GREEN MIXED SURFACTANT NANOEMULSION FORMULATION OF CYTOKININ NPK FERTILIZER FOR CULTIVATING CEYLON SPINACH *(BASELLA ALBA)*

By

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ABSTRACT

GREEN MIXED SURFACTANT NANOEMULSION FORMULATION OF CYTOKININ NPK FERTILIZER FOR CULTIVATING CEYLON SPINACH *(BASELLA ALBA)*

Eyvonne Shee Yu Qian

The soil applied granular and liquid fertilizers contain three important nutrients which are nitrogen, phosphorus and potassium. Plants only absorb 10-40% of the fertilizers applied while 60-90% of the fertilizers is lost due to washed off from the ground and leaching into the waterways during rain, therefore cause eutrophication of water bodies. In order to solve these problems, the nanoemulsion formulations of liquid fertilizer that absorb through leaves into the plants are capable of maximizing nutrients uptake via foliar spray. This study aims to formulate nanoemulsion cytokinin NPK fertilizer and investigate the nanoemulsion system with cytokinin NPK fertilizer on the cultivation of Ceylon spinach *(Basella Alba)*.

The phase behaviours of emulsions were studied using palm oil fatty methyl ester 1218K and the mixed surfactants, Tween 80 and alkyl polyglucosides and water. The emulsions of transparent isotropic phase were obtained at the surfactant:oil ratio of 9:1 and mixed surfactant ratio of 9:1 and 8:2. These stable emulsions were added into the mixed cytokinin NPK fertilizer through phase inversion composition

(PIC) method to produce nanoemulsion formulation. The nanoemulsion formulations of cytokinin NPK fertilizer were stable under heat stress, 54℃ for two weeks. The result of High Resolution Transmission Electron Microscope (HRTEM) indicates spherical particles are observed in the nanoemulsion formulations. The nanoemulsion were incorporated well into the cytokinin NPK fertilizer solution. The particle sizes are in the range of 20 nm to 250 nm.

The formulated nanoemulsion fertilizers were assessed by the measurement of chlorophyll content, total phenolic content, fresh mass, surface area of leaves and the number of leaves of Ceylon Spinach. The plants that treated with cytokinin nanoemulsion fertilizers grow the healthiest which is indicated by 35.0 SPAD of chlorophyll content, 10.4459 mgGAE/L of total phenolic content, 4.0 leaves per plant, 2.6715 g of fresh weight and 12.44 cm^2 surface area of leaves in comparison to the non-formulated cytokinin NPK fertilizer.

ABSTRAK

FORMULASI HIJAU NANOEMULSI SURFAKTAN CAMPURAN UNTUK BAJA NPK SITOKININ BAGI MENANAM BAYAM CEYLON *(BASELLA ALBA)*

Eyvonne Shee Yu Qian

Baja tanah butiran dan cecair mengandungi tiga unsur kimia utama iaitu nitrogen, fosforus dan kalium. Tumbuhan hanya menyerap 10-40% baja yang digunakan manakala 60-90% baja terlesap akibat dihanyutkan dari permukaan tanah dan larut lesap ke dalam saluran air semasa hujan, oleh itu menyebabkan eutrofikasi badan air. Bagi menyelesaikan masalah ini, formulasi nanoemulsi baja cecair diserap melalui daun ke dalam tumbuhan mampu memaksimakan penyerapan unsur baja melalui semburan daun. Kajian ini bertujuan untuk merumus dan menyiasat sistem nanoemulsi baja NPK sitokinin dalam penanaman bayam Ceylon *(Basella Alba)*.

Kelakuan fasa emulsi telah dikaji menggunakan ester metil lemak minyak sawit 1218K dan surfaktan campuran, Tween 80 dan alkil poliglukosa dan air. Emulsi fasa isotropik lutsinar diperolehi pada nisbah surfaktan-minyak (SOR) 9:1 dan nisbah surfaktan campuran (MSR) 9:1 dan 8:2. Emulsi stabil itu telah ditambah ke dalam baja NPK sitokinin campuran melalui kaedah fasa penyongsangan komposisi (PIC) untuk menghasilkan formulasi nanoemulsi. Formulasi nanoemulsi

dengan baja NPK sitokinin adalah stabil pada tekanan haba 54 ℃ selama dua minggu. Keputusan Mikroskopi Transmisi Elektron Resolusi Tinggi (HRTEM) menunjukkan zarah sfera diperhatikan dalam formulasi nanoemulsi. Nanoemulsi telah digabung baik ke dalam larutan baja NPK sitokinin. Saiz zarah berada dalam julat daripada 20 nm hingga 250 nm.

Baja nanoemulsi yang dirumus telah dinilai dengan pengukuran kandungan klorofil, jumlah kandungan fenolik, jisim segar, luas permukaan daun dan bilangan daun bagi sayur bayam Ceylon. Tumbuhan yang dirawat dengan baja nanoemulsi sitokinin tumbuh paling sihat dengan menunjukkan kandungan klorofil pada 35.0 SPAD, jumlah kandungan fenolik pada 10.4459 mgGAE/L, bilangan daun per tumbuhan pada 4.0, jisim segar pada 2.6715 g dan luas permukaan daun pada 12.44 cm^2 berbanding dengan baja NPK sitokinin yang tidak dirumus.

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First and foremost, I would like to express my special thank of gratitude to my Final Year Project supervisor, Dr. Lim Chaw Jiang, for his guidance, patience, constant encouragement for me, as well as enthusiasm throughout my research journey. Besides, his passion and perseverance have lead this project to a success as he meticulously supervised my work from the beginning to the end with much care by giving many advices after every hurdle that I had faced during this project. His dedication and willingness to strive for the best have not only imparted all the theoretical knowledge regarding to the field of study, but also research methodologies and lab skills needed to execute the study well and efficiently. Lastly, I would like to thank Dr. Lim for giving me the opportunity to work on the project by selecting me as his FYP student.

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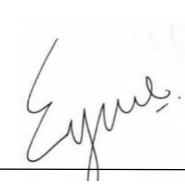
DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.



EYVONNE SHEE YU QIAN

Date:26 May 2023

APPROVAL SHEET

The project report entitled '**GREEN MIXED SURFACTANT NANOEMULSION FORMULATION OF CYTOKININ NPK FERTILIZER FOR CULTIVATING CEYLON SPINACH *(BASELLA ALBA)*'** was prepared by Eyvonne Shee Yu Qian and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Chemical Science at Universiti Tunku Abdul Rahman.

Approved by:

CJLim

(Dr. Lim Chaw Jiang)

Date: 26 May 2023

Supervisor

Department of Chemical Science

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UNIVERSITI TUNKU ABDUL RAHMAN

Date: 26 May 2023

PERMISSION SHEET

It is hereby certified that Eyvonne Shee Yu Qian (ID No: 19ADB01637) has completed this final year project entitled 'GREEN MIXED SURFACTANT NANOEMULSION FORMULATION OF CYTOKININ NPK FERTILIZER FOR CULTIVATING CEYLON SPINACH *(BASELLA ALBA)*' under the supervision of Dr Lim Chaw Jiang from the Department of Chemical Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

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(EYVONNE SHEE YU QIAN)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

LIST OF ABBREVIATIONS

AFM	Atomic Force Microscope
ANOVA	Analysis of variance
APG	Alkyl polyglucoside
CMC	Critical Micelle Concentration
CPO	Crude Palm Oil
DNA	Deoxyribonucleic acid
DOBI	Deterioration of bleachability index
EIP	Emulsion inversion point
FAME	Fatty Acid Methyl Ester
FAO	Food and Agricultural Organisation
FC	Folin-Ciocalteu
FFA	Free fatty acids
GAE	Gallic acid equivalent
HLB	Hydrophilic-lipophilic balance
HRTEM	High resolution transmission electron microscope
HSD	Honestly Significant Difference

K	Potassium
K_2O	Potassium oxide
KCl	Potassium Chloride
MKP	Monopotassium Phosphate
MSR	Mixed surfactant ratios
NPK	Nitrogen, Phosphorus and Potassium
O/W	Oil-in-water
P	Phosphorus
P_2O_5	Phosphorus pentoxide
PCCS	Photon Cross Correlation Spectrometer
PDI	Polydispersity Index
PFAD	Palm fatty acid distillate
PIC	Phase Inversion Composition
PIT	Phase Inversion Temperature
RBDPO	Refined Bleached Deodorised Palm Oil
RCBD	Randomized Completely Block Design
RNA	Ribonucleic acid
SOR	Surfactant-to-oil ratio

SPAD	Soil Plant Analysis Development
W/O	Water-in-oil
W/W	Weight to weight

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The United Nations had made an assumption that the world's population will reach 9.7 billion in 2050, continuously growing, and eventually reaching to 10.4 billion in year 2100. Sustainable development is set to establish gender equality, to minimize hunger and poverty, as well as to enhance education and health systems when the world's population growing rapidly (Population Matters, 2023). Due to overpopulation problem, the demand for transportation, water, energy, food, housing, and healthcare become greater impact in the society. Besides, the energy consumption leads to greater probability of disasters, and conflicts are more likely to happen, as well as ecological degradation (Bish, 2020). Therefore, an overwhelming pressure to urge the agriculture sector to make sure that the supply of food is sufficient to the society. The global food demand has made expectation which the percentage increases from 35% to 56% in 40 years (van Dijk et al., 2021). In addition, consumers request for high nutritional value crops. Hence, it is vital for agriculture sector to put more efforts in producing crops that satisfy the requirements of the consumers. Nevertheless, the production of crops is affected by the climate changes. The increase in temperature causes an increase in insect disease and pests. This results in the reduction of crop yields (Edgerton, 2009).

Moreover, the labour shortages, breakdown of transport and logistics, shutdown of processing and supply food chain (OECD, 2020).

In order to improve the yield of crops, as well as its nutritional values, the application of fertilizers is encouraged. There are two types of fertilizers, which are organic and mineral fertilizers. The fertilizers are categorized according to their functions and compositions. Organic fertilizers are those contain natural biological materials. For example, insects, bacteria, worms and so on. They have organic carbon that is required for development of healthy soil. In order to provide nutrients to the plants, the organisms that live in the soil, like bacterial transforms organic fertilizers into nutrients. On the other hand, the mineral fertilizers are those produced from chemical process. There is no any conversion process needed and the plants can absorb the nutrients directly from mineral fertilizers. The mineral fertilizers contain more nutrients which are needed for plant growth than organic fertilizers (Kooij, 2020).

Thus, the demand of fertilizer in the market increases by 12% on year 2021 which has reached the amount of 193 billion dollars. In 2023, the demand of fertilizer in the market is expected to reach 240 billion dollars (Statista Research Department, 2023). This is due to the fact that the fertilizers mainly supply the essential nutrients to facilitate plant growth. A statement explains that the plants face the growing problem when fertilizers are absent when the soil is depleted. The plants are not able to grow on water alone. They require additional nutrients for better plant growth (Yara, 2022). Therefore, a lot of studies have been conducted to determine the effect of fertilizers on the plant growth, as well as different materials and techniques to improve the effectiveness of fertilizers.

1.2 Research Problems

Plants need water, sunlight and nutrients to grow. Soil contains micronutrients and macronutrients. For instance, potassium, phosphorus, nitrogen, calcium, and so on. However, plants will stop growing when they are facing nutrient deficiency problem. Therefore, the farmers usually apply fertilizer on the plants. This is due to the fact that fertilizers contain three important nutrients, like nitrogen, potassium, and phosphorus. Besides, it also contains micronutrients, such as zinc which are required for plant growth. Traditionally, the farmers apply fertilizers by scattering solid powders/granules or spraying liquid directly onto the soil. The nutrients and water are taken up by the root system, and then transport to the parts of plant through their conductive tissues.

Nevertheless, long-term use of soil-applied fertilizers had brought some problems to the environment. According to Janzen (1987), the application of nitrogen fertilizer decreased the pH of soil. The long-term application of nitrogen fertilizer released H^+ ion into the soil through nitrification. The higher the amount of nitrogen, the more acidic the soil. Besides that, the application of fertilizer causes the accumulation of phosphorus and potassium in the soil. Other than that, the bacterial diversity affects the pH of soil. Compared to other types of fertilizer, the application of nitrogen fertilizer affects the microorganisms in soil seriously. By applying nitrogen fertilizer continuously, the bacterial community structure of soil had boosted (Du et al., 2019). Hence, the high concentration of nitrogen in the soil increases the acidity of soil, as well as alters the bacterial community of soil. The application of fertilizers provides nutrients to the plants, and promotes their microbial activities and soil nutrient cycling as well (Janzen, 1987). Conversely, long-term application of fertilizer on soil causes accumulation of nutrients in the soil, such as potassium, nitrogen and phosphorus. These eventually leads to decrease in the rate of fertilizers absorption and obstruct the growth of plants. Furthermore, the deficiency of nutrients leads to the effect of unhealthy roots development. According to Fageria and Moreira (2011), the development of root length, as well as surface area are correlated with application of nitrogen fertilizer. By applying fertilizers, the root length increases with surface area. At the same time, it decreases the mass per unit of the root. The deficiency of nitrogen leads to the decrease in root hairs and branching of plants. Therefore, the correction must be made because the plants will become more and more deficient in nutrient due to the long-term application of nitrogen fertilizer.

Moreover, the nitrogen fertilizer runoffs from farmland in the water streams during rainy day and leaching into groundwater when excessive nitrogen-rich fertilizer is applied. This results in the formation of toxic algal blooms in the ocean, rivers and poisoning to the marine life. According to Chakraborty et al. (2017), the number of the nutrients drain cases into the water bodies via agricultural runoff depends on how much the fertilizers have been applied to increase the yields of crops. The residues of agricultural crops and dead algae increase the amount of detritus, as well as provide nutrients in the water streams. This eventually leads to algal blooms and eutrophication. The phosphorus and nitrate eutrophication results in death of aquatic animal, as well as dense growth of algal plants causes lack of oxygen. The fertilizer application enhances the production of agricultural yields to an extent. However, the large amount of fertilizer applied and some fertilizers depletes naturally. So, there is only small amount of fertilizers that are absorbed into the plants.

Last but not least, plants usually absorb small portion of total fertilizers applied, which means that most of fertilizer is not in target when soil fertilizers are applied. The absorption of nutrients into the plants is limited because the soil applied fertilizers leach easily into the deep soil, and eventually reach the underground waters, as well as rivers. Thus, it ends up with applying larger amount of fertilizer than the recommended amount. According to Yamaguchi (1994), there is only 30 to 70% of nitrogen content in the soil-applied fertilizer can be absorbed by the plants through the tracing of ^{15}N isotopic nitrogen-based fertilizers. The large portion of fertilizers are leached into the water bodies and eventually causes eutrophication in rivers and lakes to happen. Therefore, the long-term application of nitrogen fertilizers affects and harms to aquatic ecosystem.

In order to solve these problems, scientists had found out that foliar-applied fertilizer is suitable to be applied on the leaves of plants. Recently, foliar fertilization is becoming alternatively use in agriculture field. The foliar-applied fertilizer for an example, nanoemulsion formulation of liquid fertilizer which allows the nutrients to be absorbed efficiently into the plants by spraying it directlly onto the leaves. The nutrients are absorbed by above-ground tissues, which are stomata and cuticle, as well as trichomes. (Li et al., 2018). The plant growth regulators had been introduced into foliar fertilizer to promote the metabolism of plants, like seed germination and cell elongation. It stimulates the signals that allow the nutrients to be translocated to the demanding sites as much as possible (Llanes et al., 2018). This thesis focuses on the nanoemulsion formulation of phytohormone mixed fertilizer and application on plants.

1.3 Objectives

The objectives of this project are:

1. To study the phase behaviour of emulsion constructed from mixed surfactant and oil
2. To develop nanoemulsion from stable emulsion through emulsion inversion point method
3. To formulate nanoemulsion cytokinin NPK fertilizer

4. To assess the biological activites of nanoemulsion formulation on Ceylon Spinach *(Basella Alba)*

CHAPTER 2

LITERATURE REVIEW

2.1 Emulsion

Emulsion is a dispersive mixture in which partially immiscible or immiscible liquids, like milk, are present in the continuous phases. One of the liquids acts as discrete droplets that disperse in a continuous medium of another liquid. Phase separation happens when two pure liquids are mixed together to form an emulsion, especially when the concentration of the dispersed part is high. This is due to the fact that high interfacial tension exists when two liquids are mixed together. In order to prepare a stable emulsion which oil and water can be mixed and formed into a single phase, an emulsifying agent is required. For instance, solid powder, surfactant, or co-surfactant (Chang, 2016). The addition of emulsifying agent gives stability and high thermodynamics, as well as improving other physicochemical properties to the emulsion (Ashaolu, 2021). The surfactant consists of hydrophilic head and hydrophobic tail (Figure 2.1). The amphiphilic properties of the surfactant play the important role to lower the interfacial tension between two liquids and make oil and water into a single phase.

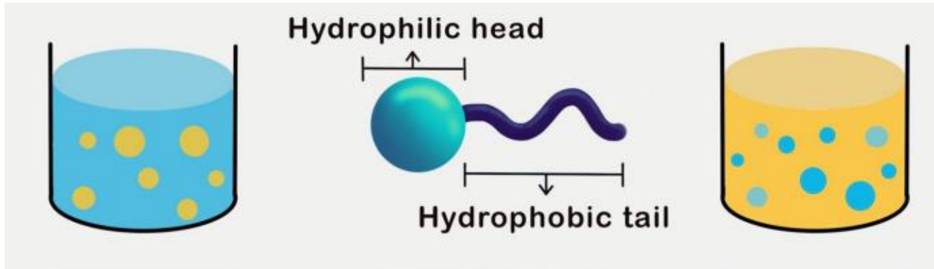


Figure 2.1 Structure of surfactant (Adapted from Ashaolu, 2021)

Generally, the phases of emulsion are constructed of aqueous and oil. Hence, they are categorized into two types according to the properties of phases which are water-in-oil (W/O) and oil-in-water (O/W) emulsion (Figure 2.2) (Ashaolu, 2021). The O/W emulsion forms when oil acts as dispersed phase. Conversely, W/O emulsion forms when water acts as dispersed phase (Chang, 2016). In another words, the liquid that occupies larger volume and has higher percentage acts as continuous phase while another liquid that occupies smaller volume in the system acts as dispersed phase.

