PRODUCT DEVELOPMENT OF PLANT ESSENTIAL OIL NANOEMULSIONS FOR CONTROLLING *PHYLLOTRETA SINUATA* STEPHENS, *PLUTELLA XYLOSTELLA* LINNAEUS AND *SPODOPTERA LITURA* FABRICIUS

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หัวข้อวิทยานิพนธ์	การพัฒนาผลิตภัณฑ์น้ำมันหอมระเหยนาโนอิมัลชันจากพืช ในการควบคุมด้วงหมัดผัก, หนอนใยผักและหนอนกระทู้ผัก
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บทคัดย่อ

พืชตระกูลกะหล่ำ (Cruciferous vegetables) ถือเป็นกลุ่มพืชที่ได้รับการขนานนามว่าเป็นราชาแห่งพืชผัก ซึ่งได้รับการยอมรับว่าเป็นกลุ่มพืชที่มีคุณค่าทางโภชนาการสูง ซึ่งปัญหาหลักของความเสียหายของผลผลิตทางการเกษตรคือการเข้าทำลายของแมลงศัตรูพืช ซึ่งส่งผลต่อปริมาณและคุณภาพของผลผลิต โดยเฉพาะด้วงหมัดผัก หนอนใยผักและหนอนกระทู้ผักสามารถสร้างความเสียหายต่อผลผลิตทางเศรษฐกิจสูงถึง 100% เกษตรกรจึงมีการใช้สารเคมีกำจัดแมลงศัตรูพืชอย่างกว้างขวาง ส่งผลการเป็นอันตรายและมีสารพิษตกค้างในสภาพแวดล้อมและสิ่งมีชีวิตที่ไม่ใช่เป้าหมาย ด้วยเหตุนี้งานวิจัยฉบับนี้จึงมีวัตถุประสงค์เพื่อการพัฒนาสารกำจัดแมลงศัตรูพืชในรูปของน้ำมันหอมระเหยนาโนอิมัลชันจากพืช เพื่อใช้ในการควบคุมแมลงศัตรูพืชที่เป็นมิตรต่อสิ่งแวดล้อม

การทดสอบเบื้องต้นของน้ำมันหอมระเหยจากพืช 12 ชนิดที่มีคุณสมบัติในการป้องกันกำจัดแมลงศัตรูพืชได้แก่ กระวาน ยูคาลิปตัส กะเพรา เทียนข้าวเปลือก พลู อบเชย ตะไคร้บ้าน จันทร์แปดกลีบ กานพลู ดีปลี พริกไทยและขมิ้นชัน ด้วยวิธีจุ่มใบ โดยใช้ใบกวางตุ้งที่ตัดเป็นวงกลมขนาดเส้นผ่านศูนย์กลาง 3 เซนติเมตร นำใบที่ตัดแล้วจุ่มลงในน้ำมันหอมระเหยดังกล่าว และตากให้แห้งที่อุณหภูมิห้องเป็นเวลา 15 นาที หลังจากนั้นปล่อยระยะตัวเต็มวัยของด้วงหมัดผักและระยะหนอนวัยที่ 2 ของหนอนใยผักและหนอนกระทู้ผักจำนวน 10 ตัว ลงในกล่องทดสอบแต่ละชนิดของแมลง ตรวจนับอัตราการตายที่ 24 ชั่วโมง ผลการศึกษาพบว่าที่ความเข้มข้น 1% ของน้ำมันหอมระเหยจันทร์แปดกลีบและกานพลูมีผลต่ออัตราการตายที่ 100% ของด้วงหมัดผัก และน้ำมันหอมระเหยจันทร์แปดกลีบและขมิ้นชันมีผลต่ออัตราการตายที่ 100% ของหนอนใยผักและหนอนกระทู้ผัก ดังนั้นน้ำมันหอมระเหยจันทร์แปดกลีบ ขมิ้นชันและกานพลูถูกคัดเลือกเพื่อนำมาพัฒนาเป็นน้ำมันหอมระเหยนาโนอิมัลชัน

จากนั้นนำน้ำมันหอมระเหยนาโนอิมัลชันจากจันทร์แปดกลีบ ขมิ้นชัน กานพลูและสารออกฤทธิ์หลักทางเคมีของพืชดังกล่าว นำมาทดสอบการควบคุมด้วงหมัดผัก หนอนใยผักและหนอนกระทู้ผัก เพื่อประเมินในรูปแบบของสารฆ่า สารยับยั้งการกิน สารยับยั้งการเจริญเติบโตและสารไล่ น้ำมันหอมระเหยนาโนอิมัลชันถูกเตรียมโดยการผสมน้ำมันหอมระเหยจากพืชกับสารลดแรงตึงผิวหลักและ

สารลดแรงตึงผิวร่วมโดยใช้วิธี aqueous titration method จากนั้นนำมาวัดขนาดอนุภาคและค่า zeta potential ของน้ำมันหอมระเหยนาโนอิมัลชันโดยใช้เครื่อง Nanoplus Zeta/Nano Particle Analyzer โดยขนาดอนุภาคของน้ำมันหอมระเหยนาโนอิมัลชันมีขนาดเล็กกว่า 100 นาโนเมตร และนำน้ำมันหอมระเหยนาโนอิมัลชันของจันทร์แปดกลีบ ขมิ้นชันและกานพลูมาทดสอบโดยใช้วิธีจุ่มใบและเก็บผลการทดลองที่ 24 ชั่วโมง พบว่าน้ำมันหอมระเหยนาโนอิมัลชันของจันทร์แปดกลีบที่ความเข้มข้น 0.35%, 0.20%, 0.20% และ 0.03% มีความเป็นพิษสูงสุดต่อด้วงหมัดผักโดยมีอัตราการตาย, อัตราการยับยั้งการกินแบบมีทางเลือก, อัตราการยับยั้งการกินแบบไม่มีทางเลือกและอัตราการไล่เมื่อเปรียบเทียบกับกลุ่มควบคุมเท่ากับ 100%, 100%, 100% และ 81.67% ตามลำดับ และพบว่าโดยทั่วไปน้ำมันหอมระเหยนาโนอิมัลชันของจันทร์แปดกลีบที่ความเข้มข้น 0.35%, 0.20%, 0.20%, 0.30% และ 0.20% มีความเป็นพิษสูงสุดต่อหนอนใยผักโดยมีอัตราการตาย, อัตราการยับยั้งการกินแบบมีทางเลือก, อัตราการยับยั้งการกินแบบไม่มีทางเลือก, อัตราการยับยั้งการเจริญเติบโตของการพัฒนาเป็นระยะดักแด้และอัตราการยับยั้งการเจริญเติบโตของการพัฒนาเป็นระยะตัวเต็มวัยเมื่อเปรียบเทียบกับกลุ่มควบคุมเท่ากับ 100% และพบว่าน้ำมันหอมระเหยนาโนอิมัลชันของจันทร์แปดกลีบที่ความเข้มข้น 0.35%, 0.15%, 0.20%, 0.30% และ 0.20%มีความเป็นพิษสูงสุดต่อหนอนกระทู้ผักโดยมีอัตราการตาย, อัตราการยับยั้งการกินแบบมีทางเลือก, อัตราการยับยั้งการกินแบบไม่มีทางเลือก, อัตราการยับยั้งการเจริญเติบโตของการพัฒนาเป็นระยะดักแด้และอัตราการยับยั้งการเจริญเติบโตของการพัฒนาเป็นระยะตัวเต็มวัยเมื่อเปรียบเทียบกับกลุ่มควบคุมเท่ากับ 100% จากผลการทดลองน้ำมันหอมระเหยนาโนอิมัลชันจากจันทร์แปดกลีบที่มีประสิทธิภาพสูงและเดลตร้าเมทรินซึ่งเป็นสารเคมีกำจัดแมลงศัตรูพืชนำมาทดสอบกับระยะตัวเต็มวัยของด้วงหมัดผัก และระยะหนอนวัยที่ 2 ของหนอนใยผักและหนอนกระทู้ผักด้วยวิธีจุ่มใบ โดยดูอัตราการตายที่ 24 ชั่วโมง จากผลการทดลองพบว่าที่ความเข้มข้น 0.35% ของน้ำมันหอมระเหยนาโนอิมัลชันจากจันทร์แปดกลีบ มีอัตราการตายของด้วงหมัดผัก หนอนใยผักและหนอนกระทู้ผักที่ 100% จากผลการทดลองข้างต้นพบว่าน้ำมันหอมระเหยนาโนอิมัลชันจากจันทร์แปดกลีบมีประสิทธิภาพในการควบคุมด้วงหมัดผัก หนอนใยผักและหนอนกระทู้ผักได้ดีในห้องปฏิบัติการ เมื่อนำน้ำมันหอมระเหยนาโนอิมัลชันจากจันทร์แปดกลีบและสารเคมีกำจัดแมลงศัตรูพืชเดลตร้าเมทรินมาทดสอบโดยวิธีการฉีดพ่นในสภาพโรงเรือนและแปลงทดสอบพบว่าน้ำมันหอมระเหยนาโนอิมัลชันจากจันทร์แปดกลีบที่ความเข้มข้น 0.35% มีการควบคุมสูงสุดโดยควบคุมได้100% และ 85.45% ตามลำดับ ดังนั้นน้ำมันหอมระเหยนาโนอิมัลชันจากจันทร์แปดกลีบสามารถนำไปใช้เป็นผลิตภัณฑ์เพื่อควบคุมด้วงหมัดผัก หนอนใยผักและหนอนกระทู้ผักได้อย่างมีประสิทธิภาพและปลอดภัยต่อสิ่งแวดล้อม

Thesis	Product Development of Plant Essential Oil Nanoemulsions for Controlling *Phyllotreta sinuata* Stephens, *Plutella xylostella* Linnaeus and *Spodoptera litura* Fabricius
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ABSTRACT

Cruciferous vegetables were considered as the king of vegetables and super food. The main cause of the damage to the production of cruciferous vegetables for export was insect pests affecting the quality and quantity of crop production. Especially, flea beetle (*Phyllotreta sinuata* Stephens), diamondback moth (*Plutella xylostella* Linnaeus), and cutworm (*Spodoptera litura* Fabricius) in which may cause 100% of economic yield loss. Therefore, in case of plant production agriculturists widely used chemical insecticides to control the insect pests. Chemical insecticide and its residue remaining in environment could pose risks to non-target organism. Therefore, this study aimed to develop eco-friendly and less toxic insecticides from plant essentials oil in form of nanoemulsions for controlling flea beetle, diamondback moth and cutworm.

The initial screening test of plant essential oils (EOs) obtained from 12 selected plants, namely cardamon, eucalyptus, holy basil, sweet basil, betel vine, cinnamon, lemongrass, star anise, clove, long pepper, black pepper, and turmeric against those insect pests was performed by using leaf dipping bioassay method. Organic Chinese cabbage leaves of 3 cm in diameter were dipped into EOs and dried at room temperature for 15 minutes. After that 10 adult stages of flea beetles and second stage

of instar larvae of diamondback moth and cutworm were put in the testing box. The mortality rates of these adults and larvae were observed at 24 hours. The results showed that at 1% concentration of star anise and clove essential oil gave the mortality rates of flea beetle with 100%. Whereas, 1% concentration of star anise and turmeric essential oil presented the mortality rates of diamondback moth and cutworm with 100%. Therefore, star anise, turmeric, and clove were subjected to develop as essential oil nanoemulsions.

Essential oil nanoemulsions (nEOs) from star anise, turmeric, clove and their main chemical compounds were tested against flea beetle, diamondback moth, and cutworm to evaluate their mortality, antifeedant, growth inhibition, and repellent activities. Essential oil nanoemulsion (nEO) was prepared by mixing the selected plant essential oils with various surfactants or co-surfactants by aqueous titration method. After that particle sizes and zeta potential of nanoemulsions were measured by Nanoplus Zeta/Nano Particle Analyzer. As a result, the nEO particle sizes were smaller than 100 nm. The nEOs of star anise, turmeric and clove were tested against those insects by using leaf dipping method with the observation at 24 hours. The results showed that nEO of star anise at concentrations of 0.35%, 0.20%, 0.20%, and 0.03% had the highest level of toxicity against the flea beetle with choice and no-choice antifeedant activities, as well as repellent activities at 100%, 100%, 100%, and 81.67%, respectively. The results also demonstrated that nEOs of star anise at 0.35%, 0.20%, 0.20%, 0.30% and 0.20% had the highest level of toxicity against diamondback moth with mortality, in choice and no-choice antifeedant activities, and growth inhibitions in pupae and adults at 100%. The nEOs of this plant at concentrations of 0.35%, 0.15%, 0.20%, 0.30% and 0.20% also gave the highest level of toxicity against cutworm with mortality, in choice and no-choice antifeedant activities, and growth inhibition in pupae and adults at 100%. The nEOs of star anise and deltamethrin (chemical insecticide) were aimed to test with adult stage of flea beetles and second stage of instar larvae of diamondback moth and cutworm by the same method. The results showed 100%

mortality of flea beetles, diamondback moth, and cutworm at 0.35% concentration of star anise nanoemulsions where, deltamethrin at recommendation rate gave only 96.70% (flea beetle), 98.30% (diamondback moth) and 95.00% (cutworm) mortality. In addition, when star anise nEO and deltamethrin were applied by using direct spraying method against the flea beetle, diamondback moth, and cutworm in greenhouse and field conditions, it revealed that at 0.35% concentration of star anise nEO could control by 100% and 85.45%, respectively. When deltamethrin at recommendation rate performed only 65.38% (flea beetle in greenhouse conditions), 46.91% (diamondback moth in greenhouse conditions), 58.55% (cutworm in greenhouse conditions) and 49.39% (field conditions) controls. Therefore, star anise nEO seem to be used as a highly-effective and environmentally friendly pesticide to control flea beetle, diamondback moth, and cutworm.

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CHAPTER 1
INTRODUCTION

1.1 Statement and significance of the problems

Cruciferous vegetables, including Chinese cabbage, cabbage, cauliflower, etc. are the biggest leafy crops, and are commonly cultivated in various climate conditions worldwide, and the cultured land is extended year (Liu *et al*. 2021). Cruciferous crop cultivation is affected by various fungi, bacteria, viruses, and pests, which can cause club roots, wilt, downy mildew, etc. The main cause of the damage to cruciferous crop cultivation is pest destruction, which affects the quality and quantity of production. The main destructive pests of cruciferous vegetables include flea beetle (*Phyllotreta sinuata* Stephens), diamondback moth (*Plutella xylostella* Linnaeus), cutworm (*Spodoptera litura* Fabricius). In addition, such destructive pests also affect the growth and development of cruciferous crop, and greatly reduce the production of cruciferous crop. They can spread rapidly throughout the year causing damage to crop production which can lead to large commercial losses. These pests can infest every stage of growth (Krishnamoorthy, 2004). Hence, many farmers used more than two chemical insecticides and used them up to 4 times a month to control and reduce pests (Ahouangninou and Fayomi, 2011). As a result, there will have a negative effect on the environment, non-target organisms, and mammals (Ondieki, 1996).

Using chemical insecticides for a long time will result in an increase in insecticide resistance, and can cause difficulty in controlling insect pests. Moreover, due to insecticide resistance, farmers who choose to continue using chemical insecticides will suffer the cost resulting from an increase in insecticide quantity. Moreover, they have to use different insecticides to control pests. Chemical insecticide residues also have a harmful effect on the environment and non-target organism (Ortega *et al.* 2021). Some farmers used botanic insecticide as an alternative to reduce the negative effect of using chemical insecticides.

Botanic insecticides can be used to control pests and are environmentally friendly (Patel *et al*. 2022). In many countries, botanic insecticides are used to control pests in field and storages (Begna, 2015). Botanic insecticides are used as an alternative to chemical insecticides because of its properties as repellent, antifeedant, and growth inhibition, as well as the ability to reduce pest resistance while rapidly degraded (Isman, 2006; Mochiah *et al*. 2011). For this reason, herb nanoemulsion is used as an alternative to solve the agricultural problems. Nanoemulsions are secondary metabolites that plants produce to defend themselves from stimuli in the unsuitable environment and destroy pests and pathogen that cause disease. Most nanoemulsions are stable and have low volatility and solubility property during storage and formulation process. So, nanoemulsions can be developed into highly- effective insecticides (Noichinda and Suppavorasatit, 2019).

The purpose of this study was to develop eco-friendly insecticides in form of plant essential oil nanoemulsions for controlling flea beetle (*Phyllotreta sinuata* Stephens), diamondback moth (*Plutella xylostella* Linnaeus), and cutworm (*Spodoptera litura* Fabricius). Therefore, it might be imperative to practically apply botanic insecticide in the farmers' fields for the success of using integrated pest management (IPM) in the country.

1.2 Objectives of the study

1.2.1 To evaluate plant essential oil, plant essential oil nanoemulsions and main chemical compound of plants in controlling flea beetle, diamondback moth, and cutworm in terms of the insecticidal properties (mortality activity, antifeedant activity, growth inhibited activity, repellent activity (only flea beetle) in laboratory conditions.

1.2.2 To evaluate the effectiveness of plant essential oil nanoemulsions and Deltamethrin (chemical insecticide) against flea beetle, diamondback moth, and cutworm in laboratory conditions, green house, and field conditions.

1.3 Scope of the study

This research study aimed to develop the plant essential oil nanoemulsions for controlling flea beetle, diamondback moth, and cutworm by assessing their mortality activity, antifeedant activity, growth inhibition activity, and repellent activity (against only flea beetle) by using leaf dipping method and compared the results with those of chemical insecticides. The most highly-effective plant essential oil nanoemulsion was tested against flea beetle, diamondback moth, and cutworm in laboratory conditions, green house and field conditions.

1.4 Expected output of the study

1.4.1 Obtaining plant essential oil nanoemulsions showing highly-effective toxicity against flea beetle, diamondback moth, and cutworm in laboratory conditions, green house and field conditions

1.4.2 Obtaining the guidelines for using the plant essential oil nanoemulsions to control insect pests of cruciferous vegetables instead of chemical insecticides.

CHAPTER 2

LITERATURE REVIEW

This chapter showed the review of the relevant literature on plant essential oil and plant essential oil nanoemulsions for controlling flea beetle, diamondback moth, and cutworm which are important insect pests of cruciferous vegetables. The review included nanoemulsions and the effectiveness of plant essential oil nanoemulsions.

2.1 Insect pests

2.1.1 Flea beetle

Scientific name: *Phyllotreta sinuata* Stephens

Common name: Flea beetle

Family: Chrysomelidae

Biology and life cycle

Eggs, larvae, and pupae stages live mostly in the soil. Female adults lay on the surface of root plants (Knodel, 2017). Most growth and propagation of flea beetles are in warm weather. These are flown and destroyed throughout the host plant (Burgress and Wiens, 1980). These breed repeatedly. Female adults lay eggs approximately 100 eggs in a group. Eggs are yellow with an oval shape, and they live at root plants in moist soil (Ulmer and Dosdall, 2006). After 12 days, they hatch and develop into the larvae stage. These larvae are 3-4 mm white body, brown head and anal. They can molt twice. Growth of the larvae stage takes 25-34 days and twice molting. Larvae eat mostly root hair and taproot. After fully developing, larvae develop into pupae in small soil. Pupae have a white body and take 7-9 days for the development period. After that, pupae develop into the adult stage (Figure 2.1). The adult stage is a beetle that eats the stem, leaves, and pods of the crop (Wylie, 1979).

Destruction, outbreak and pest management

Phyllotreta sinuata or flea beetle is the most economically important insect pest that destroys crucifer crops (Zimmer *et al*. 2014). Flea beetle in the adult stage has a small size, strong pest, and large hind legs to jump and move far and fast. The larvae in the adult stage eat by chewing, creating loss of seedlings to total crops (Cranshaw, 2006) during the emergence of crops with a high infestation (Williams, 2010), and much more than 65% of damaged crops were found (Alves *et al*. 2015). The problem of resistance to pests from chemical insecticides has long time (Willis and Davies, 2020). This resulted in pest mutation and increased resistance to the insecticide. Finally, it's difficult to control. Therefore, chemical insecticides were more harmful, with a low level of control of pests and residue pesticides in the environment and non-target organisms (Ortega *et al*. 2021).

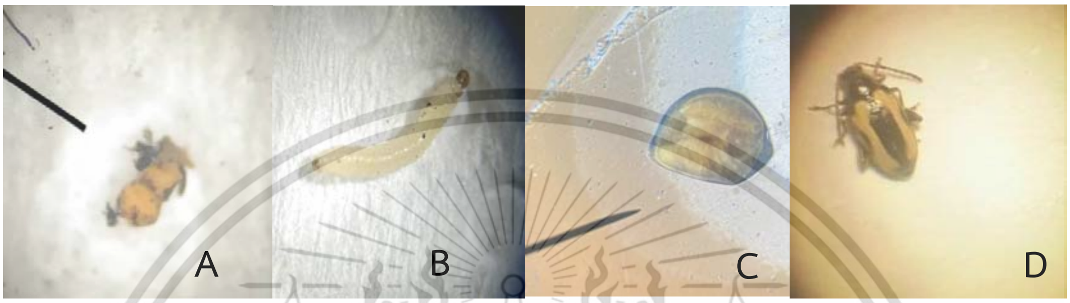

Figure 2.1 The flea beetle (*Phyllotreta sinuata* Stephens), A: Egg, B: Larva, C: Pupa and D: Adult

2.1.2 Diamondback moth

Scientific name: *Plutella xylostella* Linnaeus

Common name: Diamondback moth

Family: Yponomeutidae

Biological and life cycle

These eggs are yellow with an oval shape. They are laid under leaves and take 2 - 4 days to grow. Female adults can lay eggs approximately 150 eggs in 2-3 days on surface leaves (Capinera, 2002). Eggs are yellow with an oval shape. Then, eggs hatch into larvae and these eat the foliage. These larvae have bitten mouth, three true legs on thoracic segments, and five pseudo-legs on abdominal segments. They take 8-10 days and four molting in the development period. After that, pupae are developed from the larvae stage to be wrapped in a thin web. When the new beginning of pupae has a light green color, the color changes to light yellow and cream. They take 3-4 days of period development. After that, they develop into the adult stage having greyish brown and yellow-white stripes. Male adults are darker and smaller than females (Figure 2.2). They can live 4-21 days of development period.

Destruction, outbreak and pest management

The diamondback moth (*Plutella xylostella*) is a moth in Yponomeutidae family (Li *et al*. 2016). They are most deadly in pest crucifer crops in the world (Begna and Damtew, 2015). In the destruction, these larvae can feed the vein, midribs, and lower surface of leaves crucifer crop (Justus and Mitchell, 1996). This included the heads of cabbages, broccoli and cauliflower, resulting in growth stagnation and leading to poor yield quality (Timbilla and Nyarko, 2004). It was found that these larvae stage spread severely in all seasons. Therefore, they caused enormous damage to the quality and dropped ability of the export market in crucifer crops (Waiganjo *et al*. 2011). At present, these larvae can cause a loss of approximately sixteen million dollars and damage much more than 90% to the product of cruciferous crops in summer. Therefore, a control needs to be developed for reducing threat from diamondback moths (Charleston *et al*. 2006). The smallholder farmer at present used more excessive chemical pesticides, resulting in water and soil pollution, pesticide residues, resistance inducing and ecosystem destruction (Schulz *et al.* 2001). So, eco-friendly biopesticide that reduced negative problems in the environment was discovered.

