PREPARATION, CHARACTERISATION AND EFFECTIVENESS OF NANOCOMPOSITES BASED ON GO-Fe_3O_4 AS NANOCARRIERS FOR INSECTICIDE COMPOUNDS

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

This research aimed to prepare, characterise and study the effectiveness of two magnetic graphene oxide-based nanocomposites, namely gellan gum-graphene oxide (GG-GO-Fe_3O_4) and pectin-graphene oxide (PEC-GO-Fe_3O_4) as nanocarriers for permethrin and cinnamaldehyde insecticide compounds. This research is divided into three parts, namely preparation, characterisation and effectiveness studies. Nanocomposites with amorphous structure were successfully produced based on the existence of conjugation mechanism between GG and PEC with iron oxide at wavenumber peak 650 cm^{-1}. Results from thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) haveshown that the nanocomposites loaded with insecticides are thermally stable. Performancestudy of nanocomposites loaded with insecticides towards *Aedes aegypti* larvae was analysed through Abbott's and Probit's formula via *in vitro* at different pH using ultraviolet-visible (UV-Vis) spectrometer. The encapsulation of insecticide to nanocarrierswas successfully carried out based on the change in intensity and wavenumber of absorption bands from Fourier Transform Infrared Spectrometer (FTIR) analysis and size increment of nanocarriers in the range of 18.2 to 70.1%. The formulated nanocarriers have successfully prolonged the release duration of permethrin and cinnamaldehyde by 15 and 24 hours, respectively. The release profiles of insecticide compounds were best fitted by Korsemeyer-Peppas kinetic model. Formulation of GG-GO-Fe_3O_4 nanocarrier loaded with cinnamaldehyde was found as the most effective formulation to control *A. aegypti* larvae. In conclusion, both nanocarriers studied have shown outstanding performance in loading and releasing permethrin and cinnamaldehyde in larvicide formulations. In implication, theapplication of both environmental friendly nanocarriers in larvicide formulations could reduce mosquito borne diseases and sustain the environment.

PENYEDIAAN, PENCIRIAN DAN KEBERKESANAN NANOKOMPOSIT BERASASKAN GO-Fe_3O_4 SEBAGAI NANOPEMBAWA UNTUK SEBATIAN RACUN SERANGGA

ABSTRAK

Kajian ini bertujuan untuk menyedia, menciri dan mengkaji keberkesanan dua nanokomposit berasaskan magnetik grafin oksida, iaitu gam gellan-grafin oksida (GG-GO- Fe_3O_4) dan pektin-grafin oksida (PEC-GO-Fe_3O_4) sebagai nanopembawa untuk sebatian racun serangga permethrin dan sinamaldehid. Kajian ini dibahagikan kepada tiga bahagian, iaitu penyediaan, pencirian dan mengkaji keberkesanan. Nanokomposit dengan struktur amorfus berjaya dihasilkan berdasarkan kewujudan mekanisma konjugatan antara GG dan PEC dengan ferum oksida pada puncak nombor gelombang 650 cm^{-1}. Keputusan penganalisis termogravimetri (TGA) dan kalorimeter pengimbasan pembezaan (DSC) menunjukkan bahawa nanokomposit yang dimuatkan dengan racun serangga adalah stabil secara terma. Kajian prestasi nanokomposit yang dimuatkan dengan racun serangga terhadap jentik-jentik *Aedes aegypti* dianalisis melalui Formula Abbott dan Probit melalui *in vitro* pada pH berbeza menggunakan spektrometer ultralembayung-nampak (UV-Vis). Pengkapsulan sebatian racun serangga ke dalam nanopembawa berjaya dilaksanakan berdasarkan perubahan dalam keamatan dan nombor gelombang jalur penyerapan dari analisis spectrometer penyerapan inframerah transformasi Fourier Fourier (FTIR) dan peningkatan saiz nanopembawa dalam julat 18.2 hingga 70.1%. Nanopembawa yang diformulasi berjaya memanjangkan tempoh pelepasan permethrin dan sinamaldehid masing-masing selama 15 dan 24 jam. Profil pelepasan sebatian racun serangga telah dipadankan dengan baik oleh model kinetik Korsemeyer-Peppas. Formulasi nanopembawa GG-GO-Fe_3O_4 yang dimuatkan dengan sinamaldehid didapati sebagai formulasi yang paling berkesan untuk mengawal jentik-jentik *A. aegypti*. Kesimpulannya, kedua-dua nanopembawa yang dikaji menunjukkan prestasi yang baik dalam memuatkan dan melepaskan permethrin dan sinamaldehi dalam formulasi racun jentik-jentik. Implikasinya, penggunaan kedua-dua nanopembawa mesra alam ini dalam formulasi racun jentik-jentik dapat mengurangkan penyakit bawaan nyamuk dan melestarikan alam sekitar.

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LIST OF ABBREVIATIONS

DSC	Differential Scanning Calorimetry
DVSS	Dengue Virus Surveillance System
EDX	Energy Dispersive X-Ray Spectroscopy
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
GG-GO-Fe_3O_4	Gellan Gum-Magnetic Graphene Oxide Nanocomposite
^{1}H NMR	Proton Nuclear Magnetic Resonance Spectroscopy
NDSP	National Dengue Strategic Plan
OCHA	United Nations for the Coordination of Humanitarian Affairs
PEC-GO-Fe_3O_4	Pectin-Magnetic Graphene Oxide Nanocomposite
STEM	Scanning Transmission Electron Microscope
TGA	Thermogravimetic Analysis
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
WHO	World Health Organization
XRD	X-ray Diffraction Spectroscopy

CHAPTER 1

INTRODUCTION

1.1 Nanocarriers

Nano comes from the Greek word *nanos* which indicates dwarf or extremely small (Din et al., 2017). It is used as a prefix by having meaning of a billionth of the unit. For instance, a nanometer is a billionth of a meter which is 1.0×10^{-9} m.

