Communication in Physical Sciences, 2025, 12(2): 208-215

The Potential of Arginine-Modified Nanoclay Suspension Against Fungi and Bacteria Infestation in Maize and Groundnut

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Received: 21 October 2024/Accepted 18 January 2025/Published 05 February 2025
https://dx.doi.org/10.4314/cps.v12i2.4

Abstract: Maize and groundnut crops are highly vulnerable to fungal and bacterial contamination, and can lead to significant post-harvest losses, reduced quality, and health risks for consumers. This study explores the potential of an arginine-modified nanoclay suspension as an antimicrobial treatment for these crops. A working solution was prepared using a 1.43 mg/mL concentration of the nanoclay suspension, which was applied in vitro to test its effects against selected fungal strains (*Aspergillus niger, Penicillium notatum*, and *Mucor pusillus*) and bacterial strains (*Bacillus subtilis, Bacillus cereus*, and *Pseudomonas aeruginosa*). Results demonstrated that the arginine-modified nanoclay effectively inhibited microbial growth, with inhibition zones ranging from 1.30 to 8.80 mm for fungi and 1.70 to 4.20 mm for bacteria. Treated maize and groundnut seeds exhibited significantly reduced microbial growth three days after application. This eco-friendly, cost-effective approach shows promise for controlling fungal and bacterial infestations in maize and groundnut, thereby supporting food security, sustainability, and a safer food supply chain. Findings suggest that arginine-modified nanoclay could serve as an effective alternative for managing agricultural pathogens.

Keywords: Bacteria, Fungi, Groundnut, Hybrid-Suspension, Infestation, Maize, Organoclay

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1.0 Introduction

Several reports have confirmed that approximately 1.7 billion people in the world are suffering from various forms of fungal infections, with an estimated death proportion approaching 1.5 million yearly (Bongomi *et al.*, 2017; Wambaugh *et al.*, 2020). Although anti-fungal drugs are effective against fungal infections, some literature have ascertained certain side effects such as renal, and kidney malfunctioning have been extensively documented. Also, significant is the development of resistance to some of the familiar antibiotics (Lockhart *et al.*, 2017). Transmission could come from the food value chain due to fungal/bacterial contamination from the farm or during storage. Clay minerals are hydrophilic natural phyllosilicates effective in immobilizing both toxic and non-toxic pollutants due to their adsorptive properties, low cost, biocompatibility, eco-friendliness, and stability (Acevedo *et al.*, 2017; Sarkar *et al.*, 2010; Jadoon *et al.*, 2019; Zaidi *et al.*, 2019; Massaro *et al.*, 2021). They are classified into three main types: Illite (mica), smectite, and kaolinite (Zaidi *et al.*, 2019). Among these,

smectite, especially montmorillonite (MNT), is particularly beneficial due to its low cost, natural abundance, non-toxicity, and excellent dispersion properties in composites (Acevedo *et al.,* 2017; Zaidi *et al.,* 2019). Humans have been familiar with these materials since prehistoric times (Rocha *et al.,* 2014; Azeh *et al.,* 2021). They are used in various consumer goods and potential therapeutic systems (Elele *et al.,* 2020; Azeh *et al.,* 2021), often after purification or chemical modification. These materials also contain minerals like carbonates, feldspar, quartz, iron and aluminium hydroxides (Elele *et al.,* 2020), along with possible organic matter (Acevedo *et al.,* 2017). Nanomaterials are new materials with highly robust properties for domestic and industrial functional uses. Nanoclay is not an exception, with its large surface area coupled with other properties that are normally found with nanoscale matter, it shows enhanced capacity for the adsorption of fungi, bacteria, or viruses on different surfaces, including the lining of the human stomach. More importantly, modification using the organo-cation of the amino acids further enhances its adsorptive performance and interactions with the organisms' cell contents by bonding via –NH, ─COOH, ─NH_2, ─CO, ─N─R_3, ─N─CO─, and $^{+}NR_4$ and so, disable them. It is expected that an insertion of an amino group into the amino acid sequence may alter the configuration of the helix chain sequence, leading to the eventual death of the bacteria/viruses. This research aims to develop a novel, cost-effective, and environmentally sustainable nano-hybrid suspension for the protection of Maize or Groundnut seeds against fungal or bacterial contamination.

