

Development and Characterization of Nano-fertilizer using Pseudo-Ternary Phase Diagram

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ABSTRACT

This study aims to develop and characterize a nano-fertilizer for foliar application on rock melon (*Cucumis melo*). The formulation of the nano-fertilizer was based on a ternary phase diagram, which showed the behavior of NPK-TE within emulsion components made up of surfactants, oil, and water, with the isotropic region identified as the most stable. In addition, the formulation was prepared using a high-energy emulsification method, consisting of 25.04% Tween 80, 4.36% neem oil, 20.60% water, and 50% NPK-TE. The results showed that the optimized nano-emulsion had a particle size of 92.58 nm, with a polydispersity index of 0.156, a zeta potential of -39.8 mV, a surface tension of 40.67 mN m⁻¹, and a viscosity of 100.46 mPa s⁻¹. Morphological analysis of the optimized nano-fertilizer showed spherical particles, showing good stability. The formulation showed no phase separation during the centrifugation test and maintained stability at 3 different temperatures (4, 25, and 54 °C) with turbidity reduction values of 23.18%, 8.65%, and 42.90%, respectively, over a period of 60 days.

Keywords: *Cucumis melo*; emulsion; foliar; nano-fertilizer; nanotechnology

INTRODUCTION

Improving agricultural productivity has been a significant challenge due to factors such as nutrient availability, environmental conditions, and the inefficient uptake of nutrients by plants. Fertilizers, either chemical or from agricultural wastes, play a crucial role in enhancing crop yields and maintaining soil fertility (Alege et al., 2022). However, conventional fertilizers

often have low nutrient use efficiency because of losses that occur through leaching, volatilization, and poorly regulated root uptake (Ibrahim, 2022). This inefficiency not only raises production costs but also contributes to environmental issues, including groundwater contamination and greenhouse gas emissions. Therefore, developing new fertilizer formulations that enhance nutrient uptake and reduce losses is an important study goal. Foliar fertilizers, an innovative approach in

which nutrients are absorbed directly through the leaf surface, have recently attracted significant attention for improving nutrient-delivery efficiency by avoiding soil losses, allowing finer control of release, and enhancing plant uptake (Janmohammadi et al., 2016).

To further improve the performance and precision of foliar nutrient delivery, recent advances have turned to nanotechnology. Several studies have shown that nanotechnology plays a significant role in the production of nano-fertilizers, which offer enhanced stability, improved bioavailability, and controlled delivery systems. The process of creating emulsion-based nano-fertilizers involves forming nanoparticles within the nanometer size range. This size reduction increases surface area and reactivity, which can result in better adhesion, penetration, and nutrient uptake by plants (Rajonee et al., 2017). The stability of emulsified nano-fertilizers is greatly influenced by the emulsification methods and surfactant systems used in their formulation. It is prepared using a system in which a dispersed phase is present in a well-defined state, producing a uniform and stable emulsion. Pseudo-ternary phase diagrams are useful for determining the optimal ratios of surfactant, oil, and water required to generate stable emulsions with desirable chemical properties. The choice of surfactants and oil phases is crucial to prevent phase separation, droplet growth, and instability of the system during storage and use (Kumari et al., 2019).

Neem oil, a well-known biopesticide, has been reported to possess surfactant activity and is proposed for use in emulsion production. It contributes to the stability of the emulsion and offers additional benefits, such as protecting plants from pests and stimulating plant growth. Non-ionic surfactants, including Tween 80, are commonly used in emulsion systems as these chemicals reduce surface tension, enhance dispersion, and promote the formation of stable droplets. When used in combination, these chemicals create a formulation with the desired physicochemical properties for nutrient delivery in agricultural production (Choupanian & Omar, 2018).

