PRODUCT DEVELOPMENT OF PLANT ESSENTIAL OIL NANOEMULSIONS FOR CONTROLLING THE AFRICAN RED MITE (*Eutetranychus africanus* (Tucker)) AND OTHER MITE PESTS

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หัวข้อวิทยานิพนธ์	การพัฒนานาโนอิมัลชันน้ำมันหอมระเหยจากพืชเพื่อการควบคุมไรแดงทุเรียน *Eutetranychus africanus* (Tucker) และไรศัตรูพืชอื่น
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บทคัดย่อ

ไรแดงแอฟริกัน (*Eutetranychus africanus* (Tucker)) เป็นศัตรูพืชที่สำคัญทางเศรษฐกิจของพืชเศรษฐกิจหลายชนิด เช่น ส้มเขียวหวาน ส้มโอ มะนาว มะกรูด มะละกอ และทุเรียน ตัวอ่อนและตัวเต็มวัยของไรแดงแอฟริกัน จะดูดของเหลวที่ด้านหน้าหรือด้านบนของใบ โดยเฉพาะอย่างยิ่งในทุเรียน ทำให้เกิดจุดสีขาวจำนวนมากบนใบ หากการระบาดรุนแรงอาจทำให้ใบและผลผลิตเสียหายในที่สุด นอกจากนี้ยังพบว่าไรศัตรูพืชอื่นๆ สามารถก่อความเสียหายให้แก่พืชเป็นอย่างมาก ทำให้เกษตรกรต้องสูญเสียรายได้เป็นจำนวนมากจากการระบาดของไรศัตรูพืช การใช้สารเคมีเป็นวิธีที่เกษตรกรมักนำไปใช้ในการควบคุมแมลงหรือไรศัตรูพืช แต่การใช้สารเคมีส่งผลโดยตรงต่อเกษตรกร ผู้บริโภค และสิ่งแวดล้อม นอกจากนี้ยังส่งผลให้ไรศัตรูพืชเกิดความต้านทานต่อสารฆ่าไรเพิ่มขึ้นและเพิ่มความเสี่ยงต่อสิ่งแวดล้อม ปัจจุบันมีการค้นคว้าในการใช้สารเคมีสังเคราะห์ด้วยวิธีการควบคุมอื่นๆ เพิ่มมากขึ้น โดยเฉพาะอย่างยิ่งผลิตภัณฑ์จากธรรมชาติเนื่องจากมีความเป็นพิษต่ำ และปลอดภัยต่อผู้บริโภค นอกจากนี้ยังเป็นมิตรต่อสิ่งแวดล้อมเนื่องจากมีคุณสมบัติย่อยสลายได้ทางชีวภาพ ดังนั้นจึงมีการศึกษาเพื่อพัฒนาสารฆ่าไรที่เป็นมิตรต่อสิ่งแวดล้อมในรูปแบบของ น้ำมันหอมระเหยนาโนอิมัลชันจากพืชในการควบคุมไรแดงแอฟริกัน (*Eutetranychus africanus* (Tucker)) และไรศัตรูพืชอื่นๆ

การทดสอบความเป็นพิษของน้ำมันหอมระเหยจากพืช 13 ชนิด ได้แก่ ตะไคร้หอม (*Cymbopogon nardus* Rendle), ตะไคร้บ้าน (*Cymbopogon citratus* (DC. Ex Nees) Stapf), อบเชย (*Cinnamomum bejolghota* Sweet), กานพลู (*Syzygium aromaticum* (L.) Merr.&L.M. Perry), พริกไทยดำ (*Piper nigrum* Linn), ยูคาลิปตัส (*Eucalyptus globulus* Labill), โป๊ยกั๊ก (*Illicium verum* Hook. f.), ขิง (*Zingiber officinale* Roscoe), เทียนข้าวเปลือก (*Foeniculum vulgare* Miller subsp. var. vulgare), มะกรูด (*Citrus hystrix* DC), ไพล (*Zingiber cassumunar* Roxb), กระวาน (*Amomum cardamomum* L) และโหระพา (*Ocimum basilicum* L) ในรูปแบบของอิมัลชันต่อตัวเต็มวัยของไรแดงแอฟริกัน (*Eutetranychus africanus* [Tucker]) โดยวิธีจุ่มใบ โดยใช้ใบหม่อนที่ตัดเป็นวงกลมขนาดเส้นผ่านศูนย์กลาง 2.7 cm. นำใบที่ตัดจุ่มลงในน้ำมันหอมระเหย

จากนั้นตากให้แห้งเป็นเวลา 15 นาที ทำการเขี่ยตัวเต็มวัยเพศเมีย 10-15 ตัว ลงบนใบหม่อนที่แช่บนสำลีในจานเพาะเชื้อ โดยใช้น้ำมันหอมระเหยจากพืชที่ความเข้มข้น 2% ตรวจนับอัตราการตายที่ 24 ชั่วโมง ผลการศึกษาพบว่าน้ำมันหอมระเหยอิมัลชันของตะไคร้หอม ตะไคร้บ้าน อบเชย และกานพลู ที่ความเข้มข้น 2% มีประสิทธิภาพในการฆ่าไรแดงแอฟริกันได้สูง ซึ่งมีผลต่อการตายของไรแดงแอฟริกันมากกว่า 70-90%

น้ำมันหอมระเหยนาโนอิมัลชันจากตะไคร้หอม ตะไคร้ กานพลู และอบเชย และสารออกฤทธิ์หลักทางเคมีของน้ำมันหอมระเหย เพื่อประเมินความเป็นพิษ การยับยั้งการวางไข่ และการไล่ ต่อไรแดงแอฟริกัน น้ำมันหอมระเหยนาโนอิมัลชัน (nEOs) ถูกเตรียมโดยการผสมน้ำมันหอมระเหยกับสารลดแรงตึงผิว และสารลดแรงตึงผิวร่วม โดยใช้สารลดแรงตึงผิว ที่ความเข้มข้นต่างๆ ทำการลดขนาดอนุภาคด้วยเครื่อง High Pressure Homogenizer จากนั้นวัดอนุภาคน้ำมันหอมระเหยโดยใช้เครื่อง Nano plus Zeta / Nano Particle Analyzer ตรวจสอบอัตราการตายของไร และตรวจนับจำนวนไข่ ที่ 24 ชั่วโมง ส่วนการทดสอบในรูปของสารไล่ การทดสอบแบบมีทางเลือก โดยการจุ่มใบที่ผ่าครึ่งลงในน้ำมันหอมระเหยนาโนอิมัลชัน nEO ในขณะที่อีกครึ่งหนึ่งถูกจุ่มลงในกลุ่มควบคุม (น้ำ) จากนั้นตรวจนับอัตราการไล่ที่ 24 ชั่วโมง ผลการศึกษาพบว่าน้ำมันหอมระเหยนาโนอิมัลชันของตะไคร้หอม มีความเป็นพิษสูงต่อไรแดงแอฟริกัน ที่มีความเข้มข้น 0.6 % สามารถฆ่าไรได้ 100% ส่วน Geraniol หนึ่งในสารประกอบหลักทางเคมี ที่ความเข้มข้น 0.2% พบว่ามีการยับยั้งการวางไข่ได้สูงโดยมีไข่เฉลี่ย 0.1 ฟอง เมื่อเทียบกับกลุ่มควบคุม มีไข่เฉลี่ย 4.7 ฟอง นอกจากนี้น้ำมันหอมระเหยนาโนอิมัลชันของตะไคร้หอม ที่ความเข้มข้น 0.1 % มีคุณสมบัติในการไล่ไรแดงแอฟริกันเป็นอย่างมาก สามารถไล่ได้ถึง 95% ที่ 24 ชั่วโมง ผลลัพธ์ที่ได้ยังพบว่าน้ำมันหอมระเหยจากพืชที่ผสมกับสารลดแรงตึงผิวแสดงให้เห็นถึงคุณสมบัติในการเสริมฤทธิ์กันของสารฆ่าไร ที่มีฤทธิ์ในการต้านทานต่อไรแดงแอฟริกันอีกด้วย

จากการทดสอบประสิทธิภาพด้วยสูตรน้ำมันหอมระเหยนาโนอิมัลชันของตะไคร้หอม nEO พบว่าไม่มีความเป็นพิษต่อพืชอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับกลุ่มควบคุม โดยเฉพาะอย่างยิ่งน้ำมันหอมระเหยนาโนอิมัลชันของตะไคร้หอม (nEOs) ที่ความเข้มข้น 0.2-0.4% พบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญในการเปลี่ยนสีและการเปลี่ยนแปลงทางสรีรวิทยาของพืช ในขณะที่ความเข้มข้น 0.4% ของน้ำมันหอมระเหยนาโนอิมัลชันของตะไคร้หอม โดยวิธีการฉีดพ่นโดยตรง ต่อไรแมงมุมเทียมกล้วยไม้ (*Tenuipalpus pacificus*) และไรขาวพริก (*Polyphagotarsonemus latus* (Banks)) ในห้องปฏิบัติการ สามารถควบคุมไรศัตรูพืชได้ดี นอกจากนี้เมื่อทดสอบที่ความเข้มข้น 0.4 % ต่อไรแดงแอฟริกันโดยวิธีการฉีดพ่นโดยตรง ในภาคสนาม พบว่าน้ำมันหอมระเหยนาโนอิมัลชันของตะไคร้หอม มีเปอร์เซ็นต์การควบคุมสูงสุดที่ 82.31% เมื่อเทียบกับกลุ่มควบคุมและยาฆ่าแมลง (Amitraz) มีเปอร์เซ็นต์การควบคุมเพียง 20.76-55.09% ดังนั้นน้ำมันหอมระเหยนาโนอิมัลชันจากตะไคร้หอมสามารถนำไปใช้เป็นผลิตภัณฑ์เพื่อการควบคุมไรศัตรูพืชทั้งไรแดง ไรแมงมุมเทียม ไรกล้วยไม้ และไรขาวพริก ได้อย่างมีประสิทธิภาพและปลอดภัยต่อสิ่งแวดล้อม

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ABSTRACT

The African red mite (*Eutetranychus africanus* (Tucker)) is an economic important pest of many economic crops, such as tangerines, grapefruit, lime, bergamot, papaya and durian. The nymphs and adults of the African red mite suck the fluid on the front or top of the leaves, particularly in durian plant, it causes many white spots on the leaves. If a severe outbreak can cause leaf and fruit loss eventually. It was also found that other mite pests can cause a lot of damage to plants, it was causality farmers to lose a lot of income out of mite pests. The use of chemicals is the way that farmers often widely apply to control the insect or mite pests. But the use of chemicals directly affect to the farmers, consumers and the environment. It also results in increased resistance of mite to acaricide and increased environmental risks. The search for displacement the use of synthetic chemicals by other control methods is increasing nowadays, especially natural products because of their low toxicity, and consumer approval. Moreover, they are considered to be environment friendly due to their biodegradable characteristics. Therefore, it also study was to develop eco-friendly acaricide of the in form of plant essential oil nanoemulsions for controlling the African red mite (*Eutetranychus africanus* (Tucker)) and other mite pests.

The primary test was to evaluate the toxicity of essential oil emulsions (EOs) obtained from 13 selected medicinal plants, namely; citronella grass (*Cymbopogon nardus* Rendle), lemongrass (*Cymbopogon citratus* (DC. Ex Nees) Stapf), cinnamon (*Cinnamomum bejolghota* Sweet), clove (*Syzygium aromaticum* (L.) Merr.&L.M. Perry), black pepper (*Piper nigrum* Linn), eucalyptus (*Eucalyptus globulus* Labill), star anise

(*Illicium verum* Hook. f.), ginger (*Zingiber officinale* Roscoe), sweet fennel (*Foeniculum vulgare* Miller subsp. var. vulgare), kaffir lime (*Citrus hystrix* DC), cassumunar ginger (*Zingiber cassumunar* Roxb), siam cardamom (*Amomum cardamomum* L) and sweet basil (*Ocimum basilicum* L) in forms of their emulsions against adults of the African red mite (*Eutetranychus africanus* [Tucker]) was investigated by using leaf dipping method. The mulberry leave used as the mites host was cut into a 2.7 diameter circle and then cut leave was dipped in 2% concentration of essential oil emulsions and air dried for 15 minutes. About 10-15 female mites were introduced to the cut leave placed on soaked cotton kept in Petri disc. The mortality of mite was checked at 24 hours. The result presented that essential oil emulsions (EOs) of citronella grass, lemongrass, cinnamon and clove at 2% were highly effective in kill the African red mite, more than 70-90% mite mortality.

