Formulation and Characterization of Nanoemulsion Biopesticide from *Balanite aegyptiaca* Seed Kernel Extract

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Abstract: Consumers' growing awareness and desire for food free of pesticide residues is what led to the incubation of this research work. The study aimed at developing a nanoemulsion formulation of *Balanite aegyptiaca* seed kernel extract for use as a biopesticide. A preliminary study was carried out on the solubility and miscibility of surfactant, carrier, and deionized water utilizing the low-energy spontaneous emulsification method. Information from the study was then used to plot a ternary phase diagram (TPD) showing both the isotropic zone and the anisotropic zones, from which a point with a surfactant, oil, and water ratio (20:20:60) was selected from the isotropic zone for the formulation. The developed nanoemulsion was tested for stability and thermostability, and both results revealed the system was stable. Similarly, the physiochemical properties of the developed nanoemulsion system were characterized, and the results showed that the system showed the following properties: mean particle size of 173.2 ± 21.6, polydispersity index (PDI) of 0.403 ± 0.038, surface tension of 32.53 ± 0.71, viscosity of 37.33 ± 0.67 mPas, and a pH of 4.92 ± 0.02. The formulation appears homogeneous, clear, and transparent, which further indicates it's a nanoemulsion formulation. Therefore, the nanoemulsion formulation of *B. aegyptiaca* seed kernel can serve as a safe and environmentally friendly substitute for synthetic pesticides in the management of plant diseases.
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1.0 Introduction

The exports and consumption of fresh fruits and vegetables are hampered by the widespread of postharvest diseases in fresh fruits. According to Idris *et al*. (2015), the diseases result in up to 37% financial losses from the transportation and marketing of fresh fruits in impoverished nations. Alvindia *et al*. (2002) reported losses of up to 86% of marketable chemically untreated bananas because of crown rot. Jayasheela *et al*. (2015) reported diseases management in crops and agricultural products as one of the key components of global food security. According to Bokhari *et al*. (2013), apparently, the synthetic pesticides are reportedly used throughout the world to treat both pre and postharvest diseases on crops. This act directly or indirectly predisposes humans to chemical residues consumption, environmental pollutions, and a supposedly higher cost of production (Abed-Ashtiani *et al*., 2018). Farmers especially producers of fruits and vegetables are forced to look for safe alternative techniques like the use of botanical pesticides for the management of disease in crops especially fresh fruits. Sequel to this situation, a growing customer demand for pesticide-free products is hatched (Bokhari *et al*., 2013).

According to Coping and Menn (2000), botanical pesticides or biopesticides are organic products (pesticides) produced from higher plants, animals, and microorganisms. In USA, there are over 245 recognized biopesticide. Of the hundreds of the existing developed biopesticides, about 20% of the formulation is derived from bioactive compounds. Furthermore, 19 of the 33 of the Korean are fungicides biopesticides are registered (Yoon *et al*., 2013). Due to its widespread distribution in Southern Asia and Africa (Northern Guinea, Sudan, and the Sahel savannah), Balanites aegyptiaca (L.) Del is regarded as a truly dry and semi-arid evergreen tree with spiny branches (Ishaku et al., 2020). Elfeel and Warrag (2011) state that it is used as food, oil, fodder, traditional medicine, agroforestry species, and possibly as a shelterbelt. It has been determined that several tree components have been used as traditional remedies to treat a wide range of illnesses (Sagna *et al*., 2014). According to AlThobaiti and Zeid (2018), the fruit's mesocarp, endocarp, and kernel are the terms for the outer skin, woody shell, and interior seed, respectively. All of the fruit's layers are used to make various industrial and pharmacological products

The use of nanotechnology in the control of plant diseases through nanoemulsion formulations is an alluring and promising drug delivery strategy that offers farmers the safe and effective alternative formulations for controlling fruit disease (Mishra *et al*., 2014). According to Rodrigues *et al*. (2014), a nanoemulsion is a heterogeneous drug delivery system made up of two immiscible liquid constituents (oil and water) and a surfactant(s), which become thermodynamically and kinetically stable, dispersed by an interfacial film provided by the surfactant. Because they are composed of generally recognized as safe chemicals (GRAS), nanoemulsion systems are seen as promising for safe and environmentally friendly pesticide delivery system (Palou *et al*., 2016). The systems were appealing or desirable for a variety of industrial applications due to their effective and efficient delivery nature (Deen *et al*., 2016). Their uses range from the delivery of pesticides in agriculture to the delivery of drugs in pharmaceuticals, body care formulations in the cosmetics industry, and polymerization in the chemical industry (Qian *et al*., 2017).