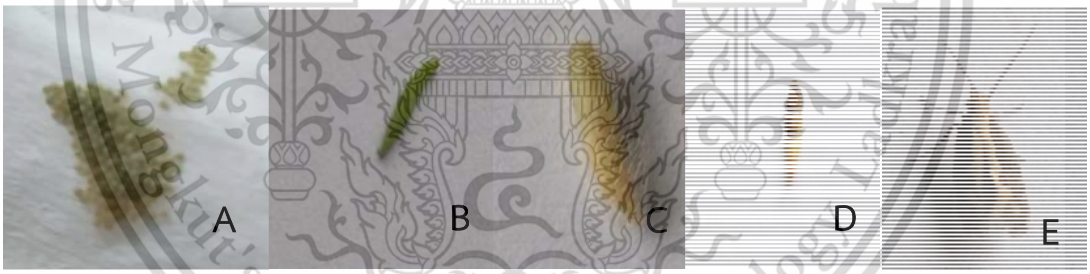

Figure 2.2 The diamondback moth (*Plutella xylostella* Linnaeus), A: Egg, B: Larva, C, D: Pupa and E: Adult

2.1.3 Cutworm

Scientific name: *Spodoptera litura* Fabricius

Common name: Cutworm

Family: Noctuidae

Biology and life cycle

Eggs are laid under the leave around 200-300 eggs. The eggs are flat, and oval with a diameter of 0.6 mm. Eggs are covered with brown hair-like scales from the abdomen of the female moth to protect them from hunters (Ranga *et al*. 1993). A female moth lays more than two thousand eggs within 4–8 days and lay in days 2-4 (Shekhawat *et al*. 2018). After 14 days, eggs hatch at 35°C. The larvae stage can develop within 27 days at 20 °C. A length can up to 45 mm. The pupa lives in the soil at 25 °C. The pupa can grow up to 15–20 mm long with red-brown color. It can develop into the adult stage after 12 days (Gupta *et al*. 2016). These moths have a greyish brown body with a length of 15-20 mm length and 30–38 mm of wingspread (Figure 2.3). Female moths can mate 3-4 times within 6–8 days but they aren't mated within the first night of emergence (Etman and Hooper, 1979). Female moths can attract males using tetradecadienyl acetate as a sex pheromone compound (Brown and Dewhurst, 1975).

Destruction, outbreak and pest management

The instar larvae eat the upper layers of leaves while the old instar larvae eat the whole leave. In the larvae stage, the beet armyworm destroyed young crops. These early instar-larvae eat the soft and easy digestion tissue from the lower to the upper surface of leaves. Then, the nearby instar larvae can eat the leaf but avoid the veins and midrib of leaves. Mature larvae eat the whole leaf, buds, fruit, and flowers. They eat plant regions. The mature larvae eat less and live together in a group. Larvae can live in the summer, but mostly be found in late summer (Murata *et al*. 2002).

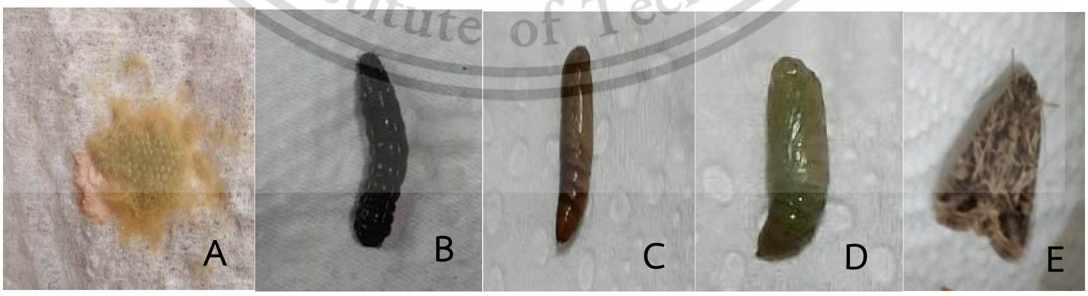

Figure 2.3 The cutworm (*Spodoptera litura* Fabricius), A: Egg, B: Larva, C, D: Pupa and E: Adult

2.2 The effect of using chemical and botanic insecticides for control insect pest

2.2.1 Chemical insecticide

Agriculturists in Thailand normally use chemicals to prevent and eliminate pests. Chemical insecticides are hazardous toxin that affects human and mammal health and can absorb the direct and indirect of the human body. Farmers used many chemical insecticides more than 4 times each month and mixed several of them to control and reduce infested pests (Ahouangninou and Fayomi, 2011). This resulted in a polluted environment and a negative effect on non-target organisms and mammals (Ondieki, 1996). So, many countries prioritize water and soil pollution. At present, serious infestation by diamondback moths, cutworms, and flea beetles caused severe damage to economic vegetables. The residues of pesticides can negatively affect health and the environment (Cox, 2001) as well as the environment, non-target organisms, and mammals (Ondieki, 1996). Therefore, many countries control the use of insecticide that affects water and soil. Chemical insecticides are highly effective in controlling pests, but these induce pest resistance for long-term use (Duhan *et al*. 2017). The concentration of insecticide is important for control and effect on insect pests (Lutz *et al*. 2018). These are key issues that need to be solved quickly for sustainable pest control (Moshi and Matoju, 2017). Botanic insecticides are considered alternative agents for integrated pest management since it is quickly resolved in an environment with less toxicity to mammals and low-risk resistance to insect pests (Ahmad, 2013).

2.2.2 Botanic insecticide

At present, botanic insecticides are the most effective method for integrated pest management to control antifeedant, growth, and development (Wang *et al*. 2016). Using botanic insecticides mixed with chemical insecticides is useful for reducing chemical insecticides in integrated pest management programs. The attract and kill form are a possible strategy in pest management that is a good combination of attracting and killing agents (Gregg *et al*. 2018). The selection of botanic insecticides is highly effective and environmentally friendly to replace or reduce the use of chemical insecticide that is harmful to agricultural production. Botanic insecticides were used to control pests in many countries (Isman, 2006). At present, using botanic insecticides to control agricultural pests has been popular among many farmers (Gerken *et al*. 2022).

This alternative has solved the problem for agriculture with plant nanoemulsion essential oils.

At present, botanic insecticides are the most effective for integrated pest management. The antifeedant effect was activated to deterrent receptor in the medial sensillum styloconicum (Liner *et al.* 1995). It could inhibit the transmission of cholinergic nerve signaling and calcium channel in suboesophageal ganglion and reduced the frequency of miniature excitatory postsynaptic currents (mEPSCs). Nerve cell conductivity is decreased, resulting in abnormal of central nervous system affecting to antifeedant, growth and development in insect pests. Botanic insecticide can inhibit the biosynthesis of ecdysteroids and juvenile hormones (Lai *et al*. 2014). It blocked the release of prothoracicotropic hormone (PTTH) from neuroendocrine cells that develop and molt inhibition (Cortez *et al*. 2012) in addition to blocking the function of endocrine and neuroendocrine systems (Sayah *et al*. 1998) and it included the reduced fecundity (Nathan and Kalaivani, 2006). This affected physiology. including larva, pupa and adult form. Also, the protein synthesis of pupae was reduced as well as the lymph volume of the last larvae, the enzyme activity of the gut in larvae, and these changes cuticular protein levels in larvae (Yooboon *et al*. 2015). Moreover, it caused damage to the plasma membrane and organelle atrophy in plasmatocytes and granular hemocytes (Sharma *et al*. 2003). The growth development of pests showed that the function of the midgut was important for digestion and absorption during insect growth and development. Botanic insecticides could reduce the midgut digestive enzymes level and activity (Khosravi and Sendi, 2013). In vivo, botanic insecticide induced apoptosis. Apoptosis is a cell death activity regarding negative stimuli (Huang *et al*. 2013). Insecticides can activate apoptosis (Gregorc and Ellis, 2011). In vitro, it regulated the antifeedant and growth effect of pests (Xu *et al*. 2016). Botanic insecticide induced inhibited effect of α-amylase and a digestive enzyme was produced by midgut epithelial cells (Rharrabe *et al*. 2008).

2.3 Plant extracts and essential Oils

Essential oils (EOs) are perfumed and evaporated agents that were extracted through hydrodistillation, steam distillation, dry distillation, and others from various parts of plants (Boukroufa *et al*. 2015). Specific aromatic components were products of the metabolism of each plant referred to as volatile secondary metabolites of plant, which could be found in glandular hairs or secretory cavities of plant-cell walls as droplets of fluid in various plants. The specific aroma in EOs has various functions, such as attracting or repellent pests and protecting them from the environment. EOs were found in 0.01 - 10% of total plants. At room temperature, these EOs are liquid but they can transform to gas at a higher temperature without decomposing (Opender *et al*. 2008). Terpenoids and phenylpropanoids are key in EOs, which have antioxidant, antibacterial, and insecticidal. They were used in pharmaceuticals, food, and agriculture (Pavela, 2015). Using EOs botanic insecticides increased considerably since they are accepted in sustainable agriculture. The Food and Drug Administration of the United States (FDA) reported that EOs were safer than chemical pesticides. EOs were used for repellent, insecticidal, ovicidal, antifeedant, growth inhibition, and others (Chaudhari *et al*. 2021). The methods of testing in EOs were used in terms of the fumigant, absorbing, ingesting, inhaling, and contact to control pests (Lira *et al.* 2015). The modes of EOs in control pests inhibited the functions of gamma-aminobutyric acid (GABA) receptors. They are the first inhibition in the neurotransmitter of the central nervous system of pests (Tampe *et al*. 2015). The function of acetylcholinesterase (AChE) inhibited hydrolyzes acetylcholine which is the response of neurotransmitters in the central nervous system for signal transmission (López *et al*. 2019). This included the contracted muscles of the legs and abdomen. Octopamine receptor binding sites were blocked, related to the modulatory influence of the nervous-muscular system (Plata *et al*. 2018). The expression of cytochrome P450 gene increased to control the release of toxins in pests. It resulted in the death of pests (Hussain *et al*. 2017). Os has many advantages for effectively controlling pests. Since it has a complex composition, it is highly variable and easily deteriorated under environmental conditions. They have the effect under the condition of oxidation and photolytic, resulting in quality loss and reduced biological effects (Bilia *et al.* 2014). So nanoencapsulation techniques can solve those disadvantages (Chaudhari *et al*. 2021).

2.4 Nanoemulsion

Essential oil nanoemulsions (nEOs) and nanoemulsions are EOs that are encapsulated with materials. The range dimension of a nanometer is between 1-100 nm. (Kumar *et al*. 2019). The EOs research reported the oil phase had the released ingredient from the core of oil and it was more effective properties of agents than oil alone (Nasr *et al*. 2020). Nanoemulsions mixed two phases: water in oil (W/O) or oil in water (O/W) for the ratio of substances to be stable. Nanoemulsions have three phases (oil, surfactant, and water). Oil and water phases were separated by surfactants inducing. Surfactants in form emulsifiers in nEOs have four types: cationic, anionic, amphoteric, and nonionic (Devarajan and Ravichandran, 2011). The range of HLB of O/W formulations in insecticides is used 10 -16 in agriculture since it was kinetically stable nanoemulsions. Using surfactants can change the electrostatic charge in the nanoemulsion which caused low aggregation (Feng *et al*. 2016). HLB in mixtures of nonionic surfactants have similar value to supplement stability (Du *et al*. 2016). For the reduced surface tension, water and oil can use surfactants and the size of the nanoemulsion has physical stability and can protect the environment (Narawi *et al*. 2020). In many reports, microemulsions are referred to as nanoemulsions but they have more surfactant concentrations and the size was in nanometers (Nasr *et al*. 2020). Nanoemulsions can prepare from the aqueous titration method with surfactants as emulsifiers for the distribution of droplet size (Ariyaprakai, 2017). This method was cheap when compared to other methods and it was easy method to stir the mixture of essential oils, surfactant, and water together (McClements and Rao, 2011). The Zeta potential of nanoemulsion was an indicator of stability. A negative zeta value could induce repulsive forces more than the attracted forces in each droplet and protect coagulated coalescences (Mohammadi *et al*. 2022). Nanoemulsion can coat the cuticles of pests and increase absorbed ingredients which destroys the wax cuticular layer in pests, resulting in dehydration. Finally, it can mortality (Omar and Kordali, 2019). Moreover, ingredients can absorb the cuticles of plants and can spread well on leaves. Many studies showed nanoemulsions control various pests and many insects can relate to higher mortality from using nanoemulsions against pests, compared to commercial pesticides and crude extracts. In the future, nanotechnology can change the research of medical and veterinary science as well as entomology that developed insecticides in the form of nanoemulsion since it can slowly release into the environment to control pests (Sharma *et al*. 2020).

2.4.1 Star anise

Star anise (*Illicium verum*) is mostly grown in tropical areas of Asia and is less toxic to the human body. So, it is useful in food, medicine (Ohira *et al*. 2009), and pest control (Peter *et al*. 2022). Dried fruit of star anise EO contained 81.4% of trans-anethole, 6.5% of limonene, 2.1% of chavicol, and 1.8% of anisaldehyde (Gholivand *et al*. 2009). Trans-anethole in star anise EO was an effective pesticide with an effect on AChE activity in pests (Cruz *et al*. 2013). Metabolism of EO affected the inhibited growth, dehydration, and death of lipid components in the epidermis, laying inhibited, antifeedant and neuron toxicity (Park *et al*. 2016). Acetylcholinesterase (AChE) and glutathione S-transferase (GST) enzymes increased the specific mechanism of toxicity at low concentrations in star anise. EO used AChE inhibited and finally died. So that this EO can use to control pests (Peter *et al*. 2022). Trans-anethole had a strong repellent effect on pests applied with polyethylene terephthalate (PET) film and its coated layer (Choi *et al*. 2022). The previous study showed that a low dose of trans-anethole had high potential sensitivity in a repellent of *Tribolium confusum* (Alkana and Erturk, 2020). Star anise EO had insecticidal effects on larvae and adults of *Callosobruchus chinensis, Botrytis cinerea*, and *Colletotrichum gloeosporioides* (Shukla *et al*. 2009). Star anise had 100% mortality of cat fleas and juvenile snails (Freitas *et al*. 2021). From this study, the comparison of chemical insecticides showed that star anise had a higher effect on *Blattella germanica* than Deltamethrin and hydramethylnon using direct contact and fumigation methods (Chang *et al*. 2002). So, botanic insecticide is an alternative to chemical insecticides for pest control since it is safe, easily degraded and eco-friendly (Sukumar *et al*. 1991).

2.4.2 Turmeric

Turmeric (*Curcuma longa*) is an herb that humans used a long time ago (Ravindran *et al*. 2007). The main component of turmeric oil contained 34.9% of ar-turmerone (Jantan *et al*. 2008), found in a growth period, harvest time, climate factors, cultivated area, extracted part of a plant, and extracted methods, resulting in a difference in quality and quantity of constituents in oil (Hu *et al*. 2017). A high level of ar-turmerone in turmeric oil has a repellence effect on pests (Tavares *et al*. 2013). This included antibacterial, antifungal, and other effects (Singh *et al*. 2010).

Ar-turmerone in turmeric EO affected pesticidal which has a mortality of female adults among *Nilaparvata lugens*, larvae of *Plutella xylostella* and *Spodoptera litura* while a low concentration had no effects (Lee *et al*. 2001). Ar-turmerone in turmeric affected Acetylcholine esterase (AChE) and Butyrylcholine esterase (BChE) which are the main molecular targets for induced larval mortality (Rao *et al*. 2022). The inhibited AChE affected abnormal neurotransmission, resulting in pest mortality (Hirata, 2016). In the nervous system, Acetylcholine receptors can negatively affect growth and induce palsy, and lead to pest fatality (Rattan, 2010).

2.4.3 Clove

Clove oil is extracted from the dry flower bud of *Syzygium aromaticum.* Clove can grow well in sandy loam and lateritic soil (Bhattacharya, 2016). Clove oil can be extracted by distillation method using bud, leaf, or stem. Yield and quality of oil were affected by the cultivation area, the process of postharvest, the distillation method, and storage. The bud and stem were crushed before distillation since oil cells could break and spread the surface for easy release from cells. The leaf was not crushed due it its thinness. Water or steam distillation took 8-24 hours. Bud distillation is the highest quality yield at 20% contained eugenol at 85 - 89% in water distillation (Purseglove *et al*. 1981). Compounds of EOs affected neuron toxicity, GABA mechanism, octopamine synapse, and acetylcholinesterase inhibited. Eugenol acted as octopamine receptors, and GABA modulated to bind to the membrane which contained nicotinic acetylcholine receptors (nAChRs), resulting in decreased effects on the nerve system and inhibition of acetylcholinesterase (AChE). Finally, these affected apoptoses, abnormal nutrition, and reproductive pests. Previous studies showed that eugenol could control pests, mites, ticks, and spiders with different chemical pyrethroids due to insecticide resistance. In the study, main constituents of clove buds EOs using hydro-distillation method included 88.61% of eugenol, 8.89% of eugenol acetate, and 1.89% of β-caryophyllene. Gas chromatography-mass spectrometry analysis had potential of natural insecticides in laboratory and field (Tian *et al.* 2015). Clove EOs were insecticidal and repellent effects that control various pests (Chaieb *et al.* 2007). In this study, the main components of clove oil were blended with EOs which showed a comparable repellent effect to the main oils (Omolo *et al.* 2004). Eugenol and trans-caryophyllene in clove oil controlled *Tribolium castaneum*. Botanic insecticides were more environmentally friendly than chemical insecticides (Ikawati *et al.* 2022). Eugenol had a neurotoxic mechanism affecting acetylcholinesterase and octopamine synapses inhibited which control pests (Regnault *et al.* 2012). The effect of eugenol interrupted octopamine energy, resulting in damage to nervous pests (Dayan *et al.* 2009). Clove oil-regulated oxidative phosphorylation resulted in a reduced respiratory rate (Plata *et al*. 2017). Botanic products had alternative control pests which has advantages over synthetic chemical products (Gitahi *et al.* 2021) due to pesticide resistance and toxicity in non-target organisms (Batiha *et al.* 2020). At present, boosted eco-friendly management was found.

These studies showed that botanic insecticides could control pests and they were much safer for humans and mammals and more eco-friendly than chemical insecticides since the previous studies clearly showed the high effectiveness of botanic insecticides against pests and they can reduce chemical insecticides in the future. Therefore, these are better alternatives to chemical pesticides and justify further development into commercial bio-insecticidal products.

CHAPTER 3

METHODOLOGY

The methodology showed the processes of the test on flea beetle, diamondback moth and cutworm cultures, preparation of plant essential oils, plant essential oil nanoemulsion and main chemical compound, the leaf dipping and spraying methods and the experiment of mortality, antifeedant, growth inhibition and repellent effects on the flea beetle, diamondback moth and cutworm in laboratory conditions. Finally, these nanoemulsions were applied in greenhouse and field conditions to examine their effectiveness for insect pests in cruciferous crops control and management.

3.1 Insect culture

3.1.1 *Phyllotreta sinuata* Stephens (Flea beetle)

The samples of *Phyllotreta sinuate* were collected from *Brassica rapa (*Chinese cabbage), *Brassica olracea* (Chinese kale) plots in Nakhon Pathom, Thailand (Figure 3.1). After that, they were reared in insect boxes at room temperature (25±2 °C) and 12: 12 light-dark cycle, at Department of Plant Production Technology, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. New food was changed every 1-3 day using wholes of *Brassica rapa* for larvae and adults as well as using soil for egg laying and pupae living.

Figure 3.1 The flea beetle (***Phyllotreta sinuata* Stephens)** culture preparation, A: The organic Chinese cabbage field infested with the flea beetle, B: The flea beetle reared in box

3.1.2 *Plutella xylostella* Linnaeus (Diamondback moth)

The samples of *Plutella xylostella* were collected from *Brassica rapa* (Chinese cabbage) plot in Nakhon Pathom and Nonthaburi, Thailand. After that, they were reared in insect boxes at room temperature (25±2 °C) and 12: 12 light-dark cycle, at Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand (Figure 3.2). New food was changed every 1-3 day using leaves of *Brassica rapa* for larvae rearing and honey for adults rearing.

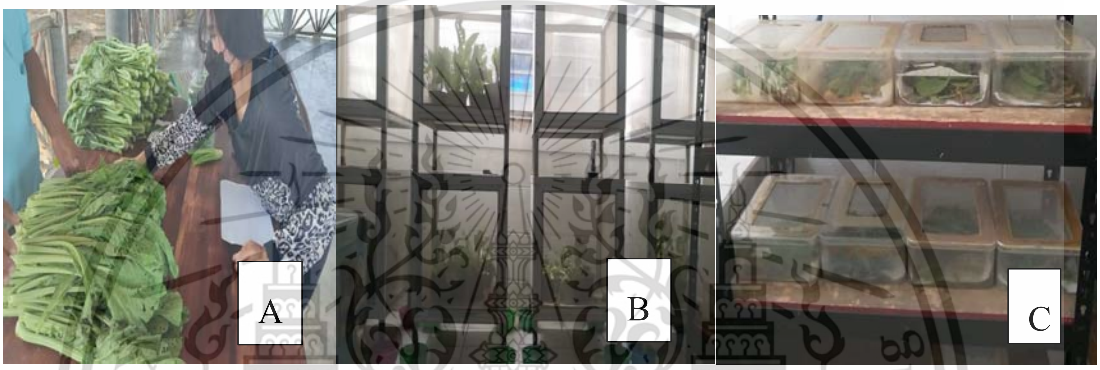

Figure 3.2 The Diamondback moth (*Plutella xylostella* Linnaeus) culture preparation, A: The larvae of diamondback moth from organic Chinese cabbage, B: The adult of diamondback moth laying eggs in insect cages, C: The larva and pupa of diamondback moths reared in boxes

3.1.3 *Spodoptera litura* Fabricius (Cutworm)

The samples of *Spodoptera litura* were collected from Brassica rapa (Chinese cabbage), Brassica olracea (Chinese kale) plots in Nakhorn Pathom, Chachoengsao and Nonthaburi, Thailand. After that, they were reared in insect boxes at room temperature (25±2 °C) and 12: 12 light-dark cycle, at Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand (Figure 3.3). New food was changed every 1-3 day using leaves of *Brassica rapa* larvae rearing and honey for adults rearing.

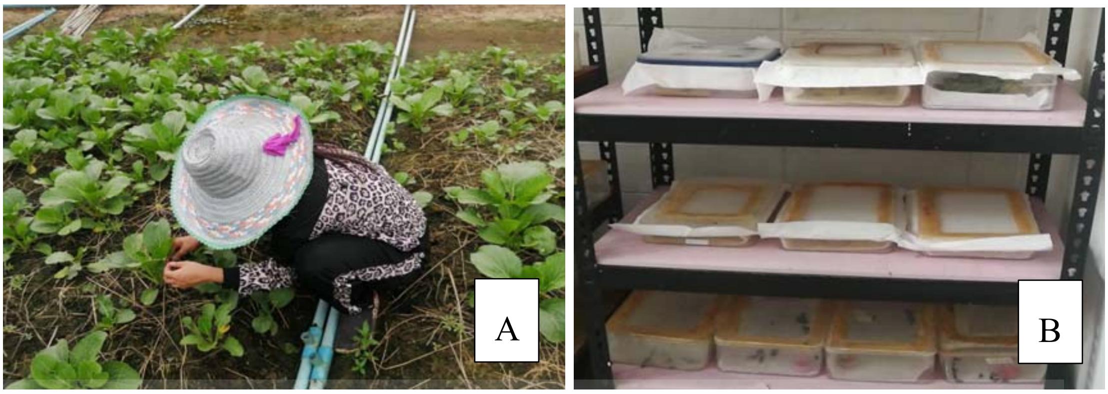

Figure 3.3 The cutworm (***Spodoptera litura*** **Fabricius**) culture preparation, A: The larvae of cutworm randomly collected from an organic Chinese cabbage field, B: The cutworm reared in box

3.2 Chinese cabbage cultivating

Chinese cabbages (*Brassica rapa* Linnaeus) were cultivated for insect food at Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand (Figure 3.4). After planting, fertilizer formula 46-0-0 was used. They watered twice a day (morning and evening).