The concept of nanocarrier comes from the emergence of nanoparticles. The first nanoparticle has been recorded in the ninth century of Mesopotamia and it is used in ancient pottery (Heiligtag & Niederberger, 2013). Application of nanoparticles as carrier agents has been proposed by Richard Feynman in "There's Plenty Room at the Bottom". The theory states that a more powerful form of synthetic chemistry is possible

by direct manipulation of individual atom and this is how nanotechnology start (Foster, 2006; Hulla, Sahu, & Hayes, 2015).

Nanocarriers are drug delivery systems that having size of less than 500 nm which the active compounds are dissolved, entrapped, encapsulated, absorbed or attached on it (Din et al., 2017). The delivery of drugs can be enhanced by modification of surface characteristics of the nanoparticles without causing any change in active compound. Nanocarriers act as transportation for therapeutic agents to the target species. There are variety types of nanocarriers encapsulated active constituents for respective drug delivery.

The ideal nanocarrier size is highly dependent on application and desired drug load capacity. For instance, nanocarriers which have size less than 400 nm will not be recognised by phagocyte as invader towards body. If the size of nanocarrier is less than 200 nm, i*n vivo* distribution in liver will be affected because the nanoparticles will be removed by the sinusoidal fenestrations. Sinusoidal fenestrations are the pores in liver and they facilitate the transfer of substrates in the liver. If the drug loaded nanocarriers are removed by the liver, the liver uptake the less amount of drugs. Thus, the enhance permeability and retention effect will be better if the size is within 100-200 nm (Xia, Li & Zhao, 2017). If the size is smaller than 100 nm, rate of endocytosis and lymphatic transport increase sharply. As size decreases, the loading capacity of the nanocarrier decreases and more nanocarriers to be needed to deliver same dosage of drug. Furthermore, composition, size and surface charge are also key factors that contributing release behaviour and accumulation (Singh & Lillard Jr, 2009).

Single, fused, aggregated or agglomerated form with spherical, tubular and irregular shapes are the ways of nanoparticles show themselves under magnification. Nanoparticles are classified by Hadjipanayis and Siegel (2012) into four groups based on their ultra-fine size (< 50 nm) or by a dimensionality limited to 50 nm and the groups are zero dimensional nanoparticles (atomic clusters, filament and cluster assemblies), one dimensional nanoparticles (multilayers), two dimensional nanoparticles (ultrafine-grained over-layers or buried layers) and three dimensional nanoparticles (nano-phase materials consisting of equiaxed nanometer sized elements). Table 1.1 shows type of nanocarriers, their properties and examples according to classification of nanoparticle.

Nanocarriers have advantages in drug delivery due to their physical properties. They are small in size, large surface area to volume ratio and easier surface manipulation using intended functional moiety for high selectivity (Mukherjee, Satapathy, Bhattacharya, Chakraborty, & Mishra, 2017). The explanation for every advantage has shown in Table 1.2. Other than that, pharmacokinetics, biodistribution, solubility and stability of nanocarriers increases. Decline of toxicity and well controlled site-specific are also features of nanocarriers. Dissolvation, absorption, entrapment, encapsulation or attachment either on or inside nanocarriers are ways of drug delivery of nanocarriers.

Table 1.1

Type of Nanocarriers, Properties and Examples

Classification of nanoparticle	Type of nanocarrier	Properties	Examples
Zero dimensional	Nanoparticles	Diameters ranged 0-50 nm	Quantum dots
One dimensional		Diameter size are between 1 and 100 nm	Carbon nanotubes (CNT) Nanowires
Two dimensional	Carbon based	Composed mostly of carbon	Fullerenes, graphene thin film
Three dimensional	Organic based dendrimer nanoparticles	Nanosized polymers built from branched unit	Poly (amido amine)
	Polymer	Polymer	Polyacrylamide, chitosan, gelatin
	Polymer based micelles	Amphiphilic polymer	Polyethylene glycol (PEG)
	Hydrogels	Do not dissolve but can swell in water	Sodium alginate
	Inorganic based nanoparticles	Displays localised surface resonance	Gold nanoparticles
	Magnetic nanoparticles	Show magnetic properties	Nanogold, metal oxide, metal alloys
	Semiconductor nanoparticles	Exhibit optical and electronic properties	Quantum dots
	Ceramics	Have high resistance and chemical inertness	Iron oxide nanoparticles

Note. Adapted from Foster (2006) and Malhotra and Ali (2018)

Table 1.2

Advantages of Nanocarrier and Its Explanation

Advantage	Explanation	Reference
Large surface area to volume ratio	Area for drug to react increases, hence less dosage will be needed and lower the toxicity.	Din et al. (2017)
Tuneable surface	Extends the duration of drug release sustained, therefore the bioavailability of drug on target species increases. Besides that, the drug is shield from undue degradation by efficient navigation *in vivo* environment.	Ding and Li (2017)
Flexible in drug formulation and administration routes	Direct the drug to the target species but also to parts where enhanced intracellular trafficking being carried out.	Mukherjee et al. (2017)

1.2 Gellan gum

Fermentation process through *Sphingomonas elodea* (previously known as *Pseudomonas elodea*) produces gellan. The process is fulfilled under sterile condition with controlled temperature, air flow, pH and agitation. After pasteurisation, the recovery of gellan gum will be done either by alcohol precipitation or with alkali before alcohol precipitation to obtain high acyl (HA) or low acyl (LA) gellan gum (Nussinovitch, 2012; Cho & Dreher, 2001). In this study, HA gellan gum is used as a material of nanocarrier.

The molecular structure of HA gellan gum is shown in Figure 1.1. It has a linear structure of heteropolysaccharide which consists of two glucose, one glucaronic acid

and one rhamnose. It is anionic. The repeating unit is (1,3)β-D-Glcp-(1,4)-β-D-GlucpA-(1,4)-β-D-Glc-(1,4)-α-L-Rha-(1→) (Sworn & Stouby, 2009).