Nanoemulsions of plant essential oils from citronella grass, lemon grass, clove and cinnamon and their main chemical compounds were examined against the African red mite in order to assess their toxicity, egg-laying inhibition and repellent property effect on the mite. Essential oil nanoemulsion (nEOs) were prepared by mixing the essential oils with different surfactants and co-surfactants via High Pressure Homogenizer. Then essential oil particles were measured by using Nano plus Zeta / Nano Particle Analyzer. The mortality of mite as well as number of eggs laid were checked at 24 hours. As for repellent test, the choice test was accessed by dipping an half cut leaf into the nEOs, whereas the other half leaf was dipped in control (water). Then, the repellent rate was observed at 24 h. The result revealed that nEO of citronella grass showed highly toxic effect to the mite that only 0.6% concentration proposed 100% mite mortality. Geraniol, one of the main chemical compounds consisting in citronella grass at 0.2% concentration proposed the highly egg- laying inhibition with average of 0.1 egg compared with 4.7 eggs found in the control. Besides, nEO from citronella grass at 0.1% showed extremely repellent property against the mite, when presented with 95% repellent at 24 h. Obtained result also informed that plant essential oils mixed with surfactants showed of some synergistic acaricidal property against the African red mite.

The phytotoxic effect of the citronella grass nEO, was also evaluated and revealed with no rather effect on plants when compared to the control. Especially, the nanoemulsion at 0.2-0.4% presented no significant differences in color changes

and physiological changes. While the concentration at 0.4% of citronella grass was applied by direct spray method against the orchid flat mite (*Tenuipalpus pacificus*) and the broad mite (*Polyphagotarsonemus latus* (Banks)). The result implied that it was able to control mite pest well in laboratory conditions. In addition, when tested at a concentration of 0.4% was applied by direct spray method against the African red mite, in field conditions, it was found that citronella grass nanoemulsion essential oil the maximum control percentage was 82.31% compared to the control and the acaricide (Amitraz) had the control percentage of only 20.76-55.09%. Therefore, citronella grass essential oil nanoemulsion can be used as a product for the control of all mites, the African red mite, the spider mites, the orchid flat mite and the broad mite were effective and secured for the environment.

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CHAPTER 1
INTRODUCTION

1.1 Statement and significance of the problems

Plant mites are small and with the naked eyes and they are hardly visible as small reddish or brown dots on the leave surface. The small size of mites makes it difficult for farmers or field workers to recognize them. Correct identification is also important because not all mites are pests. Several predatory mites can be found on the leaves these are beneficial as natural enemies of the plant mites (Mite and Spider Research Section, 2013; Jeppson et al., 1975). The African red mite (*Eutetranychus africanus* (Tucker)) (Actinedida; Tetranychidae) is relatively the most important mite pest species attacking the greatest of plants particularly citrus, apple, peach, grape, guava, papaya, cotton, eggplant, jackfruit, breadfruit, horse-radish, cassava, soybean, cowpea, castor bean, watermelon, garden pea and a variety of ornamentals. It is also remarkably an important pest of durian in Thailand, especially during the cool season (late October to early March). The larvae, nymphs and adults of the African red mite suck the fluid on the front or top of the leaves, remarkably, in the durian plant, it causes many white spots on the leaves. If a severe outbreak can cause leaf and fruit loss eventually (Jeppson et al., 1975; Meyer, 1974; Meyer, 1987).

The chemical method is often widely applied to control the insect and mite pests because of convenient application and high efficacy. But it brought harm on users, consumers, and the environment. It also results in increased resistance of mite to acaricide and increased environmental risks (Kulpiyawat and Chandrapatya. 1989). With this reason, the search for replacing the use of synthetic chemicals by other control methods is increasing nowadays, especially the use of natural products which shows of their low toxicity, and consumer approval. Moreover, they are considered to be environment friendly due to their biodegradable characteristics (Katz et al., 2008; Lertsutthiwong and Rojsitthisak, 2011; Chung et al., 2013). Insung and Pumnuan (2008) stated that the essential oil of citronella grass (*Cymbopogon nardus*) was remarkably toxic to *Dermatophagoides pteronyssinus* with LD_{50} value at 0.935 μg/cm^3 24 hrs after fumigation. When, Insung et al. (2008) reported that extract from *Piper retrofractum* at the concentration of 1% could completely control the African red mite. Moreover, essential oil of *Piper nigrum* showed acaricidal effect on the spider mite, *Eutetranychus cendanai* Rimando with LC_{50} at 23.6

ml/l (Sornlek, 2001). There are few studies regarding using of nanotechnology available in insect or mite pest controls. This research focused on if nanoparticles of essential oils in term of nanoemulsions with high potential for use in mite pest management. Plant extracts or essential oils would be one of the main components of safety control strategies. Nanotechnology is helpfully used to improve the essential oil efficiency. The nanoemulsions are oil-in-water dispersions having in different variety of droplet size. The nanoemulsions are stable for a long time. Furthermore, the stability depending on the individual system or mixture of ingredients, low viscosity and droplet size make them better looking system for many industrial applications (Solans et al., 2003; Bouchernal et al., 2004: Ehrlich et al., 2008). Sarapothong et al. (2017) presented that nano-essential oil of black pepper and surfactants showed high effectiveness against African red mite at concentration of 1% caused the mite mortalities of 96 and 92% and gave LC_{50} at 0.34 and 0.54%, respectively. Nano-essential oil of black pepper presented moderately repellency effect, with 42.39-51.67 %RI at 24 hrs. Accordingly, Lakyat et al. (2017) revealed the repellent activity of nano essential oils from lemon glass at 0.02% concentration showed the most repellent effect to adult brown planthopper, *Nilaparvata lugens*, with 43.83 %RI.

The purpose of this study was to develop eco-friendly acaricide in form of plant essential oil nanoemulsions for controlling the African red mite (*Eutetranychus africanus* (Tucker)) and other mite pests. Therefore, it might be imperative to actually apply in the farmer's fields and to fulfill the successful of integrate pest management (IPM) in the country

1.2 Objectives of the study

1.2.1 To evaluate the essential oils obtained from various plant species with high effectiveness in killing the African red mite, *Eutetranychus africanus* (Tucker).

1.2.2 To evaluate the effectiveness of various plant essential oils in form of nanoemulsions in controlling the African red mite interm of the acaricidal property, egg laying inhibition and repellent property.

1.2.3 To evaluate the effective essential oil nanoemulsion on the orchid flat mite (*Tenuipalpus pacificus*) and the broad mite (*Polyphagotarsonemus latus* (Banks)) in the laboratory conditions.

1.2.4 To study the phytotoxic effect of essential oil nanoemulsions on the tested plant.

1.2.5 To evaluate of effectiveness of essential oil nanoemulsions formulas on the African red mite (*Eutetranychus africanus* (Tucker)) in field conditions.

1.3 Scope of the study

This research study aimed to develope the natural product of plant essential oils for controlling the African red mite (*Eutetranychus africanus* (Tucker)) and other mite pests based on nanotechnology, or in form of nanoemulsion by assessing their toxicity, egg-laying inhibition and repellent effect on the African red mite by leaf dipping method and compared with chemical acaricide. Then highest effective essential oil nanoemulsion was tested against other economic mite pests with different families as Tenuipalpidae and Tarsonemidae in laboratory conditions. Whereas, the African red mite was also examined in field conditions. Moreover, phytotoxic effect of this essential oil nanoemulsion would be conducted. Hopefully, such essential oil nanoemulsion of medicinal plant can be developed as a product for the control or prevention of the African red mite and other mite pests especially, to replace the use of chemicals that are currently in highly apply.

1.4 Expected output of the study

1.4.1 Obtaining essential oil nanoemulsions formulas which present extremely effective mortality effect against the African red mite.

1.4.2 Knowing the phytotoxicity effect of the selected essential oil nanoemulsions formula which may present to the tested plant.

1.4.3 Obtaining effective essential oil formula to control the African red mite, orchid flat mite and broad mite, that could be developed as a product for controlling mite pests.

1.4.4 Obtaining the guidelines of using the essential oil nanoemulsion formula to control those mite pests.

CHAPTER 2
LITERATURE REVIEW

This chapter presents a review of the relevant literature on plant essential oil for controlling the mite pests which an economically important pest of many economic crops, such as tangerines, grapefruit, lime, bergamot, papaya durian, orchid flower and vegetable. Control methods of pest mites management were also demonstrated as well as the information dealing with plant extract or essential oil used for mite pest control. Besides, nanoemulsion and the effectiveness of some plant essential oil nanoemulsion are reviewed.

2.1 Mite pests

2.1.1 The African red mite

The African red mite (*Eutetranychus africanus* (Tucker)) (Actinedida; Tetranychidae) is relatively the most important mite pest species attacking the greatest of plants particularly citrus, apple, peach, grape, guava, papaya, cotton, eggplant, jackfruit, breadfruit, horse-radish, cassava, soybean, cowpea, castor bean, watermelon, garden pea, a variety of ornamentals. It is remarkably an important pest of durian in Thailand, especially during the cool season (late October to early March).

Female African red mite is flat and oval, about 0.42 mm in length and 0.35 mm wide it is dark brown in color. The developmental period is consisting with 5 stages including: egg stage 4-5 days, larva stage has 3 molts, 1st nymph, 2nd nymph and 3rd nymph, it takes an average of 1.20, 0.67 and 0.98 days, respectively. The average time from egg to adult is 6-9 days. The one female can lay 13-15 eggs, with an average of 3-4 eggs per day (Figure 2.1). The male will fertilize the female as soon as the adult molts 1 day will start spawning. The male African red mite reportedly completes its life cycle within 9.2 days. The larvae, nymphs and adults of the African red mite suck the fluid on the front or top of the leaves, particularly in durian plant, it causes many white spots on the leaves. If a severe outbreak can cause leaf and fruit loss eventually. Optimum growth temperature (25-35 °C) with high humidity (>70-90%) affects the infestation rate. (Jeppson et al., 1975; Meyer, 1974; Meyer, 1987; Mite and Spider Research Section, 2013).

Figure 2.1 The African red mite (*Eutetranychus africanus* (Tucker)), A: Egg, B: Female, C: Male

2.1.2 The Orchid flat mite

The Orchid flat mite *Tenuipalpus pacificus* Baker, is considered one of the main orchid, strawberry, vegetables, flowers, and fruit trees pests. It attacks a temperate zone species, but it is also found in the subtropical regions. (Bostanian et al. 2003; Gerson and Weintraub 2012; Park and Lee 2005). Because of their small size, barely visible with the naked eye as reddish or greenish spots on leaves and stems; the adult females measure about 0.4 mm long in length with an elliptical body. The male is elliptical with the caudal end tapering and smaller than the female (Figure 2.2) (Thomas and Denmark. 2009). Had habit of feeding on the underside of foliage, this species may go undetected until a population has caused serious damage to a plant. (Park and Lee, 2005; Fraulo et al. 2008).

Figure 2.2 The Orchid flat mite (*Tenuipalpus pacificus* Baker), A: Egg, B: Nymph, C: Female

2.1.3 The Broad mite

The broad mite, *Polyphagotarsonemus latus* (Banks) (Acarina: Tarsonemidae) this species has a large host range and is distributed worldwide. It is an important pest of vegetables, and peppers which have a particularly low tolerance for the mite (de Coss-Romero and Peña, 1998). Female mites are about 0.2 mm long and oval in outline. Their bodies are swollen in profile and a light yellow to amber or green in color. Males are similar in color but lack the stripe (Figure 2.3) (Thomas. 2019; Peña

and Campbell. 2005). Which give attacks usually feeds on the lower leaf surface and causes leaf edges to become rigid and roll under, and causes distortion and/or discoloration of flowers and blistering of fruits. Less than five mites on a young pepper plant can cause severe damage resulting in significantly fewer fruit per plant and lower fruit weight (Cho et al., 1996). Therefore, once the broad mite gains entry into a greenhouse, it can spread rapidly and have severe economic impact. Due to its high reproductive potential, it can reach damaging densities within a very short time.