(a) (b)

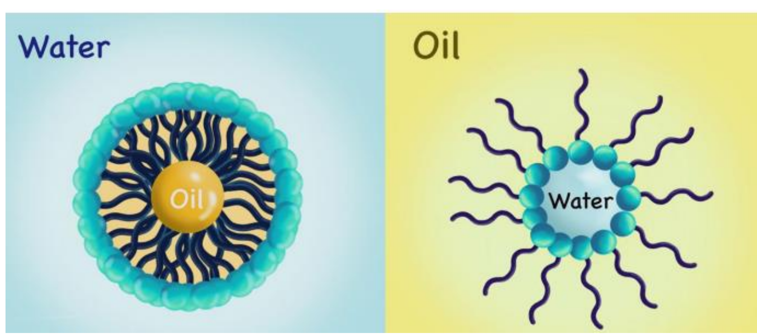


Figure 2.2 (a) oil-in-water (b) water-in-oil emulsions (Adapted from Ashaolu, 2021)

2.1.1 Oil

Oils, also known as oleochemicals are derived from animal fats, or vegetable oils. For examples, methyl esters, fatty amines, free fatty acids, fatty alcohols and glycerols. There is only 20% of the world's production of fats and oils are of animal origin. The soybean is accounted for around a quarter of the world production, followed by palm oil, rapeseed oil, and sunflower oil. The palm kernel and coconut oils are vital for the manufacture of surfactants. Besides, they consist of large amount of saturated C-12 and C-14 fatty acids. They provide high purity of fatty acids which can be utilized in chemical conversions, and in the synthesis of chemical compounds. For instance, linoleic acid which is produced from soybean, linolenic acid from linseed, oleic acid from sunflower, and so on (Metzger and Bornscheuer, 2006).

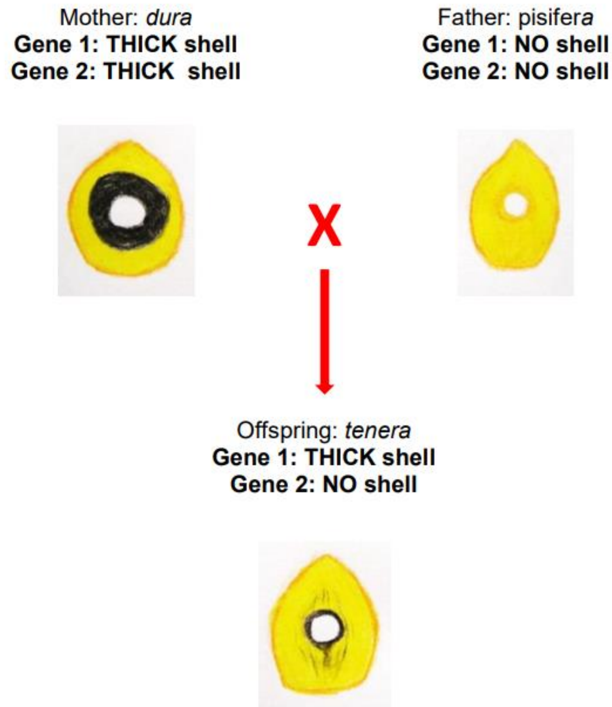
According to Mustafa and Hussein (2020), oils are categorized into two groups, which are non-essential oils, as well as essential oils. The essential oils are derived from various parts of plant which are volatile, and concentrated. They are biodegradable, having eco-friendly, as well as high efficiency characteristics. On the other hand, the non-essential oils are carrier oils which are not volatile. The nanoemulsion plays an important role to act as the carrier for essential oil that act as organic phase that deliver the active ingredients. This is due to the fact that the essential oils are unstable because of their characteristics which are biodegradable and complex structure (Flores et al., 2011). The essential oils are more likely to be exposed to reactions, like isomerization, oxidation, rearrangement, as well as polymerization regarding to their environmental factors, such as light, temperature, and atmospheric oxygen. These instability issues affect its effectiveness. Besides, they are insoluble in water, volatile and short half-life.

Table 2.1 examples of oils used in nanoemulsion

Dispersion Compositions	Purposes
Carvone, cinnamaldehyde, citral, geraniol, pulegone oils (5 v/v%)	Antibacterial activity against *Pectobacterium carolovorum* and *Ralstonia solanacearum*
Neem oil (0.5-3 w/v%)	Antifungal activity against *Aspergillus flavus* and *Penicillium citrinum*
Sweet basil, marjoram, peppermint, spearmint and thyme essential oils (10 v/v%)	Antifungal activity against *Fusarium oxysporum*
Thyme essential oil (10 v/v%)	Antifungal activity against *Sclerolinia sclerotiorum*
Citronella and lemongrass oil (10 v/v%)	Antifungal activity against *Botrytis cinerea*
Sweet flag oil or Acorus Calamus (6 v/v%)	Insecticidal activity against rice weevil, *Sitophilus oryzar*
Clove oil (1 w/v%)	Antifungal/cytotoxicity activity against *Glomerella cingulata*
	Ecotoxicity and genotoxicity effect against *Folsomia candida*
Eugenol oil (3.3 v/v%)	Antifungal activity against *Glomerella cingulata*
Vegetable oil and fatty acid methyl ester (1.9-7.5 w/w%)	Herbicidal activity against *Eleusine indica*
Clove and lemongrass oil (5 w/w%)	Antifungal activity against *Fusarium oxysporum*
Eucalyptus oil (6-10 w/w%)	Insecticidal activity against *Tribolium castaneum* and *Sitophilus oryzae*
Rosmarinus officinalis oil (5 w/w%)	Larvicidal activity against *Aedes aegypti*
Linseed oil (10.5 w/v%)	Pesticide applications
Cinnamon oil, thyme oil, Manuka oil and tea tree oil (1%-3%)	Antimicrobial activity

These poor physio-chemical properties cause essential oils hard to apply and handle. Therefore, the nanocarriers are used to boost the bioactivity of essential oils because they achieve deeper tissue penetration, easier cellular uptake as well. In addition, they are also able to control and stimulate the active ingredients towards the targeted site (Table 2.1). So, the physcio-chemical properties of essential oils have been improved, as well as their stability through decreasing their volatility, making them resist to the environmental factors, and making them capable of dispersing in water (Pavoni et al., 2020).

The palm oil and palm kernel oil are the largest oleochemical production that are available in Malaysia. Malaysia had produced 25.8% of palm oil while exported 34.3% of palm oil in year 2020. There are two kinds of oil that are produced from the oil palm which are palm oil and palm kernel oil. The palm oil is produced from the flesh while palm kernel oil is extracted from kernel or seed (Malaysian Palm Oil Council, 2023). In oil palm, 'hybrids' species produces the best yield. Their parents are different due to the thickness of fruit and shell (Woittiez et al., 2016). They are many types of oil palm species, but *tenera* species is selected due to it produces yields of palm kernel oil and palm oil that are in a good ratio. It is a hybrid between *pisifera* and *dura* species (Malaysian Palm Oil Council, 2023). The *dura* species acts as the mother palm while *pisifera* species acts as the father palm. The fruit of *dura* consists of thick shell while the fruit of *pisifera* does not contain any shell. These had been shown in Scheme 2.1. According to Woittiez et al. (2016), *tenera* fruits consist of 30% more oil than the fruits of *dura*. Table 2.2 shows the Plantation 1 which contains 100% *tenera* that are produced with more oil than other plantations.



Scheme 2.1 Production of *tenera* palm (Adapted from Malaysian Palm Oil Council, 2023)

Table 2.2 Oils produced from different plantation (Adapted from Malaysian Palm Oil Council, 2023)

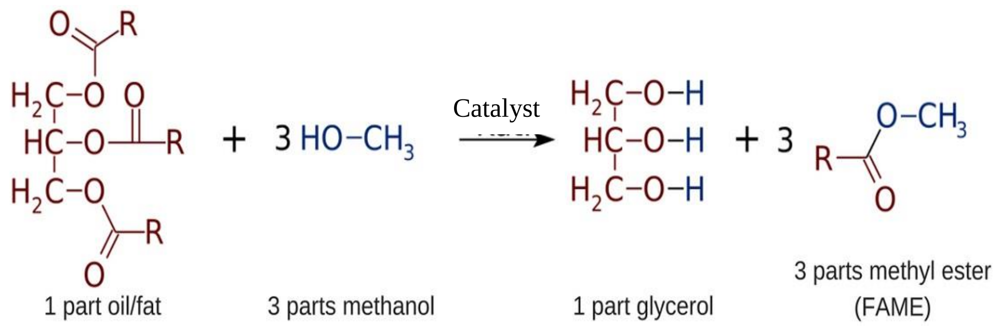
	Plantation 1	**Plantation 2**	**Plantation 3**
Planting material:	100% *tenera*	50% *tenera* 25% *dura* 25% *pisifera*	100% *dura*
Situation:	Good quality certified seeds	Seeds taken and planted from the plantation	Only *dura* seeds planted
FFB yield (t/ha)	24.0	18.0	24.0
Oil from *tenera* (23%)	5.5	2.8	0.0
Oil from *dura* (16%)	0.0	1.0	3.8
Oil from *pisifera* (sterile)	0.0	0.0	0.0
TOTAL oil yield (t/ha)	**5.5**	**3.8**	**3.8**
Selling price for farmers[1]	3600 US$/ha	2700 US$/ha	3600 US$/ha
Selling price for mill[2]	4290 US$/ha	2964 US$/ha	2964 US$/ha
Profit for mill	**690 US$/ha**	**264 US$/ha**	**-636 US$/ha**

1) FFB price = 150 US$/tonne; 2) CPO price = 780 US$/tonne

There are several steps to be taken to obtain palm oil. At first, digestion process which involves the heating of fruits takes place, meanwhile exocarp of fruits is digested. Then, the kernel and endocarp are splitted from the mesocarp. After that, pressing process occurs. The pressing or extraction machine presses the mesocarp in order to liberate the crude palm kernel oil (CPO). The CPO is obtained after the kernel and endocarp have been separated (Edupalm, 2023). Then, CPO undergoes degumming, bleaching, filtration, deodorization, fractionation and lastly the packaging. The details of palm oil processing are shown in the Scheme 2.2 (Gar, 2022).

The CPO is delivered out from the mills to the refineries by using ships or trucks. Then, it will be separated into tanks according to its quality. The quality is measured by referring to the parameters, like moisture and impurities, free fatty acids, deterioration of bleachability index (DOBI). By assessing its quality, the oil is categorized into biodiesel use or food preparation use. Then, CPO is transferred into the storage tanks by pumping with pipelines from ships or trucks. After the quality check process is done, degumming of CPO takes place by heating under vacuum in the presence of phosphoric acid. The addition of phosphoric acid is aimed to separate the gums. In the bleaching stage, the impurities are removed from CPO. The activated clay as bleaching earth tend to adsorb the color pigments. In order to remove the activated clay, filtration takes place and eventually obtains bleached palm oil. The odour and free fatty acids (FFA) in the bleached palm oil are removed in deodorization stage. The vapor, palm fatty acid distillate (PFAD) is obtained when the oil is heated. Since it is being removed, Refined Bleached Deodorised Palm Oil (RBDPO) is obtained. In this case, PFAD is able to be applied in biodiesel/oleochemical production. RBDPO is sold as industrial frying fat. The fractionation of the bulk is then conducted by cooling the oil gradually and passing it through membrane filters. At this stage, palm stearin forms because it crystallizes upon cooling. In the market, it is sold as margarine. On the other hand, the liquid part of the oil is palm olein and cooking oil.

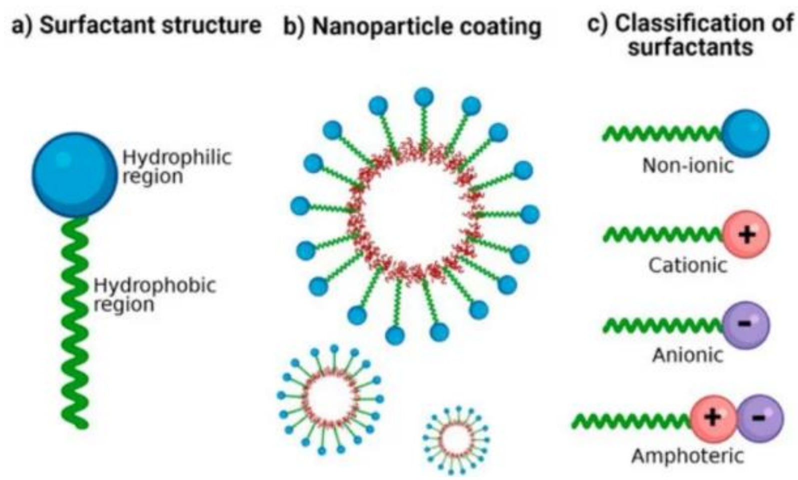
The production of palm oil brings many advantages. For example, fatty acid methyl ester (FAME) is applied as biodiesel. The production of biodiesel had replaced gasoline and mineral diesel which are utilized to start the engines (Crown Oil, 2022). Compared to diesel, biodiesel is biodegradable, renewable, releases less sulfur which is harmful to the environment (Adipah, 2018). According to Olutoye, Lee and Hameed (2011), FAME is synthesized through transesterification of animal fats or vegetable oils, along with alcohol. In order to speed up the reaction, the catalyst is added (Scheme 2.2). The feedstock which contains oil or fat derived from soybean, palm, sunflower oils. An excessive alcohol is added to play the important role for increasing the yields (Olutoye, Lee and Hameed, 2011).



Scheme 2.2 Transesterification of oil/ fat into FAME (Adapted from Crown Oil, 2022)

2.1.2 Surfactant

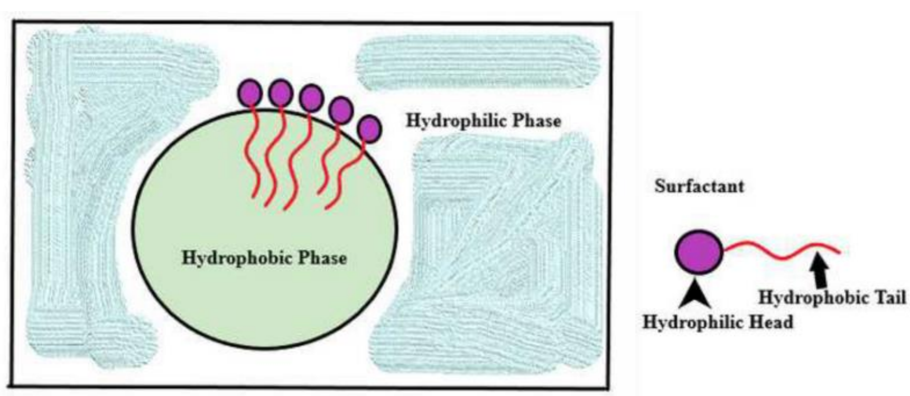
Surfactants are the chemical substances that form micelles which self-assemble as clusters in a solution, either oil or water phase, as well as adsorb onto the interface between other phases, like gases, solids and solutions. This is due to the fact that it consists of different functional groups in its chemical structure. Generally, surfactants contain hydrophobic and hydrophilic groups. The hydrophobic group is an alkyl chain that contains 8-22 carbons, and they do not interact with water. When surfactants are used in water, they are named as hydrophobic. However, they are named as lipophilic group when they are used in lipid. On the other hand, the hydrophilic group interacts with water. This hydrophilic and hydrophobic behavior is known as amphiphilic structure (Nakama, 2017).



Scheme 2.3 Structure of surfactant (Adapted from Cortés et al., 2021)

According to Nakama (2017), the surfactants are categorized into nonionic and ionic surfactants. Besides, anionic, cationic, and amphoteric surfactants are under the group of ionic surfactants. They are classified based on the charge of polar head, which is hydrophilic head (Scheme 2.3). If the polar head is uncharged, it is named as nonionic surfactant and vice versa (Cortés et al., 2021). The anionic surfactants consist of negatively charged heads which cause the hydrophilic head to dissociate into the anions in solution. Furthermore, cationic surfactants contain positively charged hydrophilic head that dissociate into cations. Lastly, the amphoteric surfactants are able to dissociate into anions, as well as cations according to pH. On the other hand, the nonionic surfactants are not able to dissociate into any ions when they are in solutions (Nakama, 2017). They are solubilized due to the weak hydrophilic groups, like hydroxyl groups, and ether-type bonds (Cortés, 2021). The ionic surfactants are categorized according to their hydrophilic groups. For instance, carboxylate, sulfate, carboxybetaine, sulfobetaine and so on (Nakama, 2017).

Surfactants are added into emulsion to act as emulsifier. They adsorb onto liquid-gas interface to reduce its surface tension. Besides that, they can adsorb onto liquid-liquid interface to reduce interfacial tension. By having the ability to reduce the surface tension, the water-immiscible phase of emulsion is able to disperse into aqueous phase. This makes the separated layer in the emulsion to develop into single phase (Assadi, Farajzadeh and Bidari, 2012). The surfactants deform and break the emulsion to the droplets to prevent coalescence. According to Safaya and Rotliwala (2020) (Scheme 2.4), when surfactants are added, they coat the dispersed oil droplets. A thin layer of water forms between the dispersed oil droplets when they come closer to each other. This causes the oil droplets to repel each other due to the charge of surfactant layer that coated on the droplets. Therefore, the surfactants have stabilized the oil droplets and prevent the droplets from developing coalescence.



Scheme 2.4 Droplets stabilization by surfactant (Adapted from Safaya and Rotliwala, 2020)

According to Perinelli et al. (2020), the surface tension decreases when concentration of surfactant increases. Referring to the Figure 2.3, two peaks are shown. The first peak shows that surface tension is not changed when the concentration of surfactant is low. However, the second peak shows that CMC is achieved when the surfactant molecules are saturated and aggregated at the air-water surface. Since the interaction is present between the hydrophobic tail of surfactant, primary micelles are formed. The micelles are eventually assembled into larger aggregates, which are the secondary micelles.