Previous studies focus on conventional fertilization techniques, while the current study develops an emulsion-based foliar nano-fertilizer through a high-energy emulsification method that improves both nutrient solubility and bioavailability. The single study examining nano-fertilizer effects on rock melon (*Cucumis melo*) growth was carried out by the same team (Fadzil et al., 2024), reporting that application frequency directly impacts vegetative growth parameters. The current study focuses on developing both a high-efficiency nutrient delivery system and a scalable, stable nano-fertilizer formulation to support the precision

agriculture needs of rock melon cultivation, addressing a critical gap in existing literature. The novelty lies in its systematic application of pseudo-ternary phase diagrams to optimize a nano-fertilizer formulation containing NPK-TE for foliar application, specifically tailored for rock melon cultivation. It uniquely integrates detailed characterization, including particle size, PDI (polydispersity index), zeta potential, surface tension, and viscosity to establish a stable and scalable emulsion-based delivery system, which has not been previously reported in the context of this crop.

METHODS

Materials

Formic acid, acetonitrile, methanol, sodium acetate, Tween 20, Tween 40, Tween 60, Tween 80, and all reagents for HPLC were obtained from Merck (Germany). Neem oil at a concentration of 30,000 ppm was supplied by Agrow Synergy Sdn. Bhd. (Malaysia), while commercial fertilizer containing NPK-TE was sourced from MayHoshct Sdn. Bhd. (Malaysia). All experiments were conducted in a controlled laboratory environment at a temperature of 25 °C and relative humidity between 60% and 80%.

Construction of Pseudo-Ternary Phase Diagrams

The diagrams of pseudo-ternary phase (oil, aqueous, and surfactant) were constructed using aqueous titration or the spontaneous emulsification method (Mazonde et al., 2020). Selected surfactants comprising Tween 20, Tween 40, Tween 60, and Tween 80 were dissolved separately with 3% of neem oil in the oil phase with ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:10 (w/w) in a 10 mL screw cap glass tube at room temperature. The produced mixtures were left for a minute to reach equilibrium. Subsequently, 5% (w/w) water was titrated into the mixes until the emulsion system attained a water content of 95%. Each mixture was vortexed using an IKA MS 3 basic mixer and centrifuged at 4000 rpm for 15 minutes at 25 °C using an Eppendorf 5810R centrifuge (Germany).

Samples were visually evaluated at 25 °C for spontaneous emulsification based on clarity, stability, and transparency. The obtained proportions of surfactants, neem oil, and water were showed on the apices ternary phase diagram. The sample exhibiting a transparent/translucent appearance was categorized as isotropic (Li), while the opaque sample, prior to the formation of separation layers, was classed as multilayer (Mi). Similar procedures were conducted using various surfactants. The experimental results were used to create pseudo-

Table 1. Composition of minerals in NPK-TE

Minerals (NPK-TE)	Mass in 1 litre (L) of water (g)
Magnesium sulphate	56.4
Potassium nitrate	100.0
MKP	26.3
Iron chelates	1.9
Manganese (II) sulphate	0.17
Hibor	0.33
Zinc sulphate	0.12
Copper sulphate	0.02
Molybdate	0.02

ternary phase diagrams using the Chemix version 3.5 phase diagram plotter (UK).

Selection of Formulation Composition Based on Pseudo-Ternary Phase

Formulations were selected from the isotropic region of the pseudo-ternary diagrams based on the following criteria, namely optical isotropy and transparency, physical stability, and single-phase consistency at room temperature (25 °C). Several points with different surfactant/oil ratios within the isotropic region were also selected and incorporated with NPK-TE. Formulations miscible with NPK-TE as the active ingredient (AI) and maintained a monophasic form were chosen since the system was dynamically stable, making scale-up of the volumes easier. Among the selected formulations, those miscible with NPK-TE (Table 1) were selected and subjected to optimization of the nano-fertilizer formulation.

Preparation of Nano-Fertilizer Formulations

Homogenization and ultrasonication were used to prepare nano-fertilizer formulations using a combination of low and high-energy emulsification procedures. Oil, surfactant, and water systems were used based on the selected points from the ternary-phase diagram constructed and incorporated with the composition of NPK-TE. Initially, neem oil was mixed with surfactant to generate an oil phase. NPK-TE and water were then added, and a high-shear homogenizer was used at 1500 rpm for 25 minutes (Ika T25 digital Ultra Turrax, USA). High shear homogenization provides sufficient mechanical energy to break down larger droplets into smaller ones efficiently, contributing to improved

physical stability by preventing premature destabilization mechanisms such as flocculation and sedimentation (Preeti et al., 2023). This uniformity prevents localized concentration gradients, leading to a more consistent and reproducible emulsion. The mixture was subjected to 30 minutes of probe sonication (Q500 sonicator, Qsonica, USA) at 500 Watts and room temperature (Kumari et al., 2019; Saharan, 2010).