The material is easy to produce and involves a green-chemistry approach (Eco-friendly) and it is easy to handle. Topical application of the formulations on stored grains, processed/unprocessed food by wet-spray or dry-spray could be adapted for sustainable food safety throughout the food-value-chain, from harvesting to storage to retailing to processing to packaging and consumption. It is imperative to deploy this simple and sustainable technology for application in our traditional food markets, where food retailers serve as middlemen or women in the food value chain. This will reduce food losses or poisoning usually caused by undetected microorganisms especially, fungi producing aflatoxins (Silent killers). Therefore, it is imperative to look into the readily available, cheaper, and eco-friendly alternative materials for developing new antifungal/antibacterial adsorbents that could stop the growth of dangerous microbes that colonize the food value chain to ensure food security and consumer safety. The research aims to develop and deploy a novel, cost-effective and environmentally sustainable nano-hybrid suspension technology for use in our local food markets for enhanced food security and consumer protection. The specific objectives of the proposed research are (1) the formulation of hybrid nanoclay suspension from locally mined Nigerian clay, (2) the inoculation of the Maize or Groundnut seeds with selected fungi or bacteria; and (3) the application of the hybrid nanoclay suspension on Maize or Groundnut seeds and evaluation of their efficacy.

2.0 Materials and Methods

2.1 Materials and Reagents

All the reagents and chemicals used in this work were obtained from a reliable chemical vendor in Nigeria and were BDH chemicals. The Arginine-modified nanoclay used was a product of our previous research. Other chemicals used include; Hypo (NaOCl) 35 % active chlorine, and ethanol (95%). Others were Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA), Muella Hinton Agar (MHA), slant bottles, Cork borer, Wire loop, incubator and petri dish. Antibacterial and antifungal profiling of the Arginine-modified nanoclay was carried out using *Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, and Penicilliums notatum, Mucor*

Pusilus, and *Aspergillus niger*. Nutrient agar (NA) or nutrient broth (NB) were general-purpose media for 80-88% of bacteria used in this experiment. Native clay was collected from two local mining sites, Kaffin-Koro and Dogon-Ruwa, in Paikoro and Bosso Local Government Areas, respectively in Niger State, Nigeria.

2.2 Preparation of Clay Sample

The preparation of clay, nanoclay, or arginine-modified nanoclay samples followed the procedure described in our previous work (Azeh and Mohammed, 2024). In brief, 2000 g of oven-dry native clay was weighed, transferred in a mortar, and ground into fine powder using a pestle. The fine powdered clay was sieved using a 30 µm mesh size sieve. \

2.3 Preparation of Nanoclay

Nanoclay was synthesized according to the method described in our previous work (Azeh and Mohammed, 2024). In brief, fine particles of crushed layered mixed clay minerals with particle size less than 30 µm, sieved through a 30 mm mesh sieve were mixed with 1:30 times by weight of water. The mixture was stirred and allowed to stand still for 24 hours for the layered clay mineral and water to undergo hydration. The suspension was decanted, stirred, and allowed to stand for 48 h to sediment followed by decantation. Into this was added 1.0 $mold^{-3}$ of a saturated solution of sodium acetate followed by treatment with 90 % solution of hydrogen peroxide and 3.5 % solution of hypochlorite system at pH of 8.2 to eliminate organic matter that may be present and was allowed sediment and then, saturated with NaCl. Afterwards, the mixed clay slurry and the suspension were centrifuged and washed with deionized water (2-3 times) and dried at 100-110 ^oC. The dried nanoclay cake was crushed using a mortar and pestle into a fine powder, sieved, and collected as nanoclay. The prepared nanoclay was extensively characterized using Spectro-analytical techniques like FT-IR, XRD, SEM, TGA, and Particle size analytical techniques.

2.4 Modification of Nanoclay

The modification of nanoclay using the amino acid cation followed the procedure described in our previous work (Azeh and Mohammed, 2024). In brief, 10 g of nanoclay was dispersed into 250 mL deionized water followed by the addition of an excess amount of different molar equivalents of the organo-cations generated from amino acids at a pH range of 1-2. The resulting mixture of organocation and nanoclay suspension was stirred on a mechanical stirrer at room temperature (^oC) for 3 h until precipitation occurred. The reaction product (Precipitate) was filtered, washed, dried, and ground into fine powder and kept in plastic sample bottles for further analyses. The characterization of the modified nanoclay derivatives was carried out using FT-IR, XRD, SEM, TGA, BET, and XRF Spectro-analytical techniques. The equation below shows the modification reaction of clay with an organo-cation under acidic conditions.