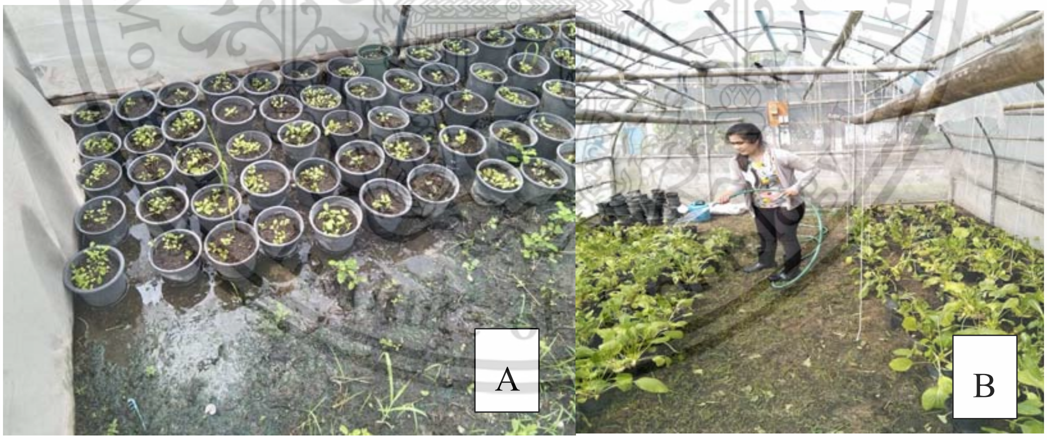

Figure 3.4 The organic Chinese cabbage preparation, A and B: the organic Chinese cabbages cultivated for insect food

3.3. Preparation of plant essential oils, main chemical compounds, selected plant essential oil and chemical compound nanoemulsions

3.3.1 Plant essential oils screening

Twelve selected plant essential oils (EOs) in preliminary test included Cardamon, Eucalyptus, Holy basil, Sweet fennel, Betel vine, Cinnamon, Lemon grass, Star anise, Clove, Long pepper, Black pepper, Turmeric. These 12 EOs in this study were prepared in keeping with principles after the hazard analysis and critical control point (HACCP) and procured from the Thai - China Flavors and Fragrances Industry Co. Ltd., Thailand. These EOs were diluted in water by using Tween 20 as an emulsifier (ratio 1:1). These botanical information and plant parts used to gain essential oils were shown in Table 3.1. They were screened to evaluate their insecticidal property by leaf dipping bioassay. Organic Chinese cabbage leaves with a diameter of 3 cm were dipped into 1% and 10% concentrations of EOs (1 minute), and dried at room temperature (15 minutes). After 10 seconds, stage larvae of cutworm, diamondback moth and adult flea beetle were put in the testing boxes. The mortality test was observed 24 hours. The most effective plant essential oils were selected for mortality, antifeedant and growth inhibition test at 0.00%, 0.25%, 0.50%, 0.75%, 1.00% and 1.25% concentrations. After that, they were developed into nanoemulsions with further bioassay test.

Table 3.1. Plant essential oils used for control test of flea beetle, diamondback moth and cutworm

Scientific name	Common name	Family	Plant part
1. *Elletaria cardamomum* Linn.	Cardamon	Zingiberaceae	Seed
2. *Eucalyptus globulus* Labille	Eucalyptus	Myrtaceae	Leaf
3. *Ocimum tenuiflorum* Linn.	Holy basil	Lamiaceae	Leaf
4. *Foeniculum vulgare* Miller Subsp. Var. Vulgare	Sweet fennel	Umbelliferae	Seed
5. *Piper betel* Linn.	Betel vine	Piperaceae	Leaf
6.*Cinnamomum bejolghota* Sweet.	Cinnamon	Lauraceae	Leaf
7.*Cymbopogon citratus* (DC.) Stapf.	Lemon grass	Poaceae	Leaf
8. *Illicium verum* Hook. f.	Star anise	Illiciaceae	Seed
9. *Syzygium aromaticum* (L.) Merr. & L.M. Peery.	Clove	Myrtaceae	Leaf
10. *Piper longum* Blume.	Long pepper	Piperaceae	Fruit
11. *Piper nigrum* Linn.	Black pepper	Piperaceae	Fruit
12. *Curcuma longa* Linn.	Turmeric	Zingiberaceae	Rhizome

3.3.2. Preparation of essential oil nanoemulsions (nEOs)

3.3.2.1 Star anise and trans-anethole

Illicium verum (star anise) and trans-anethole (main compounds in star anise essential oil) were prepared for nanoemulsions. The selected EOs of star anise and trans-anethole were diluted with water and surfactant. The surfactants were tween 20, tween 80, NP 9, PEG 400 and Span 20 (Table 3.2). In this process, the star anise and trans anethole nanoemulsions contained star anise essential oil and surfactant (Smix). The variation of Smix was investigated as followed: 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, and 1:4. The first selected star anise and trans-anethole nanoemulsions were observed with naked eyes. It was nonsediment, nonseparation and got a homogeneous emulsion (Figure 3.5). Smix of star anise and trans-anethole nanoemulsions were used to measure particle sizes and zeta potential with a particle analyzer (NanoPlus Zeta, Otsuka Electronic Co., Ltd., Osaka, Japan). The nEOs particle sizes analysis was found to be smaller than 100 nm (Table 3). The most effective star anise and trans anethole nanoemulsion were selected for further bioassay tests.

3.3.2.2. Turmeric and ar-turmerone

Curcuma longa (turmeric) and ar-turmerone (main compounds in turmeric essential oil) were prepared for nanoemulsions. The selected EOs of turmeric and turmerone were diluted with water and surfactants. The surfactants included tween 20, tween 80, NP 9, PEG 400 and Span 20. In this first process, the turmeric and ar-turmerone nanoemulsions contained turmeric and ar-turmerone essential oil and surfactant (Smix). The variation of Smix was investigated as followed: (Table 3.2) 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, and 1:4. The first selected of turmeric and ar-turmerone nanoemulsions were observed with naked eyes. It was nonsediment, nonseparation and got a homogeneous emulsion (Figure 3.5). Smix of turmeric and ar-turmerone nanoemulsions were used to measure particle sizes and zeta potential with a particle analyzer (NanoPlus Zeta, Otsuka Electronic Co., Ltd., Osaka, Japan). The particle sizes analysis of nEOs was found to be smaller than 100 nm (Table 3). The most effective turmeric and ar-turmerone nanoemulsion were selected for further bioassay tests.

3.3.2.3. Clove and eugenol

Syzygium aromaticum (clove) and eugenol (main compound in clove essential oil) were prepared for nanoemulsions. The selected EOs of clove and eugenol were diluted with water, surfactant and co-surfactant. The surfactants and co-surfactants included tween 20, tween 80, NP 9, PEG 400 and Span 20. In this process, the clove and eugenol nanoemulsions contained clove and eugenol essential oil, surfactant and co-surfactant (Smix). The variation of Smix was investigated as followed: 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, and 1:4. The variation of Smix (clove, eugenol essential oil, surfactant and co-surfactant) was investigated as followed: 1:1:1, 1:1:1.5, 1:1:2, 1:1:2.5, 1:1:3, 1:1:3.5, 1:1:4, 1:1.5:1, 1:1.5:1.5, 1:1.5:2, 1:1.5:2.5, 1:1.5:3, 1:1.5:3.5, 1:1.5:4, 1:2:1, 1:2:1.5, 1:2:2, 1:2:2.5, 1:2:3, 1:2:3.5, 1:2:4, 1:2.5:1, 1:2.5:1.5, 1:2.5:2, 1:2.5:2.5, 1:2.5:3, 1:2.5:3.5, 1:2.5:4, 1:3:1, 1:3:1.5, 1:3:2, 1:3:2.5, 1:3:3, 1:3:3.5, 1:3:4, 1:3.5:1, 1:3.5:1.5, 1:3.5:2, 1:3.5:2.5, 1:3.5:3, 1:3.5:3.5, 1:3.5:41:1:1, 1:4:1.5, 1:4:2, 1:4:2.5, 1:4:3, 1:4:3.5, and 1:4:4. The first selected clove and eugenol nanoemulsions were observed with naked eyes.

It was nonsediment, nonseparation and got a homogeneous emulsion (Figure 3.5). Smix of clove and eugenol nanoemulsions were used to measure particle sizes and zeta potential with a particle analyzer (NanoPlus Zeta, Otsuka Electronic Co., Ltd., Osaka, Japan). The nEOs particle sizes analysis was found to be smaller than 100 nm (Table 3). The most effective clove and eugenol nanoemulsions were selected for further bioassay tests.

To prepared the combinated nanoemulsions (star anise: turmeric, star anise: clove, trans-anethole: ar-turmerone, trans-anethole: eugenol). In this process, these combinated nanoemulsions were investigated the variational ratios. The first selected ratio of combinated nanoemulsion was observed with naked eyes which it's non sediment, nonseparation and get a homogeneous emulsion. Choosing combinated nanoemulsions are measured particles sizes and zeta potential with a particle analyzer (NanoPlus Zeta, Otsuka Electronic Co., Ltd., Osaka, Japan) The combinated nEOs particle sizes analysis were found with smaller than 100 nm (Table 3). The most effective combinated nanoemulsion were selected to further bioassay test.

Table 3.2. Surfactants and co-surfactant in the Study

Surfactant / Co-surfactant	Hydrophile Lipophile balance (HLB)
Polysorbate 20 (Tween 20)	16.7
Polysorbate 80 (Tween 80)	15.0
Nonyl Phenol Ethoxylate (NP 9)	12.9
Polyethylene glycol (PEG400)	13.0
Sorbitan Monolaurate (Span 20)	8.6

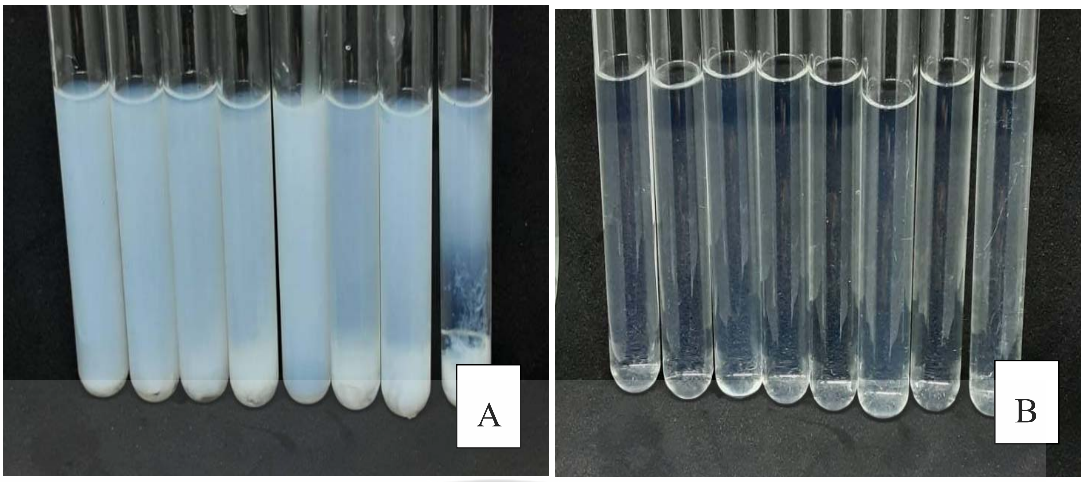

Figure 3.5. The essential oil nanoemulsions with different ratios of surfactant and/or co-surfactants, A: precipitate, B: with no precipitate

3.4 Bioassay

3.4.1 Bioassay test in laboratory conditions

3.4.1.1 Mortality test

For the no choice in mortality pretest, the prepared five Chinese cabbage leaves were dipped into the selected nEOs at variation of concentrations as 0.00% (surfactant, control), 0.20%, 0.40%, 0.60%, 0.80% and 1.00% (Figure 3.6). Then, for the no choice in mortality test at appropriate range of concentration, the prepared five Chinese cabbage leaves were dipped into the EOs, nEOs and main compound at variation of concentrations as 0.00% (surfactant, control), 0.10%, 0.15%, 0.20%, 0.25%, 0.30% and 0.35% for 1 minute, and dried at room temperature for 15 minutes (Figure 3.6). Then, ten 2nd stage larvae of cutworms, diamondback moth or adults of flea beetles (3 hours of diet) were put on Chinese cabbage leaves and kept in the box. The mortality was observed after 24 hours and the mortality rates were calculated and compared with the control group (0.00% concentration) using Abbott's formula (Abbott, 1925).

$$\text{Mortality (\%)} = [T - C/ 100 - C] \times 100$$

Where T is test mortality (%) with EO, nEO, main compound and C is control mortality (%)

3.4.1.2. Antifeedant effect test

The no choice in antifeedant test, antifeedant effect caused by the selected EOs, nEOs and main compound were determined by the abovementioned method. The Chinese cabbage leaves with the diameter of 3 cm were dipped in the selected EOs, nEOs and main compound at variation of concentrations of 0.00%

(surfactant, control), 0.10%, 0.15%, 0.20%, 0.25%, 0.30% and 0.35% for 1 minute, and dried at room temperature for 15 minutes (Figure 3.6). Then, ten 2nd stage instar larvae of cutworms, diamondback moth or adults of flea beetles (3 hours of diet) were released in the test boxes with 1 sample test and 3 replications. Antifeedant effect was observed after 24 hours, and the consumed area was measured and compared with the control group. The percentage of antifeedant effect was calculated and represented by the antifeedant index (AFI) (Escoubas *et al.* 1992) as shown below:

$$AFI = [\%T / (\%T + \%C)] \times 100$$

where C and T are controlled and treated leaf area consumed

As for the choice antifeedant test, the prepared one Chinese cabbage leaf was dipped into various concentrations of EOs, nEOs and main compounds at different concentrations: 0.10%, 0.15%, 0.20%, 0.25%, 0.30% and 0.35%, using one leaf dipped 0.00% (surfactant, control for compared test for 1 minute, and dried at room temperature for 15 minutes (Figure 3.6). Each concentration was placed in the opposite site with the control in round plastic box with a diameter of 13 centimeters and a height of 3 centimeters, supported by tissue paper and the lid and the net padded were cut for ventilation. Then, ten 2nd stage instar larvae of cutworms, diamondback moth or adults of flea beetles (3 hours of diet) were released in the test boxes with 1 sample test and 3 replications. Antifeedant effect was observed after 24 hours, and the consumed area was measured and compared with the control group. The percentage of antifeedant effect was calculated and represented by the antifeedant index (AFI) (Escoubas *et al*. 1992) as shown below:

$$AFI = [\%T / (\%T + \%C)] \times 100$$

where C and T are controlled and treated leaf area consumed

3.4.1.3 Growth inhibition test

As for the no choice in growth inhibition test, the study of growth inhibition effect of EOs, nEOs and main compound were determined by the abovementioned method. The Chinese cabbage leaves with a diameter of 3 cm were dipped in the selected EOs, nEOs and main compound at variation of concentrations: 0.00% (surfactant, control), 0.10%, 0.15%, 0.20%, 0.25%, 0.30% and 0.35% for 1 minute, and dried at room temperature for 15 minutes (Figure 3.6). Ten 2nd stage instar larvae of cutworms and diamondback moths (3 hours of diet) were released in the test boxes with 1 sample test and 3 replications. The development of growth from larvae to pupa and adult were observed and compared with the control group. The number of pupa and adult survival were recorded.

3.4.1.4 Repellent test:

In choice repellent test, the prepared one Chinese cabbage leaf was dipped into various concentrations of EOs, nEOs and main compounds at variation of concentrations: 0.01, 0.02 and 0.03%, using one leaf dipped 0.00% (surfactant, control) for compared test for 1 minute, and dried at room temperature for 15 minutes. Each concentration was placed in the opposite site with the control in round plastic boxes with a diameter of 13 cm, a height of 3 cm, supported by tissue paper and the lid and the net padded were cut for ventilation (Figure 3.6). Then, the adults of flea beetles were put on treated Chinese cabbage leaves and kept in the box. The repellent effect was observed after 24 hours. When compared with the control group, the percentage of repellent effect was calculated and represented by the repellent index (RI) (Pascual and Robledo, 1998) as shown below:

$$RI = [(C - T) / (C + T)] \times 100$$

where C and T are controlled and treated repellent test

3.4.1.5. Efficacy of nanoemulsions and chemical insecticide in laboratory conditions

The most effectively of nanoemulsion and Deltamethrin (chemical insecticide) were tested with cutworms, diamondback moths and flea beetles using mortality test at appropriate concentrations of nanoemulsion and deltamethrin. These mortality test showed the abovementioned method in laboratory (Figure 3.6). The data were calculated with statistical analysis. The treatments were tested as

T1 = Control

T2 = Star anise nanoemulsion (at 0.25% concentration)

T3 = Star anise nanoemulsion (at 0.35% concentration)

T4 = Deltamethrin (recommended ratio)

T5 = Deltamethrin (half of recommended ratio) and star anise nanoemulsion (at 0.15% concentration)

T6 = Deltamethrin (half of recommended ratio) and star anise nanoemulsion (at 0.25% of concentration)

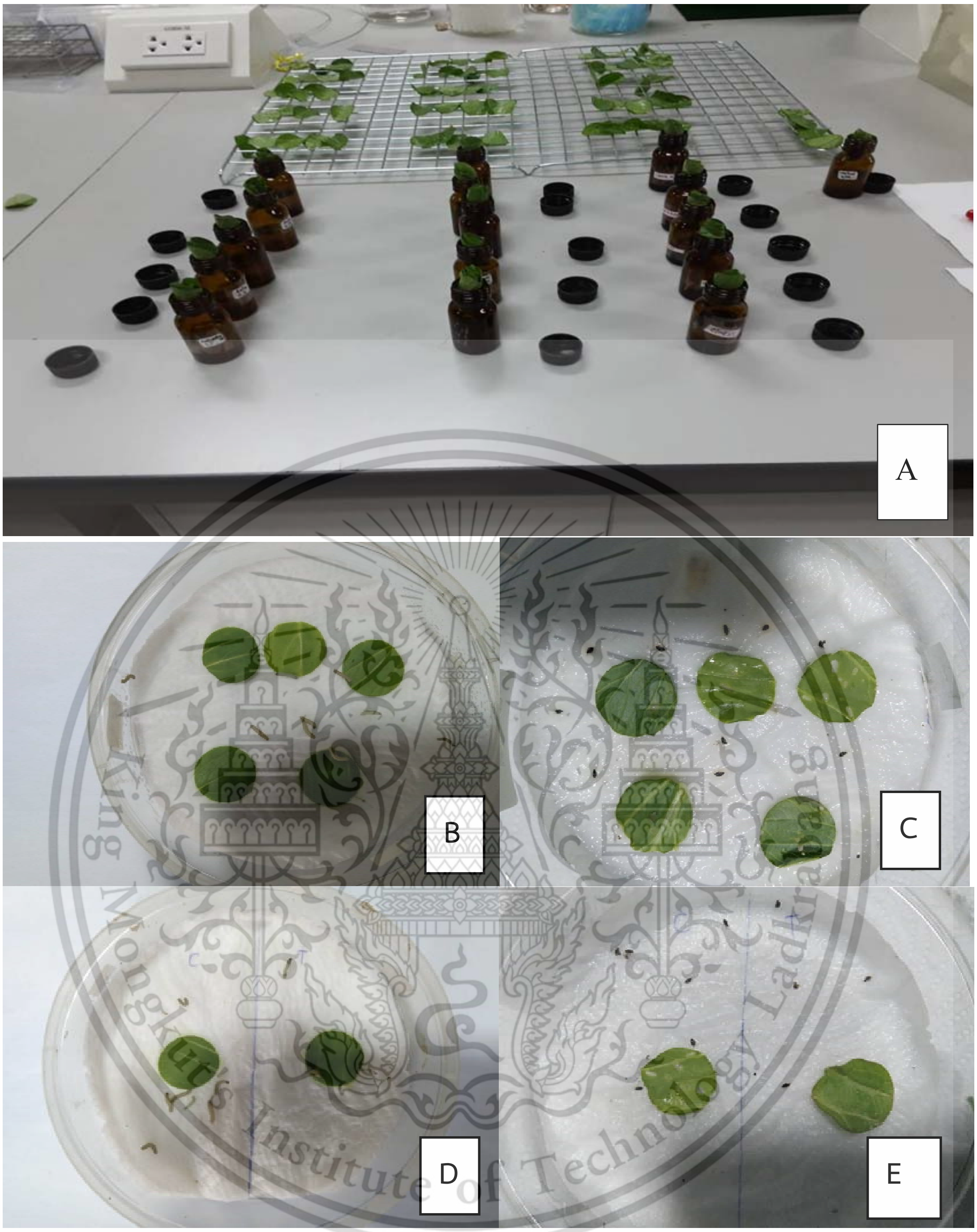

Figure 3.6 The insecticidal property test of essential oil and nanoemulsion against the flea beetle, diamondback moth and cutworm in laboratory by using leaf dipping method, A: Leaf dipping preparation, B, C: Leaf dipping method in no choice test, D, E: Leaf dipping method in choice test

3.5 Efficacy of nanoemulsions in greenhouse conditions

The efficacy of the nanoemulsion was tested in comparison with the Deltamethrin and control group in the greenhouse conditions. The efficacy of the star anise nanoemulsion was tested at these concentrations against the cutworms, diamondback moths and flea beetles as follows:

T1 = Control

T2 = Star anise nanoemulsion (at 0.25% concentration)

T3 = Star anise nanoemulsion (at 0.35% concentration)

T4 = Deltamethrin (recommended ratio)

T5 = Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.15% concentration)

T6 = Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.25% of concentration)

Planting plots were designed with a size of 1.5×1.5 meters and a distance of 10 cm between sub plots. The total of experiment plots were 24 plots, divided into 6 trials and 4 replications. In one small plot, 20 Chinese cabbages were planted at King's Mongkut institute of technology Ladkrabang (KMITL), Bangkok, Thailand (Figure 3.7). After planted, fertilizer formula 46-0-0 was used. They were watered every day with 2 times per day (morning and evening). To imitate natural outbreaks, 500 adults of cutworms and diamondback moths were released during day 20 and 2,000 adults of flea beetles during day 25 of Chinese cabbages growth. For spraying methods, the air blast sprayer was performed 2 times every 7 days and using water of 120 liters/rai. After that the larvae of cutworms and diamondback moths and the adults of flea beetle in whole plant were counted (20 trees/sub plot). The count was performed as follows: before and after spraying insecticides every 3, 5 and 7 days. After that, these data were analyzed by Duncan's Multiple Range Test (DMRT).

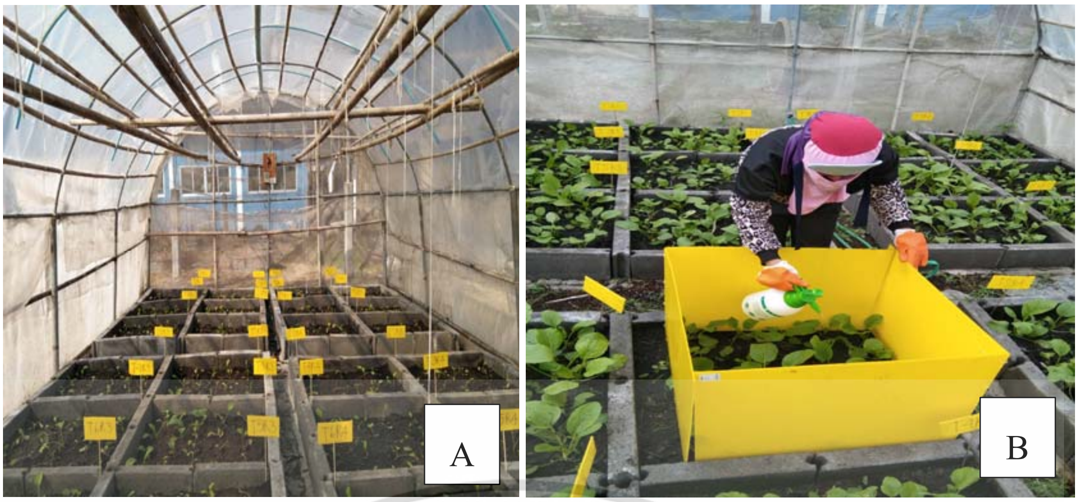

Figure 3.7 The insecticidal property test of essential oil and nanoemulsion against the flea beetle, diamondback moth and cutworm in greenhouse conditions by direct spray method, A: Treatment of greenhouse conditions, B: Direct spray method

3.6 Efficacy of nanoemulsions in field conditions

The efficacy of the nanoemulsion was tested in comparison with the deltametrin and control group in the farmer's field conditions (Figure 3.8). The efficacy of the nanoemulsion was tested at these concentrations against the cutworms, diamondback moths and flea beetles as follows:

T1 = Control

T2 = Star anise nanoemulsion (at 0.25% concentration)

T3 = Star anise nanoemulsion (at 0.35% concentration)

T4 = Deltamethrin (recommended ratio)

T5 = Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.15% concentration)

T6 = Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.25% of concentration)

Chinese cabbages were planted in the farmer's fields, Dontum District, Nakornpathom, Thailand. Planting plots were designed with a size of 4 x1.5 meters and a distance of 20 cm between sub plots. The total of experiment plots were 24 plots, divided into 6 trials and 4 replications. After planted, fertilizer formula 46-0-0 was applied at 30 kg/1 rai. Watering was performed every day with 2 times per day (morning and evening). Watering was performed every day with 2 times per day (morning and evening). In spraying method, the air blast sprayer was performed 2 times every 7 days using water of 120 liters/rai. After that, the larvae of cutworms and diamondback moths and the adults of flea beetle in whole plant were randomly counted (20 trees/sub plot). The count was performed as follows: before and after spraying insecticides every 3, 5 and 7 days. After that, these data were analyzed by Duncan's Multiple Range Test (DMRT).