Physicochemical Characterization of Nano-fertilizer

The particle size and polydispersity index (PDI) of the nano-fertilizer were measured using a Zetasizer (Brookhaven Instrument, USA), and the zeta potential was also evaluated with the same instrument at 25 °C. The morphology of the nano-fertilizer was examined using Scanning-TEM (STEM) mode with a Hitachi CFE-SEM Regulus 8230 (Japan). The surface tension of the nano-fertilizer was measured using the Du Noüy ring method with a Krüss K6 tensiometer (Krüss, UK).

To evaluate the stability of the optimized formulation under centrifugal force, the sample was centrifuged at 4000 rpm for 15 minutes, and any precipitated materials were observed. The stability concerning particle size and PDI was tested at 3 different temperatures (4, 25 and 54°C) over a period of 60 days. After this period, the formulation's transparency and the presence of emulsion were assessed (Choupanian & Omar, 2018). Any changes in the sample's physical appearance were documented visually.

The turbidity of the nano-fertilizer was also measured at 3 different temperatures (4, 25, and 54°C) over a period of 60 days using a portable turbidity meter (Hanna Instruments, USA). The turbidimetry method provides a rapid, cost-effective, and accurate assessment of emulsion stability (Alade et al., 2021).

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using IBM SPSS Statistics Version 25.0 (Armonk, NY: IBM Corp). In addition, the means were compared by the Post Hoc Tukey test at the significance level of ($p \leq 0.05$) to verify the significant differences among samples.

RESULTS AND DISCUSSION

Construction of Pseudo-Ternary Phase

A pseudo-ternary phase diagram is one of the most well-established methods for describing the phase behavior of emulsion systems. The pseudo-ternary phase diagram of non-ionic surfactants comprising

