemical fertilizers, old-generation pesticides, and other industrial inputs to enhance crop yields, have raised significant concerns regarding their environmental and ecological impacts. The overuse and improper application of these chemicals have been linked to declining biodiversity, reduced soil fertility, and disruption of ecosystems. As such, there is an urgent need to explore sustainable alternatives to conventional agricultural practices (Singh *et al.*, 2021).

Nanotechnology has emerged as a promising solution to these challenges, offering innovative approaches to improving agricultural productivity while mitigating environmental harm. Unlike traditional agricultural inputs, nanotechnology represents a targeted approach, minimizes the quantity of active ingredients required, significantly reducing environmental contamination and enhancing efficiency (Ashraf *et al.*, 2021). Traditional/old-generation pesticides and fertilizers often fail to reach their intended targets, with a significant portion lost through leaching or runoff, contributing to water and soil pollution (Rajmohan *et al.*, 2020). Nanotechnology addresses these inefficiencies by utilizing nanoparticles, which possess unique properties such as compact size, high surface area-to-volume ratio, and superior transportability. These attributes make nanoparticles highly effective in delivering agrochemicals to their target sites (Haris *et al.*, 2023; Hazarika *et al.*, 2022).

Applications of nanotechnology in agriculture include the development of nano-pesticides, nano-fertilizers, and nutrient use efficiency systems. These innovations enhance plant health and control phytopathogens using eco-friendly products that are effective even at lower dosages (Yadav *et al*., 2024). Nanoparticles also find use in industries such as medicine and environmental management due to their diverse chemical and physical properties, which enable wide-ranging applications (Mitra *et al*., 2023). In agriculture, these properties are leveraged to improve pest control and nutrient uptake, making nanotechnology a key tool in sustainable farming practices (Francis *et al*., 2024).

Nanoparticles are typically synthesized using either the "top-down" or "bottom-up" approaches. While the top-down method involves breaking down bulk materials into nanoparticles and is often energy-intensive, the bottom-up approach, which relies on old-generation chemical or biological treatments, is more efficient and cost-effective (Abid *et al*., 2022). However, chemical synthesis methods have a significant drawback: they often produce toxic by-products that can harm the environment. In contrast, biological synthesis methods, using plant extracts, fungi, or bacteria, are eco-friendly and increasingly preferred for their minimal environmental impact (Tripathy *et al*., 2023).

2.2. Effect of nematodes on crop yields

Plant-parasitic nematodes (PPN) significantly reduce crop yields across various regions, with the severity of the impact varying depending on local conditions such as soil type, climate, and crop species. For example, in Ethiopia, research by Abebe *et al.* (2015) demonstrated that *Meloidogyne javanica* (Treub) caused severe damage to tomato and pepper crops under glasshouse conditions, with increasing nematode population densities leading to higher root galling and reduced yields. Similarly, in India, Hema and Khanna (2018) reported 35.2% and 37.4% yield losses in tomatoes (*Solanum lycopersicum*) due to *M. incognita* during two consecutive growing seasons.

In South Africa, root-knot nematodes have been associated with substantial yield losses in tomato production. According to Jones *et al*. (2017), nematode infections in the 1980s resulted in a 21% reduction in tomato yields, leading to monetary losses of ZAR 35.3 million. The extent of yield loss is often underestimated, as many farmers are unaware of nematode infestations due to their microscopic nature and absence of above-ground symptoms (Ansari *et al.*, 2023). Moreover, secondary infections from fungi and bacteria, which often accompany nematode damage, can exacerbate yield losses, further complicating the management of plant-parasitic nematodes (Sikora and Roberts, 2018). Globally, crop losses attributed to PPN are estimated to exceed US$ 157 billion annually (Islam, 2024). These figures highlight the importance of developing effective nematode management strategies, including the use of botanical pesticides and nanotechnology, to mitigate the economic impact of these pests on agricultural productivity.

2.3. Plant-parasitic nematodes, with emphasis on root-knot nematodes

Plant-parasitic nematodes (PPN), particularly root-knot nematodes (*Meloidogyne* spp.), are among the most economically damaging agricultural pests. These nematodes infest a wide variety of crops, causing significant damage to plant roots, reducing water and nutrient uptake, and ultimately lowering crop yields (Sikandar *et al.*, 2020). In South Africa, 14 *Meloidogyne* species have been identified, with eight known to affect tomato crops, a staple in many farming systems (Jones *et al.*, 2017). The reproductive strategy of root-knot nematodes, which involves mitotic and meiotic parthenogenesis, allows their populations to proliferate rapidly under favourable conditions, completing their life cycle in as little as 20–30 days (Azlay *et al.*, 2023; Tefu, 2020).

The economic impact of nematode infestations is immense, with global crop yield losses attributed to plant-parasitic nematodes estimated at $157 billion annually (Ansari *et al.*, 2023). These losses are further exacerbated by secondary infections from fungi and bacteria, which reduce both the quality and quantity of affected crops (Sikora and Roberts, 2018).

Meloidogyne incognita, the root-knot nematode species is a microscopic, soil-dwelling roundworm classified within the Animal Kingdom (Kingdom: Animalia; Phylum: Nematoda; Class: Secernentea; Order: Tylenchida; Family: Heteroderidae; Genus: *Meloidogyne*). It is one of the most damaging plant-parasitic nematodes globally and is particularly important in South African agriculture and horticulture, where it significantly reduces yields in crops such as tomatoes, potatoes, maize, and various fruits. *Meloidogyne incognita* invades plant roots, forming galls or "knots" that interfere with water and nutrient uptake. It reproduces primarily through parthenogenesis, a form of asexual reproduction where females produce offspring without male fertilization, enabling rapid population build-up under favorable conditions. This species' adaptability, broad host range, and high reproductive potential make it a persistent and economically damaging pest in both commercial and small-scale farming systems.

2.4. Current management strategies and their limitations

The management of plant-parasitic nematodes is influenced by farming practices and the availability of effective control tools. Once nematode populations establish in a field, eradication becomes nearly impossible, making it essential to integrate multiple management strategies into a comprehensive approach. These strategies include chemical and biological control, resistant cultivars, crop rotation, fallowing, and soil amendments (Fourie *et al*., 2017; Mnyambo *et al*., 2023).

2.4.1. Chemical control of nematodes

Chemical nematicides have traditionally been the primary method for managing *M. incognita* nematode populations. Pre-plant fumigants, granular nematicides, and soluble formulations effectively reduce plant-parasitic nematode densities in infected soils (Sikora and Roberts, 2018). For example, fenamiphos treatments have shown efficacy against mixed populations of

M. incognita and *M. javanica* on tomato crops (Daiber, 1990). However, the use of old-generation chemical nematicides has declined due to their adverse environmental and health impacts. Many old-generation chemicals have been banned or restricted, while withdrawing of those still being available on markets are also under scrutiny: and those that remain available often require skilled operators to ensure safe application (Desaeger, 2020).

2.4.2. Biological control of plant-parasitic nematodes

The exploration of biological antagonists as a means to manage plant-parasitic nematodes (PPN) gained traction following the identification of predatory nematodes, nematode-trapping fungi, and nematode parasites (Ahmad *et al*., 2021). Various biological control agents (BCAs) that exhibit activity against PPN are naturally prevalent in cultivated soils across the world, offering a sustainable approach to nematode suppression (Abd-Elgawad, 2020). Among these, fungi and bacteria are the most extensively employed biocontrol agents (Abd-Elgawad and Askary, 2020 Tefu, 2020). By the late 1990s, over 30 genera and 80 species had been identified as natural parasites of root-knot nematodes. Since then, researchers in nematology have continued to isolate and study natural enemies, delving into their biology, ecology, and potential applications in biological control programs (Annapurna *et al*., 2018).

Research into the practical application of these BCAs has yielded promising results. For instance, Huang *et al*. (2016) demonstrated that a combination of *Syncephalastrum racemosum* and *Purpureocillium lilacinus* significantly decreased *M. incognita* populations in cucumber (*Cucumis sativus* L.) during pot and greenhouse trials conducted in China. Similarly, Hari (2014) assessed the nematicidal efficacy of *Pseudomonas fluorescens* against *M. incognita* on pepper (*Capsicum annuum* L.) in India and found that the bacterium outperformed conventional chemical treatments. Moreover, *P. lilacinus* successfully mitigated *M. incognita* infestations in eggplant (*Solanum melongena*) (Khan and Tanaka, 2023).

In the South African context, Viljoen *et al*. (2019) documented a significant reduction in root galling and egg mass formation on tomato roots following treatment with the plant growth-promoting rhizobacterium *Bacillus aryabhattai* strain A08 in glasshouse trials. However, despite these advancements, the biological control of soil-dwelling pests remains a complex challenge due to the intricate and dynamic nature of soil ecosystems (Neher, 2010) . An in-depth understanding of agricultural practices, including soil amendments and crop management strategies, is essential to enhance the effectiveness of naturally occurring biocontrol agents.

Certain bacterial species, including *Pseudomonas* spp. and *Serratia* spp., exert nematicidal effects by secreting bioactive compounds that directly target nematodes (Diyapoglu *et al*., 2022; Gamalero and Glick, 2020). The nematicidal and antimicrobial attributes of natural compounds derived from either bacterial metabolites or plant extracts present a viable alternative to synthetic pesticides. Consequently, there has been a growing emphasis on reducing chemical pesticide use and encouraging farmers to adopt environmentally sustainable pest management approaches (Khosa *et al*., 2020). Biocontrol strategies incorporating microorganisms and entomopathogenic nematodes (EPN), such as *Steinernema feltiae*, *S. glaseri*, and *S. riobrave*, have undergone extensive evaluation against root-knot nematodes (*M. incognita* and *M. javanica*) under controlled laboratory, glasshouse, and field conditions (Sushma *et al*., 2024; Caccia *et al*., 2018). The successful deployment of various EPN species in both field and greenhouse settings underscores their potential as effective biological control agents (Koppenhöfer *et al*., 2020). Integrated Pest Management (IPM) incorporating biological control is gaining recognition as a pivotal strategy in optimizing agricultural productivity while promoting sustainability (Angon *et al*., 2023).

Another notable biocontrol agent, *Bacillus subtilis*, has demonstrated nematicidal activity against a range of nematode species, including *Aphelenchoides besseyi*, in *in vitro* trials (Pires *et al*., 2022). Furthermore, *Bacillus firmus*, when applied as an aqueous suspension, exhibited a remarkable 98-100% suppression of *M. incognita* juvenile egg hatch within 24 days post-treatment. Glasshouse experiments revealed a concurrent reduction in gall formation, nematode populations, and egg counts on tomato seedlings (El-Nagdi *et al*., 2021).

2.4.3. Resistant cultivars in nematodes

Only a limited number of widely cultivated vegetable varieties possess inherent resistance or tolerance to plant-parasitic nematodes (PPN), making the development of resistant cultivars a crucial aspect of sustainable vegetable production. The selection of resistant or tolerant varieties can substantially lower production costs and enhance yield stability. Advances in breeding programs have successfully identified and developed tomato cultivars resistant to *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* (Aydınlı and Mennan, 2019). The cultivation of resistant plant varieties plays a pivotal role in reducing nematode population densities in the soil (Abd-Elgawad, 2022).

In tomato plants, several resistance genes have been identified, including the Mi-1 gene, which confers resistance to multiple root-knot nematode species (El-Sappah *et al*., 2019). However, resistance breakdown has been reported, as evidenced by Kaloshian *et al*. (1996), who identified *M. incognita* populations capable of parasitising Mi-1-containing tomato plants in California (Hajihassani *et al*., 2022). This underscores the need for integrating resistance genes with complementary control measures. Also, vertical resistance (one gene conferreing the resistance) is risky and hence horizontal resistance (several genes conferring the resistance trait) is a better option to ensure the resistance is sustainable (Simmonds, 1991). In South Africa, tomato cultivars such as Rhapsody, MFH 9324, FA 1454, and FA 593 exhibited resistance to *M. incognita*, as confirmed through microplot trials involving varying initial nematode densities (Fourie *et al*., 2012). Given the continuous evolution of agricultural markets, it is imperative to routinely screen new tomato cultivars for resistance to economically significant root-knot nematodes, including *M. enterolobii* (Tefu, 2020).

2.4.4. Fallowing in nematode management

Plant-parasitic nematodes are obligate parasites that rely exclusively on plant hosts for sustenance (Kumar and Yadav, 2020). Consequently, plant-parasitic nematode population densities decline in the absence of host plants (Feyisa, 2022). Bare fallowing which is leaving soil free of vegetation can be an effective strategy for controlling *Meloidogyne* spp., particularly during hot and dry intercropping periods when alternative weed hosts are scarce (Yigezu and Gelena, 2019). However, while bare fallowing can reduce nematode populations by up to 90% in certain conditions (Schloemer, 2024), it negatively impacts soil health by depleting organic carbon levels, thereby compromising essential physical, chemical, and biological soil properties (Ramesh *et al*., 2019).

2.4.5. Tillage controlling nematodes

Soil tillage influences nematode populations by altering soil structure and nematode distribution. Reduced tillage can restrict nematode reproduction by limiting their dispersal through soil movement on farming implements (Schmidt *et al*., 2017). Conversely, soil disturbance has been linked to increased soybean cyst nematode (SCN; Heterodera schachtii) egg populations due to horizontal redistribution of inoculum (Wendimu, 2022). No-tillage systems can concentrate nematodes in deeper soil layers, while minimal tillage in compacted soils may reduce available root growth space, thereby exacerbating nematode-induced stress on crops (Zheng *et al*., 2023).

2.4.6. Crop rotation and cover crops in nematode management

Crop rotation is a long-standing agricultural practice that serves as an effective strategy for managing plant-parasitic nematodes (PPN). By alternating susceptible crops with resistant or non-host crops, farmers can reduce the population densities of nematodes, thus minimising yield losses and decreasing the reliance on chemical pesticides. The effectiveness of crop rotation largely depends on the compatibility of the chosen rotation crops with the nematode species present in the soil (Phani *et al*., 20210).

Several studies have demonstrated the potential of crop rotation in nematode suppression. For instance, the incorporation of American joint vetch (*Aeschynomene americana* L.), castor bean (*Ricinus communis* L.), hairy indigo (*Indigofera hirsuta* L.), partridge pea (*Cassia fasciculata* L.), sesame (*Sesamum indicum* L.), and velvet bean (*Mucuna deeringiana* (Bort) Merr. H) has led to significant reductions in *Meloidogyne* spp. populations while enhancing the yields of peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* L. Merr) (Tefu, 2020). Similarly, in Maryland, USA, Sorghum sudangrass (*Sorghum* × *drummondii* L.) rotation successfully curtailed *M. incognita* and root-lesion nematode (*Pratylenchus*) populations when followed by susceptible potato (*Solanum tuberosum* L.) or cucumber (*Cucumis sativus* L.) (Gleason, 2021). In Southern California, USA, incorporating rotation crops such as broccoli (*Brassica oleracea* L.), carrot, marigold (*Tagetes patula* L.), and strawberry (*Fragaria ananassa* Duch) in *M. incognita*-infested fields resulted in a 36% reduction in root galling in tomato and an impressive 19% yield increase (Lopez-Perez *et al*., 2010). These findings underscore the crucial role of strategic crop rotation in enhancing soil health and improving crop productivity. South African research has also confirmed the effectiveness of specific rotational crops in nematode suppression. Berry *et al*. (2011) reported that black oat (*Avena strigosa* Schreb.), wheat (*Triticum aestivum* T. durum), forage peanut, and marigold significantly reduced *M. javanica* populations. However, one of the primary challenges with crop rotation is the economic tradeoff. While it is a sustainable strategy, many rotation crops do not generate direct income, creating a financial burden for farmers. The cultivation of cover or rotational crops, particularly those from the Brassicaceae family, has shown promise in suppressing soil-borne diseases, including PPN. Well-studied examples include cowpea (*Vigna unguiculata* L.), marigold, Indian mustard (*Brassica juncea* L.), mustard (*Eruca sativa* L.), radish (*Raphanus sativus* L.), and sunn hemp (*Crotalaria juncea* L.) (Ralmi *et al*., 2016).

Cowpea residues, when incorporated into the soil before planting tomato, were shown to promote tomato growth and reduce *Meloidogyne* spp. damage, even in infested soils (Roberts *et al*., 2005). Many *Crotalaria* species, valued for their nematode-suppressive properties and nitrogen-fixing capabilities, have been successfully used as cover crops. Sunn hemp residues, in particular, substantially reduced *M. incognita* population densities and minimized root galling in yellow squash (*Cucurbita pepo* L.) (Ralmi *et al*., 2016).

Brassicaceae species have also been extensively studied for their biofumigant properties. These plants release glucosinolates, which, upon hydrolysis, generate isothiocyanates compounds known for their nematicidal activity (Ntalli and Caboni, 2017). Studies have demonstrated that several Brassicaceae species effectively reduce nematode populations while increasing crop yields (Ziouche *et al*., 2023). South African research has further validated these findings. Gardner and Caswell-Chen (1994) investigated the host status of Arugula (*Eruca sativa* L.), Brown mustard (*Brassica juncea* L.), and Radish (*Raphanus sativus* L.) to *M. incognita* and *M. javanica* through glasshouse and field trials. While most evaluated cover crops demonstrated resistance to *Meloidogyne* spp., *B. juncea* was susceptible to *M. incognita*. Biofumigation using these crops significantly reduced nematode egg counts and J2 populations, leading to increased tomato yields. However, these same Brassicaceae species unexpectedly increased *M. incognita* populations by 115% in a potato field trial (Gardner and

Caswell-Chen, 1994). These results highlight the complexity of biofumigation strategies, necessitating careful crop selection based on nematode species and environmental conditions.

2.4.7. Organic amendments in nematodes management

Organic soil amendments have gained attention as a viable alternative for nematode control, particularly within IPM frameworks (Sikora and Roberts, 2018). Organic amendments include various organic materials, such as compost, animal manure, plant residues, and oil cakes, which not only suppress nematodes but also improve soil health by enhancing its physical, chemical, and biological properties (Chaudhari *et al.*, 2021).

Manure, compost, and plant waste materials are critical components of soil fertility. They improve soil structure, water retention, and microbial diversity while reducing soil compaction and erosion (Gurmu, 2019; Usharani *et al.*, 2019). Furthermore, organic amendments act as slow-release nutrient sources, reducing the need for synthetic fertilisers (Verma *et al.*, 2020). Organic matter containing chitin releases ammonia into the soil, which can directly suppress PPN populations while encouraging beneficial soil microbes (Treonis *et al.*, 2018).

Several studies have confirmed the efficacy of organic amendments in nematode management. For instance, the incorporation of chicken manure, cattle manure, and compost significantly reduced nematode populations in different environmental conditions (Karuri, 2022). In one study, chicken manure application led to a 92% reduction in *Meloidogyne* population densities (Maina *et al.*, 2020). Poultry refuse at a rate of 3 t/ha effectively decreased *M. incognita* egg counts and increased tomato yields by 43.37% in Bangladesh (Tefu, 2020). Similarly, swine and green manure amendments demonstrated positive effects by improving plant growth parameters on tomato plants infected with root-knot nematodes (Grabau *et al.*, 2018).

Plant-based amendments have also been widely explored. Neem (*Azadirachta indica* L.), cassava (*Manihot esculenta* Crantz L.) peel, and sunflower (*Helianthus annuus* L.) leaf composts significantly reduced *M. incognita* J2 populations in okra (*Abelmoschus esculentus* L.) fields in Nigeria (Olabayi and Oladeji, 2014). In South Africa, small-scale farmers frequently use organic waste, including cattle manure, chicken manure, and compost, as their primary fertilizers. Mashela *et al*. (2017) observed that amending soil with marigold and compost reduced root-knot nematode (*M. incognita*) populations by up to 92%, with significant reductions of 52% observed with chicken manure.

Essential oils and plant extracts

Botanical nematicides derived from plants such as *Maerua angolensis* DC., *Cissus cactiformis* Gilg., *Lippia javanica* L., and *Lantana camara* L. have shown significant nematicidal properties (Malahlela *et al*., 2019; Dube *et al*., 2018; Mashela *et al*., 2017). Plant extracts from neem (*Azadirachta indica* J.), moringa (*Moringa oleifera* L.), lantana (*Lantana camara* L.), and liquorice (*Glycyrrhiza glabra*) has effectively inhibited *Meloidogyne* spp. J2 hatch (Haroon *et al*., 2018). Additionally, citrus (*Citrus*) peel extracts, rich in phytochemicals like saponins and flavonoids, have demonstrated strong nematicidal effects (Diab, 2016). These findings highlight the growing potential of botanical alternatives in sustainable nematode management strategies.

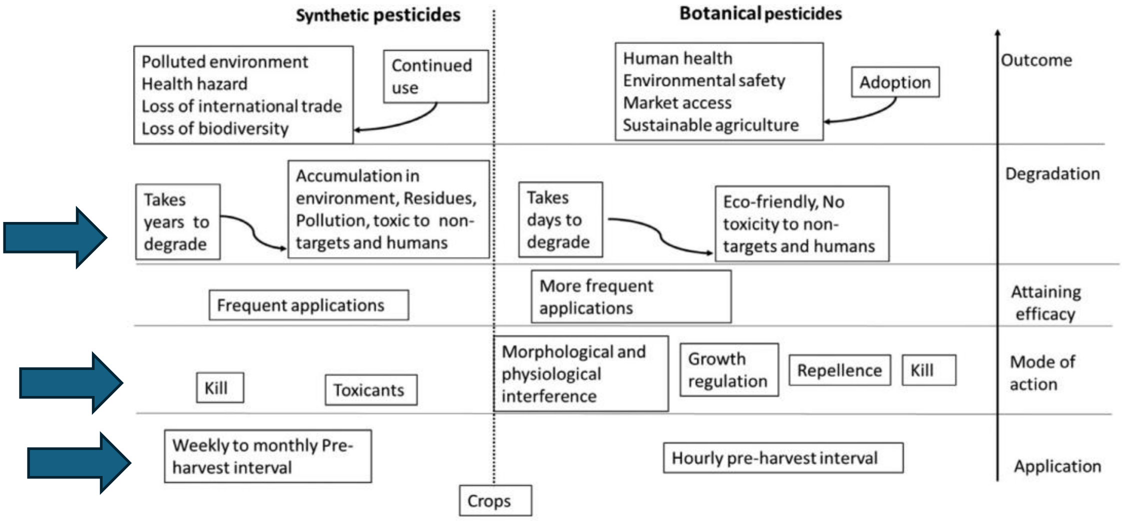

Figure 2.1: A model illustrating differences between systematic pesticides and botanical pesticides with respect to use, mode of action, persistence and effect on ecosystem (Lengai *et al*., 2020).

2.5. Nanoparticles as novel nematode management tools

2.5.1. Nanoparticles and their applications in agriculture against nematodes

Plant-parasitic nematodes (PPN) are among the most significant threats to global agriculture, causing extensive damage to a wide range of crops and significantly reducing yields. Root-knot nematodes (*Meloidogyne* spp.) are particularly destructive, with *M. incognita* identified as one of the most economically important nematode species globally (Tapia-Vázquez *et al*., 2022). These nematodes invade plant roots as J2 creating feeding sites known as giant cells that disrupt the plant’s ability to absorb water and nutrients. This leads to stunted growth, reduced productivity, and economic losses estimated at $100 billion annually in horticultural crops (Páez, 2023). The situation is especially critical in countries like South Africa, where tomatoes, a staple crop, are significantly affected by nematodes, leading to substantial economic losses (Khosa *et al*., 2020).

Conventional methods for managing PPN include the use of chemical nematicides, crop rotation, resistant cultivars, and biological control agents. However, each of these strategies has limitations that make them less than ideal. Chemical nematicides, while effective in reducing nematode populations, often result in environmental contamination, health risks, and the development of pesticide-resistant nematode strains (Abd-Elgawad, 2021). Crop rotation can reduce nematode densities but is time-intensive and economically unsustainable for many farmers, particularly when rotation crops do not generate income (Sikora and Roberts, 2018). Similarly, resistant cultivars, although promising, face challenges due to the emergence of virulent nematode populations that overcome resistance genes, rendering the method ineffective over time (Hajihassani *et al.*, 2022).

Nanotechnology has emerged as a revolutionary approach to addressing these challenges. Nanoparticles (NPs), which are materials with dimensions between 1–100 nm, possess unique physicochemical properties that make them highly effective for agricultural applications. Their small size, large surface area-to-volume ratio, chemical stability, and ability to be engineered for targeted delivery enable NPs to serve as precision tools in pest and pathogen management (Khan *et al.*, 2022). These properties allow NPs to enhance the efficacy of active ingredients while minimizing their environmental impact. For example, nanotechnology enables controlled release and site-specific delivery of pesticides, reducing the risk of non-target exposure and environmental contamination (Shivashakarappa *et al.*, 2022).

2.5.2. Inefficiencies in conventional pesticide applications

Traditional pesticide application methods are highly inefficient, with studies estimating that up to 90% of applied pesticides are lost due to volatilization, leaching, surface runoff, and adsorption onto non-target areas (Tudi *et al.*, 2021). This inefficiency not only leads to environmental contamination of water bodies and soil but also increases the economic burden on farmers, who must apply higher doses to achieve desired results. These chemicals were effective at killing a wide range of pests, but they often lacked specificity, leading to the unintended death of beneficial insects, soil organisms, and even wildlife. The persistent use of such methods exacerbates issues like soil degradation and reduced biodiversity. Additionally, pesticides that do reach their targets often degrade quickly due to environmental factors such as UV radiation and microbial activity, necessitating frequent reapplication (Rajmohan *et al*., 2020). These new-generation pesticides are designed with greater selectivity, lower environmental persistence, and reduced toxicity to non-target organisms. These include neonicotinoids, insect growth regulators (IGRs), and biopesticides such as those derived from microbial sources (e.g., *Bacillus thuringiensis*). Many of these newer compounds act on specific biochemical pathways in target pests, reducing unintended impacts on other species. They are often more biodegradable, less prone to bioaccumulation, and can be integrated more effectively into sustainable pest management systems. However, concerns still exist, such as the link between some neonicotinoids and pollinator decline and resistance can still develop if new-generation pesticides are overused or misapplied.

Nanotechnology addresses these inefficiencies by offering controlled-release formulations and protecting active ingredients from degradation. For instance, encapsulating nematicides within nanoparticles ensures that the active compounds remain stable and effective over longer periods, reducing the frequency of application and overall usage (Hazarika *et al*., 2022). Furthermore, nanoparticles can be engineered to interact specifically with plant parasitic nematodes, ensuring precise delivery of the active ingredient while minimizing off-target effects (Khan *et al*., 2022).

2.6. Green nanotechnology: A sustainable alternative

The synthesis of nanoparticles can be achieved through physical, chemical, or biological methods. While old-generation chemical nematicides are widely used, such as organophosphates and carbamates, are often broad-spectrum, they often produce toxic by-products that pose risks to human health, beneficial soil microorganisms, non- target organisms and the environment (Goyal *et al.*, 2019). Their persistence in the environment contributes to soil and water contamination, leading to long-term ecological damage. Even new-generation chemical alternatives, while typically more targeted and less toxic, may still accumulate in ecosystems over time, and their long-term effects are not always fully understood. Moreover, chemical resistance among pest and nematode populations remains a growing concern, potentially diminishing the efficacy of both old and new formulations.

Nanoparticles, on the other hand, while promising, are not without limitations. For example, the chemical synthesis of silver nanoparticles (AgNPs), which are known for their strong antimicrobial properties, generates hazardous waste that contaminates soil and water ecosystems (Castillo-Henríquez *et al.*, 2020). Similarly, the persistence of metal nanoparticles in the environment raises concerns about their accumulation in the food chain and potential toxicity to non-target organisms, including beneficial soil microbes and aquatic species (Punniyakotti *et al.*, 2024). Some concerns include the cost of large-scale production, potential cytotoxicity to non-target organisms, and uncertainties around their behavior in natural environments, such as soil and water systems. The risk of nanoparticle accumulation and unintended ecological interactions must be carefully assessed. However, despite these concerns, the application of nanoparticles in agriculture can offer distinct advantages over chemical nematicides. Their high surface area-to-volume ratio allows for more efficient delivery of active compounds, often at lower dosages, which can reduce environmental load.

Additionally, nanoparticles can be engineered for controlled release and targeted action, increasing their specificity and minimizing collateral damage to non-target species

Biological synthesis, or "green nanotechnology," offers an eco-friendly alternative. This method uses natural materials such as plant extracts, fungi, and bacteria to produce nanoparticles without generating harmful by-products. Plant extracts, in particular, are rich in bioactive compounds that act as reducing and stabilizing agents during nanoparticle synthesis, eliminating the need for toxic chemicals (Ma, 2022). For example, nanoparticles synthesized using *Jatropha curcas* L. and *Calotropis gigantea* L. extracts have demonstrated strong nematicidal and antimicrobial properties, making them effective in managing agricultural pests (Kiriyanthan *et al*., 2020; Rajkuberan *et al*., 2015).

2.6.1. Application of green nanoparticles in nematode management

In this study, green nanoparticles were synthesized from *T. elegans* and *L. camara* plant extracts using *A. vera* gel as a natural reducing and stabilizing agent. This approach combines the nematicidal properties of the plant extracts with the enhanced efficacy of nanoparticles, offering a novel solution for managing root-knot nematodes. To the best of our knowledge, this is the first study to explore the nanoscale conversion of these specific plant extracts for nematode management.

Previous research has highlighted the potential of green nanoparticles in agricultural pest control. For instance, nanoparticles synthesised from *Hevea brasiliensis* M. reduced nematode populations in greenhouse trials, while those from *Euphorbia confinalis* L. exhibited strong biocidal activity against multiple pathogens (Muchanyereyi *et al*., 2017; Guidelli *et al*., 2011). Similarly, studies on nanoparticles derived from *Achras sapota* L. and *Calotropis gigantea* L. have demonstrated significant reductions in nematode activity and improved plant health under controlled conditions (Kiriyanthan *et al*., 2020; Rajkuberan *et al*., 2015).

Green nanoparticles offer several advantages over traditional pest control methods. Their low toxicity and biodegradability make them safe for non-target organisms and the environment. Additionally, their small size and high reactivity enable efficient interaction with nematodes, disrupting their physiology and reducing populations effectively. For example, nanoparticles can penetrate the nematode's cuticle and interfere with its metabolic processes, ultimately leading to mortality (Khosa *et al*., 2021). These characteristics make green nanoparticles a promising alternative to chemical nematicides, particularly in sustainable agriculture systems.

2.7. Noble metal nanoparticles and their interactions with plants

2.7.1. Silver nanoparticles

Silver nanoparticles (AgNPs) have become one of the most extensively studied nanoparticles due to their remarkable physical and chemical properties, making them highly versatile across multiple domains. In agriculture, AgNPs have shown potential applications as fungicides, insecticides, and pesticides. Their broad-spectrum antimicrobial activity against bacterial, fungal, and nematode pathogens makes them a valuable tool in sustainable pest management strategies (Siddiqi and Husen, 2021).

AgNPs have demonstrated variable effects on plants, influenced by factors such as plant species, particle size, and concentration. In some cases, AgNPs promote plant growth by increasing biomass production (Salachna *et al*., 2019) and stimulating seed germination (Xin *et al*., 2020). Positive effects on root and shoot development have also been observed, although other studies report inhibitory impacts at higher concentrations, such as reduced root elongation and stunted shoot growth (Kong *et al*., 2021). AgNPs can induce oxidative stress in plants, characterized by the overproduction of reactive oxygen species (ROS), which can damage cellular structures if not mitigated by antioxidant mechanisms (Horie and Tabei, 2021).

Chlorophyll content, a critical determinant of photosynthetic efficiency, has been shown to decrease in some plants exposed to AgNPs, potentially due to chlorophyll quenching or damage to chloroplasts (Sharma *et al*., 2019; Queiroz *et al*., 2016). However, other studies suggest that AgNPs can activate ROS-scavenging enzymes, enhancing a plant's ability to tolerate abiotic stress (Salachna *et al*., 2019). Despite their potential benefits, the toxicity of AgNPs at high concentrations remains a concern, necessitating careful optimization of their dosage to ensure their safe and effective use in agriculture.

2.7.2. Gold nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) are another important class of noble metal nanoparticles with emerging applications in agriculture. While AuNPs are considered chemically inert under most environmental conditions, their interactions with plants primarily depend on their size, surface charge, and coating properties (McGivney *et al*., 2019). AuNPs are mainly absorbed by plant roots and translocated to aerial parts, with uptake efficiency varying significantly across plant species and nanoparticle formulations.

AuNPs have shown predominantly positive effects on plants, including improved seed germination, enhanced photosynthesis, and increased scavenging of free radicals (Joshi *et al*., 2022). For example, AuNPs in the size range of 5–60 nm has been found to promote root and shoot growth in various plant species, including *Arabidopsis thaliana* L., where exposure to lower concentrations of AuNPs stimulated the formation of lateral roots and improved overall plant health (Pradhan *et al*., 2023). However, higher concentrations or smaller particles, such as 10 nm AuNPs, have been associated with adverse effects, including reduced primary root elongation and lateral root development (Geng *et al*., 2022; Siegel *et al*., 2018).

Despite these findings, the environmental implications of AuNPs in agriculture remain understudied. One potential pathway for AuNPs to enter agricultural soils is through biosolids derived from wastewater treatment plants, where gold residues can accumulate. Studies suggest that while AuNPs are generally less reactive than other metal nanoparticles, their long-term behavior and potential transformation in agricultural soils warrant further investigation (McGivney *et al*., 2019).

2.7.3. Platinum nanoparticles (PtNPs)

Platinum nanoparticles (PtNPs) have attracted interest due to their catalytic properties and low phytotoxicity compared to other metal nanoparticles. Studies on PtNPs have shown their potential for uptake by plants such as *Sinapis alba* L. and *Lepidium sativum* L., with no adverse effects on plant growth or physiology at moderate concentrations (Asztemborska *et al*., 2015). PtNPs were absorbed primarily by roots, with limited translocation to aerial parts, suggesting their accumulation in below-ground tissues.

In hydroponic systems, PtNPs have been shown to enhance root growth and flavonoid content, potentially activating plant defense mechanisms against oxidative stress (Astafurova *et al*., 2017). For example, cucumber plants exposed to PtNPs exhibited no phytotoxic effects, with Pt accumulation restricted to roots and leaves while fruits remained unaffected. This selective accumulation reduces the risk of PtNPs entering the human food chain, making them a safer option for agricultural applications compared to ionic platinum compounds.

2.8. Methods of nanoparticle synthesis

2.8.1. Chemical synthesis

Chemical synthesis is one of the most widely used methods for nanoparticle production due to its efficiency and scalability (Figure 2). This method involves the reduction of metal ions to their corresponding nanoparticles using reducing agents such as sodium borohydride ($NaBH_4$), ascorbate ($C_6H_7O_6$), or citrate ($C_6H_5O_7^{3-}$). Protective agents, or capping agents, are added to stabilize the nanoparticles and prevent aggregation, ensuring uniform size and dispersion (Kumar *et al*., 2021).