Figure 2.3 The Broad mite (*Polyphagotarsonemus latus* (Banks)), A: Egg, B: Female, C: Male

2.2 Control

Presently, control of these mites depends mainly on chemical applications with novel modes of action are available and these are becoming increasingly important in modern agriculture as a component of integrated pest management and resistance management strategies. Kulpiyawat et al. (2002) reported the resistance of the African red mite to several insecticides such as amitraz, dicofol, bromopropylate and propakite. They found that all African red mite species in some northern regions were resistant to all chemicals. The use of chemicals to control mites increases production costs and risks to the environment. Although, combined application of pesticides is a labour - saving short cut method, but an understanding and knowledge of pesticide compatibility is essential in order to avoid problems which may arise from combinations of some pesticides. However, a growing concern about the impacts of chemicals on the environment, increased resistance of arthropods to these pesticides is appeared (Furuhashi, 1994; Masui et al., 1995; Goka, 1998; Aiki et al., 2005), and may cause unacceptable phytotoxicity of many miticides to plant. For another controls, Lo. (2002) has stimulated interest in a more integrated pest management approach. Biological control, in particular, may offer greater permanence of pest suppression in this system. Compared to field situations, it is

easier to establish populations of natural enemies in enclosed environments so that desired levels of pest control can be achieved without the detrimental effects of chemical pesticides.

In recent years, many researchers have focused on the use of aromatic and medicinal plants as control agents for pests in greenhouses and stored products (Aslan et al., 2004; Calmasur et al., 2006; Afify et al., 2012; Zandi-Sohani, 2012; Azimzadeh et al., 2013). Essential oils, which are obtained by the steam distillation or water distillation of aerial plant parts, are one of the potential alternative sources for pest control. They have several modes of action and can affect pests as repellents and antifeedants, as fumigants of contact toxins, and as disruptors of cuticles, and they can reduce growth and fecundity (Isman, 2000, 2006; Akhtar and Isman, 2004; Sertkaya et al., 2010).

2.3 Plants extract and essential oils

The essential oil (EO) has been used for centuries for various purposes and was regarded with great intrigue. However, many uses have disappeared with time. It is known that people have extracted it from aromatic plants since the very beginning of humankind. Its use for various purposes will gradually change, not just for culinary purposes. It is also used in cosmetics, perfumery, aromatherapy, the food and drinks industry, pharmaceuticals and agriculture as well (Sonwa, 2000).

Essential oils (EOs) are organic compounds with a complex composition obtained from the extraction of essential oil stored in different parts of medicinal plants such as seeds, flowers, leaves, fruits, bark, stems or roots and rhizomes of plants. The essential oils have the property of clear, colorless or lightly colored liquids with a characteristic odor that evaporates at normal temperature, the oil will evaporate better as heated and the fragrance of essential oils has different properties. It depends on the chemical composition of the essential oils contained in each medicinal plant such as citronella grass oil, which has insect repellent properties, including geraniol, citronella and borneol. The lemon grass oil, which has properties that help in expelling wind, and cure colic contain citral, linalul and geraniol. The scent of essential oils in the part of the flower an important role in attracting pollinators. Essential oils in other parts of the plant were believed to have a protective effect against external enemies such as insects, pathogenic bacteria and

fungi to destroy those plants. Plants that produced essential oils belong to a variety of about 60 different families, the most important of which were Labiatae such as mint / Rutaceae such as orange / Zingiberaceae such as ginger / and Gramineae such as lemon grass (Viuda-Martos et al., 2008; Yepez et al., 2002; López-Cortés et al., 2013; Maurya et al., 2013)

Essential oils (EOs) can be classified several based on their various methods of extraction, chemical composition, notes, fragrance etc. According to their extraction method, various methods of extraction were currently used, but the most common and prevalent methods were steam distillation, cold pressing and solvent extraction. Those essential oils can be classified into four various types: steam-distilled, expressed, solvent-extracted and absolutes or concretes (Herman et al., 2019). In general, plant EOs were recognized as an important natural source of monoterpenoids, which are well-known substances that showed kill properties, repellent and antifeedant against insects and mites (Ketoh et al., 2005; Isman, 2006; Kumar et al., 2011). Naturally, plants release a variety of including various volatile substances alcohols, terpenes, and aromatic compounds in order to protect that self from animals and intruders (Khater, 2012). These compounds were largely active in the vapor phase via the respiratory system (Tripathi et al., 2009; Khater et al., 2011), These compounds were largely active in the vapor phase via the respiratory system and may be played an inhibition of acetylcholinesterase (AChE) (Tripathi et al., 2009; Khater et al., 2011; Seo et al., 2014; Abou-Taleb, et al., 2015; Yeom et al., 2015) or adenosine triphosphatases (ATPases) as well as intervene with the GABA-gated chloride channels or neuromodulator octopamine of insects (Abou-Taleb, et al., 2015; Priestley et al, 2003; Kostyukovsky et al., 2002; Enan, 2005). These compounds were normally pest selective and harmless to non-target organisms and mammals (Isman, 2000; Barnard and Xue, 2004). Whence, plant EOs have been studied as potential alternative insect controlling agents for their highly insect control properties, but low mammalian toxicity and persistence in the environment (Isman, 2000; Erler, 2005; Isikber et al., 2006).

There are some reports on the insecticidal and acaricidal effects of the essential oils of some plants belonging to the Lamiaceae family (Aslan et al., 2004; Miresmaili et al., 2006; Sertkaya et al., 2010; Zandi-Sohani, 2011, 2012). Previous research has focused on the insecticidal effects of the EO of clove showed

insecticidal property against fruit fly (*Ceratitis capitata* (Wiedemann)), head louse (*Pediculus humanus capitis* (De Geer)), maize weevil (*Sitophilus zeamais* Motschulsky), rice weevil (*S. oryzae*) and pear psyllid (*Cacopsylla chinensis* (Yang & Li)) (Arancibia et al., 2013; Choi et al., 2010; Pumnuan et al., 2012; Ahmed and Salam, 2010; Tian et al., 2015). The EO of lemon grass showed insecticidal property against larger grain borer (*Prostephanus truncates* (Horn)), rice weevil (*S. oryzae*), cowpea bruchid (*C. maculatus*) and house fly (*M. domestica*) (Masamba et al., 2003; Ahmed and Salam, 2010; Paranagama et al., 2003; Pinto et al., 2015). The EO of cinnamon showed toxicity against rice weevil (*S. oryzae*) and cowpea weevil (*C. maculatus*) and maize weevil (*S. zeamais*) (Ahmed and Salam, 2010; Pumnuan et al., 2012). In addition, The essential oils of cinnamon, clove and lemon grass also expressed highly insecticidal activity against adults of thrips (*Frankliniella schultzei*) mealybug (*Pseudococcus jackbeardsleyi*) and stored product mites (*Suidasia pontifica Oudemans*) (Pumnuan and Insung, 2011; Pumnuan and Insung, 2016). Whereas research has focused on the acaricidal effectiveness on two spotted spider mites, *Tetranychus urticae* Koch (Aslan et al., 2004; Choi et al., 2004; Miresmaili et al., 2006). Aslan et al. (2004) investigated the toxicity of essential oil vapours from *Thymus vulgaris* L., *Ocimum basilicum* L., and *Satureja hortensis* to *T. urticae*. Choi et al. (2004) tested 53 essential plant oils for their toxicity against the eggs and adults of *T. urticae* as well as the adults of *Phytoseiulus persimilis* Athias-Henriot. Miresmaili et al. (2006) compared the toxicity of *Rosemarinus officinalis* L. essential oil and a blend of its major constituents against *T. urticae* on two different host plants. Insung et al. (2008) reported that extract from *piper retrofractum* at the concentration of 1% could completely control the African red mite. Insung and Pumnuan (2008) showed that the essential oil of citronella grass (*Cymbopogon nardus*) was greatly toxic to *D. pteronyssinus* with LD_{50} value at 0.935 μg/cm^3 at 24 hrs after fumigation. Pumnuan et al. (2009) found that Lemon grass and citronella grass essential oils at the concentration of 75 μg/cm^3 were the most effective in inhibiting hatching of *Luciaphorus perniciosus* Rack, and resulted in 97.3 and 95.8% mortality. Pumnuan et al. (2010) tested the essential oil of *Litsea cubeba* and it was found to be the most toxic to *Luciaphorus perniciosus* by both contact and fumigation methods with LD_{50} values equaling to 0.932 and 0.166 μg/cm^3, respectively, followed by essential oil of *Litsea salicifolia* and *Melaleuca cajuputi.* Previous study by Danarun et al. (2015)

reported that essential oil from turmeric and lemon grass were highly effective to adult of African red mite with EC_{50} of 1.66 and 2.43%, respectively. Moreover, essential oil of *P. nigrum* showed acaricidal effect on the spider mite, *Eutetranychus cendanai* Rimando with LC_{50} at 23.6 ml/l (Sornlek, 2001).

2.4 Nanoemulsion

Nanotechnology had emerged as a hopeful for development of products in a wide range of applications, inclusive of pesticide agents. Considering that various insecticides known today were organic compounds with low water solubility, the development of nanoproducts appears to solve this main problem, enhancing water solubility, and bioavailability and resulting in stable formulations without the utilization of organic toxic solvents (Margulis-Goshen and Magdassi, 2013. Nanoemulsion formulations have various advantages, including more effectiveness in pest control. The lower toxic effect on non-target organisms and the high stability of the substance (Anjali et al., 2012). There are few studies regarding using of nanotechnology available in insect or mite pest controls. This research focused on if nanoparticles of essential oils with high potential for use in mite pest management. Plant extracts or essential oils would be one of the main components of safety control strategies. Nanotechnology is helpfully used to improve the essential oil efficiency. Nanoemulsion was type of emulsions with the particle size ranging from 5 to 200 nm (Shah et al., 2010). The nanoemulsions are oil-in-water dispersions having in different variety of droplet size and can be stable for a long time. Furthermore, the stability depending on the individual system or mixture of ingredients, low viscosity and droplet size make them better looking systems for many industrial applications. (Solans et al., 2003; Bouchernal et al., 2004: Ehrlich et al., 2008). Therefore, the formulation screening stage was crucial if the development of a stable nanoformulation was desired. Tang et al. (2013) showed that the nanoemulsion effectiveness can be reached up to 60% with an average size of 11 nm of nanoemulsion with have a good insecticidal activity and low environmental effect (Bian, 2013; Shen et al., 2012). Bian (2013) presented that the product had an improving insecticidal activity of nanoemulsion against the diamondback moth. Passara et al. (2022) showed the effect of essential oil nanoemulsion (nEOs) of turmeric and star anise against to the cutworm (*Spodoptera litura*), in term of

insecticide properties and growth development effect as well as antifeedant properties. The complete mortality rate was shown at 0.35 % concentration of turmeric and star anise nEOs, when 100% antifeedant effective was found at concentrations of 0.20%, and 100% growth inhibition was observed at concentrations of 0.30%. Sarapothong et al. (2017) presented that nano-essential oil of black pepper and surfactants showed high effectiveness against the African red mite at concentration of 1% caused the mite mortalities of 96 and 92% and gave LC_{50} at 0.34 and 0.54%, respectively. Nano-essential oil of black pepper presented moderately repellency effect, with 42.39-51.67 %RI at 24 hrs. Mwandila et al. (2013) found that the Syringa (*Melia azedarach*) seed extracts (SSE) performed acaricidal properties against adults, egg and nymphs of the red spider mite (*Tetranychus* spp.). Unfortunately, any reports regarding the interference of surfactants to mite egg-laid was not found. Beside, Lakyat et al. (2017) revealed the repellent activity of nano essential oils from lemon glass at 0.02% concentration showed the most repellent effect to adult brown planthopper, *Nilaparvata lugens*, with 43.83 %RI.

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CHAPTER 3
METHODOLOGY

The research methodology entailed the processes of tested mite cultures preparation of plant essential oils and their main chemical compounds. In case of the leaf dipping and direct spray method assays, different experiments were conducted to obtain the best essential oil nanoemulsion formulas which presented high mortality rate against the tested mites, while keeping low phytotoxic effect on the treated plants. Finally, the formulas were applied in field conditions to examine its effectiveness by direct spray for mite pest control and management.

3.1 Mite culture and preparation

3.1.1 The African red mite

The African red mite (*Eutetranychus africanus* [Tucker]) adults were randomly collected from an organic durian (*Durio zibethinus* Murray) orchard in Chanthaburi province, Thailand. They were initially reared on mulberry (*Morus alba* L.) leaves placed on a cotton swab soaked with water on a tray to serve as their food source. Thousands of African red mite adults were reared on mulberry leaves and maintained at room temperature (25 ± 2 °C) and 85 ± 2% relative humidity (RH). Each leaf was removed every 4 to 5 days and a fresh leaf was provided (Figure 3.1). Mite rearing and all the bioassay experiments were conducted at the laboratory of Natural Products for Pest Control Research Center (NPCRC) project, Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand.