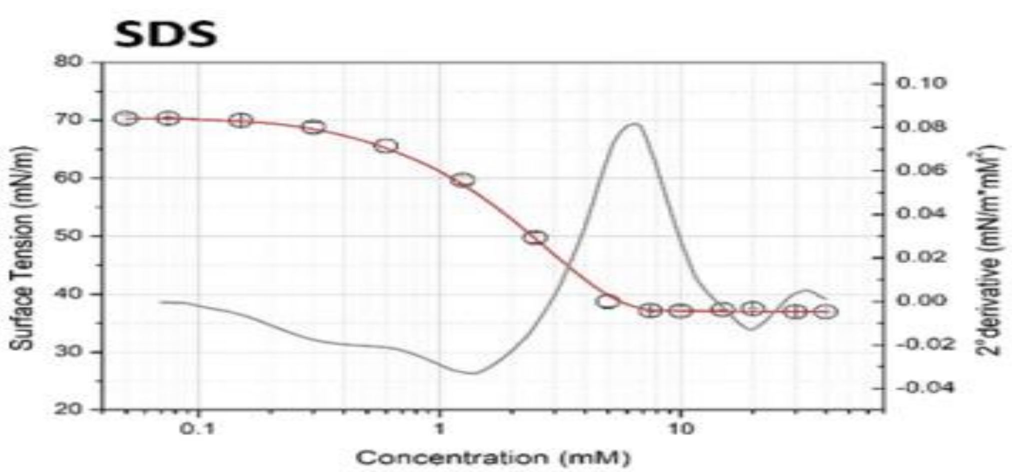


Figure 2.3 Surface tension vs concentration of ionic surfactant (Adapted from Perinelli et al., 2020)

2.1.2.1 Tween 80

Tween 80, also known as polysorbate 80, is a non-ionic surfactant. It is widely used as stabilizer, emulsifier in pharmaceuticals, cosmetics, as well as in food products. Tween 80 influences the biofilm growth and planktonic growth (Nielsen et al., 2016). According to Karjiban et al. (2012), Tween 80 is derived from oleic acid and polyethoxylated sorbitan, which has a tasteless and odourless nonionic surfactant. With four expanded hydrophilic components and a linked alkyl chain, it consists of a multi-headed structure.

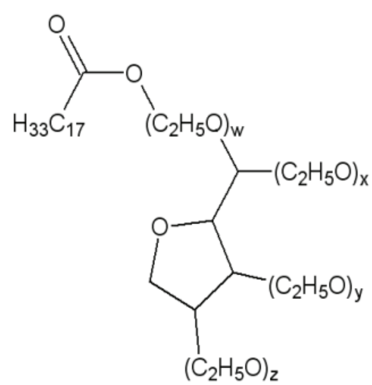


Figure 2.4 Molecular structure of Tween 80 (Adapted from Chebbi et al., 2018)

2.1.2.2 Alkyl Polyglucosides

Alkyl polyglycoside is a sugar-based surfactant. It consists of hydrophobic alkyl chain and a saccharide unit (Figure 2.5) (van Ginkel, 2007). It is a dispersing and wetting agent which is biodegradable and has low toxicity. Besides that, it is stable in alkaline, as well as in neutral conditions. It endures high concentration of salt (Glittenberg, 2012). It contains hydrophilic head and hydrophobic tail in its structure. The hydrophilic group has formed from one to five parts of condensed glucoside. On the other hand, the hydrophobic tail is an alkyl chain that consists of six to eighteen of carbon atoms. It acts as additive to reduce the wear and resistance of motion. Besides that, it can improve the antiseizure properties (Sułek et al., 2013).

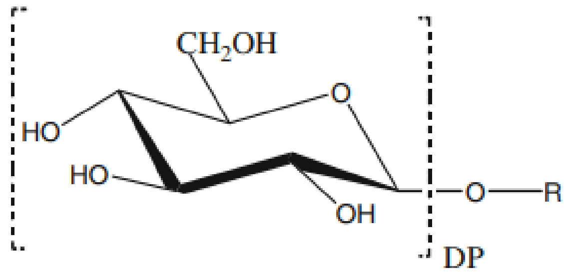
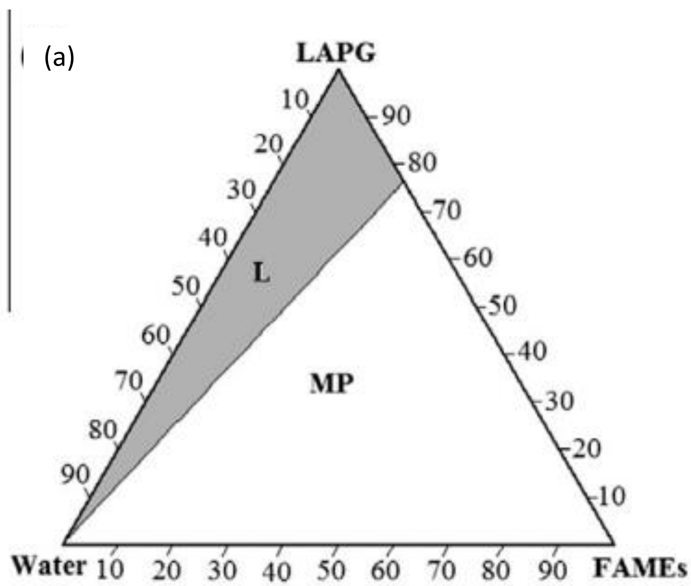


Figure 2.5 Molecular structure of alkyl polyglucoside (Adapted from Sułek et al., 2013)

2.1.2.3 Mixed Surfactant

According to Szymczyk, Zdziennicka and Janczuk (2018), Tween 80 has been widely applied to enhance the wetting properties in drug delivery by solubilizing, and stabilizing hydrophobic drugs. The alkyl polyglucoside is used as base to be mixed with Tween 80 because it gives high compatibility of FAMEs (fatty acid methyl esters) and consists of high hydrophobicity due to its long alkyl chain. Therefore, Gibbs free energy for its adsorption at the interface of surfactant and oil decreases, and eventually enhance the solubilization of FAMEs in the mixture. Tween 80 acts as co-surfactant to assist APG in the stabilization of emulsion. According to the pseudoternary phase diagrams, the application of single surfactant gives a small L region. However, the application of mixed surfactant gives a larger isotropic L region (Figure 2.6(a)). This indicated that the concentration of FAMEs that are incorporated is higher than single-surfactant system. This is due to the effect of interaction between the surfactant molecules which is resulted from the formation of mixed micelles. Hence, it increases capacity of oil solubilization, due to higher hydrophobicity. Moreover, mixed surfactants give optimized HLB number which causes the surfactant layer to work more efficiently (Lim et al., 2012).



(b)

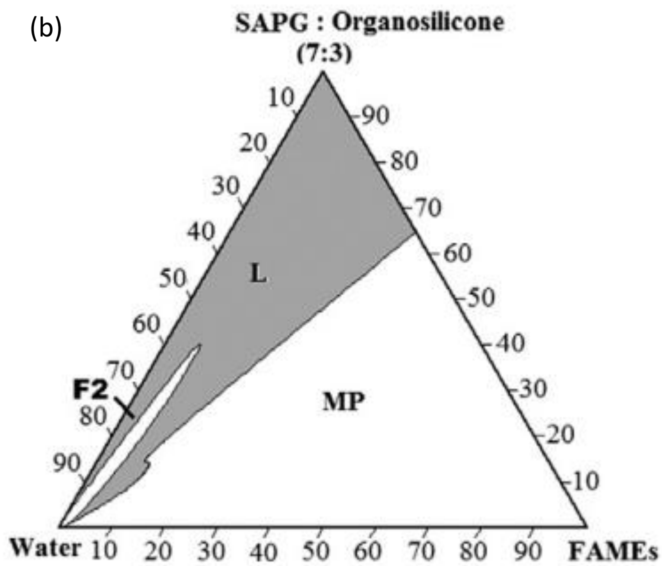


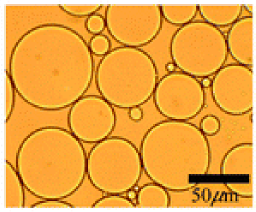
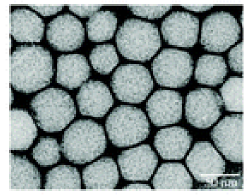
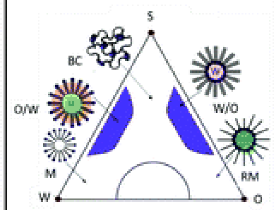
Figure 2.6 Pseudoternary phase diagram of (a) single surfactant (b) mixed surfactant (Adapted from Lim et al., 2012).

2.2 Nanoemulsion

Nanoemulsions are colloidal systems contain the droplet sizes range in the system between 20 to 200 nm. A kind of stable dispersion is created when surfactant is added. It is known as nanoemulsion. Kinetic stability is obtained when the gravitational forces of the emulsions are overcome by Brownian motion of the nanodroplets. This result in avoiding the particles from aggregating into clusters (Jaiswal, Dudhe and Sharma, 2014). The droplets of nanoemulsions are in spherical shape with smooth surfaces. Besides that, the droplets are in narrow size distribution which the polydispersity index is less than 0.25 (Su et al., 2017). Due to its very small particle size, the stability, rheology, optical transparency, as well as surface area of the nanoemulsions are enhanced. These result in association with the innovative technologies use in the production of food and beverage (Ashaolu, 2021).

There are different forms of emulsion, which are macro-, micro-, and nanoemulsion. The forms of emulsions depend on the size of droplets and its stability characteristics. The droplet sizes range which falls between 1 $x10^{-6}$ to $100x10^{-6}$ m are classified as macroemulsions while the droplets sizes of 2010^{-9} to $500x10^{-9}$ m are known as nanoemulsions. The droplet sizes range that falls between $10x10^{-9}$ to $100x10^{-9}$m are microemulsions (Scheme 2.5) (Gupta et al., 2016).

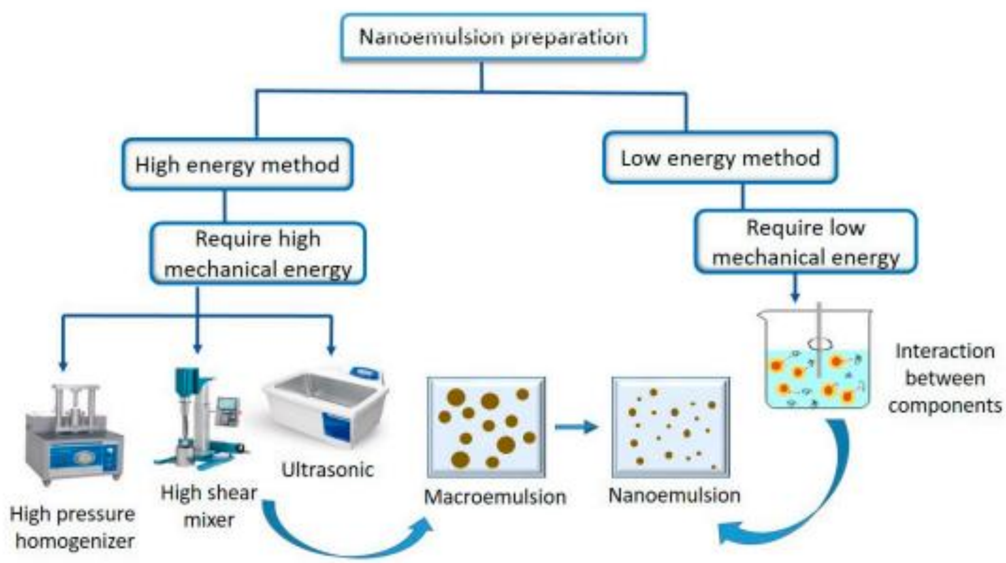
According to Gupta et al. (2016), nanoemulsions and macroemulsions are thermodynamically unstable, which indicate that the systems do not exist in equilibrium. Nevertheless, due to the small droplet sizes of nanoemulsions, it is able to achieve kinetically stable over a period of time. In contrast, microemulsions are sensitive to temperature and composition changes because they are thermodynamically stable. Thus, nanoemulsions are more likely to be studied because they are the least sensitive to chemical, as well as physical changes.

	macroemulsions	nanoemulsions	microemulsions
Structure			
size	1-100 μm	20-500 nm	10-100 nm
shape	spherical	spherical	spherical, lamellar
stability	thermodynamically unstable, weakly kinetically stable	thermodynamically unstable, kinetically stable	thermodynamically stable
method of preparation	high & low energy methods	high & low energy methods	low energy method
polydispersity	often high (>40%)	typically low (<10-20%)	typically low (<10%)

Scheme 2.5 Comparison between macro-, micro-, and nanoemulsion (Adapted from Gupta et al., 2016)

Moreover, in order to prepare macroemulsions and nanoemulsions, there are two methods to be applied which are high- and low-energy methods. In comparison, there is only one method to prepare microemulsion, which is low-energy method. From the aspect of polydispersity index, nanoemulsions and microemulsions have better potential to be produced in low dispersity index. Hence, nanoemulsions are the best choice because it produces low polydispersity index, and it can be prepared by applying high- and low-energy methods that require low concentration of surfactants (Gupta et al., 2016).

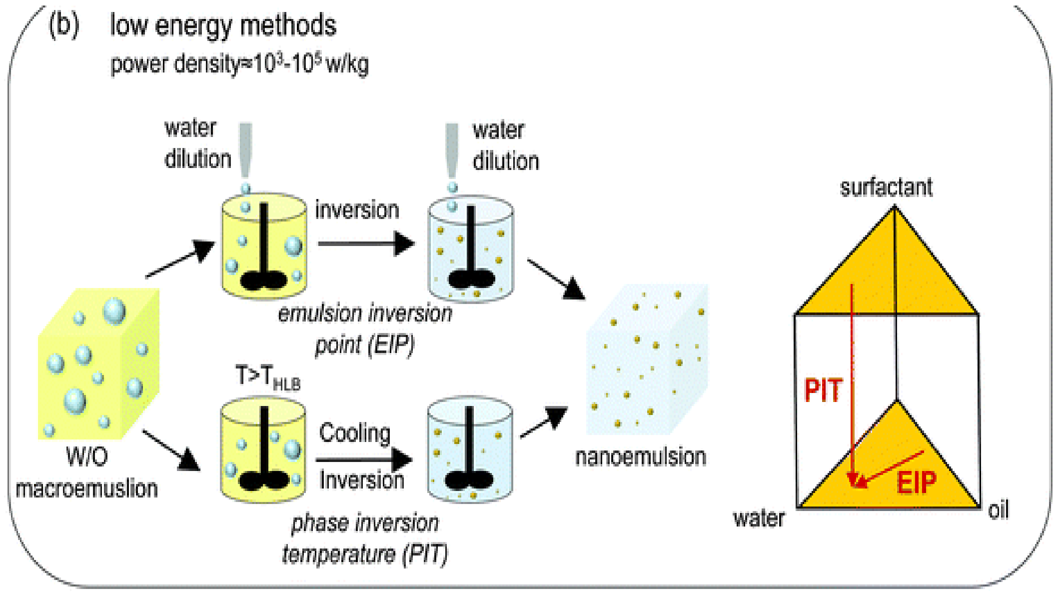
According to Safaya and Rotliwala (2020) (Scheme 2.6), the application of low-energy method is able to change the physicochemical properties of nanoemulsions by creating nanosized particles. By using low-energy method, less mechanical energy is required. However, the high mechanical energy is required to start up the stress level in order to achieve over Laplace pressure with 10 to 100 atm pressure range. In order to transform the nanoemulsion into more stable, droplets are ruptured by high mechanical energy (Mustafa and Hussein, 2020). Thus, low energy method has been studied to prepare nanoemulsion recently.



Scheme 2.6 Preparation method of nanoemulsion (Adapted from Mustafa and Hussein, 2020)

The high energy methods include high pressure homogenization and microfluidization (Shah, Bhalodia and Shelat, 2010). Meanwhile, the low-energy methods include self-emulsifying and phase inversion method. The self-emulsification is stimulated when the surfactants or solvent diffuses rapidly without changing the surfactant curvature. It can be achieved by oil phase titration, as well as aqueous phase titration. On the other hand, phase inversion method is stimulated when a change in the surfactant curvature during emulsification process. The phase transitions happen due to a change in composition and temperature. These two factors are known as phase inversion composition (PIC) and phase inversion temperature (PIT), respectively (Mustafa and Hussein, 2020).

Since the high energy method requires a machine or device to generate strong forces in order to produce smaller droplets in formulations, higher cost is required in the investment and operating system. The strong forces that produced by device are exerted to break the droplets, and eventually create nanoemulsions. Besides that, the longer time of sonication produces more kinetic energy and reduces the particle size (Mustafa and Hussein, 2020). In contrast, in low-energy method, the W/O macroemulsion is formed first, and then subjected to phase transformation during agitation, and through slowly diluting the mixture with dropwise addition of water (Emulsion Inversion Point EIP/PIC), or by preparing initial emulsion at a higher temperature and gently cool it down to form O/W nanoemulsion (PIT) (Scheme 2.7) (Gupta et al., 2016). Hence, low-energy method has greater potential to be adapted in preparation of nanoemulsion.