The chemical synthesis process typically includes two stages: nucleation and growth. During nucleation, metal ions form small atomic clusters, which act as seeds for further growth. Controlled growth of these nuclei results in nanoparticles with specific sizes and shapes, depending on the reaction conditions such as temperature, pH, and reducing agent concentration (Yaqoob *et al*., 2020). While chemical methods offer precise control over nanoparticle properties, they often generate toxic by-products, raising environmental and health concerns.

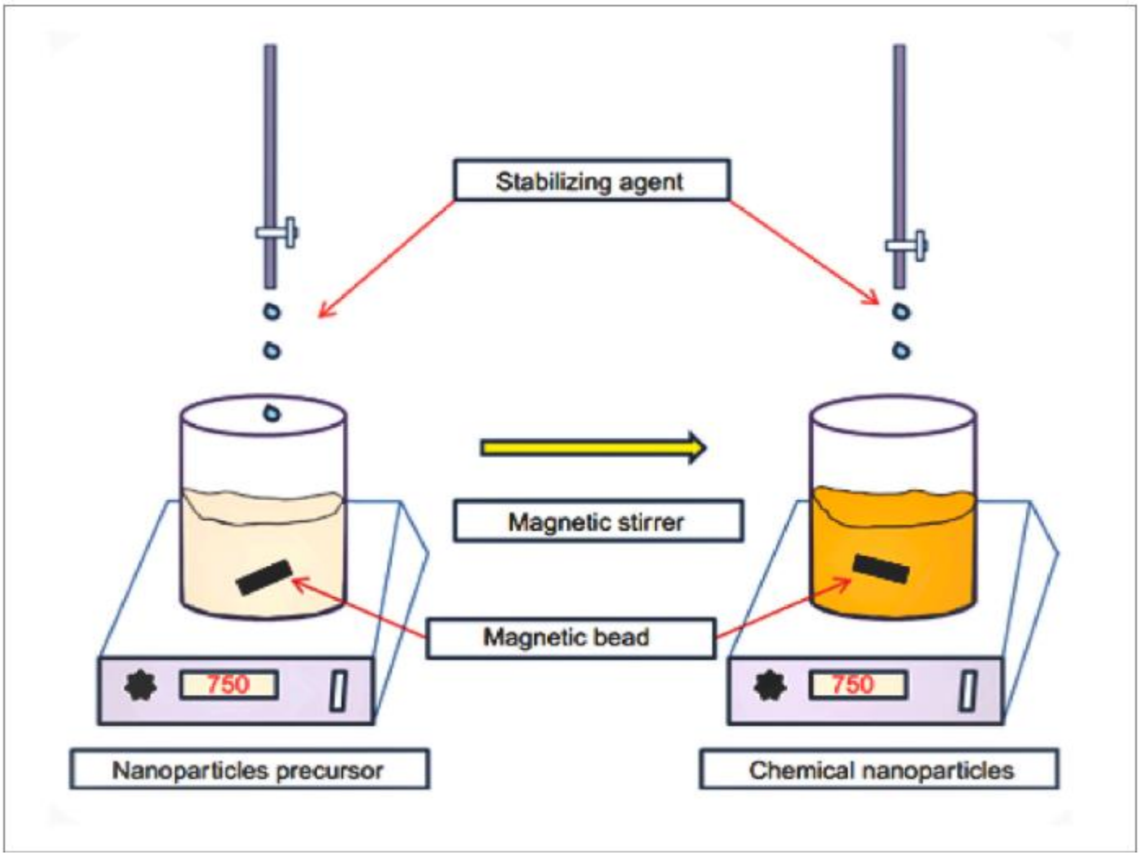

Figure 2.2: Chemical method for synthesis of Nanoparticles (Deepak *et al*., 2019)

2.8.2. Green synthesis

Green synthesis methods have emerged as a sustainable alternative to chemical synthesis, leveraging biological materials such as plant extracts, fungi, and bacteria to produce nanoparticles. This approach eliminates the need for toxic reducing agents and solvents, making it environmentally friendly and cost-effective (Ma, 2022). Plant extracts, in particular, are rich in natural bioactive compounds that act as reducing and stabilizing agents. For instance, nanoparticles synthesized using *Jatropha curcas* L. or *Calotropis gigantea* L. extracts have demonstrated effective biocidal activity against pathogens while minimizing environmental risks (Kiriyanthan *et al*., 2020; Rajkuberan *et al*., 2015).

The green synthesis of nanoparticles not only reduces environmental risks but also enhances their biological activity. For example, the use of plant extracts ensures the incorporation of phytochemicals into the nanoparticles, potentially amplifying their antimicrobial and nematicidal properties (Saratale *et al*., 2018). This makes green-synthesized nanoparticles particularly suitable for agricultural applications, where safety and sustainability are critical.

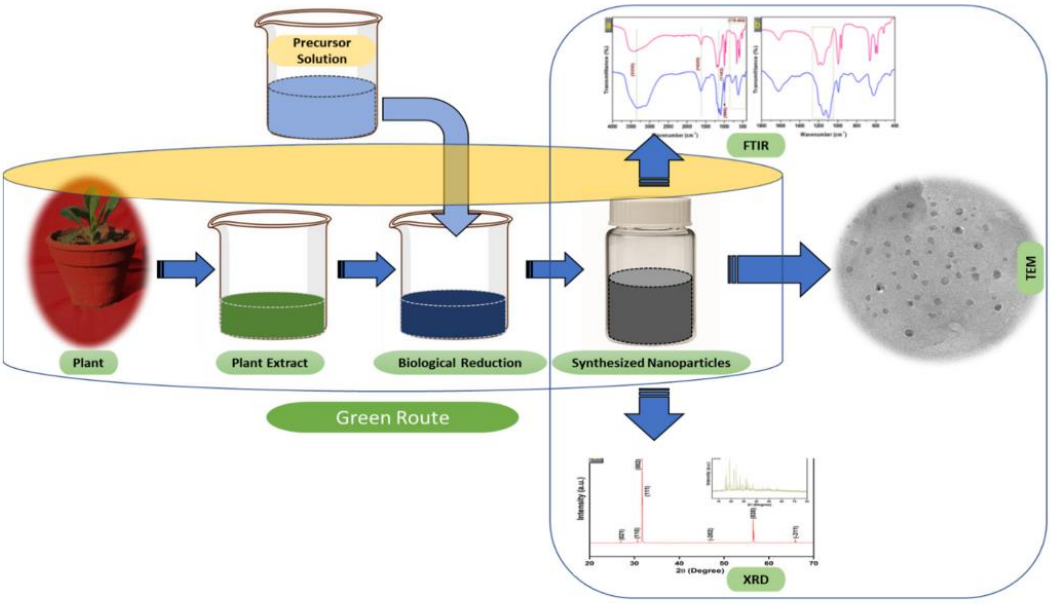

Figure 2.3: The schematic diagram for the biosynthesis of nanoparticles via a green route using plant extract (Khan *et al*., 2022)

2.9. Importance of reducing and capping agents

Nanoparticles have emerged as a transformative tool in agriculture, offering innovative solutions for crop protection, nutrient delivery, and pest management. The synthesis of nanoparticles involves the use of reducing and capping agents, which play crucial roles in determining the properties and effectiveness of these materials. In agricultural applications, selecting appropriate reducing and capping agents is vital for ensuring the nanoparticles' environmental safety, biocompatibility, and efficiency in addressing specific challenges.

Reducing agents are essential in nanoparticle synthesis as they facilitate the conversion of metal ions into their reduced, nanoscale form. In agriculture, the choice of reducing agents significantly impacts the size, morphology, and reactivity of nanoparticles. Green reducing agents derived from plant extracts or natural compounds are gaining attention due to their eco-friendly and sustainable nature. These agents not only minimize environmental toxicity but also add value by functionalizing nanoparticles with bioactive molecules that enhance their antimicrobial or pest-repellent properties. This green approach aligns with the global shift towards sustainable agricultural practices, reducing reliance on synthetic chemicals that may have harmful ecological consequences.

Capping agents, on the other hand, stabilize nanoparticles by preventing aggregation and controlling their growth during synthesis. They play a critical role in defining the surface chemistry, solubility, and interaction of nanoparticles with target organisms or systems. For agricultural purposes, biodegradable and non-toxic capping agents are preferred to avoid adverse effects on soil health, plant growth, and non-target organisms. By choosing the right capping agents, researchers can tailor nanoparticles to deliver fertilizers or pesticides in a controlled manner, enhancing efficiency while reducing waste and environmental contamination.

Moreover, the combined use of sustainable reducing and capping agents can significantly influence the cost-effectiveness and scalability of nanoparticle synthesis. As agriculture often requires large-scale applications, ensuring that the synthesis process is both economically viable and environmentally friendly is crucial. This dual focus not only benefits farmers by lowering costs but also protects ecosystems, ensuring long-term agricultural sustainability.

2.10. Physical and photochemical methods for nanoparticle synthesis

2.10.1. Physical method

The physical synthesis of nanoparticles primarily involves condensation or vaporization techniques, where high-energy inputs transform bulk materials into nanoscale particles. Two commonly employed approaches are evaporation-condensation and laser ablation.

2.10.2. Evaporation-condensation

In the evaporation-condensation process, bulk materials are heated in a furnace tube to evaporate the material, which is then carried by an inert gas to a cooler zone where it condenses to form nanoparticles. This method is versatile and can be used to synthesize nanoparticles from metals such as silver (Ag), zinc (Zn), copper (Cu), lead (Pb), and tin (Sn) (Dippong *et al.*, 2021). However, this approach has significant drawbacks. The process requires high temperatures, resulting in elevated energy consumption and environmental heat emissions. Moreover, achieving thermal stability within the furnace can take extended periods, such as 10 minutes or longer, which further increases energy costs and limits its scalability for industrial applications (Dąbrowska *et al.*, 2018). Additionally, nanoparticles produced through this method may exhibit cylindrical shapes and agglomeration, reducing their uniformity and potential applications.

2.10.3. Laser ablation

Laser ablation offers a more refined alternative for nanoparticle synthesis, particularly for silver nanoparticles (AgNPs). In this technique, a pulsed laser beam is directed at bulk material immersed in a liquid medium, causing ablation of the material and subsequent nanoparticle formation. Unlike evaporation-condensation, laser ablation does not require chemical reducing agents, ensuring that the nanoparticles are free from contaminants. This method is advantageous for applications demanding high-purity nanoparticles, such as medical devices and diagnostics (Sohal *et al.*, 2021). Key factors influencing the size, shape, and properties of nanoparticles synthesized via laser ablation include the laser's power, the irradiation duration, and the physical properties of the liquid medium (Naser *et al.*, 2019). For example, adjusting the laser intensity and exposure time can produce nanoparticles with fine size distributions, tailored for specific agricultural or biomedical applications.

2.10.4. Photochemical method

The photochemical synthesis of nanoparticles is gaining traction as a cost-effective and environmentally friendly alternative to traditional methods. This approach uses UV or visible light to reduce metal precursors in a liquid medium, eliminating the need for harmful chemicals or sophisticated equipment.

2.10.5. Mechanism of photochemical synthesis

In photochemical processes, light acts as an energy source to trigger the reduction of metal ions (Mn^{+}) into their metallic state (Mn^{0}). This method employs a variety of reagents, including metal salts and complexes, as precursors. The absence of toxic stabilizers and harsh reducing agents makes photochemical synthesis safer and more sustainable compared to conventional methods (Kumar *et al.*, 2022).

2.11. Comparison of Top-Down and Bottom-Up approaches

2.11.1. Top-Down approach

The top-down approach involves breaking down bulk materials into nanoparticles through mechanical or physical processes, such as ball milling, ultrasonic machining, and laser ablation. This method is advantageous for producing large quantities of nanoparticles. However, it often requires high energy inputs, specialized equipment, and may result in irregular particle shapes and sizes due to mechanical stress (Tripathy *et al.*, 2023).

2.11.2. Bottom-Up approach

In contrast, the bottom-up approach builds nanoparticles from atomic or molecular precursors. This method is energy-efficient and produces nanoparticles with uniform size and fewer structural defects. Green synthesis, a subset of the bottom-up approach, employs biological entities like plant extracts or microorganisms to reduce metal ions and form nanoparticles in an eco-friendly manner (Tripathy *et al.*, 2023).

2.12. Biological methods of nanoparticle synthesis

2.12.1. Microbial-mediated synthesis

Microbial synthesis involves using bacteria, fungi, or algae as natural biofactories for nanoparticle production. These organisms can reduce metal ions through their metabolic processes, producing nanoparticles intracellularly or extracellularly. For example, bacteria like *Pseudomonas* and fungi like *Verticillium* species have been shown to synthesize silver nanoparticles through enzymatic reduction, where nitrate reductase plays a key role in reducing Ag^+ ions to Ag^0 (Zhao *et al*., 2022; Tripathi and Goshisht, 2022).

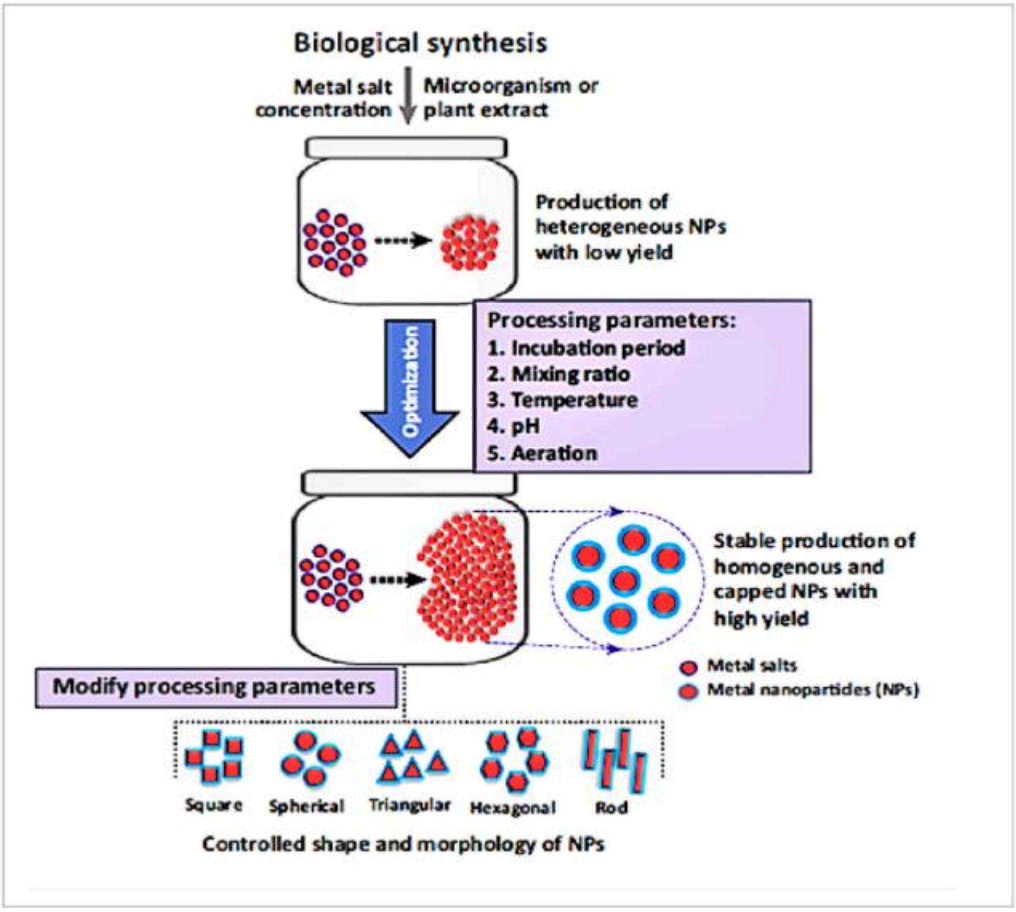

Figure 2.4: Biological method for nanoparticles synthesis (Singh *et al*., 2016)

2.12.2. Plant-mediated synthesis

Plant extracts provide a simpler and more scalable alternative for nanoparticle synthesis. Biomolecules such as phenols, flavonoids, and alkaloids in plant extracts act as reducing and stabilizing agents, enabling the eco-friendly synthesis of nanoparticles. For example, silver nanoparticles synthesized using *Jatropha curcas* L. extracts exhibited strong antimicrobial and nematicidal properties (Rajkuberan *et al*., 2015). The conditions under which the plant extracts interact with metal ions such as pH, temperature, and sunlight exposure affect the size and morphology of the nanoparticles, highlighting the need for precise control during synthesis (Khan and Arasu, 2022).

2.13. Future prospects of green nanotechnology

Green nanotechnology, particularly plant-mediated synthesis, offers a sustainable pathway for nanoparticle production with minimal environmental impact. This approach is ideal for large-scale industrial applications due to its cost-effectiveness, scalability, and compatibility with various climates and resources. Future research should focus on optimizing reaction conditions and exploring the functionalization of nanoparticles to enhance their efficacy in targeted applications such as pest control, water purification, and drug delivery (Wani and Suresh, 2022).

By integrating advanced synthesis techniques with sustainable practices, green nanotechnology holds promise for revolutionizing agriculture, healthcare, and environmental management. For instance, nanoparticles synthesized from *L. camara* and *T. elegans* extracts using *A. vera* as a capping agent offer a novel solution for controlling root-knot nematodes while reducing the reliance on chemical nematicides. The development of such eco-friendly technologies underscores the importance of innovation in achieving sustainable development goals.

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CHAPTER 3

DEVELOPING A PROTOCOL FOR THE SYNTHESIS OF *TABERNAEMONTANA ELEGANS* AND *LANTANA CAMARA* NANOPARTICLES USING *ALOE VERA* GEL AS A REDUCING AND STABILIZING AGENT

3.1 Introduction

Among the plants with promising nematicidal potential are *Tabernaemontana elegans* and *Lantana camara* (Ncube *et al*., 2020; Adegbite and Adesiyan, 2005). Previous studies have highlighted the efficacy of *T. elegans* in reducing nematode populations, with reports indicating a reduction of up to 97% in *M. incognita* eggs and J2 (Ncube *et al*., 2020). Similarly, *L. camara* rich in allelochemicals has been observed to be capable of reducing nematode induced root galls and egg masses in infested plants (Adegbite and Adesiyan, 2005). Despite these findings, the phytochemical composition of *T. elegans* remains underexplored, limiting its broader application. This study aims to address this gap by characterizing the phytochemical profiles of *T. elegans* and *L. camara* and evaluating their potential in nanoparticle synthesis.

Nanotechnology offers promising solutions to enhance the efficacy of botanical pesticides and nematicides (Abd-Elgawad, 2024). Nanoencapsulation of botanical compounds improves their stability, bioavailability, and efficiency in pest control (de Oliveira *et al*., 2019; de Oliveira *et al*., 2018). Green synthesis of nanoparticles using plant extracts is a promising approach, combining safety advantages with enhanced antioxidant, antimicrobial, and anticancer properties (Myint *et al*., 2021). Silver nanoparticles (AgNPs) synthesised using *L. camara* leaf extracts demonstrated antioxidant, antibacterial, and cytotoxic properties (Shriniwas and Subhash, 2017). El-Qurashi *et al*. (2023) also demonstrated effectiveness of biologically or green synthesized nanoparticles in suppressing PPN, offering an environmentally sustainable alternative to conventional nematicides. Even though nanotechnology-based formulations of botanical pesticides show great potential for improving agricultural productivity and reducing negative environmental impacts, technological challenges still need to be addressed for wider adoption in agri-food production especially the use of heavy metals like silver and zinc (de Oliveira *et al*., 2018). *Aloe vera*, known for its bioactive components like polyphenols and flavonoids, has emerged as a promising candidate for NP synthesis (Khaldoune *et al*., 2024). The bioactive compounds in plant extracts act as reducing agents, facilitating the conversion of precursor molecules into nanoparticles, while agents like *A.* vera stabilize the nanoparticles, preventing aggregation and enhancing nanoparticle activity (Logaranjan *et al*., 2016).

Conventional nanoparticle synthesis methods often involve heavy metal-based components, which pose environmental risks such as soil contamination and disruption of microbial ecosystems (Kumar *et al*., 2021). Metals like silver (Ag) and zinc (Zn), commonly used in nanoparticle formulations, can exhibit toxicity to soil microorganisms and aquatic systems (Keller *et al*., 2013). To mitigate these risks, this study employs *A. vera* gel as a natural reducing and stabilising agent, eliminating the need for hazardous chemicals. By integrating the phytochemicals of *T. elegans* and *L. camara* with *A. vera* gel, this research seeks to develop sustainable nanoparticles for effective nematode management. For these agents to be effective, they must possess specific properties, including biocompatibility, non-toxicity, and high reducing and stabilizing efficiency (Javed *et al*., 2020). Plant extracts, such as those from *A. vera*, are particularly attractive as dual reducing and capping agents due to their rich composition of bioactive compounds like polysaccharides, flavonoids, and phenolic acids, which contribute to effective and eco-friendly nanoparticle synthesis.

This study represents the first documented use of *A. vera* gel in the green synthesis of nanoparticles from *T. elegans* and *L. camara*. The objective of the study was to develop a protocol for the synthesis of *Tabernaemontana elegans* and *Lantana camara* nanoparticles using *Aloe vera* gel as a reducing and stabilizing agent.

3.2. Materials and methods

3.2.1. Description of the study

The research was conducted under controlled laboratory conditions at the University of Mpumalanga, South Africa, located at latitude 25.4365° S and longitude 30.9818° E. This setting provided an environment conducive for careful preparation, analysis, and synthesis of plant-based nanoparticles.

3.2.2. Reagents used

High-purity chemicals were utilized, including glacial acetic acid, ferric chloride solution (2%), concentrated sulfuric acid (98%), sodium hydroxide, anhydrous sodium carbonate, and trisodium citrate. Copper (II) sulfate pentahydrate and chloroform were also utilized. All reagents were analytical grade and procured from Merck, South Africa. Distilled water served as the solvent for nanoparticle synthesis.

3.2.3. Collection and preparation of plant material

Leaves of *T. elegans* and *L. camara* were collected from Nkomazi Municipality, Mpumalanga, South Africa. The freshly harvested leaves were washed with distilled water to remove debris and contaminants. The leaves were then cut into 5 cm-long sections and air-dried at room temperature for 24 h to remove surface moisture before further drying in an oven. Subsequently, the leaves were dried in a hot air oven set at 52°C for four days to ensure complete moisture removal. The dried leaves were ground into a fine powder using a high-speed blender, operating for 3 m to achieve uniform particle size. The powdered leaf meals were put in labelled glass jars and stored at room temperature (25 °C) with 55 relative humidity until required.

Fresh *A. vera* preparation: fresh leaves were harvested from the University of Mpumalanga, Mbombela campus. The leaves were washed thoroughly with distilled water to eliminate contaminants and microorganisms. The outer layer of the leaves was peeled away using a knife that was sterilised with distilled water to extract the inner gel, which was collected in labelled sterile containers. To prepare an aqueous extract, the gel was blended with distilled water (10 g gel: 100 mL water) to form a colloidal solution. The resulting mixture was filtered through Whatman No. 42 filter paper and stored at 4°C until required (Sohal *et al.*, 2019).

3.2.4. Preparation of aqueous extracts from plant powders

Aqueous extracts were prepared by mixing 1 g of powdered plant material with 10 mL of distilled water in 100 mL conical flasks. The mixtures were agitated on a rotary shaker at 150 rpm for 48 h at room temperature. The resulting solutions were filtered and stored at 4°C for subsequent analysis.

3.2.5. Green synthesis of nanoparticles using *A. vera* gel

Nanoparticles were synthesised by mixing four ratios (25:75, 50:50, 75:25, and 0:100 v/v) of *A. vera* gel with each plant extract. Each individual plant extract mixture prepared was stirred for 12 h at room temperature using a magnetic stirrer. All procedures were performed under aseptic conditions, with triplicates for each ratio to ensure reproducibility.

3.3. Experimental procedure

The experiment was a 2 x 4 x 3 following a randomised complete design with three replicates. The first factor was made up of 2 plants extracts (*Tabernaemontana elegans* and *Lantana camara*), the second factor was made up of 4 temperatures (10 ℃, 15 ℃, 20 ℃, 25 ℃) and the last factor was made up of three ratios (25:75, 50:50, 75:25).

3.3. Characterization of synthesized nanoparticles

3.3.1. Qualitative phytochemical screening

Standard qualitative tests were used to detect key phytochemicals. The appearance of characteristic colors or precipitates confirmed the presence of specific compounds.

Glycosides: The Keller-Kiliani test was employed by adding 2 mL of plant extract to 2 mL of glacial acetic acid and two drops of 2% $FeCl_3$ solution, followed by layering with concentrated H_2SO_4. The appearance of a brown ring at the interface indicated cardiac glycosides.

Flavonoids: The alkaline reagent test involved mixing 2 mL of 2% NaOH solution with the extract. A bright yellow color that faded upon acid addition confirmed the presence of flavonoids.

Reducing sugars: Benedict’s test was conducted by mixing 5 ml of Benedict's reagent with 0.5 ml of the extract in a test tube. The mixture was thoroughly blended and heated for 2 m. After cooling under running tap water, the final color was observed. The appearance of a brick-red precipitate or green coloration indicates the presence of reducing sugars.

Alkaloids: Mayer’s test involved adding Mayer’s reagent to 1 mL of extract mixed with 2 mL of concentrated H_2SO_4. The appearance of a green precipitate indicated alkaloids.

Saponins: The foam test was performed by shaking 2 mL of extract with 5 mL of distilled water. Persistent foam indicated the presence of saponins.

Phenols and Tannins: Mixing 1 mL of extract with 2 mL of 2% $FeCl_3$ produced a blue-green or black tint, confirming their presence.

Terpenoids: A reddish-brown interface resulting from the addition of chloroform and concentrated H_2SO_4 to the extract indicates presence of terpenoids.

Steroids: The formation of a red or green color upon treatment with H_2SO_4 and chloroform confirmed the presence of steroids.

3.3.2. Visual observation and UV-Vis spectra analysis

UV-Vis spectroscopy was conducted using a Perkin Elmer Lambda 265 spectrophotometer (Perkin Elmer Lambda, Shimadzu, Japan). Spectra were recorded between 200 and 700 nm using a 1 cm pathlength quartz cuvette, with distilled water as the blank. Data smoothing was performed using the Savitsky-Golay method in Origin Lab software.

3.3.3. Transmission electron microscopy (TEM)

Nanoparticle morphology was analysed using a TEM (JEM-2100, Joel, Japan) operated at 200 kV. Samples were prepared by drop-casting onto holey carbon-coated copper grids and drying at 60 °C overnight in a desiccator. Particle size distribution was determined using ImageJ (NIH, USA) software. Origin Lab 2024 software was used to plot the histogram graphs.

3.3.4. Scanning electron microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDS)

Morphological characteristics were observed using SEM (FEGSEM Zeiss Ultraplus, Japan). Samples were mounted on carbon tape, gold-coated, and analysed at 20 kV. EDS was performed using AZTEC software to determine elemental composition.

3.3.5. Selected area electron diffraction (SAED) patterns

Selected Area Electron Diffraction (SAED) is a critical technique used to analyse the crystallographic properties of nanoparticles during their formation (Czigány. and Kis, 2023). A small drop of this suspension was deposited onto a TEM grid made of copper and coated with a thin carbon film to provide mechanical stability. Excess liquid was removed using filter paper, and the sample was left to dry at room temperature. This preparation ensures that nanoparticles are well-dispersed on the grid, minimizing agglomeration and overlap. The SAED patterns obtained were analysed to determine the crystallographic properties of the nanoparticles. The diffraction spots or rings correspond to specific lattice planes, and their spacing (d-spacing) is calculated using Bragg's Law. The radius of each ring in the diffraction pattern is measured and correlated with the constant camera, which is determined during the instrument calibration. Using these measurements, the lattice parameters and crystalline of the nanoparticles are inferred. To ensure reliability, the SAED measurements were repeated for multiple regions on the sample grid and for different samples prepared under the same conditions. Consistency in the diffraction patterns validates the reproducibility of the nanoparticle synthesis process and the SAED measurement.

3.3.6. Energy-dispersive X-ray spectroscopy

An Energy-dispersive X-ray spectroscopy (EDX) analysis was carried out to determine the chemical purity and elemental composition of the synthesized NPte, NPlc and the individual extracts.

3.4 Results

3.4.1 Phytochemical screening

The Tables (3.1-3.3) provide a detailed phytochemical analysis of *L. camara*, *T. elegans* and their respective combinations with *A. vera* gel across different concentrations and temperatures during nanoparticle synthesis. These results highlight the presence, abundance, and variability of bioactive compounds critical for nematicidal applications.

Table 3.1 illustrates that *L. camara* exhibited a high abundance (+++) of glycosides, flavonoids, alkaloids, tannins, phenols and moderate presence (++) of saponins, reducing sugars and terpenoids. Similarly, *T. elegans* displayed abundant levels of alkaloids, tannins, phenols, and saponins, with moderate levels of flavonoids, reducing sugars and glycosides. Notably, terpenoids were absent in *T. elegans*, which may limit its range of bioactive effects compared to *L. camara*. *Aloe vera*, used as a stabilising and reducing agent, demonstrated abundant steroids, saponins, and phenols, which contribute to its role in nanoparticle synthesis and stabilization. *Aloe vera* had all the phytochemicals tested in abundance (+++) except for alkaloids (++), glycosides (+) and terpenoids (+).

Table 3.2 focuses on the phytochemical profile of *L. camara* nanoparticles synthesised with *A. vera* gel at various concentrations and temperatures. Generally, adding *A. vera* increased the levels of phytochemicals such as saponins, terpenoids and reducing sugars on the *L. camara* nanoparticles (Table 3.2). Across all concentrations, glycosides, flavonoids, tannins, phenols, saponins, and terpenoids were consistently abundant (+++). Steroid levels, however, varied with concentration and temperature, being more prominent at 50:50 and 25°C. The abundance of reducing sugars was concentration-dependent, increasing with higher levels of *A. vera* in the formulation, however, temperature did not have a significant effect on their presence.

Table 3.3 presents the phytochemical screening of nanoparticles synthesized using *T. elegans* and *A. vera*. As with *L. camara* nanoparticles, adding *A. vera* improved the levels of glycosides, flavonoids, steroids, terpenoids and reducing sugars in *T. elegans* synthesised nanoparticles (Table 3.3). Glycosides, flavonoids, tannins, and phenols were consistently abundant (+++) across all formulations and conditions, indicating their stability during the synthesis process. However, variability was observed in saponins, steroids, reducing sugars and terpenoids. Saponins and terpenoids showed moderate presence (++) in certain formulations, while steroids displayed significant variation, being traceable (+) in some cases and abundant (+++) in others, particularly at 25°C. The presence of reducing sugars was influenced by the concentration of *A. vera*, with higher proportions, such as a 25:75 ratio (extract to *A. vera*), showing a greater presence of reducing sugars. Temperature had no significant impact on the presence of reducing sugars.

Table 3.1: Phytochemical screening profile of leaf meal extracts of *Lantana camara*, *Tabernaemontana elegans* and *Aloe vera*

	Plant species				
Phytochemical constituent	LC	TE	*Aloe vera*	Observation/ color change	Reference
Glycosides	+++	+	+	Brown ring	Keller-kilani test (Prakash *et al*. 2017)
Flavonoids	+++	++	+++	Bright yellow color	Alkaline reagent test (Bista *et al*. 2020)
Alkaloids	+++	+++	++	Green colour	Mayer's test (Vijayalakshmi *et al*., 1979)
Tannins	+++	+++	+++	Black or blue-green tint	Bista *et al*. (2020)
Phenols	+++	+++	+++	Black or blue-green tint	Ferric chloride test (Bista *et al*. 2020)
Saponins	++	+++	+++	Development of foam	Foam test (Ehiowemwenguan *et al*. 2014)
Steroids	+	+	+++	Red colour and green	Bista *et al*. (2020)
Terpenoids	++	-	+	Reddish brown monolayer	Bharathi *et al*. (2017)
Reducing sugars	++	++	+++	brick-red precipitate or green coloration	Shubhan *et al*. (2019)

(+++) = abundantly, (++) = moderate present, (+) traceable (-) = not detected, LC = *Lantana camara*, TE = *Tabernaemontana elegans*

Table 3.2: Phytochemicals screening for concentration of *Lantana camara*: *Aloe vera* nanoparticles (NPlc)

Phytochemicals	Temperature											
	10°C			15°C			20°C			25°C		
	Concentration of *Lantana camara*: *Aloe vera*											
	25:75	75: 25	50:50	25: 75	75: 25	50: 50	25: 75	75: 25	50: 50	25:75	75:25	50:50
Glycosides	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Flavonoids	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Tannins	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Phenols	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Saponins	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Steroids	++	+	+++	++	+	++	++	++	++	+++	++	+++
Terpenoids	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Reducing sugars	+++	+	++	+++	+	++	+++	+	++	+++	+	++

(+++) = abundantly, (++) = moderate present, (+) traceable (-) = not detected

Table 3.3: Phytochemicals screening of *Tabernaemontana elegans* and *Aloe vera* nanoparticles (NPte)

Phytochemicals	Temperature											
	10°C			15°C			20°C			25°C		
	Concentration of *Tabernaemontana elegans*: *Aloe. vera*											
	25:75	75: 25	50:50	25: 75	75: 25	50: 50	25: 75	75: 25	50: 50	25:75	75:25	50:50
Glycosides	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Flavonoids	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Tannins	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Phenols	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Saponins	+++	++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
Steroids	++	-	+	+++	+	++	+++	+++	+++	+++	+++	+++
Reducing sugars	+++	+	++	+++	+	++	+++	+	++	+++	+	++
Terpenoids	++	+	++	++	+	++	+++	++	+++	+++	++	+++

(+++) = abundantly, (++) = moderate present, (+) traceable (-) = not detected

3.4.2 UV-Visa spectrophotometer

The UV-Vis spectra illustrate distinct absorbance trends and spectral shifts among the *L. camara* extract (LC), *A. vera* extract (Aloe), and the nanoparticle (50:50) (LC: Aloe) (Figure 3.1). Each sample exhibits characteristic peaks, reflecting differences in their chemical composition and interaction with UV light. The *L. camara* extract displays peaks at 215 nm, 240 nm, and 300 nm, with smaller peaks at 325 nm and 370 nm**.** The intensity of absorbance is moderate across the spectrum (Figure 3.1).

The *A. vera* extract has peaks at 230 nm, 255 nm, and 275 nm, with additional peaks at 315 nm and 370 nm (Figure 3.1). Compared to *L. camara*, the peaks for *A. vera* appear at slightly longer wavelengths, and absorbance intensities are higher than those of *L. camara*. In the 50:50 NPlc, peaks appear at 215 nm**,** 240 nm**,** 300 nm**,** 325 nm**,** and 370 nm, with additional broader peaks at 400 nm and 420 nm (Figure 3.1). The presence of broader and more intense peaks, especially in the visible region (400–420 nm). The nanoparticle spectrum also shows peak shifts and overlaps between the individual extracts (Figure 3.1). When comparing the three spectra, NPlc exhibits higher overall absorbance and broader peaks, especially in the UV-visible range.