Figure 3.1 The African red mite (*Eutetranychus africanus*) culture preparation, A-B: The organic durian orchard infested with The African red mite, C: Mite rearing on plant leaves

3.1.2 The orchid flat mite culture

The orchid flat mite (*Tenuipalpus pacificus* Baker.) adults were randomly collected from an orchid (*Dendrobium* Sonia 'Earsakul') from insecticide or acaricidal free orchard application in Nakorn Pathom province, Thailand (Figure 3.2).

Figure 3.2 The organic orchid orchard infested with the orchid flat mite (*Tenuipalpus pacificus*)

3.1.3 The broad mite culture

The broad mite (*Polyphagotarsonemus latus* (Banks)) reared on the seedling of chilli pepper in the insectary. The adults were randomly collected from an chilli pepper (*Capsicum* spp.) from insecticide or acaricidal free garden in Nakhon Ratchasima province, Thailand (Figure 3.3).

Figure 3.3 The broad mite (*Polyphagotarsonemus latus*) culture preparation, A-B: The organic chilli pepper orchard infested with the broad mite, C: The adults of broad mite randomly collected from an organic chilli pepper orchard.

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

3.2.1 Preparation of plants Essential oils

The essential oils (EOs) were obtained from 13 species of medicinal plants and preliminary examined for their effectiveness against the African red mite. The EOs were obtained from the Thai-China Flavors and Fragrances Industry Co. Ltd, Thailand which were produce according to the principles of hazard analysis and critical control point (HACCP). The selected plant EOs were citronella grass, lemongrass, cinnamon, clove, black pepper, eucalyptus, star anise, ginger, sweet fennel, kaffir lime, cassumunar ginger, Siam cardamom, and sweet basil. Those botanical information and plant parts used to gain essential oils were shown in Table 1.

3.2.2 Analysis of chemical compounds

The four most effective essential oils were future analyzed by column chromatography in order to study on their chemical compositions. The silica gel G-60 was used for chromatography as the stationary phase, while the petroleum and ethyl alcohol solvent mixture were selected as the mobile phase. The aliquot of each fraction was analyzed by thin layer chromatography (TLC) (Saenmanot et al., 2018). The pure fraction examples of EOs were analyzed for their major components by gas chromatography-mass spectrometry (GC-MS) according to Pumnuan and Insung (2016b). The main chemical compounds appearing in effective EOs, geraniol, citral, and eugenol, were ordered from Sigma-Aldrich (St. Louis, Missouri, USA).

Table 3.1. Plant essential oils used to test against the African red mite (*Eutetranychus africanus* (Tucker)).

Family/Scientific name	Common name	Plant part
GRAMINEAE		
1. *Cymbopogon nardus* Rendle.	Citronella grass	Fresh leaf
2. *Cymbopogon citratus* (DC. Ex Nees) Stapf.	Lemon grass	Fresh leaf
LAURACEAE		
3. *Cinnamomum bejolghota* Sweet.	Cinnamon	Fresh leaf
MYRTACEAE		
4. *Syzygium aromaticum* (L.) Merr.&L.M. Perry	Clove	Dried flower bud
5. *Eucalyptus globulus* Labill.	Eucalyptus	Fresh leaf
PIPERACEAE		
6. *Piper nigrum* Linn.	Black pepper	Dried seed
RUTACEAE		
7. *Citrus hystrix* DC.	Kaffir lime	Fresh peel
LABIATE		
8. *Ocimum basilicum* L.	Sweet Basil	Fresh leaf
ZINGIBERACEAE		
9. *Zingiber officinale* Roscoe	Ginger	Fresh rhizome
10. *Zingiber cassumunar* Roxb	Cassumunar ginger	Fresh rhizome
11. *Amomum cardamomum* L.	Siam cardamom	Dried seed
UMBELLIFERAE		
12. *Foeniculum vulgare* Miller subsp. var. vulgare	Sweet fennel	Dried seed
ILLICIACEAE		
13. *Illicium verum* Hook. f.	Star anise	Dried flower bud

3.3. Preparation of Essential oil emulsions and main chemical compound nanoemulsions

Those 13 essential oils were diluted in water by using Tween20 as emulsifier or surfactant. The essential oil emulsions (EOs) contained different concentrations of surfactant according to properly dissolving that would be used for further bioassay experiment.

Selected effective essential oils and main chemical compounds of those essential oils as geraniol, citral and eugenol were diluted in water by using surfactants. Then the essential oil particles appearing in the emulsions were resized to be nanoparticle by adding the co-surfactant at different concentrations. In this experiment, tween 20, tween 40, tween 60, tween 80, span 20, span 80, span 83, span 85, neopelex, sunsoil and acacia were used as primary surfactants whereas,

polyethylene glycol 400 (PEG400) used as co-surfactant in which could describe as following steps. Firstly, each essential oil or the main chemical compound was merged with the primary surfactant, tween20 or tween60 as follows 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4 and 1:4.5 (1:1 means 100 µL:100 µL) for 10 mL. Consequently, each solution was added with co-surfactant polyethylene glycol (PEG400) called Smix as followed 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4 and 1:4.5. The prepared EOs were then put in to a nano particle size analyzer (NanoPlus Zeta, Otsuka Electronice Co., Ltd., Japan) to measure the particle size. In case of essential oil particles contained in the emulsion were still bigger than 100 nm, it must be passed into high pressure homogenizer to minimize the particle, and then particle sizes were measured again (Figure 3.4). These prepared essential oil nanoemulsions (nEOs) and their main chemical compound nanoemulsions (nMC) were used for further toxicity assessments.

Table 3.2. Surfactants and co-surfactant used in the study

Surfactant	hydrophile-lipophile balance (HLB)	Co-surfactant	hydrophile-lipophile balance (HLB)
Tween 20	16.7	polyethylene glycol 400 (PEG400)	13
Tween 40	15.6		
Tween 60	14.9		
Tween 80	15		
Span 20	8.6		
Span 80	4.3		
Span 83	3.7		
Span 85	1.8		
Neopelex	N/A		
Sunsoil 1	13		
Acacia	11.87		

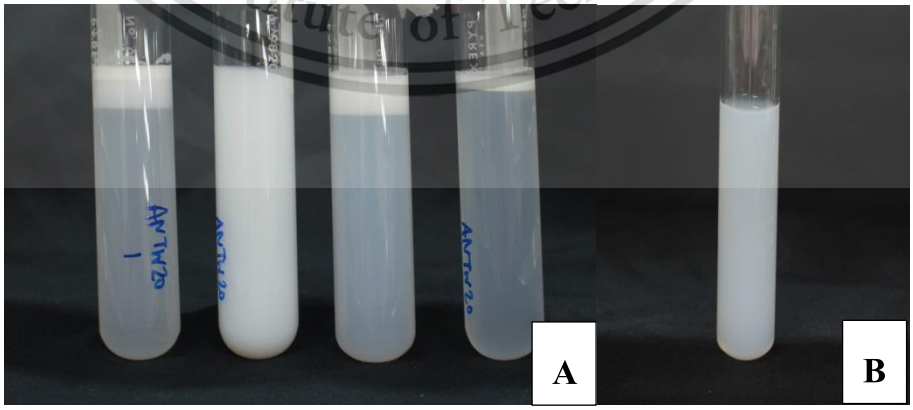


Figure 3.4 The essential oil nanoemulsions at various rations of surfactant and co-surfactants, A: precipitate, B: with no precipitate.

3.4 Bioassay

3.4.1 Toxicity tests.

The effectiveness of 13 EOs against the African red mite was primary assessed by using leaf dipping method. The mulberry leaf was cut into 2.7 cm diameter circle and dipped for 1 min with each of these EOs at 2% and discharged to air-dry at room temperature (25 ± 2 ℃, 85 ± 2% RH) during 15 min. About 10-15 adult females mites were selected under microscope and introduced to the treated leaf. The mite mortality was observed at 24 h. Mite determined as death when they did not move even if probed with a small hairbrush. The EOs showing highly effectiveness in killing the mites with more than 70% mortality were subjected to test for further experiment in forms of nEOs with various concentrations. Besides, the main chemical compounds contained in those effective essential oils verified by using column chromatography, in form of nanoemulsions were also performed.

The toxicity properties of nEOs and nMCs against the African red mite were evaluated by using leaf dipping method as well. The mulberry leaf was cut into 2.7 diameter circle and dipped for 1 min in each nanoemulsion at various concentrations: 0.0% (water as control), 0.0% (surfactants), 0.2%, 0.4%, 0.6%, 0.8% and 1.0%, as well as imidacloprid insecticide at (1X) and (2X) recommendation rates. All treatments were allowed to air dried for 15 min. About 10-15 adult female mites were transferred to the treated leaves on soaking cotton and kept in a Petri dish (Figure 3.5). Then, mite mortality was observed at 24 h. In case of death mite occurred in control, Abbott's formula (Abbott, 1987) was applied according to the actual death rate with the formula as follows:

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

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

3.4.2 Egg-laying inhibition test

Egg-laying inhibition caused by nEOs and nMCs was performed by the same method. The mulberry leaves were dipped into various concentrations of nEOs and nMCs at 0.0 (water), 0.0 (surfactant and co-surfactant or blank), 0.02, 0.04, 0.06, 0.08, 0.10 and 0.20%, as well as imidacloprid at (1X) and (2X) will be dipped for 1

min. Treated leaves were left at room temperature to air-dry for 15 min. Later on 10-15 adult females were released to treated leaves. The number of egg laid per female mite was observed under microscope at 24 h (Figure 3.5).

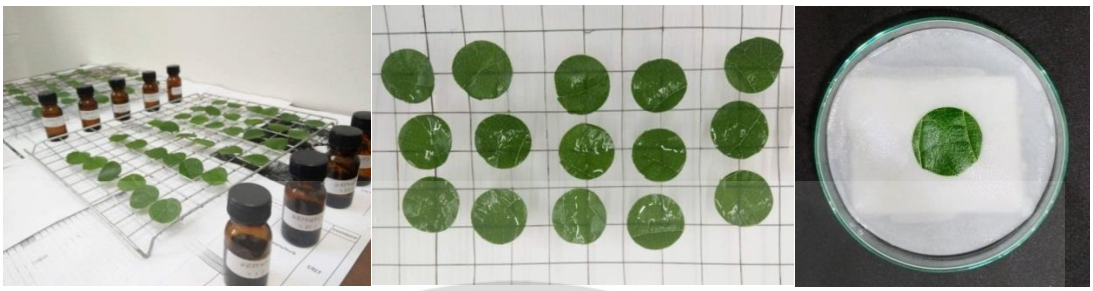


Figure 3.5 The toxicity property test of essential oil nanoemulsion to against the African red mite by using leaf dipping method.

3.4.3 Repellent and attract test

Response of the African red mite to seven nEOs and nMCs was evaluated in term of repellent and attract activities by using similar method and the choice test was performed. Then the circle cut leaves were separated into two equal parts and the first part was dipped in nEOs and nMCs at 0.02%, 0.06%, and 0.1%. Whereas, the other half was dipped with control (water or surfactant). Both parts were discharged to air-dry at room temperature for 15 min. About 10-15 adults females of the African red mite were transferred, then the repellent and attract response percentages were checked at 24 h (Figure 3.6).

Figure 3.6 The repellent and attract activity test by leaf dipping method and the choice test.

3.5 Effectiveness of essential oil nanoemulsions on the African red mites in laboratory conditions.

Different nano essential oil formulas were constructed and then the acaricidal property of those nano plant essential oil formulas against the African red mite were evaluated by using leaf dipping method. The mulberry leaf was cut into 2.7 diameter circle and dipped for 1 min in each nanoemulsion formulas at various concentrations: 0.0% (water as control), 0.0% (surfactants), 0.2%, 0.4%, 0.6%, 0.8% and 1.0%, as well as imidacloprid insecticide at (1X) and (2X) recommendation rates, and were left at room temperature to air-dry. About 10-15 adult female mites were placed on the mulberry leaves. The mortalities of african red mite were observed at 24 hrs after treatment and compared with control. The experiment was designed in completely randomized replicates (CRD).