Deacrylated gellan is a commonly used thickening agent in food industry. Variety of texture of jellies produced by combining HA and LA gellan gum. In addition, gellan gum is used as encapsulation and *in vitro* cell cultures in tissue engineering due to its high resistance of heat (Sworn & Stouby, 2009).

In pharmaceutical field, gellan gum is used to deliver drug. It is capable for drug immobilisation and withhold in a target organ by closer connection over an extended period of time (Bhatia, 2016).

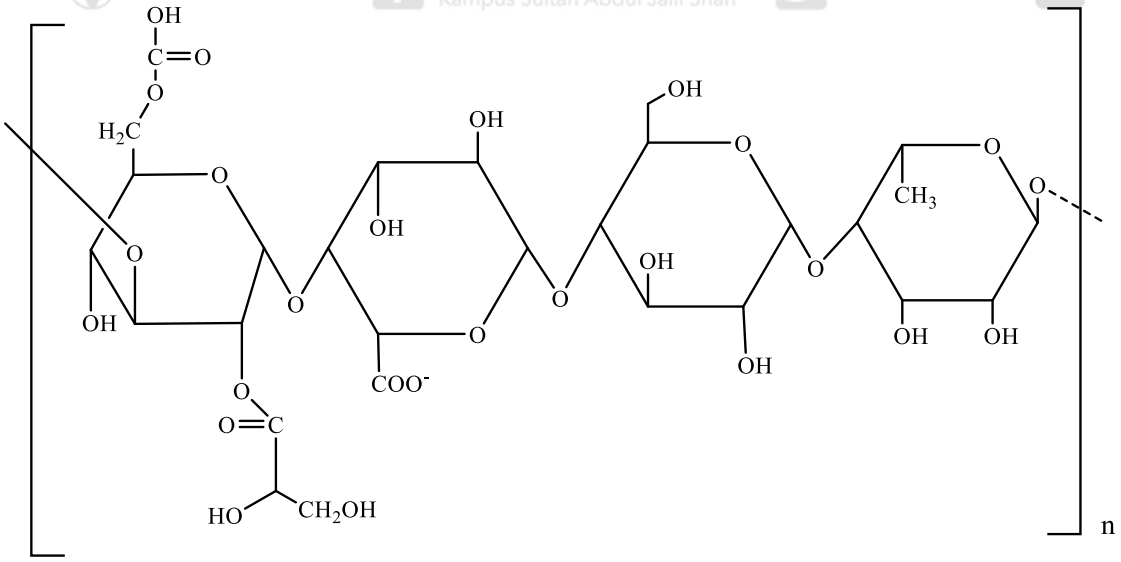

Figure 1.1. Chemical Structure of High Acyl (HA) Gellan Gum

1.3 Pectin

Greek word *pektos* which means firm and hard and it is the origin of word for "pectin". This indicates the capability of pectin to form gels. Pectin is obtained from plants in acidic condition. Pectin acts as cement of plant cell wall and hydrating agent in the form of protopectic (Nussinovitch, 2012; Edwards, 2019).

The chemical structure of pectin is shown in Figure 1.2. Monomer of pectin is D-galacturonic acid and the monomers are bonded via α-1,4-glycosidic acid (Sundar Raj, Tripathy, Patil & Majeed, 2012). Pectin can be categorised into two types: high methoxyl (HM) and low methoxyl (LM) according to their degree of methyl esterification (DE) (Burapapadh et al., 2016). HM pectin has good emulsion properties as it has high content of hydrophobic molecules and surface-active molecules also contribute to emulsion properties (Burapapadh et al., 2016). Those hydrophobic molecules absorb strongly to the interface of oil while the hydrophilic sites could be extended in aqueous sites (Ngoúemazong, Christiaens, Shpigelman, Van Loey & Hendrickx, 2015).

Pectin is soluble in water but not soluble in organic solvent. Temperature, pH and ionic strength of solution contribute to the rate of dissolution of pectin. Pectin has the properties of high heat resistance when sugar is added into the solvent. However, pectin depolymerises at pH 3.5 when heat applied (Burapapadh et al., 2016; BeMiller, 1986).

Pectin is a common gelling agent, thickener and stabiliser in food industry. The use of pectin is an emerging issue in pharmaceutical field as well. Other than these, pectin is also used as emulsifier in cosmetics product to prevent separating of oil and liquid components (Cho & Dreher, 2001).

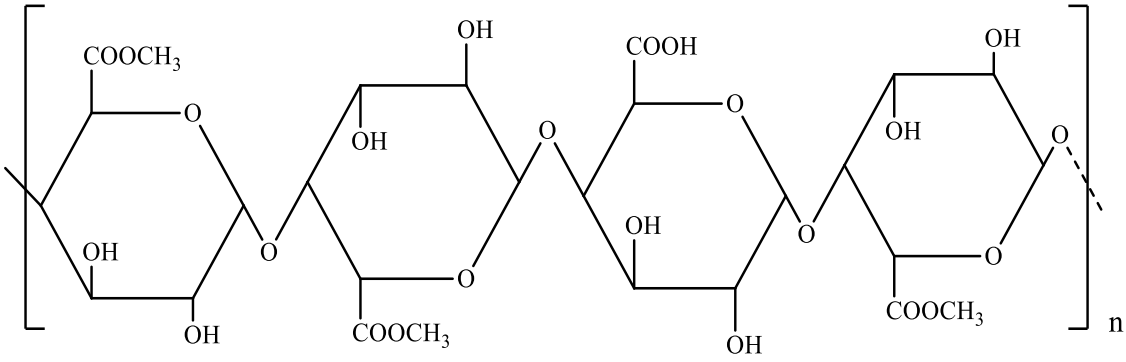

Figure 1.2. Chemical Structure of Pectin

1.4 Graphene Oxide

Graphene oxide (GO) consists of monomolecular sheets that produced by a bulk of materials that disperses spontaneously in basic solutions or disperses by sonication in polar solvents. GO is chemically modified from graphite oxide. The major difference between graphite oxide and GO is the number of layers. In GO dispersion, there are monolayer and few layered flakes (Dreyer, Park, Bielawski, & Ruoff, 2010; Marcano et al., 2010; Zhu et al., 2010).