3.7 Statistical analysis

In this study, Abbott's formula was applied to obtain the flea beetle, diamondback moth and cutworm mortality rate. The experiment was performed in a completely randomized design (CRD) with three replicates per treatment. The obtained data were analyzed by ANOVA program. The different treatments were tested by using Duncan's multiple range test (DMRT) in SAS program at 95% confidence level ($P<0.05$). In the results of the greenhouse and field conditions, in the RCBD (randomized completely block design) experiment was planned while the values of LC_{50} (50% lethal concentration) was calculated by using the probit analysis in SPSS.

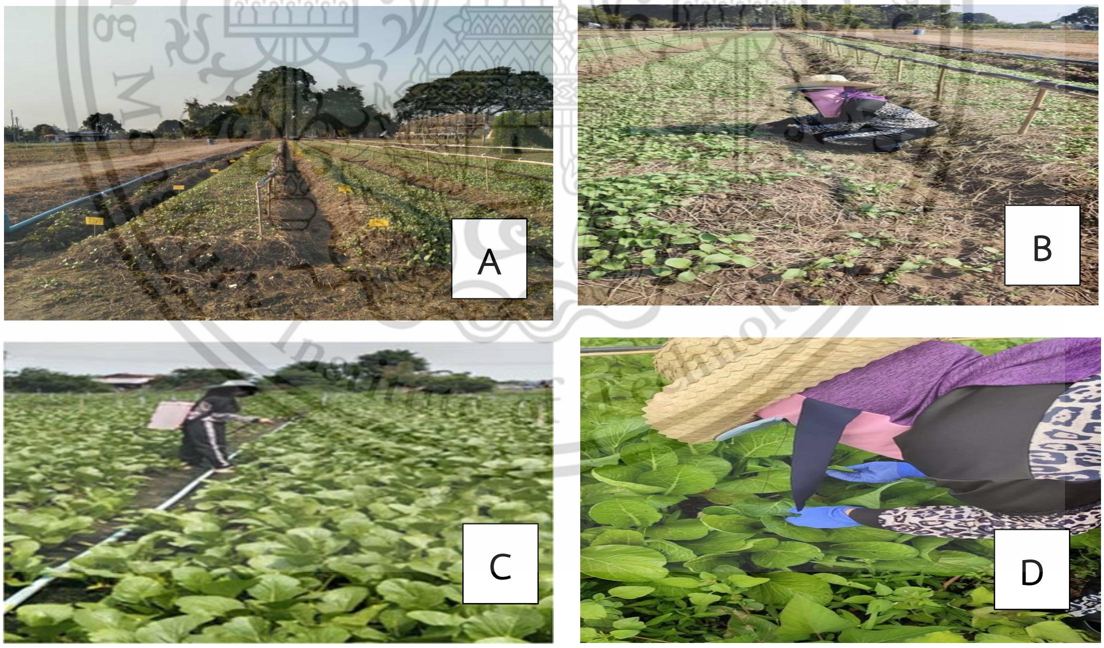

Figure 3.8 The insecticidal property test of essential oil and nanoemulsions against the flea beetle, diamondback moth and cutworm in field conditions by direct spray method, A: Treatment of field conditions, B: The removed grasses, C: The direct spray test, D: the insect test's observation and counting

CHAPTER 4

RESULTS

4.1 Examination of plant essential oils with high insecticidal activity

4.1.1 Toxicity of essential oils against flea beetle, diamondback moth and cutworm

Toxicity of 12 selected plant essential oils, namely cardamon, eucalyptus, holy basil, sweet basil, betel vine, cinnamon, lemongrass, star anise, clove, long pepper, black pepper, and turmeric against adult stage of the flea beetle showed that the star anise and clove essential oils at 1% concentration were most effective in 100% mortality of flea beetle (Figure 4.1). In second instar larva stage of the diamondback moth and cutworm testing, it showed that star anise and turmeric essential oils at 1% concentration were most effective in 100% mortality of diamondback moth (Figure 4.2) and cutworm (Figure 4.3).

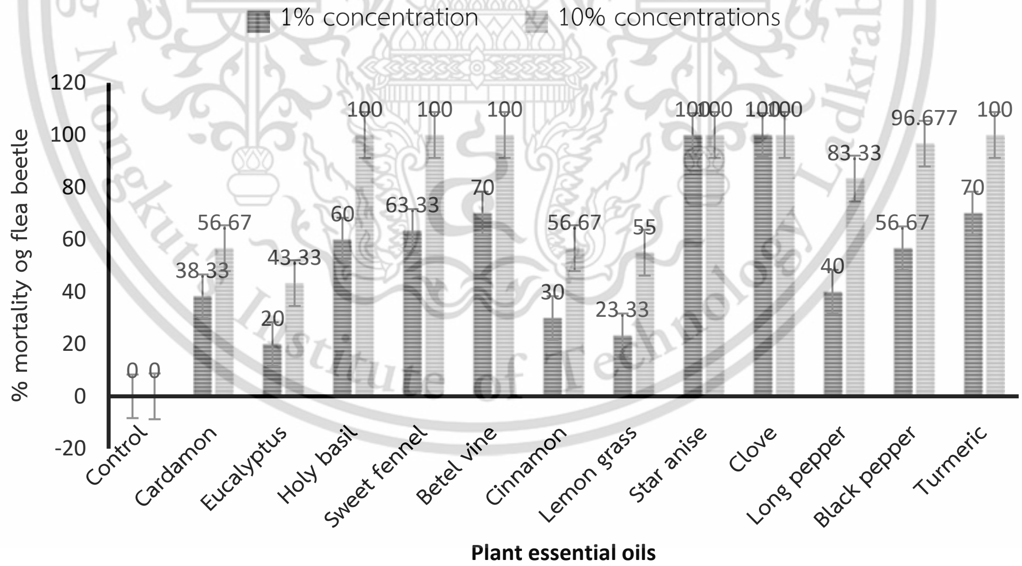

Figure 4.1. Mortality percentage of the flea beetle caused by different plant essential oils at 1% and 10% concentrations at 24 hours by leaf dipping method

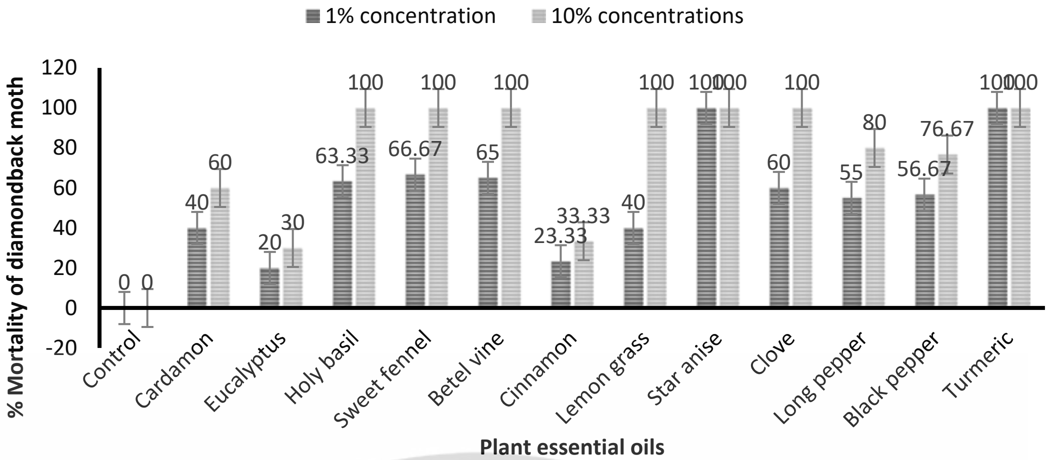

Figure 4.2. Mortality percentage of the diamondback moth caused by different plant essential oils at 1% and 10% concentrations at 24 hours by leaf dipping method

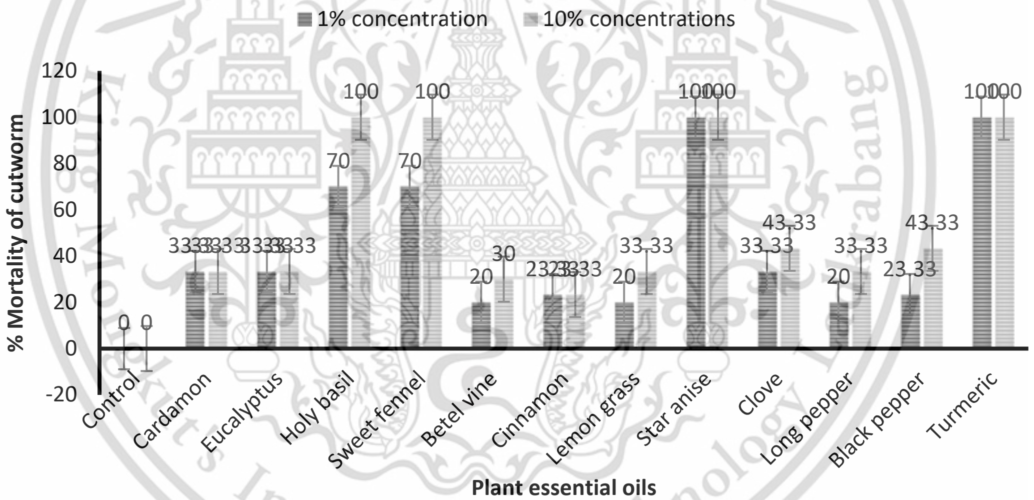

Figure 4.3 Mortality percentage of the cutworm caused by different plant essential oils at 1% and 10% concentrations at 24 hours by leaf dipping method

4.1.2 Toxicity of selected essential oils against flea beetle, diamondback moth and cutworm

The two selected plant essential oils as star anise were the highest effective insecticidal activity in flea beetle, such as 100% of mortality, and antifeedant at 0.75%. concentrations. While the star anise essential oils were the highest insecticidal effect in diamondback moth. It was found that 100% of mortality, antifeedant, and growth inhibition activities were at 1.00%, 0.75%, and 1.25% concentrations respectively whereas 0.75%, 0.25%, and 0.50% concentrations of star anise showed 100% mortality, antifeedant and growth inhibition activities, respectively.

Table 4.1 Insecticidal activity of plant essential oils against flea beetle, diamondback moth and Cutworm

Plant essential oils	**Average of mortality percentage**					
	Concentrations (%)					
	0.00	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**
Flea beetle						
Star anise	$0.00±0.00^{Ad}$	$20.00±0.41^{Ac}$	$53.33±0.58^{Ab}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$
Clove	$0.00±0.00^{Ae}$	$16.67±0.68^{Bd}$	$36.67±0.67^{Bc}$	$96.67±0.76^{Bb}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$
Diamondback moth						
Star anise	$0.00±0.00^{Ae}$	$8.33±0.53^{Ad}$	$30.00±0.44^{Ac}$	$66.67±0.89^{Ab}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$
Turmeric	$0.00±0.00^{Ae}$	$8.33±2.32^{Ad}$	$23.33±0.58^{Bc}$	$63.33±0.58^{Bb}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$
Cutworm						
Star anise	$0.00±0.00^{Ad}$	$36.00±1.53^{Bc}$	$56.67±0.44^{Bb}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$
Turmeric	$0.00±0.00^{Ad}$	$60.47±1.52^{Ac}$	$75.45±1.00^{Ab}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$
Plant essential oils	**Average of antifeedant percentage**					
	Concentrations (%)					
	0.00	0.25	0.50	0.75	1.00	1.25
Flea beetle						

Star anise	0.00±0.00^{Ae}	6.83±2.52^{Ac}	58.59±2.00^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Clove	0.00±0.00^{Ae}	3.33±0.58^{Bd}	37.89±1.00^{Bc}	62.11±3.21^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Diamondback moth						
Star anise	0.00±0.00^{Ad}	20.00±0.44^{Ac}	60.00±0.21^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ad}	13.40±0.58^{Bc}	53.31±0.78^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Cutworm						
Star anise	0.00±0.00^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Plant essential oils	**Average of growth inhibition of pupa percentage**					
	Concentrations (%)					
	0.00	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**
Diamondback moth						
Star anise	0.00±0.00^{Af}	20.00±0.00^{Ae}	23.33±0.98^{Bd}	46.67±0.67^{Ac}	71.67±0.58^{Ab}	100.00±0.43^{Aa}
Turmeric	0.00±0.00^{Af}	20.00±0.44^{Ae}	31.67±0.58^{Ad}	36.67±0.67^{Bc}	53.33±0.98^{Bb}	73.33±0.67^{Ba}
Cutworm						
Star anise	0.00±0.00^{Ac}	40.00±0.54^{Ab}	100.00±0.00^{Aa}	100.00 ±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ac}	40.00±0.54^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Plant essential oils	**Average of growth inhibition of adult percentage**					
	Concentrations (%)					
	0.00	**0.25**	**0.50**	**0.75**	**1.00**	**1.25**

Diamondback moth						
Star anise	$0.00±0.00^{Af}$	$26.67±0.31^{Ae}$	$33.33±0.28^{Ad}$	$66.67±0.58^{Ac}$	$83.33±0.58^{Ab}$	$100.00±0.00^{Aa}$
Turmeric	$0.00±0.00^{Af}$	$23.33±0.55^{Be}$	$33.33±0.44^{Ad}$	$61.67±0.76^{Bc}$	$81.67±0.78^{Bb}$	$100.00±0.58^{Aa}$
Cutworm						
Star anise	$0.00±0.00^{Ac}$	$50.00±0.00^{Ab}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$
Turmeric	$0.00±0.00^{Ac}$	$50.00±0.00^{Ab}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$	$100.00±0.00^{Aa}$

4.2 Plant essential oil and main chemical compound nanoemulsions preparation

The star anise, turmeric and clove essential oils and their main chemical compounds were selected to prepare emulsions with 5 surfactants (Tween 20, Tween 80, NP 9, PEG 400 and Span20) for nanoemulsion. It was found that their surfactants with hydrophile-lipophile balance (HLB) value between 12.9-16.7 with various ratios showed no precipitate and high solubility. The star anise essential oil with Tween 20 as ratios 1:4 showed the highly soluble and non – precipitated outcomes while turmeric essential oil with NP 9 as ratio 1:2 and clove essential oil with NP9 and tween20 as ratio 1:2.5:3 respectively showed highly soluble and non – precipitated outcomes. In addition, their main chemical compounds used the surfactant ratios similar to their plant essential oils. The trans-anethole emulsion with Tween 20 as ratio 1:2.5, the ar-turmerone emulsion with NP 9 as ratio 1:2 and the eugenol emulsion with NP 9 and tween 20 as ratio 1:1:2.5 showed highly soluble and non – precipitated outcomes. From the formula result, it was found that the star anise with turmeric and clove nanoemulsion formulas as ratio 1:1 showed highly soluble and non – precipitated outcomes while the nanoemulsion formulas of trans-anethole with ar-turmerone and eugenol at 1:1 ratio showed highly soluble and non – precipitated outcomes (Table 4.2).

Table 4.2 Capability of surfactants with essential oils and main chemical compound at different ratios to obtain emulsion (P: Precipitated)

Emulsion	Surfactant	Appropriate ratio
Essential oil		
Control	Tween 20	(1:1),(1:1.5),(1:2), (1:2.5), (1:3), (1:3.5), (1:4)
	Tween 80	(1:3)
	NP 9	(1:1),(1:1.5),(1:2),(1:2.5),(1:3), (1:3.5), (1:4),(1:4.5)
	PEG 400	(1:1),(1:1.5),(1:2),(1:2.5),(1:3), (1:3.5), (1:4),(1:4.5)
	Span 20	P
Star anise	Tween 20	(1:4)
	Tween 80	P
	NP 9	P
	PEG 400	P
	Span 20	P
Turmeric	Tween 20	P
	Tween 80	P
	NP 9	(1:2)
	PEG 400	P

	Span 20	P
Clove	Tween 20	P
	Tween 80	P
	NP 9	(1:2.5)
	PEG 400	P
	Span 20	P
Main Chemical compound		
Trans-anethole	Tween 20	(1:2.5)
	Tween 80	P
	NP 9	P
	PEG 400	P
	Span 20	P
Ar-turmerone	Tween 20	P
	Tween 80	P
	NP 9	(1:2)
	PEG 400	P
	Span 20	P
Eugenol	Tween 20	P
	Tween 80	P
	NP 9	(1:1)

PEG 400	P
Span 20	P

After that those emulsions were selected and measured in particle size and zeta potential by using a particle analyzer. It was found that the particle sizes of star anise, turmeric and clove nanoemulsions were 93.7, 16.1 and 19.5 nm, respectively whereas the trans-anethole, ar-turmerone and eugenol nanoemulsion were 13.4, 10.0 and 19.9 nm, respectively. The star anise with turmeric and clove nEO formulas were 15.5 and 15.8 nm, respectively while the trans-anethole with ar-turmerone and eugenol nanoemulsion formulas were 13.0 and 27.6 nm, respectively. The zeta potential of star anise, turmeric and clove nanoemulsions showed -29.41, -19.53 and -26.30 mV, respectively. Three main chemical compounds selected showed that the particle sizes of trans-anethole, ar-turmerone and eugenol nanoemulsions showed -21.52, -21.82 and -24.96 mV, respectively. The zeta potential of star anise with turmeric and clove formulas showed -19.09 and -22.18 mV, respectively while the zeta potential of trans-anethole with ar-turmerone and eugenol formulas had -21.82 and -18.39 mV, respectively. This study showed that three plant essential oils, three main chemical compounds, and these nanoemulsion formulas had small particle sizes lower than 100 nm (Table 4.3).

Table 4.3 The particle sizes of essential oils and main chemical compound emulsions with different ratios of surfactants

Emulsion	**Surfactant**	**Co-surfactant**	**Particle size (nm)**	**Zeta potential (mV)**
Essential oils				
Star anise			1,057.7	-0.57
Turmeric			468.5	-1.47
Clove			1,850.3	18.91
Trans-anethole			435.0	-7.15
Ar-turmerone			570.0	-13.06
Eugenol			1,919.5	1.37
Essential oil nanoemulsions				
Star anise	Tween 20		93.7	-29.41
Turmeric	NP 9		16.1	-19.53
Clove	NP 9	Tween 20	19.5	-26.30
Trans-anethole	Tween20		13.4	-21.52
Ar-turmerone	NP 9		10.9	-21.82
Eugenol	NP 9	Tween 20	19.9	-24.96
Essential oil nanoemulsion formulas				
Star anise : Turmeric (Formula II)			15.5	-19.09
Star anise : Clove (Formula I)			15.8	-22.18

Trans-anethole : Ar-turmerone (Formula IV)	13.0	-21.82
Trans-anethole : Eugenol (Formula III)	27.6	-18.39

4.3 Examination of essential oil and nanoemulsions with high insecticidal activity

4.3.1 Mortality effect of plant essential oils and nanoemulsions against the flea beetle, diamondback moth and cutworm

The mortality activity of the selected plant essential oil nanoemulsion against the flea beetle, diamondback moth and cutworm showed the 100% mortality at 0.40% concentration (figure 4.4). Then, the insecticidal activity evaluation revealed that plant essential oil nanoemulsions (nEOs) of star anise and clove at 0.35 % concentration could completely kill the adult of flea beetle while the star anise and clove emulsions at 0.35 concentration showed the 41.67% and 23.33% mortality of flea beetle, respectively. The larvae of diamondback moth and cutworm test showed that the star anise and turmeric nanoemulsions at 035 % concentration could 100% kill them whereas EOs of star anise and turmeric had 13.33% and 8.33% of mortality diamondback moth and 63.33% of cutworm at 0.35% concentration (Table 4.5).

The previous results showed that the higher concentration of nEOS could result in 100% mortality of flea beetle, diamondback moth and cutworm. Therefore, the concentration of nanoemulsion was lower than the previous test. It was found that the star anise and clove nanoemulsions at 0.35% concentration showed 100 % mortality of the flea beetle whereas the trans-anethole and eugenol were main chemical compounds (nMCs) of these plant nEOs. It was found that the trans-anethole and eugenol nanoemulsions had the highest mortality rate at 38.33% and 33.33%, respectively, at 0.35% concentration of the flea beetle while the formula of star anise with clove nEO showed that the highest flea beetle mortality rate at 75.00% of 0.35% concentration and the formula of trans-anethole with eugenol nanoemulsion had highest mortality at 36.67% of 0.35% concentration. The diamondback moth and cutworm test showed that the star anise and turmeric nanoemulsions at 0.35% concentration had the highest 100% mortality while the trans-anethole and ar-turmerone were main chemical compounds (nMCs) of these plant nEOs. It was found that the trans-anethole and ar-turmerone nanoemulsions had the highest mortality rate at 8.33% and 3.33% of diamondback moth and 38.33% and 33.33% of cutworm at 0.35% concentration of diamondback moth. Diamondback moth and cutworm test results showed that the star anise with turmeric and trans-anethole with ar-turmerone nanoemulsion formulas had the highest mortality at 63.33% and 3.33% of diamondback moth and 46.67% and 23.33% of cutworm, respectively.

However, for the flea beetle results, the LC_{50} values of EO, nEO and nMC star anise were 0.361, 0.176 and 0.357, respectively. The LC_{50} values of EO, nEO and nMC clove were 0.420, 0.192 and 0.390, respectively. The LC_{50} values of the star anise with clove nEOs and the trans-anethole with eugenol nEOs formulas were 0.279 and 0.396, respectively. In diamondback moth results, it was found that the LC50 values of EO, nEO and nMC star anise were 0.457, 0.183 and 0.546, respectively. The LC_{50} values of EO, nEO and nMC turmeric were 0.505, 0.193 and 0.518, respectively. The LC_{50} values of the star anise with turmeric nEOs and the trans-anethole with ar-turmerone were 0.272 and 0.546, respectively. In cutworm results, it was found that the LC50 values of EO, nEO and nMC star anise were 0.306, 0.245 and 0.358, respectively. The LC_{50} values of EO, nEO and nMC turmeric were 0.246, 0.246 and 0.367, respectively. The LC_{50} values of the star anise with turmeric nEOs and the trans-anethole with ar-turmerone were 0.343 and 0.396, respectively. From these results, the star anise nEO were mostly effective insecticidal activity of flea beetle, diamondback moth and cutworm at the lowest concentration

Table 4.4. Mortality percentage of the pretest flea beetle, diamondback moth and cutworm caused by various essential oil nanoemulsions at different concentrations by leaf dipping method under laboratory conditions.

