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DEVELOPMENT OF SAPONIN-BASED NANO EMULSION FORMULATION FROM SELECTED PLANTS TO CONTROL COTTON APHID Aphis gossypii GLOVER

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DEVELOPMENT OF SAPONIN-BASED NANO EMULSION FORMULATION FROM SELECTED PLANTS TO CONTROL COTTON APHID *Aphis gossypii* GLOVER

By

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DEVELOPMENT OF SAPONIN-BASED NANO EMULSION FORMULATION FROM SELECTED PLANTS TO CONTROL COTTON APHID, *Aphis gossypii*, Glover

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Chairperson: Prof. Rita Muhamad Awang, PhD
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This study focused on development of saponin based nano-emulsion formulation from 10 locally available medicinal plants to control *Aphis gossypii*. Saponin was extracted and quantified using HPLC. The results revealed that all plants tested in the study were rich in saponin. However, the highest saponin contents were observed in *Porterandia anisophylla* (53063 mg/L), *Antidesma cuspidatum* (42829 mg/L) and *Clidemia hirta* (42510 mg/L). Plant extracts were further tested for toxicity on *A. gossypii* through mortality and repellency tests. Based on mortality and repellency tests, *C. hirta*, *Phaleria macrocarpa*, *P. anisophylla*, and *A. cuspidatum* showed strong pesticidal effects and were selected to be formulated with non-ionic surfactants (Termul 1284, Emersense AM8025 and Tween 80) and oils (rapeseed oil and methyl oleate). The nano-emulsion formulations were successfully developed using ternary phase diagram system. Eleven formulations namely TR3PM, TR3CH, TR3AC, TR3PA, TM1PM, TM1CH, TM2CH, TM3PM, TM3CH, TM3AC and TM3PA (T= Termul 1284, R= Rapseed oil, M= Methyl oleate; PM= *P. macrocarpa*, CH= *C. hirta*, AC= *A. cuspidatum* and PA= *P. anisophylla*) passed stability tests and further proceeded for physical characterization, results showed that all formulations having particle size ranging from 52.1 to 216.4 nm and fit into nano-emulsion group. The mean zeta potential and mean surface tension of these nano-emulsion formulations ranging from -9.39 to -23 mV and 32.414 to 35.409 mN/m respectively. These 11 formulations were further proceeded to evaluate their toxicity on cotton aphid in laboratory condition and results revealed that after 72 hours the highest mortality percentage was achieved at 10000 mg/L concentration in TR3CH (100%), TR3PM (96%), TR3AC (96%), TR3PA (95%) and TM1PM (94%). The shortest LC_{50} at 72 hours and LT_{50} at 10000 mg/L concentration was observed in TR3CH (1391 mg/L; 24.32 hours), TR3PM (1516 mg/L; 27.50 hours), TR3AC (1644 mg/L; 31.30 hours), TR3PA (1766 mg/L; 30.83 hours) and TM1PM (1832 mg/L; 34.43 hours). The highest repellency percentage was also recorded for TR3CH (76%) followed by TR3PM (73%), TR3PA (69%), TR3AC (64%) and TM1PM (64%). These formulations were further evaluated for their efficacy against cotton aphid in glasshouse and results revealed

that lowest LC_{50} (1480 mg/L) after 120 hours and shortest LT_{50} (29.1 hours) at 20000 mg/L concentration was recorded for TR3CH. TR3CH (25% Termul 1284, 5% rapeseed oil with *C. hirta* as an active ingredient) nano-emulsion formulation overall performed better than all other tested nano- emulsion formulations, therefore, it could be considered as an eco-friendly alternative approach in pesticides technology.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGHASILAN FORMULASI NANO-EMULSI SAPONIN DARI TANAMAN TERPILIH UNTUK MENGAWAL KUTU KAPAS, *Aphis gossypii.* Glover