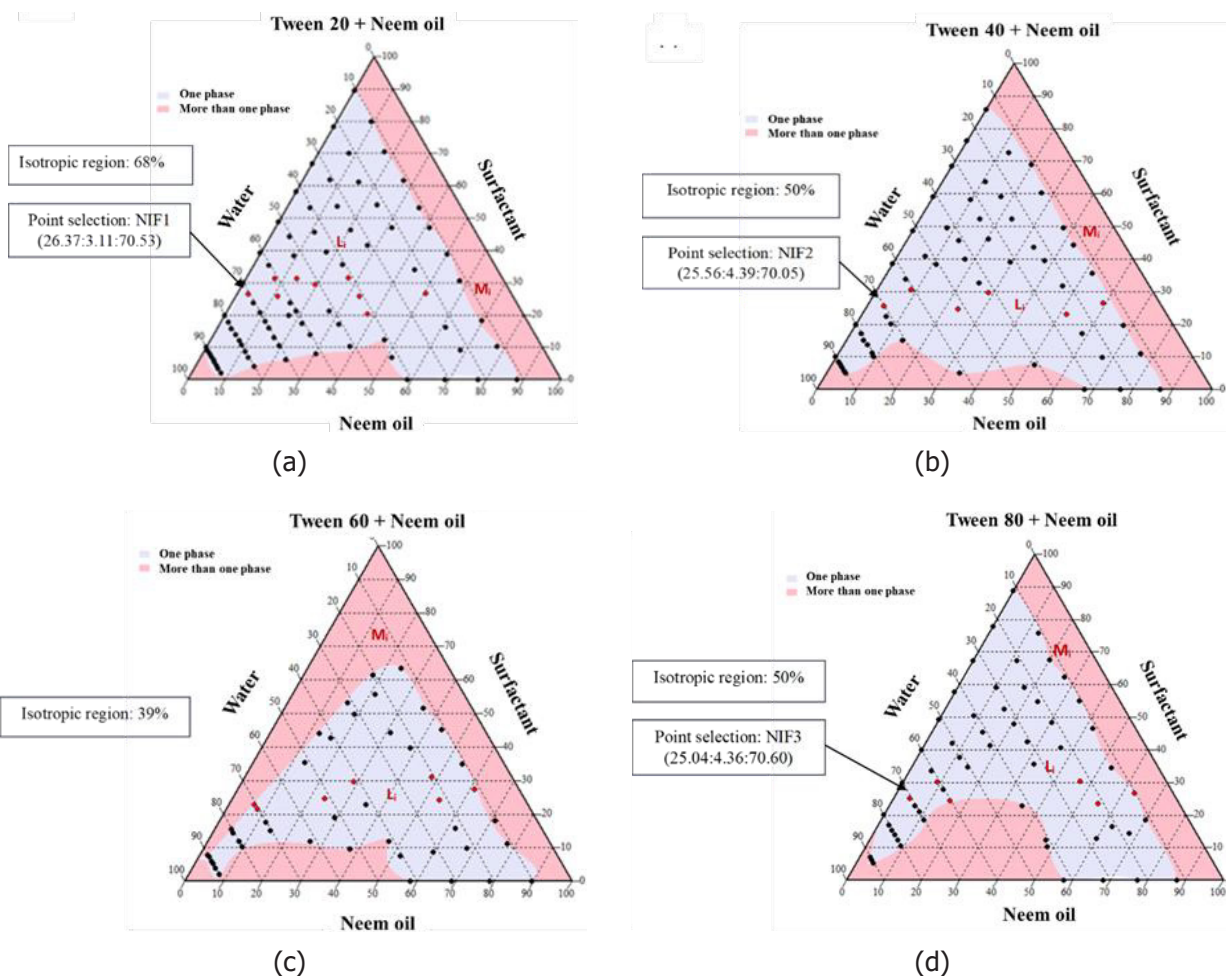


Figure 1. Ternary phase diagrams of surfactant/neem oil/water system with different surfactants (a) Tween 20, (b) Tween 40, (c) Tween 60, and (d) Tween 80. L_1 = Isotropic region, and M_1 = Multiphase region

Tween 20, Tween 40, Tween 60, and Tween 80 as surfactant, neem oil as oil phase, and water phase were constructed at different HLB values (16.72, 15.6, 14.9, and 15, respectively) to obtain the one-phase (isotropic) emulsion region.

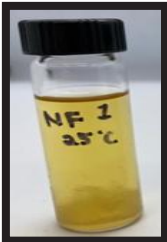

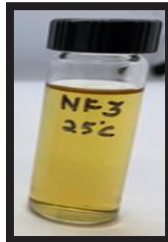
Figure 1 (a-d) presents the phase diagrams showing the emulsion system's two-phase areas (isotropic and multiphase). Based on the results, all the constructed phase diagrams showed a range of 39% to 69% in the isotropic region, where the percentage area of the isotropic region in Figure 1 (a) showed the largest and Figure 1 (c) at the smallest. Figure 1 (a), (b), and (d) phase diagrams were relatively similar.

As the concentration of surfactants increased, the phase diagram systems exhibited a wider isotropic region. A consistent triangular pattern was observed across all diagrams, where the isotropic areas gradually narrowed toward the water apex as oil content increased, showing reduced miscibility and phase stability at higher oil-to-water ratios.

This showed that the surfactants used are more hydrophilic and dissolve better in water. Fatty acid-based surfactants, as non-ionic surfactants, were chosen to create a stable emulsion for neem oil because of their known properties that are less affected by pH and have good stability. In addition, stability is not only dependent on pH but also on ionic strength, oil-water ratio, and environmental conditions (Azeem et al., 2009).

All phase diagrams were examined, and 3 points were selected from the isotropic regions based on the low amount of surfactant used in the formulations ($\leq 30\%$ w/w) (Azeem et al., 2009). The chosen formulations are labeled as NIF1, NIF2, and NIF3 in Table 2. Each formulation contained 25-26% surfactants, 3-5% neem oil, and 20% water. For each selected point, NPK-TE was added at 50% as the AI and as part of the aqueous phase in the formulations. The effect of adding NPK-TE to the emulsion systems in the phase diagram was analyzed. The results showed that the NIF3 formulation mixed well with NPK-TE, while NIF1 and NIF2 exhibited

Table 2. Surfactant, neem oil, and water percentage (w/w) compositions in the formulations, together with observation following NPK-TE addition

Composition	NIF1 (%, w/w)	NIF2 (%, w/w)	NIF3 (%, w/w)
Surfactant	26.37	25.56	25.04
Oil	03.11	04.39	04.36
Water	20.52	20.05	20.60
NPK-TE	50.00	50.00	50.00
Observation			

flocculation and phase separation upon the introduction of NPK-TE, as shown in Table 2.