The nanoemulsion system are effective/efficient pesticide delivery technique because of their extremely small droplet size (nano size), which is crucial for pesticide availability and penetration (Margulis-Goshen & Magdassi, 2013). Owing to the use of natural oils and surfactants, nanoemulsions have been found to be extremely selectively toxic to microbes while yet being safe for humans, according to Chime *et al*. (2014). Majority of the antimicrobial nanoemulsions are water-dilutable systems since they are mostly oil-in-water emulsions. They have a broad range of effects on bacteria, viruses with envelopes, and fungi (Chime *et al*., 2014).

As its mechanism of action, the systems' nanoparticles are thermodynamically compelled to combine with the microbial organisms' lipids. When enough nanoparticles are fused to the pathogens, a portion of the energy stored within the emulsion system is released, which causes both the released energy and the active ingredient to disrupt the pathogen's lipid membrane, ultimately causing cell lysis and death. The fusion is perfected by the electrostatic attraction between the anionic charge on the pathogen and cationic charge of the nanoemulsions. However, in the case of spores, the system has to be supplemented with a germination enhancer (Wang *et al*., 2007).

Significant research efforts have recently been focused on formulation of nanoemulsion pesticide using a variety of active ingredients. Therefore, the purpose of this study was to prepare and characterize a nanoemulsion formulation of *Balanite aegyptiaca* for use against important phytopathogens.

2.0 Materials and Methods

2.1 Experimental site and materials

The University Central Laboratory as well as the Department of Crop Protection Laboratory, Faculty of Agriculture, Modibbo Adama University, Yola, Adamawa State Nigeria were used for the preparation of nanoemulsion formulations. Materials used were surfactant, oil and deionized water; (Tween80 and MBL510H, Edenor SP 100 oil, deionized water).

2.2 Preparation and extraction of *B. aegyptiaca* seed kernel

We purchased dried *B. aegyptiaca* fruits from Jimeta market in Yola, Adamawa State, Nigeria. The seed epicarps were manually removed, and the endocarps were broken with a hammer to reveal the kernel. Collected seed kernels were grounded into a fine powder using local grinding machine then kept at room temperature until needed. A non-polar solvent (methanol), was used in a serial exhaustive extraction approach (Abaka *et al*., 2020; Sharma & Rana, 2018). A 1:10 w/v extract powder to solvent ratio was achieved by mixing 5g of the seed kernel powder in 50 mL of the solvent (Zhang *et al*., 2018). Using the method described by Gayathri and Sahu, (2015), the mixture was vigorously shaken for three to five minutes, then placed on an orbital shaker at 150 rpm for twenty-four hours. Initially, mixture was filtered through a double layer of muslin cloth, then second stage of filtering was through Whatman No. 1 filter paper, then lastly lysate was filtered through 0.45-micro syringe filter, PS (Gurjar *et al.*, 2012). Using a rotary evaporator (EYELA-CCA-1111), filtrate was first evaporated until jelly-like, and then transferred to a beaker, covered with perforated foil paper, and stored in a 40–45 ^{o}C oven (Memmert, Germany) to entirely evaporate into powdered form. Then 100g of the powdered extract was re-dissolved in 1 mL of 99% purity methanol to achieve a 100 % solution of extracts, which were then kept at - 4 ^{o}C until needed (Gayathri & Ramesh 2013).