3.6 Effectiveness of essential oil nanoemulsions on the African red mite in greenhouse conditions.

Acaricidal test in greenhouse conditions of essential oil nanoemulsion formulas of citronella grass the most effective essential oils on African red mite was performed by direct spray method (Figure 3.7). With various concentrations as well as with the insecticide (Amitraz), at recommendation rates were examined as followed treatment:

T1 = Control (water)

T2 = nEO of Citronella grass (0.2%)

T3 = nEO of Citronella grass (0.4%)

T4 = nEO of Citronella grass (0.6%)

T5 = Amitraz (1X)

The experiment was performed on mulberry seedling planted 2 months in a single pot (1 plant per pot), About 100 adults of African red mite were released and randomly counted was made after 3 day as number of mites/trees before and after spraying on days 0, 1, 3, 5 and 7 days (Figure 3.7). The surviving African red mites were counted on branches and leaves and compared with the control. The experiments were conducted based on RCBD and five replications

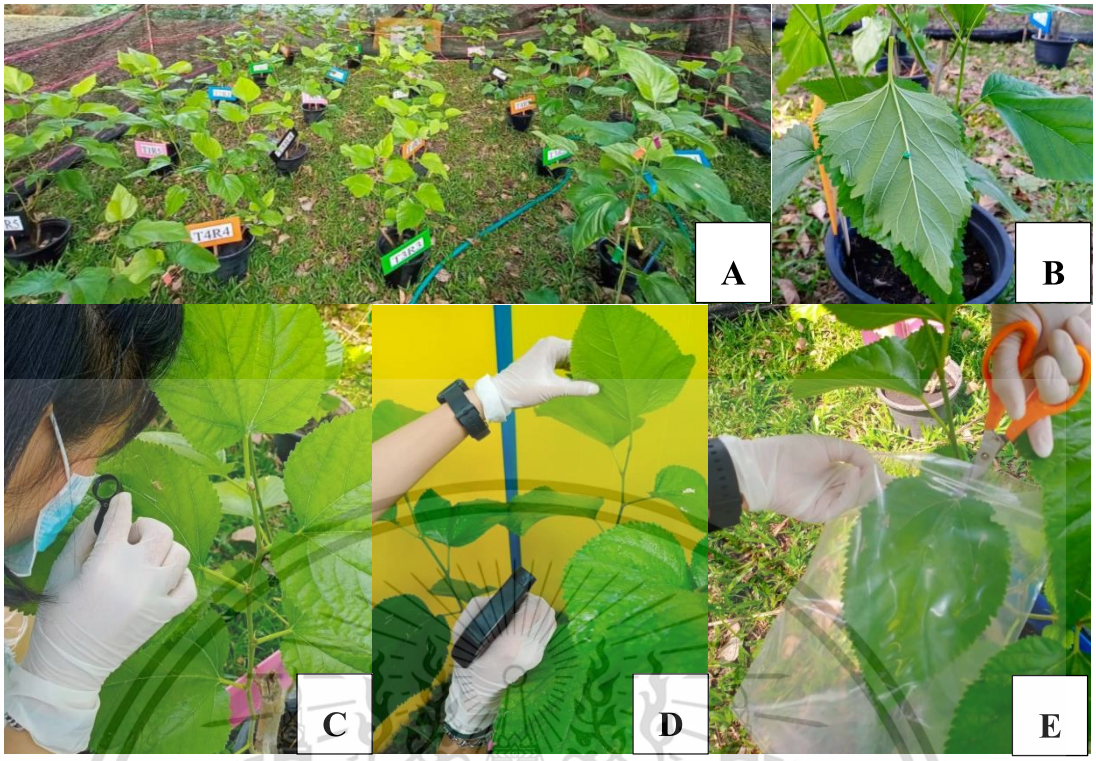


Figure 3.7 Greenhouse experiment, A-C: The adults of African red mite released and randomly counted, D: The direct spraying, E: The number of surviving African red mites recording.

3.7 Study on phytotoxicity of essential oil nanoemulsions

3.7.1 The phytotoxicity of essential oil nanoemulsion compared with amitraz insecticide.

The nEO of citronella grass at 0.2, 0.4, and 0.6 % were applied and compare with control (water) and amitraz at recommendation rates as follows:

T1 = Control (water)

T2 = nEO of Citronella grass (0.2%)

T3 = nEO of Citronella grass (0.4%)

T4 = nEO of Citronella grass (0.6%)

T5 = Amitraz (1X)

The test was done on the mulberry trees age 2 months and plants were sprayed at volume of 50 ml/trees. After spraying on 0, 1, 3, 5 and 7 days, the phytotoxicity characteristics of the mulberry trees were examined, according to affected mulberry leaf area (according to Boonplain, 2014) (Figure 3.8). Which can be graded as follows:

5= very wilted leaves, black scorched leaves

4= 7-8 leafed with brown leaf bands

3= 4-5 leafed with burnt leaves

2 = 2-3 leafed with burned leaves and bruises

1= 1-2 leafed with slightly brown stripes

0=normal leaves

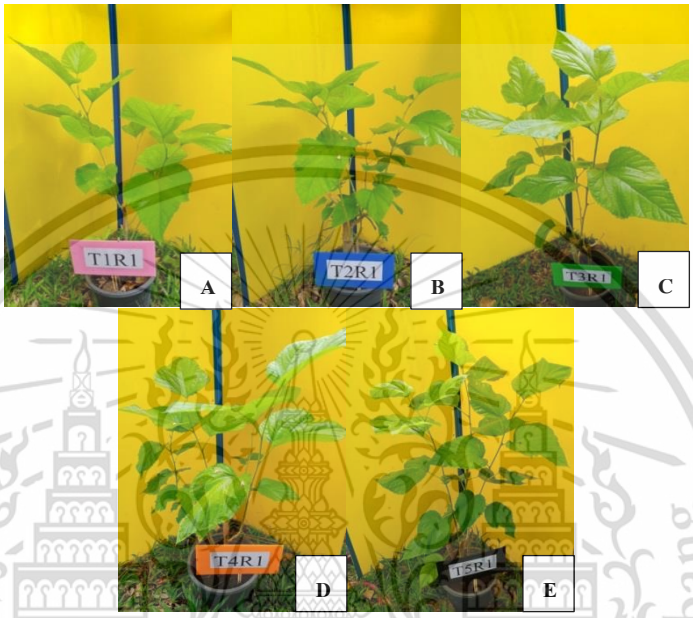


Figure 3.8 Characteristics of plant test by direct spray with essential oil nanoemulsion formulas at various concentrations compared to the insecticide (Amitraz), at recommendation rate. A: Water, B: nEO citronella grass (0.2%), C: nEO citronella grass (0.4%), D: nEO citronella grass (0.6%), E: insecticide (Amitraz) recommendation rate.

3.7.2 The phytotoxicity of essential oil nanoemulsion compared with surfactants.

The nEO of citronella grass at previous concentration were also tested for their phytotoxicity and compared with control (water) and the surfactants as described below:

T1 = Control (water)

T2 = nEO of Citronella grass (0.2%)

T3 = nEO of Citronella grass (0.4%)

T4 = nEO of Citronella grass (0.6%)

T5 = Surfactant A (A; Tween20:PEG400(4:3.5)) (0.2%)

T6 = Surfactant A (0.4%)

T7 = Surfactant A (0.6%)

The test was also performed on the mulberry trees age 2 months and was sprayed with above items for the volume of 50 ml/trees. After spraying on 0, 1, 3, 5 and 7 days, the phytoxicity characteristics of the mulberry trees were examined by the same criteria as referred in item 3.7.1.

3.8 The efficacy test of selected essential oil nanoemulsion on other mite pests in laboratory conditions.

3.8.1 The Orchid flat mite

The acaricidal property of selected essential oil nanoemulsions formulas against the orchid flat mite (*Tenuipalpus pacificus* Baker.) was evaluated by direct spray method. The 3x4 inch orchid leaves were sprayed with selected nEO at concentrations: 0.0% (water as control) and 0.4%, as well as pyridaben insecticide at (1X) by using a volume of 1.5 ml. It was left at room temperature to air-dry. Amount of 20-60 the orchids flat mites were randomly collected from infested orchid leaves. The number of surviving orchid flat mite was observed at 24 hrs after treatment and compared with control. The experiment was designed in completely randomized replicates (CRD).

3.8.2 The Broad mite

The acaricidal property of selected essential oil nanoemulsions formulas against the broad mite (*Polyphagotarsonemus latus* (Banks)) was evaluated by using also direct spray method. The bioassay was applied by spraying the selected nEO on the chili leaves at concentrations: 0.0% (water as control), 0.4%, as well as amitraz insecticide at (1X) for 1.5 ml. It was left at room temperature to air-dry. Amount of 10 the broad mite adults were placed on the chili leaves. The mortalities of broad mite were observed at 12 and 24 hrs after treatment. The experiment was designed in completely randomized replicates (CRD).

3.9 The efficacy of selected essential oil nanoemulsion formulas on the African red mite in field conditions.

Following the laboratory and greenhouse conditions studies, effective of selected essential oil nanoemulsion formula was evaluated in field conditions. In this assay, the direct spray was applied to organic durian orchard (in Nakhon Ratchasima

province) which was infested by the African red mite. The orchard was applied with selected nEO at the 0.4% concentration and compared with insecticide (Amitraz) at the recommendation rate. The African red mites were randomly collected from durian leaves before and after spraying on days 0 and 1days (Figure 3.9). The surviving African red mites were counted on durian leaves, compared with the control. The experiment was conducted on RCBD and 5 replications.

Figure 3.9 Field experiment in Nakhon Ratchasima province. A-C: durian leave attacked by the African red mites, D: The direct spraying, E-F: The observe of surviving African red mite.

3.10 Statistical analysis

In this study, Abbott's formula was applied to obtain the mite actual death rate. The experiment was performed in a completely randomized design (CRD) with five replicates per treatment. The data obtained were analyzed by ANOVA, and the differences among various treatments were tested by Duncan's multiple range test (DMRT) at 95% confidence level ($P<0.05$) in SAS programme. As for percent response in term of repellent and attract test frequencies of mite in the choice test were analyzed by the $\chi 2$ test in SPSS. For the experiment in the greenhouse conditions, in the RCBD (randomized completely block design) experiment was planned.

Besides, the values of LC_{50} (50% lethal concentration) and LC_{90} (90% lethal concentration) will be calculated by the probit analysis in SPSS.

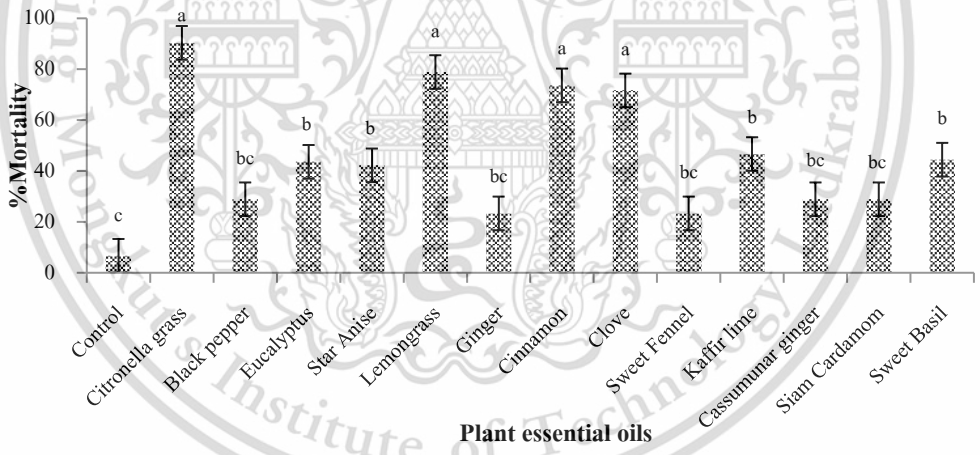
CHAPTER 4

RESULTS

4.1 Examination of essential oil and main chemical compound with high acaricidal activity.

4.1.1 Toxicity of essential oils against the African red mite

Toxicity of essential oils obtained from 13 selected medicinal plants, namely; citronella grass, lemongrass, cinnamon, clove, black pepper, eucalyptus, star anise, ginger, sweet fennel, kaffir lime, cassumunar ginger, siam cardamom and sweet basil in forms of their emulsions against adults of the African red mite revealed that essential oil emulsions (EOs) of citronella grass, lemongrass, cinnamon and clove at 2% were highly effective in killing the African red mite with more than 70-90% mite mortality obtained (Figure 4.1).



The same common letter above each column indicates nonsignificant difference according to Duncan's multiple range test.

Figure 4.1. Mortality percentage of the African red mite caused by various plant essential oils at 2% concentrations at 24 h by leaf dipping method.

Un-satisfactory result was found on the essential oils of black pepper, ginger, sweet fennel, cassumunar ginger and siam cardamom when they expressed with low acaricidal activity.