NIF3, as shown in Table 2, was selected for a comprehensive physicochemical characterization of the emulsion formulation containing NPK-TE. This formulation was chosen because it uses a lower percentage of surfactants within the isotropic region. The use of a low concentration of surfactants has several benefits, including reduced irritation, lower toxicity, and decreased environmental pollution. In addition, the reduced charge on the non-ionic Tween 80 surfactant head groups is expected to enhance compatibility with hydrophobic compounds. Neem oil was incorporated into the formulation to improve droplet spreading on leaves, leveraging its numerous beneficial properties. Based on the results, NF3 was selected as the nano-fertilizer formulation to be applied to rock melon.

Physicochemical Characterization of Nano-Fertilizer

The droplet size distribution in Figure 2 showed that the size of nano-fertilizer droplets was obtained at 92.58 nm and spherical in shape. In the colloidal system, the oil droplet was showed in a vivid color surrounded by NPK-TE. The image showed definite phase boundaries of the particle shells, suggesting the system's excellent physical stability. Instability, for instance, aggregates and particle deformation could be seen from the STEM image if the formulation's excess surfactant or imbalance composition was in the system. In the study of Kumari et al. (2019) using Cryo-FESEM examination of thymol nano-emulsion against bacterial

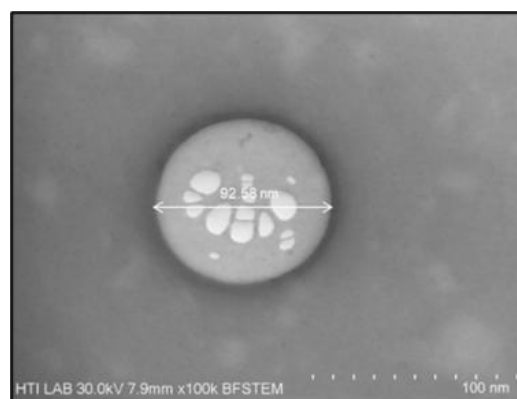


Figure 2. The nano-fertilizer droplet morphology analyzed using TEM

pustule disease, the same bright and smooth-surfaced spherical droplets of 80-150 nm were observed.

The PDI was 0.156, showing a monodisperse system with a zeta potential of -39.80 mV. Emulsions are typically considered stable when their zeta potential values exceeding 40 mV are associated with enhanced electrostatic stabilization and reduced aggregation risks (Massarweh & Abushaikha, 2020). The relationship between the amount of oil and surfactant in the formulation could impact the zeta potential value (Sarheed et al., 2020). By managing surface charge due to electrostatic repulsion between particles, a sufficient amount of surfactant relative to the amount of oil can influence the formulation's stability (Massarweh & Abushaikha, 2020). A high zeta potential shows a high electrostatic repulsion energy between the emulsion

particles that prevents the particles from aggregating. This parameter gives an idea about the surface charge density, where the strong repulsive force prevents particles from agglomeration. Stability is found to improve when the repulsive forces are greater than the attractive Van der Waals forces, resulting in a deflocculated and well-dispersed system.

These are consistent with Choupanian & Omar (2018), who worked on the development of neem oil nano-emulsions for the management of the pathogens *Sitophilus oryzae* and *Tribolium castaneum*. The study showed 4 formulations from the isotropic region of the phase diagram, namely Neem oil, surfactant, and water ratios of 48:30:22 (NeemPolys1 and NeemAPG1) and 40:27:33 (NeemPolys2 and NeemAPG2). The droplet size was related to the zeta potential and the PDI of the formulations, where NeemPolys1 (208 nm) and NeemPolys2 (253 nm) had higher zeta potentials of 39.1 and 37.9 mV, respectively. Meanwhile, NeemAPG1 (328 nm) and NeemAPG2 (507 nm) had lower zeta potentials of 32.5 and 31.3 mV, respectively. When the zeta potential decreases below a certain limit, colloidal particles tend to fuse together by means of attractive forces. High zeta potential values are also central to the improved stability of the nano-emulsion system and maintenance of a well-dispersed nano-emulsion system (Choupanian et al., 2017).