Emulsion	Average of mortality percentage of flea beetle					
	Concentrations (%)					
	0.00	0.20	0.40	0.60	0.80	1.00
Essential oil nanoemulsions						
Star anise	0.00±0.00^{Ac}	71.67±0.55^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Clove	0.00±0.00^{Ac}	61.67±0.35^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Emulsion	Average of mortality Percentage of diamondback moth					
	Concentrations (%)					
	0.00	0.20	0.40	0.60	0.80	1.00
Essential oil nanoemulsions						
Star anise	0.00±0.00^{Ac}	76.67±0.84^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ac}	73.33±0.55^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Emulsion	Average of mortality Percentage of cutworm					
	Concentrations (%)					
	0.00	0.20	0.40	0.60	0.80	1.00
Essential oil nanoemulsion						
Star anise	0.00±0.00^{Ac}	30.00±0.56^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ac}	25.00±1.00^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}

Table 4.5 Mortality percentage of the flea beetle, diamondback moth and cutworm caused by various essential oil and main chemical compound nanoemulsions at different concentrations by leaf dipping method under laboratory conditions

Emulsion	Average of mortality percentage of flea beetle							
	Concentrations (%)							
	0.00	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**	LC_{50}
Essential oils								
Star anise	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ed}	0.00 ± 0.00^{Ed}	20.00 ± 0.44^{Dc}	21.67 ± 2.56^{Cc}	30.00 ± 0.44^{Cb}	41.67 ± 0.64^{Ca}	0.361
Clove	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ed}	0.00 ± 0.00^{Ed}	6.67 ± 0.79^{Ec}	18.33 ± 0.69^{Db}	21.67 ± 0.58^{Da}	23.33 ± 0.32^{Ea}	0.420
Essential oil nanoemulsion								
Star anise	0.00 ± 0.00^{Ag}	11.67 ± 0.53^{Af}	33.33 ± 0.58^{Ae}	71.67 ± 0.98^{Ad}	83.33 ± 0.58^{Ac}	96.67 ± 0.23^{Ab}	100.00 ± 0.00^{Aa}	0.176
Clove	0.00 ± 0.00^{Ag}	6.67 ± 0.58^{Bf}	25.00 ± 1.00^{Be}	63.33 ± 0.76^{Bd}	80.00 ± 0.44^{Ac}	91.67 ± 0.58^{Ab}	100.00 ± 0.00^{Aa}	0.192
Tran-anethole	0.00 ± 0.00^{Ag}	0.00 ± 0.00^{Ef}	13.33 ± 0.89^{Ce}	23.33 ± 0.32^{Dd}	33.33 ± 0.88^{Bc}	36.67 ± 0.44^{Cb}	38.33 ± 0.44^{Da}	0.357
Eugenol	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ee}	11.67 ± 0.76^{Cd}	21.67 ± 0.58^{Dc}	23.33 ± 0.75^{Cc}	31.67 ± 0.56^{Cb}	33.33 ± 0.55^{Da}	0.390
Essential oil nanoemulsion formulas								
Formula I	0.00 ± 0.00^{Ag}	8.33 ± 0.57^{Bf}	13.33 ± 0.58^{Ce}	30.00 ± 0.41^{Cd}	36.67 ± 0.98^{Bc}	53.33 ± 0.58^{Bb}	75.00 ± 1.00^{Ba}	0.279
Formula III	0.00 ± 0.00^{Ag}	5.00 ± 0.00^{Cf}	10.00 ± 0.23^{De}	20.00 ± 0.00^{Dd}	25.00 ± 1.00^{Cc}	28.33 ± 0.94^{Db}	36.67 ± 0.39^{Da}	0.396

Emulsion	Average of mortality percentage of diamondback moth							
	Concentrations (%)							
	0.00	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**	**LC50**
Essential oils								
Star anise	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Dc}	0.00 ± 0.00^{Dc}	0.00 ± 0.00^{Dc}	3.33 ± 0.38^{Db}	8.33 ± 0.38^{Db}	13.33 ± 0.58^{Ca}	0.457

Turmeric	0.00±0.00^{Ac}	0.00±0.00^{Dc}	0.00±0.00^{Dc}	0.00±0.00^{Dc}	3.33±0.45^{Db}	8.33±0.38^{Db}	8.33±0.38^{Da}	0.505
Essential oil nanoemulsion								
Star anise	0.00±0.00^{Ag}	10.00±0.00^{Af}	30.00±0.4^{Ae}	76.67±0.84^{Ad}	80.00±0.44^{Ac}	86.67±0.87^{Ab}	100.00±0.0^{Aa}	0.183
Turmeric	0.00±0.00^{Ag}	8.33±0.58^{Bf}	25.00±1.0^{Be}	73.33±0.79^{Bd}	77.67±0.58^{Bc}	80.00±0.00^{Bb}	100.00±0.0^{Aa}	0.193
Tran-anethole	0.00±0.00^{Ac}	0.00±0.00^{Dc}	0.00±0.00^{Dc}	0.00±0.00^{Dc}	0.00±0.00^{Ec}	3.33±0.74^{Eb}	8.33±0.48^{Da}	0.546
Ar-turmerone	0.00±0.00^{Ac}	0.00±0.00^{Dc}	0.00±0.00^{Dc}	0.00±0.00^{Dc}	0.00±0.00^{Ec}	3.33±0.58^{Eb}	3.33±0.58^{Ea}	0.518
Essential oil nanoemulsion formulas								
Formula II	0.00±0.00^{Ah}	3.33±0.58^{Cg}	16.67±0.50^{Cf}	36.67±0.34^{Cd}	53.33±0.58^{Cc}	58.33±0.58^{Cb}	63.33±0.76^{Ba}	0.272
Formula IV	0.00±0.00^{Ac}	0.00±0.00^{Dc}	0.00±0.00^{Dc}	0.00±0.00^{Dc}	0.00±0.00^{Ec}	3.33±1.24^{Ea}	3.33±0.58^{Ea}	0.546

Emulsion	Average of mortality percentage of cutworm							
	Concentrations (%)							
	0.00	0.10	0.15	0.20	0.25	0.30	0.35	LC50
Essential oils								
Star anise	0.00±0.00^{Ad}	0.00±0.00^{Ad}	0.00±0.00^{Bd}	0.00±0.00^{Cd}	30.00±0.44^{Bc}	56.66±0.78^{Bb}	63.33±0.51^{Ba}	0.306
Turmeric	0.00±0.00^{Ad}	0.00±0.00^{Ad}	0.00±0.00^{Bd}	0.00±0.00^{Cd}	30.00±0.23^{Bc}	51.67±0.54^{Bb}	63.33±0.75^{Ba}	0.246
Essential oil nanoemulsion								
Star anise	0.00±0.00^{Af}	0.00±0.00^{Af}	20.00±0.44^{Ae}	30.00±0.00^{Ad}	40.00±0.41^{Ac}	70.00±0.21^{Ab}	100.00±0.00^{Aa}	0.245
Turmeric	0.00±0.00^{Af}	0.00±0.00^{Af}	20.00±0.56^{Ae}	26.67±0.58^{Bd}	40.00±0.32^{Ac}	70.00±0.78^{Ab}	100.00±0.00^{Aa}	0.246
Tran-anethole	0.00±0.00^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Bc}	0.00±0.00^{Cc}	0.00±0.00^{Dc}	26.67±0.38^{Db}	38.33±0.75^{Da}	0.358

Ar-turmerone	0.00±0.00^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Bc}	0.00±0.00^{Cc}	0.00±0.00^{Dc}	23.33±0.76^{Db}	33.33±0.48^{Da}	0.367
Essential oil nanoemulsion formulas								
Formula II	0.00±0.00^{Ae}	0.00±0.00^{Ae}	0.00±0.00^{Be}	23.33±0.75^{Bd}	28.33±0.23^{Cc}	33.33±0.44^{cb}	46.67±0.98^{Ca}	0.343
Formula IV	0.00±0.00^{Ac}	0.00±0.00^{Ac}	0.00±0.00^{Bc}	0.00±0.00^{Cc}	0.00±0.00^{Dc}	13.33±0.78^{Eb}	23.33±0.58^{Ea}	0.396

Star anise with clove nEO formula: formulas I, star anise with turmeric nEO formula: formula II, Trans-anethole with Eugenol nEO formula: formula III, Tran-anethole with Ar-turmerone nEO formula: formula IV

4.3.2 Antifeedant effect of plant essential oils and nanoemulsion against the flea beetle, diamondback moth and cutworm

The antifeedant effects in no choice test were tested with nE0s and nMCs. The star anise and clove nEOs were the most effective antifeedant against the flea beetle at 0.25% concentration. The nMCs of star anise and clove showed the highest antifeedant rate at 20.00% of 0.35% concentration. While the star anise with clove and trans-anethole with eugenol nanoemulsion formulas had the highest antifeedant effect at 70.00% and 20.00% of 0.35%concentration. In the diamondback moth and cutworm results, the star anise and turmeric nEOs were the most effective antifeedant at 0.20% concentration against the diamondback moth and cutworm respectively. The nMCs of star anise and turmeric showed 100% antifeedant rate at 0.35% and 0.30% concentration in diamondback moth and cutworm respectively. While the star anise with turmeric and trans-anethole with ar-turmerone nanoemulsion formulas had the highest antifeedant effect at 0.35% concentration of diamondback moth and 0.20 and 0.30 concentrations of cutworm (Table 4.6).

The antifeedant in the choice test measured feeding area when compared to the control. The star anise and clove showed 100% of feeding inhibition of flea beetle at 0.20% concentrations. The feeding inhibition activity of flea beetle was mostly effective at 38.33% and 33.33%, respectively at 0.35% concentrations of nMCs of star anise and clove. The flea beetle antifeedant property of star anise with clove and their nCMs formulas was mostly effective at 75.00% and 36.67%, respectively, at 0.35% concentrations. The diamondback moth and cutworm antifeedant effect showed the highest effectiveness of 0.20% concentration of diamondback moth and 0.15% concentration of cutworm of star anise and clove nEOs, 0.30% concentration of their cMS nEOs and 0.25% and 0.30% concentrations in diamondback moth and 0.15% and 0.30% concentrations in cutworm of their nEOS and nCMs formulas. These results showed that the star anise nEOs were mostly effective antifeedant activity at the lowest concentration (Table 4.7).

Table 4.6 Antifeedant percentage of the flea beetle, diamondback moth and cutworm in no choice tests caused by various essential oil and main chemical compound nanoemulsions at different concentrations by leaf dipping method under laboratory conditions

Emulsion	Average of antifeedant percentage of flea beetle						
	Concentrations (%)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Essential oils							
Star anise	0.00 ± 0.00^{Af}	6.60 ± 1.03^{Be}	6.60 ± 1.03^{De}	10.00 ± 0.00^{Cd}	20.00 ± 0.00^{Cc}	26.60 ± 1.03^{Cb}	33.40 ± 0.52^{Ca}
Clove	0.00 ± 0.00^{Ag}	1.60 ± 1.03^{Df}	3.31 ± 0.00^{Ee}	5.00 ± 0.00^{Dd}	6.60 ± 1.03^{Dc}	20.00 ± 0.98^{Db}	30.00 ± 0.91^{Ca}
Essential oil nanoemulsions							
Star anise	0.00 ± 0.00^{Ad}	33.40 ± 3.93^{Ac}	53.40 ± 3.93^{Ab}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Clove	0.00 ± 0.00^{Ad}	5.00 ± 0.00^{Cc}	27.54 ± 2.83^{Bb}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Tran-anethole	0.00 ± 0.00^{Af}	0.00 ± 0.00^{Ef}	3.40 ± 2.04^{Ee}	5.00 ± 0.91^{Dd}	6.66 ± 1.33^{Dc}	10.00 ± 0.00^{Eb}	20.00 ± 0.91^{Da}
Eugenol	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ed}	1.60 ± 1.03^{Fc}	1.60 ± 1.03^{Fc}	6.66 ± 1.33^{Bb}	6.66 ± 1.33^{Fb}	20.00 ± 0.91^{Da}
Essential oil nanoemulsion formulas							
Formula I	0.00 ± 0.00^{Ah}	1.60 ± 1.03^{Dg}	13.40 ± 2.04^{Cf}	26.60 ± 0.34^{Bd}	33.40 ± 2.04^{Bc}	50.00 ± 0.91^{Bb}	70.00 ± 0.00^{Ba}
Formula III	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ee}	3.31 ± 3.17^{Ed}	3.31 ± 3.17^{Ed}	6.60 ± 1.03^{Dc}	20.00 ± 0.44^{Da}	20.00 ± 0.68^{Da}

Emulsion	Average of antifeedant percentage of diamondback moth						
	Concentrations (%)						
	0.00	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
Essential oils							
Star anise	0.00 ± 0.00^{Ag}	2.27 ± 2.84^{Ef}	11.36 ± 3.27^{De}	26.14 ± 3.89^{Ad}	43.18 ± 3.41^{Cc}	65.91 ± 2.66^{Cb}	100.00 ± 0.00^{Aa}
Turmeric	0.00 ± 0.00^{Ag}	1.14 ± 3.98^{Ef}	9.09 ± 1.83^{Ee}	17.05 ± 1.94^{Dd}	31.86 ± 5.50^{Dc}	54.55 ± 2.88^{Db}	100.00 ± 0.00^{Aa}
Essential oil nanoemulsions							
Star anise	0.00 ± 0.00^{Ad}	30.45 ± 1.34^{Ac}	67.39 ± 2.65^{Ab}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Turmeric	0.00 ± 0.00^{Ad}	24.20 ± 2.12^{Bc}	60.23 ± 1.03^{Bb}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Tran-anethole	0.00 ± 0.00^{Ag}	4.55 ± 2.45^{Df}	18.18 ± 3.21^{De}	26.14 ± 3.89^{Cd}	48.86 ± 2.04^{Cc}	73.86 ± 5.06^{Bb}	100.00 ± 0.00^{Aa}
Ar-turmerone	0.00 ± 0.00^{Ah}	2.27 ± 0.68^{Ef}	17.05 ± 2.93^{De}	24.20 ± 1.46^{Cd}	43.18 ± 3.41^{Cc}	69.32 ± 3.54^{Cb}	100.00 ± 0.00^{Aa}
Essential oil nanoemulsion formulas							
Formula II	0.00 ± 0.00^{Af}	17.05 ± 0.24^{Ce}	24.20 ± 0.34^{Cd}	53.41 ± 6.09^{Bc}	72.73 ± 3.46^{Bb}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Formula IV	0.00 ± 0.00^{Af}	4.55 ± 1.31^{Ef}	13.64 ± 2.17^{De}	19.32 ± 1.05^{Dd}	31.82 ± 4.78^{Dc}	56.82 ± 5.07^{Db}	100.00 ± 0.00^{Aa}

Emulsion	Average of antifeedant percentage of cutworm						
	Concentrations (%)						
	0.00	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
Essential oils							

Star anise	0.00±0.00^{Ae}	23.33±0.62^{Bd}	44.13±3.27^{Bc}	77.15±0.73^{Cb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ae}	35.15±0.78^{Ad}	51.67±0.58^{Ac}	85.12±2.11^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Essential oil nanoemulsions							
Star anise	0.00±0.00^{Ad}	24.30±0.00^{Bc}	45.30±0.45^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ad}	26.52±0.00^{Bc}	47.51±0.98^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Tran-anethole	0.00±0.00^{Af}	5.69±0.78^{Ce}	23.33±0.61^{Dd}	49.54±0.35^{Ec}	76.15±0.26^{Cb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Ar-turmerone	0.00±0.00^{Af}	7.87±2.16^{Ce}	31.15±0.15^{Cd}	55.12±1.03^{Dc}	81.23±1.34^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Essential oil nanoemulsion formulas							
Formula II	0.00±0.00^{Ad}	3.67±2.03^{Cc}	33.12±2.01^{Cb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Formula IV	0.00±0.00^{Af}	5.33±1.56^{Ce}	23.25±1.57^{Dd}	47.33±2.08^{Ec}	69.16±0.54^{Db}	100.00±0.00^{Aa}	100.00±0.00^{Aa}

Star anise with clove nEO formula: formulas I, star anise with turmeric nEO formula: formula II, Trans-anethole with Eugenol nEO formula: formula III, Tran-anethole with Ar-turmerone nEO formula: formula IV.

4.3.3. Growth inhibition of plant essential oils and nanoemulsions against the diamondback moth and cutworm

From Table 4.7, the star anise nEO had the highest effectiveness of growth inhibition of diamondback moth at 0.30% and 0.20% concentrations of pupa and adult, respectively. The highest growth inhibition of pupa and adult in cutworm showed 0.30% and 0.20% concentration, respectively, of star anise. The diamondback moth and cutworm results from these star anise nEO had the highest growth inhibition at low concentrations when compared with the other treatments (Table 4.8).

Table. 4.7 Antifeedant percentage of the flea beetle, diamondback moth and cutworm in choice test caused by various essential oil and main chemical compound nanoemulsions at different concentrations by leaf dipping method under laboratory conditions

Average of antifeedant percentage of flea beetle							
Emulsion	**Concentrations (%)**						
	0.00	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
Essential oils							
Star anise	0.00±0.00^{Ae}	0.00±0.00^{Ce}	0.00±0.00^{Ee}	20.00±0.00^{Cd}	21.67±0.33^{Cc}	30.00±0.00^{Db}	41.67±0.86^{Ca}
Clove	0.00±0.00^{Ae}	0.00±0.00^{Ce}	0.00±0.00^{Ee}	6.67±2.76^{Dd}	18.33±1.04^{Dc}	21.67±0.33^{Bb}	23.33±1.23^{Ea}
Essential oil nanoemulsions							
Star anise	0.00±0.00^{Ad}	11.67±5.23^{Ac}	53.33±2.84^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Clove	0.00±0.00^{Ad}	6.67±2.55^{Bc}	25.00±1.00^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Tran-anethole	0.00±0.00^{Af}	0.00±0.00^{Cf}	13.33±0.27^{Ce}	23.33±1.23^{Cd}	36.33±1.67^{Bc}	33.67±2.76^{Cb}	38.33±1.35^{Da}
Eugenol	0.00±0.00^{Af}	0.00±0.00^{Cf}	11.67±0.56^{Ce}	21.67±0.33^{Cd}	23.33±1.23^{Cc}	31.67±0.33^{Cb}	33.33±1.23^{Da}
Essential oil nanoemulsion formulas							
Formula I	0.00±0.00^{Ag}	8.33±2.86^{Bf}	13.33±2.44^{Ce}	30.00±0.44^{Bd}	36.67±2.11^{Bc}	53.33±2.04^{Bb}	75.00±1.03^{Ba}
Formula III	0.00±0.00^{Ag}	5.00±0.44^{Bf}	10.00±0.23^{De}	20.00±0.00^{Cd}	25.00±1.00^{Cc}	28.33±2.21^{Eb}	36.67±3.11^{Da}
Average of antifeedant percentage of diamondback moth							
Emulsion	**Concentrations (%)**						

	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Essential oils							
Star anise	0.00±0.00^{Af}	9.09±0.06^{De}	20.45±0.81^{Ed}	54.55±3.44^{Dc}	71.59±2.38^{bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Af}	3.41±2.88^{De}	18.18±1.56^{Fd}	31.82±3.21^{Fc}	65.91±2.76^{Cb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Essential oil nanoemulsions							
Star anise	0.00±0.00^{Ad}	60.23±1.21^{Ac}	88.64±2.10^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ad}	54.55±0.94^{Bc}	82.95±3.24^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Tran-anethole	0.00±0.00^{Af}	4.55±0.42^{De}	35.23±3.29^{Dd}	54.55±1.67^{Dc}	71.59±2.38^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Ar-turmerone	0.00±0.00^{Af}	2.27±0.64^{De}	32.95±3.11^{Dd}	48.86±1.54^{Ec}	69.59±1.12^{Cb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Essential oil nanoemulsion formulas							
Formula II	0.00±0.00^{Ae}	31.82±0.47^{Cd}	54.55±0.32^{Bc}	71.59±0.38^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Formula IV	0.00±0.00^{Af}	3.41±0.21^{De}	48.86±3.24^{Cd}	60.23±1.11^{Cc}	71.59±0.73^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}

Emulsion	Average of antifeedant percentage of cutworm						
	Concentrations (%)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Essential oils							
Star anise	0.00±0.00^{Ad}	46.67±0.83^{Cc}	83.33±1.15^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ad}	52.33±2.03^{Bc}	71.63±1.02^{Cb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Essential oil nanoemulsions							

Star anise	0.00±0.00^{Ac}	86.67±0.90^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ac}	88.33±1.28^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Tran-anethole	0.00±0.00^{Ag}	33.33±0.38^{Df}	41.67±0.81^{De}	75.00±1.00^{Bd}	91.67±0.81^{Bc}	98.33±0.41^{Bb}	100.00±0.00^{Aa}
Ar-turmerone	0.00±0.00^{Ag}	33.33±0.38^{Df}	41.12±0.35^{De}	63.33±0.55^{Cd}	88.33±0.79^{Cc}	96.67±0.64^{Cb}	100.00±0.00^{Aa}
Essential oil nanoemulsion formulas							
Formula II	0.00±0.00^{Ac}	83.33±0.71^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Formula IV	0.00±0.00^{Af}	25.00±1.09^{Ee}	45.00±1.10^{Dd}	55.00±1.00^{Dc}	83.33±2.16^{Cb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}

Star anise with clove nEO formula: formulas I, star anise with turmeric nEO formula: formula II, Trans-anethole with Eugenol nEO formula: formula III, Tran-anethole with Ar-turmerone nEO formula: formula IV.

4.3.4 Repellent activity of plant essential oils and nanoemulsions against flea beetle

For the repellent result, the star anise nEO at 0.03% concentration had the highest repellent effect of 81.67% while the nCMs of star anise at 0.03% had the highest repellent effect of 71.67%. At 0.03% of nEO formulas had the highest effectiveness of repellent activity at 71.67% concentrations (Figure 4.4).

4.4. Examination of essential oil nanoemulsions and chemical insecticide with high insecticidal activity

The highest effectiveness of star anise nEO showed that the star anise nEO was extremely effective at 100% killing adults of flea beetles and larvae of diamondback moth and cutworm at 24 hours when compared with the control while deltamethrin had a lower mortality rate (Table 4.9).