2.5 Collection and Selection of Sample

2.5.1 Maize Sample

Maize (*Zea may*) seeds were purchased from a certified agricultural supplier. The selected seeds were uniform in size and weight, ensuring consistency in experimental conditions.

2.5.2 Groundnut Sample

Groundnut *(Arachis hypogaea)* seeds were similarly obtained, and chosen for their quality and uniformity. The seeds were visually inspected to exclude any damaged or diseased specimens.

2.5.3 Sample Pre-treatment

Before applying the modified Nanoclay suspension, maize and groundnut seeds underwent a series of pre-treatment steps to ensure a controlled experimental environment.

2.5.4 Cleaning and Drying

Seeds were thoroughly cleaned using sterile distilled water to remove surface debris and potential contaminants to minimize background microbial load. After washing, the seeds were air-dried on sterile filter paper in a laminar flow hood for 30 min to prevent recontamination.

2.6 Preparation of Modified Nanoclay Suspension

The modified nanoclay, 1.43 mg was suspended in 250 mL or 500 mL of sterile deionized water to afford a concentration of 1.43 mg/mL, which was used for the experiments.

2.7 Preparation of Fungal Media

Potato Dextrose Agar (PDA) or Savoured Dextrose Agar (SDA) was used for the fungal growth within 24-48 h. Chloramphenicol was to inhibit bacterial growth.

2.8 Preparation of Bacterial Media

Antimicrobial profiling of the maize and groundnut samples was carried out using *Bacillus subtilis, Bacillus cereus* and *Pseudomonas aeruginosa, and Penicilliums notatum*, *Mucor Pusilus* and *Aspergillus niger*. Standard biochemical tests were employed for the isolation and identification of bacterial isolates (Azeh *et al.*, 2021).

2.9 Application of Arginine-Modified Nanoclay Suspension

In a typical experiment, 1 mL concentrated hypo mixed with 4 mL CH_3CH_2OH (95%) was sprayed on 10 g of Maize or Groundnut, packed and kept in a container to kill the pre-existing microorganisms. After 24 h, 10 mL of the prepared Arginine-modified nanoclay suspension was sprayed on 10 g of the pretreated maize or groundnut seeds.

2.9.1 Treatment Group

Three treatment groups of seeds were sprayed with various concentrations of the Arginine-modified nanoclay suspension.

2.9.2 Control Group

Seeds treated with sterile distilled water. Untreated seeds were included for comparison.

2.9.3 Low Concentration Group

Seeds treated with a 0.5% Argine-modified nanoclay suspension.

2.9.4 High Concentration Group

Seeds treated with a 1 % Arginine-modified nanoclay suspension.

2.9.5 Application Method

The seeds in each treatment group were immersed in their respective suspension for 30 min. After treatment, seeds were placed on sterile Petri dishes lined with sterile filter paper to dry for 15 min.

2.9.6 Inoculation of Seeds

To assess the efficacy of the Arginine-modified nanoclay suspension, both treated and control seeds were inoculated with fungi (*Penicilliums notatum, Mucor Pusilus* and *Aspergillus niger*) and bacteria (*Bacillus subtilis, Bacillus cereus* and *Pseudomonas aeruginosa)* pathogens known to affect maize and groundnut.

2.9.7 Inoculation Procedure

A standardized suspension of each pathogen was prepared at a concentration of 1 x 10^6 cfu/mL. Each seed was inoculated by dipping it into the pathogen suspension for 5 min. After inoculation, seeds were again placed on sterile filter paper to remove excess moisture.

2.9.8 Storage Conditions

After sample inoculation, they were stored in a controlled environment chamber at 25 °C with 70 % relative humidity. This environment was maintained for 7 days, simulating conditions conducive to fungal and bacterial growth.

2.10 Microbial Load Assessment

The colony-forming unit (CFU) count was employed to determine the efficiency of the Arginine-modified nanoclay suspension in reducing microbial growth.

3.0 Results and Discussion

Based on the presented data in **Table 1** and the observed results in **Fig. 2**, the antimicrobial

activity of the arginine-modified nanoclay suspension against selected fungal (*Aspergillus niger*, *Penicillium notatum*, *Mucor pusillus*) and bacterial strains (*Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*) was visually and quantitatively assessed. The clear zones around the wells in the Petri dishes illustrate the extent of microbial inhibition caused by the nanoclay treatment.