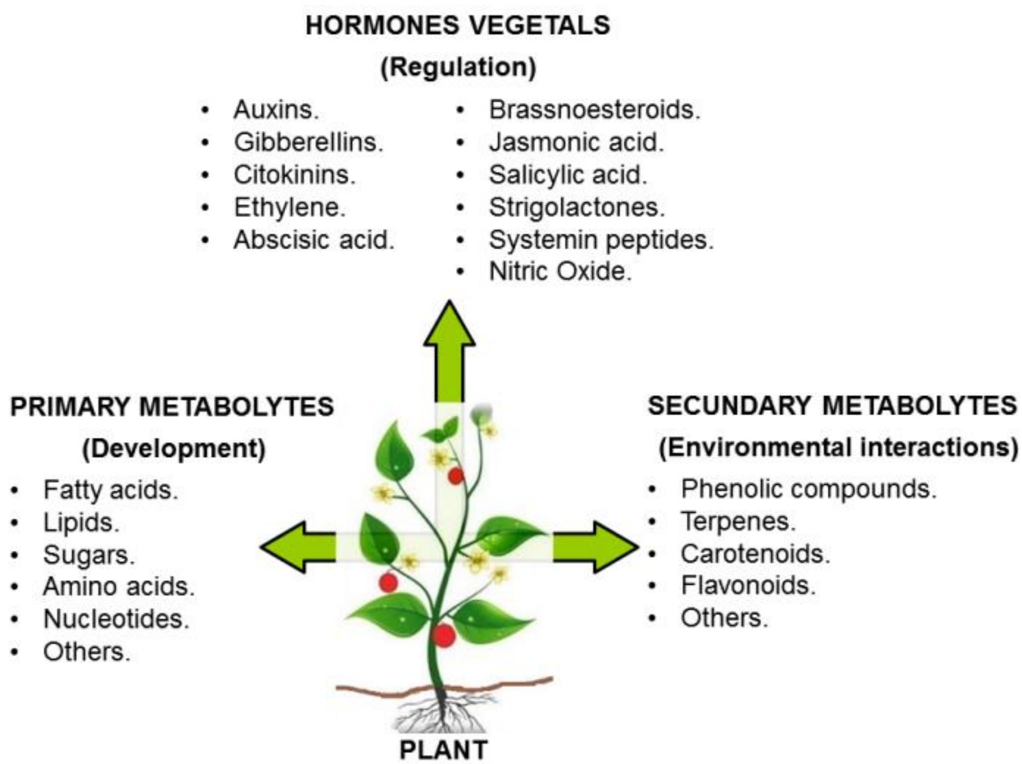


Scheme 2.7 Low-energy method (Adapted from Gupta et al., 2016)

2.3 Phytohormone

The coordination and regulation of metabolism, morphogenesis, as well as the growth of plants depend on the chemical signals that are produced by plants. The chemical messengers that mediate intercellular communication in animals are known as hormone while phytohormones are defined as the chemical messengers delivered by plants (Agudelo-Morales et al., 2021). The glands in organisms secrete hormone, and then the hormones are transported to the target organs through circulatory system in order to regulate plant behavior and physiology. After that, hormones tend to bind with specific receptor proteins in the targeted cells. This causes the function of cell changes and the signal transduction pathway is activated. However, in plants, they do not have specific hormone-secretion gland. Naturally, there are small signal molecules which are at very low concentration are generated by the plants. These are known as plant hormone (Asami and Nakagawa, 2018). Phytohormones are plant growth regulators. They bring good effect on plant metabolism, as well as to stimulate defense response of plant when they are facing stresses.

The environmental stresses are harmful to the plants growth include flooding, drought, heavy metal contamination, salinity, temperature, and ultraviolet radiation. These factors limit the plant growth to a point where decrease in the yield production. Besides that, the presence of abiotic stresses results a decrease in water intake, and the change in biochemical and physiological processes of plants. There are heavy metals, such as mercury and cadmium which are toxic and it is present in the soil although it is at low concentrations. The heavy metals are absorbed by the plants and transported into the shoot because of its high mobility. However, high concentration of heavy metals results in retardation of growth and eventually causes a decrease in enzyme activity, necrosis, change in nutrient uptake and phytoxicity (Egamberdieva et al., 2017). In order to solve these problems, the environmental conditions must be changed. Therefore, phytohormones, such as gibberellins, salicylic acid, auxins, cytokinin, and so on are synthesized to act as the organs that bring effects on the metabolism in plants (Scheme 2.8).



Scheme 2.8 example of phytohormones in plant (Adapted from Agudelo-Morales et al., 2021)

2.3.1 Nitrogen

The most deficient nutrient in the soil is nitrogen. Therefore, the rate of application of nitrogen fertilizer is high (Duguma and Aga, 2019). The addition of nitrogen into the fertilizers influence the senescence of plants and transportation of various nutrients throughout the parts of plants, like vegetative to generative parts. For instance, the nicotianamine-based chelates and various types of organic acids are responsible for transportation and remobilization of micronutrients, such as Mn, Fe, Zn and Cu. Besides that, the synthesis of nitrogenous chelates are controlled by the amount of nitrogen (Bojtor et al., 2021).

The urea is introduced into the fertilizer because it consists of 46% of nitrogen. It exists in prilled form with highest concentration of nitrogen content. The urea is transformed into the ammonium carbonate when it is present in the soil. This results in high pH of soil. When the urea is used as top-dressing, the nitrogen in the form of ammonia is easily lost from the soil's surface due to the hot weather. It works well when the nitrogen is washed into the soil, just like other nitrogen fertilizer. Other than that, it works in the best way when the moisture content of soil is sufficient. So, the gaseous ammonia can be dissolved rapidly (Finch, Samuel, and Lane, 2014).

2.3.2 Phosphorus

The phosphorus is crucial part of ribonucleic acid (RNA) that plays role for regulating the synthesis of proteins. On the other hand, it acts as carrier of genetic information in deoxyribonucleic acid (DNA). On the other hand, the phospholipid is the structural component of cellular membranes. Hence, the phosphorus is also a vital element for plant growth. The uptake of phosphorus stimulates the development of root, growth of stem and stalk strength, formation of flower, production of seed as well. Besides that, improves the N-fixing capacity in legumes, quality of crop, ability of plants to resist diseases, and so on (Kahsay, 2019). The commercial monopotassium phosphate consists of 52% of P_2O_5 (275g P) and 34% K_2O (28% K) (Ma et al., 2021). Hence, it is added to contribute the phosphorus to the fertilizer.

2.3.3 Potassium

The introduction of potassium affects the transportation of carbohydrate, nutrients and water, regulates the formation of protein, stimulate the growth of plants at early stage, as well as improves its resistance to insect and diseases. Besides that, it regulates the opening of stomata, transportation of assimilates, activate the enzymatic activity in the biological process, like formation of starch, sugars, energy transfer, and so on. The potassium that present in soil is water soluble. In addition, they are exchangeable and fixed in soil. The uptake of potassium from soil happens through soil exchangeable potassium (Gebreslassie, 2016).

The selectively permeable of plant membrane allows the passage of potassium ions through high-affinity and low-affinity K^+ channels. The electrochemical difference in the external medium and cytosol is crucial for operation of low-affinity channels. When the electrochemical potential of external medium is higher than the internal cytosol, the potassium ions tend to be transported into the cell. This result in increases in electrochemical potential in cytosol until it reaches equilibrium with the external medium by having the equal electrochemical potential on both sides. Hence, there is no net movement of potassium ions across the membrane. The H^+ ions in the cytosol are removed by plasmalemma H^+ pump and diffuse into the apoplast. Therefore, the negative charge in the cytosol increases. The electrochemical difference in apoplast and cytosol become greater. When the negative charges in the cytosol decreases, the potassium ions are diffused into the apoplast from cytosol through low-affinity channel. The flow of potassium ions into the soil happens through facilitated diffusion which is controlled by rectifying channels. The closure and opening of channels depend on the electrochemical difference in apoplast and cytosol (Gebreslassie, 2016). The potassium chloride contributes different grades of potassium to the fertilizer, which are 50% of potassium, 41% of potassium, as well as 33% of potassium (Scherer, 2005).

2.3.4 Cytokinin

The addition of cytokinin influences the characteristics of leaves through improving photosynthesis and cell expansion. In order to enhance photosynthesis and stomatal opening, chloroplast differentiation is promoted. Besides, regular senescence is delayed, ability of organs and tissues to uptake nutrients are improved (Egamberdieva et al., 2017). Furthermore, formation of pigments, and new shoots are initiated. According to Agudelo-Morales et al. (2021), the application of cytokinin (Figure 2.7) stimulates lateral meristems and apical activity. They promote growth, stimulate lateral branching, germination and shoot breakage, as well to improve yield. The chlorophyll content decreases when the amount of cytokinin reduces. This affect the photosynthetic activity of plants. According to the study, the yield of grain increases even though the vegetables are facing stress problems. Cytokinin helps to regulate immunity of plant when host-pathogen interactions exist. Thus, the addition of cytokinin into foliar fertilizer is a good choice to be studied on vegetables.

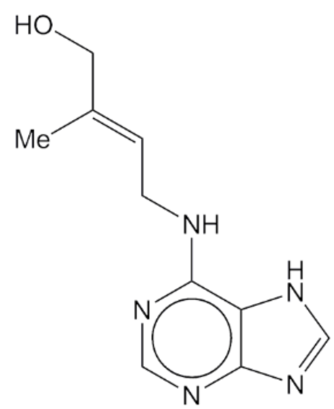


Figure 2.7 Structure of cytokinin (Adapted from American Chemical Society, 2014)

2.4 Nanoemulsion formulation

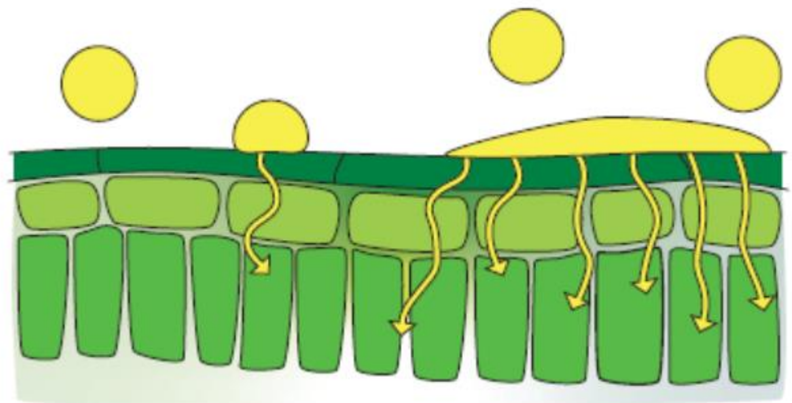
Nanoemulsion plays an important role in cosmetics and pharmaceutics industry by reducing drug resistance, as well as enhancing anticancer activities. Due to small particles size in nanoemulsion, it increases the effectiveness of drug encapsulation (Safaya and Rotliwala, 2020). The application of nanoemulsion in pharmaceutical industry promotes the development of nanotechnology in agricultural industry. The pesticides with nano-scale particles enhance the dispersion and adsorption of active ingredients onto the surface of leaves. Besides that, the development of nanoemulsion promotes the delivery process of water-soluble herbicide (Lim, et al., 2012).

2.4.1 Delivery mechanism through nanoemulsion

The foliar fertilizer uses water as solvent in the formulation, when it is being sprayed onto the leaves, the fertilizer tends to bound up and difficult to disperse on the surface of leaves. Thus, the liquid fertilizer is easy to repel onto the soil. Due to the fact that the surface of leaves is hydrophobic, the great interfacial tension exists between the liquid fertilizer and leaves' surfaces (Wei et al., 2018). This results in weak wettability and inefficient absorption of nutrients into the leaves.

The application of nanoemulsion in foliar-applied fertilizer improves the efficiency of nutrients absorption by plants. The nanoemulsion formulation of foliar-applied fertilizer penetrates into the leaves through the stomata's pores, bases of trichomes, or cuticular nanopores. The absorption of nutrients is facilitated by opening of stomata (Borak et al., 2022). The nanoemulsion contains oil and surfactant. The addition of surfactant allows well dispersion of formulation, emulsification, and sticking of fertilizer on leaves through the reduction of surface tension. This results in the stronger and longer of contact time between the surface of leaf and fertilizer

(Hock, 2022). The oil allows the fertilizer to stick on the leaves' surface because the leaves are hydrophobic (Scheme 2.9).



Scheme 2.9 Surfactants contains formulation interact with leaves' surfaces (Adapted from Hock, 2022)

2.4.2 Fertilizer composition

Plants require plenty of elements to grow, such as hydrogen, oxygen and carbon to conduct photosynthesis. The nutrients are categorized into micronutrients and macronutrients. There are seven essential elements under micronutrients. For instance, zinc, boron, manganese, iron, molybdenum, copper and chlorine. These elements are only required in small amount. On the other hand, the macronutrients like nitrogen, phosphorus and potassium are required in large quantities for plant growth. The elements that are required for plant growth are shown in the Table 2.3 (White and Brown, 2010).

Table 2.3 Essential elements for plant growth

Element	Essentiality		Critical leaf concentrations (mg g^{-1} DM)	
	Plant	Animal	Sufficiency	Toxicity
Nitrogen (N)	yes	yes	15 – 40	
Potassium (K)	yes	yes	5 – 40	>50
Phosphorus (P)	yes	yes	2 – 5	>10
Calcium (Ca)	yes	yes	0·5 – 10	>100
Magnesium (Mg)	yes	yes	1·5 – 3·5	>15
Sulphur (S)	yes	yes	1·0 – 5·0	
Chlorine (Cl)	yes	yes	0·1 – 6·0	4·0 – 7·0
Boron (B)	yes	suggested	5 – 100 × 10^{-3}	0·1 – 1·0
Iron (Fe)	yes	yes	50 – 150 × 10^{-3}	>0·5
Manganese (Mn)	yes	yes	10 – 20 × 10^{-3}	0·2 – 5·3

The nitrogen, potassium and phosphorus are the most important elements for plant growth. Nitrogen are required for building blocks in the growth of plant and chlorophyll. The greater the amount of nitrogen is absorbed, the darker the color of leaves, while phosphorus are used for energy storage, as well as transfer energy in the form of adenosine triphosphate. The potassium is required for stimulation of enzyme in assimilation and transpiration process (Barita et al., 2018).

2.4.3 Formulation Additives

Other than surfactant and oil, as well as fertilizer, the additives are introduced to boost the performance of formulations, like humectant and wetting agent. The addition of additives plays role of modifying the physical properties of fertilizer, and improving the biological efficiency and the yield of crops (Hazen, 2000). The wetting agents are surfactants that are applied with water (Ruiter et al., 2003). Strong surface tension results in the formation of spherical shape droplets and eventually leads to decrease in the contact of hydrophobic leaves' surfaces. Once the wetting agent is applied, the droplets are able to spread and incorporate onto the large area of leaves by decreasing the sphericity of droplets and making it spreading on the leaves' surfaces (Hazen, 2000). The application of wetting agent reduces the surface tension of formulation and enhances the spreading effect of fertilizer onto the leaves' surface (Ruiter et al., 2003).

On the other hand, the incorporation of humectant into the formulation enhances the moisture content on the surfaces of leaves (Ruiter et al., 2003). In other words, the equilibrium moisture content is increased and results in longer moisturizing time of fertilizer (Hazen, 2000). The humectant acts as anti-evaporant that tends to form film on the surfaces of leaves to reduce the rate of evaporation (Ruiter et al., 2003). The humectant, like glycerin consists of hydroxyl group and forms the hydrogen bonding between the fertilizers and water molecules (Fu et al., 2021). Once the droplets are dried, the active ingredients are crystallized. This results in the decrease in nutrients absorption and uptake. In order to preserve high humidity level, the humectant obtains moisture from the atmosphere (Hazen, 2000). Hence, it is important to introduce humectants into the formulation of fertilizer.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

In order to prepare emulsion, palm kernel oil, fatty acid methyl ester (FAMEs) 1218K (12 to 18 carbon chain length) was used as oil phase while Tween 80 and APG were used as surfactants. The materials to prepare fertilizer were potassium chloride (KCl), urea, monopotassium phosphate (MKP), micronutrient mixture that consists of copper, iron, magnesium, zinc, boron, molybdenum, and cobalt, as well as epsom salt (magnesium sulphate pentahydrate) and commercial fertilizer Ganlek. The additives, glycerine acted as humectant while KENPO 888 acted as spreading agent were introduced into the formulations. The cytokinin (6-benzylaminopurine) was used as phytohormone in the formulations to regulate the plant growth.

3.2 Preparation and Selection for Emulsion

3.2.1 Phase Behaviour Study for Emulsion

The mixed surfactants (Tween 80:APG) were prepared in 15 mL in the mass ratios of 9:1, 8:2, 7:3, and 6:4. The mixed surfactants-to-oil mass ratios of 9:1, 8:2, 7:3, and 6:4 were prepared by mixing FAMEs oil and mixed surfactants 9:1, 8:2, 7:3 and 6:4 in 15 mL centrifuge tubes. About 25% w/w of distilled water was added into the mixtures. All the chemicals were weighed by using an analytical balance

(Mettler Toledo, United States), homogenized with Gemmy VM-300 vortex mixer (Taiwan) for 2 to 3 minutes, and centrifuged at 4000 rpm using Dynamica Velocity 14/14R Pro refrigerated centrifuge (United Kingdom), operating at room temperature (25 ℃) for 15 minutes. The appearances of the emulsions were visually observed and recorded. The above steps were repeated with incremental addition of water until the emulsions achieved at 50, 75 and 90% w/w of water.

3.2.2 Selection for Emulsion

From the phase behaviour study, two emulsions were selected based on the following criteria:

(i) Optically translucent or transparent and one phase at room temperature (25℃) after centrifugation at 4000 rpm for 15 minutes

(ii) containing the highest oil-to-surfactant in mass ratio

3.3 Development and Optimisation of Nanoemulsion Fertilizer

3.3.1 Preparation of Water-Soluble Solution of Fertilizer and Additives

A bulk solution of fertilizer and water-soluble additives were prepared in different chemical compositions, as shown in Table 3.1. All of the components were mixed together and dissolved in 500 mL of distilled water.