2.3 Preparation of oil-in-water nanoemulsions formulation of *B. aegyptiaca*

For the purpose of choosing the surfactants, preliminary solubility and miscibility investigations were carried out. Edenor oil was used as a carrier to test Tween 80 and MBL510H independently before the two surfactants (Tween 80 and MBL510H) were combined in a 70:30 % (w/w) blend. Based on the results of the preliminary test, the formulations' components included a blend of surfactants (70:30 % (w/w) Tween80 and MBL510H, Edenor SP 100 oil (carrier), and deionized water as the aqueous phase.

Using low-energy spontaneous emulsification approach, the formulation was accomplished by gradually adding deionized water drop by drop into the mixture of blended surfactant and oil in 15 ml falcon tubes with the following surfactant to oil ratios (SOR): 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, and 10:90 w/w (Lee *et al*., 2016). Each tube was thoroughly homogenized following each titration by vortexing and centrifuging it for five minutes at 3,500 rpm at room temperature. After that, the emulsion system of each tube composition was visually observed for its phase separation, transparency, and homogeneity, and results were noted. Utilizing the CHEMIX School version 11.7 phase diagram plotter (UK), a pseudo ternary diagram was created utilizing data on the percentages of surfactant, oil, and water as well as information from the visual evaluation. Based on the distinct colourations on the three-axis ternary phase diagram (TPD), clearly showed are the isotropic and anisotropic zones for the compositions. The isotropic zone had many points that varied in formulation composition ratios of surfactant, oil, and water. The point with 20 % each, of surfactant and oil, and 60% water (20:20:60) was then selected. Thereafter, the composition was then reduced to 80% and 20% active ingredient (ai) of *B. aegyptiaca* seed kernel extract (100% methanol stuck solution) was loaded.

2.4 Characterization of emulsions formulations

2.4.1 Stability test

The formulations 20:20:60 was centrifuged at 3500 rpm for 15 minutes, after which their capacity to keep a transparent, single phase was visually evaluated (Asib *et al*., 2015).

2.4.2 Thermostability test

As recommended by the Food and Agricultural Organization (FOA), formulations were kept in an oven (Memmert, Germany) at a temperature of 54 ºC for two weeks and at 28 ± 2 ºC for two months before being checked for potential phase separation (Asib *et al*., 2015).

2.4.3 Droplet size and Polydispersity index (PDI)

Zetasizer Nano series ZS (Malvern, UK) was used to measure the mean droplet diameter and mean polydispersity index (PDI) for the formulation over the course of 60 seconds at a scattering angle of 90º and a temperature of 25 ºC (Lee *et al*., 2016). Deionized water was used to dilute the sampled formulation (2 drops in 1 ml of deionized water) in a 2 ml syringe. The sample was then gently mixed by gently inverting the syringe 2-3 times. A folded 1 cm^3 capillary cell cuvette with two screw caps was filled with approximately 0.75ml of the diluted samples. To prevent bubbles from forming inside the cuvette, which could affect the measurement, the sample was diligently filled into the cuvette. In order to develop nanoemulsion with desirable droplet sizes, the spontaneous emulsification method was used along with 10 cycles of homogenization and centrifugation.

2.4.4 Surface tension

Measurement of the surface tension of the formulations was done using the Du Nuoy ring method with the Kruss K6 tensiometer (Kruss, UK) instrument (Lee *et al*., 2016). The apparatus had previously been calibrated using deionized water with a 70.23 mN/m surface tension. The ring was completely cleaned with methanol after each sample measurement, then flamed before the next measurement for the triplicate observation.

2.4.5 Viscosity

The viscosity of emulsion formulations was assessed using a viscometer (Mishra *et al*., 2014). 40 ml of the formulation was filled into the viscometer cup, the cover was tightened and measurements were recorded in triplicates. After each measurement, the cup was washed thoroughly using distilled water to evade contamination. Measurements were carried out at room temperature.

2.4.6 Acidity

A glass electrode pH meter, the GLP 21 CRISON, was used to measure the acidity of each emulsion formulation in triplicate at room temperature.

2.5 Statistical Analysis

Data were expressed as mean ± SD of triplicate samples by one-way of variance (ANOVA) and significant difference between the means were separated using LSD at (P< 0.05) using SAS software Version 9.4.