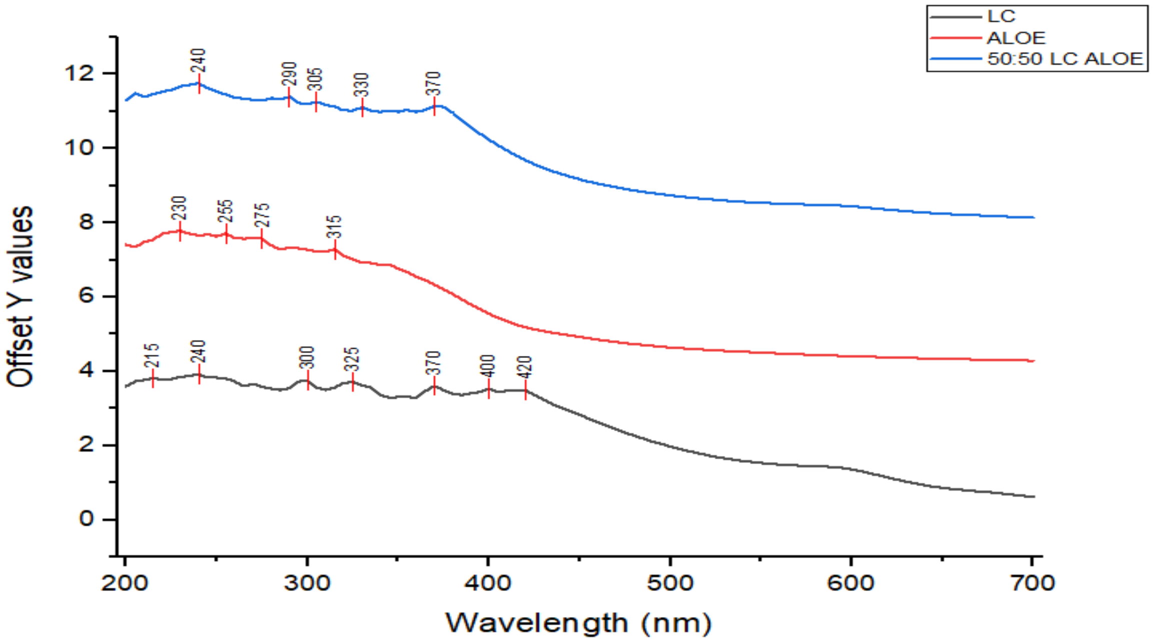


Figure 3.1: UV-Vis spectra of synthesised NPlc at 50:50 (LC: *A. vera*), Aloe (*Aloe vera*) 100% and LC (*Lantana camara*) 100%.

For the 25:75 (LC: Aloe) nanoparticle (NPlc), the spectrum shows peaks at 235 nm, 285 nm, and 365 nm, with broader peaks in the visible region around 400 nm and 420 nm (Figure 3.2). Compared to the individual extracts, the peaks have shifted and broadened. The absorbance intensity is the highest among the three samples, particularly in the UV-visible range (Figure 3.2).

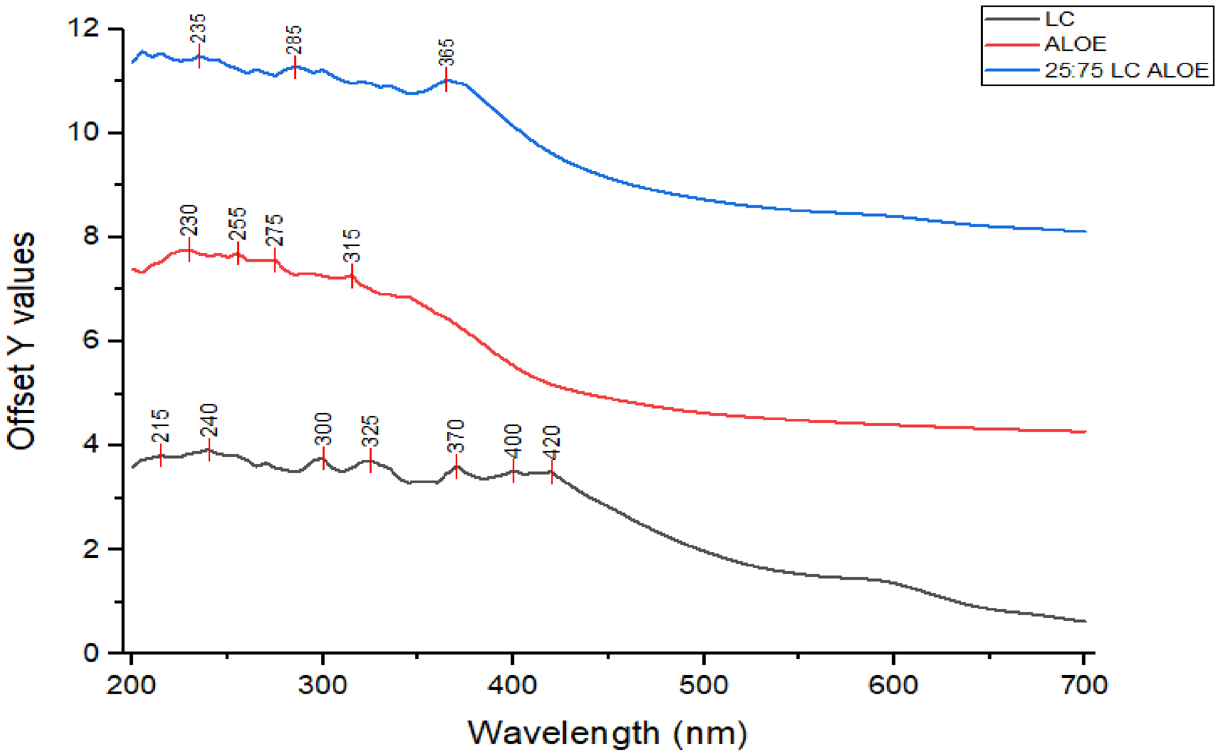


Figure 3.2: UV-Vis spectra of synthesised NPlc at 25:75 (LC: *A. vera*) ratio, Aloe (*Aloe vera*) 100% and LC (*Lantana camara*) 100%.

The 75:25 (LC: Aloe) synthesised nanoparticle shows both shifts in the positions of existing peaks and the appearance of new peaks (Figure 3.3). The peaks observed in the *L. camara* and *A. vera* extracts are slightly shifted, suggesting an interaction between the biomolecules from both extracts during the nanoparticle formation process (Figure 3.3). Additionally, new peaks in the NPlc spectrum indicate the formation of distinct molecular species or structural changes due to nanoparticle synthesis (Figure 3.3).

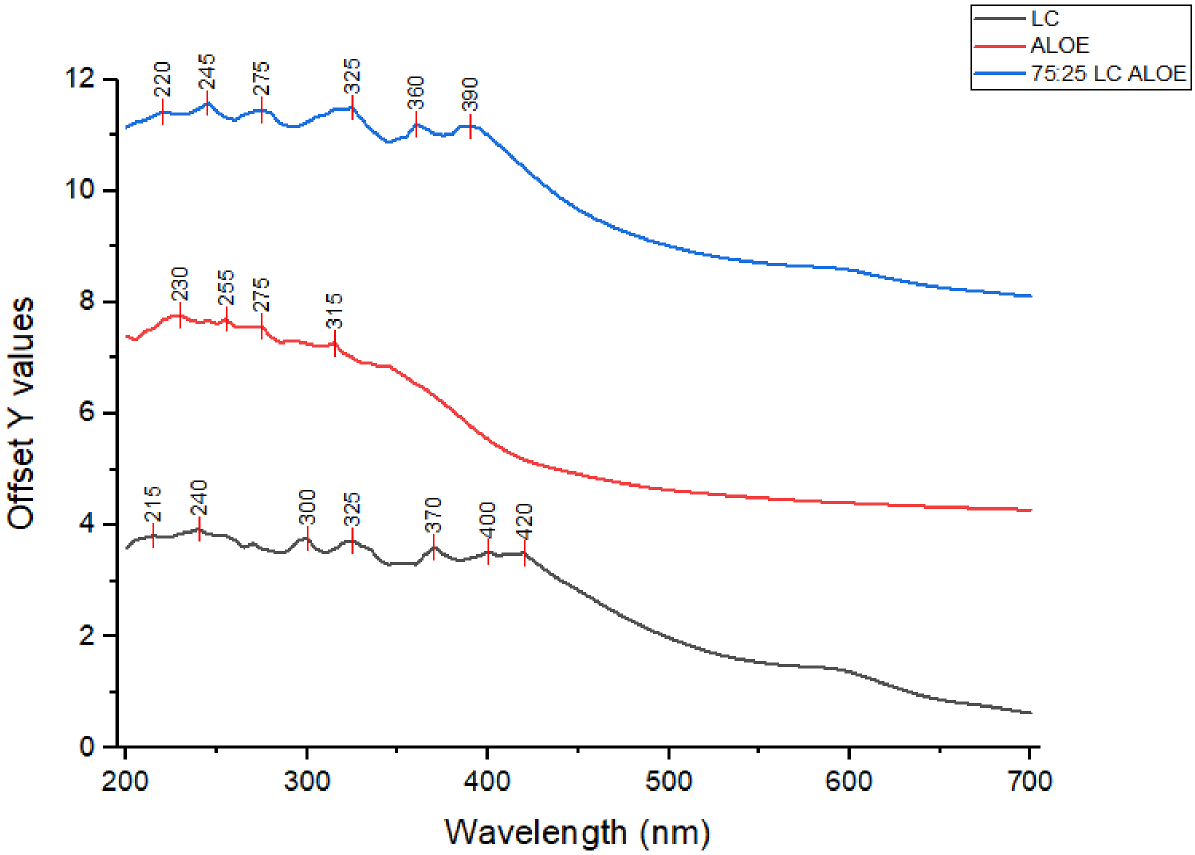


Figure 3.3: UV-Vis spectra of synthesised NPlc at 75:25 (LC: *A. vera*) ratio, Aloe (*Aloe vera*) 100% and LC (*Lantana camara*) 100%.

The UV-Vis spectra show distinct trends and shifts in absorption peaks across the three samples: *T. elegans* (TE) extract, *A. vera* (Aloe) extract, and the synthesised NPte formed using a 50:50 combination of *T. elegans* and *A. vera* (Figure 3.4). These changes provide insights into the molecular interactions and potential nanoparticle formation mechanisms (Figure 3.4). The *T. elegans* extract exhibits prominent peaks at approximately 235 nm, 280 nm, 315 nm, 360 nm, and 405 nm (Figure 3.4). These peaks are characteristic of specific phytochemicals, such as aromatic compounds and conjugated systems, which are commonly found in plant extracts (Figure 3.4). The *A. vera* extract displays absorption peaks at 230 nm, 275 nm, 315 nm, 350 nm, 400 nm, and 420 nm. Compared to *T. elegans*, the peaks for *A. vera* are slightly shifted, with additional peaks at higher wavelengths (400 nm and 420 nm) (Figure 3.4). The 50:50 TE:Aloe nanoparticle sample shows a combination of features from both extracts, but with notable changes in peak intensity and wavelength shifts. Peaks are observed at 230 nm, 260 nm, 295 nm, 330 nm, 400 nm, and 420 nm (Figure 3.4). Compared to the individual extracts, the nanoparticle spectrum shows new peaks at 260 nm and 295 nm, indicating the formation of new molecular species or interactions between the phytochemicals of *T. elegans* and *A. vera* during nanoparticle synthesis (Figure 3.4). The increased intensity of the peaks at 400 nm and 420 nm reflects the successful stabilization of nanoparticles by *A. vera*, which provides reducing agents to facilitate the process. Overall, the shifts in peak positions and the appearance of new peaks in the nanoparticle spectrum confirm the successful integration of *T. elegans* and *A. vera* phytochemicals and the formation of a stabilized nanoparticle system. These results highlight the synergistic effects of combining *T. elegans* and *A. vera* in nanoparticle synthesis, with *A. vera* contributing significantly to reduction and stabilization.

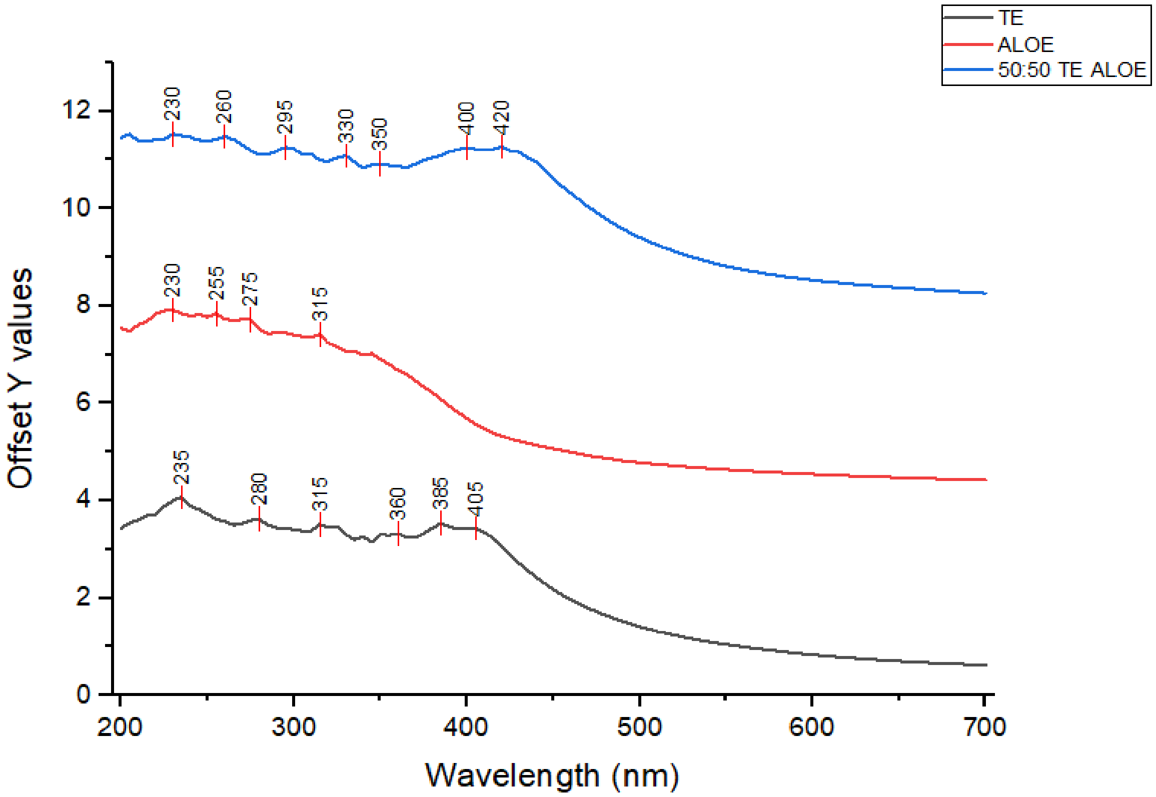


Figure 3.4: UV-Vis spectra of synthesised NPte at 50:50 (TE: *A. vera*) ratio, Aloe (*Aloe vera*) 100% and TE (*Tabernaemontana elegans*) 100%.

The synthesized nanoparticles using a 25:75 (TE: Aloe) show further shifts and the appearance of new peaks. Peaks are observed at 240 nm, 305 nm, 330 nm, 360 nm, and 385 nm (Figure 3.5). Compared to both *T. elegans* and *A. elegans*, the nanoparticle spectrum features a new peak at 240 nm and significant shifts in peaks previously seen at 315 nm (shifted to 305 nm) and 385 nm (enhanced intensity) (Figure 3.5). The shift of peaks and the appearance of new absorption features confirm the structural and electronic modifications resulting from the reduction and stabilization processes facilitated by *A. vera* (Figure 3.5).

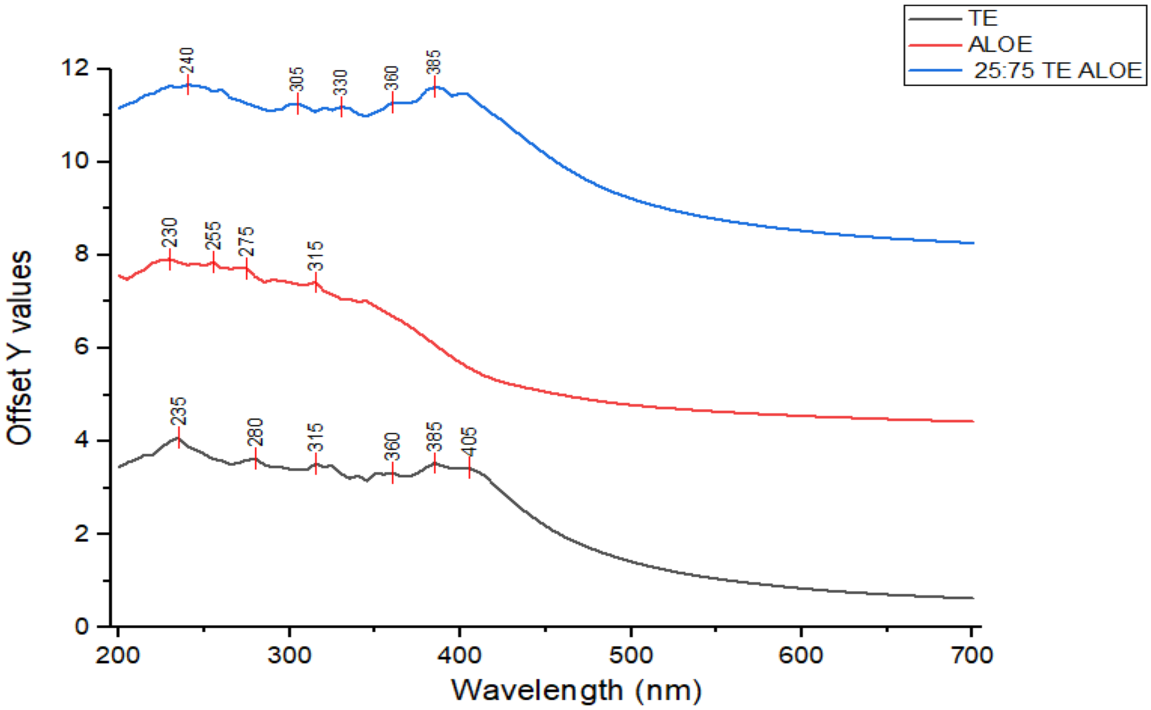


Figure 3.5: UV-Vis spectra of synthesised NPte at 25:75 (TE: *A. vera*) ratio, Aloe (*Aloe vera*) 100% and TE (*Tabernaemontana elegans*) 100%.

The 75:25 (TE: Aloe) nanoparticle spectrum demonstrates significant modifications (Figure 3.6). The peaks are observed at 240 nm, 305 nm, 330 nm, 360 nm, and 385 nm. A new peak at 240 nm, alongside the shifts of existing peaks (315 nm to 330 nm and 385 nm to 370 nm), indicates the formation of new molecular species during nanoparticle synthesis (Figure 3.6).

These changes suggest strong interactions between *T. elegans* and *A. vera* phytochemicals during the reduction and stabilization process (Figure 3.6). The 405 nm peak, which remains consistent but exhibits increased intensity, suggests the stabilization of the nanoparticles and the retention of some original *T. elegans* chromophores (Figure 3.6).

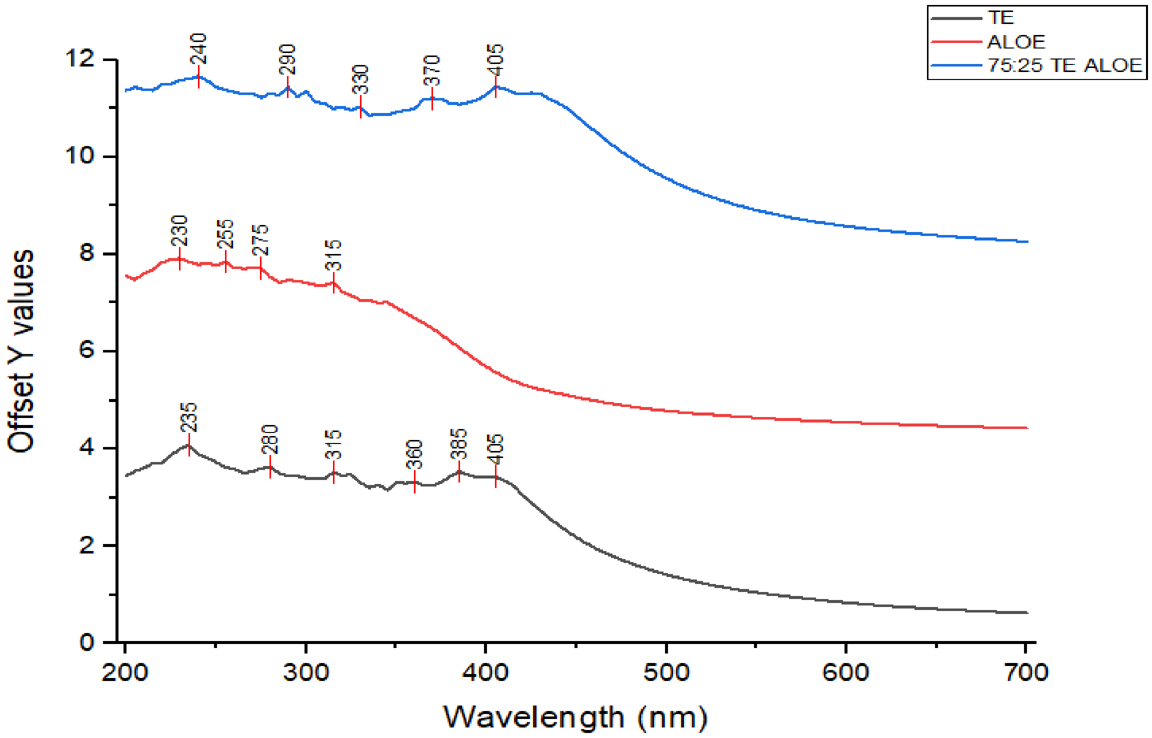


Figure 3.6: UV-Vis spectra of synthesised NPte at 75:25 (TE: A. vera) ratio, Aloe (*Aloe vera*) 100% and TE (*Tabernaemontana elegans*) 100%.

3.4.3 Transmission electron microscopy (TEM)

The Transmission Electron Microscopy (TEM) analysis provided detailed visualization of the structural and morphological properties of the synthesized nanoparticles. Nanoparticles derived from *T. elegans* (NPte) and *L. camara* (NPlc) exhibited a variety of shapes, including cubic, triangular, platelet, and irregular forms (Figures 3.7–3.16). For NPte, particle size varied significantly with the concentration ratios of the plant extracts. The 50:50 concentration

(Figure 3.7) exhibited the largest particle size, averaging 13.04 nm, followed by the 25:75 concentration (Figure 3.8) at 10.30 nm, while the 75:25 concentration (Figure 3.9) produced the smallest average particle size at 5.85 nm.

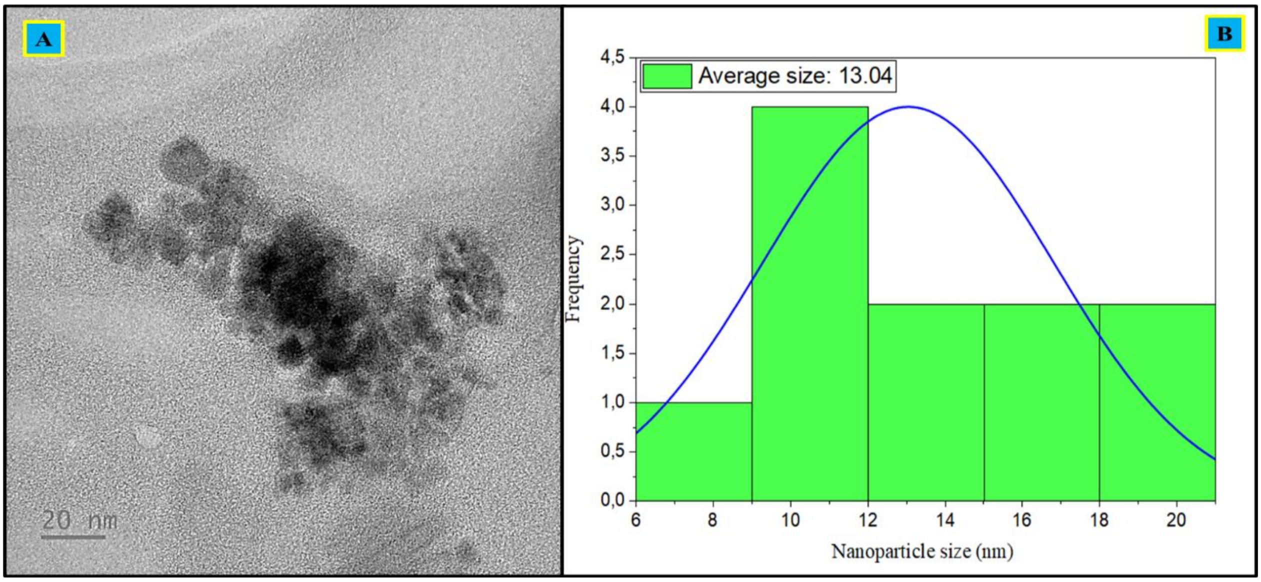


Figure 3.7: Transmission electron microscopy (TEM) images displaying *T. elegans* nanoparticles (NPte) at a 50:50 concentration ratio of *T. elegans* to *A. vera*, captured at magnifications of 20 nm (A). Particle size distributions curve (B) of NPte with an average of 13.04 nm

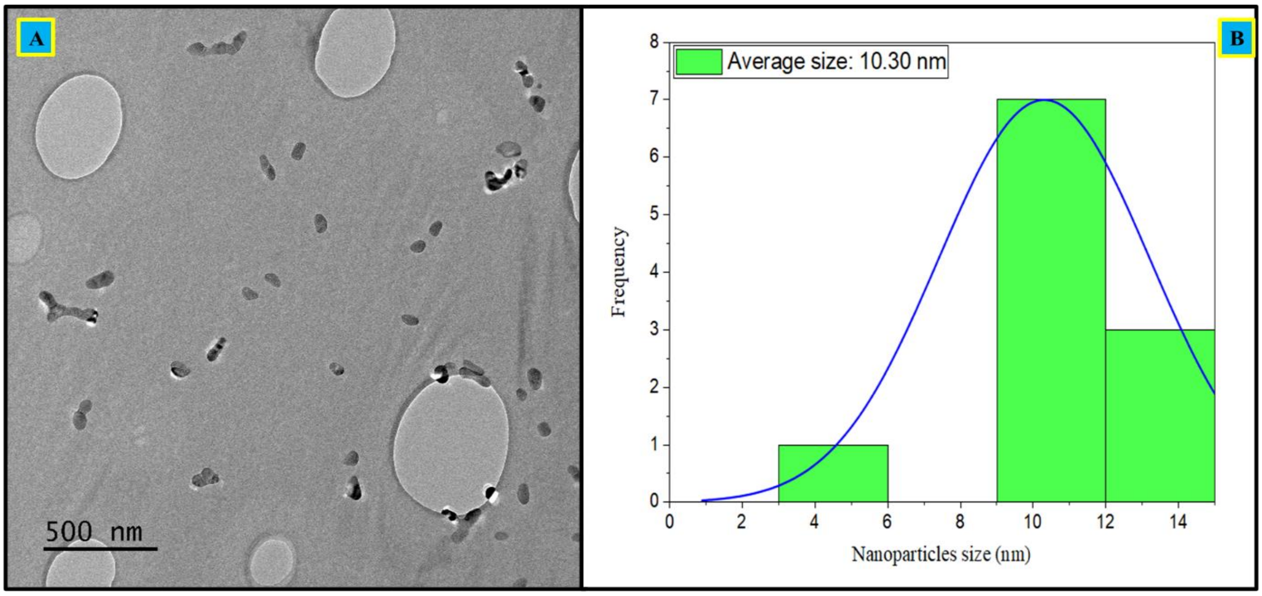


Figure 3.8: Transmission electron microscopy (TEM) images displaying *T. elegans* nanoparticles (NPte) at a 25:75 concentration ratio of *T. elegans* to *A. vera*, captured at magnifications of 500 nm (A). Particle size distributions curve (B) of NPte with an average of 10.30 nm

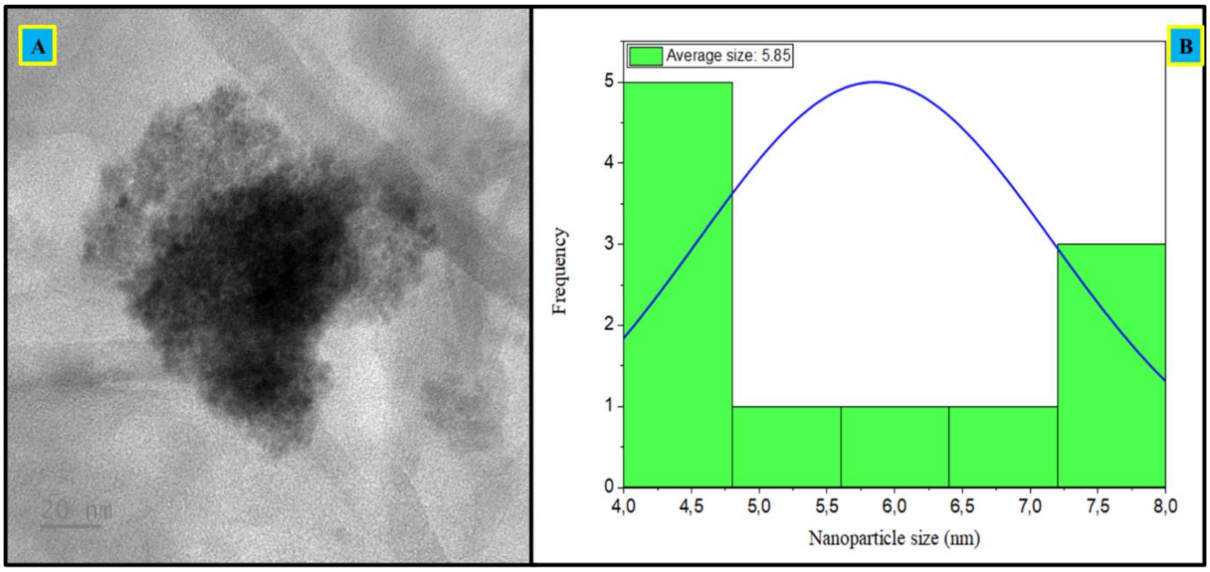


Figure 3. 9: Transmission electron microscopy (TEM) images displaying *T. elegans* nanoparticles (NPte) at a 75:25 concentration ratio of *T. elegans* to *A. vera*, captured at magnifications of 20 nm (A). Particle size distributions curve (B) of NPte with an average of 5.85 nm.

For NPlc, the particle sizes followed a similar trend, with the 75:25 concentration (Figure 3.10) producing the largest average particle size at 21.02 nm, followed by the 25:75 concentration (Figure 3.11) at 10.37 nm, and the smallest average particle size of 5.03 nm, observed in the 50:50 concentration (Figure 3.12).