Oxygenated functionalities are introduced into the structure of the graphite when strong oxidising agents are used to oxidise graphite. Hence, the compound will be hydrophilic and the layer separation will be expanded. This make graphite oxide to

be exfoliated in water by sonication and GO which is single layered or few layered oxygen functionalised graphene produced (Loh, Bao, Ang, & Yang, 2010; Zhu et al., 2010). Chemically modified graphene (CMG) is well known for its excellent electrical, mechanical and thermal properties, thus it is used in polymer composites, sensors and biomedical applications (Xu & Shi, 2011).

GO has a range of reactive oxygen functional groups and these make GO can be dispersed easily in organic solvents, water and different matrices. The chemical structure of GO is shown in Figure 1.3. The mechanical and electrical properties will be enhanced if the graphene oxide combines with ceramic or polymer matrices (Dreyer et al., 2010). There is a method suggested by Xu and Shi (2011) to make sure CMG disperses easily in organic solvents that use amines through organic covalent functionalisation. This brings graphene oxide more suitable to be used in biodevices, optoelectronics and drug delivery.

The distorted network of sp^2 bonding provides an electrically insulated property to GO. However, the electricity can be conducted once the π-network in honeycomb hexagonal lattice is resumed.

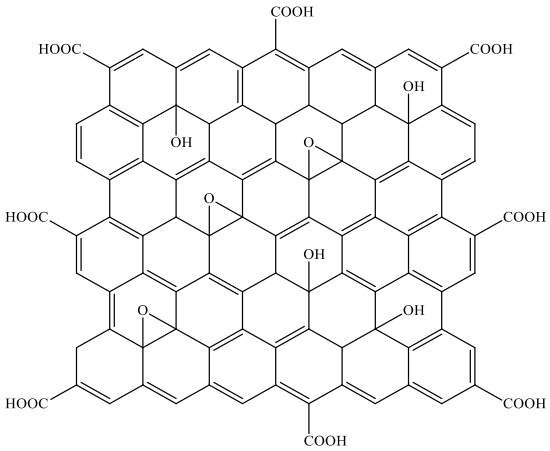

Figure 1.3. Chemical Structure of Graphene Oxide

1.5 Research Background

1.5.1 Overview of Mosquito-Borne Diseases

Mosquito-borne diseases (MBD) are defined as the diseases that spread by the bite of an infected mosquito. Examples of MBD virus are dengue, malaria, Zika, Chikungunya, and West Nile (Figure 1.4). Mosquito is a well-known vector carrying diseases that might bring fatality to human being. Moreover, the potential of the diseases spread increases when it comes to global travel, urbanisation and rapid population growth. This rises alarm of worldwide when the number of mortality cases increases sharply in short while.

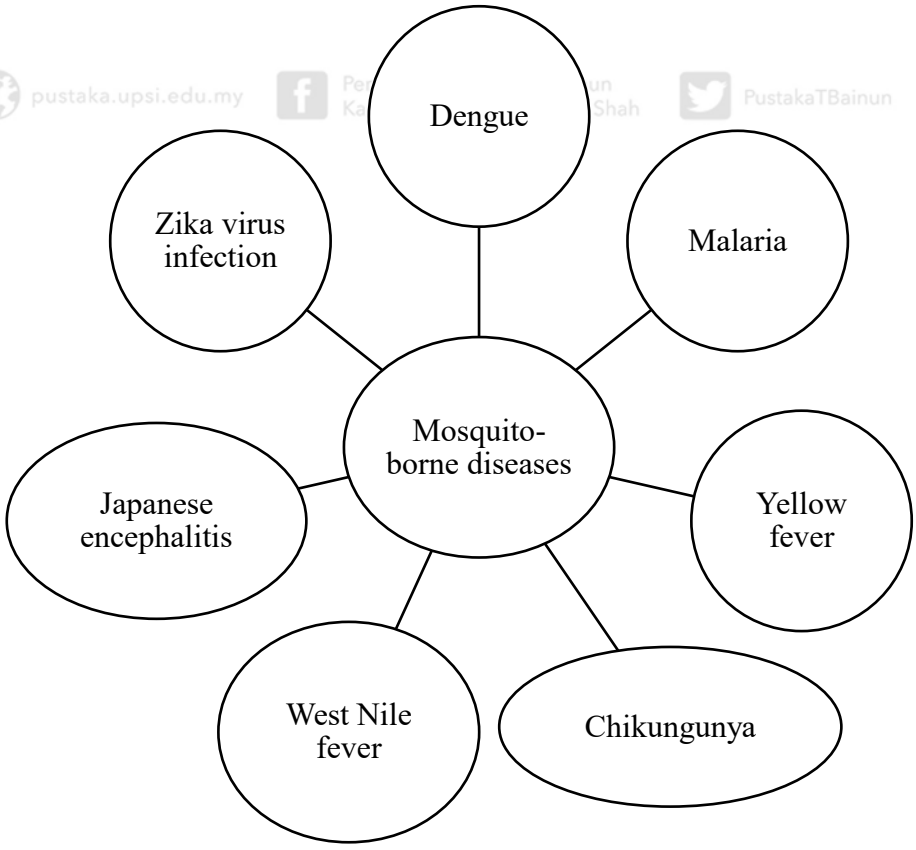

Figure 1.4. Examples of Mosquito-Borne Diseases (MBD)

1.5.2 Mosquito-Borne Diseases in Worldwide

According to a report by United Nations for the Coordination of Humanitarian Affairs (OCHA) in 2019, 40% of world population is at risk of exposed to malaria. The highest number of cases happened in Africa. As a matter of fact, there are 500 million cases occur and there are 2.7 million deaths every year. At the same time, 2500 million people are at infection risk and 20 million cases are reported for dengue. In Latin America and Caribbean, the most severe dengue infection case was recorded in 1995 with more than 200,000 cases of dengue fever and nearly 6000 cases of dengue haemorrhagic fever. This struck more than 14 countries for fifteen years [World Health Organization (WHO), 2011].