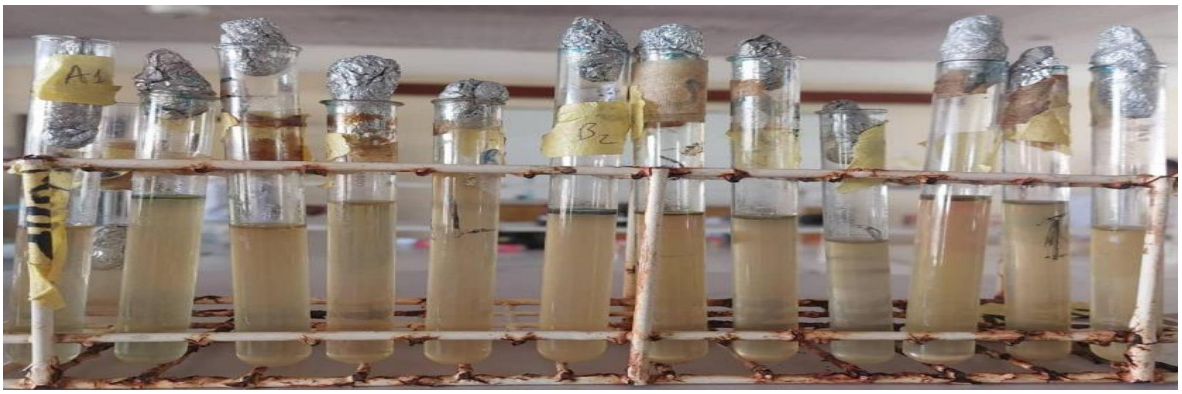
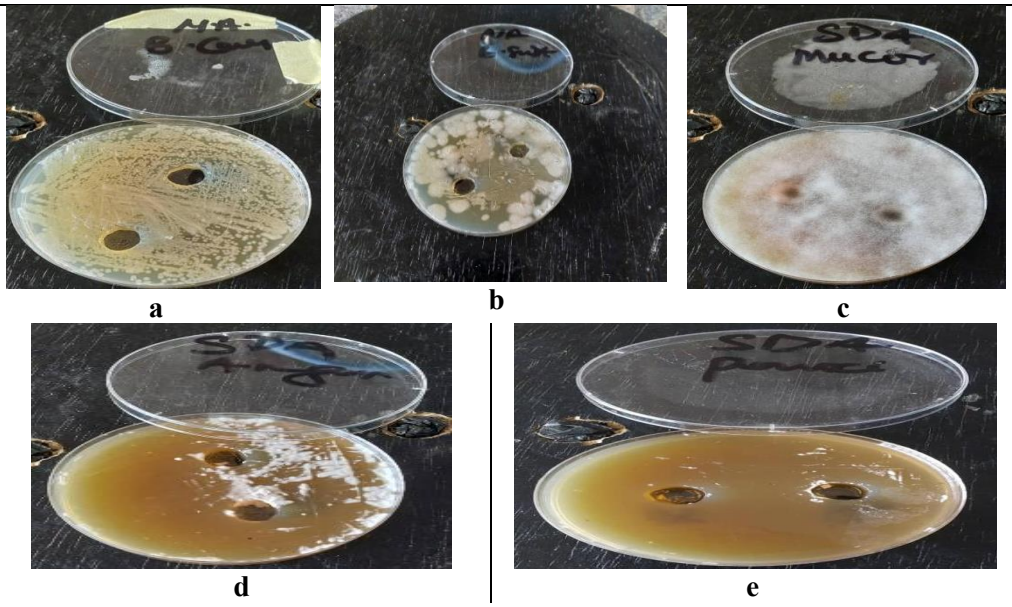


Fig. 1: Arginine-Modified Nanoclay Suspension

Table 1: Diameter Zone of Inhibition (mm)

Microorganisms	**Zone of Inhibition (mm)**		**Key: A = 250 mL; B = 500 mL of 1.43 mg Arginine-Modified Nanoclay Suspension**
	A	**B**	
Aspergillus niger	6.50	8.80	
Penicillium notatum	7.02	3.40	
Mucor pusilus	1.30	Nill	
Bacillus subtilis	4.20	1.90	
Bacillus cereus	3.40	1.70	
Pseudomonas aeruginosa	NIL	Nill	



a **b** **c**

d **e**

Fig. 2: Inhibition Zones for (a) *Basilus cereus*, (b) *Basilus subtilis*, (c) *Mucor*, (d) *Aspergillus niger*, and (e) *Penicillium notatum*

3.1 Antifungal Activity Analysis

Fig. 2 (Top Row) shows inhibition results for the fungal strains. The plates reveal noticeable inhibition zones for *Aspergillus niger* (left), *Penicillium notatum* (middle), and *Mucor pusillus* (right). For the *Aspergillus niger*, a clear inhibition zone with reduced fungal growth surrounding the well indicates strong antifungal activity, aligning with the 8.80 mm inhibition zone reported in **Table 1.** This reflects the nano clay's effective interaction with *A. niger*. *Penicillium notatum*: Moderate growth inhibition is evident, with the nanoclay treatment generating an inhibition zone of 6.30 mm. This result suggests partial sensitivity, which could be attributed to the cell structure of *P. notatum*.