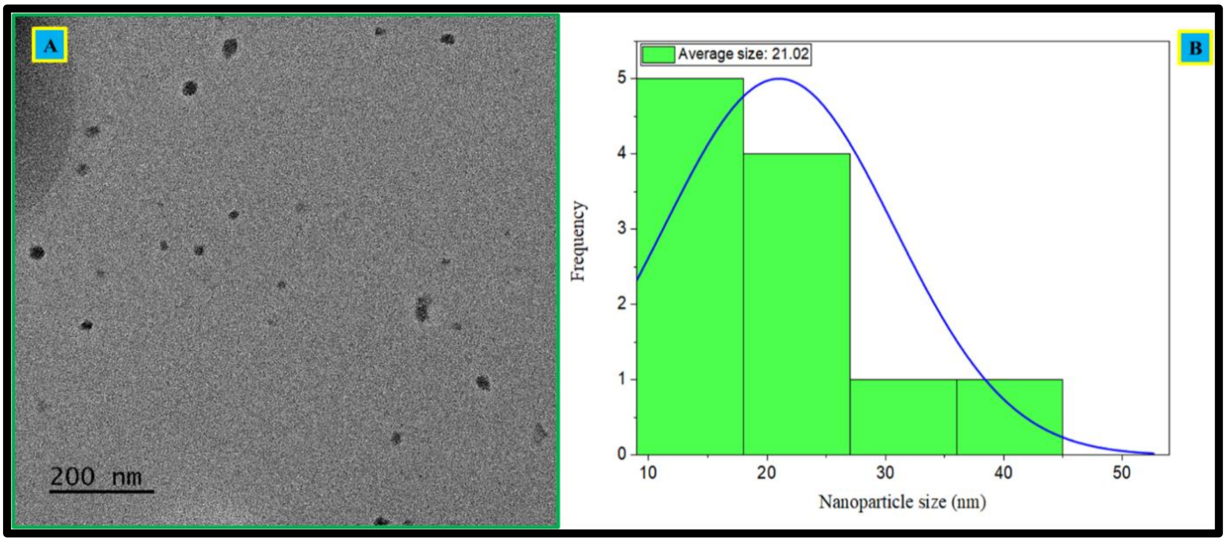


Figure 3.10: Transmission electron microscopy (TEM) images displaying *L. camara* nanoparticles (NPlc) at a 75:25 concentration ratio of *L. camara* to *A. vera*, captured at magnifications of 200 nm (A). Particle size distributions curve (B) of NPlc with an average of 21.02 nm

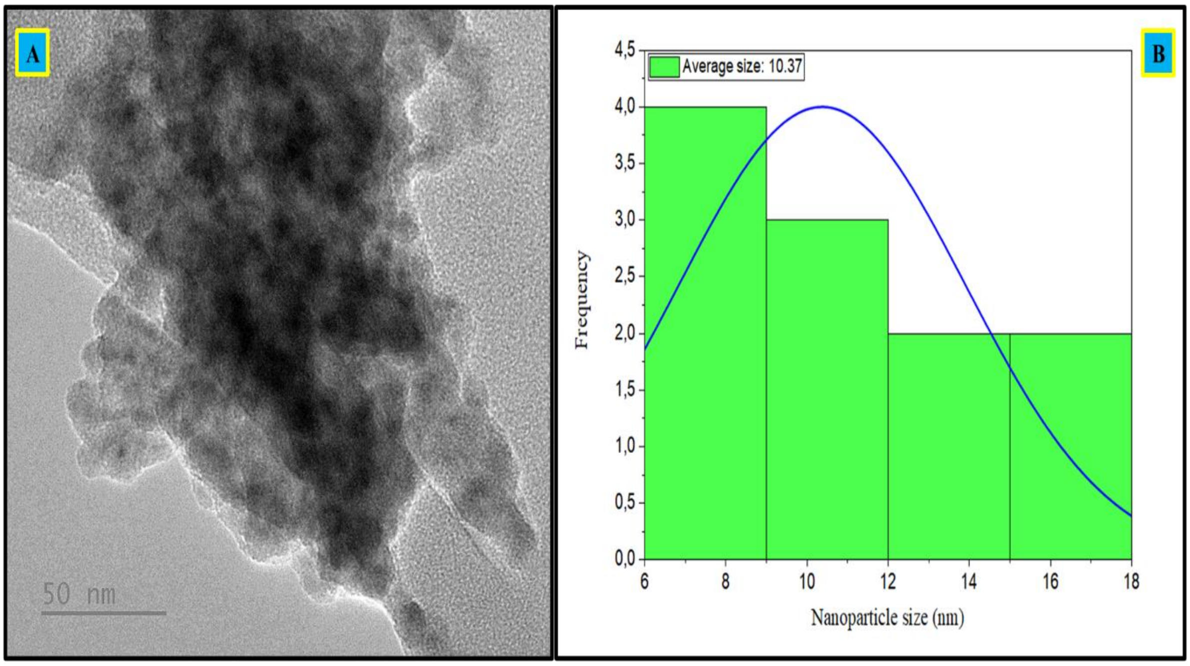


Figure 3.11: Transmission electron microscopy (TEM) images displaying *L. camara* nanoparticles (NPlc) at a 25:75 concentration ratio of *L. camara* to *A. vera*, captured at magnifications of 50 nm (A). Particle size distributions curve (B) of NPlc with an average of 10.37 nm

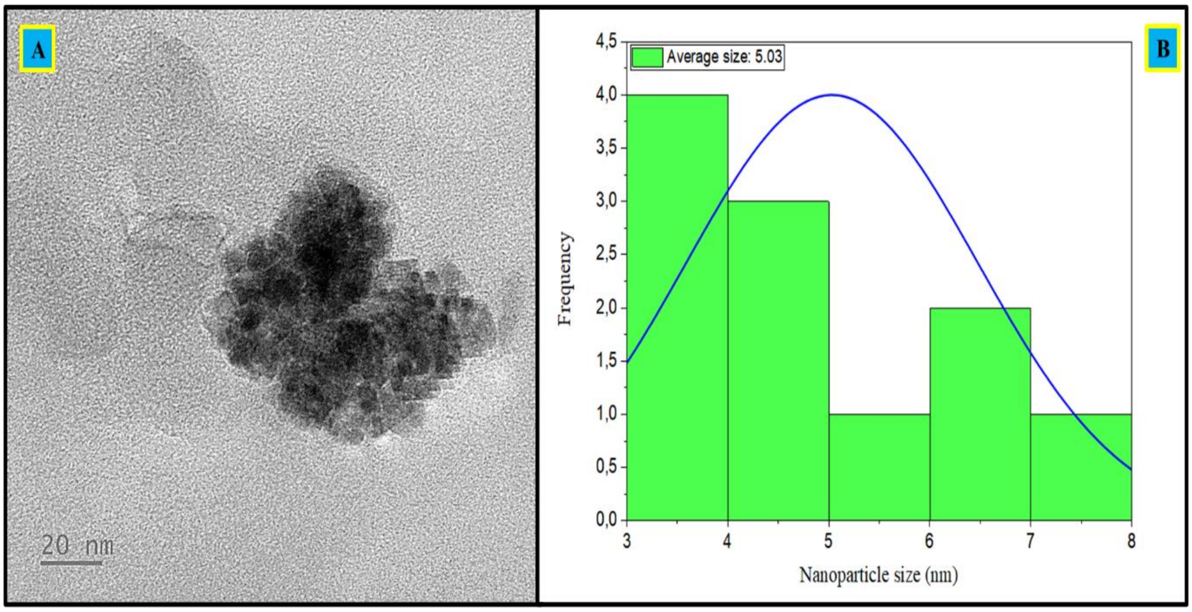


Figure 3.12: Transmission electron microscopy (TEM) images displaying *L. camara* nanoparticles (NPlc) at a 50:50 concentration ratio of *L. camara* to *A. vera*, captured at magnifications of 20 nm (A). Particle size distributions curve (B) of NPlc with an average of 5.03 nm

Particle size from *L. camara* (Figure 3.13) and *A. vera* (Figure 3.14) exhibited significantly larger average particle sizes, measuring 482 nm and 507.52 nm, respectively. In comparison, particles size from *T. elegans* (Figure 3.15) were notably larger, averaging 7290 nm.

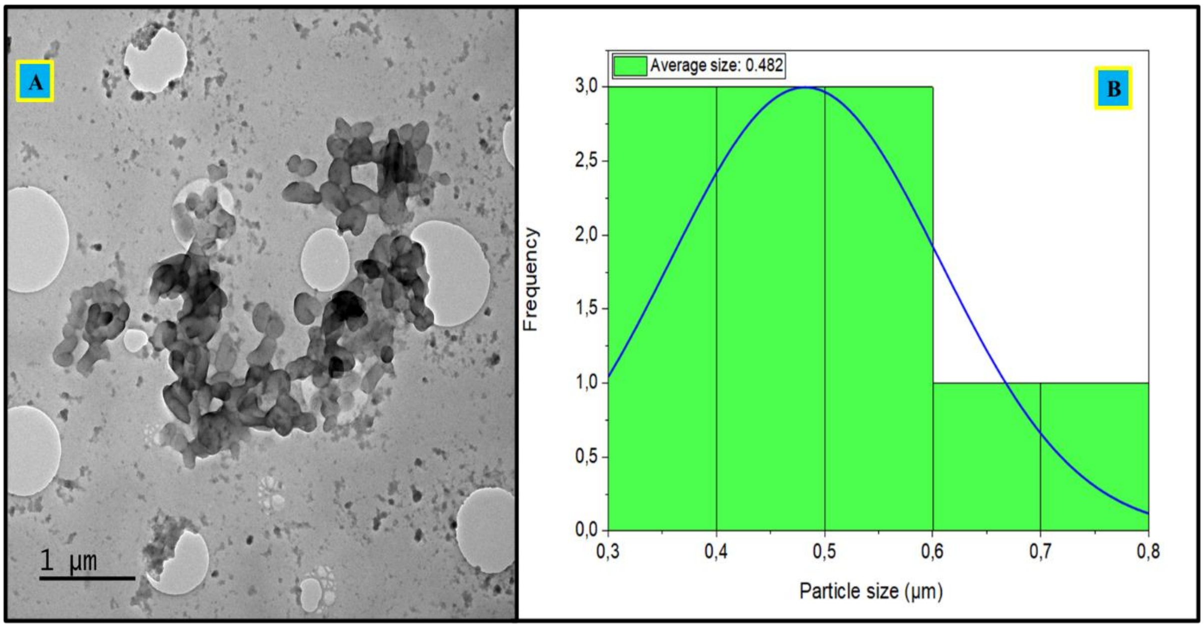


Figure 3. 13:TEM images of *L. camara* particles at a 100% concentration, captured at

magnifications of 1 µm (A). Particle size distributions curve (B) with an average of 0.482 µm (482 nm).

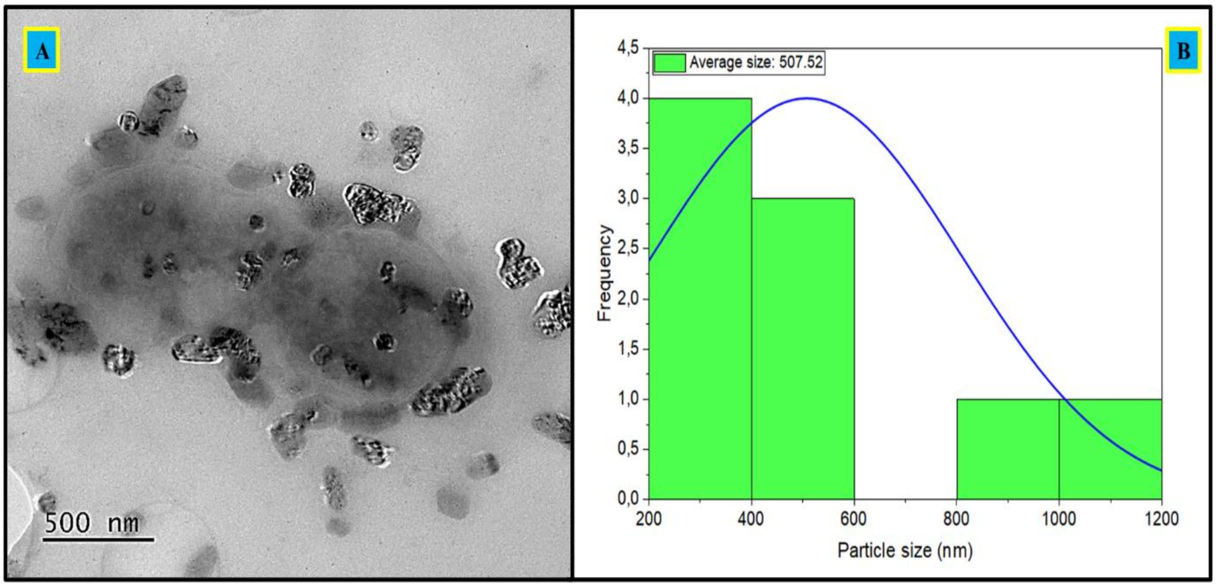


Figure 3.14: TEM images of *A. vera* particles at a 100% concentration, captured at magnifications of 500 nm (A) and (B). Particle size distributions curve (B) with an average of 507.52 nm.

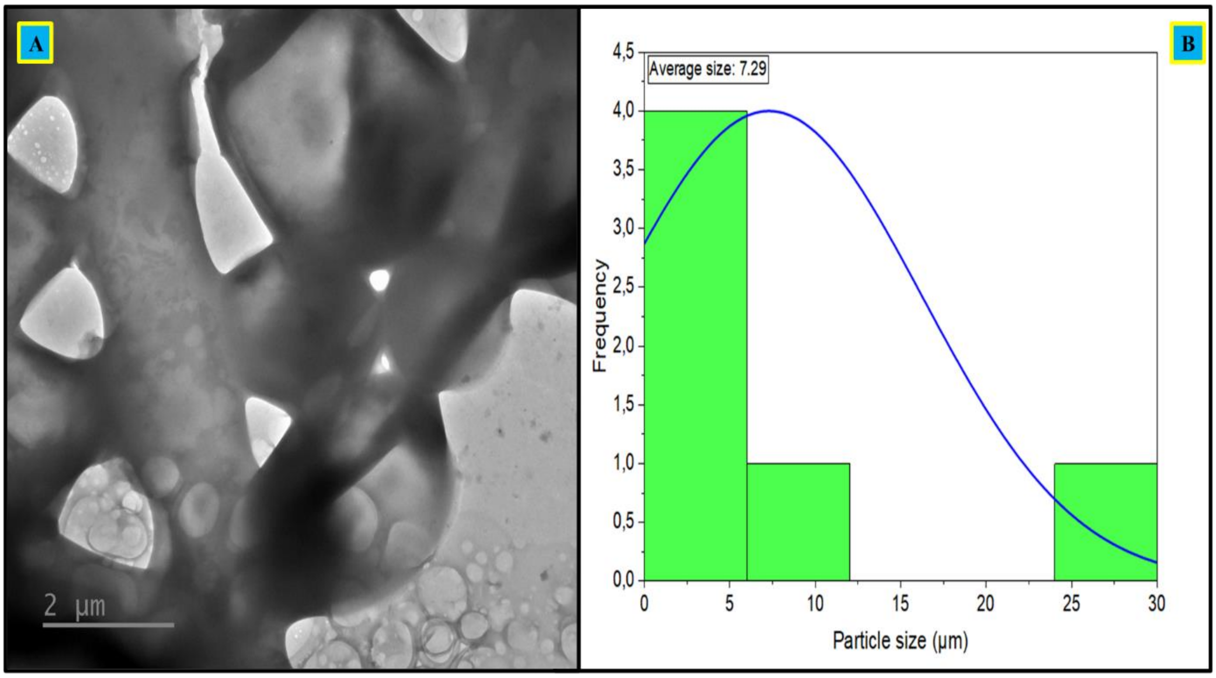


Figure 3.15: TEM images of *T. elegans* particles at a 100% concentration, captured at magnifications of 500 nm (A). Particle size distributions curve (B) with an average of 7.29 µm (7290 nm).

3.4.4. Selected area electron diffraction (SAED) patterns

The Selected Area Electron Diffraction (SAED) patterns confirmed the polycrystalline nature of the synthesized nanoparticles, indicating their structural stability and crystallinity. The SAED analysis revealed that the ratio of plant extracts used during synthesis played a critical role in determining the structural characteristics of the nanoparticles. For the NPlc samples, the 50:50 ratio exhibited a highly defined ring pattern with bright and distinct spots, indicative of well-developed polycrystalline structures (Figure 3.16). However, at the lower proportion of *A. vera* (75:25), the ring patterns became less defined with reduced sharpness and uniformity. A similar trend was observed in the NPte samples. The 50:50 ratio of NPte and NPlc displayed sharp and bright SAED patterns.

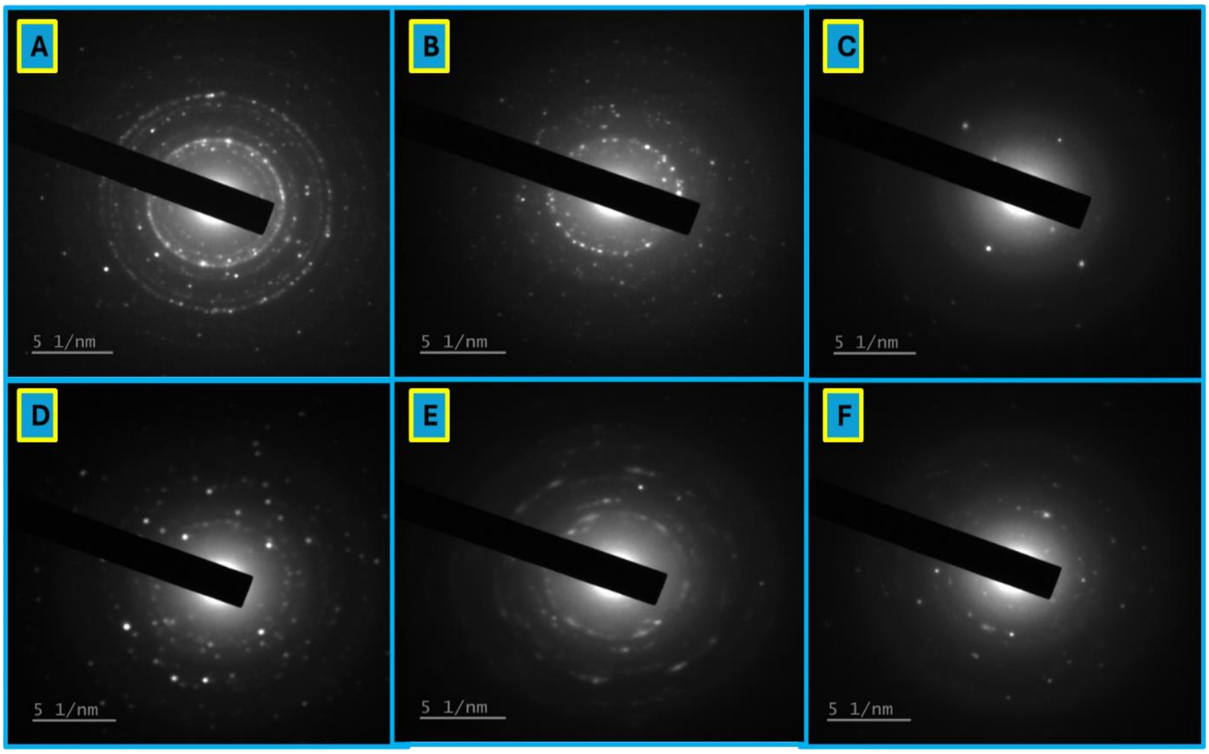
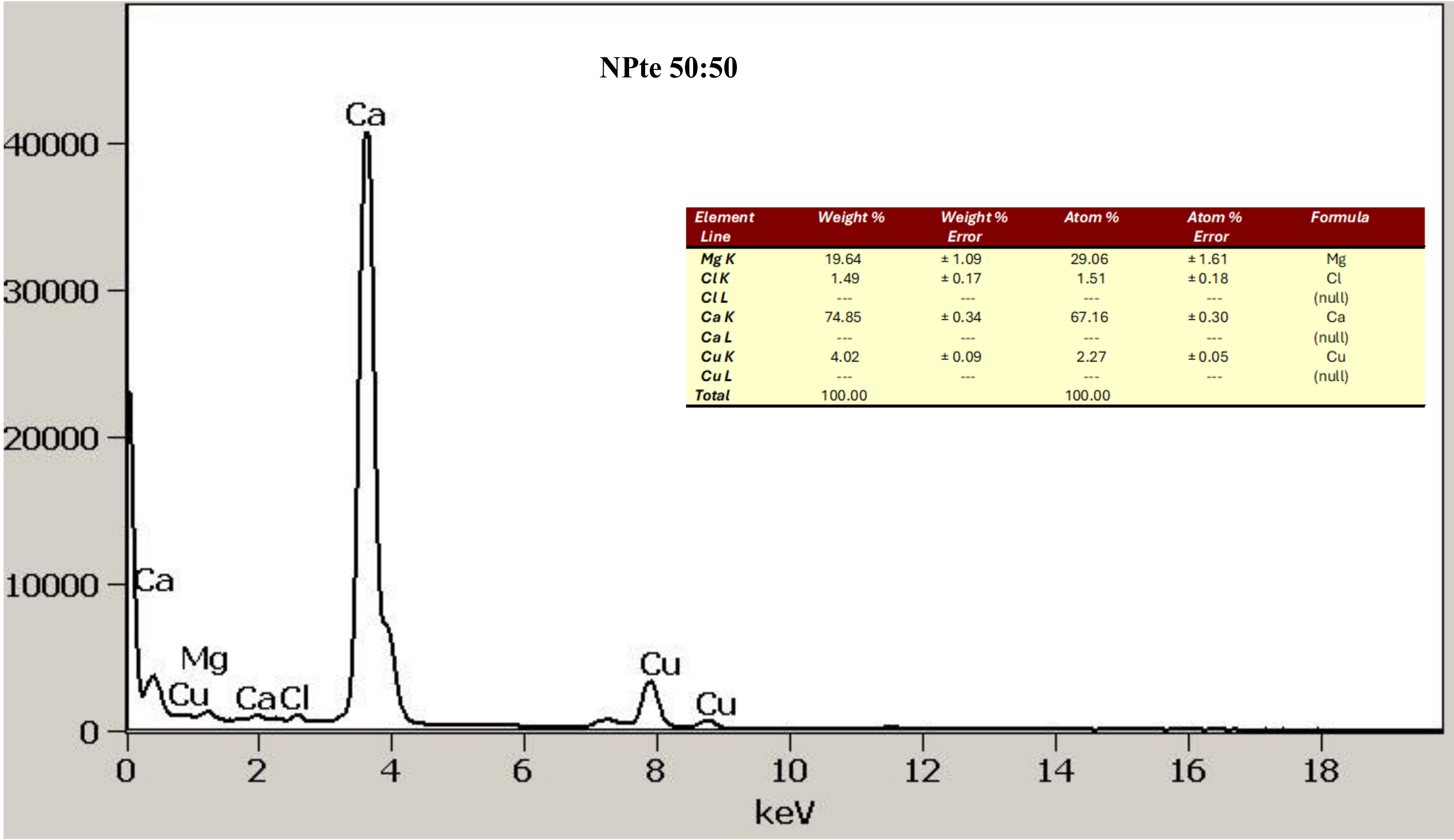


Figure 3. 16: Ring SAED pattern of polycrystalline of extract *A. vera*: (A) 50:50 NPlc; (B) 25:75 NPlc; (C) 75:25 NPlc; (D) 50:50 NPte; (E) 25:75 NPte; (D) 75:25 NPte

3.4.5. Energy-dispersive X-ray spectroscopy

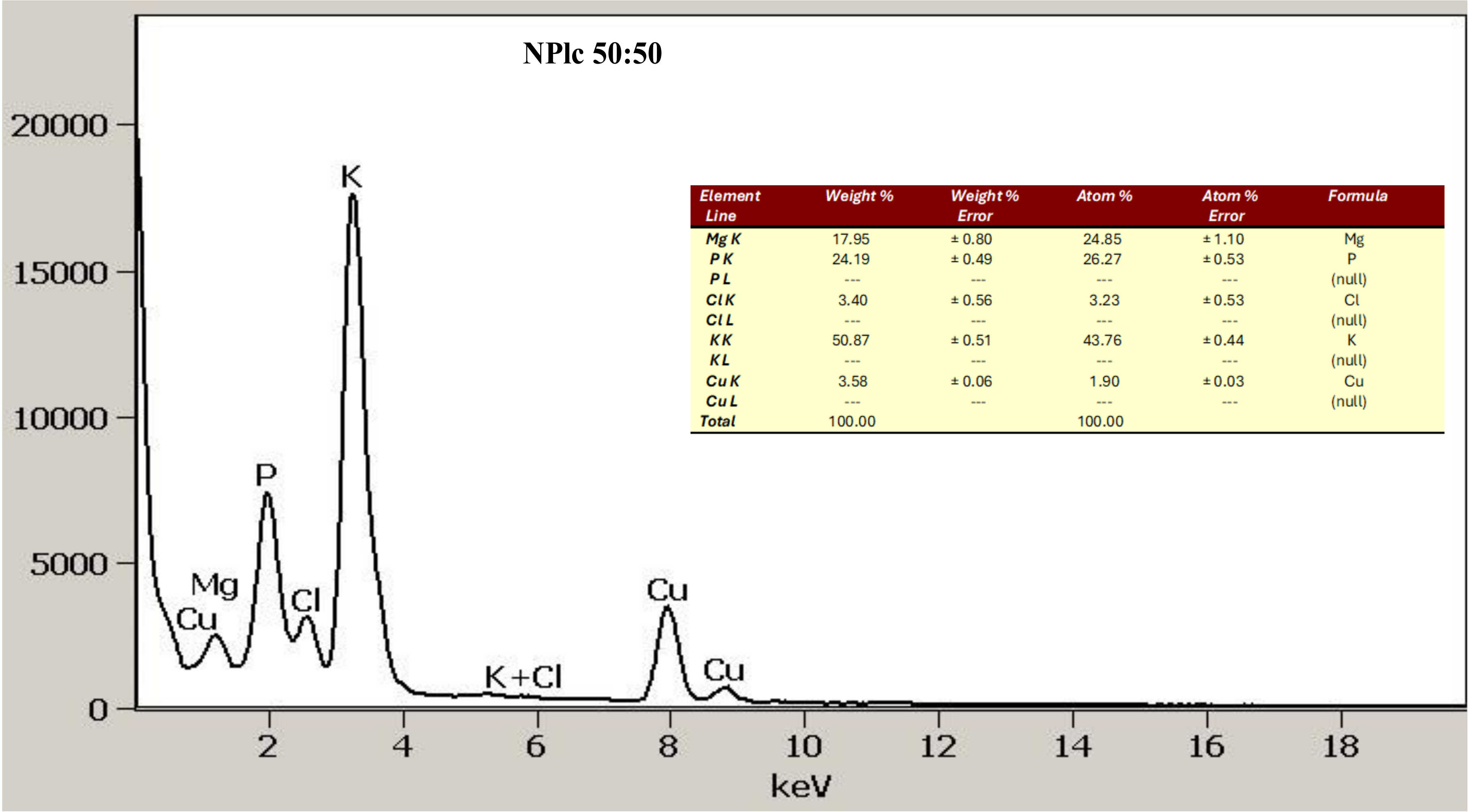
The Energy-dispersive X-ray spectroscopy (EDS) spectrum shown provides a detailed elemental composition of the sample labelled NPte 50:50 (Figure 3.17). The spectrum indicates prominent peaks for calcium (Ca), magnesium (Mg), copper (Cu), and chlorine (Cl), suggesting the presence of these elements in varying quantities. The quantitative table reveals that calcium dominates with a weight percentage of 74.85% and an atomic percentage of 67.16%. Magnesium follows with a weight percentage of 19.64% and an atomic percentage of 29.06%. Copper, though present in smaller quantities, has a weight percentage of 4.02% and an atomic percentage of 2.27%. The trace presence of chlorine is indicated by its minor weight and atomic percentages. The data, alongside the associated error margins, confirm a sample composition primarily dominated by calcium, followed by magnesium and minor amounts of copper and chlorine.



Element Line	Weight %	Weight % Error	Atom %	Atom % Error	Formula
Mg K	19.64	± 1.09	29.06	± 1.61	Mg
Cl K	1.49	± 0.17	1.51	± 0.18	Cl
Cl L	---	---	---	---	(null)
Ca K	74.85	± 0.34	67.16	± 0.30	Ca
Ca L	---	---	---	---	(null)
Cu K	4.02	± 0.09	2.27	± 0.05	Cu
Cu L	---	---	---	---	(null)
Total	100.00		100.00		

Figure 3. 17: Energy-dispersive X-ray spectroscopy (EDX) analysis showing weight % and atomic % of elements present in the NPte 50:50.

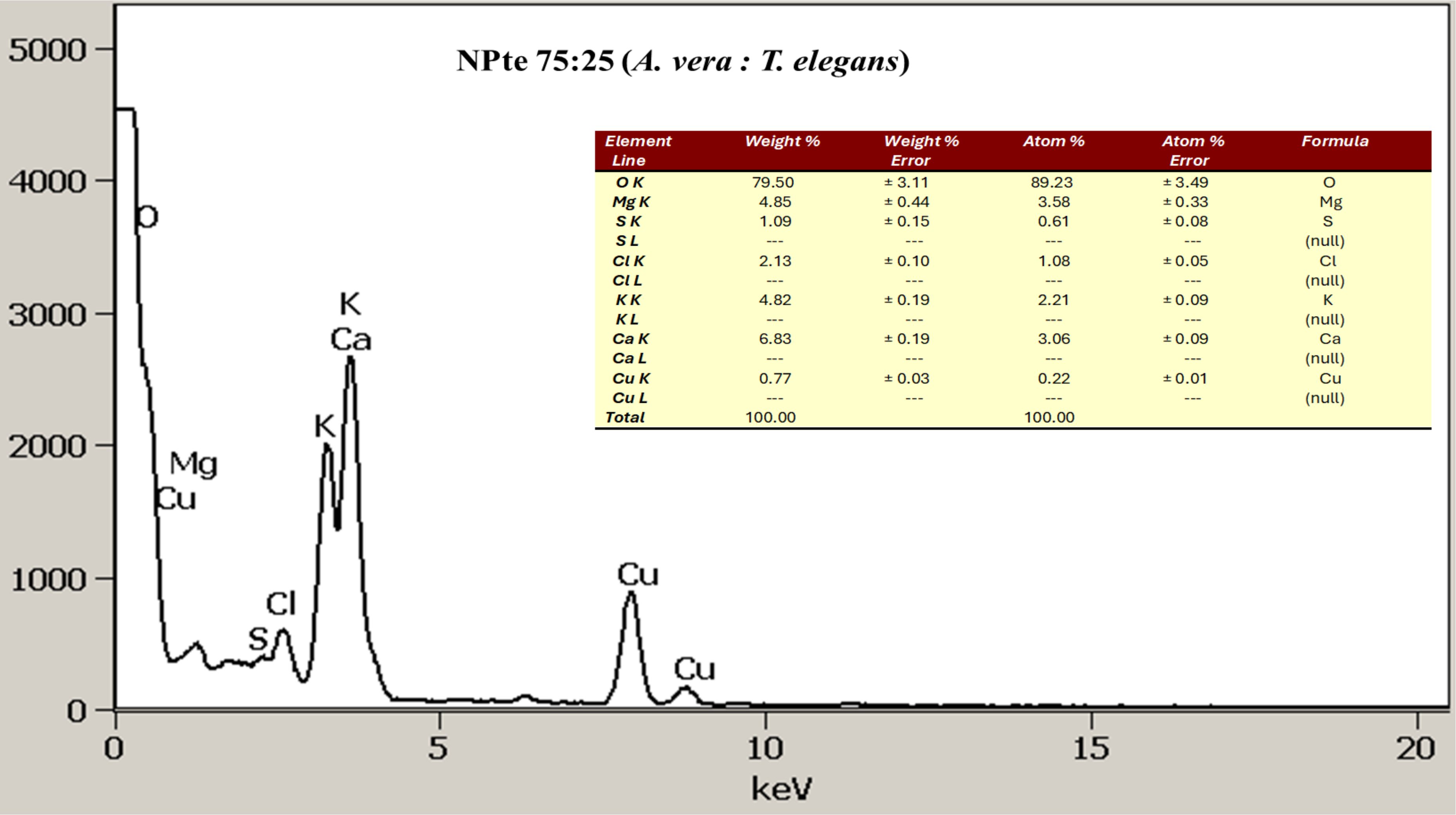
The NP1c 50:50 indicates the presence of key elements such as potassium (K), phosphorus (P), magnesium (Mg), chlorine (Cl), and copper (Cu) (Figure 3.18). Potassium dominates the sample, with a weight percentage of 50.87% and an atomic percentage of 43.76%, making it the most abundant element. Phosphorus follows with a weight percentage of 24.19% and an atomic percentage of 26.27%, while magnesium contributes 17.95% by weight and 24.85% by atomic percentage. Chlorine and copper are present in smaller amounts, with chlorine at 3.40% by weight and 3.23% by atomic percentage, and copper at 3.58% by weight and 1.90% by atomic percentage. The spectrum highlights the sample's elemental composition, with potassium and phosphorus being the primary contributors, supported by magnesium and trace amounts of chlorine and copper.



Element Line	Weight %	Weight % Error	Atom %	Atom % Error	Formula
Mg K	17.95	± 0.80	24.85	± 1.10	Mg
P K	24.19	± 0.49	26.27	± 0.53	P
P L	---	---	---	---	(null)
Cl K	3.40	± 0.56	3.23	± 0.53	Cl
Cl L	---	---	---	---	(null)
K K	50.87	± 0.51	43.76	± 0.44	K
K L	---	---	---	---	(null)
Cu K	3.58	± 0.06	1.90	± 0.03	Cu
Cu L	---	---	---	---	(null)
Total	100.00		100.00		

Figure 3. 18: Energy-dispersive X-ray spectroscopy (EDX) analysis showing weight % and atomic % of elements present in the NPlc 50:50.

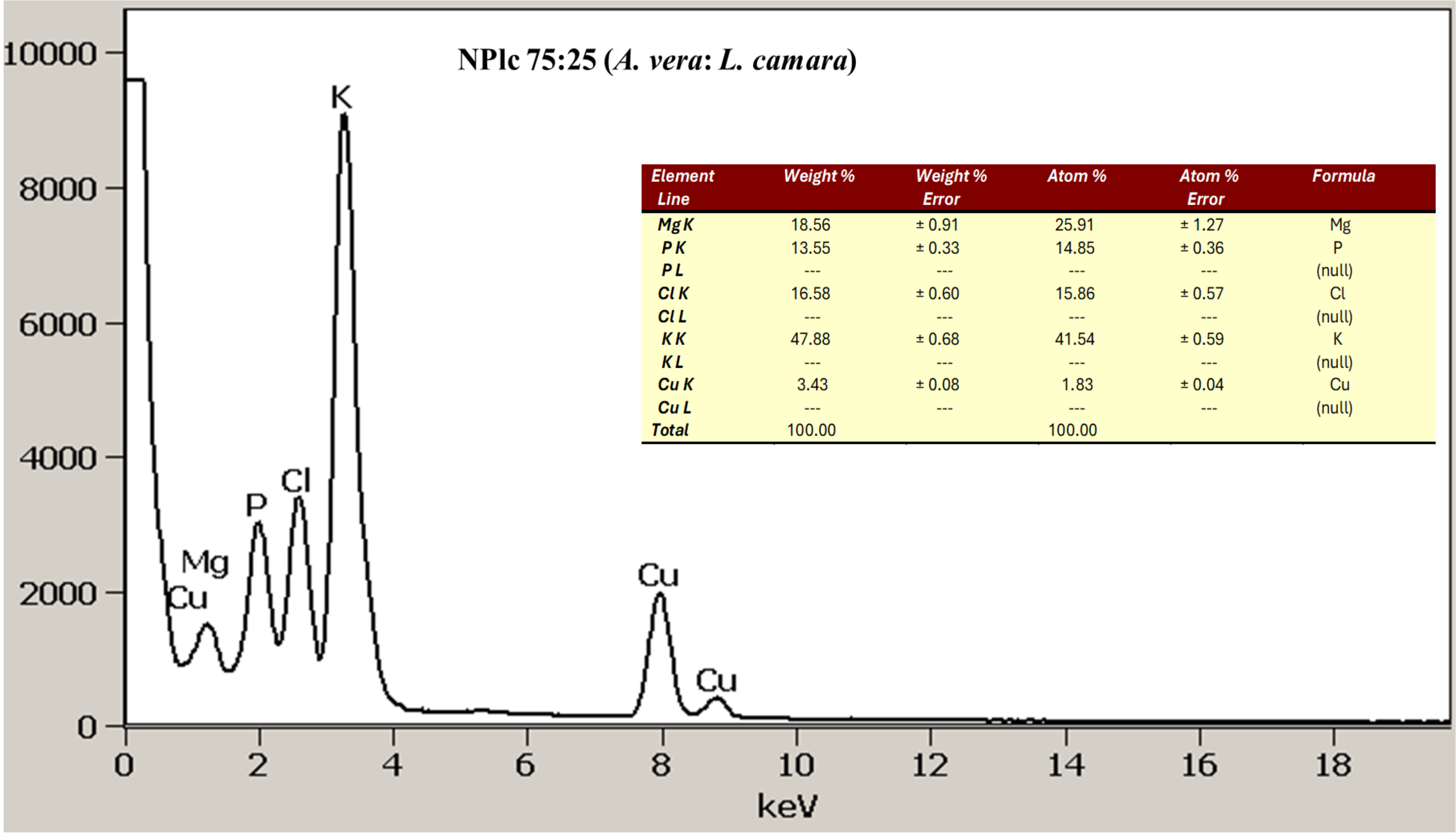
The EDS spectrum for the sample for NPte 75:25 (*A. vera* : *L. camara*) reveals the elemental composition dominated by oxygen (O), which constitutes 79.50% by weight and 89.23% by atomic percentage (Figure 3.19). Magnesium (Mg) contributes 4.85% by weight and 3.58% by atomic percentage, followed by potassium (K) at 4.82% by weight and 2.21% by atomic percentage. Calcium (Ca) is present at 6.83% by weight and 3.06% by atomic percentage, with sulfur (S) and chlorine (Cl) showing minor contributions of 1.09% and 2.13% by weight, respectively. Copper (Cu) appears in trace amounts, contributing 0.77% by weight and 0.22% by atomic percentage. The data indicates that oxygen is the primary element, followed by significant contributions from magnesium, calcium, and potassium, with smaller amounts of sulfur, chlorine, and copper.