3.0 Results

3.1 Formulation of *B. aegyptiaca* nanoemulsion of *B. aegyptiaca* seed kernel extract

The ternary phase diagram (TPD) for the two blended surfactants in ratio of 70:30% (Tween80: MBL 510H), then mixed with Edenor oil displayed miscibility and generated an isotropic zone when mixed in varied ratios from 90:10, 80:20, 70:30, 60:40, 50:60, 30:70, 20:80, and 10:90. The resulting ternary diagram for the Tween80/MBL 510H, carrier oil (Edenor SP 100), and deionized water showed a distinct smaller isotropic zone that was the transparent and single phase zone as well as large anisotropic zone that was the multiphase and foggy (Figure 1). Then point with the compositions 20:20:60, was selected from the isotropic zone of the TPD.

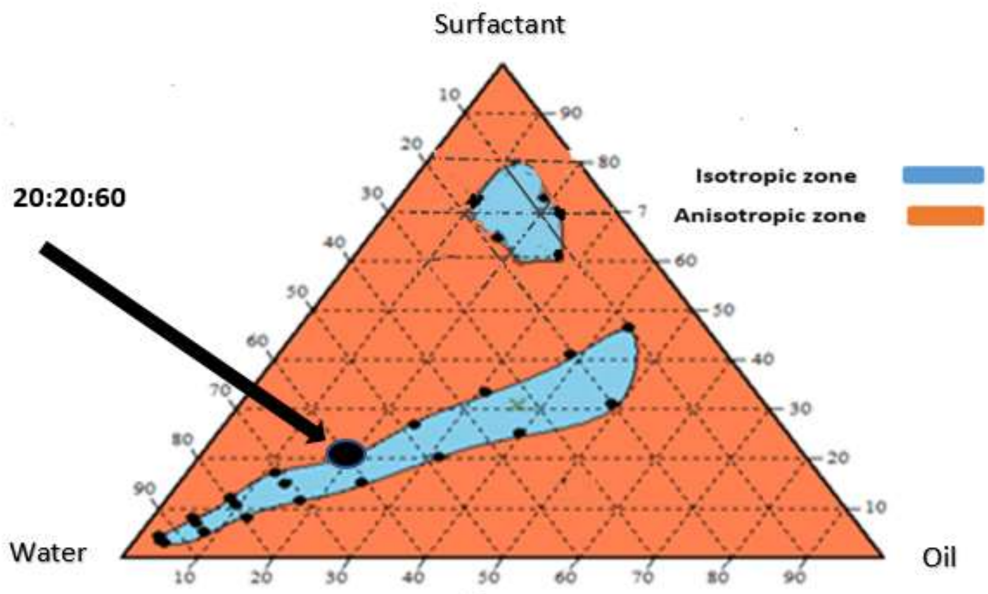


Figure 1. Ternary phase diagram for MBL 510H, oil and water showing small and narrow isotropic region.

Tween80 is a common non-ionic surfactant and emulsifier. It has a variety of uses, including those of a surfactant, co-surfactant dispersant, solubilizing agent, and even a piercing agent and are found in foods, cosmetics, medications and agrochemicals. This substance is a thick, yellow liquid that is soluble in water. Tween 80 is a non-ionic oil with a density of 1.06 g/cm³ at 25 ºC and a hydrophile–lipophile balance (HLB) of 15, and within the range of HLB 8–18 for oil-in-water (O-in-W) emulsifiers, emulsifiable concentrate (EC), suspension emulsion (SE), and other formulations use it in the agrochemical industry. In a similar vein, Agnique (Germany) sells MBL 510H, a non-ionic, hydrophilic high-performance emulsifier for ECs with an HLB of 1030 to 1080 at 25 ºC (g/mL). They are mostly employed as dispersants and emulsifiers.

Figure 2. Oil-in-water nanoemulsion formulation of *B. aegyptiaca* seed kernel extract at 20:20:60 surfactant, oil, and water (SOW) ratio.