Other than that, citizens in cities especially Americans are highly exposed to yellow fever. A minimum of 120 million people and 73 countries in Asia, Africa, the Western Pacific and some areas in America have been infected by elephantiasis (Fox & King, 2013).

In year 2005, WHO introduced vaccines for dengue. However, this introduction was pulled off because the concern regarding the threat of new type of dengue. However, this was resumed in 2019 by U.S. Food and Drug Administration (FDA) [Centre for Diseases Control and Prevention (CDC), 2021; Ong, 2016].

1.5.3 Mosquito-Borne Diseases in Malaysia

Malaysia stays hot and humid all year round. According to Malaysian Meteorological Department (2019), the average amount of annual precipitation in Kuala Lumpur is 2630.0 millimetres. Possibility of diseases transmission increases due to the increase in relative humidity, temperature and precipitation (Mudin, 2015). If compared to 50 years ago, the number of cases rises approximately by 30 percent (Ong, 2016). The lag period between heavy rainfall and dengue cases increment is two to three months (Li, Lim, Han, & Fang, 1985). However, Cheong, Burkart, Leitao, and Lakes (2013) found that this is shorten to three weeks after precipitation. Bigger human population and more breeding sites after rainfall are the factors of the shorten lag period (Ong, 2016).

Mudin (2015) reported that first dengue outbreak in Malaysia was discovered in Penang with 41 cases and 5 deaths in 1962. Dengue fever and dengue haemorrhagic fever are hot issues among Malaysia citizens. There are four types of dengue virus serotypes, which are DENV-1, DENV-2. DENV-3 and DENV-4. All four dengue virus serotypes found in Malaysia and the predominant serotypes changes from year to year. The serotype shift leads to increment of cases and death because immunisation of the community towards new virus declines (Mudin, 2015; Tee et al., 2009).

The Malaysian government is committed to control the mosquito breeding at larva and adult stage of mosquitoes. At adult stage, chemical approach such as thermal or ultra-low volume (ULV) fogging is the most common way to control the mosquitoes from breeding. For larval stage control, temephos, an active ingredient of Abate is commonly used.

Other than that, Malaysian government carried out Dengue Virus Surveillance System (DVSS) in 13 states and 3 federal territories for weekly basis since 2009. Hence, Destruction of Disease-Bearing Insect Act 1975 is enforced in order to conduct this program more successfully by giving the inspectors of the program to enter household for carrying out investigation (Mohd-Zaki, Brett, Ismail, & L'Azou, 2014).

Based on statistics from the Department of Statistics Malaysia, DOSM (2021), the estimated population of Malaysia in 2020 is 32.7 million and its population density is 99 per kilometre square. Department of Statistics Malaysia has released a report of demographic in every state in Malaysia for second quarter of 2021 (April to June) and the data is presented in Table 1.3. In Table 1.3, the densest population is in Selangor which has 6,555.4 thousands citizens. There are 3,833.0 thousands citizens in Sabah and this is the second densest state in Malaysia. Among states in Malaysia, Perlis has the smallest population which is only 255.4 thousands people. Labuan has the smallest population among three federal territories of Malaysia, which is 100.1 thousands only.

Table 1.3

Demographic Statistics in Malaysia from April to June of 2021

State/ Federal territory	Population (thousands)
Selangor	6,555.4
Sabah	3,833.0
Johor	3,794.0
Sarawak	2,822.2
Perak	2,508.9
Kedah	2,194.1
Kelantan	1,928.8
Penang	1,774.4
Kuala Lumpur	1,746.6
Pahang	1,684.6
Terengganu	1,275.1
Negeri Sembilan	1,129.1
Malacca	937.5
Perlis	255.4
Putrajaya	116.1
Labuan	100.1

Note: Adapted from Department of Statistics Malaysia (2021)

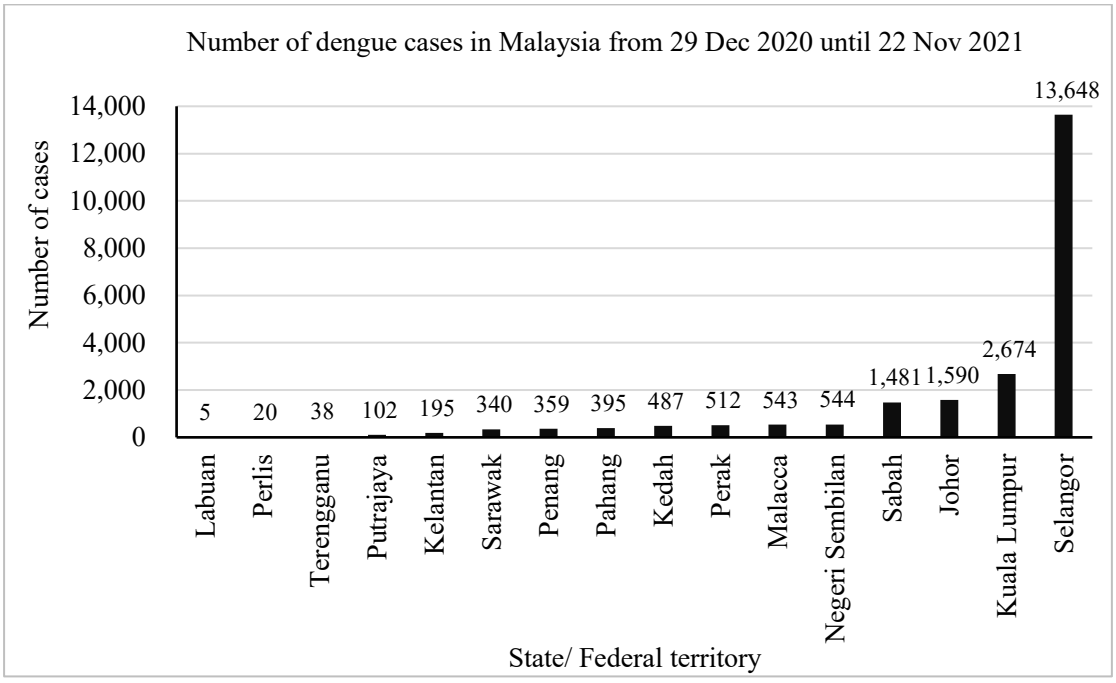

Figure 1.5. Number of Dengue Cases in Malaysia from 29 December 2020 to 22 November 2021. Adapted from Ministry of Health Malaysia (2021)