Element Line	Weight %	Weight % Error	Atom %	Atom % Error	Formula
O K	79.50	± 3.11	89.23	± 3.49	O
Mg K	4.85	± 0.44	3.58	± 0.33	Mg
S K	1.09	± 0.15	0.61	± 0.08	S
S L	---	---	---	---	(null)
Cl K	2.13	± 0.10	1.08	± 0.05	Cl
Cl L	---	---	---	---	(null)
K K	4.82	± 0.19	2.21	± 0.09	K
K L	---	---	---	---	(null)
Ca K	6.83	± 0.19	3.06	± 0.09	Ca
Ca L	---	---	---	---	(null)
Cu K	0.77	± 0.03	0.22	± 0.01	Cu
Cu L	---	---	---	---	(null)
Total	100.00		100.00		

Figure 3. 19: Energy-dispersive X-ray spectroscopy (EDX) analysis showing weight % and atomic % of elements present in the NPte 75:25 (*A. vera*: *L. camara*).

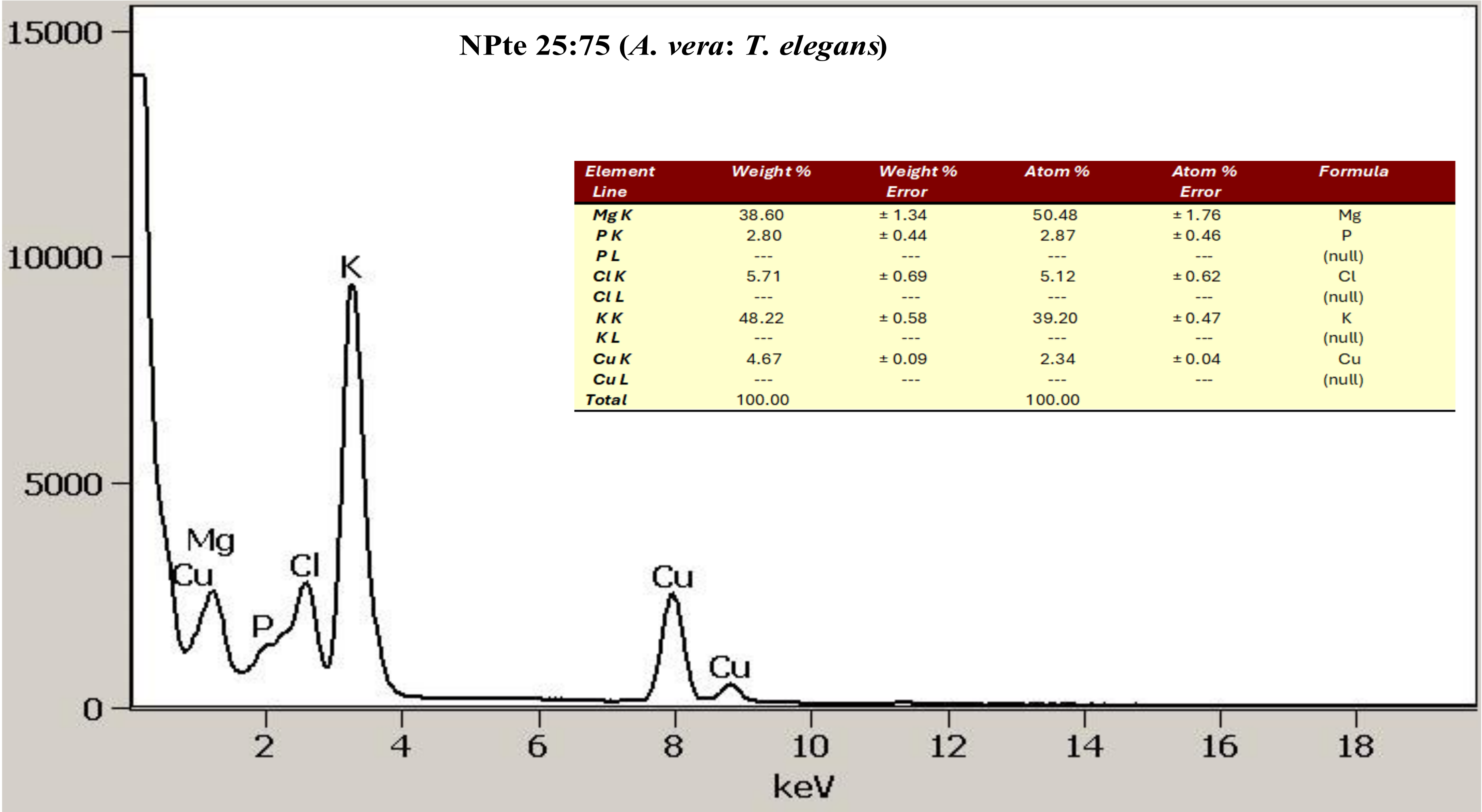
The EDX analysis provides a detailed compositional analysis of the sample NPlc 75:25 (*A. vera: L. vera*). The spectrum indicates the presence of key elements, including magnesium (Mg), phosphorus (P), chlorine (Cl), potassium (K), and copper (Cu) (Figure 3.20). The most prominent peak in the spectrum corresponds to potassium (K). The weight percentage and atomic percentage confirm potassium as the most abundant, with 47.88% by weight and 41.54% by atom. Magnesium is the next significant element, with 18.56% by weight and 25.91% by atom. Phosphorus and chlorine also contribute notably, with weight percentages of 13.55% and 16.58%, respectively. Copper appears as a trace element, with a lower weight percentage of 3.43%. The analysis indicates a heterogeneous distribution of elements, with potassium being predominant and copper being the least represented.



Element Line	Weight %	Weight % Error	Atom %	Atom % Error	Formula
Mg K	18.56	± 0.91	25.91	± 1.27	Mg
P K	13.55	± 0.33	14.85	± 0.36	P
P L	---	---	---	---	(null)
Cl K	16.58	± 0.60	15.86	± 0.57	Cl
Cl L	---	---	---	---	(null)
K K	47.88	± 0.68	41.54	± 0.59	K
K L	---	---	---	---	(null)
Cu K	3.43	± 0.08	1.83	± 0.04	Cu
Cu L	---	---	---	---	(null)
Total	100.00		100.00		

Figure 3. 20: Energy-dispersive X-ray spectroscopy (EDX) analysis showing weight % and atomic % of elements present in the NPlc 75:25 (A. vera: *L. camara*).

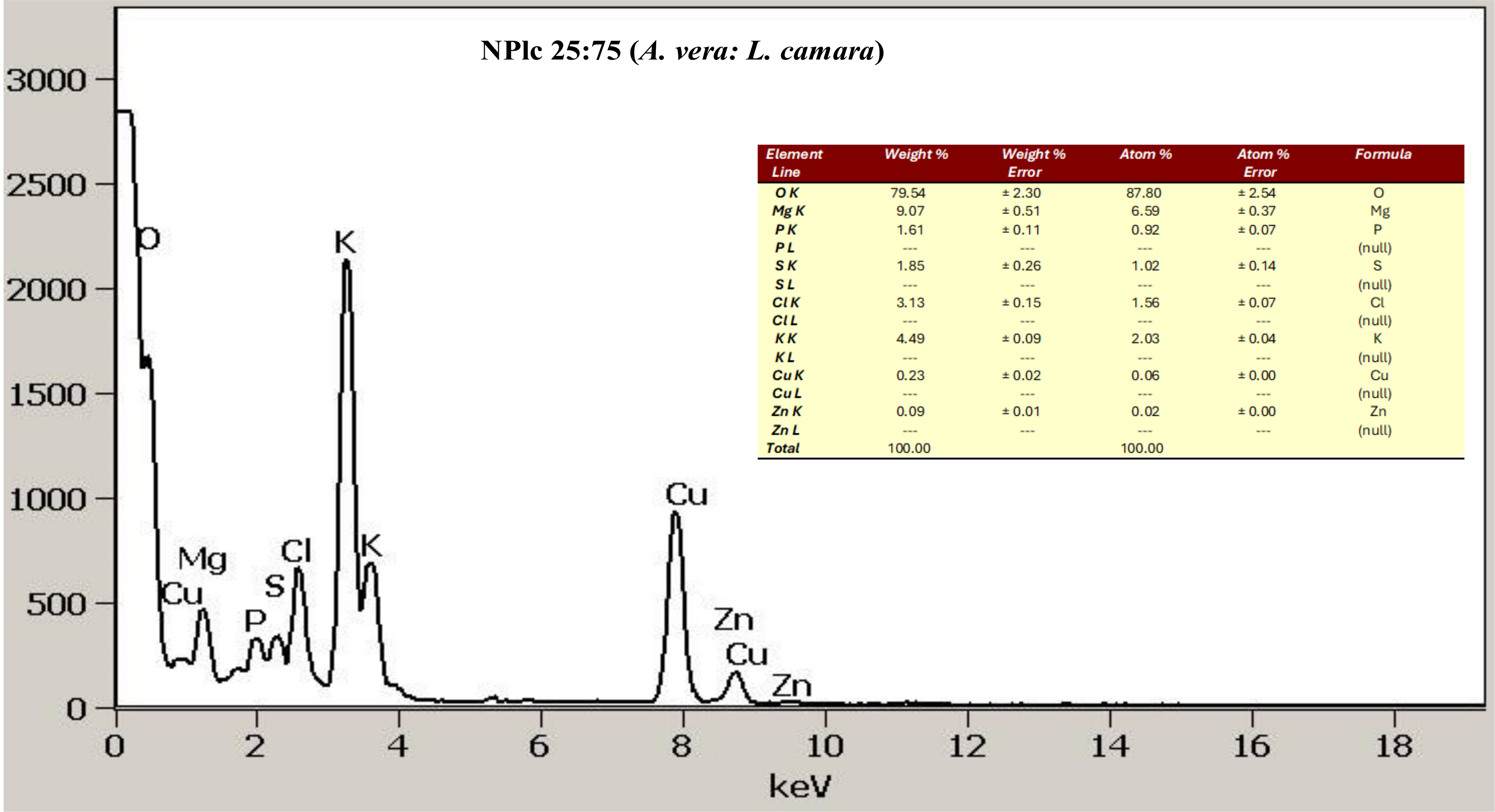
The EDX analysis of the sample NPte 25:75 (*A. vera*: *T. elegans*) reveals a diverse elemental composition. The spectrum highlights potassium (K) as the most abundant element, with a weight percentage of 48.22% and an atomic percentage of 39.20% (Figure 3.21). Magnesium (Mg) is also present in significant quantities, contributing 38.60% by weight and 50.48% by atom. Chlorine (Cl) is identified with a moderate presence of 5.71% by weight and 5.12% by atom, while phosphorus (P) is detected in smaller amounts, comprising 2.80% by weight and 2.87% by atom. Copper (Cu) appears as a trace element, accounting for 4.67% by weight and 2.34% by atom. The low error margins in the measurements ensure the accuracy and reliability of the nanoparticle data, indicating a predominantly potassium- and magnesium-rich sample with minor contributions from other elements.



Element Line	Weight %	Weight % Error	Atom %	Atom % Error	Formula
Mg K	38.60	± 1.34	50.48	± 1.76	Mg
P K	2.80	± 0.44	2.87	± 0.46	P
P L	---	---	---	---	(null)
Cl K	5.71	± 0.69	5.12	± 0.62	Cl
Cl L	---	---	---	---	(null)
K K	48.22	± 0.58	39.20	± 0.47	K
K L	---	---	---	---	(null)
Cu K	4.67	± 0.09	2.34	± 0.04	Cu
Cu L	---	---	---	---	(null)
Total	100.00		100.00		

Figure 3. 21: Energy-dispersive X-ray spectroscopy (EDX) analysis showing weight % and atomic % of elements present in the NPte 25:75 (*A. vera*: *T. elegans*).

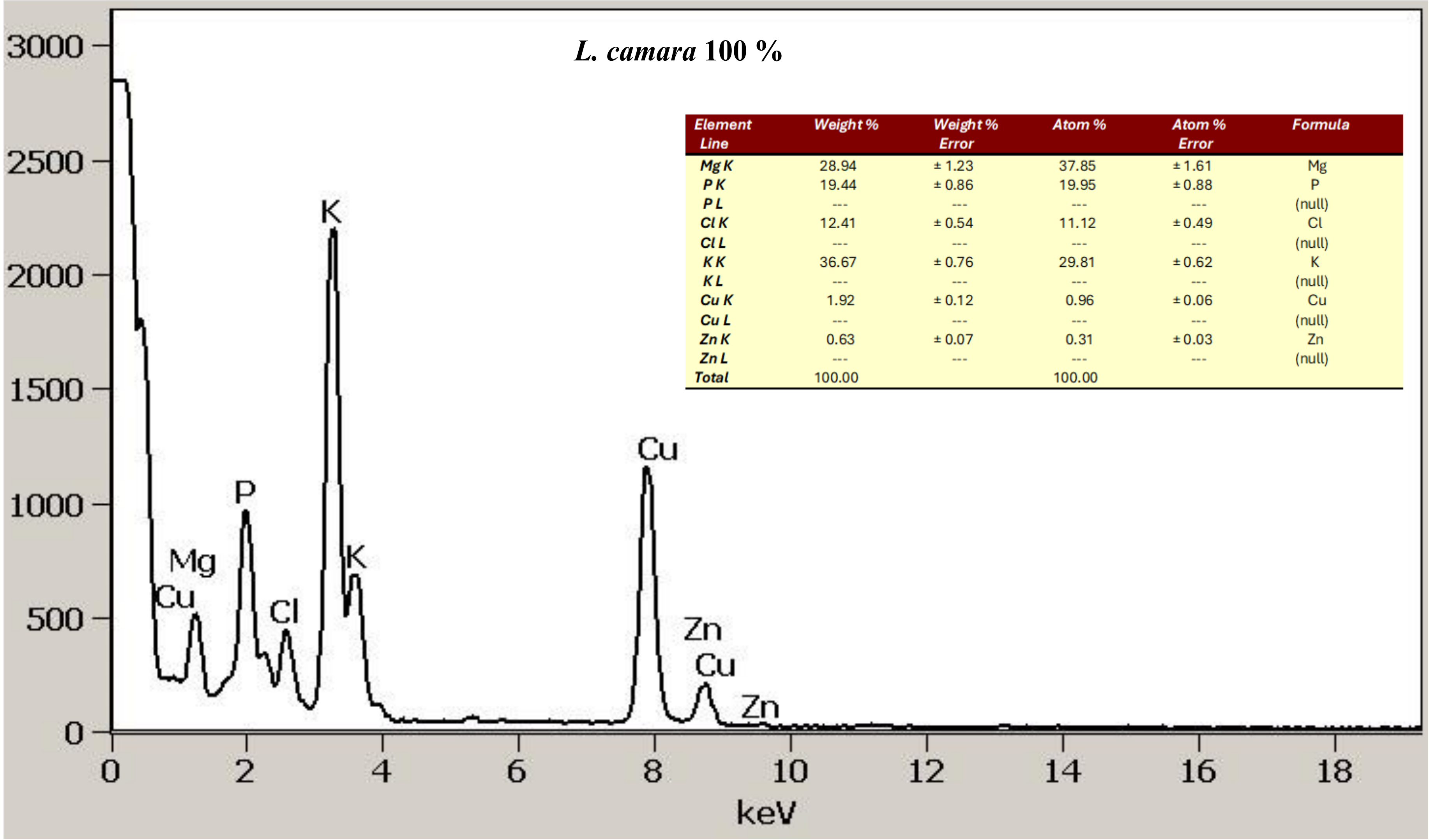
The EDX analysis of NPlc 25:75 (*A. vera: L. camara*) indicates a composition dominated by oxygen (O), which accounts for 79.54% by weight and 87.80% by atom (Figure 3.22). Magnesium (Mg) is the next significant element, contributing 9.07% by weight and 6.59% by atom. Potassium (K) and chlorine (Cl) are in smaller amounts, with weight percentages of 4.49% and 3.13%, respectively. Sulfur (S) and phosphorus (P) are detected as minor constituents, comprising 1.85% and 1.61% by weight, respectively. Copper (Cu) and zinc (Zn) are trace elements with negligible contributions to the overall composition.



Element Line	Weight %	Weight % Error	Atom %	Atom % Error	Formula
O K	79.54	± 2.30	87.80	± 2.54	O
Mg K	9.07	± 0.51	6.59	± 0.37	Mg
P K	1.61	± 0.11	0.92	± 0.07	P
P L	---	---	---	---	(null)
S K	1.85	± 0.26	1.02	± 0.14	S
S L	---	---	---	---	(null)
Cl K	3.13	± 0.15	1.56	± 0.07	Cl
Cl L	---	---	---	---	(null)
K K	4.49	± 0.09	2.03	± 0.04	K
K L	---	---	---	---	(null)
Cu K	0.23	± 0.02	0.06	± 0.00	Cu
Cu L	---	---	---	---	(null)
Zn K	0.09	± 0.01	0.02	± 0.00	Zn
Zn L	---	---	---	---	(null)
Total	100.00		100.00		

Figure 3. 22: Energy-dispersive X-ray spectroscopy (EDX) analysis showing weight % and atomic % of elements present in the NPlc 25:75 (*A. vera*: *L. camara)*

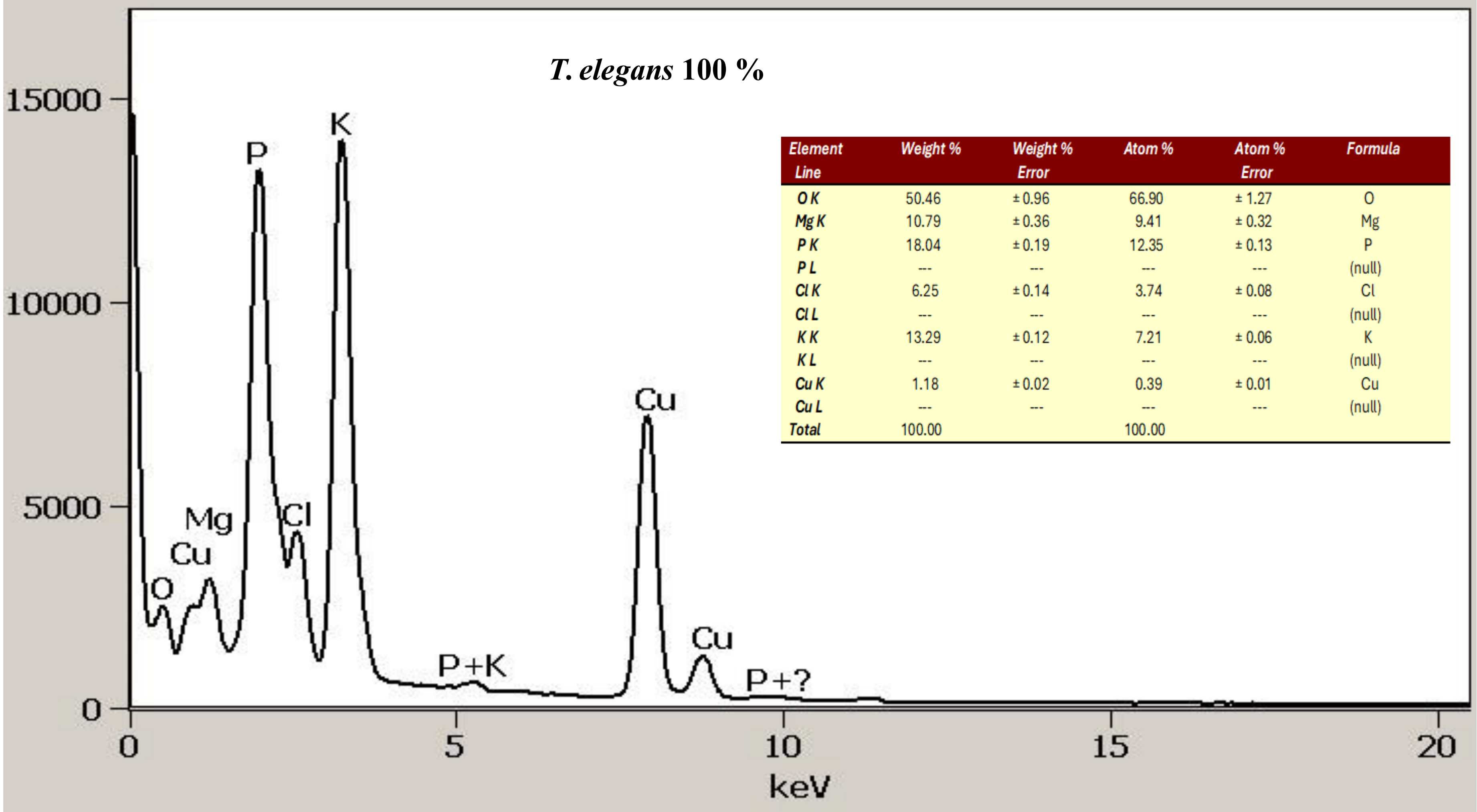
The EDS spectrum reveals the elemental composition of the sample, showing prominent peaks for magnesium (Mg), phosphorus (P), chlorine (Cl), potassium (K), copper (Cu), and zinc (Zn) (Figure 3.23). The relative abundance of these elements is indicated by the peak intensities: magnesium (28.94% weight, 37.85% atomic), phosphorus (19.44% weight, 19.95% atomic), chlorine (12.41% weight, 11.12% atomic), potassium (36.67% weight, 29.81% atomic), copper (1.92% weight, 0.96% atomic), and zinc (0.63% weight, 0.31% atomic).



Element Line	Weight %	Weight % Error	Atom %	Atom % Error	Formula
Mg K	28.94	± 1.23	37.85	± 1.61	Mg
P K	19.44	± 0.86	19.95	± 0.88	P
P L	---	---	---	---	(null)
Cl K	12.41	± 0.54	11.12	± 0.49	Cl
Cl L	---	---	---	---	(null)
K K	36.67	± 0.76	29.81	± 0.62	K
K L	---	---	---	---	(null)
Cu K	1.92	± 0.12	0.96	± 0.06	Cu
Cu L	---	---	---	---	(null)
Zn K	0.63	± 0.07	0.31	± 0.03	Zn
Zn L	---	---	---	---	(null)
Total	100.00		100.00		

Figure 3. 23: Energy-dispersive X-ray spectroscopy (EDX) analysis showing weight % and atomic % of elements present in the 100 % *L. camara*.

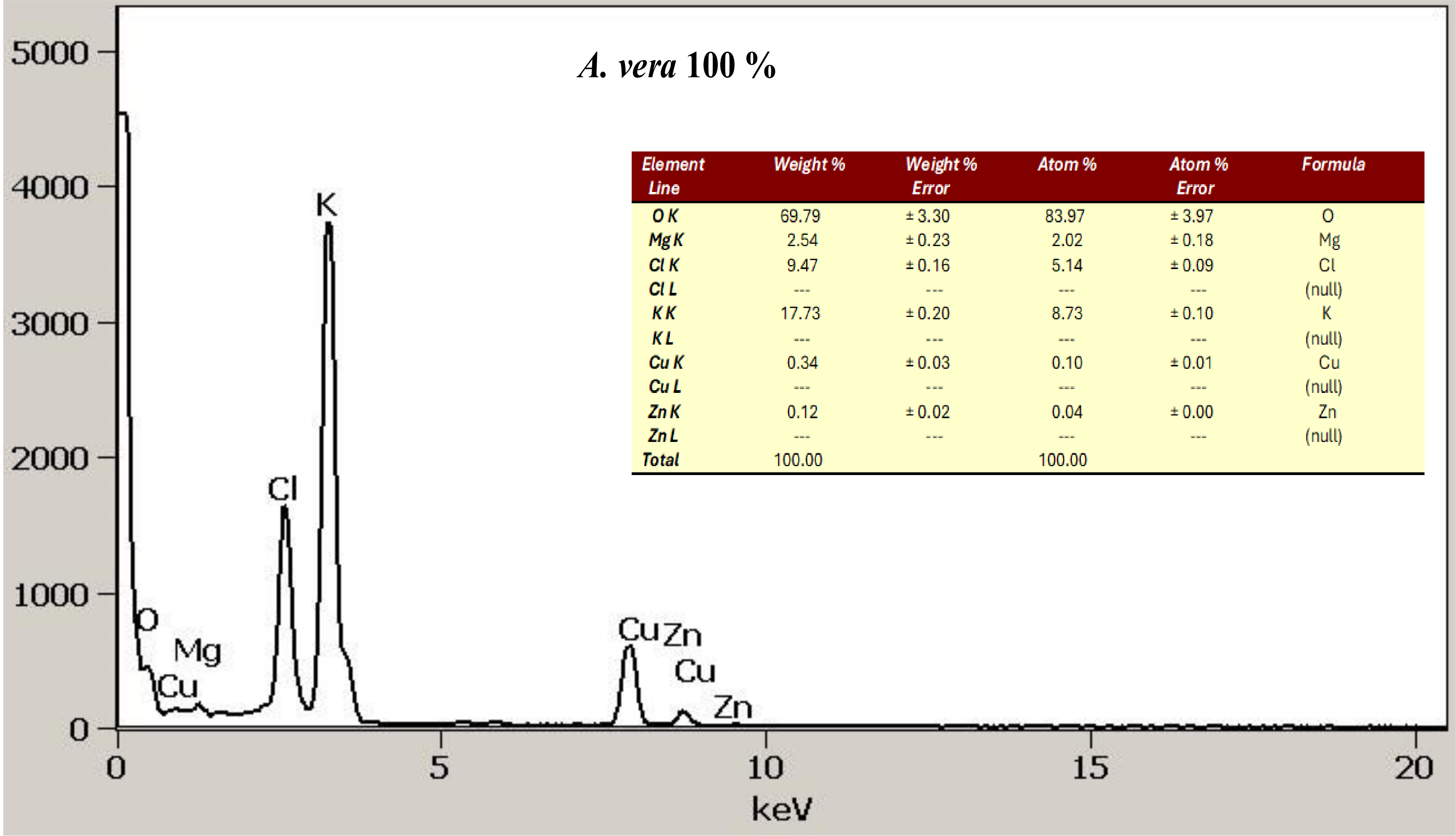
The EDS spectrum reveals the elemental composition of the *T. elegans* sample, showing prominent peaks for oxygen (O), potassium (K), magnesium (Mg), phosphorus (P), chlorine (Cl), and copper (Cu) (Figure 3.24). The relative abundance of these elements is indicated by the peak intensities: oxygen (50.46% weight, 66.90% atomic), potassium (13.29% weight, 7.21% atomic), magnesium (10.79% weight, 9.41% atomic), phosphorus (18.04% weight, 12.35% atomic), chlorine (6.25% weight, 3.74% atomic), and copper (1.18% weight, 0.39% atomic).



Element Line	Weight %	Weight % Error	Atom %	Atom % Error	Formula
O K	50.46	± 0.96	66.90	± 1.27	O
Mg K	10.79	± 0.36	9.41	± 0.32	Mg
P K	18.04	± 0.19	12.35	± 0.13	P
P L	---	---	---	---	(null)
Cl K	6.25	± 0.14	3.74	± 0.08	Cl
Cl L	---	---	---	---	(null)
K K	13.29	± 0.12	7.21	± 0.06	K
K L	---	---	---	---	(null)
Cu K	1.18	± 0.02	0.39	± 0.01	Cu
Cu L	---	---	---	---	(null)
Total	100.00		100.00		

Figure 3. 24: Energy-dispersive X-ray spectroscopy (EDX) analysis showing weight % and atomic % of elements present in the 100 % *T. elegans*.

The EDS spectrum reveals the elemental composition of the *A. vera* sample, showing prominent peaks for oxygen (O), potassium (K), chlorine (Cl), magnesium (Mg), copper (Cu), and zinc (Zn) (Figure 2.25). The relative abundance of these elements is indicated by the peak intensities: oxygen (69.79% weight, 83.97% atomic), potassium (17.73% weight, 8.73% atomic), chlorine (9.47% weight, 5.14% atomic), magnesium (2.54% weight, 2.02% atomic), copper (0.34% weight, 0.10% atomic), and zinc (0.12% weight, 0.04% atomic).



Element Line	*Weight %*	*Weight % Error*	*Atom %*	*Atom % Error*	*Formula*
O K	69.79	± 3.30	83.97	± 3.97	O
Mg K	2.54	± 0.23	2.02	± 0.18	Mg
Cl K	9.47	± 0.16	5.14	± 0.09	Cl
Cl L	---	---	---	---	(null)
K K	17.73	± 0.20	8.73	± 0.10	K
K L	---	---	---	---	(null)
Cu K	0.34	± 0.03	0.10	± 0.01	Cu
Cu L	---	---	---	---	(null)
Zn K	0.12	± 0.02	0.04	± 0.00	Zn
Zn L	---	---	---	---	(null)
Total	100.00		100.00		

Figure 3. 25: Energy-dispersive X-ray spectroscopy (EDX) analysis showing weight % and atomic % of elements present in the 100 %b *A. vera*.

3.5. Discussion

3.5.1. Qualitative phytochemical screening

The moderate presence of steroids and terpenoids in specific formulations further enhances the bioactivity of these nanoparticles. Steroids have been implicated in modulating nematode reproduction, while terpenoids exhibit direct nematotoxic effects by impairing nematode metabolism and nervous system function (Hernández-Carlos and Gamboa-Angulo, 2019). Oka *et al*. (2000) found that 12 out of 27 essential oils immobilized over 80% of *M. javanica* J2 j at 1000 µl/liter, with oils from *Carum carvi* L., *Foeniculum vulgare* L., and *Mentha* species showing the highest activity. Ntalli *et al*. (2011) reported synergistic nematicidal interactions between terpene pairs, particularly trans-anethole/geraniol and transanethole/eugenol. Ohri and Pannu (2009) reviewed various terpenoids effective against *M. incognita* and *M. javanica*, including carvacrol, thymol, and geraniol. Ntalli *et al*. (2013) evaluated essential oils from *Rosmarinus officinalis*, *Citrus sinensis*, *Lavandula angustifolia*, and *Dittrichia viscosa*, finding that *D. viscosa* oil was most effective in paralysing *M. incognita* J2 and inhibiting juvenile hatch. These studies demonstrate the potential of essential oils and terpenoids as natural nematicides, offering alternatives to conventional chemical control methods.

The phytochemical profiles of *L. camara* and *T. elegans* are consistent with studies on other plants with nematicidal properties. For example, extracts of *Azadirachta indica* L. are rich in saponins, tannins, and alkaloids, which have been successfully employed in managing nematodes (Abbassy *et al*., 2017). Similarly, *Urtica urens* extracts, which contain phenols and flavonoids, have demonstrated significant nematicidal activity, particularly when formulated into nanoparticles (Nassar, 2016). The enhanced efficacy of *L. camara* and *T. elegans* nanoparticles, as evidenced by their stable phytochemical composition, suggests their potential to rival or complement these established biocontrol agents. According to War *et al*. (2012), tannins are involved in plant resistance to insect herbivory, and a handful of studies have also found a correlation between tannin accumulation and nematode resistance by the host plant.

Plant extract-mediated synthesis of nanoparticles offers an eco-friendly, cost-effective alternative to traditional chemical methods (Makarov *et al*., 2014; Mittal *et al*., 2013). The nano-formulation process increases stability, bioavailability, and targeted delivery of bioactive compounds, enhancing their efficacy while minimizing environmental risks (Kumari *et al*., 2022). For instance, nanoparticles can be applied as soil amendments to control plant-parasitic nematode populations directly in the rhizosphere or as foliar sprays to protect above-ground plant parts from pest infestations. They can enhance plant resilience to abiotic stresses by modulating phytohormone content (Kumari *et al*., 2022).

The nanoparticles synthesised from *L. camara* and *T. elegans*, rich in glycosides, flavonoids, and phenols, can disrupt nematode life cycles by inhibiting J2 hatch and J2 e development. Additionally, these nanoparticles could enhance plant resistance by acting as elicitors of plant defense mechanisms. Cassava roots (*Manihot esculenta*) contain varying levels of glucosides, which, when plant cells are damaged, are hydrolysed by enzymes. During cassava root processing, the resulting liquid has been traditionally used in Brazil for the control of *M. javanica* and *M. incognita* for decades (Easson *et al*., 2021). Additionally, the antioxidant properties of flavonoids and phenols may help reduce oxidative stress in plants caused by plant-parasitic nematode infections, thereby supporting overall plant health. Desmedt *et al*. (2020) highlighted the role of the flavonoid glyceollin I in enhancing soybean resistance against *M. incognita*. However, Kirwa *et al*. (2020) noted that flavonoids influence nematode behavior in a complex manner, either attracting or repelling *M. incognita* J2 depending on their molecular composition and concentration.

Research by Chin *et al*. (2018) indicates that phytochemicals, particularly flavonoids, can induce quiescence in nematodes by reducing their movement and altering their migration patterns toward plant roots, ultimately repelling and killing them. Moreover, flavonoids contribute to root development and plant defence mechanisms against various microorganisms. Root exudates, which contain flavonoids, cytokinins, and alkaloids, trigger concentration-dependent responses in nematodes, influencing their behavior (Kirwa *et al.*, 2018). Plants release an array of bioactive secondary metabolites through their roots, which function as nematode attractants, repellents, hatching stimulants, or inhibitors (Sikder and Vestergård, 2020).

Phenolic compounds play a crucial role in plant resistance against numerous pests and diseases (Oszmiański *et al.*, 2015), with their involvement in plant-parasitic nematode resistance being recognized since the early 1960s. Research by Dhakshinamoorthy *et al.* (2014) and Hölscher *et al.* (2014) has shown that higher baseline or induced levels of phenolic compounds are linked to nematode resistance across various plant-nematode interactions. Additionally, phenolics have been found to inhibit *M. incognita*-induced gall formation in cotton (*Gossypium* L.) under greenhouse or micro-plot conditions (Bauske *et al.*, 1994).

Beyond nematode management, the bioactive properties of these nanoparticles suggest potential applications in managing other pests and pathogens. For example, the antimicrobial activity of tannins and phenols could be harnessed to control fungal and bacterial diseases in crops. The insecticidal properties of saponins and terpenoids make these nanoparticles suitable for integrated pest management (IPM) strategies. Furthermore, the sustainable nature of these formulations, which rely on plant-based synthesis without the use of toxic metals, aligns with global efforts to reduce the ecological footprint of agricultural practices (Castillo-Henríquez *et al.*, 2020). According to Rice-Evans *et al.* (1996), the phenolic compounds containing free hydrogen are responsible for the antioxidant activities. It became evident that the antioxidant activities of all the extracts are due to the presence of flavonoids and polyphenols in all the plants (Giri *et al*., 2014). However, the natural products profile and consequently the bioactivity are known to vary with the climate and geographic location of the plants.