Surface Tension and Viscosity Analysis

The capability of a foliar spray to distribute effectively on plant leaves varies with surface tension to a large extent. The optimized nano-fertilizer was found to exhibit a surface tension of $40.67 \pm 1.53 \text{ mN m}^{-1}$, which is much lower than that of water (72 mN m^{-1}). This results in absorption through the leaf surface more effortlessly. Surfactants are long known to play a crucial role in the reduction of the surface tension in an emulsion system. This is achieved by positioning at the liquid interface, orienting the hydrophilic heads in the aqueous phase and their hydrophobic tails in the oil phase. The reduction in surface tension improves the deposition of the formulation on the leaf surfaces, lowering the contact angle and enhancing the spreading

and wetting properties. Consequently, these attributes help to augment the penetration of active ingredients through the plant's stomata and increase the uptake of nutrients (Díaz-Blancas et al., 2016).

A previous study found that Gemcitabine is effectively preserved in nano-emulsion due to the inclusion of non-ionic polyoxymethylene sorbitan esters (Tween 80 and Span 80). These compounds stabilize the emulsion by reducing the surface tension between particles and preventing coalescence (Wahgiman et al., 2019). In addition, Chaw Jiang et al. (2012) showed that nano-emulsion formulations exhibited a lower surface tension ($<30 \text{ mN m}^{-1}$) compared to commercial herbicide formulations, which had a surface tension of 47.8 mN m^{-1} . The study focused on the use of green nano-emulsion interventions for water-soluble glyphosate isopropylamine (IPA) formulations in the control of *Eleusine indica*. The remarkably low surface tension values observed could lead to an increased number of excess droplets that disperse and form a thin liquid film, resulting in less deposition and improved absorption.

The viscosity analysis showed that the nano-fertilizer had a viscosity of $100.46 \pm 0.11 \text{ Pa}\cdot\text{s}^{-1}$. Emulsion viscosity has been found to be influenced by various key factors, including dispersed phase volume fraction, surface charge, particle size, and colloidal interactions (Guerra-Rosas et al., 2016). However, the amplified viscosity of the nano-fertilizer may be largely credited to the enhanced concentration of non-ionic surfactants that facilitate water to be confined within the surfactant cross-linking chains (de Castro e Silva et al., 2019). This occurs because the hydrophilic parts of the surfactants get hydrated, thereby retaining water in the system.

Previous studies on nano-emulsions have reported similar viscosity trends in their discoveries. Bilia et al. (2017) and De Oca-Ávalos et al. (2017) reported that micelle formation, surfactant self-assembly, and hydration effects in PEG-based non-ionic surfactants were the chief causes of an increase in viscosity through the retention of water in the micellar phase and augmentation of intermuscular interactions. Costa

Table 3. Physical stability assessment of the nano-fertilizer

Temperature (°C)	Stability storage assessment (days)					Centrifugation test
	1	7	21	30	60	
4	Yes	Yes	Yes	Yes	Yes	No separation observed
25	Yes	Yes	Yes	Yes	Yes	No separation observed
54	Yes	Yes	Yes	Yes	Yes	No separation observed