Table.4.8 Growth inhibition percentage of the diamondback moth and cutworm in choice tests caused by various essential oil and main chemical compound nanoemulsions at different concentrations by leaf dipping method under laboratory conditions

Emulsion	Average of growth inhibition percentage of pupa diamondback moth						
	Concentrations (%)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Essential oils							
Star anise	0.00 ± 0.00^{Ae}	13.67 ± 0.56^{Bd}	13.67 ± 0.76^{Dd}	23.33 ± 0.89^{Dc}	23.33 ± 0.58^{Dc}	26.67 ± 0.76^{Db}	33.33 ± 0.89^{ca}
Turmeric	0.00 ± 0.00^{Af}	6.67 ± 0.78^{Ee}	11.67 ± 0.76^{Dd}	16.67 ± 0.41^{Ec}	16.67 ± 0.75^{Ec}	21.67 ± 0.39^{Db}	23.33 ± 1.21^{Da}
Essential oil nanoemulsions							
Star anise	0.00 ± 0.00^{Af}	16.67 ± 0.76^{Ae}	50.00 ± 0.44^{Ad}	86.67 ± 0.76^{Ac}	90.00 ± 0.23^{Ab}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Turmeric	0.00 ± 0.00^{Ae}	10.00 ± 2.32^{Cd}	35.67 ± 0.45^{Bc}	80.00 ± 0.44^{Bb}	80.00 ± 0.44^{Bb}	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Tran-anethole	0.00 ± 0.00^{Af}	13.33 ± 0.58^{Be}	23.33 ± 0.82^{Cd}	23.33 ± 0.45^{Dd}	26.67 ± 0.76^{Dc}	31.67 ± 0.75^{Cb}	36.67 ± 0.34^{Ca}
Ar-turmerone	0.00 ± 0.00^{Af}	6.67 ± 0.76^{Ee}	16.67 ± 0.76^{Dd}	23.33 ± 0.92^{Dc}	23.33 ± 0.58^{Dc}	26.67 ± 0.87^{Db}	33.33 ± 0.58^{Ca}
Essential oil nanoemulsion formulas							
Formula II	0.00 ± 0.00^{Ag}	8.33 ± 0.58^{Df}	23.33 ± 0.58^{Ce}	30.00 ± 0.44^{Cd}	61.67 ± 0.75^{Cc}	63.33 ± 0.86^{Bb}	83.33 ± 0.87^{Ba}
Formula IV	0.00 ± 0.00^{Ag}	3.33 ± 0.58^{Ff}	6.67 ± 0.76^{Ee}	10.00 ± 2.45^{Fd}	11.67 ± 0.32^{Ec}	18.33 ± 0.44^{Eb}	26.67 ± 0.76^{Da}

	Average of growth inhibition percentage of adult diamondback moth						
	Concentrations (%)						
Emulsion	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Essential oils							
Star anise	0.00 ± 0.00^{Ae}	26.67 ± 0.76^{Bd}	33.33 ± 0.58^{Cc}	33.33 ± 0.58^{Cc}	33.33 ± 0.58^{Cc}	51.59 ± 0.70^{Cb}	50.00 ± 0.44^{Ca}
Turmeric	0.00 ± 0.00^{Ag}	13.33 ± 0.58^{Gf}	18.18 ± 0.19^{Ee}	31.82 ± 0.29^{Dd}	33.33 ± 0.75^{Cc}	$40.00\pm0.^{Db}$	43.33 ± 0.23^{Da}

Essential oil nanoemulsions							
Star anise	0.00±0.00^{Ad}	30.00±0.44^{Ac}	73.33±0.55^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ad}	20.00±0.00^{Dc}	62.95±1.45^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Tran-anethole	0.00±0.00^{Ag}	11.67±0.75^{Ff}	21.67±0.86^{De}	31.67±0.76^{Dd}	33.33±0.58^{Cc}	36.67±0.36^{Eb}	46.67±0.76^{Da}
Ar-turmerone	0.00±0.00^{Af}	16.67±0.43^{Ee}	21.67±0.65^{Dd}	31.67±0.89^{Dc}	31.67±1.46^{Dc}	36.67±0.23^{Eb}	43.33±0.58^{Da}
Essential oil nanoemulsion formulas							
Formula II	0.00±0.00^{Ag}	23.33±0.58^{Cf}	33.33±0.56^{Ce}	41.67±1.16^{Bd}	53.33±0.59^{Bc}	73.33±0.67^{Bb}	90.00±0.00^{Ba}
Formula IV	0.00±0.00^{Ag}	6.67±0.76^{Hf}	11.67±0.46^{Fe}	16.67±1.23^{Ed}	21.59±2.08^{Ec}	36.67±0.43^{Eb}	41.67±0.76^{Da}
	Average of growth inhibition percentage of pupa cutworm						
	Concentrations (%)						
Emulsion	**0.00**	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
Essential oils							
Star anise	0.00±0.00^{Aa}	16.67±0.98^{Aa}	16.67±1.15^{Aa}	23.33±0.58^{Aa}	23.33±0.58^{Aa}	46.67±0.76^{Aa}	83.33±0.48^{Aa}
Turmeric	0.00±0.00^{Aa}	16.67±0.56^{Aa}	21.67±0.87^{Aa}	26.67±0.34^{Aa}	26.67±0.76^{Aa}	31.67±1.23^{Aa}	83.33±0.69^{Ba}
Essential oil nanoemulsions							
Star anise	0.00±0.00^{Af}	10.00±0.00^{Ae}	20.00±0.44^{Bd}	36.67±0.76^{Bc}	70.00±0.75^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Af}	10.00±0.44^{Ae}	25.67±0.35^{Ad}	40.00±0.65^{Ac}	70.00±0.44^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Tran-anethole	0.00±0.00^{Ag}	3.33±0.45^{Ef}	13.33±0.58^{De}	23.33±0.55^{Dd}	33.33±0.98^{Bc}	61.67±0.79^{Bb}	66.67±0.76^{Da}
Ar-turmerone	0.00±0.00^{Ag}	6.67±0.79^{Ce}	16.67±0.76^{Cd}	23.33±0.58^{Dc}	23.33±0.52^{Cc}	66.67±0.42^{Bb}	73.33±0.44^{Ca}
Essential oil nanoemulsion formulas							
Formula II	0.00±0.00^{Ag}	8.33±0.97^{Bf}	23.33±0.28^{Ae}	30.00±0.75^{Cd}	31.67±0.56^{Bc}	53.33±0.34^{Cb}	75.00±1.00^{Ca}
Formula IV	0.00±0.00^{Ag}	5.67±0.34^{Df}	11.67±0.75^{Ee}	20.00±0.44^{Ed}	25.00±1.00^{Cc}	38.33±0.58^{Db}	46.67±0.76^{Ea}
	Average of growth inhibition percentage of adult cutworm						

Emulsion	Concentrations (%)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Essential oils							
Star anise	0.00±0.00^{Ae}	26.67±0.78^{Bd}	33.33±0.58^{Bc}	33.33±0.68^{Cc}	33.33±0.28^{Ec}	71.59±0.46^{Bb}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Af}	3.41±2.05^{De}	18.18±2.34^{Dd}	31.82±1.20^{Cc}	65.91±1.56^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Essential oil nanoemulsions							
Star anise	0.00±0.00^{Ad}	90.00±0.23^{Ac}	93.33±0.58^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ad}	90.00±0.44^{Ac}	92.95±0.41^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Tran-anethole	0.00±0.00^{Ag}	11.67±0.69^{Cf}	21.67±0.75^{Ce}	31.67±0.56^{Cd}	43.33±0.58^{Dc}	66.67±0.54^{Cb}	76.67±0.92^{Ca}
Ar-turmerone	0.00±0.00^{Ag}	16.67±0.76^{Cf}	31.67±0.34^{Be}	33.33±0.75^{Cd}	51.67±0.76^{Cc}	76.67±0.44^{Bb}	91.67±0.71^{Ba}
Essential oil nanoemulsion formulas							
Formula II	0.00±0.00^{Ag}	23.33±0.91^{Bf}	33.33±0.58^{Be}	41.67±0.79^{Bd}	53.33±0.45^{Cc}	73.33±0.58^{Bb}	100.00±0.00^{Aa}
Formula IV	0.00±0.00^{Ag}	16.67±0.67^{Cf}	21.67±0.67^{Ce}	26.67±0.55^{Dd}	41.59±0.73^{Dc}	56.67±0.75^{Db}	61.67±0.76^{Da}

Star anise with clove nEO formula: formulas I, star anise with turmeric nEO formula: formula II, Trans-anethole with Eugenol nEO formula: formula III, Tran-anethole with Ar-turmerone nEO formula: formula IV.

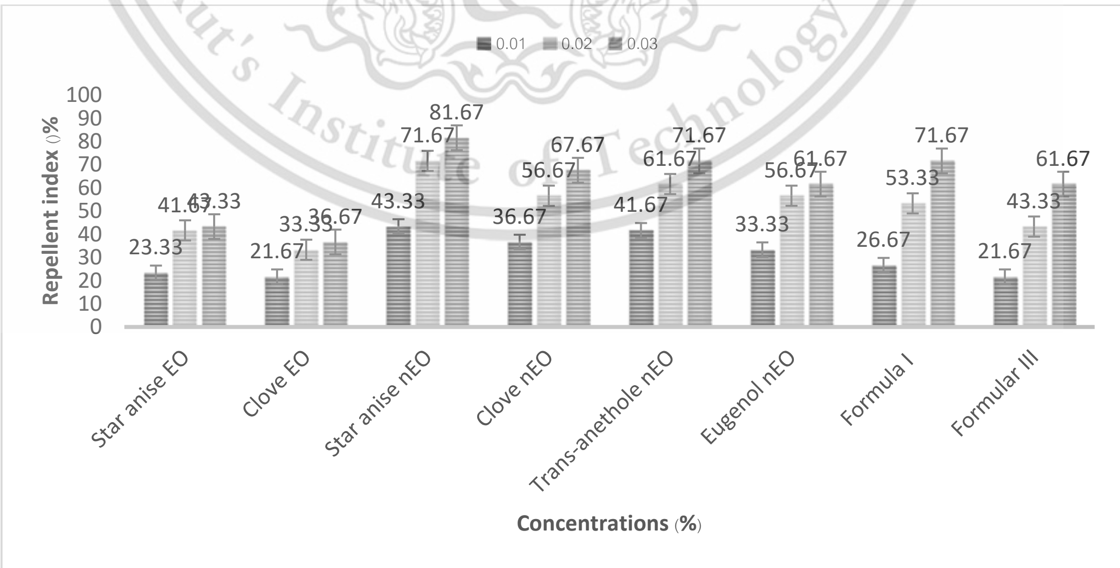

Figure 4.4 Repellent percentage of the flea beetle caused by various concentrations of emulsion at 24 hours by leaf dipping method

Table 4.9 The mortality percentage of flea beetle, diamondback moth and cutworm caused by different treatments of essential oil nanoemulsions and chemical insecticide at 24 hours

Essentail oil nanoemulsion / Chemical insecticide	**Average of mortality Percentage of flea beetle at 24 hours**
T 1 (Control)	0.00 ± 0.00[F]
T 2 (Star anise nanoemulsion (at 0.25% concentration))	81.70 ± 1.52[E]
T 3 (Star anise nanoemulsion (at 0.35% concentration))	100.00 ± 0.00[A]
T 4 (Deltamethrin (recommended ratio))	96.70 ± 0.52[B]
T 5 (Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.15% concentration))	83.33 ± 1.86[D]
T 6 (Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.25% of concentration))	86.70 ± 0.82[C]

Essentail oil nanoemulsion / Chemical insecticide	**Average of mortality Percentage of Diamondback moth at 24 hours**
T 1 (Control)	0.00 ± 0.00[E]

T 2 (Star anise nanoemulsion (at 0.25% concentration))	85.00 ± 1.87^{C}
T 3 (Star anise nanoemulsion (at 0.35% concentration))	100.00 ± 0.00^{A}
T 4 (Deltamethrin (recommended ratio))	98.30 ± 0.41^{B}
T 5 (Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.15% concentration))	52.20 ± 0.71^{D}
T 6 (Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.25% of concentration))	95.00 ± 0.55^{B}

Essentail oil nanoemulsion / Chemical insecticide	**Average of mortality Percentage of cutworm** at 24 hours
T 1 (Control)	0.00 ± 0.00^{E}
T 2 (Star anise nanoemulsion (at 0.25% concentration))	42.10 ± 0.89^{B}
T 3 (Star anise nanoemulsion (at 0.35% concentration))	100.00 ± 0.00^{A}
T 4 (Deltamethrin (recommended ratio))	95.00 ± 0.55^{B}

T5 (Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.15% concentration))	48.5 ± 0.71[D]
T 6 (Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.25% of concentration))	68.7 ± 0.65[C]

4.5. Greenhouse conditions

In the greenhouse conditions, the experiment using 0.25% and 0.35% concentrations of star anise essential oil nanoemulsion to control the flea beetle, diamondback moth, and cutworm in a Chinese cabbage field, compared to chemical insecticide (Deltamethrin) was performed by direct spray method. At 0.35% concentration, star anise showed the lowest number of adult flea beetle, larvae of diamondback moth, and cutworms at 3, 5, and 7 days after treatment observation while Deltamethrin showed a higher number of these insect tests when compared with control treatment (Figure 4.5, 4.6, 4.7).

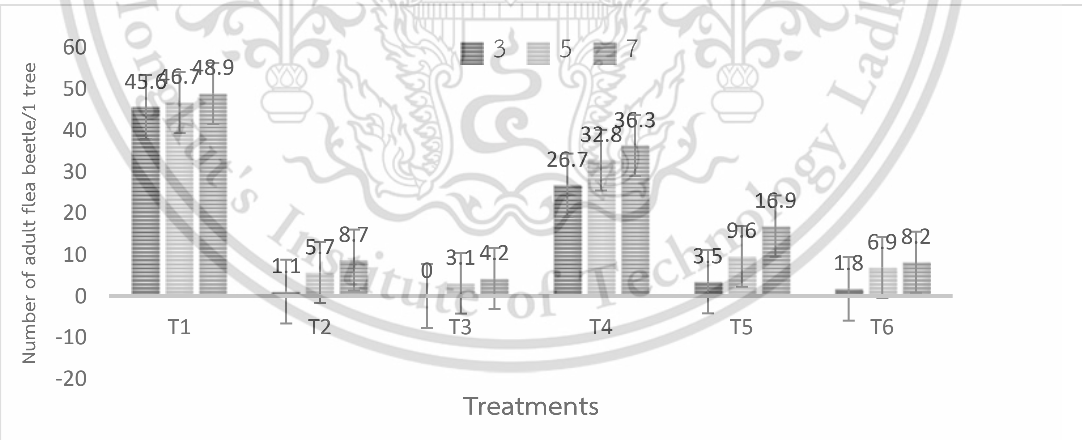

Figure 4.5 Number of adult flea beetle caused by star anise essential oil nanoemulsion and chemical insecticides by direct spray method. (T1 : Control, T2 : Star anise nanoemulsion (at 0.25% concentration), T3 : Star anise nanoemulsion (at 0.35% concentration), T4 : Deltamethrin (recommended ratio), T5 : Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.15% concentration), T6 : Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.25% concentration).

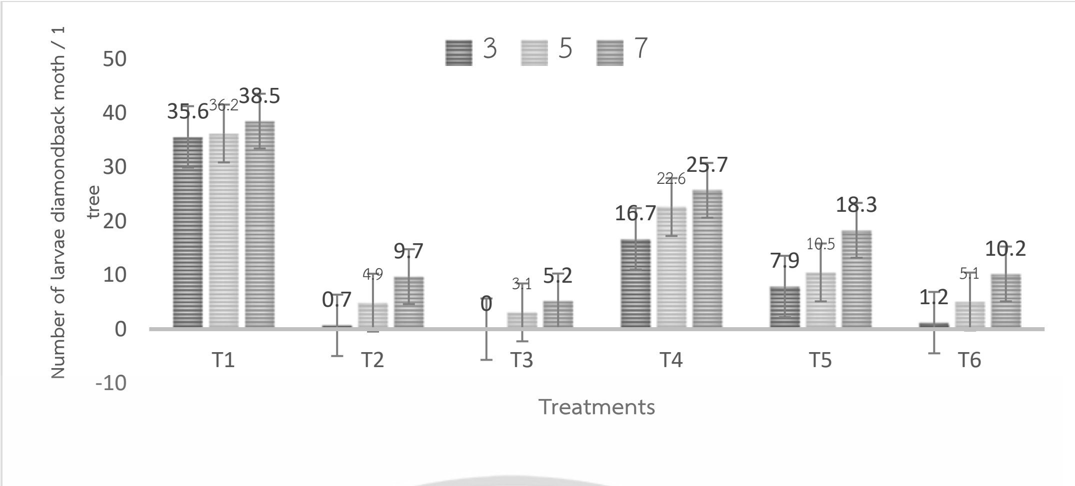

Figure 4.6 Number of larva diamondback moth caused by star anise essential oil nanoemulsion and chemical insecticide by direct spray method. (T1 : Control, T 2 : Star anise nanoemulsion (at 0.25% concentration), T3 : Star anise nanoemulsion (at 0.35% concentration), T4 : Deltamethrin (recommended ratio), T5 : Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.15% concentration), T6 : Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.25% concentration).

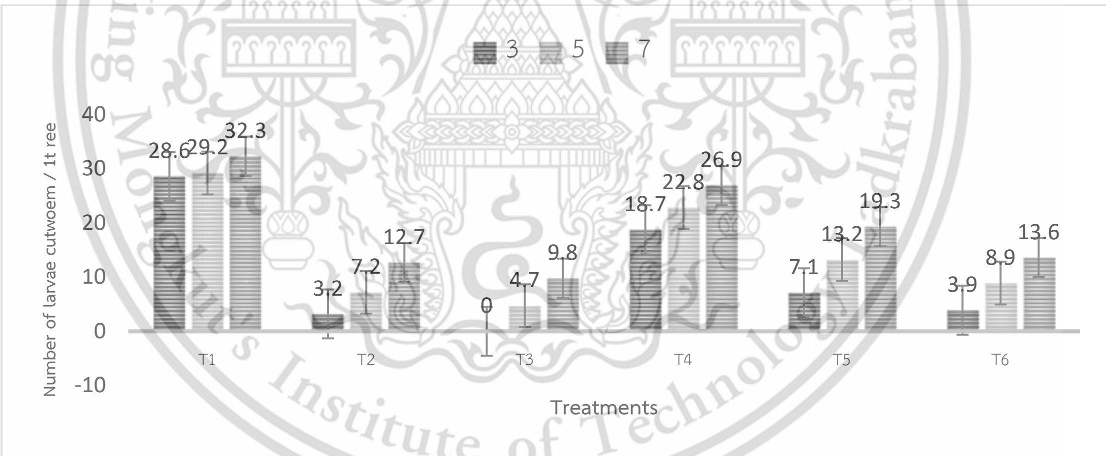

Figure 4.7 Number of larva cutworm caused by star anise essential oil nanoemulsion and chemical insecticide by direct spray method. (T1 : Control, T2 : Star anise nanoemulsion (at 0.25% concentration), T 3 : Star anise nanoemulsion (at 0.35% concentration), T4 : Deltamethrin (recommended ratio), T5 : Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.15% concentration), T6 : Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.25% concentration).

4.6 Field conditions

In the field conditions, the experiment using 0.25% and 0.35% concentrations of star anise essential oil nanoemulsion to control the flea beetle, diamondback moth, and cutworm in a Chinese cabbage field, compared to chemical insecticide and deltamethrin was performed by direct spray method at 3, 5 and 7 days. After observation, it was revealed that the star anise nanoemulsion at 0.35% star anise concentrations showed the highest control percentage of 85.45 % at 3 days while deltamethrin insecticide showed a lower control percentage when compared with the control (Figure 4.8).

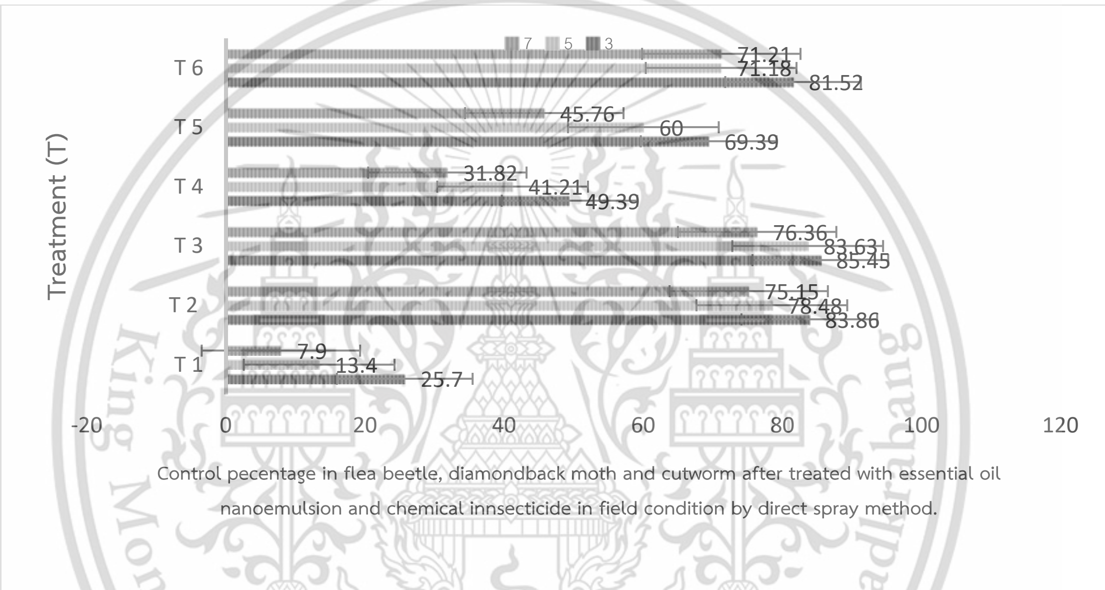

Figure 4.8 The Control percentage in the flea beetle, diamondback moth and cutworm after tested with essential Oil nanoemulsion in field conditions. (T1 : Control, T2 : Star anise nanoemulsion (at 0.25% concentration), T3 : Star anise nanoemulsion (at 0.35% concentration), T4 : Deltamethrin (recommended ratio), T5 : Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.15% concentration), T6 : Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.25% concentration).

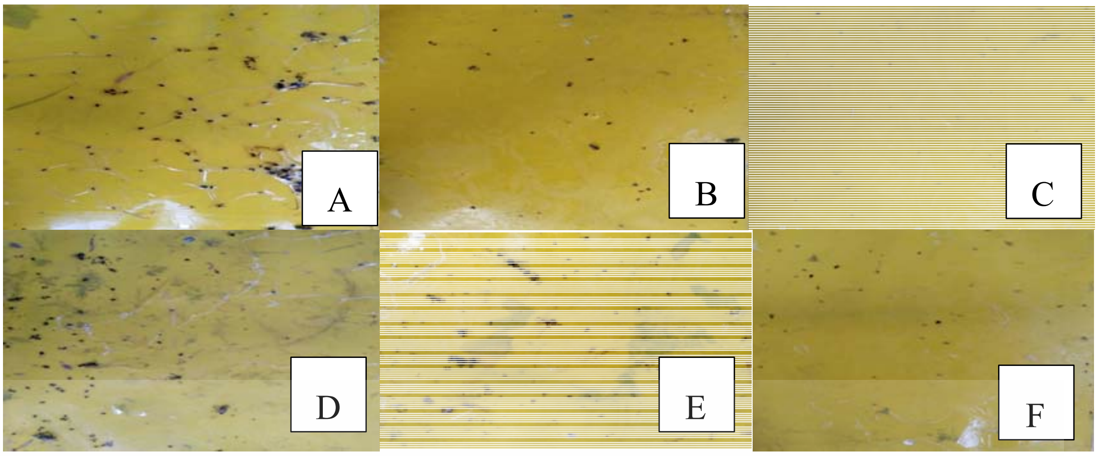

Figure 4.9 The yellow sticky trap in seven days after tested in the field conditions. A: T1: Control, B: T2: Star anise nanoemulsion (at 0.25% concentration), C: T3: Star anise nanoemulsion (at 0.35% concentration), D: T 4: Deltamethrin (recommended ratio), E: T5 : Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.15% concentration). and F: T6: Deltamethrin (half of recommended ratio) and star anise nanoemulsion (0.25% concentration).

CHAPTER 5
DISCUSSION

5.1. Effective essential oil emulsion and main chemical compound with highly insecticidal activity

In the present study, of 12 selected plant essential oils in this study, only 3 essential oils: star anise, turmeric, and clove showed highly insecticidal activities against adults of flea beetle and instar larvae of diamondback moth and cutworm. These essential oils (EOs) presented insecticidal properties such as mortality, antifeedant, growth inhibition, and repellent activities (Isman, 2000). The three selected EOs: star anise, turmeric and clove showed a high insecticidal effect on flea beetle, diamondback moth, and cutworm. The main chemical compound of star anise, turmeric, and clove showed trans-anethole, Ar-turmerone and eugenol. It was found that the star anise EO had trans-anethole, caryophyllene, and limonene at 82.7%, 4.8% and 2.3% based on GC/MS analysis (Aly *et al.* 2016). While the chemical compound of star anise EO showed 9.7%, 7.5%, and 10.1% of trans-anethole in methyl alcohol, ethyl acetate, and petroleum ether extraction, respectively (Wei *et al.* 2014). The main chemical compounds of star anise EO included phenylpropanoids, flavonoids, neolignans, monoterpenoids, and sesquiterpenoids. The phenylpropanoid trans-anethole was the main component of star anise EO. The content of trans-anethole had 70.61% of solvent extraction and 74.96% of steam distillation (Sharafan *et al.* 2022). The main chemical compounds of turmeric EO from fresh rhizome included aromatic-turmerone, alpha-turmerone, and beta turmerone at 24.4%, 20.5%, and 11.1% respectively whereas dried rhizome included aromatic-turmerone, alpha-turmerone, and beta turmerone at 53.4%, 18.1%, and 6.2% respectively. The alpha-turmerone was the major component of dried and fresh rhizomes (Singh *et al.* 2010). The turmeric EO was extracted through steam distillation. It was found that the main components included alpha-zingiberence, aromatic-turmerone, beta-sequiphellandrene, alpha turmerone, beta-turmerone, and beta-bisabolene at 27.70-36.75%, 19.54-32.24%, 13.14-18.23%, 3.72-5.60%, and 2.50-3.46%, respectively by using GC-MS (Hwang *et al.* 2016). Eugenol is the main chemical compound of fresh clove (Salvador *et al.* 2014). The main chemical compounds of clove EO from the leaf extracted included eugenol, beta-caryophyllene, alpha-humulene, and eugenyl acetate at 76.8%, 17.4%, 2.1%, and 1.2%, respectively (Jirovetz *et al.* 2006). It was found that their main chemical compounds had high insecticidal activity.