3.2 Stability and thermostability tests of the nanoemulsion of B. aegyptiaca seed kernel extract.

Result for the stability and thermostability tests of the nanoemulsion formulation are presented in Table 1. From the result, the nanoemulsion formulation expressed stability after being centrifuged at 3,500 rpm for 15 and also expressed thermostability after being kept at 54 ºC for 2 weeks and at 25 ºC for 2 months. These means that the formulation retained its single and transparent phases after the two tests, which indicates good and stable nanoemulsion systems.

Table 1: Mean thermostability and stability tests of nanoemulsion formulation of *B. aegyptiaca* seed kernel extract.

Formulations	**Thermostability test**		**Stability**
	54 ºC for 2 weeks	**25 ºC for 2 months**	**3500 rpm/15 min**
20:20:60	√	√	√

Key: √=stable.

3.3 Physiochemical characterizations of nanoemulsion formulations of *B. aegyptiaca* seed kernel extract

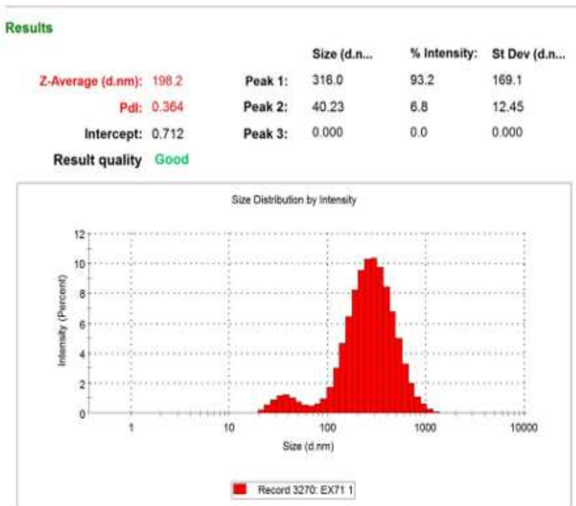
Result in table 2 and figure 3, showed the mean droplet sizes for the formulation (20:20:60) was 173 .2 ± 21.6 nm, which indicates that particle size of the system is within nano range (20-200 d nm). According to the result, no significant difference was found between the droplet diameters of the replicates formulations study at (P< 0.05). Ability to preserve its transparent single phase is the crucial component in the of nanoemulsion formulation.

The degree of homogeneity of dispersion is determined by the polydispersity index (PDI), which is a measurement of particle size distribution intensity within the emulsion system. Result from the characterization shows that formulation was a stable and monodispersed emulsion system, as evidenced by the low mean PDI value close to zero, 0.403 ± 0.038. Value of the triplicate readings did not significantly differ among each other at (P<0.05. Therefore, formulation is considered to be a stable system (Table 2 and Figure 3).

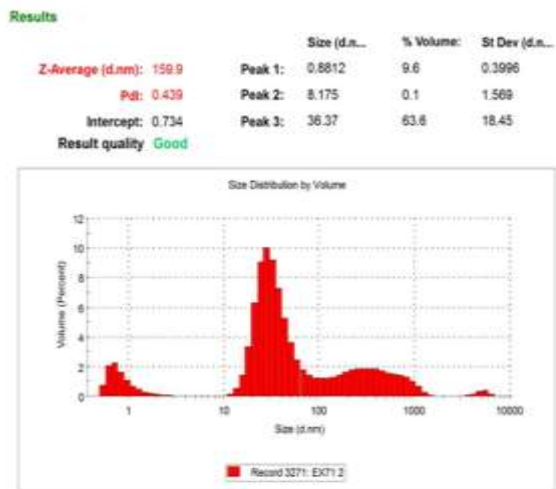
Table 2: Mean Physiochemical characterization of nanoemulsion formulation of *B. aegyptiaca* seed kernel extract

	Physiochemical properties				
Formulations	**Diameter of droplets (nm)**	**PDI**	**Viscosity mPas**	**Surface tension (Mn /m)**	**pH**
20:20:60	173.2 ± 21.6	0.403± 0.038	37.33 ± 0.67	32.53 ± 0.71	4.92 ± 0.02