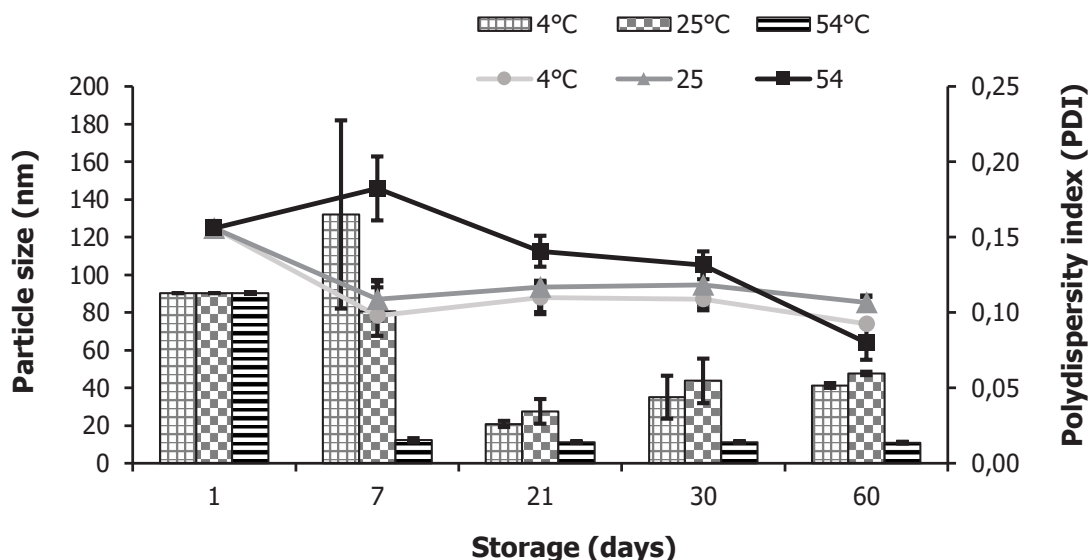


Figure 3. Effect of temperatures on the stability of the nano-fertilizer

et al. (2019) stated that those emulsions developed more stability with an increase in surface charge. This is because the repulsive forces between the droplets avoid flocculation and coalescence, which also increases the stability of the nano-fertilizer formulation.

Stability and Turbidity Analysis

The nano-fertilizer was subjected to various conditions to test its stability against phenomena, such as flocculation, Ostwald ripening, coalescence, and gravitational separation (which includes creaming and sedimentation). As shown in Table 3, the results show that the storage stability maintained a homogeneous mixture, even after 60 days at 3 different temperatures. In addition, the centrifugation tests showed that the nano-fertilizer was resistant to gravitational separation and remained physically stable throughout the experiments.

During this period, a reduction in both particle size and PDI values was observed, as shown in Figure 3 that the particles were monodisperse and homogeneous in size with no physical changes.

The consistently small particle size and low PDI values showed that the particles were monodisperse and homogeneous in size, with no physical changes occurring. This observation was consistent with the results of Mustafa and Hussein (2020), who state that a stable system was characterized by the absence of apparent changes, such as phase separation, creaming, flocculation, coalescence, or sedimentation. The formulation's stability can be attributed to the appropriate selection of surfactants and the small droplet size, which mitigate factors such as Ostwald ripening. The high curvature of nano-emulsion helps to inhibit

flocculation and coalescence, while the Laplace pressure prevents droplet deformation. Over time, the decrease in particle size and PDI correlated with an increase in droplet elasticity. The use of Tween 80 as a surfactant effectively reduces interfacial free energy, thereby forming a mechanical barrier against coalescence (Rana et al., 2024). Quantitative data on storage stability have been included to support these results.

The developed turbidimetry method is a simple, accurate, and fast method of evaluating the stability of emulsions. This method grants an indirect measurement of stability by observing the changes in particle size distribution and turbidity in colloidal systems (Alade et al., 2021). In the nano-fertilizer, the turbidity was reduced by 23.18% at 4 °C, 8.65% at 25 °C, and 42.90% at 54 °C over a 60-day storage period. This shows the different levels of stability at different temperatures. Therefore, comparing turbidity values with particle size measurements made it clear that it is possible to produce a more stable nano-fertilizer formulation with smaller droplet sizes over the long term. The results of the study show that formulations having lower turbidity values appear to be more stable than those with higher turbidity values. This phenomenon is due to reduced aggregation and phase separation. In addition, the result of the system with ultrafine droplets showed lower turbidity after 60 days, showing the need to optimize droplet size for better nano-fertilizer stability (Figure 4).