Table 3.1 Chemical compositions and masses of fertilizer components

Chemical composition	Ratio (parts)	Fertilizer components	Mass (g)
Nitrogen	16	Urea	86.9440
Phosphorus (in form P_2O_5)	16	MKP	76.9269
Potassium (in form K_2O)	16	KCl	21.9126
Magnesium	8	Epsom salt	242.7040
Sulphur	10.54	Micronutrient	27.0784
Iron	0.44	Glycerine	4.1867
Zinc	0.55	KENPO 888	4.1810
Copper	0.055	Iron	1.0831
Manganese	0.22	Zinc	1.3539
Boron	0.22	Copper	0.1354
Cobalt	0.011	Magnesium	0.5416
Molybdenum	0.011	Manganese	0.5416
		Boron	0.5416
		Cobalt	0.0271
		Molybdenum	0.0271

3.3.2 Preparation of Emulsion

The selected emulsions were produced in bulk according to the oil-to-surfactant ratios and mixed surfactant ratios assigned and 75% w/w distilled water was used to create a stable, one phase and optically transparent emulsion. The masses of oil, surfactant and water are listed in Table 3.2.

Table 3.2 Mass of emulsion components

Emulsions	Mass (g)		
	Oil	Mixed surfactant	Water
Tween 80:APG (9:1) and FAME 1218K (9:1)	0.2128	1.7922	6.0429
Tween 80:APG (8:2) and FAME 1218K (9:1)	0.3668	1.8144	6.5436
Tween 80:APG (7:3) and FAME 1218K (9:1)	0.4099	1.8043	6.6426
Tween 80:APG (6:4) and FAME 1218K (9:1)	0.1060	0.9380	3.1320
Tween 80:APG (9:1) and FAME 1218K (8:2)	0.4025	1.6441	6.1398
Tween 80:APG (8:2) and FAME 1218K (8:2)	0.4026	1.6222	6.0744
Tween 80:APG (7:3) and FAME 1218K (8:2)	0.2040	0.8155	3.0585
Tween 80:APG (6:4) and FAME 1218K (8:2)	0.2050	0.8186	3.0708

3.3.3 Preparation of Cytokinin (6-benzylaminopurine) Solution

The cytokinin powder was dissolved in 0.1 M hydrochloric acid (Tim and Arditti, 2017). Approximately 5 mL of 0.1 M hydrochloric acid was required to dissolve 0.0030g of cytokinin. The cytokinin solution was mixed with NPK fertilizer at 1:1111 ratio using IKA RW20 digital overhead stirrer at low speed mode and room temperature for 15 minutes.

3.3.4 Preparation of Nanoemulsion Formulation of Cytokinin NPK Fertilizer

The samples preparations of diluted emulsions are mixed and stirred with fertilizer solution containing cytokinin and without cytokinin using IKA RW20 digital overhead stirrer operating at low speed mode for 30 minutes (Table 3.3). If the formulation was clear after mixing, it was subjected to another round of centrifugation at 4000 rpm for 15 minutes.

Table 3.3 Preparation of Nanoemulsion Formulation of Cytokinin NPK

Label	Treatment
F1	Nanoemulsion contains 9:1 surfactant to oil ratio with mixed surfactant of 9:1 + NPK fertilizer solution
F2	Nanoemulsion contains 9:1 surfactant to oil ratio with mixed surfactant of 8:2 + NPK fertilizer solution
F3	Nanoemulsion contains 9:1 surfactant to oil ratio with mixed surfactant at 9:1 + NPK fertilizer solution + cytokinin solution
F4	Nanoemulsion contains 9:1 surfactant to oil ratio with mixed surfactant at 8:2 + NPK fertilizer solution + cytokinin solution

3.3.5 Preparation of Samples

The emulsions were prepared at 9:1 SORs with different MSRs at 9:1 and 8:2. The 75% w/w of water was weighed and added dropwise into the emulsions while stirring with IKA RW20 digital overhead stirrer operating at low speed mode. The NPK fertilizer solution was then added into the sample dropwise at 9:1 NPK fertilizer to emulsion ratio and stirrer for 30 minutes. In order to prepare the samples with cytokinin, the cytokinin NPK fertilizer solution was added into the diluted samples dropwise with stirring with IKA RW20 digital overhead stirrer operating at low speed mode for 30 minutes. Therefore, the samples had been prepared were 9:1 SOR at mixed surfactant ratios of 9:1 and 8:2 by adding NPK fertilizers and cytokinin NPK fertilizers respectively.

3.4 Characterization of Nanoemulsion Fertilizer Formulation

3.4.1 Thermostability Test

The formulation samples were placed in the oven at 54 ℃ for two weeks in accordance with the guidelines established by the Food and Agricultural Organisation (FAO) as a standard assessment for agrochemical products to demonstrate good physical stability in the tropical weather (Lim et al., 2012). The appearances of the formulation samples were observed and recorded visually at the end of two weeks. The formulation samples which were one phase, no creaming and sedimentation were selected.

3.4.2 Freeze-thaw cycle

The formulation samples were placed in the freezer at -20℃ for 24 hours and placed at the room temperature for another 24 hours. The freeze-thaw processing were conducted for three cycles. The appearances of the formulation samples were visually observed and recorded after every cycle. The selected formulation samples were no phase separation, no creaming and sedimentation.

3.4.3 Morphology

The formulation samples and unformulated fertilizer solution were characterized using HRTEM (JEOL JEM-2100F, Japan) for comparison. 1mL of samples were diluted with 200 mL of distilled water and the samples were subjected to ultrasonication for 15 minutes. The samples were then dropped onto copper grids and negatively stained with 1% uranyl acetate solution. The copper grid was then dried in open air under a table lamp for a day, before the samples being characterized using the HRTEM at 200 kV.

3.4.4 Particle Size Distribution and Polydispersity index (PDI)

Malvern particle size analyser coupled with zetasizer (United Kingdom), that equipped with Photon Cross Correlation Spectrometer (PCCS), was applied for measuring the particle size distribution and PDI of the formulation samples and fertilizer solution. 100 µL of samples were diluted 200-fold with distilled water and underwent ultrasonification for 5 minutes. 1000 μL of the diluted samples were then loaded into the cuvettes and subjected to the instrument for characterizations.

3.5 Biological Activities Study of Ceylon Spinach against Nanoemulsion Cytokinin NPK Fertilizer Formulation

3.5.1 Seed germination

The purchase of Ceylon spinach (*Basella alba*) seeds were soaked in tap water for one day. After that, the tap water was poured out and the seeds were rinsed with tap water to remove the impurities. Two seeds were sown in each hole of seedling tray (40 holes per tray) which contains germination soil. The seeds were placed on the surface of the soil by using forceps. The seedling trays loaded with Ceylon spinach seeds were irrigated with tap water twice a day to ensure the moisturization of soil for the seed growth.

3.5.2 Cultivation of Ceylon Spinach

After two weeks, each growing plant was transplanted into 12 cm diameter and area of 113 cm^2 plastic pots filled with 3-in-1 soil on 16th February, 2023 at the afternoon. A total of 70 pots were placed in the green house with the condition of temperature ranging from 25 to 38.1℃ with humidity of 34-48% RH (16 hrs/ light) , as well as 24.5-30.39 ℃ with humidity 69-99% RH (8 hrs/ dark) at Block E, Universiti Tunku Abdul Rahman. The plants were watered with tap water twice a day.

3.5.3 Preparation of Treatments

The experiment was conducted with seven treatments and ten replications per each treatment. The samples were mentioned in Section 3.3.4:

T_1 = NPK formulation

T_2 = commercial leaf applied fertilizer

T_3 = 9:1 mixed surfactant (9:1 Tween 80:APG) to oil nanoemulsion fertilizer formulation without cytokinin

T_4 = 9:1 mixed surfactant (8:2 Tween 80:APG) to oil nanoemulsion fertilizer formulation without cytokinin

T_5 = 9:1 mixed surfactant (9:1 Tween 80:APG) to oil nanoemulsion fertilizer formulation with cytokinin

T_6 = 9:1 mixed surfactant (8:2 Tween 80:APG) to oil nanoemulsion fertilizer formulation with cytokinin

T_7 = water as control

Before spraying, the NPK fertilizer was diluted at 1:215. The formulations of T_3, T_4, T_5, T_6 treatments were diluted with dilution factor of 1:60. The commercial foliar-applied fertilizer was diluted with a dilution factor of 1:100 by following the commercial application recommendation. The dilution factors of all treatments were different because the water content in concentrated NPK formulations was different in all treatments. Hence, the concentration of NPK was fixed in all treatments, which is 0.0005%.

3.5.4 Application of Treatments

All the treatments were applied by using 1L pressure sprayer operating at the flow rate of 5.15 mL per second. The application rate of treatments was 77.38722 mL/m^2. The walking speed was 0.14787 m/s and sprayed at 50 cm height from the ground. The ten pots of each treatment was arranged within 5 m. The distance between each pot was 0.5 m. After spraying. the pots were arranged through randomized completely block design (RCBD) in the greenhouse. The application of treatments was conducted once a week. The plants were irrigated twice a day.

3.5.5 Data Collection

After 36 days of planting (30^{th} March, 2023), the plants were harvested. Three pots were selected randomly from each treatment to conduct bioassay evaluation. There were five growth response parameters, which were fresh mass, chlorophyll content, total phenolic content, surface area of leaves, and number of leaves per plant were assessed. The fresh mass of each plant was weighed by using analytical balance (Mettler Toledo, United States) while the chlorophyll content was measured by using chlorophyll meter, CM-B model, (Shandong, China). On the other hand, the total phenolic content was measured by applying Folin-Ciocalteu colorimetric assay. The surface areas of leaves were measured by using leaf area meter, LAM-B model, (Shandong, China).

3.5.5.1 Measurement of Total Phenolic Content (TPC) by Applying UV-visible Spectrometry

3.5.5.1.1 Preparation of 10% Folin-Ciocalteu Reagent

10 mL of reagent was added into 100 mL volumetric flask. Folin-Ciocalteu reagent was diluted with distilled water until it reaches the meniscus mark. The volumetric flask was inverted for a few times to mix the solution well.

3.5.5.1.2 Preparation of 5% Sodium Carbonate Solution

Five grams of solid sodium carbonate was weighed into a beaker. Ten millimeters of distilled water were added to dissolve the Na_2CO_3. Then, it was transferred into 100 mL volumetric flask and mark up with distilled water. The volumetric flask was inverted for a few times to mix the solution well.

3.5.5.1.3 Preparation of Gallic Acid Stock Solution

0.5 g of solid gallic acid was dissolved into 10 mL of ethanol. Then, it was transferred into 100 mL volumetric flask. The distilled water was used to mark up until the meniscus mark of volumetric flask and inverted for a few times.

3.5.5.1.4 Gallic Acid Calibration Curve

The different concentrations of gallic acid (0-100 mg/L) solution was used as standard (Table 3.4). 0.5 mL of standard solution was pipetted into different tubes. 2.5 mL of 10% FC reagent was added and the solutions were mixed thoroughly with vortex mixer. After 5 minutes, 2 mL of 5% Na_2CO_3 solution was added into each tube and mixed again with vortex mixer. They were incubated in the dark at room temperature for 90 minutes. The absorbance of different concentration of gallic acid solution were measured at 765 nm. The calibration curve which absorbance against concentration of gallic acid standard solutions was plotted.

Table 3.4 Preparation of Gallic Acid Standard Solution

Concentration of gallic acid standard solution (mg/L)	Volume of gallic acid stock solution added (mL)
0	0
20	0.4
40	0.8

60	1.2
80	1.6
100	2.0

3.5.5.1.5 Extraction of Leaves

The leaves were weighed and grinded into fine powder, then the samples were transferred into a dry glass vial and soaked in 30 mL of AR grade methanol. After that, the samples were mixed using Gemmy VM-300 vortex mixer (Taiwan) and were soaked for one day. The sample solutions were centrifuged at 4000 rpm at room temperature for 10 minutes to separate and remove the insoluble material.

3.5.5.1.6 Measurement of Total Phenolic Content

A three milliliters of extract were transferred into a vial. 1.5 mL of 10% FC reagent was added into the vial and mixed thoroughly with vortex mixer. The samples were placed in the dark for 5 minutes. Then, 1.5 mL of 5% Na_2CO_3 solution was added and shaked using vortex mixer. The vials were filled with 6 mL of distilled water for dilution. The samples were kept in dark at room temperature for 2 hours to ensure that the reaction was completed. The 1 cm^2 quartz cuvette was filled with sample solution. After that, the intensities of blue colors of the samples were measured based on the absorbance. The absorbance was determined spectrophotometrically by using Genesys 50 UV-visible spectrophotometer (Selangor, Malaysia) at a wavelength of 750 nm. The methanol acted as blank in absorbance measurement. The total phenolic content of each sample was determined by referring to the gallic acid standard calibration curve.

3.5.6 Statistical Analysis

All the data collected were subjected to ANOVA technique (analysis of variance), and then Post hoc assessment with Tukey's HSD test to distinguish the significant variations among the mean values of different treatments at $p<0.05$. The standard errors of means were shown.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Phase Behaviour Study and Selection of Emulsion

The results of phase behavior studies between different compositions of oil and mixed surfactants are shown in Figure 4.1 and Table 4.1. The ratios between Tween 80 and APG were studied as well. After centrifugation, the appearances of the emulsions were observed and recorded. The optically translucent or transparent single phase solutions were formed or separation of phases happened which were creaming and flocculation (white color precipitate floated on the surface of solution).

(a) (b) (c)

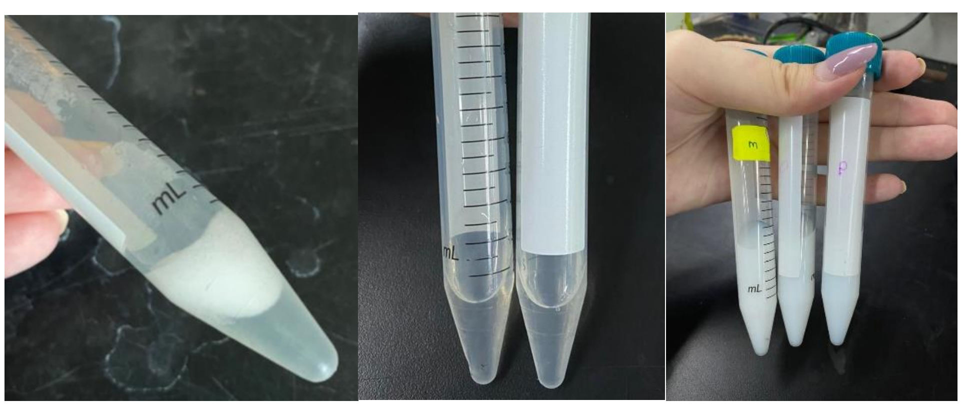


Figure 4.1 Observation of emulsions after centrifugation formed (a) creaming, (b) transparent single phase or (c) opaque

Table 4.1 Phase behaviour of different compositions of oil and mixed surfactants in the emulsion systems

<table>
<tr><th>Tween 80:APG / Surfactant :oil</th><th>9:1</th><th>8:2</th><th>7:3</th><th>6:4</th></tr>
<tr><td>9:1</td><td rowspan="2">One phase (transparent)</td><td rowspan="2">One phase (transparent)</td><td rowspan="2">One phase (transparent)</td><td rowspan="2">One phase (transparent)</td></tr>
<tr><td>8:2</td></tr>
<tr><td>7:3</td><td colspan="4">One phase (opaque)</td></tr>
<tr><td>6:4</td><td colspan="4">Two phases (creaming)</td></tr>
</table>

Based on the result that obtained, the emulsion with higher mixed surfactant-to-oil ratios at 7:3 and 6:4 were unstable. The higher surfactant-to-oil ratio tends to decrease the size of droplets by stabilizing the oil and water interface (Mustafa and Hussein, 2020). When the composition of oil is greater while the concentration of mixed surfactant is lower, the water-oil interfacial tension becomes greater. The higher energy and more surfactant are required to break down the oil/water interface to form droplets. This results in the reduction of micellar structure formation of surfactant to stabilize oil-water interface (Church et al., 2021). Hence, the phase separation takes place at higher oil-to-mixed surfactant ratio in the emulsion system (7:3 and 6:4).

The phase separation happened due to destabilization mechanism, such as flocculation and creaming, as well as Ostwald ripening. An opaque single phase solution was formed at mixed surfactants:oil ratio of 7:3 due to Ostwald ripening process. The Ostwald ripening takes place in a continuous water phase results in creation of larger oil droplets (Safaya and Rotliwala, 2020). The small sizes of oil droplets are transformed into large and low curvature radius of oil droplets and eventually leads to increase in particle size (Mustafa and Hussein, 2020).

After centrifugation, the mixed surfactant-to-oil ratio (7:3) in the emulsion system produced two phases which flocculation and creaming happened. The flocculation takes place when the globules are bind together and produce floccules which are large clumps. They tend to float or sink in the emulsion system rapidly instead of individual globules. Due to the sinking and floating process of the dispersed globules, it gives rise to the formation of concentrated layer. This concentrated layer is called as creaming. Thus, flocculation process is eventually leads to creaming (Jaiswal, Dudhe and Sharma, 2014).

The emulsion systems that consisted of 9:1 and 8:2 of mixed surfactant-to-oil ratios produced the most stable emulsions which were optically transparent and single phase solutions. According to Lim et al. (2012), the mixed surfactants system (Tween 80 and APG) enhances the solubilization of FAMEs in the emulsion system and resulting in the formation of transparent monophase. This explains that more

FAMEs were soluble in the emulsion system. This is because the application of mixed surfactants leads to the formation of mixed micelles and resulting in greater hydrophobicity in binary surfactant systems. Hence, the stabilization capacity of oil is increased. The particles are dispersed evenly in the emulsion system while the mixed micelles formed are randomly distributed. Besides, the application of mixed surfactants optimized the HLB (hydrophilic-lipophilic balance) number. This phenomenon improves the flexibility of the layer of surfactant to enable the effective partitioning between oil and water phases. Hence, by increasing the concentration of Tween 80 in the mixed surfactant, more oil is stabilized in the emulsion system and reduces the probability of phase separation to happen. The optimum mixed surfactant-to-oil ratios were observed at 9:1 and 8:2. The concentration of APG does not affect the solubilization of oil because it acts as co-emulsifier to assist Tween 80 to stabilize the emulsion systems.

