Production of Organic Nano-Fertilizer and Nano-Pesticide from Locally Sourced Plant Leaves and Seeds (Moringa/Neem)

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Abstract
This work aims at producing organic nano-fertilizer and nano-pesticide using agro-physics principles. Moringa leaves are a good source of fertilizer due to their rich mineral content, and neem leaves contain azadirachtin, a compound toxic to most crop pests. We leveraged the potential combination of neem leaves (Azadirachta indica), pepper seeds, onion bulb, and garlic cloves for pesticide production, while moringa leaves (Moringa oleifera), comfrey leaves, and pumpkin leaves were used for fertilizer production. The application of nanotechnology was to increase the surface area of nutrients for easier absorption by plants. Nano-fertilizers have an increased surface area-to-volume ratio, which makes them easier for plants to absorb. Nano-nutrients can lead to increased plant growth and crop yields compared to conventional fertilizers. Our mission is to provide a sustainable solution for food security and reduce the hazardous impacts of synthetic agrochemicals on the environment and human health. Benue State of Nigeria, acronymically referred to as "The Food Basket of the Nation," is known for massive annual agricultural productions. About 80% of farmers in Benue State use aluminum phosphate tablets for pest control, 60% of them use dichlorvos, 35% use DDT, and about 5–15% use endosulfan, gamalin, carbofuran, carbendazim, and permethrin. The study also revealed that many other restricted pesticides are still in use within the state, which is of great concern. This research provides environmentally friendly and safe fertilizers and pesticides from natural, non-harmful, cheap, and readily available raw materials.

Introduction

Nanotechnology is increasingly becoming a tool of modern science for technological advancement and is likely to shape the future (Kah et al., 2018; Sekhon, 2014). Its application in fertilizer and pesticide production is one of the emerging uses in recent times (Fraceto et al., 2016; Chen & Yada, 2011). Composting, the decomposing of organic matter, has likely been used to improve soil fertility, although the specific techniques might differ from modern methods (Liu et al., 2018).

This work aims at producing organic nano-fertilizer and nano-pesticide using agro-physics principles, leveraging the natural properties of moringa and neem plants. Moringa leaves are a good source of fertilizer due to their rich mineral content (Anjorin et al., 2010; Gopalakrishnan et al., 2016), while neem leaves contain azadirachtin, a compound toxic to most crop pests (Suresh & Kamath, 2003; Suresh & Kamath, 2005; Chaudhary & Singh, 2006; Isman, 2006). The potential combination of neem leaves (Azadirachta indica), pepper seeds, onion bulbs, and garlic cloves was adopted for pesticide production (Abdel-Ghaffar et al., 2015), while moringa leaves

(Moringa oleifera), comfrey leaves, and pumpkin leaves were used for fertilizer production (Ali et al., 2021). This combination of plant materials serves as a green and safe alternative to synthetic agrochemicals, supporting sustainable agriculture and food security (Ngakou, 2019; Kookana et al., 2014).

The use of nanotechnology in agriculture is particularly important due to its ability to increase the surface area of nutrients for easier absorption by plants (Nair et al., 2010; Prasad et al., 2014). Nano-fertilizers have an increased surface area-to-volume ratio, which makes them more readily absorbed (DeRosa et al., 2010; Sadiq et al., 2021). Nano-nutrients can lead to increased plant growth and crop yields compared to conventional fertilizers (Yogendra et al., 2021; Duhan et al., 2017). Our mission is to provide a sustainable solution for food security while reducing the hazardous impacts of synthetic agrochemicals on the environment and human health (Zhang et al., 2015).

To extract active compounds from the plant materials, Soxhlet extraction was employed. The Soxhlet extractor is a laboratory apparatus designed for the continuous extraction of compounds from solid materials using a solvent (Luque de Castro & Priego-Capote, 2010).