4.1 Examination of essential oil and main chemical compound with high acaricidal activity.

4.1.2 Chemical components of essential oils with high acaricidal activity.

The four selected plant essential oils as citronella grass, lemon grass, cinnamon and clove which showed high efficiency in killing the mite were analyzed by CG-MS to find out their chemical compounds. The identified chemical compositions were shown in Table 4.1. It was found that the essential oils of citronella grass contained 37.965% citronella, followed by geraniol 18.604%. While the lemon grass essential oils had the highest amount of geranyl acetal at 29.034%, followed by citral 20.209%. In addition, the essential oils of clove and cinnamon had relatively the same main active ingredient which specifically was eugenol at 81.545 and 98.452%, respectively.

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Table 4.1. Chemical components of plant essential oils based on GC-MS assay.

Compound	Plant/ Percentage (%)			
	Citronella grass	Lemon grass	Cinnamon	Clove
α-Phellandrene	-	-	0.075	-
α-Pinene	1.237	-	0.712	-
Camphene	-	-	0.223	-
Benzaldehyde	-	-	0.110	-
β-Pinene	-	-	0.189	-
6-methyl-5-hepten-2-one	-	1.222	-	-
β-Myrcene	-	4.518	-	-
α-Fellandrene	-	-	0.561	-
p-Cymene	-	-	1.070	-
Limonene	4.014			
β-Phellandrene	-	-	0.495	-
1,8-Cineole	-	-	0.129	-
cis-Ocimene	-	0.551	-	-
β-Ocimene	-	0.321	-	-
Terpinolene	-	-	1.630	-
Gamma.-Terpinene	-	0.707	-	-
Linalool	0.734	-	-	-
Citronella	37.965	0.234	-	-
cis-Verbenol	-	0.406	-	-
Cyclohexanone	-	0.769	-	-
α-Terpineol	-	-	0.172	-
Citronellol	13.175	0.250	-	-
Neral	-	19.777	-	-
Nerol	-	3.389	-	-
Geraniol	18.604	-	-	-
Citral	-	20.209	-	-
Cinnamaldehyde	-	-	0.753	-
Safrole	-	-	1.028	-
3-(2-Oxopyridyl) propanal	-	14.920	-	-
Geranyl acetal	-	29.034	-	-
cis-2,6-dimethyl-2,6-octadiene	3.130	-	-	-
Eugenol	1.390	-	81.545	98.452
Geranyl acetate	2.702	-	-	-
cis-Geraniol	-	1.309	-	-
α-Copaene	-	-	0.546	-
β-elemene	2.145	-	-	-
β-Caryophyllene	-	0.272	2.598	-
Caryophyllene	-	-	-	0.430
trans.-alpha.-Bergamotene	-	0.221	-	-
Cinnamyl alcohol	-	-	1.422	-
α-Humulene	-	-	0.418	0.207
Germacrene-D	3.158	-	-	-
α-muurolene	1.112	-	-	-
γ-cadinene	0.736	-	-	-
Phenol	-	-	2.870	0.397
Delta-Cadinene	3.157	-	-	-
Elemol	2.998	-	-	-
Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1S-cis)-(cas)	0.818	-	-	-
1,2-Benzenedicarboxylic acid	-	-	0.553	-
Cyclohexane	-	-	-	0.439
Spiro[4.4]nonan-2-one	-	-	-	0.074
1H-Cycloprop[e]azulene	-	0.434	-	-
Cadinene	0.433	-	-	-
Torreyol	0.968	-	-	-
t-muurolol	1.523	-	-	-
Benzyl Benzoate	-	-	2.901	-
9-Octadecenamide	-	1.459	-	-

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4.1 Examination of essential oil and main chemical compound with high acaricidal activity.

A total of 12 surfactants were used in order to the prepare emulsions for the reduction of essential oil particles from citronella grass, lemon grass, cloves, cinnamon and their main chemical compounds to be nanoparticles appeared in the emulsion. It was found that surfactants with hydrophile-lipophile balance (HLB) values between 13-16.7 in different ratios showed good solubility with no precipitate. In this case, citronella grass essential oil emulsion at the mix ratio of Tween 20, Tween 40 and Tween 60 as ratios 1:3, 1:3.5, 1:4 and 1:4.5, was found with good solubility and no precipitate. The mixed lemon grass essential oil with Tween 60, Tween 80 and Neopelex ratios with 1:4, 1:3.5 and 1:1.5 were highly soluble and non-precipitated. While of cinnamon and clove were highly soluble and non-precipitated at Tween 40, Tween 60 and Tween 80, ratio 1:4, 1:4.5, 1:4, 1:4.5 and 1:4.5, respectively, while the mixed ratios of geraniol Tween 20, Tween 40 and Tween 60 ratios 1:3, 1:4 and 1:4.5 were highly soluble and did not form precipitate, respectively. Citral was highly soluble at the Tween 20, Tween 60 and Tween 80 ratios of 1:2, 1:2.5, 1:3 and 1:4.5, and without precipitate. In addition, eugenol was highly soluble and no precipitate at the mixture among Tween 40 Tween 60 and Tween 80 at the ratios of 1:1.5, 1:3, 1:4 and 1:4.5, respectively. While the essential oil of plants, main chemical compound mixed other surfactant ratios presented a precipitate formed, that implied a difficulty to make nanoparticles (Table 4.2)

Table 4.2. Capability of 12 surfactants mixed with essential oils and main chemical compounds at various ratios to obtain emulsions (P=precipitate).

Emulsion	Surfactant	Appropriate ratio
Essential oil		
Control	Tween 20	(1:1),(1:2),(1:3),(1:4)
	Tween 40	(1:1),(1:2),(1:3),(1:4)
	Tween 60	(1:1),(1:2)
	Tween 80	(1:3)
	Span 20	P
	Span 80	P
	Span 83	P
	Span 85	P
	PEG 400	(1:1),(1:2),(1:3),(1:4)
	Neopelex	(1:3),(1:4)
	Sunsoil	P
	Acacia	(1:1),(1:2)
Citronella grass	Tween 20	(1:3.5),(1:4)
	Tween 40	(1:3),(1:4)
	Tween 60	(1:4),(1:4.5)
	Tween 80	p
	Span 20	P
	Span 80	P
	Span 83	P
	Span 85	P
	PEG 400	P
	Neopelex	P
	Sunsoil	P
	Acacia	P
Lemongrass	Tween 20	P
	Tween 40	P
	Tween 60	(1:4.5)
	Tween 80	(1:3.5)
	Span 20	P
	Span 80	P
	Span 83	P
	Span 85	P
	PEG 400	P
	Neopelex	(1:1.5)
	Sunsoil	p
	Acacia	P
Cinnamon	Tween 20	P
	Tween 40	(1:4),(1:4.5)
	Tween 60	(1:4),(1:4.5)
	Tween 80	(1:4.5)
	Span 20	P
	Span 80	P
	Span 83	P
	Span 85	P
	PEG 400	P
	Neopelex	P
	Sunsoil	P
	Acacia	P

Clove	Tween 20	p
	Tween 40	(1:4),(1:4.5)
	Tween 60	(1:4),(1:4.5)
	Tween 80	(1:4.5)
	Span 20	P
	Span 80	P
	Span 83	P
	Span 85	P
	PEG 400	P
	Neopelex	P
	Sunsoil	P
	Acacia	p
Main chemical compound		
Geraniol	Tween 20	(1:3),(1:4)
	Tween 40	(1:4.5)
	Tween 60	(1:4.5)
	Tween 80	P
	Span 20	P
	Span 80	P
	Span 83	p
	Span 85	P
	PEG 400	P
	Neopelex	P
	Sunsoil	P
	Acacia	P
Citral	Tween 20	(1:3.5)
	Tween 40	P
	Tween 60	(1:2),(1:2.5),(1:3),(1:4.5)
	Tween 80	(1:3)
	Span 20	P
	Span 80	P
	Span 83	P
	Span 85	P
	PEG 400	P
	Neopelex	P
	Sunsoil	P
	Acacia	P
Eugenol	Tween 20	P
	Tween 40	(1:4)
	Tween 60	(1:1.5),(1:3),(1:4.5)
	Tween 80	(1:4.5)
	Span 20	P
	Span 80	P
	Span 83	P
	Span 85	P
	PEG 400	P
	Neopelex	P
	Sunsoil	P
	Acacia	P

When appropriate surfactants were specific to each essential oil of plants and main chemical compounds. Therefore, the more appropriate ratio between essential oils and surfactants was conducted. It was found that essential oils from citronella grass mixed Tween 20 and Tween 40 at ratios of 1:3.5, 1:4 and 1:4.5 gave very satisfactory result, while lemon grass with Tween 60 and Neopelex at ratios 1:4.5 and 1:2.5 were optimum. Cinnamon and clove were highly soluble for Tween 40-80 at various ratios, while geraniol mixed with Tween 20 and Tween 60 at ratios 1:4 and 1:4.5 would be optimal. In addition, citral and eugenol were highly soluble if using Tween 60 at ratio 1:4.5 (Table 4.3). In the end the particles dispersed in emulsion were then measured and reduced in particle size by mean of high pressure homogenizer.

Table 4.3. The appropriate ratio of a surfactant mixed with essential oils and main chemical compounds.

Emulsion	Surfactant	Appropriate ratio
Essential oil		
Citronella grass	Tween 20	(1:3.5),(1:4)
	Tween 40	(1:4)
	Tween 60	P
Lemongrass	Tween 60	(1:4.5)
	Tween 80	P
	Neopelex	(1:2.5)
Cinnamon	Tween 40	(1:4),(1:4.5)
	Tween 60	(1:4),(1:4.5)
	Tween 80	(1:4.5)
Clove	Tween 40	(1:4),(1:4.5)
	Tween 60	(1:4),(1:4.5)
	Tween 80	(1:4.5)
Main chemical compound		
Geraniol	Tween 20	(1:4)
	Tween 60	(1:4.5)
	Tween 80	P
Citral	Tween 20	P
	Tween 60	(1:4.5)
	Tween 80	P
Eugenol	Tween 20	P
	Tween 60	(1:4.5)
	Tween 80	P

4.1 Examination of essential oil and main chemical compound with high acaricidal activity.

The 19 water-soluble essential oils mentioned above were measured for their particle sizes. It was found that the particles were between 8.9-926.7 nm, with most of them having particle sizes greater than 100 nm. Therefore, the particles of essential oil dispersed in the emulsion were then minimized by using high pressure of 15,000 psi or continuously higher pressure as 20,000 and 30,000 psi. From the experiment, it was found that 4 essential oils from plants and 3 main chemical compounds could be applicable with Tween 20 or Tween 60 in different ratios. And the particle size was becoming nanoparticles. Where citronella grass mixed with Tween 20 at 1:4 ratio, showed the particle size 10.20±0.10 nm with good dispersion (Polydispersity Index) but still not very stable at -4.29±1.51 mV. This number should be greater than 30 or less than -30 that means the strong stable of the emulsion. Lemon grass mixed with Tween 60 at the rate of 1:4.5 under a pressure of 20,000 psi dimensions of 10.77±0.85 nm. As for cinnamon and clove mixed with Tween 60 at ratio 1:4.5 gave resulted in nanoparticle sizes of 19.97±0.32 and 24.93±0.51 nm, respectively. Whereas geraniol mixed with Tween 20 at ratio 1:4 was exactly nanoparticle, 10.17±0.06 nm. Citral and eugenol mixed with Tween 60 at ratio 1:4.5 already had a small particle size of 53.23±0.72 and 21.73±1.33 nm, respectively. (Table 4.4).

Table 4.4. The particle size of essential oils and main chemical compound emulsion with different ratios of surfactants.