In particular, the data suggests that the plant extracts, individually and in combination, possess key phytochemicals that facilitate the conversion to the nanoscale. These phytochemicals contribute significantly to the formation and stabilisation of nanoparticles, confirming that whole plant extracts, rather than isolated active compounds, may offer a broader spectrum of bioactive agents for applications such as nematode management. The use of whole plant extracts in the synthesis of nanoparticles presents several advantages over single isolated active compounds, primarily due to the inherent complexity and synergy of phytochemicals present in the extract. Whole plant extracts contain diverse bioactive compounds, including polyphenols, flavonoids, alkaloids, terpenoids, and saponins, which collectively function as reducing, capping, and stabilizing agents.

This synergistic interaction enhances the efficiency of nanoparticle formation and improves their stability and functionality (Bao *et al*., 2021). By contrast, isolated active compounds often lack the stabilising support of other phytochemicals present in the plant extract (da Silva *et al*., 2016). For instance, while flavonoids may act as effective reducing agents, the absence of co-occurring compounds such as saponins and terpenoids can result in unstable nanoparticles or higher rates of agglomeration (Singh *et al*., 2023). The whole plant extract provides a holistic chemical environment that mimics natural systems, fostering controlled nucleation and growth processes during synthesis.

Isolated compounds, while effective, may not provide this comprehensive stabilisation, resulting in nanoparticles with limited shelf life or compromised functionality. For instance, *A. vera* used in the current study not only reduced ions to nanoparticles but also acted as a potent stabiliser due to its rich content of polysaccharides and phenolic compounds. These compounds prevented nanoparticle agglomeration and ensured uniform size distribution, a key factor for applications in agriculture (Khaldoune *et al*., 2024). The results highlight the importance of maintaining the phytochemical complexity of whole plant extracts to achieve stable and functional nanoparticles.

Using whole plant extracts is also more cost-effective and scalable than isolating individual active compounds. The isolation process is labor-intensive, time-consuming, and often requires hazardous solvents, which contradicts the principles of green chemistry. In contrast, whole plant extracts can be prepared using simple and eco-friendly methods, such as aqueous or ethanolic extraction, making the process accessible for large-scale production. This advantage is particularly significant for resource-constrained settings, where cost and scalability are critical considerations. The diverse phytochemical profile in whole plant extracts imparts functional versatility to the synthesized nanoparticles. These compounds interact differently during synthesis, resulting in nanoparticles with varied shapes, sizes, and surface morphologies. Such diversity enables tailored applications, particularly in agriculture, where different nanoparticle morphologies may exhibit distinct modes of action, such as enhanced nematode penetration or controlled release of bioactive agents (Mondéjar-López *et al*., 2024).

Possible mode of nanoparticles synthesis

Reducing sugars, particularly carbohydrates, play an essential role in the green synthesis of nanoparticles, particularly when plant extracts are the sole source of reducing and stabilizing agents (Shafey, 2020). In the absence of external chemicals or metals, reducing sugars functions as natural electron donors that drive the reduction process necessary for nanoparticle formation (Guo *et al*., 2021). Their role is pivotal because they initiate and sustain the conversion of bioactive compounds in plant extracts into nanoscale particles through a series of biochemical reactions (Kumar *et al*., 2019). These sugars contain functional groups, such as aldehydes and ketones, that are highly reactive and capable of transferring electrons to the precursor molecules, facilitating the reduction of reactive species into stable nanoparticles

(Bahari *et al*., 2023). This electron transfer is central to the nucleation phase, where the initial formation of nanoparticles occurs (Mittal *et al*., 2013).

In the context of using plant extracts like *T. elegans* and *L. camara* for nanoparticle synthesis, reducing sugars act synergistically with other phytochemicals, such as alkaloids, phenolics, and flavonoids, to create an environment conducive to nanoparticle formation. For example, carbohydrates in these plant extracts provide the necessary electrons to reduce reactive compounds within the extracts themselves, forming nanoparticle nuclei (El-Seedi *et al*., 2019). Once these nuclei are formed, they act as seeds, and additional layers of bioactive molecules deposit on these seeds, facilitating nanoparticle growth. This growth process is inherently stabilised by the structural properties of the carbohydrates, which adsorb onto the surface of the nanoparticles, preventing aggregation and ensuring uniform size distribution (Sharma *et al*., 2020).

The mechanism through which carbohydrates facilitate nanoparticle synthesis involves their ability to act as capping agents. During the reduction process, carbohydrates bind to the surface of the nanoparticles, forming a protective layer (Verma *et al*., 2020). This layer serves to stabilize the high surface energy of nanoparticles, which, without stabilization, would lead to aggregation or unwanted growth patterns. The stabilisation effect is crucial in maintaining the functional properties of the nanoparticles, such as their size, shape, and bioactivity (Xu *et al*., 2018). For example, in the synthesis of nanoparticles using *A. vera* gel as a reducing agent, the polysaccharides in the gel stabilize the nanoparticles by interacting with their surface through hydrogen bonding and van der Waals forces (Sharma *et al*., 202). This interaction not only prevents particle aggregation but also enhances the dispersity of the nanoparticles, making them more effective for applications like nematode management (Yadav *et al*., 2018).

Furthermore, reducing sugars also influences the nucleation and growth phases of nanoparticle formation by modulating the kinetics of these processes. The concentration of carbohydrates and the reaction conditions, such as pH and temperature, determine the rate of nucleation versus growth, which ultimately affects the size and morphology of the resulting nanoparticles (Vijayaraghavan and Ashokkumar, 2017). Higher concentrations of carbohydrates often favor rapid nucleation, leading to smaller, more uniform nanoparticles. Conversely, slower nucleation and sustained growth can result in larger nanoparticles with diverse morphologies (Jadoun *et al*., 2021). Verma *et al*. (2020) explained that these controlled mechanisms are critical when plant extracts are used as the only source for nanoparticle synthesis, as the absence of chemical stabilizers or metal ions makes the role of bioactive compounds, including carbohydrates, even more significant.

The possible mode of action of carbohydrates in the nanoparticle synthesis process is rooted in their ability to act as natural reductants and stabilisers. According to Ovais *et al*. (2018), during the synthesis, the hydroxyl groups in carbohydrates interact with the bioactive compounds within the plant extract, reducing them into reactive intermediates that subsequently form nanoparticles. This reaction is catalysed by the inherent redox potential of carbohydrates, which allows them to donate electrons without requiring external catalysts. Moreover, the protective carbohydrate layer formed on the nanoparticles' surface enhances their colloidal stability, making them suitable for long-term storage and various

Plant-based synthesis of nanoparticles, facilitated by reducing sugars, offers several advantages over traditional chemical methods. The absence of toxic by-products ensures an environmentally friendly process, while the natural components of the plant extract contribute to the functionalization of the nanoparticles. This dual functionality, where carbohydrates reduce and stabilize nanoparticles, enhances their bioavailability and efficacy in agricultural applications. From the current study, nanoparticles synthesised from *T. elegans* and *L. camara*, the reducing sugars and other phytochemicals work in concert to produce stable nanoparticles capable of targeting nematodes effectively. These nanoparticles likely exhibit enhanced bioactivity due to their nanoscale properties, which will improve interaction with nematodes and reduce the overall amount of material needed for pest management, making the approach both cost-effective and sustainable (Sharma *et al*., 2020).

The phytochemical composition of the plant extracts was comprehensively analysed to confirm the presence of key bioactive compounds. This was achieved through an evaluation of the corresponding absorption peaks associated with the functional groups of the identified phytochemicals using a UV-vis spectrophotometer. This technique allowed for the precise detection and characterisation of compounds (Sharma *et al*., 2020). By correlating the observed peaks with known absorption patterns, the study provided insights into the molecular structure and functional groups responsible for the bioactivity of the extracts.

3.5.2. Visual observation and UV-Vis spectra analysis

Plant extracts, such as those from *L. camara* (LC), *A. vera* (Aloe), and *T. elegans* (TE), contain diverse bioactive compounds that facilitate nanoparticle synthesis. These phytochemicals act as reducing and stabilizing agents, enabling the conversion of metal salts to nanoscale particles. The UV-Vis spectra highlight unique absorption peaks for each extract, confirming the presence of such active compounds. The UV-Vis spectroscopic data obtained from the individual plant extracts and their respective nanoparticle combinations provide valuable insight into the molecular interactions and structural transformations that occur during nanoparticle synthesis.

The UV-Vis spectra of the *L. camara* and *A. vera* extracts reveal their distinct absorbance profiles, reflecting the presence of various phytochemicals with characteristic functional groups. The *L. camara* extract displays absorbance peaks at 215 nm, 240 nm, and 300 nm, which are consistent with the presence of phenolic compounds and aromatic structures commonly associated with plant polyphenols. Phenolic compounds absorb strongly in this range due to electronic transitions within their aromatic rings, as reported by Mizzi *et al.* (2020). Additionally, phenolic acids and flavonoids, often abundant in *L. camara*, are known to exhibit similar spectral characteristics, supporting their potential role in nanoparticle synthesis (Patil *et al.*, 2020).

Similarly, the *A. vera* extract shows peaks at 230 nm, 255 nm, and 275 nm, indicative of polysaccharides and flavonoid compounds. Polysaccharides are known to exhibit absorbance in the lower UV range (220–240 nm), attributable to their glycosidic linkages, as noted by Bushra *et al.* (2023). The absorbance at 255 nm and 275 nm is characteristic of flavonoids, which display absorption bands due to electronic transitions within their molecular structures (Verma *et al.*, 2020). The detection of these peaks aligns with findings from prior studies, confirming that *A. vera* is rich in bioactive compounds that contribute to its reducing and stabilising capabilities during nanoparticle formation (Bahari *et al.*, 2023).

The distinct absorbance patterns of these extracts highlight their complementary phytochemical compositions, which are crucial for their role in nanoparticle synthesis. Phenols and flavonoids from *L. camara* provide reduced power due to their ability to donate electrons, initiating the reduction of precursor molecules into nanoparticles (Patil *et al.*, 2020). Meanwhile, polysaccharides and flavonoids in the *A. vera* act as stabilising agents, forming a protective layer around nanoparticles to prevent aggregation and ensure uniformity (Logaranjan *et al.*, 2016). By correlating the observed UV-Vis peaks with known literature on the absorption characteristics of these functional groups, the presence of phenol, flavonoid, and polysaccharide compounds can be confidently attributed to the respective extracts.

The UV-Vis absorbance peaks observed in the *L. camara* and *A. vera* extracts correspond to specific functional groups associated with the bioactive phytochemicals present in these plants (Logaranjan *et al.*, 2016) . For *L. camara*, the peaks at 215 nm and 240 nm indicate the presence of phenolic compounds and aromatic rings, with functional groups such as hydroxyl groups (-

OH) attached to aromatic systems and conjugated double bonds (C=C) within these structures (Patil *et al.*, 2020). The peak at 300 nm is characteristic of extended conjugation in aromatic compounds like flavonoids and polyphenols, highlighting functional groups such as hydroxyl (-OH) and ketones (C=O) commonly found in these molecules. In *A. vera*, the peak at 230 nm is associated with polysaccharides and carbohydrates, where functional groups like hydroxyl (-OH) and ether groups (C-O-C) are predominant. The additional peaks at 255 nm and 275 nm are indicative of flavonoids, characterized by hydroxyl (-OH) and ketone (C=O) groups within their conjugated aromatic structures. These functional groups play critical roles in reduction and stabilization processes during nanoparticle synthesis.

The observed shifts and broadening of peaks in the UV-Vis spectra of the synthesized nanoparticles (e.g., the 50:50 LC: Aloe nanoparticle) further support the hypothesis that the plant extracts facilitate the formation of nanoparticles by providing a reducing environment. The presence of new peaks, especially in the visible region (400–420 nm), suggests the successful formation of nanoparticles and the interaction between the biomolecules from both extracts (Ravindran *et al.*, 2010). Ahmadi and Lackner (2024) explained that the increased absorbance intensity in the nanoparticle spectra is indicative of nanoparticle growth, with plant-derived compounds serving both as reducing agents and stabilizers during the process. The UV-Vis spectra indicates that the nanoparticles exhibit unique absorbance characteristics not observed in the individual extracts, particularly in the UV-visible range. The broader and more intense peaks in the nanoparticle spectra, especially in the 400–420 nm region, suggest the formation of surface plasmon resonance (SPR) peaks, which are a hallmark of nanoparticle formation. The peak shifts and overlap between the individual extracts in the 50:50 mixture reflect changes in the molecular structure and electronic properties of the phytochemicals upon nanoparticle synthesis.

These spectral changes point to the potential involvement of polymerisation processes, particularly the polymerisation of phenolic compounds (Guo *et al.*, 2021). Polyphenolic compounds, which are abundant in many plant extracts, are known to undergo oxidative polymerisation, leading to the formation of nanoparticles (Wang *et al.*, 2022). The presence of polysaccharides, such as those found in *A. vera*, likely plays a role in stabilising these nanoparticles through interactions with the formed metal ions or other nanoparticles. The stabilisation is likely facilitated by the hydrophilic nature of polysaccharides, which may form a protective shell around the nanoparticles, preventing aggregation and enhancing their stability in solution (Shehzad *et al.*, 2024).

In the nanoparticle synthesis process, phenolic compounds likely undergo polymerization, while polysaccharides act as stabilisers (Sahraeian *et al.*, 2024). According to Bahari *et al.* (2023), the ratio of phenols to saccharides in the mixture is crucial to the nanoparticle formation process, as it determines both the reduction and stabilisation mechanisms. The observed shifts in the UV-Vis spectra, particularly the changes in the absorbance intensity and the appearance of new peaks, suggest a balance between these two classes of compounds. The higher concentration of *A. vera* (75%) in the 75:25 (LC: Aloe) mixture leads to the formation of a more stable nanoparticle system, as evidenced by the increased intensity of the peaks at 400–420 nm and the shifts in the UV region.

While the exact ratio of phenols to polysaccharides is not directly determined from the UV-Vis spectra, the presence of both classes of compounds in the extracts is confirmed. Phenolic compounds are likely responsible for the redox reactions required for nanoparticle reduction, while polysaccharides provide the necessary stabilization and prevent aggregation of nanoparticles (Tapia-Hernández *et al.*, 2018).

In the nanoparticle spectra, new peaks and shifts suggest the interaction between phenolic compounds and polysaccharides, resulting in the formation of complex molecular species (Xue *et al*., 2024). The functional groups responsible for these interactions likely include hydroxyl groups (-OH) from phenolic compounds and ether linkages (C-O-C) from polysaccharides, which are essential for both polymerization and stabilization during nanoparticle formation (Cassani *et al*., 2023). Several parameters may influence the formation of nanoparticles, including the concentration of the plant extracts, the pH of the solution, and the temperature during synthesis. The reduction and stabilisation processes are likely influenced by the concentration of reducing agents (phenolic compounds) and stabilising agents (polysaccharides) present in the plant extracts. Moreover, the interaction between these compounds, facilitated by changes in pH and temperature, may lead to the formation of nanoparticles with specific sizes and morphologies. However, pH was not considered in the current study. The presence of polymerisation during nanoparticle synthesis is likely a result of the oxidative coupling of phenolic compounds, which is influenced by both the concentration of the plant extract and the reaction conditions. The synthesised nanoparticles were characterised, offering high-resolution imaging to analyse their morphology, size, and size distribution.

3.5.2. Transmission electron microscopy (TEM)

The synthesis results highlight the significant influence of plant extract concentration on nanoparticle size, shape, and dispersion. Ratios of 50:50 NPlc produced nanoparticles with smaller sizes. TEM analysis confirmed these nanoparticles exhibited well-defined morphologies and reduced aggregation. In contrast, deviations from this ratio led to larger, less uniform nanoparticles, indicating suboptimal nucleation and growth conditions.

Similar findings have been reported by Khan *et al*. (2022), who demonstrated that smaller nanoparticles, with sizes ranging from 5 to 15 nm, are more effective in nematode management due to their higher surface area and reactivity. The aggregation observed at 75:25 and 25:75

ratios, forming flower-like clusters, aligns with studies by Mahawar *et al.* (2018), suggesting that insufficient or excessive stabilizing agents can disrupt the synthesis process, leading to reduced functionality. The study revealed diverse nanoparticle morphologies, including cubic, triangular, platelet, and irregular shapes. Nanoparticle morphology is a critical determinant of their functional properties, influencing their behavior, efficacy, and potential applications in agriculture. These varied forms are indicative of the role plant extracts play in controlling nucleation and growth during the green synthesis process. Each morphology carries distinct advantages that impact their utility in pest management, nutrient delivery, and crop protection. Morphologies such as triangular and platelet-shaped nanoparticles exhibit high surface-area-to-volume ratios, which significantly enhance their reactivity (Sayed-Ahmed *et al.*, 2024). The sharp edges and flat surfaces of triangular nanoparticles make them particularly effective in interactions with biological systems, including pest suppression (Yu *et al.*, 2024). For example, these morphologies can disrupt nematodes' structural integrity or improve adhesion to plant surfaces, thereby enhancing their efficiency as biopesticides (Khan *et al.*, 2022). Similarly, platelet-shaped nanoparticles offer a larger exposed surface area, facilitating better absorption or release of bioactive compounds when used for nutrient delivery or soil conditioning (Dutta, 2023). Cubic-shaped nanoparticles also contribute to improved functionality. Their defined edges and stable geometry can result in predictable interactions, making them suitable for controlled release systems (Kinnear *et al.*, 2017). These shapes are advantageous for applications that require a uniform response, such as slow nutrient release or targeted pest suppression (Shang *et al.*, 2019).

While morphologies with higher surface areas, such as triangular or irregular shapes, enhance reactivity, they also increase the likelihood of aggregation. Irregular nanoparticles, in particular, are prone to clustering due to their uneven surfaces, which can reduce their functional efficiency by decreasing their bioavailability (Kankala *et al.*, 2020). This aggregation can hinder their dispersal in soil or plant environments, limiting their effectiveness (Mukherji *et al.*, 2019). Stabilisation strategies, such as the use of *A. vera* in this study, play a crucial role in minimizing such challenges by maintaining particle dispersion.

The synthesised nanoparticles were evaluated in the context of agricultural applications, particularly nematode management. Previous research has demonstrated the efficacy of nanoparticles synthesised using green methods. For instance, Khan *et al.* (2022) reported that gold and silver nanoparticles synthesised with plant extracts effectively suppressed nematode juveniles around chickpea roots. These nanoparticles exhibited enhanced interaction efficiency due to their small size and high reactivity.

Compared to these studies, the nanoparticles synthesised in the current research offer additional advantages, such as the complete absence of external metal ions and a greater reliance on bioactive plant compounds. The synthesised nanoparticles, particularly those in 50:50 and 25:75 (extract: *A. vera*) ratios, offer a promising and sustainable alternative to conventional agrochemicals. This is largely due to the phytochemicals present, which are effective in managing nematodes. Their smaller particle size compared to bulk extracts provides an added advantage, as the nanoscale amplifies the bioactive properties of *T. elegans* and *L. camara*, facilitating the creation of more efficient pesticides. Additionally, their high surface area and reactivity make them well-suited for soil conditioning, improving nutrient retention and enhancing soil fertility (Mittal *et al.*, 2020).

The crystal structure, phase, and orientation of nanoparticles were analysed using selected area electron diffraction (SAED). Examining the diffraction spots or rings generated by the interaction of a focused electron beam with the nanoparticle sample allowed for the determination of parameters, crystallinity, and symmetry (Ponce *et al.*, 2021). SAED is particularly useful for identifying the nanostructure of individual particles or specific sample regions, making it a vital technique in nanomaterials research and development (Steinhoff *et al.*, 2020).

3.5.3. Selected area electron diffraction (SAED) Patterns

Selected Area Electron Diffraction (SAED) patterns provide key information about the crystalline structure of materials (Shishir *et al.*, 2024). The presence of concentric rings in the SAED pattern indicates a polycrystalline material, where each ring corresponds to a set of lattice planes from randomly oriented grains. The tiny, bright spots superimposed on the rings are caused by individual crystalline domains contributing to the diffraction (Sastry, 2022).

The SAED patterns confirmed the polycrystalline nature of the synthesised nanoparticles, with the 50:50 ratios showing the highest crystallinity. These findings align with Zhang *et al.* (2021), who noted that well-ordered crystalline structures exhibit enhanced reactivity and stability. The superior structural properties observed in this study suggest that the 50:50 formulations could be particularly effective for agricultural applications, providing targeted pest control and nutrient delivery. In contrast, broader and more diffuse ring patterns in the 75:25 and 25:75 samples indicate reduced crystallinity and increased aggregation. These results emphasize the importance of achieving an optimal balance of reducing and stabilising agents to produce high-quality nanoparticles.

3.5.4 Energy-dispersive X-ray spectroscopy

The Energy-dispersive X-ray spectroscopy (EDS) analysis of various nanoparticle samples reveals a diverse elemental composition with unique traits that can significantly contribute to plant growth and nematode management. Nanoparticles such as NPte and NPlc exhibit specific elemental profiles that can enhance agricultural productivity and pest control mechanisms. The presence of elements like potassium (K), magnesium (Mg), phosphorus (P), chlorine (Cl), and copper (Cu) in significant amounts across the analysed nanoparticles is noteworthy. Potassium and magnesium, for instance, are essential macronutrients for plants. Potassium plays a vital role in osmoregulation, enzyme activation, and photosynthesis, contributing to improved plant vigour and resistance to biotic and abiotic stresses (El-Shetehy *et al.*, 2020). Similarly, magnesium is a core component of the chlorophyll molecule and is critical for photosynthesis and the activation of numerous enzymes involved in carbohydrate metabolism (Marschner, 2012). The high magnesium content observed in NPte 25:75 and NPte 50:50 nanoparticles suggest potential applications in enhancing photosynthetic efficiency and overall plant health. Phosphorus, a key component in energy transfer processes, is crucial for root development and flowering. The notable presence of phosphorus in NPte 25:75 and NP1c 50:50 could facilitate improved root architecture, enabling better nutrient uptake and plant stability (Vance *et al.*, 2003). Furthermore, chlorine, though required in trace amounts, plays a role in osmotic balance and stomatal regulation. Its presence in nanoparticles like NPte 50:50 and NPte 25:75 indicates potential for enhancing water-use efficiency in plants under drought conditions.

Copper is a micronutrient with significant roles in lignin synthesis, enzyme activity, and photosynthetic electron transport (Li *et al.*, 2023). The trace presence of copper in the nanoparticles, such as NPte 50:50 and NPte 25:75, highlights their potential to support enzymatic functions and strengthen plant structural integrity (Hansch and Mendel, 2009). Moreover, the presence of trace elements like zinc in NPlc 25:75 further underscores the versatility of these nanoparticles in addressing micronutrient deficiencies in crops.

In addition to supporting plant growth, the elemental composition of these nanoparticles suggests potential applications in nematode management. Potassium has been reported to enhance plant resistance to nematodes by strengthening cell walls and reducing nematode penetration (Prabhakar *et al.*, 2015). Similarly, copper's antimicrobial properties can inhibit nematode activity and propagation in the soil. The diverse elemental profile of these nanoparticles, combining macronutrients and micronutrients, could provide a dual benefit of promoting plant health while reducing nematode infestations.

The dominance of oxygen in certain nanoparticles, such as NPlc 75:25 and NPte 75:25, indicates an oxide-rich structure that could enhance their stability and efficacy in soil applications. Oxygen-rich nanoparticles may facilitate better interaction with soil components, improving nutrient availability and uptake (Zhang *et al.*, 2020). Furthermore, their oxide-based composition could support slow-release mechanisms, ensuring a sustained supply of nutrients to plants (Hafeez *et al.*, 2024).

The presence of these nutrients indicates that the nanoparticles possess dual potential for agricultural applications. These elements play vital roles in plant health and development. For instance, magnesium is an essential component of chlorophyll, which is critical for photosynthesis, while phosphorus supports energy transfer and root development (Ahmed *et al.*, 2023). Potassium contributes to water regulation and resistance against stress, and sulfur is important for amino acid synthesis (Zhu *et al.*, 2020). Trace elements such as copper and zinc act as cofactors for enzymatic activities, ensuring proper metabolic functions in plants (Kaur *et al.*, 2023). The simultaneous availability of these nutrients in the nanoparticle matrix suggests that they can not only combat plant-parasitic nematodes effectively but also enhance plant growth parameters such as nutrient uptake, biomass production, and overall vigor (Kalaiselvi *et al.*, 2019). This dual functionality makes these nanoparticles a sustainable and innovative solution for integrated crop management.

3.6 Conclusion

The current study demonstrates a significant advancement in green nanotechnology through the exclusive use of plant extracts for nanoparticle synthesis. By employing *A. vera* as both a reducing and stabilising agent, the study successfully synthesised nanoparticles with diverse morphologies and characterised their structural and functional properties. Additionally, the phytochemical composition of *T. elegans* and *L. camara* extracts, as well as their role in nanoparticle synthesis, were thoroughly evaluated. These findings underscore the potential of plant-based approaches to eliminate reliance on toxic chemicals and external metal ions, aligning with sustainable and eco-friendly practices.

The optimization of synthesis conditions, particularly the ratio of plant extracts, proved critical in producing nanoparticles with superior crystalline, stability, and functional efficiency. These nanoparticles exhibit promising potential for applications in sustainable agriculture, including pest management, nutrient delivery, and soil enhancement. Such advancements contribute to addressing critical challenges in food security and environmental sustainability. The results of this study establish a foundation for further exploration into the practical applications of these nanoparticles. The subsequent chapter will investigate their efficacy in nematode management under *in vitro* conditions, aiming to validate their functionality and effectiveness in addressing agricultural pests. This transition marks a pivotal step toward integrating green nanotechnology into real-world agricultural systems.

3.7 References

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CHAPTER 4

EXAMINING THE EFFECTS OF *TABERNAEMONTANA ELEGANS* AND *LANTANA CAMARA* PREPARED NANOPARTICLES ON HATCH AND MORTALITY OF *M. INCOGNITA* SECOND-STAGE JUVENILES (J2) UNDER *IN VITRO* CONDITIONS

4.1. Introduction

Nanotechnology has emerged as a promising tool for addressing the nematode pest challenge, offering unique advantages due to the nanoscale properties of its formulations (Pramanik *et al*., 2020). According to Abd-Elgawad (2024), the high surface area-to-volume ratio and enhanced bioactivity of nanoparticles enable precise interactions with target organisms, while reducing the overall amount of material applied. In this context, nanoparticles synthesized from plant extracts could provide an eco-friendly alternative to chemical-based nanoparticles (Hassanisaadi *et al*., 2022). Their biocompatibility, reduced toxicity, and ability to integrate multiple bioactive compounds make plant-based nanoparticles particularly suitable for agricultural applications (Singh *et al*., 2021). Plant-derived nanoparticles synthesised using reducing and stabilising agents such as *Aloe vera* could offer a sustainable solution to pest management (Khaldoune *et al*., 2024). Plant-based nanoparticles were successfully synthesised from *Tabernaemontana elegans* and *Lantana camara* plant extracts (Chapter 3). Compared to the pure whole plants, *T. elegans* and *L. camara* nanoparticle had increased phytochemical composition, with 50:50 and 25:75 plant extract to aloe, concentrations having the highest at room temperature (Chapter 3). The consistent presence and stability of these phytochemicals in the nanoparticles suggest that the synthesis process effectively preserved and enhanced their bioactive properties, making them promising candidates for nematode management. The two concentrations, 50:50 and 25:75 also had more, broader and more intense peaks indicating formation of new molecular species that are stable (Chapter 3). The two concentrations also exhibited a highly defined ring patterns with bright and distinct spots, indicative of a well-developed polycrystalline nanoparticle structures (Chapter 3). The optimization of synthesis conditions, particularly the ratio of plant extracts, proved critical in producing nanoparticles with superior crystalline, stability, and functional efficiency, however, the ability of these synthesised products to suppress plant-parasitic nematode densities has not yet been determined. Therefore, the current study seeks to assess the potential of nanoparticles synthesised from *T. elegans* and *L. camara* plant extracts, with *A. vera* gel serving as a natural reducing and stabilizing agent in juvenile hatch and mortality.

The focus of this chapter is to evaluate the effects of these plant-based nanoparticles on key parameters associated with *M. incognita*, specifically J2 hatch and mortality. These parameters are critical indicators of the nanoparticles' efficacy as nematicidal agents and their potential to disrupt the life cycle of this destructive pest. Unlike traditional approaches that often rely on chemical toxicity to the target organism, the application of biologically synthesised nanoparticles is hypothesized to combine physical and chemical mechanisms that impair nematode activity (Khan *et al*., 2021). Targeting the nematodes' ability to hatch and survive, these nanoparticles could effectively limit the plant-parasitic nematodes' capacity to infect plant roots and establish feeding sites (Alfy *et al*., 2020).

The study outcomes are expected to contribute significantly to the development of sustainable and scalable solutions for managing root-knot nematodes while supporting agricultural productivity. By advancing the understanding of how biologically synthesized nanoparticles interact with nematodes, this research aims to establish a foundation for integrating nanotechnology into holistic pest management strategies.

4.2. Materials and methods

4.2.1. Experimental site

The study was conducted at the New Research Laboratory 207, Mbombela campus, University of Mpumalanga (25.4365 ° S, 30.9818 ° E), South Africa.

4.2.2. Nanoparticles synthesis

Nanoparticles synthesised in Chapter 3 were used in this study.

4.2.3. Preparation of nematode inoculum

A population of *Meloidogyne incognita* race 2, identified through sequence-characterized amplified regions polymerase chain reaction (SCAR-PCR), was sourced from the ARC-Grain Crops Institute in Potchefstroom, South Africa. This population was propagated for two months in a glasshouse using the susceptible tomato cultivar 'Star 9009'. Egg masses collected from infected tomato plants were agitated in a 1% sodium hypochlorite (NaOCl) solution for 30 seconds to dissolve the gelatinous matrix surrounding the eggs while simultaneously surface-sterilising them (Smalley, 1988). The eggs were then thoroughly rinsed with distilled water before being used in the J2 hatch assays. Freshly hatched J2 were obtained by placing the surface-sterilised eggs in Petri dishes containing 10 ml of distilled water and incubating them at 25 ± 2°C for 24 h.

4.2.4. *Meloidogyne incognita* second-stage juveniles (J2) hatch inhibition bioassay

The hatch inhibition tests were conducted under *in-vitro* conditions. Concentrations of 75:25; 50:50; 25:75; 0:100 of NPlc and NPte were tested for J2 hatch inhibition in Petri dishes at room temperature. Three replications were performed per treatment. Egg masses were dislodged from roots into a Petri dish containing distilled water using a sterilized sharp needle. Each Petri dish was filled with 2 ml distilled water containing 100 eggs + 8 ml of respective nanoparticles. Cumulative counts of J2 hatch inhibition were recorded at 24, 48, and 72 hours of exposure. Leaf extracts without *A. vera* and sterilized distilled water (SDW) were also maintained as controls. Concentrations were considered J2 hatch inhibitive when significantly more eggs did not hatch than in the control. In all trials, three independent experiments with treatments replicated three times were conducted (Wuyts *et al*., 2006).

Second-stage juvenile hatch inhibition (%) = (Total number of eggs−Number of J2 hatched) /Total number of eggs × 100.

4.2.5. Nanoparticles on *Meloidogyne incognita* second-stage juvenile (J2) mortality assay

The mortality assays were conducted under *in-vitro* conditions the same way as with J2 hatch inhibition, except that approximately 100 *M. incognita* J2 were added into Petri dishes instead of eggs. The number of J2 after 24, 48 and 72 h exposures were considered immotile if they were not mobile after transferring them into distilled water for 30 seconds even if they were probed with needles and were counted using a stereomicroscope. Concentrations were considered mortal when significantly more nematodes were dead than in the control. In all trials, three independent experiments with treatments replicated three times were conducted (Wuyts *et al*., 2006). The percentage of J2 mortality were calculated using the formula given below:

Mortality (%) = Number of dead juveniles mortality J2 in the treatment / Total number of J2 × 100.

4.3. Data analysis

Nematode data were analysed using analysis of variance (ANOVA) with Statistix 10 software. Before conducting ANOVA, the Shapiro-Wilk normality test was applied to assess data homogeneity. Non-continuous data were transformed using $\log_{10}(x+1)$ to achieve homogeneous variance (Gomez and Gomez, 1984). For variables showing significant differences, mean separation was performed using Fisher's least significant difference (LSD) test at a 0.05 significance level.

4.4. Results

Shapiro-Wilk normality test indicated that the tested nematode variables were not normally distributed ($P \leq 0.05$) hence, they were transformed accordingly (Appendix 4.1). The interactions between time and concentration on all measured variables were no significant ($P > 0.05$) (Appendix 4.2).

4.3.1. Effects of nanoparticles on second-stage juvenile (J2) hatch.

Nanoparticles had a significant ($P \leq 0.05$) effect on *M. incognita* J2 hatch over three different exposure times, 24, 48, and 72 h (Appendix 4.2). The J2 hatch counts increased with the increase in the duration of exposure to the nanoparticles, with highest J2 hatch observed after 72 h (Table 4.1). The inhibitory effect of nanoparticles on J2 hatch was most pronounced within the first 24 h of exposure at 95% with the least inhibitory effects at 72 h of exposure (Table 4.1).

Table 4.1: Effects of nanoparticles on *Meloidogyne incognita* second-stage juvenile (J2) hatch at different duration of exposure

Time (hr)	J2 hatch	J2 hatch inhibition (%)
72	1.31^{a}(21)	79
48	1.09^{b}(12)	88
24	0.70^{c}(5)	95

F-Value	207.02
$LSD_{0.05}$	0.06
P-Value	0.00

Column means followed by the same letters are not significantly different at $P \leq 0.05$ according to Fisher's Least Significant Difference. Values in brackets are untransformed percentage J2 hatch means. J2 hatch inhibition (%) = (Total number of eggs−Number of J2 hatched) /Total number of eggs × 100.

There were significant ($P \leq 0.05$) differences observed among synthesised nanoparticles together with pure extract in relation to J2 hatch (Appendix 4.2). Both the synthesised nanoparticles and the pure plant extracts had an inhibitory effect that ranged from 37-74 % on J2 hatch relative to the negative control treatment (Table 4.2). However, the J2 hatch inhibition was higher in eggs exposed to nanoparticle (54-74%) than those exposed to individual plant extracts (37-51%) (Table 4.2). The J2 hatch effects of the synthesised nanoparticles of the two plants did not differ at corresponding concentrations, however differences were observed between different concentrations (Table 4.2). When nanoparticles were considered, the lowest juvenile hatch (6.9-7%) and highest hatch inhibitions (72-74%) were observed at 50:50 concentrations of plant extract to *A. vera*, whereas the highest J2 hatch (11-12%) and lowest hatch inhibition (54-57%) (Table 4.2). An increase in the proportion of *A. vera* in the nanoparticle concentration increased the product's J2 hatch inhibition (Table 4.2).