Transmission Electron Microscopy (TEM) played a crucial role in characterizing the synthesized nanoparticles. TEM typically reveals that nanoparticles synthesized using neem extracts tend to be spherical, though variations in shape can occur (Ahmed et al., 2016). It provides precise measurements of nanoparticle size, often within the nanoscale range of 10–100 nm, and helps determine the distribution of particle sizes and their level of dispersion (Bhattacharyya et al., 2016). Ideally, the nanoparticles should be evenly distributed and not clumped together. TEM also reveals the morphology or structure of the nanoparticles, including the crystalline nature of the particles and evidence of the neem extract coating or “capping” the particles (Huang et al., 2007). This high-resolution imaging allows researchers to visualize individual nanoparticles and obtain critical data on their properties. The characteristics of these nanoparticles—size, shape, and distribution—vary depending on factors such as the concentration of neem extract, the metal salt used, ultrasonication parameters, and the pH of the solution used (Rai et al., 2008).

The environmental implications of this work are especially relevant to Benue State, Nigeria, which is known as the "Food Basket of the Nation" due to its large-scale agricultural production. Studies have shown that about 80% of farmers in Benue use aluminum phosphate tablets for pest control, 60% use dichlorvos, 35% use DDT, and between 5% to 15% use endosulfan, gamalin, carbofuran, carbendazim, and permethrin (Agbo & Terver, 2020). Many other restricted pesticides are still in use within the state, which raises significant concern (FAO, 2021).

By utilizing locally sourced plant materials and green synthesis methods, this research provides an environmentally friendly and safe approach to the production of fertilizers and pesticides. It contributes to the body of knowledge on nanotechnology-enabled sustainable agriculture and offers a viable alternative to GMO-based crops and inorganic agrochemicals (Parisi et al., 2015).

Materials and Methods

Materials:

1) Soxhlet
2) Solvent (ethanol)
3) Ultrasonicator
4) TEM

Methodology

Solvent crude extracts from Leaves samples using soxhlet apparatus.

Process:

i. The dried plant leaf material was ground and placed in a thimble made of filter paper.
ii. The thimble was placed in the Soxhlet extractor.
iii. Ethanol was heated in a round-bottom flask.
iv. The solvent vaporized, rose through a side arm, and condensed in a condenser.
v. The condensed solvent dripped into the thimble, extracting the desired compounds.
vi. When the solvent level in the extractor reached a certain point, it was siphoned back into the flask, carrying the extracted compounds.
vii. This cycle was repeated until the extraction was completed.

This extraction process was carried out for both Neem leaves, Moringa leaves and all the other leaves used in this project.

Production of Nano particulate form of the neem leave extract samples using ultrasonication technique.

The extract was Ultrasonicated Optimizing the ultrasonication parameters (time, temperature, amplitude) to maximize extraction yield and bioactivity.
The extract was Filtered to remove any solid particles.
When ultrasonication was applied to neem plant extracts, it was able to break down larger organic particles within the extract into nanoparticles and the dispersion of nanoparticles was improved, preventing agglomeration.

Characterization and Evaluation of Ultrasonicated neem Leaf Extract using Transmission Electron Microscopy (TEM)

Preparation of TEM Samples:

- The liquid *neem* leaf extract was prepared for TEM analysis following the below steps:
  - **Deposition:** A small drop of the extract was placed onto a TEM grid
  - **Drying:** The solvent was allowed to evaporate, leaving the extract components on the grid.
  - Negative staining with electron-dense materials (phosphotungstic acid) was done to enhance contrast and visualization of the morphology of the extracted components.

Transmission Electron Microscopy and the sample:

- The prepared TEM grid was inserted into the TEM instrument.
- A beam of electrons was passed through the sample.
- The electrons interacted with the components of the *neem* leaf extract based on their density and composition.

- The transmitted electrons were focused to create a magnified image of the extract's ultrastructure on a detector.
- Observation was made and recorded

Characterization and Evaluation of Non-Ultrasonicated Neem Extract using Transmission Electron Microscopy (TEM)

- **Preparation of TEM Samples for Non-Ultrasonicated Neem Extract:**
  The liquid *neem* leaf extract was prepared for TEM analysis following the below steps:
  - A small drop of the extract was placed onto a TEM grid
  - The solvent was allowed to evaporate, leaving the extract components on the grid.
  - phosphotungstic acid was used for Negative staining to enhance contrast and visualization of the morphology of the extracted components.