Emulsion	Surfactant	Parameter	Particle size			
			After formulated	After passed through pressure at 15,000 psi	20,000 psi	30,000 psi
Essential oil						
Citronella grass	Tween20 1:4	Diameter	10.20±0.10	-	-	-
		Polydispersity Index	0.09±0.03	-	-	-
		Zeta Potential	-4.29±1.51	-	-	-
Lemon grass	Tween60 1:4.5	Diameter	223.50±44.04	173.73±23.44	10.77±0.85	-
		Polydispersity Index	0.15±0.03	0.13±0.01	0.17±0.04	-
		Zeta Potential	-8.80±3.92	-15.45±0.61	-16.85±0.63	-
Cinnamon	Tween60 1:4.5	Diameter	19.97±0.32	-	-	-
		Polydispersity Index	0.32±0.06	-	-	-
		Zeta Potential	-2.29±0.55	-	-	-
Clove	Tween60 1:4.5	Diameter	24.93±0.51	-	-	-
		Polydispersity Index	0.25±0.00	-	-	-
		Zeta Potential	-3.42±1.31	-	-	-
Main chemical compound						
Geraniol	Tween20 1:4	Diameter	10.17±0.06	-	-	-
		Polydispersity Index	0.02±0.00	-	-	-
		Zeta Potential	-4.78±5.88	-	-	-
Citral	Tween60 1:4.5	Diameter	53.23±0.72	-	-	-
		Polydispersity Index	0.36±0.00	-	-	-
		Zeta Potential	-2.29±0.55	-	-	-
Eugenol	Tween60 1:4.5	Diameter	21.73±1.33	-	-	-
		Polydispersity Index	0.06±0.05	-	-	-
		Zeta Potential	0.24±5.63	-	-	-

As previous result, the emulsions of four most effective essential oils against the African mite then were made to be nanoemulsion by using surfactants and co-surfactants at appropriate various ratios. It was found that more use of surfactants than co-surfactants caused the better stability of emulsions. If using amount of surfactant less than co-surfactant resulted in unsteadiness emulsions (Table 4.5). The particle size analysis by using dynamic light scattering technique (DLS), informed that the particle size of the citronella grass essential oils, clove and cinnamon ranged from 11.80-18.70 nm diameter, with polydispersity index of 0.04-0.15 Mn and zeta potential at (-6.23) - (-7.92) mV. While the bigger particles size of lemon grass essential oil was 188.77 nm. Then definitely this particle was reduced by High Pressure Homogenizer at 30,000 psi,

to be 34.57 nm diameter, with polydispersity index of 0.19 Mn and zeta potential at -4.39 mV. The particles of the main chemical compositions as geraniol, citral and eugenol had diameters of 11.07-18.00 nm, with polydispersity index of 0.02-0.19 Mn and zeta potential of -10.30-3.38 mV.

Table 4.5. Particle sizes of essential oil and main chemical compound emulsions.

Emulsion	Surfactant	Surfactant and Co-Surfactant ratio	Parameter	Particle size	
				After formulated	After passed through pressure at 30,000 psi
Essential oil					
Citronella grass	Tween20 1:4	Tween20:PEG400 1:4:3.5	Diameter	11.80±0.10	-
			Polydispersity Index	0.04±0.00	-
			Zeta Potential	-6.95±1.02	-
Lemon grass	Tween60 1:4.5	Tween60:PEG400 1:4.5:2.5	Diameter	188.77±15.70	34.57±2.74
			Polydispersity Index	0.24±0.05	0.19±0.02
			Zeta Potential	-15.45±0.61	-4.39±0.47
Cinnamon	Tween60 1:4.5	Tween60:PEG400 1:4.5:1	Diameter	18.70±0.20	-
			Polydispersity Index	0.15±0.02	-
			Zeta Potential	-6.23±2.33	-
Clove	Tween60 1:4.5	Tween60:PEG400 1:4.5:1	Diameter	16.63±0.12	-
			Polydispersity Index	0.14±0.00	-
			Zeta Potential	-7.92±0.74	-
Main chemical compound					
Geraniol	Tween20 1:4	Tween20:PEG400 1:4:1	Diameter	11.07±0.12	-
			Polydispersity Index	0.02±0.01	-
			Zeta Potential	3.38±1.21	-
Citral	Tween60 1:4.5	Tween60:PEG400 1:4.5:2.5	Diameter	12.37±0.06	-
			Polydispersity Index	0.03±0.00	-
			Zeta Potential	-10.30±1.45	-
Eugenol	Tween60 1:4.5	Tween60:PEG400 1:4.5:1	Diameter	18.00±0.17	-
			Polydispersity Index	0.19±0.01	-
			Zeta Potential	-6.44±0.88	-

4.2 Toxicity of essential oil emulsions and nanoemulsion against the African red mite

The acaricidal activity assessment informed that essential oil nanoemulsions (nEOs) of citronella grass at concentration 1% and 2% could control completely the African red mite followed by nEOs of cinnamon, clove and lemongrass, with mite mortalities of 4.0%-28.8% and 71.7%-90.4% at 1% and 2% concentration, respectively. It would be seen that at 1%, nEOs expressed more three-time activities than that of EOs, particularly of citronella grass essential oil at 1% which caused 28.6% mite mortality, whereas, nEOs gave 100% mite mortality. A similar result was observed on the other plant essential oils. Remarkable results were appeared when percent mortality of mite among nEOs at 1% and 2% concentrations was not significantly different (Table 4.6).

Table 4.6. Mortality percentage of the African red mite caused by plant essential oil emulsions and nanoemulsions at 1% and 2% concentrations by leaf dipping method.

	Essential oil emulsion		Essential oil nanoemulsion	
	1%	2%	1%	2%
	%		%	
Citronella grass	28.6 ± 6.2^{Ac}	90.4 ± 2.6^{Ab}	100.0 ± 0.0^{Aa}	100.0 ± 0.0^{Aa}
Lemongrass	28.8 ± 6.8^{Ac}	78.9 ± 8.4^{ABb}	86.8 ± 28.4^{Aa}	100.0 ± 0.0^{Aa}
Cinnamon	10.7 ± 6.2^{Bc}	73.7 ± 11.6^{Ab}	96.2 ± 6.3^{Aa}	100.0 ± 0.0^{Aa}
Clove	4.0 ± 0.7^{Bc}	71.7 ± 1.7^{Ab}	87.6 ± 21.9^{Aa}	100.0 ± 0.0^{Aa}
CV, %	30.77	9.32	19.63	-

Means in column followed by the same capital letter and means in row followed by the same common letter are nonsignificantly different ($P < 0.05$) according to Duncan's multiple range test.

The higher concentration of all nEOs caused the more higher mortality rate of the African red mite. Citronella grass nEOs at 0.4% could kill the mite up to 97.2%, followed by geraniol (main chemical compound nanoemulsions (nMCs)) of 96.7% mortality but with nonsignificant difference. However, the LC_{50} nEOs and nMCs of citronella grass and geraniol gave the best LC_{50} and LC_{90} values at 0.146, 0.162, 0.303 and 0.330%, respectively. All surfactants prepared by mixing main surfactants and co-surfactants with different ratios (A-D), with 0.4% concentration performed mite mortality of 60.1%-89.7%, the LC_{50} and LC_{90} values at 0.190%-0.464% and 0.400%-1.136%, respectively, while other nEOs and nMCs showed 66.5%-90.0% and 55.1%-88.3% mortalities, with LC_{50} and LC_{90} values were 0.241%-0.324%, 0.210%-0.392%,

0.406%-0.691% and 0.528%-0.659% respectively. However, there were significant differences among a group of treatments. The satisfactory result was found when the nEOs of citronella grass at 0.6%-1% could completely kill the mite with nonsignificant difference among mentioned concentrations (Table 4.7 and 4.8). Comparing the efficiency of citronella grass nEOs and geraniol nMCs with others at 0.4% implied that other treatments resulted in a lower mortality percentage of the African red mite. However, all surfactants used also performed high toxic effect to the mite, particularly at 0.4%-1.0% concentrations, they caused 60.1%-100% mite mortality. Obtained result indicated that normally many compounds existing in essential oil could work together better than a chemical compound alone as well as there was some synergistic effect according to mixing between the precursor and surfactant. Disappointed result occurred when the performance of nEOs and nMCs formulas gave normally lower mortality of mite than that of citronella grass nEOs alone. Noticeably, at 0.6%, they presented 87.2%-98.3% mortality compared to 100% caused by citronella grass nEOs. This was maybe due to the influence of surfactant and co-surfactant. Mixing of plant essential oils normally presented more toxicity to the mite tested. (Figure 4.2). There were very few reports regarding the efficiency of citronella grass essential oil in controlling mite pest. Interesting result was found on chemical insecticides (imidacloprid) which showed very low toxicity to the mite with only 5% mite mortality at recommendation rate used.

Table 4.7. Mortality percentage of the African red mite caused by different essential oil and main chemical compound nanoemulsion at various concentrations by leaf dipping method under laboratory conditions.

Emulsion formation	Nanoemulsion concentration (%)					
	0.0 (water)	0.2	0.4	0.6	0.8	1.0
	%					
Surfactant						
A = Tween20(4):PEG400(3.5)	0.0^{a}	73.3^{ab}	89.7^{ab}	98.1^{a}	98.1^{ab}	99.0^{ab}
B = Tween20(4):PEG400(1)	0.0^{a}	84.8^{a}	88.3^{abc}	97.9^{a}	98.1^{ab}	100.0^{a}
C = Tween60(4.5):PEG400(2.5)	0.0^{a}	48.2^{bcde}	63.2^{bcde}	80.9^{abcd}	85.2^{cd}	93.0^{abc}
D = Tween60(4.5):PEG400(1)	0.0^{a}	42.6^{cde}	60.1^{cde}	67.1^{de}	69.9^{e}	76.6^{d}
Essential oil nanoemulsion						
Citronella grass in A	0.0^{a}	87.2^{a}	97.2^{a}	100.0^{a}	100.0^{a}	100.0^{a}
Lemongrass in C	0.0^{a}	39.9^{de}	53.2^{de}	71.6^{cde}	84.2^{cd}	86.4^{cd}
Cinnamon in D	0.0^{a}	19.3^{ef}	56.1^{de}	73.5^{bcde}	87.5^{abcd}	96.1^{abc}
Clove in D	0.0^{a}	21.3^{def}	48.0^{e}	59.2^{e}	86.2^{bcd}	87.3^{bcd}
Main chemical compound nanoemulsion						
Geraniol in B	0.0^{a}	81.1^{a}	96.7^{a}	97.0^{a}	100.0^{a}	100.0^{a}
Citral in C	0.0^{a}	69.8^{abc}	81.8^{abcd}	90.0^{abc}	95.0^{abcd}	100.0^{a}
Eugenol in D	0.0^{a}	45.0^{bcde}	63.9^{bcde}	81.7^{abcd}	84.0^{d}	90.9^{abc}
Essential oil nanoemulsion formula						
Citronella grass:Lemongrass (3:1)	0.0^{a}	44.2^{bcde}	66.5^{bcde}	87.2^{abc}	93.3^{abcd}	98.3^{abc}
Citronella grass:Cinnamon (3:1)	0.0^{a}	43.8^{cde}	90.0^{ab}	98.3^{a}	100.0^{a}	100.0^{a}
Citronella grass:Clove (3:1)	0.0^{a}	51.3^{bcd}	85.0^{abc}	94.6^{a}	98.3^{ab}	100.0^{a}
Citronella grass:Lemongrass (2:2)	0.0^{a}	39.1^{de}	81.0^{abcd}	88.7^{abc}	90.0^{abcd}	95.0^{abc}
Citronella grass:Cinnamon (2:2)	0.0^{a}	44.2^{bcde}	85.9^{abc}	95.0^{a}	100.0^{a}	100.0^{a}
Citronella grass:Clove (2:2)	0.0^{a}	38.1^{de}	76.4^{abcd}	91.7^{abc}	93.3^{abcd}	94.7^{abc}
Main chemical compounds nanoemulsion formula						
Geraniol:Citral (3:1)	0.0^{a}	9.2^{f}	65.2^{bcde}	93.8^{ab}	94.3^{abcd}	100.0^{a}
Geraniol:Eugenol (3:1)	0.0^{a}	19.5^{ef}	55.1^{de}	88.3^{abc}	93.3^{abcd}	100.0^{a}
Geraniol:Citral (2:2)	0.0^{a}	69.7^{abc}	88.3^{abc}	91.8^{abc}	96.7^{abc}	98.3^{abc}
Geraniol:Eugenol (2:2)	0.0^{a}	33.9^{def}	74.2^{abcde}	86.3^{abcd}	96.7^{abc}	98.3^{abc}
CV, %		45.79	27.84	17.40	9.82	9.32

Chemical	Concentration	
	Recommendation rate	Double rate
Imidacloprid	5.0±12.7	5.9±6.9

Means in column followed by same common letter are nonsignificantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 4.8. LC_{50} and LC_{90} of different essential oil and main chemical compound emulsions against the African red mite by leaf dipping method.