The previous studies showed the insecticidal property of various plant essential oils. For example, the star anise EO showed growth inhibition in the Gypsy moth (Kostić *et al.* 2021). While the Chinese star anise was completely inhibited in the pupa stage of a housefly, *Musca domestica* (Guntharee, 2008). Red flour beetle results showed that the turmeric EO was a repellent and growth-inhibiting effect. Many larvae of the red flour beetle had a lower time period of development when compared with the control (Jilani and Su, 1983). The turmeric extract showed high toxicity and repellent effect against *Sitophilus zeamais* and *Spodoptera frugiperda* (Tavares *et al*. 2013). While turmeric extract had insecticidal activity against *Aedes aegypti* Linnaeus (Sukari *et al.* 2010). *Bactrocera zonata* result showed that the turmeric extract had high toxicity (Siddiqi *et al*. 2011). Ali *et al*. (2014) showed the *Curcuma longa* had antifeedant, toxicity and growth inhibited activities on red flour beetle. Chowdhury *et al.* (2000) showed that the turmeric had high growth inhibition on *Schistocerca gregaria* and *Dysdercus koenigii*. The clove, cinnamon and lemon grass EOs showed highly insecticidal effect against adult stage of thrips, mealybug and store product mites (Pumnuan *et al.* 2021; Pumnuan and Insung, 2016). The clove, citronella grass, cinnamon and lemongrass EOs had high toxicity of the European house dust mite (Pumnuan *et al.* 2020). It was found that their main chemical compounds had high insecticidal activity. Trans – anethole was the main chemical compound of thymol showing a high insecticidal activity on *Spodoptera litura* (Passreiter *et al.* 2004). Ar-turmerone was the main compound of turmeric showing an insecticidal effect on *Nilaparvata lugens* and *Plutella xylostella* (Lee, 2001). Eugenol from clove EO had potential to control *Sitophilus zeamais* (Prates *et al.* 1988).

5.2. Plant essential oil and main chemical compound nanoemulsions preparation

The plant essential oils (EOs) used botanic insecticides and low toxicity to the environment. However, these EOs were easily degraded by external factors and environment, such as air, light and temperature (Isman, 2020). Therefore, the plant essential oil nanoemulsions (nEOs) were developed for resolving disadvantages in EOs (Isman, 2020). The quality of nanoemulsions was developed, and their biological effects were dependent on the cultivated area and chemical of EOs. The good formulation of nEOs showed the good homogeneous of the distribution of droplet size. The surfactant used more than co-surfactant, resulting in the good stability of nanoemulsions (Jintapattanakit, 2018).

In this study, nEOs were prepared using a mixed surfactants and/or co-surfactants for reducing droplet size. Tweens and NP9 are water solute nonionic surfactants. They were dispersed in water to come to the surface again. They were mainly used as an emulsifier in the agricultural industry. In this nanoemulsions process, they were prepared by aqueous titration method. They used surfactant and co-surfactant at an appropriate ratio. They were self nanoemulsions and can be produced smaller than 100 nm of droplet size. The dynamic light scattering (DLS) technique was used to analyze the particle size of star anise, turmeric and clove nEOs at 93.7, 16.1 and 19.5 nm, respectively. The zeta potential of these nEOs ranged from -29.41, -19.53 and -26.30 mV, respectively. The particle size and zeta potential of these main chemical compounds ranged from 13.4, 10.9 and 19.9 nm and -21.52, -21.82 and -24.96 mV, respectively whereas the particle size of star anise with turmeric and clove nEOs formulas ranged from 15. to 15.8 nm diameter and the zeta potential ranged from -19.09 to -22.18 mV, respectively. While these particle size and zeta potential of main compound nEOs formulas ranged from 13.0 to 27.6 nm and from -21.82 to -18.39 mV, respectively. These particle sizes of nEOs in this study were lower than 100 nm and these zeta potential values were close to 30 mV. It was found that the zeta potential values about 30 mV had physical stability (Marsalek, 2014). Therefore, the nEOs with small particle size could be effective better than the large size. Additionally, using surfactant and co-surfactant to prepare the nEOs resulted in the increase insecticidal effect on flea beetle, diamondback moth and cutworm.

5.3. Effective plant essential oil and main chemical compound nanoemulsions with highly insecticidal activity

Secondary plant metabolites had behavior and biological efficiency on insect pests (Güncan and Durmuşoğlu, 2004). Alkaloids, glycosides, phenols, terpenoids, tannins, and saponins were the efficiency of compounds against insect pests (Shanker and Solanki, 2000). These compounds played a major role as defense mechanism in plant against insect pests, such as toxicity, antifeedant, growth inhibition and repellent effects. Trans-anethole is a secondary metabolite, synthesized from star anise. Its main components against stored product insect pests were very good (Hikal *et al.* 2017). It was found that trans-anethole was against the main stored product insects (Shaaya *et al.* 1991; Ho, 2000; Mondal and Khalequzzaman, 2010). It was found that it did not have residual toxicity on treated wheat grain in major stored-product insects testing (Alkan and Ertürk, 2018). Trans- anethole was the main chemical compound of star anise with high potential to inhibit acetylcholinesterase activity in *Cryptolestes ferrugineus* (Wang *et al*. 2021). Additionally, these results were in accordance with the previous researches showing that star anise had antibacterial and insecticidal effects (Zhang *et al*. 2008). Star anise had highly insecticidal effect against the rust grain beetle in larva and adult stages (Wang *et al*. 2021). The star anise oil was against mosquitoes in form of the repellent (Sinthusiri and Soonwera, 2014). The star anise was highly against *Drosophila suzukii* more than 80% of mortality rate at 48 hours (Kim *et al*. 2016). It was found that ar-turmerone compounds from turmeric EO had antioxidant, antibacterial, anti-inflammatory and insecticidal properties (Chattopadhyay *et al*. 2004, Ali *et al.* 2006, Mariyappan and Vijayaragavan, 2007). Turmeric extracts and essential oils from fresh juice had highly insecticidal effect against insect pests and repellent activity in mosquito (Sukari. *et al*. 2010, Damalas, 2011). A low dose of ar-turmerone had high toxicity on *Sitophilus zeamais* and *Spodoptera frugiperda* (Tavares *et al.* 2013). The main chemical compounds from turmeric had a good insecticidal effect on form larvicidal, pupicidal, adulticidal and repellent on mosquitoes (Matiadis *et al*. 2021). Helen *et al*. (1982) suggested ar-turmerone from turmeric EO that the main phytochemical was repelled on mosquito. Curcumin and demethoxycurcumin had larvicidal activity on dipterans (Sagnou *et al*. 2012). Curcumin and ar-turmerone of turmeric could inhibit Acetylcholine esterase (AChE) and Butyrylcholine esterase (BChE) activity for inducing larval mortality (Rao *et al*. 2021). Clove EO and its main chemical compounds had many biological effects, such as insecticidal, antifungal many antibacterial properties (Lee and Shibamoto, 2001; Velluti *et al*. 2003) Clove EO had an insecticide effect on *Zabrotes subfasciatus* (Boheman, 1833). Eugenol, one of the main chemical compounds in clove EO showed high mortality effect and reduced oviposition of females on insects (Huang *et al*., 2000). This study showed that star anise, turmeric, clove and their main chemical compound, nanoemulsion, were highly against the flea beetle, diamondback moth and cutworm in form of insecticidal, antifeedant, growth inhibition and repellent activities at a low concentration. These results were in accordance with the previous studies showing the star anise, turmeric, clove and their main chemical compounds had highly insecticidal activities, such as mortality, repellent, antifeedant, inhibition of growth and oviposition activities on insect pests, especially in the leaf feeder group.

5.4. Effective botanic and chemical insecticides with highly insecticidal activity

Chemical insecticide control is generally used for eliminating these insect pests. It was found that fenvalerate, malathion, deltamethrin, cypermethrin, phoxim and cyhalothrin were commonly used insecticides alone or with mix. The efficiency of chemical insecticide in the first generation of insects was up to 90% (Li *et al*. 2001; Dong *et al.* 2007; Sun, 2008). Deltamethrin is a synthetic pyrethroid. Mode of action of deltamethrin was causative normal nerve disturbing (Najera and Zaim, 2001). Deltamethrin could kill insect pests by contacting or eating. It was low in toxic to mammals due to their high temperature and large body size. It caused decreased sensitivity of chemical. It was a moderate hazard insecticide which gives a residual effect of 3-6 months (World Health Organization, 2007). These results showed the limit effect of deltamethrin on flea beetle, diamondback moth and cutworm. Deltamethrin had highly insecticidal effect in laboratory conditions but it had low effect in greenhouse and field conditions when compared with star anise nanoemulsion. In a previous study, Wallbank (1994) showed that the deltamethrin had lower than 48% mortality of the warehouse beetle, *Trogoderma variabile* at 6 and 120 hours on metal trays. While deltamethrin was highly toxic to honeybee in laboratory conditions but it did not control bee on field conditions. It could be repellent at 2-3 hours (Johnson *et al.* 2010). Arthur (1994) found that deltamethrin range from 5% - 45% mortality after 5 days on *S. oryzae.* It was found that in the present, deltamethrin had low insecticidal effect on field conditions since it took time in and the insect pests could develop insecticide resistance. Therefore, nEOs grabbed an attention in bioactive chemicals for controlling pests. Star anise, turmeric, clove and other plants had the different effect on insect pests in form of botanical insecticides (Li *et al* 2010). Essential oils and nanoemulsions did not cause pollution in the environment and were not chemical residue which were not harmful to non-target organisms (Regnault-Roger *et al.* 2012).

However, using nEOs for insect pest control showed a niche group compared with chemical insecticide and others. The nEOs could be useful for IPM in post-harvest since it was highly effective in controlling the insect pests. Furthermore, it could be used in combination with sustainable control insect pests for repellent, antifeedant and avoiding residues on vegetables (Palermo *et al*. 2021). In the future, a study should focus on the mechanisms of nEOs against flea beetle, diamondback moth and cutworm. Therefore, it can develop eco-friendly nEOs based insecticides under more actual operating conditions to estimate their applicable ability on the larger scale.

CHAPTER 6

CONCLUSION AND SUGGESTION

6.1. Conclusion

The first examination of 12 plant essential oils (EOs) against flea beetle showed that the 1% concentration of star anise and clove EOs revealed high insecticidal effects against these insects at 100% mortality by using leaf dipping method. Additionally, the 1% of star anise and turmeric EOs resulted in 100% mortality of diamondback moth and cutworm. The efficiency test in term of insecticidal, antifeedant, growth inhibition and repellent properties of three selected plant essential oils, main chemical compound, and nanoemulsions against flea beetle, diamondback moth and cutworm showed that nEO of star anise at concentrations of 0.35%, 0.20%, 0.20%, and 0.03% had the highest level of toxicity against flea beetle with choice and no-choice antifeedant activities, and repellent activities at 100%, 100%, 100%, and 81.67%, respectively, compared with the control group. The results suggested that nEOs of star anise at 0.35%, 0.20%, 0.20%, 0.30% and 0.20% had the highest level of toxicity against diamondback moth with mortality, in choice and no-choice antifeedant activities, and growth inhibitions in pupae and adults at 100% when compared with the control group. The results showed that nEOs of star anise at concentrations of 0.35%, 0.15%, 0.20%, 0.30% and 0.20% had highest level of toxicity against cutworm with mortality, in choice and no-choice antifeedant activities, and growth inhibition in pupae and adults at 100%, when compared with the control group.

When star anise nanoemulsion was tested at 0.35% concentration, it showed highest insecticidal activity at 100% mortality, when compared chemical insecticide (Deltamethrin) in laboratory conditions. It was found that the 0.35% concentration of star anise nanoemulsion gave the maximum control percentage when compared to the control and Deltamethrin in green house and field conditions, it revealed that at 0.35% concentration of star anise nEO could pose a control by 100% and 85.45%, respectively. Therefore, the star anise nanoemulsion could control flea beetle, diamondback moth and cutworm to replace or reduce the chemical insecticides. Plant essential oil nanoemulsions did not have toxic residue in the environment and they were low in toxic to mammals.

6.2. Suggestion

The major objective of this study was developed plant essential oil nanoemulsions for controlling the flea beetle, diamondback moth and cutworm. However, in the preparation of plant essential oil nanoemulsion, it should be chose the type of surfactant and co-surfactant for maximum efficiency on the large scale.

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APPENDIX

Effectiveness of plant essential oils derived from *Curcuma longa*, *Illicium verum*, *Ocimum tenuiflorum*, and *Foeniculum vulgare* for controlling common cutworm (*Spodoptera litura*)

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Abstract. Four essential oils of *Curcuma longa*, *Illicium verum*, *Ocimum tenuiflorum* and *Foeniculum vulgare* were selected for insecticidal activity against the tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera, Noctuidae) by using leaf dipping bioassay. Toxicity on second instar larvae was assessed after 24 hours of exposure. The result revealed that all the four essential oils effectively killed the larvae and showed high antifeedant activity. The highest killing amount for controlling the larvae and highest anti-feedant effect were observed in the essential oils of *Curcuma longa* and *Illicium verum* 0.75% concentration causing 100% mortality after 24 hours. These oils also presented the highest anti-feedant effect for controlling the larvae at 0.25% concentration. It seems that, plant essential oils derived from *Curcuma longa* and *Illicium verum* have an ability to be used as herbicide for controlling *Spodoptera litura*.

1. Introduction

Most agriculturists usually utilize synthetic insecticides since they are easy to use, fast action and convenient. However, the use of synthetic insecticides has disadvantageous result on the surrounding. In comparison some naturally occurring compounds are environmentally friendly, easy to decompose, low toxicity to non-prey organisms and normally inexpensive than inducted insecticides. The cutworm, *Spodoptera litura* is a cosmopolitan pest on fruits and vegetables with high reproductive rate [1]. The clusters of larvae eat gregariously by originally mashing the top of the leaf causing agricultural damages [2]. The pest management has been mostly depending on using the chemical pesticide; without any proper file of disadvantage surrounding, health of living things and rigorous surrounding rule on these insecticide [3]. Natural plant product extracts tend to be useful bio-pesticides. Chaaban *et al.* [4] reported the high effect of turmeric essential oil for controlling the larvae of *Lucilia cuprina*. Mukesh *et al.* [5] presented that the cumin essential oils cause death of adults and larvae of *Callosopruchus chinensis* by using them as fumigants. Sagnou.*et al.* [6] informed that the curcuminoids components from *Curcuma longa* against mosquito larvae contained high insecticidal property. Akono *et al.* [7] investigated the biologically active of *Ocimum canum* and *Ocimum basilicum* essential oil for controlling *Plasmodium falciparum* and *Anopheles nopheles funestus*. Dris *et al.* [8] revealed that *Ocimum basilicum* essential oil was extremely toxic against *Culex pipiens* larvae. The *Illicium verum* essential oil was evaluate for controlling *Aede salbopicus* and *Culex pipiens* with satisfactory results [9]. Shukla *et al.* [10] studied toxicity of *Illicium verum* destroy

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Tribolium castaneum by using them as fumigants. Gunthanee *et al.* [11] showed that the crude extract of *Illicium verum* inhibited development from larvae to pupae of house fly, *Musca domesticus*. The *Pimpinella anisum* and *Foenicum vulgare* essential oil presented high efficiency against *Paeni bacillus* larvae [12].

Herbicide play main part in defense for controlling pests. In using leaf dipping bioassay, the efficacy of plant essential oils, namely *Curcuma longa*, *Illicium verum*, *Ocimumtenui florum*, and *Foeniculum vulgare* were estimated for *Spodoptera litura*.

2. Methods

2.1. Essential oils preparation

Essential oils from *Curcuma longa*, *Illicium verum*, *Ocimum tenuiflorum*, and *Foeniculum vulgare* were chose. Essential oils were diluted at 0.25, 0.50, 0.75, 1.00 and 1.25% concentrations.

2.2. Insect samples

The second instar larvae of *Spodoptera litura* were used.

2.3. Bioassay

3 × 2 cm diameter Chinese cabbage leaves were dipped these essential oils. Then, the second instar larvae were put in each box (3 replications). After 24 hours, The death rate of larvae and amount feeding of leave were observed.

2.4. Data analysis

In this study, the results were calculated using probit analysis.

3. Results and discussion

After 24 hours, The highest killing rate against the larvae were observed in the 0.75% concentration *Curcuma longa* and *Illicium verum* treatments essential oils treatments giving a LC50 value of 0.292 and 0.392 % (v/v) and LC90 of 0.541 and 0.646% (v/v), respectively. At 1.25% concentration of *Ocimum tenuiflorum*, and *Foeniculum vulgare*essential oils treatments with LC50 of 0.604% and 0.559 (v/v) and LC90 of 1.158% and 1.054 and (v/v), respectively (Table1) with100% mortality. At 0.25% concentration *Curcuma longa* and *Illicium verum* essential oils presented the highest anti-feedant effect for controlling the larvae. At 0.75% concentration *Ocimum tenuiflorum* and *Foeniculum vulgare* essential oils of showed the highest anti-feedant effect for controlling the larvae of *Spodoptera litura* (Table 2)

Our results obtained are similar to other previous studies, curcumin from *Curcuma longa* essential oil revealed most powerful larvicide in mosquito larvae [6]. Tumeric containing pungent, odoriferous oils and oleoresins was reported to possess biological activities and the toxicity against *Aedes aegypti* [13]. Zhu *et al.* [14] presented the larvicide of turmeric essential oils for controlling mosquitoes. The pure compounds, ar-tumerone in turmeric essential oil controled larvae of *Anopheles quadrimaculatus* and *Aedes aegypti*. Additionally, essential oils from *Curcuma longa* caused the formation of highest number of abnormal pupae of common cutworm, *Spodoptera litura* [15]. Anise, *Pimpinella anisum* was reported effective against larvae and pupae of *Culex quinquefasciatus* [16]. Kim *et al.* [17] reported about contact activity of the extract from *Illicium verum* fruits at 3.5x103 mg/l by filter paper diffusion method with100% mortality after1 day treatment against *Lasioderma serricorne* (F.) and *Callosobruchus chinensis* (L.) adults; besides, it caused 100% mortality of *Sitophilus oryzae* (L.) adults after 4 days treatment.

The results of this study indicated that *Curcuma longa* and *Illicium verum* essential oils had high possible to be used as herbicide. These oils contain insecticidal qualification causing successful killing larvae and also have a prominent antifeedant effect. Future research should explore field evaluations of these compounds.

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Table 1. The death rate of larvae of *Spodoptera litura* were affected from different plant essential oils.

Essential oils	Concentrations (%), (v/v)						LC50	LC90
	Average mortality percentage							
	0	0.25	0.50	0.75	1.00	1.25		
Curcuma longa	0	60.47	75.45	100	100	100	0.292	0.541
Illiciumverum	0	36	56.66	100	100	100	0.392	0.646
Foeniculumvulgare	0	36.66	42.67	53.33	73.33	100	0.559	1.054
Ocimumtenuiflorum	0	27.66	53.83	70.67	76.66	100	0.604	1.158

Table 2. The antifeedant affect from different plant essential oils

Essential oils	Concentrations (%), (v/v)					
	Leaf eating area (%)					
	0	0.25	0.50	0.75	1.00	1.25
Curcuma longa	100	0	0	0	0	0
Illiciumverum	100	0	0	0	0	0
Foeniculumvulgare	100	16.08	10.52	0	0	0
Ocimumtenuiflorum	100	21.63	20.75	0	0	0

4. Conclusion

Essential oils from *Curcuma longa* and *Illicium verum* at the concentration of 0.75% are most successful in killing larvae with potent antifeedant effect against *Spodoptera litura*.

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The Growth Inhibition Effect of Essential Oils on *Spodoptera Litura*

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Keywords: Growth Inhibited, Growth Time during the Maturation, Essential Oil, Botanical Insecticide, *Spodoptera litura*

Abstract: In Thailand, the main problem of growing Chinese cabbage is the infestation of insect pests such as *Spodoptera litura* or cutworms. This research recognizes the importance of impact on agricultural products. Therefore, the objective of this research was to study the effect of star anise (*Illium verum*), turmeric (*Curcuma longa*), sweet fennel (*Foeniculum vulgare*), and holy basil (*Ocimum tenuiflorum*) essential oils in the form of inhibitors on the development of pupa and adult stages at concentrations of 0.00, 0.25, 0.50, 0.75, 1.00, and 1.25% (v/v) with 3 repetitions of the experiment. The results showed that at 0.50% concentrations of star anise, turmeric essential oils and at 1.00% concentrations of sweet fennel and holy basil essential oils, showed 100% growth inhibition effect on the pupa and adult stages, and the growth period during maturation of the pupa and the adult stage decreased, compared to the control group. So, the experimental result revealed that plant essential oils can be used as a basis for controlling the cutworms in the future.

1 INTRODUCTION

At present, agriculturists face various physical factors which affect their agricultural products, such as climate, moisture, as well as biological factors, such as pests, plant diseases, and weeds. Insect pest is the major cause of damages in agricultural products, both quality and quantity (Oliveira et. al., 2014). The main insect pests of Chinese cabbage (*Brassica chinesis*) are diamondback moth (*Plutella xylostella*), common cutworm (*Spodoptera litura*), beet armyworm (*Spodoptera exigua*), flea beetle (*Phyllotreta sinuata*), and leaf miner (*Liriomyza brassicae*), especially *Spodoptera litura,* which is one of the most economically important pests in Thailand. The Chinese cabbage can become damaged by the newly hatched larva of *Spodoptera litura*, and become more damaged when the cutworm becomes bigger. Moreover, it can spread rapidly during the year causing significant damages to Chinese cabbage production. In prevention of pests, agriculturists commonly use synthetic chemical insecticides because it's the most convenient, and effective way to eliminate all stages of pests (Aktar et. al., 2009). However, using synthetic chemical insecticides has an adverse impact on several things, including users, products, and environment (Mitra et. al., 2011) and most importantly it can also result in insecticide resistance (Sarwar and Salman, 2015). Using plant essential oils is another method to control insect pests and another way to mitigate the impact on the environment because secondary metabolites from plants have no toxicity to humans, animals, and environment, and because of its rapid decomposition, there is no toxic residue (Prakash et. al., 2008). The secondary metabolite has insecticidal, repellent effect, antifeedant, oviposition deterrent effect, and growth inhibition effect on a pest. So, this research was to investigate the growth inhibition effect of plant essential oils on *Spodoptera litura* so that it can be developed as alternatives for pest control and reduction of chemical use.

2 MATERIALS AND METHODS

2.1 Preparation of Plant Essential Oils

The pure essential oils of *Curcuma longa*, *Illicium verum*, *Ocimum tenuiflorum*, and *Foeniculum vulgare*, prepared according to principles of hazard analysis and critical control point (HACCP), were purchased from Thai-China Flavours and Fragrances Industry Co., Ltd., (Bangkok, Thailand). To obtain 0.00, 0.25,

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0.5, 0.75, 1.00 and 1.25% concentrations of essential oils, the essential oils and Tween-20 (1:1 ratio) were added into water to obtain 100 ml of diluted essential oils.

2.2 Preparation of Insect

The 2nd stage instar larvae of *Spodoptera litura* were cultured in laboratory conditions at 25°C and 12:12 light/dark cycle, and the Chinese cabbage leaves were served as their food.

2.3 Bioassay and Data Analysis

The Chinese cabbage leaves with the diameter of 3 cm were dipped into the essential oil emulsions at various concentrations as mentioned above, and water (0.00% concentration) was used for the control group. Treated leaves were put in the testing box. 10 second stage instar larvae were then released into the box. The experiment was performed in 3 replicates. Finally, the development stage and growth period of *Spodoptera litura* were observed and analyzed.

3 RESULTS

The results showed that the highest growth inhibition effect on pupae and adult stages of *Spodoptera litura* was at 0.50% concentration of star anise and turmeric essential oils, and at 1.00% concentration of the sweet fennel and holy basil essential oils (Table 1, 2). The development period of cutworm for pupa and adult stage at 0.25% concentration of star anise and turmeric essential oils was 8.33±5.77 and 9.00±0.00 days, respectively, while the average growth period of pupa and adult stage at 0.25 concentration of star anise and turmeric essential oils was 8.00 ±0.00 and 8.00±0.00 days respectively (Table 3, 4). Shorter growth period compared to the control group resulted in abnormal molting, including incomplete molt, and arrested molt cycle affecting a survival rate of *Spodoptera litura*.