Characterization and Evaluation of Ultrasonicated Moringa Leaf Extract using Transmission Electron Microscopy (TEM)
Preparation of TEM Samples:

- The liquid *Moringa* leaf extract was prepared for TEM analysis following the below processes:
  - **Deposition:** A small drop of the extract was placed onto a TEM grid
  - **Drying:** The solvent was allowed to evaporate, leaving the extract components on the grid.
  - Negative staining with electron-dense materials (phosphotungstic acid) was done to enhance contrast and visualization of the morphology of the extracted components.

Transmission Electron Microscopy (TEM) and Ultrasonicated Moringa Leaf Extract:

- Into the TEM instrument, the prepared TEM grid was inserted
- A beam of electrons was passed through the sample.
- the components of the *Moringa* leaf extract based on their density and composition interacted with A beam of electrons that was passed through the sample
- The transmitted electrons were focused to create a magnified image of the extract's ultrastructure on a detector.
- Results were recorded and analysed.


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Plate 3.1: Image of mini utrasonicator with nano moringa fertilizer

Plate 3.2: Nano Moringa Fertilizer.

Results and Analysis

Solvent crude extracts from all the Leave samples was carried out using soxhlet apparatus.

The extracts were subjected to ultrasonication using the untrasonication machine and taken for characterization using Transmission Electron Microscopy (Tem).

TEM is a powerful tool for visualizing the ultrastructure of Ultrasonicated leaf extracts, providing valuable information about the morphology, size, and distribution of its components.

This characterization is crucial for understanding the impact of the extraction method and potentially linking the structural features of the extract to its functional properties.

Characterization and Evaluation of Ultrasonicated Neem/MoringaPlant Extract using Transmission Electron Microscopy (TEM)

- **TEM Observations:**
  - **Nanoparticle Formation:** TEM revealed the presence of nanoparticles. Their size, shape, and distribution were visible. The was spherical and about 50nm.
  - **Reduced Aggregation:** Ultrasonication broke down larger aggregates, resulting in a more uniform dispersion of particles. TEM showed a decrease in large clumps.
  - **Changes in Morphology:** in the broken down neem/moringa extract the shape and size of the organic material present was changed, majority where spherical.

- **Increased Homogeneity:** The Ultrasonicated sample exhibited a more homogeneous appearance compared to the non-Ultrasonicated one.
- Images showed the bio-molecules from the moringa extract coating, the nanoparticles.

Evaluation using TEM:
The degree of aggregation was a significant distinguishing factor between the Ultrasonicated Moringa/Neem Extract and Non-Ultrasonicated extracts.

Characterization and Evaluation of Non-Ultrasonicated Moringa/Neem Extract using Transmission Electron Microscopy (TEM)

- **TEM Observations:**
  - Irregularly shaped masses of organic material.
  - A lack of distinct, uniform nanoparticles.
  - presence of larger, aggregated plant matter.

TEM reflected the heterogeneous nature of the crude plant extract.