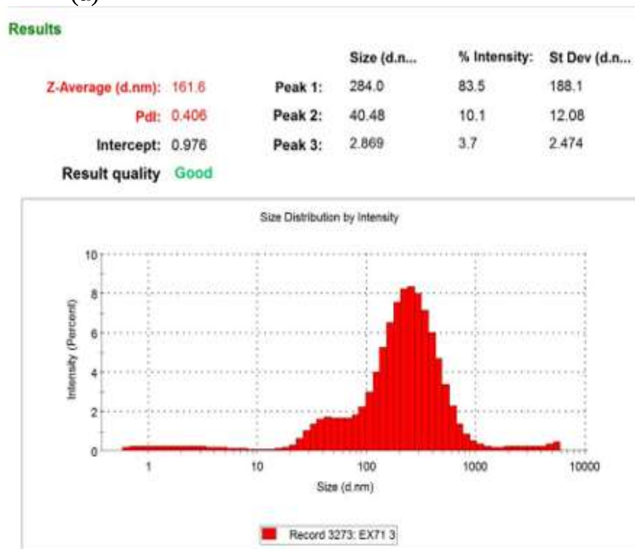
Note: Values are means of three observations for each parameter ± SD. 20:20:60 is the composition of surfactant oil and water.



(a)



(b)



(c)

Figure 3. Diameter of droplets (nm) and polydispersity index (PDI) for the formulation (20:20:60) of three observations xpressed as mean ± standard deviation (SD) in table 2 (a, b and c)

The mean viscosity value for the nanoemulsion was 37.33 ± 0.67 mPas with no discernible variation among means. Data analysis revealed no significant differences among the viscosity values of the emulsion. According to the findings, formulations had surface tensions that was much lower than the surface tension of water at ambient temperature (about 70.23 mN/m). Based on analysis, there was no significant difference between the surface tensions values of the 3 observations at (P<0.05). The formulation showed a mean surface tension for the formulations as 32.53 ± 0.71 (Table 2). Likewise, the mean pH value (4.92 ± 0.02) of the formulation showed no significant difference at (P< 0.05).

4.0 Discussions

Ternary Phase diagram of a nanoemulsion formulation is a triangular representation of the three main formulation components: surfactant, oil, and water. Each side of the triangle stands for one of these components, which are always located at the apices of the ternary plot (diagram), according to Ali *et al*. (2014). Formation of monophasic optically transparent emulsion compositions occurs in the isotropic zone of the ternary plot, while the anisotropic zone refers to the multiple phase region. Usually, the two can be identified on the plot by different coloration (Lee *et al*., 2016). The point selected (20:20:60) for the formulation is within the isotropic zone of the ternary phase diagram (TPD), which also lies in an area with low concentration of inert substance (surfactant and oil) and a lot of water. Following Lee *et al*. (2016) approach, the emulsion formulation's solubility grows with increased water content, and its loading ability scales up to an economically practical level and that surfactants are used in formulation to combine the oil and water components while oil remains the carrier (Lee *et al*., 2016).

The stability and thermostability tests of nanoemulsion, according to Tadros (2013), are conducted by mimicking the gravitational force and other physical environmental influences (speed, a wide range of temperature, 10-60 ºC, and storage period). These studies demonstrate the resilience of nanoemulsion formulations to all varying physical environmental conditions (speed, changing temperature, and storage period). All formulations maintained their translucent, clear single phases after the stability and thermostability tests, supporting this claim.

This work is in line with research by Fernandes *et al*. (2014) who found that in order to create nano-sized droplets, it is necessary to combine blends of two or more surfactants, such as hydrophilic and lipophilic non-ionic surfactants. Additionally, Mishra *et al*. (2014) noticed that 3 to 10 cycles of centrifugations are needed for low-energy spontaneous formulations in order to attain tiny and uniform particle size. Similarly, according to Rodrigues *et al*. (2014), nano-formulations are those with droplet sizes of 200 d nm or less while above 200 d nm are micron size. More so, Mishra *et al*. (2014), reported 3 to 10 cycles of homogenization are excellent for low-energy formulation, and the more homogenization cycles used, the smaller the final particle size will be. However, physiochemical properties; particle size, polydispersity index, viscosity, surface tension, and pH of the system are reflection of the compatibility of the system components (Chime *et al*., 2014).