Table 1: The average growth inhibition percentage of various plant essential oils against *Spodoptera litura* pupa.

Essential oils	Concentrations (%), (v/v) Average growth inhibition percentage (%)					
	0 (*control*)	0.25	0.50	0.75	1.00	1.25
Star anise	0.00±0.00 Ca	40.00±0.00^{Ba}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00 Ca	40.00±0.00^{Ba}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Sweet fennel	0.00±0.00 Ea	30.00±0.00^{Db}	53.33±5.77^{Cb}	60.00±0.00^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Holy basil	0.00±0.00 Da	30.00±0.00^{Cb}	53.33±5.77^{Bb}	53.33±5.77^{Bc}	100.00±0.00^{Aa}	100.00±0.00^{Aa}

Notes: Mean in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different (P<0.05) according to Duncan's multiple range test.

Table 2: The average growth inhibition percentage of various plant essential oils against *Spodoptera litura* adult.

Essential oils	Concentrations (%), (v/v) Average growth inhibition percentage (%)					
	0 (*control*)	0.25	0.50	0.75	1.00	1.25
Star anise	0.00±0.00^{Ca}	50.00±0.00^{Ba}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Turmeric	0.00±0.00^{Ca}	50.00±0.00^{Ba}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Sweet fennel	0.00±0.00^{Da}	30.00±0.00^{Cb}	60.00±0.00^{Bb}	60.00±0.00^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}
Holy basil	0.00±0.00^{Ea}	30.00±0.00^{Db}	53.33±5.77^{Cc}	60.00±0.00^{Bb}	100.00±0.00^{Aa}	100.00±0.00^{Aa}

Notes: Mean in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different (P<0.05) according to Duncan's multiple range test.

Table 3: The average growth period of pupa stage of *Spodoptera litura* caused by various plant essential oils.

Essential oils	Concentrations (%), (v/v)					
	Average growth period (days)					
	0 (*control*)	0.25	0.50	0.75	1.00	1.25
Star anise	9.33 ± 5.77^{Aa}	8.33 ± 5.77^{Bb}	0.00 ± 0.00^{Cb}	0.00 ± 0.00^{Cc}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}
Turmeric	9.33 ± 5.77^{Aa}	9.00 ± 0.00^{Aa}	0.00 ± 0.00^{Bb}	0.00 ± 0.00^{Bc}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}
Sweet fennel	9.33 ± 5.77^{Aa}	9.00±0.00Aa	9.00 ± 0.00^{Aa}	8.33 ± 5.77^{Bb}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}
Holy basil	9.33 ± 5.77^{Aa}	9.33 ± 5.77^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}

Notes: Mean in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different ($P<0.05$) according to Duncan's multiple range test.

Table 4: The average growth period of adult stage of *Spodoptera litura* caused by various plant essential oils.

Essential oils	Concentrations (%), (v/v)					
	Average growth period (days)					
	0 (*control*)	0.25	0.50	0.75	1.00	1.25
Star anise	8.33 ± 5.57^{Aa}	8.00 ± 0.00^{Aa}	0.00 ± 0.00^{Bb}	0.00 ± 0.00^{Bb}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}
Turmeric	8.33 ± 5.57^{Aa}	8.00 ± 0.00^{Aa}	0.00 ± 0.00^{Bb}	0.00 ± 0.00^{Bb}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}
Sweet fennel	8.33 ± 5.57^{Aa}	8.33 ± 5.57^{Aa}	8.33 ± 5.57^{Aa}	8.00 ± 0.00^{Aa}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}
Holy basil	8.33 ± 5.57^{Aa}	8.33 ± 5.57^{Aa}	8.33 ± 5.57^{Aa}	8.33 ± 5.57^{Aa}	0.00 ± 0.00^{Ba}	0.00 ± 0.00^{Ba}

Notes: Mean in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different ($P<0.05$) according to Duncan's multiple range test.

4 DISCUSSION

The results of this study were similar to the results of others research. For example, turmeric can act as growth inhibitor on *Candida krusei* and *Candida parapsilosis* (Roth et. al., 1998), the turmeric extract has repellent activity and growth inhibition effect on stored grain pests (Jilani and Su, 1983), the development from pupa into adult stages of house fly was affected by Chinese star anise crude extract and the development rate of pupa and adult was less than that of the control group, and the affected pupa and adult of house fly were smaller in size than the pupa and the adult of the control group (Guntharee, 2008), turmeric reduced radial growth of Mycelia in vitro, and the turmeric product was also used for pest management in crop (Damalas, 2011), turmeric extract had insecticide property against *Schistocerca gregaria* and *Dysdercus koenigii* nymphs and had mortality effect on nymphs (Chowdhury et. al., 2000), star anise extract has insecticidal effect and causes mortality of larva and adult stages on mealworm, *Alphitobius diaperinus* (Szczepanik and Szumny, 2011), anise, lime, and tangerine oils tended to have high efficiency in controlling antifungal on *Hevea brasiliensis* and anise oil is the most effective substance in inhibiting surfaced-mold, *Penicilium sp.*, and *Aspergillus niger* (Matan and Matan, 2008), and sweet fennel and pignut affected mortality on *Apis mellifera* (Abramson et. al., 2007).

5 CONCLUSION

The turmeric and star anise essential oils have high growth inhibitory potential on *Spodoptera litura*. Therefore, this study provides useful information which can be used as reference for controlling *Spodoptera litura* in the future.

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Evaluating the insecticidal effect of essential oil nanoemulsion against the cutworm, *Spodoptera litura*

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Passara, H., Pumnaun, J. and Thipmanee, K. (2022). Evaluating the insecticidal effect of essential oil nanoemulsion against the cutworm, *Spodoptera litura*. International Journal of Agricultural Technology 18(5):2161-2170.

Abstract The cutworm, *Spodoptera litura* is an important pest of cruciferous vegetables and it causes economic impact worldwide. The efficiency of turmeric and star anise essential oil nanoemulsions (nEOs) against the cutworm, including mortality, antifeedant, and growth inhibition effects was investigated. Essential oil nanoemulsions (nEOs) were prepared by mixing the essential oils with surfactants and co-surfactants. The 2nd stage instar larvae of cutworms were tested by leaf dipping method and were observed after 24 hours. The results showed that the two nEOs, which were turmeric and star anise, performing the mortality, and growth development effect on *Spodoptera litura* as well as having antifeedant property. Complete mortality rate was shown at 0.35 % concentration of turmeric and star anise nEOs, when 100% antifeedant effect was found at 0.20% concentrations of nEOs, and 100% growth inhibition was observed at 0.30% concentrations of nEOs. It could be concluded that the nEOs from star anise had antifeedant and insecticide properties.

Keywords: Nanoemulsion, Cutworm, Mortality, Antifeedant effect, Growth inhibition

Introduction

A large quantity of production losses during cultivation due to insect infestation is a main problem of crop production, which can lead to large commercial losses (Haff and Slaughter, 2004, Fornal *et al.*, 2007). Cutworm or *Spodoptera litura* is one of the most common crops pests causing economic impact. Using insecticide to control pests or cutworms is the most popular method (Kranthi *et al.*, 2002) because it is simple to use (Talekar and Shelton, 1993). However, some cutworms have developed resistance to almost all insecticide groups applicable in the field (Chen *et al.*, 2008; Ahmad *et al.*, 2008; Shad *et al.*, 2010) and the insecticide residues are found in plants, soil, and environment, and affect the non–target pests and other livings. Therefore, using plant insecticide instead of chemical insecticide can mitigate those adverse effects.

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Plant essential oils are natural elements distilled from various parts of plant containing pesticide compounds, and it can be used as an alternative to chemical pesticide to mitigate its side effect. Modes of action and properties of plant essential oils, especially poison repellents, antifeedant, and attractants can reduce the amount of insect pests (Elnabawy *et al*., 2021). Using plant essential oils for pest control causes less harm to mammalians and environment (Mossa, 2016), but plant essential oils have some limits, including physical properties, fast degradation and low water solubility. For these reasons, nanoemulsion essential oils are developed to overcome some disadvantages of essential oils by applying nanotechnology approaches.

Nanoemulsion is a class of emulsions with a particle size ranging from 5 to 200 nm (Shah *et al*., 2011). Nanoemulsions are developed to improve properties of Eos, including physical stability and solubility in the aqueous phase (Joe *et al*., 2012). Biological properties of nanomaterial play a prominent role in pest management (Abbott, 1925; Finney, 1971; Van Asperen, 1962; Habig *et al*., 1974). Nanoemulsion formulation offers several advantages, including more effectiveness on pest control, lower toxic effect on non-target organism, and high stability of substance (Anjali *et al*., 2012).

Therefore, this study aimed to evaluate the efficiency of plants nanoemulsion essential oils in the form of herbicide against cutworm, *Spodoptera litura*, and it can be used as insecticides to reduce the adverse effects of chemical pesticides in the future.

Materials and methods

Cutworm rearing

Cutworms (*Spodoptera litura*) were kept from organic Chinese cabbage (Brassica chinensis) plot in Chachoengsao province, Thailand. After that, they were reared and fed by organic Chinese cabbage in insect cage and maintained at room temperature (25 $\pm$ 2 °C) and 12:12 light - dark cycle, at the laboratory of Natural Products for Pest Control Research Center (NPCRC), Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

Selection of plant essential oils

The insecticidal effect of the essential oil emulsions (EOs) from 12 species of medicinal plants were firstly evaluated against the cutworm to select the most effective essential oil emulsions to be developed into nanoemulsions. The EOs used in this study were purchased from the Thai - China Flavors and

Fragrances Industry Co. Ltd., Thailand, the production of which is in compliance with the principles of hazard analysis and critical control point (HACCP), and Tween – 20 was used as surfactant. The 2 most effective essential oil emulsions were selected for further study.

Preparation of nanoemulsion essential oils

The turmeric and star anise EOs which performed the 2 most effectiveness were diluted with water and polysorbate (Tween - 20, Hydrophilic lipophilic balance (HLB) = 16.7) or nonyl phenol ethoxylate (NP9, HLB = 12.9) as surfactant. In this process, the turmeric essential oil nanoemulsion contained turmeric essential oil and NP9 (surfactant) at a ratio of 1:2. The star anise essential oil nanoemulsion contained Tween - 20 (surfactant) at a ratio of 1:4. The particles sizes of essential oil nanoemulsions (nEOs) were measured with a particle analyzer (NanoPlus Zeta, Otsuka Electronic Co., Ltd., Osaka, Japan). The nEOs particle sizes were found with smaller than 100 nm.

Mortality test

The preliminary efficiency of 12 EOs against the cutworm was firstly evaluated using leaf dipping method. Chineses cabbage leaves were cut into circle shape with a diameter of 3 cm, then were dipped into EOs at 1 % and 10% concentrations for 1 minute, and dried at room temperature for 15 minutes. Ten 2nd stage larvae of cutworms were released on treated Chinese cabbage leaves in the box and the mortality of cutworm was observed after 24 hours. The most effective EOs in killing cutworms were selected for the next process.

The prepared Chinese cabbage leaves were dipped into various concentrations of nEOs; at 0.00% (water + surfactant), 0.10%, 0.15%, 0.20%, 0.25%, 0.30%, and 0.35% concentrations for 1 minute, and dried at room temperature for 15 minutes. Ten 2nd stage larvae of cutworms were put on treated Chinese cabbage leaves and kept in the box. The mortality was observed after 24 hours and the mortality rates were calculated and compared with the control group using Abbott's formula (Abbott, 1925).

$$\text{Mortality (\%)} = [T – C/ 100 – C] \times 100$$

Where T is test mortality (%) with EOs, nEOs and C is control mortality (%).

Antifeedant effect test

Antifeedant effect caused by the two selected nEOs (Turmeric and Star anise) was determined by the abovementioned method. The Chinese cabbage leaves with the diameter of 3 cm were dipped in the different concentrations of selected nEOs; at 0.00%, 0.10%, 0.15%, 0.20%, 0.25%, 0.30%, and 0.35%. Ten

2nd stage instar larvae of cutworms were released in the test box. Antifeedant effect was observed after 24 hours, and the consumed area was measured and compared with the control group. The percentage of antifeedant effect was calculated and represented by the antifeedant index (AFI) (Escoubas *et al.*, 1992) as

$$AFI = [\%T / (\%T + \%C)] \times 100$$

where C and T are control and treat leaf area consumed, respectively

Growth inhibited test

The study of growth inhibition effect of turmeric and star anise in the form of nEOs was carried out at 0.00%, 0.10%, 0.15%, 0.20%, 0.25%, 0.30%, and 0.35% concentrations. The Chinese cabbage leaves were dipped into the EOs and nEOs as described above. Ten 2nd stage instar larvae were released in the test box. The development from larvae to pupa and adult were observed. And compared with the control group.

Statistical analysis

The data were analyzed by Abbott's formula, and a completely randomized design method was applied to the experiments with three replicates. The data were analyzed by ANOVA and the diverse treatments were examined by Duncan's multiple range test with a SAS software (SAS Institute, Cary, North Carolina, USA).

Results

In selection of EOs, 100 % mortality of instar larvae cutworms was observed at 1% concentration of turmeric and star anise essential oils (Table 1). The turmeric and star anise EOs had the highest effect against cutworms compared with control group and were selected to develope into nanoemulsion. The particle size in nEOs depends on the amount and type of surfactant. The surfactants in these experiments were Tween - 20 and NP9, the hydrophilic-lipophilic balance (HLB) of which were 16.7 and 12.9, respectively. The turmeric nEOs consisted of 1% turmeric oil and 2% NP9 (surfactant), and the particle size as well as zeta potential were 16.1 nm and - 6.15, respectively. The star anise nEOs in this experiment consisted of 1% star anise oil and 4% Tween - 20 (surfactant), and the particle size and zeta potential were 93.2 nm and 1.27, respectively. From the results, the turmeric and star anise had the highest insecticidal effect against cutworms. Resulting in 100% mortality of instar larvae was occurred at 0.35% concentrations of turmeric and star anise nEOs after 24 hours (Table 2). At 0.20% concentrations of turmeric and star anise nEOs showed 100% antifeedant effect after 24 hours (Table 3), The interesting result of 100% growth inhibition effect on pupa and adult stages was found at 0.30% concentration (Table 4 and 5). At 0.25% concentrations of turmeric nEOs, the developmental duration of pupa and adult stage were 8.33 ± 0.58 and 7.20 ± 0.42 days, respectively (Table 4 and 5). Where at 0.25% concentrations of star anise nEOs, the developmental duration of pupa and adult stage were 8.33 ± 0.58 and 7.30 ± 0.48 days, respectively (Table 4 and 5). The results implied a decrease in pupa and adult developments compared with control group. As a result, incomplete molting of cutworm was affected by nEOs. However, the effect of turmeric and star anise nEOs on cutworm larvae were not significantly different.

Table 1. Average mortality percentage of the cutworm larvae caused by different plant essential oils (EOs) at 1 and 10 percent concentration after 24h by leaf dipping method

EOs	**Average mortality percentage (Mean (%) ±SD)**	
	Concentrations (%)	
	1	**10**
Siam cardamon	33.33 ± 0.58^{Ac}	33.33 ± 0.58^{Ac}
Eucalyptus	33.33 ± 0.58^{Ac}	33.33 ± 0.58^{Ac}
Holy basil	70.00 ± 0.00^{Bb}	100.00 ± 0.00^{Aa}
Sweet fennel	70.00 ± 0.00^{Bb}	100.00 ± 0.00^{Aa}
Betal vine	20.00 ± 0.00^{Bd}	30.00 ± 0.00^{Ac}
Cinnamon	23.33 ± 0.58^{Ad}	23.33 ± 0.58^{Ad}
Lemon grass	20.00 ± 0.00^{Bd}	33.33 ± 0.58^{Ac}
Star anise	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Clove	33.33 ± 0.58^{Bc}	43.33 ± 0.58^{Ab}
Long pepper	20.00 ± 0.00^{Bd}	33.33 ± 0.58^{Ac}
Black pepper	23.33 ± 0.58^{Bd}	43.33 ± 0.58^{Ab}
Turmeric	100.00 ± 0.00^{Aa}	100.00 ± 0.00^{Aa}
Control	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ae}

Means in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different ($P < 0.05$) according to Duncan's multiple range test

Table 2. Average mortality percentage of the larvae cutworm caused by selected essential oil nanoemulsions (nEOs) after 24h by leaf dipping method

nEOs	**Average mortality percentage (Mean (%) ±SD)**							**LC50**
	Concentrations (%)							
	0.00	**0. 10**	**0. 15**	**0.20**	**0.25**	**0.30**	**0.35**	
Turmeric	0.00 ± 0.00^{Fa}	0.00 ± 0.00^{Fa}	20.00 ± 0.00^{Ea}	30.00 ± 0.00^{Da}	40.00 ± 0.00^{Ca}	70.00 ± 0.00^{Ba}	100.00 ± 0.00^{Aa}	0.245
Star anise	0.00 ± 0.00^{Ea}	0.00 ± 0.00^{Ea}	20.00 ± 0.00^{Da}	26.67 ± 0.58^{Da}	40.00 ± 0.00^{Ca}	70.00 ± 0.00^{Ba}	100.00 ± 0.00^{Aa}	0.246

Means in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different ($P < 0.05$) according to Duncan's multiple range test.

Table 3. Average leaf feeding area percentage of the larvae cutworm caused by selected essential oil nanoemulsions (nEOs) after 24h by leaf dipping method

nEOs	Average leaf feeding area percentage (Mean (%) ± SD)						
	Concentrations (%)						
	0.00	**0. 10**	**0. 15**	**0.20**	**0.25**	**0.30**	**0.35**
Turmeric	60.33 ± 2.37[Aa]	44.33 ± 1.96[Ba]	31.67 ± 2.23[Ca]	0.00 ± 0.00[Da]	0.00 ± 0.00[Da]	0.00 ± 0.00[Da]	0.00 ± 0.00[Da]
Star anise	60.33 ± 2.37[Aa]	45.67 ± 1.81[Ba]	33.00 ± 2.64[Ca]	0.00 ± 0.00[Da]	0.00 ± 0.00[Da]	0.00 ± 0.00[da]	0.00 ± 0.00[Da]

Means in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different (P < 0.05) according to Duncan's multiple range test.

Table 4. Average growth percentage from larval to pupal stages and duration of pupal stage of cutworm caused by selected essential oil nanoemulsions (nEOs) after 24h by leaf dipping method

nEOs	Average growth percentage from larval to pupal stages (Mean (%) ± SD)						
	Concentrations (%)						
nEOs	**0.00**	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
Turmeric	100.00 ± 0.00[Aa]	90.00 ± 0.00[Ba]	80.00 ± 0.00[Ca]	60.00 ± 0.00[Da]	30.00 ± 0.00[Ea]	0.00 ± 0.00[Fa]	0.00 ± 0.00[Fa]
Star anise	100.00 ± 0.00[Aa]	90.00 ± 0.00[Ba]	80.00+- 0.00[Ca]	60.17 ± 0.41[Da]	30.00 ± 0.00[Ea]	0.00 ± 0.00[Fa]	0.00 ± 0.00[Fa]
	Pupal duration (days) (Mean ± SD)						
nEOs	**0.00**	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	**0.35**
Turmeric	9.40 ± 0.52[Aa]	9.22 ± 0.44[Aa]	9.00 ± 0.00[Aa]	9.00 ± 0.00[Aa]	8.33 ± 0.58[Ba]	0.00 ± 0.00[Ca]	0.00 ± 0.00[Ca]
Star anise	9.40 ± 0.52[Aa]	9.22 ± 0.44[Aa]	9.00 ± 0.00[Aa]	9.00 ± 0.00[Aa]	8.33 ± 0.58[Ba]	0.00 ± 0.00[Ca]	0.00 ± 0.00[Ca]

Means in a row followed by the same capital letter and means in a column followed by the same common letter are not significant different (P < 0.05) according to Duncan's multiple range test.

Table 5. Average growth percentage from pupal to adult stages and adult duration of cutworm caused by selected essential oil nanoemulsions (nEOs) after 24h by leaf dipping method

nEOs	Average growth percentage from pupal to adult stages (Mean (%) ± SD)						
	Concentrations (%)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Turmeric	100.00 ± 0.00^{Aa}	90.00 ± 0.00^{Ba}	80.00 ± 0.00^{Ca}	50.00 ± 0.00^{Da}	20.00 ± 0.00^{Ea}	0.00 ± 0.00^{Fa}	0.00 ± 0.00^{Fa}
Star anise	100.00 ± 0.00^{Aa}	90.00 ± 0.00^{Ba}	80.00 ± 0.00^{Ca}	50.00 ± 0.00^{Da}	20.00 ± 0.00^{Ea}	0.00 ± 0.00^{Fa}	0.00 ± 0.00^{Fa}
nEOs	Adult duration (days) (Mean ± SD)						
	0.00	0.10	0.15	0.20	0.25	0.30	0.35
Turmeric	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	8.00 ± 0.00^{Ba}	7.20 ± 0.42^{Ca}	0.00 ± 0.00^{Da}	0.00 ± 0.00^{Da}
Star anise	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	9.00 ± 0.00^{Aa}	8.00 ± 0.00^{Ba}	7.30 ± 0.48^{Ca}	0.00 ± 0.00^{Ea}	0.00 ± 0.00^{Ea}

Mean in a row followed by the same capital letter and means in a column followed by the same country letter are not significant different ($P < 0.05$) according to Duncan's multiple range test

Discussion

The results of this study were in accordance with the following research as Cui *et al.* (2022) found that curcumin had a growth inhibition effect on larvae of *Spodoptera litura* and it could damage the midgut structure of *Spodoptera litura* larvae. *Brassica nigra* and *Curcuma longa* compounds had an effect on *Spodoptera exigua* (Tavares de *et al.*, 2019). Kosti ĉ *et al.* (2021) found that EOs of anise, dill, and fennel seeds were highly toxic to gypsy moth. Sea fennel EOs has an effect against *Culex quinquefasciatus* and *Spodoptera littoralis* (Pavela *et al.*, 2017). The turmeric extract showed the highest mortality effect on adult of *Tribolium castaneum* (Abida *et al.*, 2010). When the turmeric EO reduced progeny of *Rhyzopertha dominica* and *Sitophilus oryzae* by using contact and fumigant method (Tripathi *et al.*, 2002). The turmeric oil caused low weight larvae, pupae, and adults and it had repellent and growth inhibition effect on red flour beetle (Jilani *et al.*, 1998). Besides, the turmeric showed growth inhibition effect against *Schistocerca gregaria* and *Dysdercus koenigii* (Chowdhury *et al.*, 2000). The growth development of pupal and adult stages of *Musca domestica* was also inhibited by Chinese star anise (Sripongpun, 2008).

Nowadays, the plant essential oils (EOs) are used as biopesticides - the successful commercial production of plants, they are also considered eco-friendly (Isman, 2020). However, plant essential oils are easily degraded by external factors such as air, light, humidity, and high temperature. For this reason, nEOs were developed to overcome the disadvantages of EOs with nanoemulsion technology. For this reason, turmeric and star anise nEOs were developed in this study. The results also were in accordance with the previous research. Mustafa and Hussein (2020) showed that pesticide nanoemulsions had great potential to develop lipophilic active - loaded products and the advance and chance in growing nanoemulsions as carries or nano delivery system for plant conservation. Tang *et al*. (2013) showed that the efficiency of nanoemulsion could be reached up to 60% towards wild cabbage. Bian (2013) showed that the product had improved insecticidal activity of nanoemulsion towards diamondback moth. Shen *et al*. (2012) showed that the average size of 11 nm of nanoemulsion had a good killing insect effect with low environmental pollution. Also, Chen *et al*., 2012 demonstrated that the 10-100 nm of particle size of nano-emulsion had a good novel agrochemical application system.

Therefore, turmeric and star anise nEOs can increase the efficiency of cutworm control, and can be used as biopesticides to reduce or eliminate the use of chemical pesiticides which cause contamination and residues in plants and environment.

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