By referring to the Table 4.1, emulsion system at 9:1 and 8:2 mixed surfactants-to-oil ratio with different ratios of mixed surfactants (Tween 80:APG) at 9:1, 8:2, 7:3 and 6:4 were selected as they were optically transparent and produced single phase solution at room temperature. The Tween 80 is nontoxic and contains more carbon atoms in the structure, which is 18 carbons. This increases the intermolecular hydrophobic interaction between the surfactant and oil phases. So, the formation of micelles is facilitated since the surfactants are able to bind easily with the oil phase (Wang and Gao, 2017). Tween 80 is able to produce good stability. The particle sizes of APG are smaller. In APG structure, it consists of single aliphatic chain which makes them arranged themselves at the oil-water interface effectively and leads to formation of micelles rapidly due to the packing of APG. Therefore, the Tween 80 and APG were used as mixed surfactant in this project.

4.1.1 Determination of water content in the final nanoemulsion formulation

In order to prepare nanoemulsion via PIC method, which the water was added slowly in dropwise to dilute the emulsion system was applied. When the emulsion reaches 25% w/w of water, w/o emulsion is created. The small water droplets are dispersed in the continuous oil phase. It has lower stability than o/w emulsion.

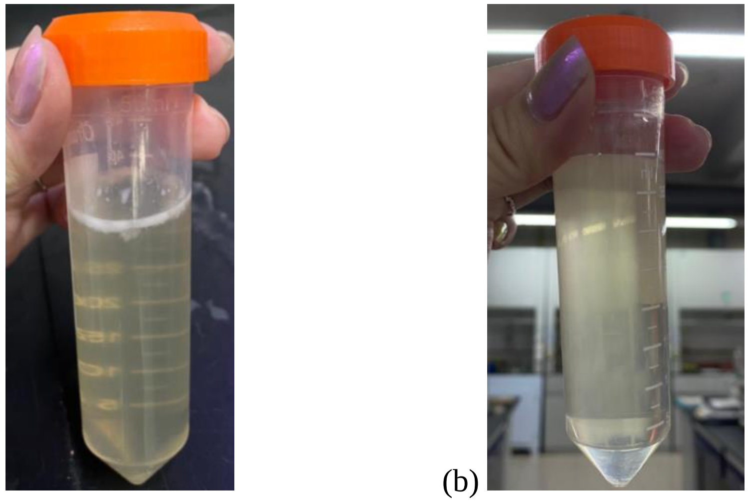
(a) (b) (c)

Figure 4.2 Observation of emulsions at different %w/w water (a) 25%, (b) 50% and (c) 75%

This is due to the fact that the water droplets are free to move in the w/o emulsion and leads to the formation of coalescence, flocculation, or sedimentation. Besides, there is only presence of steric forces is able to stabilize w/o emulsion (Colucci et al., 2020). Once reaching the 50% w/w of water, the bicontinous emulsion is produced which the ratio between oil and water is the same in the emulsion system. The surfactants start to arrange at the O/W boundary and reduce the interfacial tension. This result in the formation of smaller particles (Mustafa and Hussein, 2020). The o/w nanoemulsion forms when 75% w/w water is reached in the formulation. The oil phase is solubilized completely in the bicontinuous emulsion. The large oil-water interfacial area is due to the rapid migration of oil into the water phase, which creates intense turbulence between two phases. Once the bicontinuous phase is disrupted, it leads to spontaneous formation of small oil droplets (Safaya and Rotliwala, 2020). Hence, O/W emulsion forms when 75% w/w of water reaches in the formulation. The high water content in emulsion system was selected because oil is solubilized in the emulsion system and thereafter the nanoemulsion can be produced and the samples are indicated by transparent single phase solution. The emulsion system that contains a minimum of 75% w/w of water gave the most stable formulation.

4.2 Formulation of Nanoemulsion Fertilizer

After the addition of fertilizer into the emulsions, the stability of formulations is affected. After centrifugation, the appearances of the emulsion containing fertilizer formulation were observed and recorded (Figure 4.3 and Table 4.2). The stable formulation is indicated by the formation of optically translucent or transparent single phase solutions (Figure 4.3 (b)) whereas phase separation (Figure 4.3 (a)) happens in the unstable emulsion fertilizer formulation.



(a) (b)

Figure 4.3 Observation of nanoemulsion formulation after adding NPK fertilizer formed (a) creaming and (b) single phase

Table 4.2 Phase behaviour of nanoemulsion fertiliser formulations

<table>
<tr><th>surfactant:oil / Tween 80:APG</th><th>9:1</th><th>8:2</th><th>7:3</th><th>6:4</th></tr>
<tr><td>9:1</td><td rowspan="2">One phase</td><td rowspan="2">Two phases (creaming)</td><td rowspan="2">Two phases (creaming)</td><td rowspan="2">Two phases (creaming)</td></tr>
<tr><td>8:2</td></tr>
</table>

The liquid fertilizer is also known as salt solutions. They consist of undissociated salt molecules, ion pairs or ions. The electrolytes are the solutions that have ions and able to conduct electricity. They are able to destabilize the oil dispersed systems which reduce the steric effect between the oil particles by compressing the double layer and reducing the particles' surface potential for repulsion between particles. Moreover, the components in the fertilizer solutions compete with hydrophilic part of surfactants and affects the stabilizing effect of the surfactants. The salts in the fertilizer solution resulted in poor particles dispersion of the nanoemulsion system. The electrical conductivity of ion depends on the mobility of ions. When the ions are able to move at high speed, the electrical conductivity increases. The introduction of fertilizer into the emulsion formulation increases the electrical conductivity of the nanoemulsion, which means that can affect the stability of nanoemulsion incorporated with the fertilisers (Saltzman and Brates, 1990).

By referring to the result in Table 4.2, the formulations that consisted of surfactant-to-oil ratio of 9:1 with mixed surfactants ratios 9:1 and 8:2 gave the most stable emulsion system. There was no phase separation happened in the formulations, unlike the formulations that consisted of 7:3 and 6:4 mixed surfactant ratios which creaming took place and produced two phases. The nanoemulsions contain water and oil phase and they are stabilized by emulsifying agent which are mixed surfactants (Tween 80 and APG), eventually leads to the formation of single phase (Ashaolu, 2021). The Tween 80 and APG are non-ionic surfactants. There are no any charges on the hydrophilic head of non-ionic surfactants. The hydrogen bonds forms between the surfactant and water molecules. Thus, the compatibility of surfactants with oil phase increases (Lim et al., 2012). The surfactant molecules adsorb onto the surface of dispersed phase globules, oil droplets and form coherent film around the surface of oil droplets by reducing the interfacial tension. Therefore, the coalescence of dispersed particles is avoided (Jaiswal, Dudhe and Sharma, 2014). The oil droplets repel to each other when they are come close together due to like charge on surfactant monolayer. The emulsion system is stabilized when the steric repulsive force exerts between the oil droplets (Safaya and Rotliwala, 2020).

When the surfactant-to-oil ratio becomes larger, there is more surfactant molecules present in the emulsion system. There are more surfactant molecules adsorb onto the surface of oil droplets and result in sufficient amount of surfactant molecules to form surfactant monolayer. Thus, the higher surfactant-to-oil ratio of formulation produces stable single phase nanoemulsion formulation. In stable nanoemulsion formulation, the fertilizer ions are moving at slow speed and increases the effectiveness of surfactant molecules to adsorb onto the oil droplets. In a similar way, the cytokinin solution was added into the nanoemulsion fertilizer formulations. It gave the same result which the formulations that consisted of 9:1 and 8:2 mixed surfactants were stable. The nanoemulsion fertilizer formulations that contains 9:1 surfactant to oil ratio with mixed surfactants (Tween 80:APG) at 9:1 and 8:2 ratios are stable. Hence, they were selected to conduct physicochemical characterizations.

4.3 Characterizations of Nanoemulsion Fertilizer

The nanoemulsion fertilizers that were selected to conduct characterizations are shown in the Table 4.3 below.

Table 4.3 Samples that had been selected to conduct characterizations

Label	Treatment
F1	Nanoemulsion contains 9:1 surfactant-to-oil ratio with mixed surfactant of 9:1 + NPK fertilizer solution
F2	Nanoemulsion contains 9:1 surfactant-to-oil ratio with mixed surfactant of 8:2 + NPK fertilizer solution
F3	Nanoemulsion contains 9:1 surfactant-to-oil ratio with mixed surfactant of 9:1 + NPK fertilizer solution + cytokinin solution
F4	Nanoemulsion contains 9:1 surfactant-to-oil ratio with mixed surfactant of 8:2 + NPK fertilizer solution + cytokinin solution

The selected nanoemulsion fertilizer formulations F1 to F4 were subjected to thermostability test (54 ℃), freeze-thaw for three cycles, morphology and structure characterizations, measurement of particle sizes, and polydispersity index.

4.3.1 Thermostability Test at 54 ℃

The samples were prepared and placed in the oven at 54 ℃ for two weeks. The samples were observed visually at the end of the weeks. The stability of the samples was indicated by the appearance of the samples, like phase separation, opaque and single phase.

(a) (b) (c) (d)

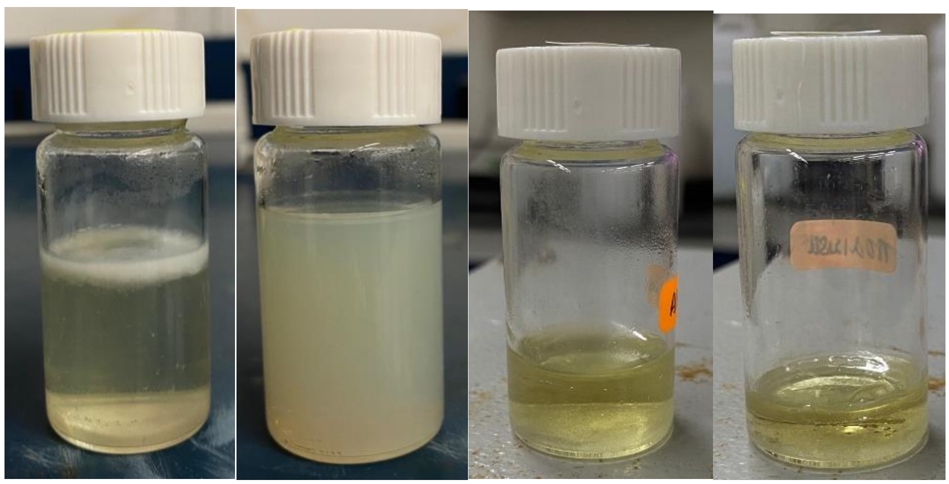


Figure 4.4 Observation of nanoemulsion fertilizer formulations under thermostability test at 54 ℃ for the samples (a) F1, (b) F2, (c) F3 and (d) F4

The thermostability test was conducted because it simulates the conditions that the emulsion may be exposed to during processing or storage. The temperature is one of the factors that affects the stability of emulsions. When the emulsions are exposed to high temperature, the collision frequency between the droplets increases. This results in large difference in densities of oil and aqueous phases. The mechanical strength of surfactant monolayer around the oil droplets becomes weaker and eventually leads to destabilization of emulsion (Adhikary and Basak, 2022). Besides that, the hydrophilic head of Tween 80 (polysorbate 80) surfactant is dehydrated due to high temperature and results in low hydrophilicity. Therefore, the stability of the emulsion system reduces since the emulsion systems become with less hydrophilic. On the other hand, the APG surfactant is not affected much by the temperature (Lim et al., 2012). Hence, phase separation took place and creaming floated on top of formulations F1 and F2.

There was no phase separation observed in the formulations F3 and F4 which contain cytokinin. The cytokinin is able to withstand extreme temperature by regulating plant growth even though it is under high temperature stress. By increasing the concentration of cytokinin, the heat stress tolerance is enhanced (Liu et al., 2020). By referring to the structure of cytokinin (6-benzylaminopurine), it consists of an aromatic ring that is attached to hydroxyl group, as well as isoprenoid side chain. The electrons delocalize in the double bond of aromatic ring and contribute resonance stabilization to the structure. In addition, the branched and long chain with the presence of double bonds on the isoprenoid side chain could result in the high stability of cytokinin structure. Due to its stable molecular structure, the cytokinin is not easily to be denatured under stress condition. The stability of emulsion systems is improved under high temperature.

4.3.2 Freeze-Thaw Cycle

The nanoemulsion fertilizers were placed in the freezer at -20℃ for one day, followed by placing at room temperature (25℃) for next day (24hr), and repeated for another two cycles. The appearances of the nanoemulsion fertilizers were observed and recorded in Figure 4.5.

(a) (b) (c) (d)

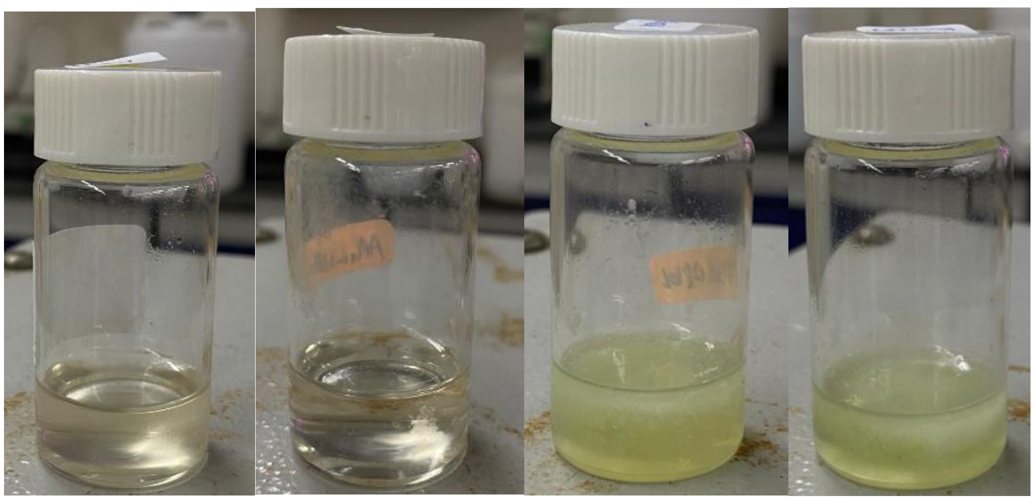


Figure 4.5 Observation of nanoemulsion fertilizer formulations after three freeze-thaw cycles for the samples (a) F1, (b) F2, (c) F3 and (d) F4

The repeated cycles of freezing and thawing affects the stability of nanoemulsion fertilizer. During freezing, the nanoemulsion fertilizer is exposed to low temperature and result in the formation of ice crystals because water molecules are frozen. During thawing process, the samples start to accumulate at certain areas and result in phase separation (Donsì, Wang and Huang, 2011). This has brought mechanical stress to the emulsion droplets and causes extensive droplet aggregation in the samples F3 and F4. Moreover, the cytokinin is not able to tolerate at freezing temperature. The structure of cytokinin is broken down and become separated in the emulsion system. The degradation of cytokinin occurs when side chain-derived aldehyde, as well as adenine are formed via oxidative cleavage of N^6 side chain (Frebort et al., 2011). Hence, the F3 and F4 samples were no longer stable at freezing temperature.

The stable nanoemulsion fertilizer formed in F1 and F2 samples. The addition of mixed surfactants adsorbs onto the surface of oil droplets and result in the formation of thick surfactant layer. The thick surfactant layer prevents the oil droplets from coalescence. Therefore, they are stable after conducting freeze-thaw for three cycles (Donsì, Wang and Huang, 2011).

4.3.3 Morphology and Structure

The formulation samples were characterized using HRTEM. The morphology and structure in the samples were observed to make comparison.

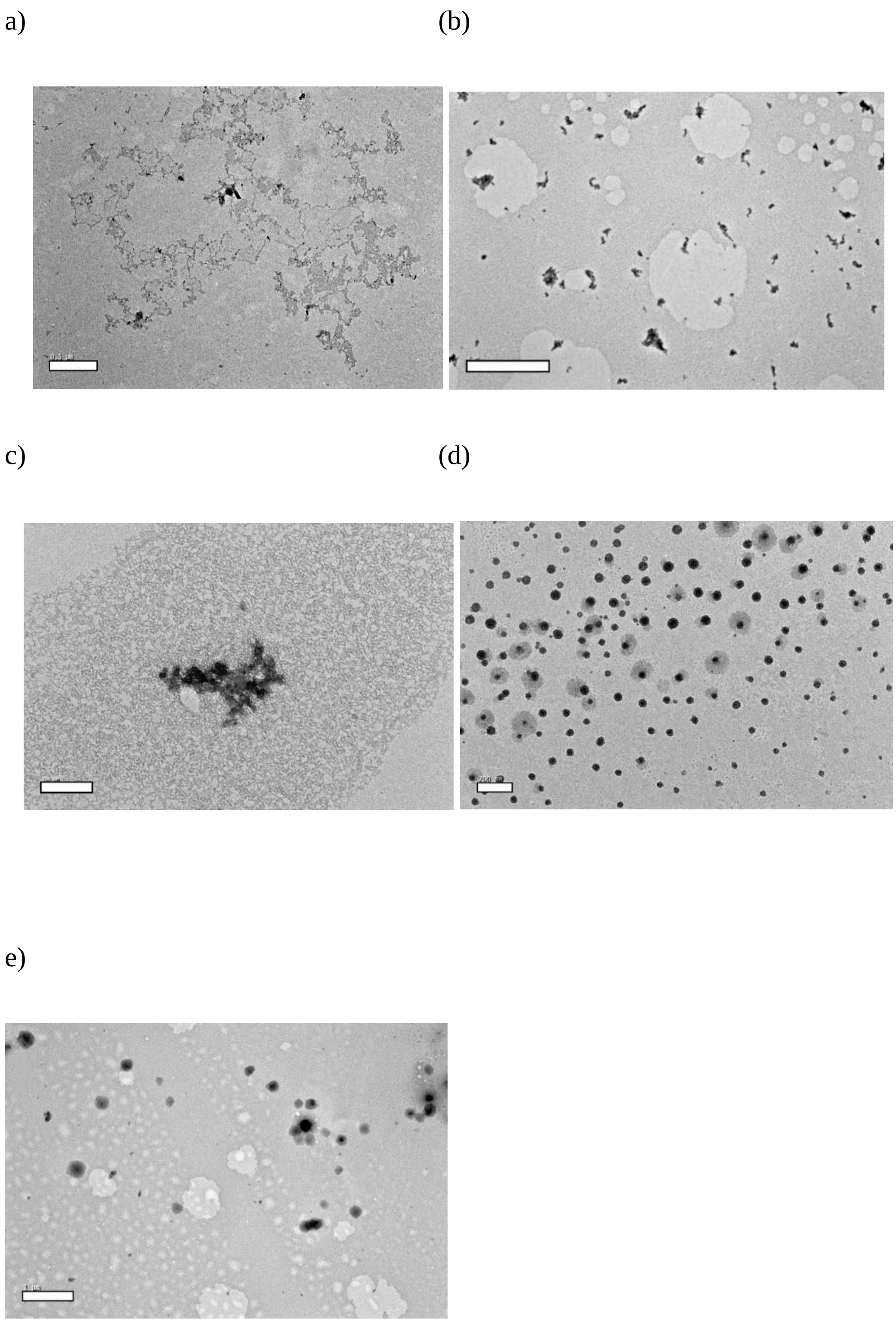


Figure 4.6 TEM images of the samples: (a) fertilizer solution, (b) F1, (c) F2, (d) F3 and (e) F4

Based on the HRTEM images of NPK fertilizer solution (Figure 4.6 (a)), the larger particle sizes in three dimensional network structure were observed. The agglomeration of particles was observed because the particles were clumped together due to the absence of nanoemulsion in the fertilizer solution. The crystallization took place. The salt ions could attract each other to form network structure.