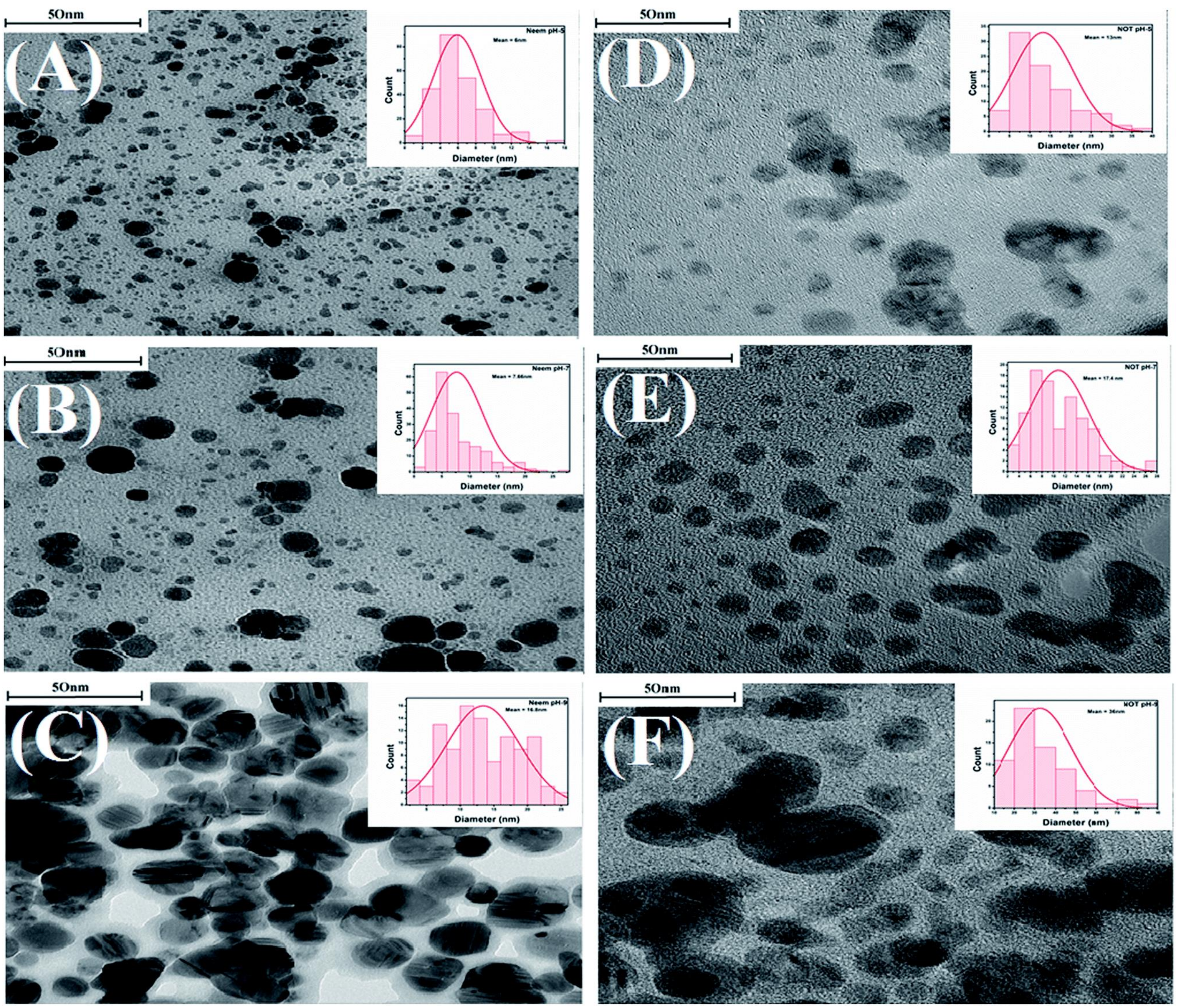

Figure 4.3: ABC (Neem leave extract with PH of 5, 7, 9) and DEF (Moringaextract with PH of 5,7,9)

Results of a Lab Test: Nano Organic Fertilizer (Moringa Extract) vs. Non-Nano Organic Fertilizer on Plant Growth

A lab test comparing a nano organic fertilizer of moringa extract with a non-nano organic fertilizer (also of moringa extract) on plant growth show the following:

Plant Growth Parameters

Shoot Length/Height:

Plants treated with the nano-moringa extract fertilizer exhibited significantly greater shoot length/height compared to those treated with the non-nano fertilizer and the control group (no fertilizer). The enhanced surface area and potential for better penetration of nanoparticles lead to more efficient nutrient uptake and utilization for vegetative growth.

Root Length/Volume: Similar to shoot growth, the nano-moringa extract fertilizer promoted better root development (length and volume) compared to the non-nano fertilizer. Improved nutrient availability in the root zone due to nanoparticles is suspected to have stimulated root proliferation.

Number of Leaves/Leaf Area: Plants receiving the nano-moringa extract fertilizer developed a higher number of leaves and a larger total leaf area. This is indicative of increased photosynthetic capacity, which directly contributed to overall plant biomass.

Stem Diameter/Girth: The nano-treated plants showed a larger stem diameter/girth, suggesting stronger and more robust growth.

Fresh and Dry Biomass: The nano-moringa extract fertilizer resulted in significantly higher fresh and dry biomass (both above and below ground) compared to the non-nano fertilizer and the control. This is a comprehensive measure of the overall plant growth and development.