The minimal inhibition zone observed aligns with the reported 1.30 mm, indicating limited susceptibility to the nanoclay suspension. This strain appears more resistant to the treatment, potentially due to its unique structural or physiological adaptations.

3.2 Antibacterial Activity Analysis

Fig 1 (Bottom Row) illustrates the bacterial inhibition results for *Bacillus subtilis*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. A prominent inhibition zone of 4.20 mm is observed, indicating significant antibacterial action, consistent with **Table 1** data. This suggests effective membrane disruption by the nanoclay against *B. subtilis*. The moderate inhibition zone of 2.70 mm suggests some susceptibility to the nanoclay, though less pronounced than *B. subtilis*. The differential response may be due to differences in cell wall composition or resistance mechanisms. The smallest inhibition zone (1.70 mm) was observed, reflecting the strain's resilience against the nanoclay. *P. aeruginosa* is known for robust defence mechanisms, including biofilm formation and efflux pumps, which could explain its minimal sensitivity.

The results on treated maize and groundnut seeds showed visibly reduced microbial growth compared to untreated controls, confirming the practical utility of the nanoclay suspension in protecting stored seeds from microbial contamination. This could enhance shelf life and maintain seed quality, reducing potential post-harvest losses.

3.3 Mechanistic Insight and Implications

The arginine-modified nanoclay appears to work by destabilizing microbial cell walls through electrostatic interactions. This results in cellular damage, as evidenced by the clear inhibition zones in **Fig. 1**. Variations in inhibition zone size reflect different susceptibility levels among microbial species, which could be optimized for broader applications. The findings from this study highlight the potential of arginine-modified nanoclay as an environmentally friendly alternative to conventional antimicrobial agents, particularly in agricultural and storage settings. Further optimization may enhance its efficacy against more resistant strains like *Mucor pusillus* and *Pseudomonas aeruginosa*.

4.0 Conclusion

The study demonstrated that arginine-modified nanoclay has effective antimicrobial properties against specific fungal and bacterial strains, showcasing its potential as a biocompatible antimicrobial agent. The nanoclay suspension inhibited the growth of *Aspergillus niger*, *Penicillium notatum*, *Mucor pusillus*, *Bacillus subtilis*, *Bacillus cereus*, and *Pseudomonas aeruginosa*, with varying inhibition zone sizes depending on the microorganism. *Aspergillus niger* and *Bacillus subtilis* were particularly susceptible, indicating that the nanoclay may be especially effective against certain pathogens in agricultural and environmental applications. The application on maize and groundnut seeds further supported its practicality, as treated seeds showed reduced microbial contamination, enhancing their storage quality.

The findings suggest that arginine-modified nanoclay could serve as a promising alternative to conventional antimicrobial agents, particularly in areas requiring sustainable and eco-friendly solutions. Its potential to improve seed storage and reduce microbial contamination could have significant benefits for agriculture, helping to extend the shelf life of seeds and protect against spoilage. Future studies should focus on optimizing the formulation to enhance its efficacy against more resilient strains like *Pseudomonas aeruginosa* and *Mucor pusillus*. Additionally, evaluating its performance across various environmental conditions and substrates could provide further insights into its broader applicability and effectiveness in real-world settings.

Acknowledgement

The authors acknowledged TETfund for the funding. The contributions of Technologist Hamidu of the Department of Biology, Federal University of Technology, Minna for the technical support is acknowledged.

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Compliance with Ethical Standards Declaration

Ethical Approval

Not Applicable

Competing interests

The authors declare that they have no known competing financial interests

Funding

The authors received external funding from TETfund IBR IBBUL, 2023.

Authors' Contributions

Azeh Yakubu, Mohammed Aliyu-Paiko: Conceptualized; Azeh Yakubu and Asseh Emmanuel: Executed the experimental work. Azeh Yakubu wrote the manuscript and proofread it.