Table 4.2: Effects of nanoparticle concentrations on *Meloidogyne incognita* second-stage juvenile (J2) hatch

Concentrations	Juvenile hatch	^{y}Relative impact (%)

(Extract: *A. vera*)		
Negative control	^{x}1. 3817^{a} (26.111)	-
L. camara (100 %)	1.1820^{b} (16.566)	-37
A. vera (100 %)	1.1424^{bc} (15.00)	-43
T. elegans (100 %)	1.0965^{bcd} (12.889)	-51
NPlc (75:25)	1.0523^{cd} (12.111)	-54
NPte (75:25)	1.0247^{de} (11.333)	-57
NPlc (25:75)	0.9363^{ef} (8.444)	-68
NPlc (25:75)	0.9025^{f} (9.222)	-65
NPlc (50:50)	0.7717^{g} (6.8889)	-74
NPte (50:50)	0.7707^{g} (7.222)	-72
F-value	19.34	
$LSD_{0.05}$	0.1159	
P-Value	0.0000	

^{x}Column means followed by the same letters are not significantly different at $P \leq 0.05$ according to Fisher's Least Significant Difference. Values in brackets are untransformed percentage J2 hatch means. ^{y}Relative impact (%) = [(treatment/control) – 1] x 100.

4.3.2. Effects on nanoparticles on second stage juvenile (J2) mortality

Time and nanoparticles had statistically significant ($P \leq 0.05$) effects on J2 mortality (Appendix 4.3). Juvenile mortality increased with the increase in duration of exposure to both the synthesised nanoparticles and pure plant extracts from 44-70 % (Table 4.3). The highest percentage juvenile mortality of 70 % was observed after *M. incognita* J2 were exposed to nanoparticles for 72 h whereas the least mortality of 44 % was recorded after 24 h of exposure to nano-plant extracts (Table 4.3). The observed trend suggests that while cumulative mortality continued to rise with prolonged exposure, the actual amount at which J2 mortality increased gradually declined over time, indicating a potential reduction in the effectiveness of the treatments beyond the initial hours of exposure (Table 4.3).

Table 4.3: Effects of nanoparticles on juvenile mortality at different duration of exposure

Time (h~~rs~~)	Juvenile mortality
72	1.7672^{a} (70.242)
48	1.6739^{b} (59.121)
24	1.5451^{c} (44.121)
F-value	85.28
$LSD_{0.05}$	0.0341
P-value	0.0000

[x] Column means followed by the same letters are not significantly different at $P \leq 0.05$ according to Fisher's Least Significant Difference. Values in brackets are untransformed juvenile mortality means

The effects of nanoparticles synthesised at varying concentrations on juvenile mortality, revealing statistically significant ($P \leq 0.05$) differences among the different concentrations (Appendix 4.3). Just like with juvenile hatch inhibition, all synthesised nanoparticles and pure extracts caused a significant juvenile mortality relative to the negative control, with nanoparticles generally performing better than the pure extracts (Table 4.4). The most pronounced *M. incognita* juvenile mortality was observed at the 50:50 and 25:75 extract-to-*A. vera* ratios for both plant extracts, at above 75 % mortality, with no significant difference between these two concentration groups from both plants (Table 4.4). Relative to negative control, juvenile mortalities of ≈ 4 000 % were observed after exposure to these concentration groups (Table 4.4). As with juvenile hatch, an increase in the proportion of *A. vera* in the nanoparticle concentration increased the product's J2 mortality (Table 4.4).

Table 4.4: Effects of nanoparticles on *M. incognita* second-stage juvenile mortality at different synthesised concentration

Treatment (Extract: *A. vera*)	Juvenile mortality	^{y}Relative impact (%)
NPlc (50:50)	1.9022^{a} (79.889)	4129
NPte (50:50)	1.9002^{a} (79.556)	4111
NPte (25:75)	1.8886^{a} (77.222)	3988
NPlc (25:75)	1.8766^{ab} (75.778)	3912
NPte (75:25)	1.8126^{bc} (65.111)	3347
NPlc (75:25)	1.7905^{cd} (62.000)	3182
T. elegans	1.7538^{cd} (57.444)	2941
L. camara	1.7321^{d} (54.889)	2806
A. vera	1.6385^{e} (43.889)	2223
Negative control	0.4147^{f} (1.889)	-
F-value	342.11	
$LSD_{0.05}$	0.0653	
P-value	0.0000	

^{x}Column means followed by the same letters are not significantly different at $P \leq 0.05$ according to Fisher's Least Significant Difference. Values in brackets are untransformed percentage juvenile mortality means. ^{y}Relative impact (%) = [(treatment/control) – 1] x 100

4.4. Discussion

Nanotechnology has emerged as a promising approach in pest management, offering enhanced delivery and efficacy of bioactive compounds through nanoscale formulations (Vishnu *et al.*, 2024). Nanoparticles synthesised using plant-based methods have garnered attention due to their eco-friendliness and potential to minimize reliance on chemical agents. The current study focused on the J2 hatch and mortality of *M. incognita* under *in vitro* conditions. The study provided valuable insights into the effects of nanoparticles synthesised from *L. camara* (NPlc) and *T. elegans* (NPte) with *A. vera* as a stabilising agent on *M. incognita* J2 hatch and mortality. The influence of exposure duration and nanoparticle concentrations revealed significant trends and variations in nematode control efficacy.

The observed pattern indicates that the inhibitory effect of nanoparticles on J2 hatch was time-dependent, with the strongest suppression occurring within the initial 24-hour period. As the duration of exposure increased, J2 hatch inhibition continued to occur, be it at slower rate. These findings align with previous research indicating that nanoparticles can effectively suppress nematode hatch and viability (Nazir *et al*., 2019).

Nazir *et al*. (2019) investigated the *in vitro* effectiveness of silver nanoparticles (AgNPs) against *M. incognita* and observed a direct relationship between nanoparticle concentration and J2 mortality. It was observed that, the higher concentrations of AgNPs resulted in increased J2 mortality rates, with significant J2 hatch inhibition observed over time, and the inhibition was directly proportional to the duration of exposure, increasing over a six-day period. Similarly, Balbaa *et al*. (2017) evaluated the effects of various nanoparticles, including silver (Ag), zinc oxide (ZnO), and silicon dioxide (SiO_2), on *M. incognita* J2 hatch and mortality. Their results indicated that all tested nanoparticles significantly reduced J2 hatch percentages and increased J2 mortality compared to controls. The current study also noted that the inhibitory effects were time-dependent, with prolonged exposure leading to greater reduction of J2 nematode.

The pronounced J2 hatch inhibition observed within the first 24 h in the current study could be attributed to the rapid interaction between the nanoparticles and nematode eggs, potentially disrupting normal embryonic development or interfering with biochemical processes required for hatching. This immediate effect is consistent with the findings of Nazir *et al*. (2019), who reported significant mortality and hatch inhibition within 24 h of AgNP exposure. As exposure time extended to 48 and 72 h, J2 hatch progressively increased, suggesting that some eggs might have required longer exposure to exhibit noticeable effects. Some of the reason can be that the eggs were not in the same stage of development and some might have developed survival mechanism. This cumulative J2 hatching trend aligns with the observations of Balbaa *et al*. (2017), who noted that while nanoparticles effectively reduced J2 hatch, the inhibitory effect diminished over time, possibly due to fewer remaining susceptible individuals. However, Khosa *et al*. (2020), demonstrated that prolonged exposure of *M. incognita* J2 to plant extracts often correlates with increased physiological responses in nematodes due to gradual activation of internal biochemical mechanisms. Longer exposure times may enhance metabolic activity and physiological processes within the eggs, enabling more efficient development and J2 hatching.

According to Ebrahimi (2015) observation on the J2 hatch of *Globodera pallida*, a cyst nematode, increased progressively with time when exposed to favourable conditions, suggesting that duration is a critical factor in the successful emergence of J2. Furthermore, Jensen (2018) found that the hatch of *Heterodera glycines* J2 was significantly higher after 72 h compared to shorter exposure durations, likely due to the extended time allowing for the accumulation of metabolic precursors required for hatching. These studies reinforce the idea that temporal factors play a crucial role in the J2 hatch process, which is consistent with the current findings.

Conversely, some studies have reported deviations from this trend, particularly under adverse environmental or chemical conditions. For instance, Ali *et al*. (2021) observed that prolonged exposure to certain nematicidal agents could inhibit J2 hatch in *M. incognita*, regardless of duration. The researchers attributed this to the toxic effects of the compounds, which disrupted embryonic development and reduced J2 hatchability over time. Similarly, Santos (2017) reported that extreme environmental factors, such as high temperatures or saline conditions, could delay or suppress *Meloidogyne* (*M. incognita*) nematode juvenile hatch even with extended durations, suggesting that hatch trends can vary based on external stressors. The increase in J2 hatch observed in the current study underlines the importance of optimising exposure durations to achieve desired outcomes in nematode management. While extended durations may favor J2 hatch under untreated or mild conditions, incorporating inhibitory agents such as nanoparticles could alter these dynamics.

Nanoparticle concentrations significantly influenced the inhibition of *Meloidogyne* juvenile hatch, as evident in the differences between untreated controls, raw plant extracts, and nanoparticle treatments. The negative control exhibited the highest J2 hatching rates, reflecting the natural hatch potential of J2 from eggs in the absence of a nanoparticle. Interestingly, treatments representing 100% *A. vera*, *L. camara*, and *T. elegans* extracts did not produce J2 hatching rates that differed significantly from the untreated control suggesting moderate nematicidal activity of the raw extracts. These findings are consistent with previous studies by Abbassy *et al*. (2017) and Rajeshkumar *et al*. (2020), who emphasized that nanoparticles exhibit enhanced bioactivity compared to bulk plant extracts due to their unique physicochemical properties.

Nanoparticles synthesised from *L. camara* and *T. elegans* at 50:50 and 25:75 ratios with *A. vera* demonstrated significant inhibition of juvenile hatch, underscoring their superior nematicidal efficacy. This enhanced activity can be attributed to the high surface area-to-volume ratio of nanoparticles, which improves their interaction with biological membranes, facilitating better delivery of bioactive compounds. Rajeshkumar *et al*. (2020) highlighted that nanoparticle derived from plant extracts showed greater bioactivity against *Meloidogyne* spp, particularly due to their ability to effectively penetrate nematode eggs and disrupt internal metabolic processes.

Several research groups are working on developing phytochemical-based approaches for nematode control (Mashela *et al*., 2017; Desmedt *et al*., 2020). Plant-nematode interactions involve various compounds that function as repellents, attractants, hatch stimulants or inhibitors, and nematotoxicants, either naturally occurring or induced in response to nematode presence (Chitwood, 2022). Phytochemical analysis of *L. camara*, *T. elegans*, and their nanoparticle formulations synthesised with *A. vera* gel identified a diverse range of bioactive compounds, including glycosides, flavonoids, tannins, phenols, saponins, steroids, and terpenoids (Chapter 3). These compounds are widely recognised for their pesticidal, nematicidal, and antimicrobial properties, highlighting the potential of these plants for sustainable agricultural use. The stable presence of these phytochemicals in the nanoparticles suggests that the synthesis process effectively preserves and enhances their bioactivity, making them promising candidates for nematode management.

These secondary metabolites are well-documented for their nematicidal properties. For example, saponins, which were moderately to abundantly present in both plant extracts and nanoparticles, are known to disrupt cell membranes by forming complexes with sterols, leading to nematode mortality (Arora *et al*., 2022). Saponins disrupt nematode membranes, leading to structural damage and mortality (Gupta *et al*., 2025). Ibrahim Srour (2013) showed that the effect of saponin on parasitic nematodes is due to its chemical composition where saponin are steroid or triterpenoid glycosides and can be used as natural nematicide. D'Addabbo *et al.* (2020) who recognised that the saponin extract has a significant role in controlling the development of insects and plant parasitic nematodes. Ibrahim and Srour (2013) reported the highest *M. incognita* J2 nematode inhibition in the treatment of 100% of saponin crude extracts. The presence of higher saponins observed in the NPte and NPlc in chapter 3 might have contributed to the effectiveness against nematodes. Chitwood (2022) also reported saponins from Ear-pod wattle (*Acacia auriculiformis* L.) and steroids from Safed moosli (*Asparagus racemosus* L.) inhibiting root galling by *M. incognita* when the compounds were applied as soil drenches or foliar sprays.

Flavonoids, on the other hand, play a dual role by providing allelopathic effects and acting as auxin precursors, which contribute to plant defence mechanisms and suppress nematode development (Zheng *et al*., 2024). Chitwood (2002) reported that flavonoids act as potent inhibitors of nematode reproduction and J2 hatch by interfering with their hormonal signalling pathways. Similarly, terpenoids and tannins inhibit *M. incognita* hatch and mobility by interfering with their biochemical pathways (Desmedt *et al*., 2020). Tannins and phenols, known for their astringent and antimicrobial properties, have been found to degrade the cuticle of nematodes, impairing their mobility and infectivity (Dutta *et al*., 2019). For instance, glycosides, particularly cardiac glycosides, have been shown to disrupt nematode cellular respiration and energy production, leading to mortality (Ntalli and Caboni, 2012). The rich presence of these compounds in NPlc and NPte positions them as effective biocontrol agents against plant-parasitic nematodes such as *Meloidogyne* spp.

The findings also corroborate studies on other nematode species. Abbassy *et al*. (2017) reported that nanoparticles synthesised from horseweed (*Conyza dioscoridis* L.) extracts and metals effectively suppressed the hatch and mobility of *M. incognita* J2, demonstrating the potential of nanoscale formulations in enhancing nematicidal activity. Similarly, Nassar *et al*. (2016) found that nanoparticles derived from Annual nettle (*Urtica urens* L.) increased nematicidal efficacy elevenfold compared to raw plant extracts. These studies collectively reinforce the conclusion that nanoparticle synthesis amplifies the bioactivity of plant-derived compounds against plant-parasitic nematodes.

Conversely, some studies suggest that the efficacy of nanoparticles may vary depending on factors such as environmental conditions, nanoparticle stability, and the specific plant-parasitic nematode species targeted. For instance, Castillo-Henríquez *et al*. (2020) raised concerns regarding the variability in nanoparticle effects under different soil compositions and moisture levels, which may influence their bioavailability. Additionally, studies by Wahab *et al*. (2024)

noted that while nanoparticles generally exhibit superior activity, their prolonged stability and potential environmental persistence warrant careful consideration in agricultural applications.

Exposure duration played a critical role in influencing J2 mortality rates in *Meloidogyne* nematodes. Mortality significantly increased with extended exposure, with the highest rates recorded at 72 h and the lowest at 24 h. This time-dependent efficacy highlights the progressive impact of nanoparticles on *M. incognita* J2, likely due to prolonged interaction and disruption of nematode physiology over time. These findings are consistent with Nassar *et al*. (2016), who reported similar time-dependent nematicidal effects in nanoparticle formulations derived from *Azadirachta indica* (Neem). The gradual release of bioactive compounds over time was noted to enhance mortality in *M. incognita*. However, Bordoloi (2019) observed that a 100% concentration of *L. camara* leaf extract was highly effective at 48 h, suggesting that specific plant extracts may exhibit rapid efficacy under certain conditions. This discrepancy emphasizes the importance of optimising exposure duration based on the source and formulation of the treatment.

Nanoparticle concentration further influenced J2 mortality, as demonstrated in Table 4.4. An increase in the proportion of *A. vera* in the nanoparticle concentration increased the product's J2 mortality with nanoparticles synthesised at 50:50 and 25:75 ratios (extract: *A. vera*) exhibiting the highest mortality rates, significantly outperforming the pure plant extracts and the negative control. This suggests a synergistic interaction between *A. vera* and the plant extracts in the nanoparticle formulations. *Aloe vera* bioactive compounds, such as polysaccharides and anthraquinones, likely contributed to this enhanced efficacy by stabilising and reducing the nanoparticles during synthesis. Prasad *et al*. (2021) highlighted similar observations, noting that *A. vera* bioactive properties amplify the nematicidal effects of nanoparticles by improving stability and bioavailability. This is further supported by Khan *et al*. (2023), who demonstrated that nanoparticles enhance the delivery of active compounds, increasing their interaction with nematodes and disrupting their biological processes more effectively.

The superior performance of synthesised nanoparticles compared to raw plant extracts underscores the advantages of nanotechnology in nematode management. Nanoparticles, with their high surface area-to-volume ratio and enhanced bioactivity, provide a more efficient delivery system for bioactive compounds. This property ensures that the active ingredients penetrate nematode cuticles and disrupt their physiological functions more effectively. In the current study, pure extracts had very large molecules compared to nanoparticles (Chapter 3). Studies on green-synthesised nanoparticles derived from plants like *Conyza dioscoridis* and *Urtica urens* similarly demonstrated significant improvements in nematicidal activity compared to their bulk counterparts (Abbassy *et al*., 2017; Nassar, 2016).

Interestingly, the proportional increase in *A. vera* in nanoparticle synthesis amplified their nematicidal activity, indicating its critical role as a reducing and stabilising agent. This aligns with findings by Castillo-Henríquez *et al*. (2020), who noted that plant-based nanoparticles exhibit increased efficacy due to the synergistic actions of their bioactive constituents. The addition of *A. vera* likely enhances the nanoparticles structural integrity, chemical composition and delivery efficiency, optimizing their nematicidal potential.

While the results align with many studies supporting the efficacy of nanoparticles in pest management, some research raises concerns about variability in performance due to environmental factors or differences in nematode species. For instance, Dang *et al*. (2018) noted that the effectiveness of nanoparticles could vary depending on soil composition and moisture levels, which influence bioavailability. Such variability underscores the need for field-based studies to validate the consistency of laboratory findings under real-world conditions.

Overall, the synthesised nanoparticles demonstrated remarkable potential as eco-friendly and efficient alternatives to conventional nematicides. By leveraging the synergistic properties of

L. camara, *T. elegans*, and *A. vera*, this study contributes to advancing sustainable pest management strategies. The results highlight the promise of green-synthesised nanoparticles in addressing the challenges of nematode control while minimizing environmental risks

4.5. Conclusion

The study demonstrated the significant potential of green-synthesised nanoparticles as an eco-friendly and effective alternative to conventional nematicides for managing *M. incognita*. By examining the effects of nanoparticles on J2 hatch and mortality, the findings revealed that both exposure duration and nanoparticle concentration played critical roles in their nematicidal efficacy. Nanoparticles synthesised using *L. camara* and *T. elegans* plant extracts, combined with *A. vera* as a reducing and stabilising agent, consistently outperformed raw plant extracts in suppressing J2 hatch and increasing J2 mortality. The enhanced performance of these nanoparticles can be attributed to their high surface area-to-volume ratio, which improved the delivery and bioavailability of bioactive compounds increased by the combination. Longer exposure durations further amplified their nematicidal effects, suggesting that sustained interaction with the nematodes disrupts their biological processes effectively.

These findings align with previous studies highlighting the superior bioactivity of nanoparticles and their ability to penetrate nematode cuticles and interfere with their metabolic pathways. Overall, this research underscores the potential of plant-based nanoparticles as a sustainable, efficient, and environmentally friendly tool for nematode management. By leveraging the synergistic properties of the selected plant extracts and *A. vera*, this approach not only provides an alternative to chemical nematicides but also contributes to the broader goals of sustainable agriculture. The next chapter focused on exploring greenhouse applications of these nanoparticles to validate their efficacy under diverse environmental conditions.

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CHAPTER 5

EXPLORING THE POTENTIAL OF PREPARED NANOPARTICLES IN MITIGATING THE IMPACT OF *MELOIDOGYNE INCOGNITA* AND ENHANCING PLANT GROWTH VARIABLES UNDER GREENHOUSE CONDITIONS

5.1. Introduction

Extracts from *Tabernaemontana elegans* and *Lantana camara* have demonstrated efficacy in the suppression of nematode populations, with aqueous and methanolic extracts of *L. camara* leaves showing significant activity against *M. javanica* juveniles (Khan *et al*., 2024) and *T. elegans* extracts exhibiting similar effects against *M. incognita* (Khosa *et al*., 2020). Despite the promising results of plant extracts, the need for bulk quantities has limited their adoption in mainstream agriculture (Hernández-Carlos and Gamboa-Angulo, 2019). This unreliability underscores the need for more stable and scalable solutions, especially in resource-constrained agricultural systems. Nanotechnology presents a transformative alternative to address the limitation (Hofmann *et al*., 2020).

Tabernaemontana elegans and *L. camara* synthesised nanoparticles consistently suppressed *M. incognita* juvenile hatch and increased their mortality (Chapter 4), the findings revealed that both exposure duration and nanoparticle concentration played critical roles in their nematicidal efficacy (Chapter 4). The inhibitory effect of nanoparticles on juvenile hatch and mortality was most pronounced within the first 24 hours of exposure with least effects at 72 hours of exposure (Chapter 4). Generally, 50:50 and 25:75 plant extract to aloe, concentrations had the highest hatch inhibition and mortality, and an increase in the proportion of *A. vera* in the nanoparticle concentration increased the product efficacy (Chapter 4). These nanoparticles exhibit promising potential for applications in sustainable agriculture, including pest management, nutrient delivery, and soil enhancement. However, for this potential to be fully realised, empirical data on their performance under less controlled environments is required to validate their laboratory performances.

Hence, the current study seeks to test the effects of nanoparticles synthesised from *T. elegans* and *L. camara* using *A. vera* as a reducing and stabilizing agent on population of *M. incognita* and tomato plant growth under greenhouse conditions. This innovative approach is expected to provide scalable and eco-friendly solutions for managing nematode infestations, addressing both the agricultural and environmental challenges associated with nematode control. Furthermore, the outcomes of this study if upscaled could significantly contribute to enhancing sustainable agricultural practices, particularly in rural and smallholder farming communities with limited access to chemical inputs.

5.2. Method and materials

5.2.1. Description of the study

The study was carried out in a controlled greenhouse environment at the University of Mpumalanga (25.4365°S, 30.9818°E) in Nelspruit, South Africa. To regulate the temperature between 25°C and 30°C, thermostatically controlled fans and a wet-wall system were installed at opposite ends of the greenhouse.

5.2.2. Plant extracts and inoculum preparation

Leaves of *T. elegans* and *L. camara* were collected from Nelspruit, Mpumalanga Province, South Africa. The leaves were separately cut into 5 cm-long sections, oven-dried at 52°C for four days, ground into fine powder using a blender, and passed through a 1-mm sieve. The resulting powdered leaf meals were stored in labelled, temperature- and light-controlled glass jars at room temperature in the dark to preserve their bioactive properties until further use. A population of southern root-knot (*M. incognita*) nematodes was obtained as previously described (Chapter 4).

5.2.3. Nanoparticle synthesis using *Aloe vera* as a reducing and stabilizing agent

Nanoparticles were synthesised as described in Chapter 4.

5.2.4. Preparation of nematode inoculum

The nematode inoculum was prepared like in achieving objective 4.

5.2.5. Treatment and experimental design

Plastic 30-cm-diameter pots were filled with a soil mixture of steam-pasteurized loamy and sandy soil at a ratio of 1:3 (v/v). The pots were spaced 0.3 m apart on greenhouse benches to ensure uniform growing conditions. Each pot was transplanted with one "Star 9009" tomato seedling. Two weeks after transplanting, seedlings were inoculated with 5000 *M. incognita* eggs and second-stage juveniles (J2) using a 20 mL plastic syringe. One-week post-inoculation, nanoparticle treatments were applied to the pots. The experiment followed a 2 x 6 x 3 factorial arrangement in a randomized complete block design (RCBD) with five replications. The first factor consisted of two plant extracts (*L. camara* and *T. elegans*). The second factor consisted of six previously developed nanoparticle concentrations (Chapter 3); a positive control [(Nemacur® 10GR at 5 g/plant) fenamiphos, AMVAC]; and a negative control (nematode-inoculated plants without treatment) were also included. The third factor comprised of three liquid nanoparticle application levels of 5 ml, 10 ml, and 15 ml.

5.3 Data collection

Plant Variables

Fifty-six days after inoculation, plant height of each tomato plant per pot was measured from the crown to the tip of the flag leaf. Stems were then cut at the soil line, and stem diameter was measured 3 cm above the cut end using a manual vernier calliper (Model no: 464-9952, RS PRO, South Africa). The chlorophyll content of the uppermost mature leaf was assessed using a chlorophyll meter (Minolta SPAD-502, Hangzhou, China). Shoots were weighed, dried in an oven at 70°C for 72 h, and reweighed to determine the dry shoot mass. Root systems were carefully removed from the soil, washed to remove any remaining soil particles, blotted dry, and weighed. Flowers were counted manually to determine the number of flowers per treatment.

Nematode Variables

Nematodes were extracted from the entire root system using the maceration and blending method described by Fourie *et al*. (2017). Soil from each pot was thoroughly mixed, and a 250 mL sample was collected. Nematodes were then extracted from the soil sample using the sugar flotation and centrifugation technique (Fourie *et al*., 2017). Eggs and second-stage juveniles (J2) from roots, as well as J2 from soil samples, were counted using under a stereomicroscope (Olympus Corporation Tokyo 163-0914, CX23RTFS2) using three independent 1 mL aliquots per sample, with the average value computed. The final nematode population density (Pf) was determined by summing the total eggs and J2 from roots with the total J2 from soil. Additionally, reproductive potential (RP), calculated as the total number of eggs and J2 per gram of fresh root mass, and the reproductive factor (RF = Pf/Pi), representing the nematode's ability to reproduce, were determined (Kayani and Mukhtar, 2018).

5.4 Data analysis

Data on plant growth and nematode variables were analysed using analysis of variance (ANOVA) in Statistix 10 software. Before ANOVA, the Shapiro-Wilk normality test was conducted to assess data homogeneity. Non-continuous data were transformed using $\log_{10}(x+1)$ to ensure homogeneous variance (Gomez and Gomez, 1984). For significantly different variables, mean separation was performed using Fisher's least significant difference (LSD) test at a 0.05 significance level.

5.5 Results

Shapiro-Wilk normality test indicated that all nematode and plant growth variables were not normally distributed ($P \leq 0.05$), hence they were transformed accordingly using $\mathrm{Log}_{10}(x+1)$ (Appendix 5.1). The presented data illustrates numerous significant ($P<0.05$) trends regarding how varying nanoparticle concentrations impacted both plant growth variables and nematode variables (Appendix 5.2 - 5.13). The second order interactions of plant x concentrations x application levels were not significant for all measured plant growth and nematode variables (Appendix 5.2 - 5.13).

5.5.1 Effects of synthesised nanoparticle on nematode variables

The only statistically significant ($P\leq0.05$) interaction was the first-order interaction between plant and nanoparticle concentration on all nematode variables, except for juvenile in root and juveniles soil (Appendix 5.3; 5.5; 5.7 and 5.8). Both the synthesised nanoparticles and the pure plant extracts had significantly lower nematode variables relative to the negative control treatment (Table 5.1; 5.2). Nanoparticles treated plants had lower nematode variables when compared to those treated with pure individual plant extracts (Table 5.1; 5.2). The highest nematode variable reductions were observed on nanoparticle concentrations of 50:50 and 25:75

for both plants (Table 5.1; 5.2), followed by 75:25 and last were the pure individual extracts (Table 5.1; 5.2). An increase in the proportion of *A. vera* in the nanoparticle concentration increased the product's effects on nematode variables (Table 5.1; 5.2). Between the pure extracts, *L. camara* outperformed *A. vera* in reducing nematode populations; however, the nanoparticle formulations combining extracts proved significantly more effective. The negative control had higher reproduction potential while the NPte and NPlc at 25:75 concentration with the highest nematode reduction demonstrated the lowest reproduction potential. Although pure extract reduced the reproduction factor compared to the negative control, they were less effective than the corresponding nanoparticle formulation.

Table 5.1: Interactive effects of plant and nanoparticles of *Lantana camara* and *Tabernaemontana elegans* concentration on *Meloidogyne incognita* nematode variables

Treatment	**Concentration**	**Eggs in root**	**Total nematodes in roots**	**Final nematode population**	**Reproduction potential**	**Reproduction factor (Pf/Pi)**
NPlc	75:25	$^{x}2.80^{c}$ (640.0)	3.01^{d} (1046.7)	3.23^{f} (1726.7)	1.97^{e} (93.76)	0.20^{e} (0.5756)
NPte	75:25	2.80^{c} (650.0)	3.03^{d} (1090.0)	3.24^{ef} (1776.7)	1.91^{e} (81.39)	0.20^{de} (0.5922)
NPlc	25:75	2.41^{de} (286.7)	2.61^{e} (426.7)	2.99^{h} (1000.0)	1.63^{fg} (46.79)	0.12^{f} (0.3333)
NPte	25:75	2.36^{de} (280.0)	2.62^{e} (453.3)	3.06^{gh} (1200.0)	1.55^{g} (36.98)	0.15^{f} (0.4000)
NPlc	50:50	2.60^{d} (380.0)	2.64^{e} (480.0)	3.02^{gh} (1106.7)	1.70^{f} (52.60)	0.14^{f} (0.3689)
NPte	50:50	2.29^{e} (300.0)	2.65^{e} (486.7)	3.07^{g} (1240.0)	1.70^{f} (56.20)	0.15^{f} (0.4133)
L. camara	100	2.81^{c} (700.0)	3.06^{cd} (1173.3)	3.32^{cde} (2146.7)	2.15^{cd} (155.99)	0.23^{bcd} (0.7156)
T. elegans	100	2.80^{c} (640.0)	3.01^{d} (1026.7)	3.27^{def} (1900.0)	2.02^{de} (107.55)	0.21^{cde} (0.6333)
Aloe vera	100	3.3557^{b} (2880.0)	3.50^{b} (3800.0)	3.71^{b} (5426.7)	2.31^{b} (213.80)	0.26^{b} (0.8422)
Negative control	-	3.65^{a} (4606.7)	3.77^{a} (6060.0)	3.87^{a} (7653.3)	2.94^{a} (637.16)	0.44^{a} (1.8089)
Nemacur® 10GR	+	2.93^{c} (900.0)	3.14^{c} (1420.0)	3.35^{cd} (2253.3)	2.20^{bc} (155.99)	0.26^{cd} (0.7511)
F-value		2.43	3.45	2.90	2.85	5.60
LSD$_{0.05}$		0.20	0.11	0.0811	0.1381	0.03
P-value		0.03	0.00	0.01	0.01	0.00

^{x}Column means followed by the same letters are not significantly different at $P \leq 0.05$ according to Fisher's Least Significant Difference. Values in brackets are untransformed means [log10 (x+1)]. NPlc: nanoparticles produced by *L. camara* + *Aloe vera*; NPte: nanoparticles produced by *T. elegans* + *Aloe vera*

5.5.2 Effects of synthesised nanoparticle on plant growth and nematode variables

There were significant differences ($P \leq 0.05$) among the synthesised nanoparticles and pure extract concerning plant growth variables and nematode populations in both roots and soil (Appendix 5.2; 5.4; 5.6; 5.9; 5.10; 5.11 and 5.12). The application of nanoparticles had a direct impact on key plant growth factors, including stem diameter, chlorophyll content, plant height, dry shoot weight, root weight, and flower production, as well as *M. incognita* egg and J2 ~~nematode~~ populations in the root and soil samples (Table 5.2). Stem diameter generally increased in treated plants compared to the negative control, which had the smallest stem diameters across all treatments (Table 5.2). Plants treated with synthesised nanoparticles, which did not differ significantly from each other, exhibited the largest stem diameters, whereas the negative control reported the smallest. Chlorophyll content showed no significant differences among plants treated with pure extract, the negative control, and the positive control (Table 5.2). However, the 25:75 and 75:25 nanoparticle treatments, which were not significantly different from each other, had higher chlorophyll content than all other treatments. Similarly, the 75:25 and 50:50 nanoparticle treatments produced taller plants compared to both the negative and positive controls. Tomato plant height was particularly improved by applying pure *L. camara*, *T. elegans*, and the 75:25 and 25:75 nanoparticles. Dry shoot mass increased with treatments using pure *A. vera*, the positive control, and the 25:75 and 50:50 nanoparticles, while the 75:25 nanoparticle treatment resulted in relatively lower dry shoot mass (Table 5.2). Flower production was higher in plants treated with pure extract and synthesised nanoparticles, which did not differ significantly from each other, while the controls produced fewer flowers per plant. Root mass increased in plants treated with the 75:25 and 25:75 nanoparticles, whereas the lowest root mass was recorded in plants treated with *L. camara* and *T. elegans*, as well as the negative control, with no significant differences among them. The 25:75 and 50:50 nanoparticles, which were not significantly different from each other, effectively reduced the number of J2 in roots compared to the positive control (Table 5.2). Additionally, nanoparticle treatments, which did not significantly differ, reduced J2 in the soil more effectively than the negative control.

Table 5.2: Effects of nanoparticle concentration on plant growth variables, *Meloidogyne incognita* second stage juvenile in root and soil

Concentration (Extract: *A. vera*)	**Stem diameter per plant**	**Chlorophyll content per upper leaf per plant**	**Plant height per plant (cm)**	**Dry mass per plant (g)**	**Number of flowers per plant**	**Root mass (g)**	**J2 numbers in roots**	**J2 numbers in soil**
75:25	$^{x}0.81^{a}$ (5.5227)	1.67^{a} (46.607)	1.97^{a} (84.86)	0.86^{a} (5.6148)	0.21^{ab} (0.8333)	1.12^{a} (12.609)	2.60^{b} (423.3)	2.77^{d} (683.3)
25:75	0.80^{a} (5.3750)	1.61^{ab} (42.875)	1.96^{ab} (82.43)	0.81^{ab} (5.7402)	0.31^{a} (1.2667)	1.08^{a} (11.393)	1.74^{c} (156.7)	2.78^{d} (660.0)
50:50	0.80^{ab} (5.2730)	1.54^{c} (35.570)	1.92^{bc} (82.53)	0.78^{bc} (5.5547)	0.25^{ab} (1.0333)	1.00^{b} (9.627)	1.76^{c} (143.3)	2.80^{d} (690.0)
100:0	0.77^{c} (4.9267)	1.54^{c} (34.483)	1.97^{a} (141.87)	0.75^{c} (5.4287)	0.26^{a} (1.0667)	0.92^{c} (7.744)	2.73^{b} (570.0)	2.98^{b} (923.3)
0:100	0.80^{a} (5.3933)	1.56^{c} (35.567)	1.90^{c} (77.02)	0.81^{b} (6.4844)	0.25^{ab} (1.0333)	1.00^{b} (9.490)	2.59^{b} (430.0)	2.92^{bc} (1020.0)
Negative	0.63^{d} (3.2603)	1.54^{c} (36.097)	1.88^{c} (74.70)	0.67^{d} (5.6675)	0.11^{c} (0.3667)	0.8^{c} (6.647)	3.00^{a} (1186.7)	3.20^{a} (1610.0)
Nemacur® 10GR	0.78^{bc} (5.0533)	1.57^{bc} (37.090)	1.92^{bc} (76.27)	0.81^{b} (5.9704)	0.15^{bc} (0.5667)	0.99^{b} (9.024)	2.70^{b} (526.7)	2.86^{cd} (763.3)
F-value	78.05	5.97	4.78	11.89	3.38	17.92	29.53	19.78
LSD$_{0.05}$	0.02	0.06	0.05	0.05	0.45	0.06	0.26	0.06
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

^{x}Column means followed by the same letters are not significantly different at $P \leq 0.05$ according to Fisher's Least Significant Difference. Values in brackets are untransformed means [log10 (x+1)].