There are 22,933 people infected with dengue from 29 December 2020 to 22 November 2021. From Figure 1.5, 13,648 people in Selangor have been infected dengue since 29 December 2020 and it is the highest number among the states. This is probably caused by rapid urbanisation of the state and dense population in the state. Besides that, there are huge number of housing areas in Selangor. Henceforth, sites for rubbish dumping will be more and the area for mosquito breeding will be more as well. This is because there are sites for stagnant water in rubbish dumping sites and these spots become potential breeding sites of mosquitoes.

When dengue virus is transmitted in an area more than 30 days, that area will be enlisted as a dengue hotspot. Hulu Langat is the area that be enlisted the most in Selangor. There are much dengue cases in different zones in Hulu Langat and the longest outbreak duration is 79 days since 3 September 2021 (MOH, 2021). It is probably because Hulu Langat has dense population density (~1,141,880 citizens in 2010) and it is urbanised rapidly (DOSM, 2017). Petaling Jaya is the second area that enlisted as dengue hotspot in Selangor. Petaling Jaya has high residential capacity in Selangor, which is around 619,925 people in the district (Petaling Jaya City Council, 2021).

Next, Johor has the second highest number of people infected dengue. Vast number of residential areas causes the mosquito breeding sites increase. Other than that, Perlis has the least number of dengue cases occur because it is not urbanised as Selangor and Johor. Perlis is also low population density as compared to other states.

1.6 Active Ingredients Used in Larvicide Formulation

1.6.1 Permethrin

Permethrin is classified to an insecticide family named pyrethroid by National Pesticide Information Centre. It can be appeared as colourless crystal or yellow or brown viscous liquid. It is hydrophobic which indicates it cannot dissolve in water. The chemical structure of permethrin has been shown in Figure 1.6 and existence of cyclopropane makes permethrin has low water solubility (5.5×10^{-3} ppm at 20 ºC). United States Environmental Protection Agency (USEPA) classified permethrin to "likely to be carcinogenic to humans" by oral consumption. Permissible limit of permethrin in drinking water is 0.3 mg/L (WHO, 2004). Permethrin can be excreted from body through urine.

There was a study conducted by WHO for investigating human exposure towards permethrin in 1990. There were four men and six women were treated with 15-40 mL of 1% v/v permethrin which is same content in head louse solution. Hair was allowed to dry naturally and washed with baby shampoo. Urine samples were collected every 24 hours for 360 hours for dermal absorption analysis. The results turned out that permethrin excretion was 1% v/v of applied dose in the first 24 hours. Besides that, there were three volunteers had symptoms of mild and patchy erythema. These symptoms took four to seven days to be faded. The cumulative maximum permethrin excretion in 14 days was 5.5 mg only (WHO, 1990).

When permethrin gets onto surface water, it will bind strongly with sediment of lakes or river. Degradation of permethrin is done by microorganisms or by photolysis. Average half-life of permethrin in aerobic soil is 39.5 days with a range of 11.6 to 113 days whereas half-life of permethrin in water is about 19-27 hours (WHO, 2004). However, it does not cause water pollution although permethrin absorbed in water sediments more than one year. Despite its hydrophobicity, it cannot evaporate easily and adsorbed strongly by soil when it is applied. It is used to kills mosquito larvae who live underneath water. Thus, it brings harm to those non-targeted aquatic lives especially invertebrates because the nervous system is affected then paralysis and death happen if the organism consume permethrin. Plus, the growth of invertebrates who live in sediments is affected seriously (WHO, 1990).

In public health mosquito abatement programmes, feed stock or feed crops, permethrin can be applied. Permethrin is also used in residential areas. In commercial, there are several products contain permethrin in mosquito control programme including repellent. There is permethrin in formulation of head lice and scabies treatment and this is regulated by United States Food and Drug Administration (USFDA).

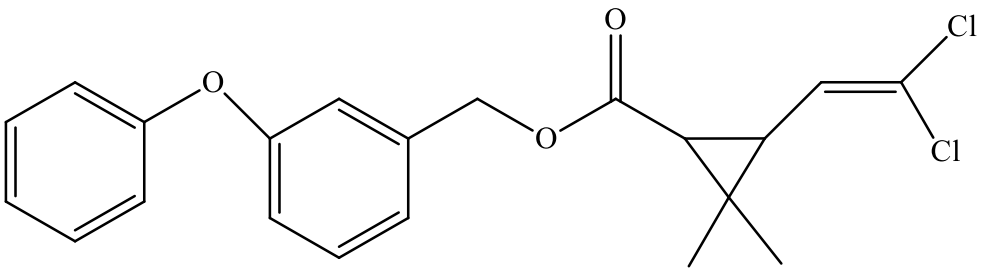

Figure 1.6. Chemical Structure of Permethrin

1.6.2 Cinnamaldehyde

Cinnamon can be seen as dried bark of the small evergreen tree *Cinnamomum zeylanicum* (genus *Lauraceae*) and originated from Sri Lanka and Southern India (Kumar & Kumari, 2019). The leaves of cinnamon tree are long and gives shinning green on surface when they are mature. There are nearly 82% of cinnamaldehyde in essential oil of cinnamon bark and it is the most abundant among the components (Li, Kong & Wu, 2013). It can be obtained in the bark of cinnamon trees or genus *Cinnamomum* such as camphor and cassia. Cinnamaldehyde is the compound that gives flavour and odour of cinnamon (Ranasinghe, 2013).