Oleh

REHMAN ABDUL

Julai 2021

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Kajian ini berfokuskan kepada penghasilan nano-emulsi saponin daripada 10 pokok herba tempatan untuk mengawal *Aphis gossypii*. Saponin diekstrak dan dikuantitikan menggunakan HPLC. Hasil keputusan mendapati kesemua tumbuhan yang diuji untuk kajian ini adalah tinggi kandungan saponin. Walau bagaimanapun, kandungan saponin tertinggi didapati daripada *Porterandia anisophylla* (53063 mg/L), *Antidesma cuspidatum* (42829 mg/L) dan *Clidemia hirta* (42510 mg/L). Ekstrak tumbuhan kemudiannya diuji dengan ujian ketoksidan terhadap *A. gossypii* melalui bioesei kematian dan penolakan (repellent). Berdasarkan ujian bioesei kematian dan penolakan, *C. hirta*, *Phaleria macrocarpa*, *P. anisophylla*, dan *A. cuspidatum* menunjukkan kesan racun perosak yang kuat dan dipilih untuk diformulasikan dengan surfaktan bukan ionik (Termul 1284, Emersense AM8025 dan Tween 80) dan minyak (minyak 'rapeseed' dan metil oleat). Formulasi nano-emulsi berjaya dikembangkan dengan menggunakan sistem rajah fasa pertigaan. Sebelas formulasi iaitu TR3PM, TR3CH, TR3AC, TR3PA, TM1PM, TM1CH, TM2CH, TM3PM, TM3CH, TM3AC dan TM3PA (T = Termul 1284, R = Minyak 'rapeseed', M = Metil oleat; PM = *P. macrocarpa*, CH = *C. hirta*, AC = *A. cuspidatum* dan PA = *P. anisophylla*) lulus ujian kestabilan dan kemudian meneruskan pencirian fizikal, hasil kajian menunjukkan bahawa semua formulasi yang mempunyai ukuran zarah antara 52.1 hingga 216.4 nm dan sesuai dengan kumpulan nano-emulsi. Min potensi zeta dan tegangan permukaan formulasi nano-emulsi ini masing-masing antara -9.39 hingga -23 mV dan 32.414 hingga 35.409 mN / m. 11 formulasi ini kemudiannya diuji untuk menilai ketoksikannya pada kutu kapas di makmal dan hasil kajian menunjukkan bahawa selepas 72 jam, kadar kematian tertinggi dicapai iaitu pada kepekatan 10000 mg/L pada TR3CH (100%), TR3PM (96%), TR3AC (96%), TR3PA (95%) dan TM1PM (94%). Keputusan kajian menunjukkan nilai LC50 terendah diperolehi pada 72 jam dan kepekatan LT_{50} pada 10000 mg/L diperhatikan dalam TR3CH (1391 mg/L; 24.32 jam), TR3PM (1516 mg/L; 27.50 jam), TR3AC (1644 mg/L; 31.30 jam), TR3PA (1766 mg/L; 30.83 jam) dan TM1PM (1832 mg/L; 34.43 jam). Kadar penolakan tertinggi juga dicatatkan bagi TR3CH (76%) diikuti oleh TR3PM (73%), TR3PA (69%), TR3AC (64%) dan TM1PM (64%). Formulasi ini selanjutnya dinilai

keberkesanannya terhadap kutu kapas di rumah kaca dan hasil kajian menunjukkan bahawa LC_{50} terendah (1480 mg/L) setelah 120 jam dan LT_{50} terpendek (29.1 jam) pada kepekatan 20000 mg/L dicatat untuk TR3CH. TR3CH (25% Termul 1284, minyak rapeseed 5% dengan *C. hirta* sebagai bahan aktif) formulasi nano-emulsi secara keseluruhannya lebih baik daripada semua formulasi nano-emulsi yang diuji, oleh itu, ia boleh dijadikan sebagai pendekatan alternatif yang mesra alam bagi teknologi racun perosak.

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Declaration by Members of the Supervisory Committee

This is to confirm that:

- the research and the writing of this thesis were done under our supervision;
- supervisory responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2015-2016) are adhered to.