Significant differences ($P < 0.05$) were recorded in plants treated with different plant extracts. *T. elegans* presence significantly enhanced chlorophyll content and root weight compared to plants treated with *L. camara* (Table 5.3). Conversely, plants treated with *L. camara* showed fewer J2 in the soil compared to those treated with *T. elegans* (Table 5.3).

Table 5.3: Effect of *Lantana camara* and *Tabernaemontana elegans* on chlorophyll content, root weight and juvenal in root

Plant	Chlorophyll	Root weight	Juvenile in root
Tabernaemontana elegans	$^{x}1.60^{a}$ (40.921)	1.02^{a} (9.9515)	2.52^{a} (534.29)
Lantana camara	1.55^{b} (35.733)	0.98^{b} (9.0582)	2.37^{b} (447.62)
F-value	12.48	6.25	5.15
$LSD_{0.05}$	0.03	0.03	0.26
P-value	0.00	0.01	0.03

^{x}Column means followed by the same letters are not significantly different at $P \leq 0.05$ according to Fisher's Least Significant Difference. Values in brackets are untransformed means [log10 (x+1)].

5.6 Discussion

Several studies have reported that plant extracts, including *T. elegans* and *L. camara*, exhibit potential for managing various plant-parasitic nematodes (Khan *et al.*, 2024; Khosa *et al.*, 2020). *In vitro* experiments have demonstrated that *M. javanica* J2 experience significant mortality compared to the untreated control, when exposed to aqueous and methanol leaf extracts of *L. camara* (Begum *et al.*, 2000). The roots of *L. camara* contain phenolic compounds and hydrogen cyanide, both of which have shown nematicidal activity (Orji *et al.*, 2024). However, at higher concentrations, allelopathic plants may induce phytotoxic effects.

Therefore, before implementing these plant extracts in field conditions for reduction of plant-parasitic nematode densities, it is crucial to determine an optimal concentration that effectively targets plant-parasitic nematodes (PPN) while safeguarding the plant and beneficial microorganisms, such as those with biocontrol and growth-promoting properties.

Above these, the widespread adoption of plant extracts for nematode management by farmers remains a significant challenge in many regions, primarily due to inconsistencies in their effectiveness and the correct or preferable formulation of plant extracts. Numerous studies have highlighted variability in the performance of plant-based treatments between *in vitro* and greenhouse or field studies, making them less reliable and limiting their appeal as a guaranteed solution in agricultural practices. For instance, Hernández-Carlos and Gamboa-Angulo (2019) noted that factors such as plant species, extraction methods, and environmental conditions can greatly influence the efficacy of phytonematicides, leading to inconsistent results. Similarly, Ntalli and Caboni (2012) argued that the variability in the bioactive compounds present in plant extracts can hinder their reproducibility and scalability, further discouraging their adoption. The unreliability associated with plant extracts underscores the urgent need for more stable and consistent strategies for nematode management, particularly within the context of sustainable agriculture. One promising alternative is the development of nanoparticle-based formulations derived from plant extracts.

The results presented in this study provide critical insights into the effectiveness of green synthesised nanoparticles in enhancing plant growth and managing plant-parasitic nematode populations. The failure of *M. incognita* J2 to reproduce may be due to the direct exposure to the nanoparticles on the soil, demonstrated strong nematicidal effects of the extracts nanoparticle. To our knowledge, this is the first report on positive efficacy of NPte and NPlc against root-knot nematodes in tomato plants without phytotoxicity. The reproduction factor of *M. incognita* further emphasised the superior efficacy of nanoparticle formulations. The untreated negative control displayed higher Rf indicating higher *M. incognita* J2 and eggs numbers in the absence of treatment. However, NPte and NPlc nanoparticle formulations at 50:50 and 25:75 (extract: *A. vera*) concentrations recorded the lowest *M. incognita* reproduction factors, confirming their significant impact on reducing nematode reproduction.

The RF quantifies nematode reproduction by comparing the initial (Pi) and final nematode populations (Pf) in rhizospheres of a plant, making it an effective indicator in controlled environments where soil nematodes can be manipulated (Kayani and Mukhtar, 2018). If the RF on a particular host is less than one, it signifies that the nematodes were unable to reproduce, indicating that the plant is not a poor host (Kayani and Mukhtar, 2018). Conversely, an RF greater than one suggests successful nematode multiplication on the host plant (Pofu *et al*., 2010). As a fundamental measure of nematode reproduction, RF serves as a reliable indicator of a plants resistance level (Pofu *et al*., 2010; Abd-Elgawad and Molinari, 2008). According to Mnyambo *et al*. (2023), RF is a key criterion in cultivar selection, with cultivars exhibiting lower RF values being considered effective against root-knot nematodes.

Meanwhile, the reproductive potential indicator assesses nematode infection severity by calculating the ratio of nematodes in roots relative to fresh root mass (Gutiérrez-Gutiérrez *et al*., 2011). The reproductive potential of a nematode is usually used for quickly screening large numbers of plants for their host status as an indicator of whether a plant is host or not. Reproductive potential is a good indicator under field conditions when comparing nematode performance among different landraces hence used mostly as the initial screening indicator of plants as it standardizes the host status per fresh root mass (Mcunu, 2023).

Using *A. vera* as both reducing and stabilizing agents for nanoparticle synthesis, the study underscores the potential of plant-based nanotechnology to offer sustainable and efficient solutions to agricultural challenges. *Aloe vera* plant contains a huge variety of vital substances, including terpenoids, flavanols, and phenols, which function as reducers and stabilizers for the formation of nanoparticles (Ahmed *et al.*, 2023; Arsène *et al.*, 2023; Munirathnam *et al.*, 2023; Vélez *et al.*, 2018). The utilization of *A. vera* for the nanoparticle synthesis enhances their biological activity (Tippayawat *et al.*, 2016) and enables regulation of the size and shape of the synthesized nanoparticles, which can enhance their therapeutic potential (Logaranjan *et al.*, 2016).

Aloe vera gel is recognized for its diverse chemical composition, comprising six specific categories of compounds. The flower of the *A. vera* plant has been employed by Karimi and Mohsenzadeh (2015) for the synthesis of copper nanoparticles reducing Cu^{2+} to Cu^0 with the presence of a peak at 578 nm confirmed the formation of copper nanoparticles. This was observed in the previous Chapter (3) where the peak of the NPlc and NPte was less than 400 nm. According to Chutrakulwong *et al.* (2024), the lower peaks of synthesised nanoparticles mean the more effective at blocking harmful UV radiation; this is because smaller nanoparticles tend to absorb more UV light due to their increased surface area and ability to interact with shorter wavelengths. The findings of the current study emphasise the superiority of specific nanoparticle formulations, particularly the 50:50 and 25:75 nanoparticle concentrations, in promoting plant health and suppressing root-knot nematode populations, while also highlighting the limitations of single-plant extract treatments and conventional nematicides. These findings align with previous research highlighting the potential of nanoparticles in agricultural applications. For instance, nanoparticles have been utilised as nano-carriers for herbicides, fertilizers, and genes, targeting specific plant parts to release their content effectively (Mondéjar-López *et al.*, 2024). This targeted delivery system allows for the slow and constant release of active substances, leading to improved plant growth and reduced environmental pollution through precision farming (Mondéjar-López *et al.*, 2024).

Nanoparticles derived through green synthesis demonstrated enhanced performance in key plant growth variables such as stem diameter, chlorophyll content, plant height, and dry weight.

For instance, the 75:25 nanoparticles concentrations consistently outperformed single extracts and controls in promoting stem diameter and plant height, corroborating previous findings that smaller and more stable nanoparticles enhance nutrient uptake and plant vigour due to their high surface-area-to-volume ratio (Khan *et al*., 2022). Similarly, chlorophyll content was significantly improved by nanoparticle formulations, particularly the 50:50 nanoparticles concentrations, indicating that these treatments likely enhance photosynthetic efficiency. Singh *et al*. (2019) reported nanoparticles application promoting and inhibiting the seedling emergence and growth rates of diverse plant species.

The improvement of plant growth variables in the current study indicates that NPte and NPlc could be taken up easily by direct penetration and transport through the stomatal opening. The ability of nanoparticles to support robust plant growth while reducing nematode populations underscores their multifunctional role in agricultural systems. The energy-dispersive X-ray spectroscopy (EDS) analysis in Chapter 3 revealed essential elements like potassium (K), magnesium (Mg), phosphorus (P), and copper (Cu) in the nanoparticles. Potassium and magnesium play vital roles in osmoregulation, enzyme activation, and chlorophyll synthesis, directly influencing plant vigour and resistance to stress (El-Shetehy *et al*., 2020; Marschner, 2012). Phosphorus enhances root development and flowering, while copper contributes to enzymatic activity and lignin synthesis, strengthening plant structural integrity (Hansch and Mendel, 2009). These elements not only support plant growth but also contribute to nematode host plant resistance. For instance, potassium strengthens cell walls, reducing plant-parasitic nematode penetration, while coppers antimicrobial properties inhibit nematode activity (Prabhakar *et al*., 2015).

The observed differences in performance between nanoparticle formulations and single extracts can be attributed to the synergistic effects of combining plant bioactive compounds with the stabilising properties of *A. vera*. The presence of secondary metabolites like polyphenols, flavonoids, and alkaloids reported in Chapter 3 facilitates the reduction of the particle size during synthesis, while *A. vera* ensures the stability and uniformity of the nanoparticles (Sharma *et al*., 2020). These synergistic interactions result in nanoparticles with superior structural and functional properties, as evidenced by the TEM and SAED analyses, which confirmed their polycrystalline nature and minimal aggregation in some concentrations as shown in Chapter 3. The findings reinforce the importance of optimising synthesis conditions, particularly the concentrations of plant extracts, to achieve nanoparticles that are both structurally robust and functionally effective.

The advantages of green synthesis using plant extracts are manifold. Unlike conventional methods that rely on toxic chemicals and metals, the green approach is environmentally friendly, cost-effective, and scalable. This is particularly relevant for resource-constrained settings, where the affordability and simplicity of the process make it accessible to smallholder farmers. Additionally, the elimination of hazardous reagents reduces environmental and health risks, aligning with global efforts to promote sustainable agricultural practices (Singh *et al*., 2018). The ability to produce nanoparticles using renewable plant resources further enhances the sustainability of this approach, contributing to the circular economy and reducing dependency on non-renewable materials.

The implications of these findings are profound. The use of green-synthesised nanoparticles offers a transformative solution to nematode management, a persistent challenge in agriculture. Nematodes, particularly *M. incognita*, cause substantial yield losses across a range of crops by disrupting root systems and nutrient uptake (Mnyambo *et al*., 2023). The ability of nanoparticles to target nematodes while simultaneously enhancing plant growth represents a significant advancement over conventional agrochemicals, which often have limited specificity and off-target effects. Moreover, the high surface reactivity of nanoparticles enables the controlled release of bioactive compounds, ensuring sustained efficacy with minimal application rates, thereby reducing costs and environmental impact.

The study also highlights the broader potential of nanotechnology in agriculture. Beyond nematode management, nanoparticles can serve as carriers for nutrients, pesticides, and growth-promoting agents, enabling precision agriculture and reducing resource wastage. For instance, nanoparticles have been shown to enhance the bioavailability of micronutrients in soils, improve water retention, and bolster plant resilience against abiotic stressors such as drought and salinity (Ahmed *et al*., 2016). The versatility of nanoparticles makes them a valuable tool in addressing the multifaceted challenges of modern agriculture, including food security and environmental sustainability.

Abbassy *et al*. (2017) reported that synthesising silver nanoparticles (Ag-NPs) from plant secondary metabolites is a rapid, large-scale, and controlled process that effectively produces formulations without causing phytotoxic effects. Wang *et al*. (2021) highlighted the promising role of nanoparticles in agriculture, with applications in crop enhancement, disease management, and environmental remediation. For instance, copper nanoparticles have been found to enhance tomato plant growth and yield by boosting nutrient uptake and photosynthetic efficiency. Metal nanoparticles also exhibit antimicrobial properties, making them valuable for plant disease control (Li *et al*., 2023). Research has shown their effectiveness against a variety of plant pathogens, suggesting their potential as alternatives to conventional pesticides, thereby reducing dependence on chemical treatments (Vera-Reyes *et al*., 2022).

Despite these advantages, metal nanoparticles also pose risks to ecosystems and non-target organisms. Studies indicate that silver and copper nanoparticles can be toxic to beneficial soil microbes, earthworms, and aquatic life (Yamini *et al*., 2023). Specifically, nanoparticles of silver, zinc oxide, and copper oxide have been found to disrupt soil microbial activity, impacting nitrogen cycling, enzyme function, and iron metabolism (Dimkpa, 2014). The continuous accumulation of metal nanoparticles in soil can harm beneficial bacteria and fungi, ultimately reducing soil fertility and plant productivity (Ameen *et al*., 2021). Additionally, these nanoparticles may interfere with arbuscular mycorrhizal fungi and nitrogen fixation in plant-microbe interactions (Dimkpa, 2014). While some studies highlight the adverse effects of metal-based nanoparticles, Castillo-Henríquez *et al*. (2020) noted that their exact environmental impact remains unclear. However, excessive exposure may help identify cytotoxicity pathways. Soil contamination with metal nanoparticles can alter microbial biomass, potentially affecting plant growth and leading to physiological, biochemical, and molecular changes (Parada *et al*., 2018; Dimkpa, 2014).

Biological indicators play a crucial role in assessing soil quality, as soil microorganisms actively contribute to key ecosystem processes such as the decomposition of inorganic matter and nutrient cycling. Consequently, any factor influencing microbial biomass will directly affect soil sustainability (Li *et al*., 2023). Research has shown that the toxic effects on microbial communities are largely dependent on their concentration in the soil. Additionally, Castillo-Henríquez *et al*. (2020) observed that silver nanoparticles (AgNPs) impact beneficial soil microorganisms that facilitate plant growth and nutrient cycling, including rhizobacteria like *Pseudomonas fluorescens* and *P. putida*. However, the ecotoxicology of silver species remains insufficiently understood, particularly regarding their potential accumulation in plants and subsequent transfer through the food chain. As a result, ongoing studies aim to assess the long-term effects of AgNPs on plants (Courtois *et al*., 2019). There is also growing concern about the buildup of metal nanoparticles in the food chain and their potential risks to human health (Bouwmeester *et al*., 2015).

Beyond nanoparticle (NP) concentration in soil, ecotoxicological research should also consider factors such as dissolution rate, particle size, surface area, electric charge, and surface chemistry, as these influence NP stability and transport, leading to more precise toxicity assessments (Castillo-Henríquez *et al.*, 2020). NPlc and NPte exhibit unique properties, including chemical stability, catalytic activity, and nematicidal effects. Despite their potential, the integration of plant-based nanoparticles in agriculture faces challenges such as scalability and regulatory constraints. Maintaining consistent quality and effectiveness at a large scale is a major obstacle, alongside the need for standardised evaluation protocols to determine their environmental and health impacts. Future studies should focus on overcoming these challenges through the development of reliable synthesis methods and extensive field trials to confirm their safety and efficiency in diverse agricultural settings.

5.7 Conclusion

This study demonstrates the bioactive performance of green-synthesized nanoparticles in enhancing plant growth and managing plant-parasitic nematode populations. By leveraging the natural bioactive properties of *A. vera*, *L. camara*, and *T. elegans*, the study offers a sustainable and effective alternative to conventional agrochemicals. The findings emphasize the superiority of specific nanoparticle formulations, particularly the 50:50 and 25:75 concentrations, in promoting plant health and suppressing plant-parasitic nematode populations, while also highlighting the limitations of single-plant extract treatments and conventional nematicides. The findings underscore the importance of optimizing synthesis conditions to maximize the structural and functional properties of nanoparticles. With further research and development, plant-based nanoparticles have the potential to revolutionize agricultural practices, contributing to global food security and environmental sustainability. The integration of plant-based nanoparticles into nematode management strategies aligns with the principles of sustainable agriculture by minimizing the reliance on synthetic chemicals while improving crop health and productivity. As noted by Khan *et al.* (2022), nanoparticle formulations not only enhance the efficacy of plant-derived compounds but also reduce the quantities required for effective pest management, making them a more resource-efficient option. This is particularly critical in regions where resource constraints and environmental concerns necessitate the adoption of low-cost, eco-friendly agricultural inputs.

The growing popularity of sustainable agricultural practices has driven the search for innovative solutions that combine environmental compatibility with consistent performance. By addressing the limitations of traditional plant extracts, nanoparticle-based technologies offer a promising path forward. Future research should focus on optimizing synthesis methods, standardizing protocols, and conducting extensive field trials to validate the reliability and scalability of these advanced formulations. Such efforts will be critical in building farmer confidence and ensuring the widespread adoption of sustainable nematode management strategies.

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CHAPTER 6

SUMMARY, SIGNIFICANCE OF THE FINDINGS, CONCLUSIONS AND RECOMMENDATION

6.1.Summary

This study investigated the potential of green-synthesised nanoparticles derived from *Lantana camara*, *Tabernaemontana elegans*, and *Aloe vera* in addressing the dual challenges of nematode management and sustainable agricultural practices. Phytochemical analyses revealed the abundance of bioactive compounds in the plant extracts, which were critical for the synthesis and efficacy of the nanoparticles. *Aloe vera* acted as a stabilising and reducing agent, enhancing nanoparticle formation and their bioactivity. The nanoparticle synthesis process was extensively optimised, focusing on variations in plant extract ratios, temperatures, and concentrations. This resulted in nanoparticles with diverse and intricate morphologies, as confirmed by TEM analysis. The smallest particle sizes were achieved with specific ratios of plant extracts and *A. vera*, underscoring the influence of synthesis conditions on nanoparticle characteristics. SAED patterns and UV-Vis spectroscopy further validated the structural stability, crystallinity, and bioactive properties of the synthesized nanoparticles. The Energy-dispersive X-ray spectroscopy (EDS) analysis of various nanoparticle samples reveals a diverse elemental composition with unique traits that can significantly contribute to plant growth and nematode management. Nanoparticles such as NPte and NPlc exhibit specific elemental profiles that can enhance agricultural productivity and pest control mechanisms.

In vitro experiments demonstrated the efficacy of the nanoparticles in significantly suppressing *M. incognita* J2 hatch. Treatments involving optimised nanoparticle formulations consistently outperformed raw plant extracts and 100% *A. vera*, highlighting the synergistic effects of combining multiple plant extracts. The nematicidal effects of the nanoparticles were further validated in greenhouse studies, where treatments not only reduced *M. incognita* populations in soil and roots but also improved key plant growth parameters, including stem diameter, plant height, chlorophyll content, and dry shoot weight. These improvements suggest that the nanoparticles have dual functionalities, acting as both nematicides and plant growth promoters. Overall, the study underscores the transformative potential of integrating green nanotechnology into agricultural practices. By leveraging the unique properties of *L. camara*, *T. elegans,* and *A. vera*, the synthesized nanoparticles present an eco-friendly and sustainable alternative to chemical nematicides. This research contributes to the growing body of knowledge on sustainable pest management solutions and lays the groundwork for future studies aimed at optimizing nanoparticle synthesis, conducting large-scale trials, and assessing the broader environmental and economic impacts of their use.

6.2.Significant of the findings

The findings of this study have far-reaching implications for sustainable agriculture and pest management practices. By demonstrating the efficacy of green-synthesised nanoparticles derived from *L. camara*, *T. elegans*, and *A. vera*, the research offers a viable alternative to conventional chemical nematicides. These nanoparticles not only exhibit potent nematicidal properties but also promote plant growth, providing a dual benefit that is critical for improving crop yields and ensuring food security.

The eco-friendly nature of the nanoparticles addresses growing concerns about the environmental impact of synthetic pesticides, offering a solution that minimizes soil and water contamination while preserving beneficial soil microbes. The use of readily available and cost-effective plant materials for nanoparticle synthesis further enhances their practicality and accessibility, particularly for resource-limited farmers in developing regions.

Moreover, the study's emphasis on optimising synthesis conditions paves the way for the production of nanoparticles with consistent quality and efficacy, a crucial factor for their widespread adoption. The findings also highlight the role of plant-based nanotechnology in bridging traditional agricultural practices with modern innovations, fostering a more sustainable and resilient agricultural system. By addressing both the economic and environmental challenges associated with pest management, this research contributes to the global effort to transition towards sustainable agricultural practices that align with the principles of environmental conservation and climate resilience.

6.3.Conclusion

This study represents the first documented use of *A. vera* gel in the green synthesis of nanoparticles from *T. elegans* and *L. camara*. The findings of this study conclusively demonstrate the potential of green-synthesised nanoparticles as a sustainable solution for nematode management in agriculture. The use of *L. camara*, *T. elegans*, and *A. vera* extracts in nanoparticle synthesis resulted in stable, bioactive nanoparticles with significant nematicidal effects. The optimisation of synthesis conditions, including extract ratios, concentrations, and temperatures, was critical in achieving nanoparticles with desired characteristics such as smaller particle sizes, enhanced bioactivity and higher structural stability. By employing *A. vera* as both a reducing and stabilising agent, the study successfully synthesised nanoparticles with diverse morphologies and characterised their structural and functional properties. Moreover, the phytochemical composition of *T. elegans* and *L. camara* extracts was extensively evaluated, revealing their significant role in nanoparticle synthesis and their bioactivity in *M. incognita* nematode suppression. The optimisation of synthesis conditions, particularly the ratio of plant extracts, proved critical in producing nanoparticles with superior crystalline, stability, and functional efficiency. These nanoparticles exhibit promising potential for applications in sustainable agriculture, including pest management, nutrient delivery, and soil enhancement. Such advancements contribute to addressing critical challenges in food security and environmental sustainability.

Among the tested formulations, nanoparticles synthesised using *L. camara* and *T. elegans* plant extracts combined with *A. vera* at ratios of 50:50 and 25:75 (plant extract: *A. vera*) demonstrated superior performance both in vitro and under greenhouse conditions. The effectiveness of these nanoparticles was attributed to enhanced phytochemical composition and stability, improved nematicidal activity, optimised structural properties for bioavailability, and the synergistic effects of *A. vera*. Phytochemical analysis revealed that the addition of *A. vera* increased the phytochemical of the synthesised nanoparticles, which are crucial in disrupting nematode metabolic pathways and interfering with their reproductive processes. Higher *A. vera* concentration (i.e., 25:75) contributed to increased nanoparticle stability, preventing aggregation and ensuring prolonged bioactivity in soil applications.

In vitro assays demonstrated that nanoparticles at 50:50 and 25:75 formulations exhibited significantly higher nematicidal activity compared to raw plant extracts and single-plant-derived nanoparticles. The 50:50 and 25:75 nanoparticles reduced *M. incognita* J2 hatch while also inducing higher juvenile mortality after extended exposure.

Greenhouse trials confirmed these findings, with plants treated with these formulations showing reduced root galling resulting from *M. incognita* J2 infection and healthier root systems compared to control groups treated with a conventional nematicide or untreated plants. The high surface area-to-volume ratio of the 50:50 and 25:75 nanoparticles facilitated greater bioavailability and enhanced penetration into cuticles of *M. incognita* J2. Structural analysis indicated that these nanoparticles possessed well-defined crystalline properties, ensuring their stability and long-term effectiveness in soil applications. The presence of *A. vera* contributed to uniform nanoparticle morphology, which is crucial in maintaining consistent delivery of bioactive compounds.

Increasing *A. vera* concentration in the nanoparticle synthesis process resulted in better dispersibility and prolonged release of bioactive compounds. The synergistic interactions between *A. vera* and plant extracts enhanced the nanoparticles' nematicidal efficacy while reducing their toxicity to non-target soil microorganisms, thereby promoting soil health and plant growth. These findings align with previous research demonstrating the superior bioactivity of nanoparticles in agricultural pest management. The ability of green-synthesised nanoparticles to penetrate nematode cuticles, interfere with their metabolic pathways, and persist in soil environments for extended periods underscores their potential as a revolutionary approach to sustainable nematode control.

By integrating traditional agricultural practices with modern nanotechnology, this study provides a viable alternative to chemical nematicides that is both environmentally friendly and economically feasible. The successful synthesis and application of plant-based nanoparticles reinforce the necessity for continued exploration of green nanotechnology in agriculture. These advancements not only reduce reliance on toxic agrochemicals but also contribute to broader goals of food security and environmental sustainability.

6.4. Recommendations

Based on the findings, it is recommended that future research focuses on scaling up the synthesis of green nanoparticles and conducting large-scale field trials to validate their efficacy in prevailing agricultural conditions in different bioclimatic zones. The optimisation of nanoparticle formulations should continue, with an emphasis on exploring additional plant extracts and their combinations to enhance nematicidal and plant growth-promoting properties. Environmental impact assessments should be integrated into future studies to evaluate the long-term effects of nanoparticle use on soil health, microbial populations, and overall ecosystem sustainability. Furthermore, economic analyses are necessary to determine the feasibility of large-scale production and application of these nanoparticles in different agricultural settings. Policymakers and stakeholders in the agricultural sector are encouraged to invest in the development and adoption of green nanotechnologies. By promoting research and development in this field, governments and organizations can support the transition to sustainable agricultural practices as part of an integrated pest management (IPM) system, reducing reliance on old-generation chemical pesticides and fostering environmental conservation. Lastly, public awareness campaigns should be initiated to educate farmers and agricultural practitioners on the benefits and application methods of green-synthesised nanoparticles, ensuring their successful integration into mainstream agricultural practices and available to farmers at an affordable rate.

6.5. APPENDICES

Appendix 4.1: Shapiro-Wilk Normality test for nematode variables.

Variable	**N**	**W**	**P**
Juvenile Mortality	99	0.9394	0.0002
Juvenile hatch	99	0.9257	0.0000

Appendix 4.2: Analysis of variance for juvenile hatch.

Source	DF	SS	MS	F	P
Rep	2	0.0280	0.01398		
Hours	2	6.2667	3.13337	207.02	0.0000
Treatment	10	2.9269	0.29269	19.34	0.0000
Hours x Treatment	20	0.3867	0.01934	1.28	0.2270
Error	64	0.9687	0.01514		
Total	98	10.5770			

Appendix 4.3: Analysis of variance for juvenile mortality.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	2	0.0170	0.00849		
Hours	2	0.8205	0.41024	85.28	0.0000
Treatment	10	16.4576	1.64576	342.11	0.0000
Hours*Treatment	20	0.1030	0.00515	1.07	0.4008
Error	64	0.3079	0.00481		
Total	98	17.7059			

Appendix 5.1: Shapiro-Wilk Normality test for nematodes and plant growth variables.

Variable	N	W	P
Stem	210	0.9417	0.0000
Chlorophyll	210	0.8853	0.0000
Flowers	210	0.7915	0.0000
Plant height	210	0.1362	0.0000
Dry weight	210	0.7030	0.0000
Root weight	210	0.9469	0.0000
Eggs in roots	210	0.6184	0.0000
Juveniles roots	210	0.8236	0.0000
Total nematodes in roots	210	0.6574	0.0000
Juvenile in soil	210	0.9521	0.0000
Total nematode per pot	210	0.7038	0.0000
Reproductive factor	210	0.7038	0.0000
Reproductive	210	0.6325	0.0000

Appendix 5.2: Analysis of variance for juvenile in root.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	2.282	0.57039		
Plant (PL)	1	1.286	1.28611	5.15	0.0246
Concentration (C)	6	44.295	7.38243	29.53	0.0000
Application rate (AR)	2	0.710	0.35521	1.42	0.2444
PL * C	6	2.897	0.48276	1.93	0.0786
PL * AR	2	0.371	0.18559	0.74	0.4775

C * AR	12	6.279	0.52324	2.09	0.0919
PL * C* AR	12	4.000	0.33331	1.33	0.2041
Error	164	40.994	0.24996		
Total	209	103.113			

Appendix 5.3: Analysis of variance for egg in root.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0.1016	0.02539		
Plant (PL)	1	0.0118	0.01183	0.15	0.6951
Concentration (C)	6	25.4291	4.23818	55.21	0.0000
Application rate (AR)	2	0.0938	0.04692	0.61	0.5439
PL * C	6	1.1190	0.18649	2.43	0.0282
PL * AR	2	0.0322	0.01612	0.21	0.8108
C * AR	12	0.2901	0.02417	0.31	0.9860
PL * C * AR	12	0.8047	0.06706	0.87	0.5750
Error	164	12.5894	0.07676		
Total	209	40.4718			

Appendix 5.4: Analysis of variance for juveniles in soil.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0.5684	0.14211		
Plant (PL)	1	0.0607	0.06074	1,73	0.1898
Concentration (C)	6	4.1580	0.69300	19,78	0.0000
Application rate (AR)	2	0.0014	0.00068	0,02	0.9807
PL * C	6	0.1989	0.03314	0,95	0.4637

PLA*AR	2	0.0106	0.00530	0,15	0.8597
C * AR	12	0.3544	0.02953	0,84	0.6062
PL * C * AR	12	0.5897	0.04914	1,40	0.1692
Error	164	5.7454	0.03503		
Total	209	11.6875			

Appendix 5.5: Analysis of variance for total nematode in soil.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0.1179	0.02947		
Plant (PL)	1	0.1025	0.10252	8.09	0.0050
Concentration (C)	6	11.7665	1.96109	154.82	0.0000
APP	2	0.0037	0.00187	0.15	0.8628
PL * C	6	0.2201	0.03668	2.90	0.0104
PL * AR	2	0.0177	0.00883	0.70	0.4994
C * AR	12	0.0676	0.00563	0.44	0.9429
PL * C *AR	12	0.1717	0.01431	1.13	0.3397
Error	164	2.0774	0.01267		
Total	209	14.5451			

Appendix 5.6: Analysis of variance for number of flowers.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0.10829	0.02707		
Plant (PL)	1	0.00907	0.00907	0,21	0.6500

Concentration (C)	6	0.89109	0.14851	3,38	0.0036
Application rate (AR)	2	0.05496	0.02748	0,63	0.5360
PL * C	6	0.25439	0.04240	0,97	0.4502
PL * AR	2	0.06394	0.03197	0,73	0.4843
C * AR	12	0.56585	0.04715	1,07	0.3851
PL * C *AR	12	0.59693	0.04974	1,13	0.3368
Error	164	7.19953	0.04390		
Total	209	9.74405			

Appendix 5.7: Analysis of variance for reproductive factor.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0.01981	0.00495		
Plant (PL)	1	0.02280	0.02280	10.05	0.0018
Concentration (C)	6	2.59950	0.43325	191.02	0.0000
Application rate (AR)	2	0.00046	0.00023	0.10	0.9035
PL * C	6	0.07625	0.01271	5.60	0.0000
PL * AR	2	0.00102	0.00051	0.22	0.7991
C * AR	12	0.01064	0.00089	0.39	0.9654
PL * C * AR	12	0.01523	0.00127	0.56	0.8718
Error	164	0.37197	0.00227		
Total	209	3.11768			

Appendix 5.8: Analysis of variance for reproductive potential.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0.48845	0.12211		
Plant (PL)	1	1.103E-05	0.00001	0.00	0.9862
Concentration (C)	6	30.4171	5.06952	138.28	0.0000
Application rate (AR)	2	0.01007	0.00504	0.14	0.8718
PL * C	6	0.62637	0.10440	2.85	0.0115
PL * AR	2	0.01306	0.00653	0.18	0.8370
C * AR	12	0.18166	0.01514	0.41	0.9570
PL * C * AR	12	0.16457	0.01371	0.37	0.9710
Error	164	6.01254	0.03666		
Total	209	37.9139			

Appendix 5.9: Analysis of variables for plant height.

Source	DF	SS	MS	F	P
Rep	4	0.05840	0.01460		
Plant (PL)	1	0.00361	0.00361	0.44	0.5059
Concentration (C)	6	0.23312	0.03885	4.78	0.0002
Application rate (AR)	2	0.00727	0.00364	0.45	0.6401
PL * C	6	0.01391	0.00232	0.29	0.9434
PL * AR	2	0.01124	0.00562	0.69	0.5025
C * AR	12	0.08838	0.00737	0.91	0.5425
PL * C * AR	12	0.07544	0.00629	0.77	0.6774
Error	164	1.33347	0.00813		

Total	209	1.82486

Appendix 5.10: Analysis of variance for dry mass.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	1.90430	0.47607		
Plant (PL)	1	0.00258	0.00258	0.28	0.5998
Concentration (C)	6	0.66507	0.11084	11.89	0.0000
Application rate (AR)	2	0.02239	0.01119	1.20	0.3036
PL * C	6	0.03721	0.00620	0.67	0.6778
PL * AR	2	0.01210	0.00605	0.65	0.5240
CV* AR	12	0.12604	0.01050	1.13	0.3418
PL * C * AR	12	0.07015	0.00585	0.63	0.8170
Error	164	1.52866	0.00932		
Total	209	4.36848			

Appendix 5.11: Analysis of variance for root mass.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0.92369	0.23092		
Plant (PL)	1	0.07455	0.07455	6.25	0.0134
Concentration (C)	6	1.28265	0.21377	17.92	0.0000
Application rate (AR)	2	0.02572	0.01286	1.08	0.3428
PL * C	6	0.05717	0.00953	0.80	0.5723
PL * AR	2	0.01003	0.00502	0.42	0.6575
C * AR	12	0.07291	0.00608	0.51	0.9067

PL * C * AR	12	0.07624	0.00635	0.53	0.8913
Error	164	1.95680	0.01193		
Total	209	4.47976			

Appendix 5.12: Analysis of variance for stem diameter.

Source	**DF**	**SS**	**MS**	**F**	**P**
Rep	4	0.02076	0.00519		
Plant (PL)	1	0.00428	0.00428	2.67	0.1041
Concentration (C)	6	0.75015	0.12502	78.05	0.0000
Application rate (AR)	2	0.00210	0.00105	0.66	0.5199
PL * C	6	0.00982	0.00164	1.02	0.4132
PL * AR	2	0.00082	0.00041	0.26	0.7744
C * AR	12	0.02425	0.00202	1.26	0.2461
PL * C * AR	12	0.01472	0.00123	0.77	0.6846
Error	164	0.26272	0.00160		
Total	209	1.08962			

Appendix 5.13: Analysis of variance for chlorophyll content.

Source	DF	SS	MS	F	P
Rep	4	0.71342	0.17836		
Plant (PL)	1	0.14576	0.14576	12.48	0.0005
Concentration (C)	6	0.41842	0.06974	5.97	0.0000
Application rate (AR)	2	0.02501	0.01251	1.07	0.3451

PL* C	6	0.09049	0.01508	1.29	0.2640
P * AR	2	0.05574	0.02787	2.39	0.0952
C* AR	12	0.04987	0.00416	0.36	0.9764
PL * C * AR	12	0.07437	0.00620	0.53	0.8926
Error	164	1.91557	0.01168		
Total	209	3.48865			