The International Union of Pure and Applied Chemistry (IUPAC) name of cinnamaldehyde is 3-phenyl-prop-2-enal. Cinnamaldehyde is also known as *trans*-cinnamaldehyde or cinnamic aldehyde. From Figure 1.7, it has the planar structure and several π bonds. However results from Friedman, Kozukue and Harden (2000) saying that there are differences on purity between *trans*-cinnamaldehyde and cinmmaldehyde. *Trans*-cinnamaldehyde has higher purity percentage which is approximately 99% than cinnamaldehyde only consists of 98%. In addition, β-phenylacrolein is another name of cinnamaldehyde because it is a derivative of acrolein (Ashakirin, Tripathy, Patil & Majeed, 2017).

Cinnamaldehyde is an oily yellow liquid at room temperature. The colour is due to transition with acrolein. Cinnamaldehyde has boiling point of 246 ºC, therefore it can be synthesised from distillation process of cinnamon bark by synthesising cinnamyl alcohol. There are findings from Cheng et al. (2009) that four compounds from

cinnamon tree which are cinnamaldehyde, cimmanyl acetate, eugenol and anethole proven their abilities to kill mosquito larvae in 24 hours. When 29 ppm of cinnamaldehyde is applied, it is able to kill larvae *Aedes aegypti* efficiently in 24 hours. But cinnamaldehyde is slightly soluble in water (1.42×10^{-3} mg/mL at 25 ºC), thus emulsification has to be done to achieve stabilisation before application in water to kill mosquito larvae.

In daily life, cinnamaldehyde is used as an antifungal agent (Wang, Chen, & Chang, 2005), a flavouring agent (Simić et al., 2004), a plant metabolite and a sensitiser (can cause allergic reaction after exposure). As an antifungal agent, cinnamaldehyde is applied to plants' root and this is effective over 40 types of crops because it contains eugenol which provides antiseptic and analgesic properties. It is ideal in agriculture because it is less toxic. On the other hand, its fragrance repels animals like cats and dogs. The cinnamon essential oil also used as scent of candles. Cinnamaldehyde is also used as corrosion inhibitor of steel in corrosive fluids.

Nevertheless, high concentration of cinnamaldehyde can cause skin irritation although it is not carcinogenic. Cinnamaldehyde is excreted in urine in oxidised form which is cinnamic acid.

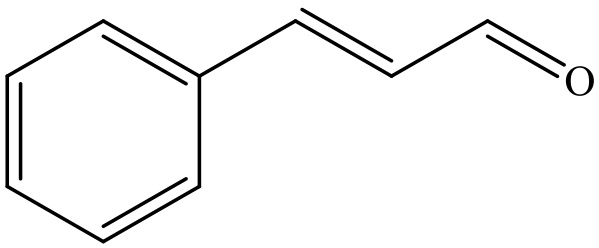

Figure 1.7. Chemical Structure of Cinnamaldehyde

1.7 Resistance Towards Insecticide: Environment Sustainability

Aedes mosquitoes is the major cause of diseases in several countries. Bad management on cleanliness in residential area leads to breeding site of mosquitoes. Accumulation of plastic bottles, tyres and rubbish contributed to higher population density of mosquitoes in the area. A mosquito does not need a large space for breed and grow. This increases the difficulty to detect the mosquito larvae. Therefore, insecticide and larvicide are applied to achieve maximum killing effects of mosquitoes in their every life stage.

Insecticide residual sprays (IRS) and space spraying usually have organophosphates, carbamates or pyrethroid to kill mosquito. Usage of IRS increases when it comes to dengue control. Temephos is a common insecticide being used in lakes, ponds and wetlands around the world. Resistance towards temephos is observed in Malaysia as well. Diagnostic dose of temephos given by WHO in 1992 is 0.012 ppm. Nonetheless, only less than 50% mortality of *Aedes* population in Kuala Lumpur and Selangor were reported in 2004 (Chen, Nazni, Lee & Sofian-Azirun, 2005). Multyano, Yamanaka, Ngadino and Konishi (2012) also reported that the larvae were not susceptible towards same dosage of temephos in Indonesia.

Dichlorodiphenyltrichloroethane (DDT) is a key element to be used to control mosquito in past several decades but it is used in controlled regulation as it could cause cancer to human and endangered life of wild animals if over usage. There are findings shows that mosquitoes are resistant to some active ingredients after several years of application. For instance, adulticide campaigns in Carribean is impacted negatively by pyrethroid resistance of mosquitoes. This resistance gives alarm to society that this

vector might be resisted to be controlled if we continue to not take this issue seriously. Instead spend more on insecticide or larvicide for increasing the dosage, environmental-friendly effective larvicide are emerging due to it degrades over time and it is less harmful to those non-target organisms.

1.8 Problem Statement

Mosquito-borne diseases remain the major source of illness and death worldwide. Various larvicides including permethrin has been used to control mosquito larvae. However, many of these larvicides have low solubility in water. Their direct applications are toxic to susceptible aquatic organisms such as fish, frogs and shrimps by bioaccumulation. There is an urgent need to overcome this issue by introducing water-solubilising carrier agents that are able to increase the solubility of larvicide in water. Therefore, this research is devised to develop an effective, innovative and eco-friendly magnetic nanocarrier for permethrin to control mosquito larvae.

1.9 Research Gaps

Based on literature review, there are studies that had successfully developed and assessed the potential of gellan gum and pectin for drug delivery, yet several significant research gaps have been found and they are relevant to be investigated.

i. Researchers did not study two active compounds at once and there is a few studies about comparison of their active compounds with commercial

larvicides. Properties of active compounds can be studied in order to make comparison with commercial larvicides. Therefore, comparison of active compounds in terms of loading capacity and entrapment efficiency can be done at the end of research.

ii. Most of the research studies did not involve loading capacity and entrapment efficiency studies. The aforementioned studies are important to investigate the relationship between amount of drug encapsulated and encapsulation efficiency. This is a stimulation about drug loading on composite with different ratio. This can save time and cost before application in real life.

iii. There are little articles on *in vitro* release studies. In this process, the experiment will be conducted in rainwater. This is to ensure drug dissolution occurs in appropriate manner in different conditions. By using certain kinetic release models, release behaviour of the drug can be predicted.

iv. Characterisation study of composite in some research studies did not sufficient to explain the chemical and physical properties of product. Several important characterisation analyses will be conducted for studying the chemical and physical properties of composite in this research.

v. There are some nanoparticles studied to control mosquito larvae. However, they are toxic to non-targeted organisms and human health as well. Environmental-friendly nanocomposite will be studied in this project.