A study by Saberi et al. (2015) on thermally reversible optically transparent emulsions yielded similar results. The analysis showed that the lack of droplet growth was likely due to these emulsions remaining well below the phase inversion temperature, making

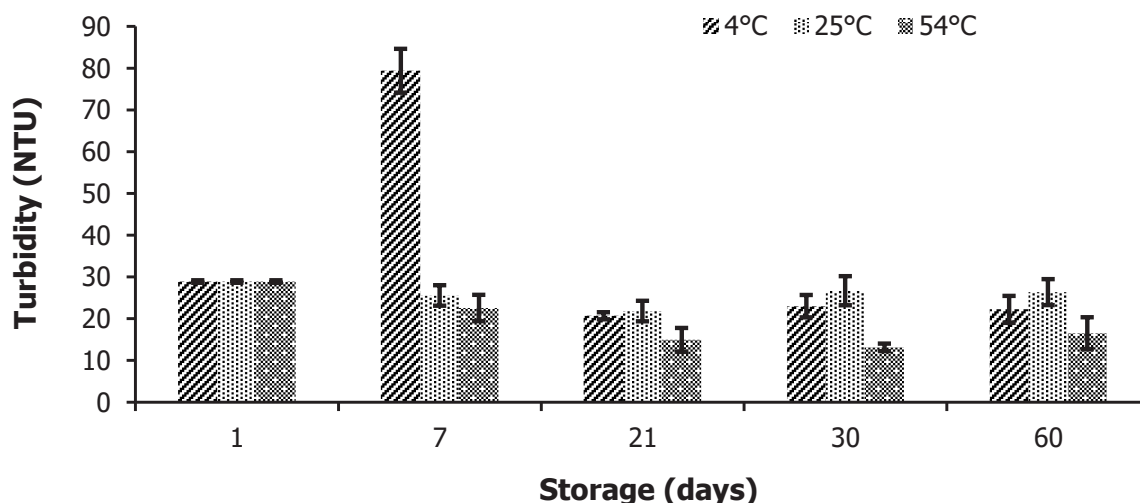


Figure 4. Effect of temperatures on the turbidity of the nano-fertilizer

the materials stable against droplet coalescence. The excellent clarity observed over extended holding periods suggests the formation of a continuous micro-emulsion, which contains small domains that do not strongly scatter light, around the phase inversion temperature (Komaiko & McClements, 2016).

Nano-fertilizers have great potential for sustainable agriculture by enhancing nutrient delivery and minimizing environmental impacts. However, there are significant concerns regarding the toxicity of nanoparticles and their environmental fate. The use of nanoparticles in fertilizers raises important questions about their potential toxicity to plants, soil organisms, and humans. Studies have shown that certain nanoparticles can be harmful if not managed properly. For example, high concentrations of some metal oxide nanoparticles, such as zinc oxide (ZnO), can induce oxidative stress in plants (Devi et al., 2023). In addition, nanoparticles can persist in the environment and accumulate in ecosystems, which poses long-term ecological risks.

While nano-fertilizers offer promising benefits for sustainable agriculture by improving nutrient efficiency and reducing reliance on chemical fertilizers, it is crucial to address concerns about toxicity. This can be achieved through rigorous testing and controlled application methods.

CONCLUSION

In conclusion, a nano-fertilizer containing NPK-TE was effectively developed using the methods involving both high-energy and low-energy emulsification. The formulation of the nano-fertilizer was based on ternary phase diagrams, which were used to optimize

the product that was called NIF3. The nano-fertilizer developed had reduced surface tension and intensified viscosity, all of which were the characteristics needed to improve the spread and absorption into plant tissues. These characteristics propose its appropriateness for use in foliar application with higher efficiency of delivering and absorbing nutrients. In addition, the nano-fertilizer exhibited good physical stability during centrifugation tests and under various storage temperatures. When stored for 60 days, it maintained a monodisperse emulsion with a stable PDI, along with a decrease in particle size corresponding to the turbidity of the emulsion. Morphological analysis showed that the nano-fertilizer droplets were spherical and had a diameter of less than 100 nm. The nano-fertilizers, to be applied through foliar, present significant potential for sustainable agriculture and are a viable option. However, some challenges must be addressed before widespread use can occur. In particular, further studies are needed to address concerns regarding toxicity, environmental residues, and to determine the optimal application concentration.

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CONFLICT OF INTEREST

The authors declare that there are no competing interests.

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