On the other hand, the HRTEM images of F1 and F2 samples (Figure 4.6 (b) and (c)) which are nanoemulsion NPK fertilizer formulations showed that the particles are less aggregated in the structure. The nanoemulsions consisted of surfactants which acts as emulsifying agent in the emulsion system. The presence of surfactant plays an important role in stabilizing the nanoemulsion system. It reduces the interfacial tension by adsorbing onto the surface of oil droplets and forming a surfactant monolayer (Jaiswal, Dudhe and Sharma, 2014). Due to the similar charges of the surfactant layer around the oil droplets, they tend to repel with each other when they come closer together. This property has prevented the oil droplets from coalescence and stabilizing them in the nanoemulsion system (Safaya and Rotliwala, 2020). Therefore, the HRTEM of nanoemulsion fertilizer shows spherical structures because cluster of nanomicelles formed during the preparation of nanoemulsion system (Mustafa and Hussein, 2020).

In comparison, the HRTEM images of F3 and F4 samples (Figure 4.6 (d) and (e)) which consisted of cytokinin in the nanoemulsion NPK fertilizer formulation show that distinctive spherical micelles formed in the formulations due to the preparation method of nanoemulsion (Su et al., 2017). The particles in F3 and F4 samples are the least aggregated among all the samples. Besides than the presence of mixed surfactants, the structure of cytokinin contains hydrophobic benzyl group which is non polar while the hydrophilic part of cytokinin is purine ring, as well as the amino group that attached to the purine ring (Pub Chem, 2023). This gives rise to the surfactant-like properties and amphiphilic character to the cytokinin. Hence, it improves the stability of the nanoemulsion. The oil droplets play a role in carrying the cytokinin particles and prevent it from the formation of clumps. Since the amount of stabilizing agent becomes greater, the stabilizing effect of the nanoemulsion is enhanced. Moreover, the cytokinin incorporated into the particles of nanoemulsion, as well as NPK fertilizer and causes the formation of large particles. The steric repulsive force becomes stronger causes the oil droplets are well dispersed in the system. The particles in F3 and F4 samples are well dispersed and repel with each other.

From the aspect of surfactant content in the nanoemulsion system, the system that contains higher ratio of surfactant (Tween 80) results in better stabilizing effect. The smaller Tween 80 ratio in the mixed surfactants indicated that low concentration of surfactant in the nanoemulsion systems, which can be observed from the samples F2 and F4. The low concentration of surfactant results in desorption of the surfactant molecules from the oil-water interface and eventually leads to larger particles sizes. In addition, the hydrophobic parts of the surfactants are reduced. This causes that the solubilization of oil phase in the system is restricted (Lim et al., 2012). The high surfactant content reduces the sizes of droplets (Mustafa and Hussein, 2020). Thus, the nanoemulsion fertilizer with Tween 80:APG at the ratio of 8:2, which are F2 and F4 samples showed that the particles clumped together while the F1 and F3 samples which contained Tween 80:APG at the ratio of 9:1 gave better dispersion of particles, as well as better stability of the system.

4.3.4 Particle Size Distribution and Polydispersity Index (PDI)

Table 4.4 shows the mean droplet size and PDI of all samples which are NPK fertilizer solution, nanoemulsion fertilizer without cytokinin, as well as nanoemulsion fertilizer that contains cytokinin. Most of the samples give average droplet sizes that fall within 20 to 250 nm in range. This indicates that the samples are present in the form of nanoemulsion. Based on the result, the F3 and F4 samples that contains cytokinin have larger average particle size compared to the samples that do not contain cytokinin. The cytokinin tends to be incorporated into the nanoemulsion particles and ions of NPK fertilizer. This result in larger particles size and increase in the steric repulsive force between the oil droplets. Hence, the average particle sizes in F3 exceeds the particles size range of nanoemulsion (20 nm-200 nm) while the average particle size in F4 sample is greater than the F1 and F2 samples.

The polydispersity index of the samples (0.241-0.673) are higher than the typical nanoemulsion. The higher polydispersity index indicates that the particles are polydisperse which means higher variability in the particle sizes. The presence of cytokinin contributes better dispersion of particles in the nanoemulsion. This is due to the stronger repulsive force between the droplets as result of an increase in stabilizing affect in the system since cytokinin has surfactant-like properties. Therefore, the samples that consisted of cytokinin (F3 and F4) have greater average particle size and lower polydispersity index. This indicates that the particles in the samples F3 and F4 samples have better dispersion which are monodisperse than the samples F1 and F2. The samples that have PDI value smaller than 0.5 are considered as good homogeneity in particle sizes (Mustafa and Hussein, 2020).

Table 4.4 Particle size distribution and PDI of samples

Samples	Average particle size (nm)	Polydispersity index
F1	38.43	0.673
F2	16.32	0.424
F3	225.0	0.399
F4	123.4	0.241

4.4 Statistical Analysis

The measurement of chlorophyll content, total phenolic content, fresh weight, surface area of leaves and number of leaves had been conducted onto the plants of different treatments.

Table 4.5 Effect of different treatments that applied onto the leaves of Ceylon spinach

Treatment	Chlorophyll content (SPAD)	Total phenolic content (mgGAE/L)	Fresh weight (g)	Surface area of leaves (cm^2)	Number of leaves
NPK fertilizer	28.0±1.0^{b*}	6.5931±1.9880^{b-d}	1.7191±0.3231^{ab}	5.25±0.48^{b}	3.0±1.0^{a}
Commercial leaves applied fertilizer	30.8±1.8^{ab*}	4.1256±2.1783^{cd}	2.0943±0.8443^{ab}	10.54±5.03^{ab}	2.7±1.2^{a}
F1	32.3±2.2^{ab*}	12.4805±0.6494^{ab*}	1.7302±0.6755^{ab}	9.92±2.59^{ab}	2.7±0.6^{a}
F2	32.4±0.6^{ab*}	15.0346±0.8450^{a*}	2.0346±0.7906^{ab}	12.98±2.49^{a*}	2.7±1.2^{a}
F3	34.0±2.5^{ab*}	8.6710±4.6105^{a-c*}	2.2806±0.4791^{ab}	10.37±0.02^{ab}	3.3±1.5^{a}
F4	35.0±3.8^{a*}	10.4459±3.9442^{a-c*}	2.6715±0.2830^{a*}	12.44±1.30^{a*}	4.0±0.0^{a}
Control	15.3±2.5^{c}	1.0519±1.0390^{d}	1.0599±0.3369^{b}	5.89±0.76^{b}	2.3±0.6^{a}

Amongst means at same column with different Roman alphabets indicate significant difference at $p < 0.05$. *Significant difference as compared to untreated control. Significant difference evaluated by Tukey's HSD test.

Based on Table 4.5, the four nanoemulsion formulations which are F1, F2, F3 and F4 gave better result than control treatment in the aspect of chlorophyll content, total phenolic content, fresh mass and surface area and amount of leaves. This is indicated by the plants that were treated with four nanoemulsion fertilizer formulations had higher chlorophyll content and total phenolic content, greater fresh mass, larger surface area of leaves, as well as more leaves grown. Due to the small particle sizes in nanoemulsion, the surface area of droplets increases. This result in higher stability, better spreading and absorption of fertilizer (Jaiswal, Dudhe and Sharma, 2014). Hence, the absorption of nutrients become effective in the plants that treated with nanoemulsion fertilizers. The plants that received only water gave the lowest chlorophyll content, total phenolic content, fresh weight, surface area of leaves, as well as the least number of leaves. There were no any additional nutrients supplied to the plants and the nutrients were only absorbed from the soil which was limited to the plants. So, the supply of nutrients was insufficient for the plant growth.

The application of commercial leaves applied fertilizer contribute low total phenolic content in plants. It does not consist of mixed surfactants, emulsion, as well as sticker and humectant, like glycerin. In the absence of humectant, the uptake of nutrients from the leaves was inhibited after the foliar applied fertilizer was dried. The introduction of humectant achieves the wetting effect on the surface of leaves. The surface tension increases and result in slow spreading of droplets. By maintaining the moisture of droplets on the surface of leaves, the availability of nutrients to be absorbed into the plants through leaves is increased (Ramsey, Stephenson and Hall, 2005). Besides, in the absence of sticker, the fertilizer solution was no longer adhere onto the leaves and it is easily washed away by rain.

The ingredients in the commercial leaves applied fertilizer did not stimulate the development of phenolic compounds which were responsible for the measurement of total phenolic content in plants. Therefore, the total phenolic content in the treatment was low. In contrast, the chlorophyll content, fresh mass, surface area and amount of leaves did not significantly affect too much.

The plants that treated by formulations F3 and F4 gave the best result among all the treatments because F3 and F4 formulations contain cytokinin, which is phytohormone that regulates plant growth. The incorporation of cytokinin into the formulations improved the growth of plants by contributing higher chlorophyll content, total phenolic content, fresh mass, greater surface area and more leaves among all the treatments. The addition of cytokinin is functioned in chloroplast differentiation (Mukherjee et al., 2022). The decrease in concentration of cytokinin reduces the chlorophyll content of plants (Agudelo-Morales et al., 2021). The chlorophyll is the component that found in the chloroplast which is a green pigment. The development of chloroplasts that is stimulated by introduction of cytokinin increases the chlorophyll content in the plants (Cackett et al., 2021). When the amount of chloroplast become greater in plants, it increases the chlorophyll content at the same time. The cytokinin is able to stimulate the production of phenolic compounds, like phenolic acid, in the plants (Yang et al., 2011). When the amount of phenolic compounds increases, there is more phenolic compounds to react with the phosphostungstate molybate and result in the formation of blue molybdenum complex, which absorbs at 765 nm. This is a redox reaction (Walker et al., 2010).

The greater the amount of blue molybdenum complex formed, the higher the intensities of blue color. The total phenolic content was determined from the gallic acid standard calibration curve based on the absorbance value. Hence, the absorbance increases and proves that the total phenolic content increases.

The cytokinin facilitates cell division and elongation in plants (Asami and Nakagawa, 2018). This results in an increase in fresh mass, surface area and amount of leaves in comparison to control treatment because the metabolism of plants is promoted. These phenomenon proves that the incorporation of cytokinin promotes the growth of plants. The nanoemulsion fertilizers that consist of higher ratio of Tween 80 provides better stabilizing effects in the system. This causes the absorption of fertilizer into the plants become more effective due to high stability of nanoemulsion. Hence, the plants that treated with F4 sample which contains cytokinin and higher Tween 80:APG ratio (8:2) produces the best result among all treatments.

CHAPTER 5

CONCLUSION

5.1 Conclusion

The 9:1 and 8:2 mixed surfactant ratios (Tween 80:APG) at 1:9 oil to surfactant ratio (FAMEs:mixed surfactants) produced the most stable emulsions. Besides, the best formulation was produced by adding the fertiliser solution dropwise into the emulsion mixture when stirring with a final 75% w/w water via Phase Inversion Composition method. The formulations produced were incorporated effectively into the fertiliser solution where:

- The particles sizes are in the particle size range of nanoemulsion
- The HRTEM images show that the particles are spherical and well dispersed in the formulations

The addition of cytokinin in the nanoemulsion formulations enhances the chlorophyll content, total phenolic content in plants, as well as improve the plant growth which is indicated by greater leaf area and fresh mass in comparison to control.

5.2 Future Research Work

5.2.1 Viscosity

The presence of surfactants increases the viscosity of the emulsion. By determining the viscosity of emulsions and formulations, the Newtonian and non-Newtonian study of the samples can be conducted. Besides that, how the shear rate influences the viscosity of emulsion can be figured out. The viscosity of nanoemulsion is able to be studied by applying shear viscometer.

5.2.2 Optimization of Chemical Composition

This project only focuses on one type of oil, which is FAMEs 1218K and mixed surfactants, which are Tween 80 and APG. However, the effect of different types of oil that are applied along with different types of surfactants on the stability of emulsion can be further studied. The most optimum of chemical compositions of nanoemulsion fertilizer can be studied to determine the best formulations which affects the growth of plant greatly.

5.2.3 Introduction of Enzyme

The study of addition of enzymes into the nanoemulsion fertilizer can be conducted. Besides the phytohormone, the effect of enzyme on the plant growth can be further studied. In addition, the interaction of enzymes and phytohormones in the nanoemulsion fertilizer can be further studied, as well as their effects on the plant growth.

5.2.4 Adsorption Study on Leaves

The absorption of nutrients into the plants is affected by the effectiveness of the nanoemulsion formulations. The stronger the adsorption of the fertilizer on the leaves, the greater the effectiveness of the formulations. The adsorption study can be conducted by using Atomic Force Microscope (AFM) to study the surface morphology of plant leaves.

References

Adhikary, T. and Basak, P., 2022. Extraction and separation of oils: The journey from distillation to pervaporation. *Advances in Oil-Water Separation*. [e-journal] pp. 511–535. https://doi.org/10.1016/b978-0-323-89978-9.00026-4

Adipah, S., 2018. Introduction of biodiesel as a sustainable resource. *Journal of Environmental Science and Public Health*. [e-journal] 03(01). https://doi.org/10.26502/jesph.96120050

Agudelo-Morales, C.E., Lerma, T.A., Martinez, J.M., Palencia, M. and Combatt, E.M., 2021. Phytohormones and Plant Growth Regulators - A Review. *Journal of Science with Technological Applications*. [e-journal] 10, pp. 27–65. https://doi.org/10.34294/j.jsta.21.10.66

American Chemical Society, 2014. *Zeatin*. [online] Available at: <https://www.acs.org/molecule-of-the-week/archive/z/zeatin.html> [Accessed 2 May 2023]

Asami, T. and Nakagawa, Y., 2018. Preface to the special issue: Brief review of plant hormones and their utilization in agriculture. *Journal of Pesticide Science*. [e-journal] 43(3), pp. 154–158. https://doi.org/10.1584/jpestics.m18-02

Ashaolu, T.J., 2021. Nanoemulsions for Health, food, and cosmetics: A Review. *Environmental Chemistry Letters*. [e-journal] 19(4), pp. 3381–3395. https://doi.org/10.1007/s10311-021-01216-9

Assadi, Y., Farajzadeh, M.A. and Bidari, A., 2012. Dispersive liquid–liquid microextraction. *Comprehensive Sampling and Sample Preparation*. [e-journal] 2, pp. 181–212. https://doi.org/10.1016/b978-0-12-381373-2.00051-x

Barita, Y., Prihastanti, E., Haryanti, S., Subagio, A. and Ngadiwiyana, 2018. The influence of granting NPK fertilizer and nanosilic fertilizers on the growth of Ganyong Plant (canna edulis ker.). *Journal of Physics: Conference Series*. [e-journal] 1025, p. 012054. https://doi.org/10.1088/1742-6596/1025/1/012054

Bish, J.J., 2020. *Overpopulation: Cause and effect*. [online] Available at: <https://www.populationmedia.org/blog/overpopulation-cause-and-effect> [Accessed 21 April 2023]

Bojtor, C., Illes, A., Mousavi, S.M.N., Szeles, A., Toth, B., Nagy, J. and Marton, C.L., 2021. Evaluation of the nutrient composition of maize in different NPK fertilizer levels based on multivariate method analysis. *International Journal of Agronomy*. [e-journal] 2021, pp. 1–13. https://doi.org/10.1155/2021/5537549

Borak, B., Gediga, K., Piszcz, U. and Scala, E., 2022. Foliar fertilization by the sol-gel particles containing Cu and zn. *Nanomaterials* [e-journal] 13(1), p. 165. https://doi.org/10.3390/nano13010165

Cackett, L., Luginbuehl, l.H., Schreier, T.B., Lopez-Juez, E. and Hibberd, J.M., 2021. Chloroplast Development in green plant tissues: The interplay between light, hormone, and transcriptional regulation. *New Phytologist*. [e-journal] 233(5), pp. 2000–2016. https://doi.org/10.1111/nph.17839

Chakraborty, S., Tiwari, P.K., Sasmal, S.K., Misra, A.K. and Chattopadhyay, J., 2017. Effects of fertilizers used in agricultural fields on algal blooms. *The European Physical Journal Special Topics* [e-journal] 226(9), pp. 2119–2133. https://doi.org/10.1140/epjst/e2017-70031-7

Chang, Q., 2016. Emulsion, foam, and gel. *Colloid and Interface Chemistry for Water Quality Control*. [e-journal] pp. 227–245. https://doi.org/10.1016/b978-0-12-809315-3.00011-6