The selection of 20:20:60 formulation was in order to minimise cost due the use of low amount of inert material and active ingredient with lot of water. Nevertheless, Anton and Vandamme (2009), said "desirable nanoemulsion formulations should exhibits low viscosity and single-phase composition in addition to having less than 30% surfactant component". The developed nanoemulsion is consistent with the report of Fernandes *et al*. (2014) which reported that for a nanoemulsion formulation to attain smaller droplets size (nano), a combination of lipophilic and hydrophilic non-ionic surfactants is required.

Gohel *et al*. (2014) and Lee *et al*. (2016) documented that the polydispersity index of nanoemulsion measures the uniformity of the size distribution of nanoparticles inside an emulsion system. They also stated that stable formulations require an emulsion system with low polydispersity index (PDI) values and that PDI values close to zero are preferred and they designate stable, monodispersed emulsion systems. Also, a wide variety of size distribution or heterogeneous systems are indicated by a PDI that is close to 1.0. However, phase separation is the most important aspect in the formulation of nanoemulsion. The mean value of PDI of the developed nanoemulsion during this study was 0.40 ± 0.04 (close to zero) and did not significantly differ from one another, indicating that formulation was stable and had homogeneous size distributions.

The form and size of a liquid's droplets are determined by the surface tension of the liquid, which is an elastic property related to the liquid's particle size (Lee *et al*., 2016). Surface tension of an emulsion system is directly related to bioavailability of the system. The surface tension of the formulation in the current investigations was 32.53 ± 0.71 mN/m, which is closer to ethanol's surface tension (29.63 Nm). Wang *et al*. (2007) reported that low surface tension displayed by a nanoemulsion system is closely related to its bioavailability. The present formulation had its surface tension significantly lower than the water used as a control, which had a surface tension of about 70.23 mN/m at 25 ºC, implying that the nanoemulsion system had a very high level of bioavailability.

Viscosity has a direct bearing of on the system's bioavailability, it is a crucial feature for consideration in any nanoemulsion systems (Wang *et al*., 2007). According to Ali *et al*. (2014), the viscosity of nanoemulsion rises as the composition's oil content rises. Similarly, Gohel *et al*. (2014) documented that the viscosity of nanoemulsion rises as the amount and concentration of oil in the composition rises. Emulsion system with low viscosity, watery, having less than 30 % surfactant and above all, having clear and transparent single-phase composition are the main requirements for nanoemulsion formulations according to Anton and Vandamme, (2009). Hence, the percentage surfactant used in the current study was 20% in an effort to formulate it in accordance with the stated specifications.

Formulation in this study recorded a mean pH value of 4.92 ± 0.02 (Table 2). The mean pH value for the formulations revealed the systems to be moderately acidic. Since the zeta potential of nanoemulsion is greatly influenced by pH, highly acidic systems may have higher zeta potentials, which further denotes an unstable and heterogenic system (Lee *et al*., 2016; Dias *et al*., 2014).

5.0 Conclusions

An unprecedented advancement in plant protection is possible because of nanoemulsion technology, which offers limitless potential for revolutions in plant disease management. The technique could be utilized to provide a safe and effective alternative bio-pesticides for the management of plants pest and diseases. It helps to improve effective drug delivery system as well as its efficacy through increased absorption/ penetration and bioavailability of the bioactive compounds (drug) which is expressed as an increased therapeutic potential as bio-pesticides. This is achieved through the function of both the antimicrobial phytochemical components present in the *B. aegyptiaca* seed kernel and the synergy provided by the nano particle as well as the inert components of the nanoemulsion formulation resulting in high efficiency of the drug delivery.

6.0 Conflict of Interests

The Authors state that there is no conflict of interest.

7.0 Acknowledgement

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