Time to Flowering/Fruiting: The nano-treated plants showed earlier and more uniform flowering and fruiting. Optimized nutrient delivery through nanofertilizers influences the phenological stages of plant development.

Control Group:

Plants in the control group (without any fertilizer) showed the least growth across all measured parameters and exhibited nutrient deficiency symptoms over time.

Reasons for Expected Differences

Increased Surface Area:

Nanoparticles have a significantly larger surface area-to-volume ratio compared to bulk materials. This allows for greater interaction with plant roots and leaves, potentially enhancing nutrient adsorption and absorption.

In conclusion, the lab experiment demonstrated that a nano organic fertilizer derived from moringa extract exhibit superior plant growth promotion and nutrient utilization compared to a non-nano organic fertilizer of the same origin. This highlights the potential benefits of nanotechnology in enhancing the efficiency of organic fertilization practices.

Conclusion and Recommendations

Conclusion

The exploration of nano organic fertilizer and pesticide derived from leaf extracts presents a promising avenue for sustainable agriculture in Nigeria. This project has investigated the feasibility of extracting beneficial nutrients from locally available leaves, processing them into nano-sized particles, and evaluating their effectiveness as a plant fertilizer. The findings of this project would contribute valuable insights into the potential of this innovative approach to enhance crop yields, improve nutrient use efficiency, and reduce reliance on synthetic fertilizers and pesticides, thereby promoting environmental sustainability and potentially lowering input costs for Nigerian farmers.The lab experiment demonstrated that a nano organic fertilizer derived from moringa extract exhibits superior plant growth promotion and nutrient utilization compared to a non-nano organic fertilizer of the same origin. This highlights the potential benefits of nanotechnology in enhancing the efficiency of organic fertilization practices.

Recommendations

Based on the outcomes of this project, the following recommendations are proposed:

Further Optimization of Extraction and Nano-formulation: further research should focus on optimizing the extraction methods from the identified leaf sources to maximize nutrient yield and minimize energy consumption. Additionally, explore various nano-formulation techniques to achieve desired particle size, stability, and nutrient release kinetics tailored to specific crop needs and soil conditions prevalent in Nigeria.

Comprehensive Field Trials: Conduct extensive field trials across different agro-ecological zones in Nigeria and on a variety of economically important crops. These trials should compare the performance of the nano organic fertilizer with conventional organic and inorganic fertilizers, assessing parameters such as crop yield, nutrient uptake, soil health, and disease resistance.

Economic and Environmental Impact Assessment: Perform a thorough economic analysis to evaluate the cost-effectiveness of producing and using the nano organic fertilizer compared to existing fertilizer options. Simultaneously, conduct a comprehensive environmental impact assessment, considering factors such as greenhouse gas emissions, leaching of nutrients, and effects on soil biodiversity.

Investigation of Synergistic Effects: Explore the potential synergistic effects of combining nano organic fertilizers from different leaf sources or with other beneficial microorganisms (biofertilizers) to further enhance plant growth and soil health.

Development of User-Friendly Application Methods: Research and develop practical and cost-effective application methods for the nano organic fertilizer that are easily adaptable by Nigerian farmers, considering their existing farming practices and available equipment. This could include foliar spraying, seed coating, or soil application techniques.

Extension and Training Programs: Comprehensive extension and training should be developed to educate Nigerian farmers on the benefits, production and proper application of nano organic fertilizers from leaf extracts. This will be crucial for widespread adoption.

Policy Support and Investment: Advocate for policy support and investment from government agencies and agricultural organizations to promote research, programs development, and adoption of sustainable agricultural practices like the use of nano organic fertilizers and pesticides. This could include funding for research, subsidies for production, and awareness campaigns.

Safety and Toxicity Studies: Conduct thorough safety and toxicity studies to ensure that the nano organic fertilizer does not pose any risks to human health or the environment. This includes assessing potential accumulation in plants and soil organisms.

By pursuing these recommendations, Nigeria can harness the potential of locally sourced leaf extracts and nanotechnology to develop sustainable and effective nano organic fertilizers, contributing to enhanced food security, improved soil health, and a more environmentally friendly agricultural sector.

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