Chebbi, S., Allouache, A., Schwarz, M. and Rabhi, S., 2018. Treatment of produced water by induced air flotation: effect of both TWEEN 80 and ethanol concentrations on the recovery of PAHS. *Nova Biotechnologica et Chimica*. [e-journal] 17(2), pp. 181-192. https://www.researchgate.net/publication/330567685_Treatment_of_produced_water_by_induced_air_flotation_effect_of_both_TWEEN_80_and_ethanol_concentrations_on_the_recovery_of_PAHS

Church, J., Willner, M.R., Renfro, B.R., Chen, Y., Diaz, D., Lee, W.H., Dutcher, C.S., Lundin, J.G. and Paynter, D.M., 2021. Impact of interfacial tension and critical micelle concentration on Bilgewater Oil Separation. *Journal of Water Process Engineering*. [e-journal] 39, p. 101684. https://doi.org/10.1016/j.jwpe.2020.101684

Colucci, G., Willner, M.R., Renfro, B.R., Chen, Y., Diaz, D., Lee, W.H., Dutcher, C.S., Lundin, J.G. and Paynter, D.M., 2020. Development of water-in-oil emulsions as delivery vehicles and testing with a natural antimicrobial extract. *Molecules.* [e-journal] 25(9), p. 2105. https://doi.org/10.3390/molecules25092105

Cortés, H., Hernandez-Parra, H., bernal-Chavez, S.A., Prado-Audelo, M.L.D., Caballero-Floran, I.H., Borbolla-Jimenez, F.V., Gonzalez-Torres, M., Magana, J.J. and Leyva-Gomez, G., 2021. Non-ionic surfactants for stabilization of polymeric nanoparticles for biomedical uses. *Materials*. [e-journal] 14(12), p. 3197. https://doi.org/10.3390/ma14123197

Crown Oil, 2022. *Guide to fame biodiesel*. [online] Available at: <https://www.crownoil.co.uk/guides/fame-biodiesel-guide/> [Accessed 7 January 2023]

Donsì, F., Wang, Y. and Huang, Q., 2011. Freeze–thaw stability of lecithin and modified starch-based nanoemulsions. *Food Hydrocolloids.* [e-journal] 25(5), pp. 1327–1336. https://doi.org/10.1016/j.foodhyd.2010.12.008

Du, Y., Wang, T., Wang, C., Anane, P., Liu, S. and Paz-Ferreiro, J., 2019. Nitrogen fertilizer is a key factor affecting the soil chemical and microbial communities in a mollisol. *Canadian Journal of Microbiology*. [e-journal] 65(7), pp. 510–521. https://doi.org/10.1139/cjm-2018-0683

Duguma, H. and Aga, M.C., 2019. Role of Nitrogen on Potato Production: A Review. *Journal of Plant Sciences*. [e-journal] 7, pp. 36–42. https://doi.org/10.11648/j.jps.20190702.11

Edgerton, M.D., 2009. Increasing crop productivity to meet global needs for Feed, food, and Fuel. *Plant Physiology*. [e-journal] 149(1), pp. 7–13. https://doi.org/10.1104/pp.108.130195

Edupalm, 2023. *Palm Oil Refining Process*. [online] Available at: <http://edupalm.org.my/web/the-palm-oil-refining-process/> [Accessed 7 January 2023]

Egamberdieva, D., Wirth, S.J., Alqarawi, A.A., Abd-Allah, E.F. and Hashem, A., 2017. Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. *Frontiers in Microbiology*. [e-journal] 8. https://doi.org/10.3389/fmicb.2017.02104

Fageria, N.K. and Moreira, A., 2011. The role of mineral nutrition on root growth of Crop Plants. *Advances in Agronomy*. [e-journal] pp. 251–331. https://doi.org/10.1016/b978-0-12-385531-2.00004-9

Finch, H.J.S., Samuel, A.M. and Lane, G.P.F., 2014. Fertilisers and Manures. *Lockhart & Wiseman's Crop Husbandry Including Grassland*. [e-journal] pp. 63–91. https://doi.org/10.1533/9781782423928.1.63

Flores, F.C., Ribeiro, R.F., Ourique, A.F., Rolim, C.M.B., Silva, C.B., Pohlmann, A.R., Beck, R.C.R. and Guterres, S.S., 2011. Nanostructured systems containing an essential oil: Protection against volatilization. *Química Nova*. [e-journal] 34(6), pp. 968–972. https://doi.org/10.1590/s0100-40422011000600010

Frebort, I., Kowalska, M., Hluska, T., Frebortova, J. and Galuszka, P., 2011. Evolution of cytokinin biosynthesis and degradation. *Journal of Experimental Botany*. [e-journal] 62(8), pp. 2431–2452. https://doi.org/10.1093/jxb/err004

Fu, L. et al. (2021) Mechanism of moisture adsorption in plant fibers surface-modified with glycerol evaluated by LF-NMR relaxation technique. *Cellulose*. [e-journal] 29, pp. 2145–2158. https://doi.org/10.21203/rs.3.rs-860149/v1

Gar, 2022. *Palm oil refining process*. [online] Available at: <https://www.goldenagri.com.sg/palm-oil-refining-process/> [Accessed 7 January 2023]

Gebreslassie, H.B., 2016. Effect of Potasium Fertilizer on Crop Production. *Journal of Natural Sciences Research*. [e-journal] 6. https://core.ac.uk/download/pdf/234656325.pdf

Glittenberg, D., 2012. Starch-based biopolymers in paper, corrugating, and other industrial applications. *Polymer Science: A Comprehensive Reference*. [e-journal] pp. 165–193. https://doi.org/10.1016/b978-0-444-53349-4.00258-2

Gupta, A., Eral, H.B, Hatton, T.A. and Doyle, P.S., 2016. Nanoemulsions: Formation, properties and applications. *Soft Matter*. [e-journal] 12(11), pp. 2826–2841. https://doi.org/10.1039/c5sm02958a

Hazen, J.L., 2000. Adjuvants—terminology, classification, and chemistry. *Weed Technology*. [e-journal] 14(4), pp. 773–784. https://doi.org/10.1614/0890-037x(2000)014[0773:atcac]2.0.co;2

Hock, W., 2022. *Spray adjuvants*. [online] Available at: <https://extension.psu.edu/spray-adjuvants> [Accessed 17 April 2023]

Jaiswal, M., Dudhe, R. and Sharma, P., 2014. Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech*. [e-journal] 5(2), pp.123-127. https://doi.org/10.1007/s13205-014-0214-0

Janzen, H.H., 1987. Effect of fertilizer on soil productivity in long-term spring wheat rotations. *Canadian Journal of Soil Science*. [e-journal] 67(1), pp. 165–174. https://doi.org/10.4141/cjss87-014

Kahsay, W.S., 2019. Effects of nitrogen and phosphorus on potatoes production in Ethiopia: A Review. *Cogent Food & Agriculture*. [e-journal] 5(1), p. 1572985. https://doi.org/10.1080/23311932.2019.1572985

Karjiban, R.A., Basri, M., Rahman, M.B.A. and Salleh, A.B., 2012. Structural properties of nonionic Tween80 Micelle in water elucidated by molecular dynamics simulation. *APCBEE Procedia*. [e-journal] 3, pp. 287–297. https://doi.org/10.1016/j.apcbee.2012.06.084

Kooij, v.d.H., 2020. *What is the difference between organic and mineral fertilizers?*. [online] Available at: <https://royalbrinkman.com/knowledge-center/crop-care/difference-organic-mineral-fertilizers> [Accessed 21 April 2023]

Li, C. et al., 2018. Absorption of foliar-applied Zn in sunflower (helianthus annuus): Importance of the cuticle, stomata and trichomes. *Annals of Botany*. [e-journal] 123(1), pp. 57–68. https://doi.org/10.1093/aob/mcy135

Lim C.J., Basri, M., Omar, D., Abdul Rahman, M., Salleh, A., Raja Abdul Rahman, R. and Selamat, A., 2012. Green nano-emulsion intervention for water-soluble glyphosate isopropylamine (IPA) formulations in controlling Eleusine indica (E. indica). *Pesticide Biochemistry and Physiology*, [e-journal] 102(1), pp.19-29. https://www.sciencedirect.com/science/article/abs/pii/S004835751100160X?via%3Dihub

Liu, Y., Zhang, M., Meng, Z., Wang, B. and Chen, M., 2020. Research progress on the roles of cytokinin in plant response to stress. *International Journal of Molecular Sciences*. [e-journal] 21(18), p. 6574. https://doi.org/10.3390/ijms21186574

Llanes, A., Iparraguirre, J., Masciarelli, O. and Maria, N., 2018. Foliar application of phytohormones enhances growth of maize and soybean seedlings. *RIA: Revista Investigaciones Agropecuarias*. [e-journal] 45, pp. 61-66. https://www.redalyc.org/journal/864/86458941005/html/

Ma, Q., Wang, X., Yuan, W., Tang, H. and Luan, M., 2021. The optimal concentration of KH2PO4 enhances nutrient uptake and flower production in rose plants via enhanced root growth. *Agriculture*. [e-journal] 11(12), p. 1210. http://doi.org/10.3390/agriculture11121210

Malaysian Palm Oil Council, 2023. *The Oil Palm Tree*. [online] Available at: <https://mpoc.org.my/the-oil-palm-tree/> [Accessed 7 January 2023]

Metzger, J.O. and Bornscheuer, U., 2006. Lipids as renewable resources: Current State of Chemical and biotechnological conversion and diversification. *Applied Microbiology and Biotechnology*. [e-journal] 71(1), pp. 13–22. https://doi.org/10.1007/s00253-006-0335-4

Mukherjee, A., Gaurav, A.K., Singh, S., Yadav, S., Bhowmick, S., Abeysinghe, S. and Verma, J.P., 2022. The bioactive potential of phytohormones: A Review. *Biotechnology Reports*. [e-journal] 35. https://doi.org/10.1016/j.btre.2022.e00748

Mustafa, I.F. and Hussein, M.Z., 2020. Synthesis and technology of nanoemulsion-based pesticide formulation. *Nanomaterials*. [e-journal] 10(8), p. 1608. https://doi.org/10.3390/nano10081608

Nakama, Y., 2017. Surfactants. *Cosmetic Science and Technology*. [e-journal] pp. 231–244. https://doi.org/10.1016/b978-0-12-802005-0.00015-x

Nielsen, C.K., Kjems, J., Mygind, T., Snabe, T. and Meyer, R.L., 2016. Effects of tween 80 on growth and biofilm formation in laboratory media. *Frontiers in Microbiology*. [e-journal] 7. https://doi.org/10.3389/fmicb.2016.01878

OECD, 2020. *Food supply chains and covid-19: Impacts and policy lessons*. [online] Available at: <https://www.oecd.org/coronavirus/policy-responses/food-supply-chains-and-covid-19-impacts-and-policy-lessons-71b57aea/> [Accessed 21 April 2023]

Olutoye, M.A., Lee, S.C. and Hameed, B.H., 2011. Synthesis of fatty acid methyl ester from palm oil (elaeis guineensis) with ky(mgca)2xo3 as heterogeneous catalyst. *Bioresource Technology*. [e-journal] 102(23), pp. 10777–10783. https://doi.org/10.1016/j.biortech.2011.09.033

Pavoni, L., Perinelli, D.R., Bonacucina, G., Cespi, M. and Palmieri, G.F., 2020. An overview of micro- and Nanoemulsions as vehicles for essential oils: Formulation, preparation and stability. *Nanomaterials.* [e-journal] 10(1), p. 135. https://doi.org/10.3390/nano10010135

Perinelli, D.R., Cepsia, M., Lorussoa, N., Palmieria, G.F., Bonacucinaa, G. and Blasi, P., 2020. Surfactant self-assembling and critical micelle concentration: One approach fits all?. *Langmuir*. [e-journal] 36(21), pp. 5745–5753. https://doi.org/10.1021/acs.langmuir.0c00420.s001

Population Matters, 2023. *Population: The numbers*. [online] Available at: <https://populationmatters.org/the-facts-numbers/?gclid=CjwKCAjw6IiiBhAOEiwALNqncdSKmRsCx9fgYQAC56eapetqNOfci7THN6-kaQVcfpFgk-hSM2wt6BoCwqcQAvD_BwE> [Accessed 21 April 21 2023]

Pub Chem, 2023. *6-benzylaminopurine*. [online] Available at: <https://pubchem.ncbi.nlm.nih.gov/compound/6-Benzylaminopurine> [Accessed 21 April 2023]

Ramsey, R.J.L., Stephenson, G.R. and Hall, J.C., 2005. A review of the effects of humidity, humectants, and surfactant composition on the absorption and efficacy of highly water-soluble herbicides. *Pesticide Biochemistry and Physiology*. [e-journal] 82(2), pp. 162–175. https://doi.org/10.1016/j.pestbp.2005.02.005

Ruiter, H.d., Kempenaar, C., Vlieger, J.J.d., Zande, J.v.d., Holterman, H.J., Mol, H.G.J., 2003. Influence of adjuvants and formulations on the emission of pesticides to the atmosphere. *International Journal of Plant Research 59*. [e-journal]. https://www.researchgate.net/publication/237660864_Influence_of_adjuvants_and_formulations_on_the_emission_of_pesticides_to_the_atmosphere

Safaya, M. and Rotliwala, Y.C., 2020. Nanoemulsions: A review on low energy formulation methods, characterization, applications and optimization technique. *Materials Today: Proceedings*. [e-journal] 27, pp. 454–459. https://doi.org/10.1016/j.matpr.2019.11.267

Saltzman, S. and Brates, N., 1990. Stability of emulsifiable concentrate pesticide formulations in liquid fertilizers. *Pesticide Science*. [e-journal] 28(2), pp. 181–195. https://doi.org/10.1002/ps.2780280206

Scherer, H.W., 2005. Fertilizers and fertilization. *Encyclopedia of Soils in the Environment*. [e-journal] pp. 20–26. https://doi.org/10.1016/b0-12-348530-4/00229-0

Shah, P., Bhalodia, D. and Shelat, P., 2010. Nanoemulsion: A pharmaceutical review. *Systematic Reviews in Pharmacy*. [e-journal] 1(1), p. 24. https://doi.org/10.4103/0975-8453.59509

Statista Research Department, 2023. *Global Fertilizer Market Size 2020-2030*. [online] Available at: <https://www.statista.com/statistics/1266004/global-fertilizer-market-size/> [Accessed 21 April 2023]

Su, R. et al., 2017. Size-dependent penetration of nanoemulsions into epidermis and hair follicles: Implications for transdermal delivery and immunization. *Oncotarget*. [e-journal] 8(24), pp. 38214–38226. https://doi.org/10.18632/oncotarget.17130

Sułek, M.W., Ogorzalek, M., Wasilewski, T., Klimaszewska, E., 2013. Alkyl polyglucosides as components of water based lubricants. *Journal of Surfactants and Detergents*. [e-journal] 16(3), pp. 369–375. https://doi.org/10.1007/s11743-012-1428-y

Szymczyk, K., Zdziennicka, A. and Janczuk, B., 2018. Adsorption and Aggregation Properties of Some Polysorbates at Different Temperatures. *Journal of Solution Chemistry*. [e-journal] 2018(47). pp. 1824-1840. https://doi.org/10.1007/s10953-018-0823-z

Tim, W.Y. and Arditti, J., 2017. Formulary. *Micropropagation of Orchids*. [e-journal] 1. https://doi.org/10.1002/9781119187080

van Dijk, M., Morley, T., Rau, M.L. and Saghai, Y., 2021. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nature Food*. [e-journal] 2(7), pp. 494–501. https://doi.org/10.1038/s43016-021-00322-9

van Ginkel, C.G., 2007. Ultimate biodegradation of ingredients used in cleaning agents. *Handbook for Cleaning/Decontamination of Surfaces*. [e-journal] pp. 655–694. https://doi.org/10.1016/b978-044451664-0/50020-6

Walker, R.B., Everette, J.D., Bryant, Q.M., Green, A.M., Abbey, Y.A. and Wangila. G.W., 2010. Reactivity of various compound classes towards the Folin-CIOCALTEU reagent. *AIP Conference Proceedings*. [e-journal] https://doi.org/10.1063/1.3419685

Wang, X. and Gao, Y., 2017. Effects of length and unsaturation of the alkyl chain on the hydrophobic binding of curcumin with Tween micelles. *Food Chem*. [e-journal] 2018(246). pp. 242 – 248. https://doi.org/10.1016/j.foodchem.2017.11

Wei, J., Tang, Y., Wang, M., Hua, G., Zhang, Y. and Peng, R., 2018. Wettability on plant leaf surfaces and its effect on pesticide efficiency. *International Journal of Precision Agricultural Aviation*. [e-journal] 1(1), pp. 30–37. https://doi.org/10.33440/j.ijpaa.20200301.62

White, P.J. and Brown, P.H., 2010. Plant Nutrition for Sustainable Development and Global Health. *Annals of Botany*. [e-journal] 105(7), pp. 1073–1080. https://doi.org/10.1093/aob/mcq085

Woittiez, L.S., Sadikin, H., Turhina, S., Dani, H., Dukan, T.P. and Smit, H., 2016. *Smallholder Oil Palm Handbook Module 1: Planting Material*. [online] Available at: <https://perennialcrops.wur.nl/smallholder-oil-palm-handbook-module-1-planting-material> [Accessed 7 January 2023]

Yamaguchi, J., 1994. Fertilizer-Nitrogen Absorption Determined by the 15N Isotopic and Difference Methods. *Japan Agricultural Research Quarterly*. [e-journal] 25(2). pp. 93-100. https://www.jircas.go.jp/sites/default/files/publication/jarq/25-2-093-100_0.pdf

Yang, H.-R., Tang, K., Liu, H.-T. and Huang, W.-D., 2011. Effect of salicylic acid on jasmonic acid-related defense response of pea seedlings to wounding. *Scientia Horticulturae*. [e-journal] 128(3), pp. 166–173. Available at: https://doi.org/10.1016/j.scienta.2011.01.015

Yara, 2022. *Five important facts about mineral fertilizer*. [online] Available at: <https://www.yara.com/knowledge-grows/five-important-facts-about-fertilizers/> [Accessed 3 May 2023]