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CHAPTER 1

INTRODUCTION

Aphis gossypii, also known as cotton aphid or melon aphid, is the most common and widely distributed aphid species throughout the world (Rostami *et al*., 2012; Wang *et al*., 2016). It belongs to Aphididae family of order Hemiptera. It is an important agricultural pest because of its polyphagous nature as it attacks around 900 plants species from 116 families; ranging from agronomical to horticultural crops to ornamental plants (Blackman and Eastop, 2000; Ma *et al*., 2019). *Aphis gossypii* cause either direct damage to its hosts as a phloem-feeder and causing plant leaves to curl and drop prematurely (Harris and Maramorosch, 2014; CABI, 2020), or indirectly by serving as vectors of many important plant viruses such as mosaic and crinkle viruses (Selvarajan *et al*., 2006; Ghosh *et al*., 2014a), and supporting the development of fungal sooty mold on its honeydew (Jaharlal, 2016; CABI, 2020). Accumulation of honeydew secretions of *A. gossypii* can cause significant losses to plant vigor by reducing the rate of photosynthesis by providing nutritional source for fungal growth.

The quantitative information on exact crop losses caused by *A. gossypii* is very limited. In the United States, during 2002 and 2003, the economic impact of *A. gossypii* was ranked sixth and seventh, respectively as most damaging pest of US cotton (William, 2003, 2004). If no control measures taken, *A. gossypii* can significantly reduce the crop yield up to 44% (Ramalho 1994; Furtado *et al*., 2009). The annual loss of the attainable cotton yield caused by *A. gossypii* reported 6% in the USA and 10-15% in China (Xia, 1997; CABI, 2020). The yield loss in cotton caused by *A. gossypii* along with other sucking insect pests reported 16-56% in Russia and Brazil, 20-40% in Pakistan and 20-25% in India (Sarwar, 2014; CABI, 2020).

Several management methods such as biological, cultural and chemical have been developed and applied throughout the world to control aphid populations, but none has yet to be proven adequately effective. Most preferred and widely practiced method is chemical control through synthetic insecticides because of their rapid action (Dedryver *et al*., 2010; Uzair *et al*., 2018). But, due to broad-spectrum properties of synthetic insecticides, they have huge adverse effects on non-target organisms including beneficial insects and humans along with their role in environmental pollution and ozone depletion (Kim *et al*., 2017; Hassaan and Nemr, 2020). However, plant-derived pesticides are reported to be relatively safe for the humans and their environment than synthetic pesticides because of their short residue effects and easy degradation (Isman, 2006; Uzair *et al*., 2018).

Many plants derived bio-active compounds have been tested and proved to be effective against various insect pests of agricultural crops (Adel *et al*., 2000; El-Wakeil, 2013). One of them is saponin that is widely distributed in monocotyledonous and dicotyledonous angiosperms. Saponins directly disturb the reproduction and growth of the insect pests because of their deterrent or repellent activities (Mokhtar, 2016; Singh and Kaur, 2018). Moreover, they also increase the mortality of target pests by lowering

their food intake due to less digestibility and toxicity of the food eaten (Adel *et al*., 2000; Singh and Kaur, 2018). However, plant extracts in unformulated form are less stable and difficult to handle (Mokhtar, 2016). Accordingly, the type of formulation applied is essential to enhance the delivery system of active ingredient as it can resolve issues concerning stability and handling process (Ali, 2018).

Therefore, the aim of this study was to formulate saponin based nano-emulsion formulation from locally available medicinal plants to control the aphids *A. gossypii* and the specific objectives of the study were:

I. To extract and quantify saponin bioactive compound from 10 selected medicinal plants.
II. To evaluate the toxicity and repellent/ feeding deterrent efficacy of crude extracts.
III. To prepare saponin nano-emulsion formulation from plant crude extracts.
IV. To evaluate the toxicity and repellent/ feeding deterrent efficacy of formulated emulsion against aphid *Aphis gossypii* in the laboratory and glasshouse.

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