1.10 Research Significance

This research is relevance to the National Dengue Strategic Plan (2015-2020) particularly on Integrated Vector Management. This research is also significance to the National Policy on the Environment particularly on Continuous Improvement in the Quality of the Environment. Gellan gum-graphene oxide-magnetic nanocomposite (GG-GO-Fe_3O_4) and pectin-graphene oxide-magnetic nanocomposite (PEC-GO-Fe_3O_4) nanocomposites will be synthesised and applied for the first time as magnetic nanocarriers for permethrin and cinnamaldehyde to control mosquito larvae. If drug loaded nanocomposites could be developed and successfully exhibited larvicidal activity against mosquito larvae of *Aedes aegypti*, MBD can be potentially reduced thus creating a significantly healthier environment to community. Therefore, the research findings are relevant to Integrated Vector Management, so that it will reduce the expenditure related to management and control mosquito breeding. It is hoped that this research could drive to an opportunity in producing effective, innovative and eco-friendly larvicide formulation.

1.11 Research Aim

The overall aim of this research project is to synthesise and evaluate the efficiency of the GG-GO-Fe_3O_4 and PEC-GO-Fe_3O_4 nanocomposites as nanocarriers for permethrin and cinnamaldehyde to control mosquito larvae.

1.12 Research Objectives

The specific objectives of the project are:

i. To prepare GG-GO-Fe_3O_4 and PEC-GO-Fe_3O_4 nanocomposites by thermal decomposition method.

ii. To characterise the physical and chemical properties of GG-GO-Fe_3O_4 and PEC-GO-Fe_3O_4 nanocomposites using several scientific instruments.

iii. To identify the loading and encapsulation mechanism of permethrin and cinnamaldehyde in GG-GO-Fe_3O_4 and PEC-GO-Fe_3O_4 nanocomposites.

iv. To access the *in vitro* release properties of permethrin and cinnamaldehyde from GG-GO-Fe_3O_4 and PEC-GO-Fe_3O_4 nanocomposites.

1.13 Hypothesis

GG-GO-Fe_3O_4 and PEC-GO-Fe_3O_4 possess two innovative strategies. Firstly, functionalisation of GO with magnetite (Fe_3O_4) will provide magnetic properties to GO. Secondly, conjugation of GO-Fe_3O_4 with GG and PEC will increase stability and biocompatibility of GO-Fe_3O_4. Magnetic properties could be the key feature of GG-GO-Fe_3O_4 and PEC-GO-Fe_3O_4 nanocomposites in enhancing their abilities to carry permethrin and cinnamaldehyde. Meanwhile, application of GG and PEC could prevent aggregation and this will increase the stability of the nanocomposites to load and release permethrin and cinnamaldehyde. Both strategies are important in order to increase the solubility and dispersion of permethrin in water, and therefore will reduce toxicity risk of permethrin and cinnamaldehyde to other aquatic life.

1.14 Research Scope and Limitations

This research comprised mainly four sections, namely synthesis, characterisation, application and larvicidal studies. There were two nanocomposites which are produced from different sources. Gellan gum is fermented from bacteria while pectin is extracted from apple. Graphene oxide and iron oxide are selected to enhance the abilities of GG and PEC in order to prevent aggregation.

There are a few characterisations study to be conducted on the nanocomposites in studying chemical structure, the presence of the functional groups, internal morphology, thermal properties and crystallography of the materials. There were two types of drugs, namely permethrin and cinnamaldehyde have been tested on these two nanaocomposites for their effectiveness in drug loading. The physiochemical properties of these two drugs that have been discussed in Sections 1.6.1 and 1.6.2 are summarised in Table 1.4.

The *in vitro* release of the permethrin and cinnamaldehyde from the nanocomposites was conducted in rain water to evaluate the controlled release properties. The larvicidal studies was performed to access the effectiveness of the drug loaded nanocomposites towards *Aedes aegypti* larvae.

Table 1.4

Physiochemical Properties of Permethrin and Cinnamaldehyde

Properties	Active ingredient	
Common name	Permethrin	Cinnamaldehyde
Chemical name	(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate	(E)-3-phenylprop-2-enal
Molecular formula	$C_{21}H_{20}Cl_2O_3$	C_9H_8O
Molecular weight (g/mol)	391.28	132.16
Boiling point (°C)	200	246
Melting point (°C)	34	-7.5
Solubility in water (mg/mL)	6.0×10^{-3} (20 °C)	1.42×10^{-3} (25 ºC)
Solubility in other solvent	Soluble in most organic solvents except ethylene glycol	Soluble in most organic solvents

1.15 Thesis organisation

The thesis comprises five chapters and is structured as follows:

i. Chapter one gives a brief information about nanocarrier, research background including information on mosquito-borne diseases and the active ingredients used to overcome the mosquito-borne diseases. Research gaps, the significance of the study, and the research aim and objectives are described in this chapter.

ii. Chapter two summarises the literature review on mosquitoes, larval source management and nanocarriers for drug delivery.

iii. Chapter three presents the methodology employed to achieve the objectives of the research. It gives a detailed description of materials, methods, apparatus, equipment and other related information used to conduct the experiments.

iv. Chapter four presents the outcomes of the experiments together with discussion of the research findings. The findings of the work were discussed and the results of previous studies were also discussed with a detailed explanation in this chapter.

v. Chapter five implies the conclusions of the current research and recommendation for future studies.