Emulsion formation	Regression eqnation	Chi-square	LC_{50} (Low-Up)	LC_{90} (Low-Up)
Surfactant				
A = Tween20(4):PEG400(3.5)	Y= -0.972+5.129x	693.389	0.190 (-)	0.439 (-)
B = Tween20(4):PEG400(1)	Y= -0.936+5.539x	203.567	0.169 (-)	0.400 (-)
C = Tween60(4.5):PEG400(2.5)	Y= -0.996+2.823x	39.103	0.353 (0.37-0.55)	0.807 (0.59-1.52)
D = Tween60(4.5):PEG400(1)	Y= -0.886+1.909x	46.679	0.464 (-0.19-0.90)	1.136 (0.77-5.03)
Essential oil nanoemulsion				
Citronella grass in A	Y= -1.193+8.176x	31.963	0.146 (-0.02-0.27)	0.303 (0.20-0.77)
Lemongrass in C	Y= -1.103+2.580x	31.980	0.427 (0.17-0.62)	0.924 (0.70-1.61)
Cinnamon in D	Y= -1.605+3.645x	14.352	0.440 (0.33-0.53)	0.792 (0.67-1.00)
Clove in D	Y= -1.497+2.983x	19.083	0.502 (0.36-0.63)	0.931 (0.76-1.27)
Main chemical compound nanoemulsion				
Geraniol in B	Y= -1.227+7.595x	230.220	0.162 (-)	0.330 (-)
Citral in C	Y= -0.905+3.994x	63.401	0.227 (-0.43-0.46)	0.548 (0.34-1.98)
Eugenol in D	Y= -0.994+2.737x	41.168	0.363 (0.02-0.57)	0.831 (0.60-1.63)
Essential oil nanoemulsion formula				
Citronella grass:Lemongrass (3:1)	Y= -1.232+3.806x	26.478	0.324 (0.15-0.45)	0.660 (0.51-0.99)
Citronella grass:Cinnamon (3:1)	Y= -1.866+7.752x	12.597	0.241 (0.17-0.30)	0.406 (0.33-0.54)
Citronella grass:Clove (3:1)	Y= -1.350+5.425x	37.271	0.249 (0.05-0.39)	0.485 (0.35-0.91)
Citronella grass:Lemongrass (2:2)	Y= -1.073+3.408x	57.769	0.315 (-0.11-0.54)	0.691 (0.47-1.61)
Citronella grass:Cinnamon (2:2)	Y= -1.631+6.364x	18.385	0.256 (0.16-0.34)	0.458 (0.36-0.65)
Citronella grass:Clove (2:2)	Y= -1.151+3.639x	63.736	0.316 (-0.12-0.56)	0.668 (0.45-1.68)
Main chemical compounds nanoemulsion formula				
Geraniol:Citral (3:1)	Y= -2.19+5.718x	38.790	0.383 (0.21-0.52)	0.607 (0.47-0.99)
Geraniol:Eugenol (3:1)	Y= -1.885+4.806x	12.512	0.392 (0.31-0.47)	0.659 (0.56-0.81)
Geraniol:Citral (2:2)	Y= -0.848+4.036x	94.247	0.210 (-)	0.528 (-)
Geraniol:Eugenol (2:2)	Y= -1.413+4.318x	29.245	0.327 (0.16-0.46)	0.624 (0.48-0.95)

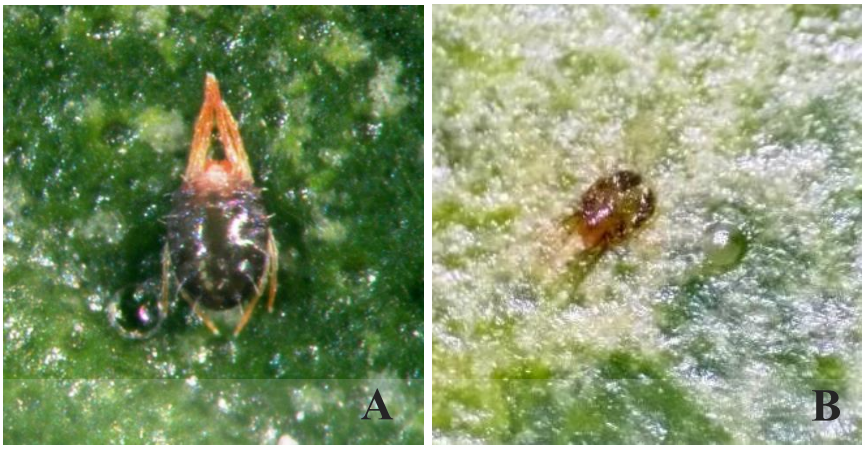

Figure 4.2. A**:** Live adults of the African red mite before bioassay

B**:** Death adult of the African red mite after bioassay.

Result obtained from the experiment of egg-laying inhibition caused by nEOs, nMCs and surfactants at 0.02%-0.2% concentrations by leaf dipping method revealed that the high concentration expressed the high reduction of egg-laid. Clove nEOs showed the minimum number of egg-laid with 2.7 eggs per adult female at 0.02% concentration, followed by eugenol, citral, cinnamon and citronella grass oil with 3.1, 3.2, 3.4 and 3.4 eggs, respectively, but there was nonsignificant difference among them. In addition, geraniol at 0.2% represented highly egg-laying inhibition effect with 0.1 egg and followed by citronella grass oil with 0.3 egg with nonsignificant difference between them. The higher concentration of surfactant performed more effect on egg-laid. Whereas, control (water) resulted in number of egg-laid with 4.7 eggs, about nearly six times more than that of surfactant at 0.2% (Table 4.9). However, when it was compared to nEOs, the bigger number of egg laid obtained. Additionally, nEOs with low concentration could inhibit egg number similar to surfactant. For nMCs result, generally they tended to have more activity with higher concentration, especially, geraniol which showed the best performance. Surprisingly, imidacloprid showed no effect on egg inhibition of the mite.

Table 4.9. Egg laid number of the African red mite after treated with essential oil and main chemical compound nanoemulsions (nMCs) at various concentrations by leaf dipping method under laboratory conditions.

Concentration (%)	Number of egg (s)/female							
	Surfactant	Essential oil nanoemulsion				Main chemical compound nanoemulsion		
	A	Citronella grass	Lemongrass	Cinnamon	Clove	Geraniol	Citral	Eugenol
0.0 (water)	4.7^{Aa}	4.7^{Aa}	4.7^{Aa}	4.7^{Aa}	4.7^{Aa}	4.7^{Aa}	4.7^{Aa}	4.7^{Aa}
0.02	2.8^{Ab}	3.4^{Ab}	3.8^{Aab}	3.4^{Ab}	2.7^{Ab}	3.8^{Aab}	3.2^{Ab}	3.1^{Ab}
0.04	2.8^{Ab}	2.2^{Ac}	3.3^{Abc}	3.3^{Abc}	2.6^{Ab}	3.0^{Abc}	2.0^{Abc}	3.0^{Ab}
0.06	2.1^{Ab}	2.2^{Ac}	2.5^{Acd}	2.8^{Abcd}	2.1^{Ab}	2.1^{Acd}	1.9^{Abc}	2.3^{Abc}
0.08	1.8^{Abc}	1.8^{Ac}	2.2^{Ad}	2.3^{Acd}	2.2^{Ab}	1.6^{Ade}	1.7^{Abc}	2.1^{Abc}
0.10	1.6^{ABbc}	1.7^{Ac}	1.9^{Ad}	1.9^{Ad}	2.2^{Ab}	1.5^{ABde}	0.5^{Bc}	2.1^{Abc}
0.20	0.8^{ABCc}	0.3^{Cd}	1.8^{Ad}	1.9^{Ad}	1.8^{Ab}	0.1^{Ce}	0.5^{BCc}	1.5^{ABc}

Chemical	Concentration	
	Recommendation rate	Double rate
Imidacloprid	4.0±1.2	4.9±0.9

Means in column followed by the same capital letter and means in row followed by the same common letter are nonsignificantly different ($P < 0.05$) according to Duncan's multiple range test

A: Tween20:PEG400(4:3.5)

For the repellent test, it was found that citronella grass and lemongrass nEOs at 0.06%-0.1% had the highest repellent efficacy with 85.0%-95.0% repellent, which were nonsignificantly different when compared to other treatments. But it was apparent that at lower concentration as 0.02%, clove nEOs and citral nMCs both showed 66.67% and 71.67% repellent. They were significantly different with others at 95% confidence ($P < 0.05$), in which responded as the attractant to the African red mites up to 28.33% and 33.33% (Table 4.10).

Table 4.10. Response percentage of the African red mite to various concentrations of essential oil and main chemical compound nanoemulsions (nEOs and nMCs) under laboratory conditions.

Emulsions	Concentration (%)	Response (%)		X^2	P
Essential oil nanoemulsion		- (repellent)	+ (attraction)		
Citronella grass	0.02	81.67	18.33	13.375	0.000**
	0.06	93.33	6.67	27.743	0.000**
	0.10	95.00	5.00	30.740	0.000**
Lemongrass	0.02	81.67	18.33	13.375	0.000**
	0.06	85.00	15.00	16.752	0.000**
	0.10	95.00	5.00	30.470	0.000**
Cinnamon	0.02	85.00	15.00	16.752	0.000**
	0.06	86.67	13.33	18.639	0.000**
	0.10	90.00	10.00	22.857	0.000**
Clove	0.02	66.67	33.33	3.429	0.641ns
	0.06	80.00	20.00	11.868	0.001**
	0.10	81.67	18.33	13.375	0.000**
Main chemical compound nanoemulsion					
Geraniol	0.02	81.67	18.33	13.375	0.000**
	0.06	83.33	16.67	15.000	0.000**
	0.10	85.00	15.00	16.752	0.000**
Citral	0.02	71.67	28.33	5.911	0.150ns
	0.06	73.33	26.67	6.910	0.009**
	0.10	83.33	16.67	15.000	0.000**
Eugenol	0.02	70.00	30.00	5.000	0.025*
	0.06	73.33	26.67	6.910	0.009**
	0.10	76.67	23.33	9.187	0.002**
Control		30.00	30.00		

Note: '+' represent of essential oil nanoemulsion of response in the tested of attract, '-' response to essential oil nanoemulsion in the repellent.

** Represented significant difference at $P < 0.01$, * represented significant difference at $P < 0.05$, ns nonsignificant difference.

For the experiment of nEOs formula of citronella grass at concentrations of 0.2, 0.4, and 0.6% in influencing the mite population and compared with the insecticide (Amitraz) at recommendation rate by the way of after planting mulberry for 2 months and about 100 adults per tree of mite was released, and the number of live mite were randomly counted after every 1, 3, 5, and 7 days. It appeared that the number of mite after spraying with nEOs formula of citronella grass, the mite decreased from day 1. It is seen that concentrations were able to control the mite effectively with the average mite number per plant even to 0.4 adults per tree. While the insecticide (Amitraz) found that the average african red mite per tree was even to 0.2 adults per tree on

day 3 with no statistical difference. But when compared with the control (surfactant A), it showed that the average number of the mites per tree were 1.6-22.6 adults per tree, which was not effective in controlling this African red mite (Table 4.11 and Figure 4.3).

Table 4.11. Number of the African red mite caused by various concentrations of essential oil nanoemulsions at 0, 1, 3, 5 and 7 days by direct spray method under greenhouse conditions.

Essential oil nanoemulsion	Day (Means±SD)					F-test	%C.V.
	0 (Before)	1	3	5	7		
Surfactant A	151.8±4.2^{Aa}	22.6±22.7^{Ab}	11.4±14.9^{Ab}	7.0±11.8^{Ab}	1.6±3.0^{Ab}	**	58.47
Citronella grass 0.2%	134.8±16.8^{Aa}	0.4±0.9^{Bb}	0.0±0.0^{Bb}	0.0±0.0^{Bb}	0.0±0.0^{Bb}	**	27.82
Citronella grass 0.4%	156.2±25.2^{Aa}	0.0±0.0^{Bb}	0.0±0.0^{Bb}	0.0±0.0^{Bb}	0.0±0.0^{Bb}	**	36.06
Citronella grass 0.6%	164.2±51.2^{Aa}	0.0±0.0^{Bb}	0.0±0.0^{Bb}	0.0±0.0^{Bb}	0.0±0.0^{Bb}	**	69.70
Amitraz	141.2±11.3^{Aa}	0.0±0.0^{Bb}	0.2±0.4^{Bb}	0.0±0.0^{Bb}	0.0±0.0^{Bb}	**	17.81
%C.V.	21.87	221.29	287.21	377.96	426.19		
F-test	ns	**	*	ns	ns		

Means in column followed by the same capital letter and means in row followed by the same common letter are nonsignificantly different (P < 0.05) according to Duncan's multiple range test

** Represented significant difference at P < 0.01, * represented significant difference at P < 0.05, ns nonsignificant difference.

A: Tween20:PEG400(4:3.5)

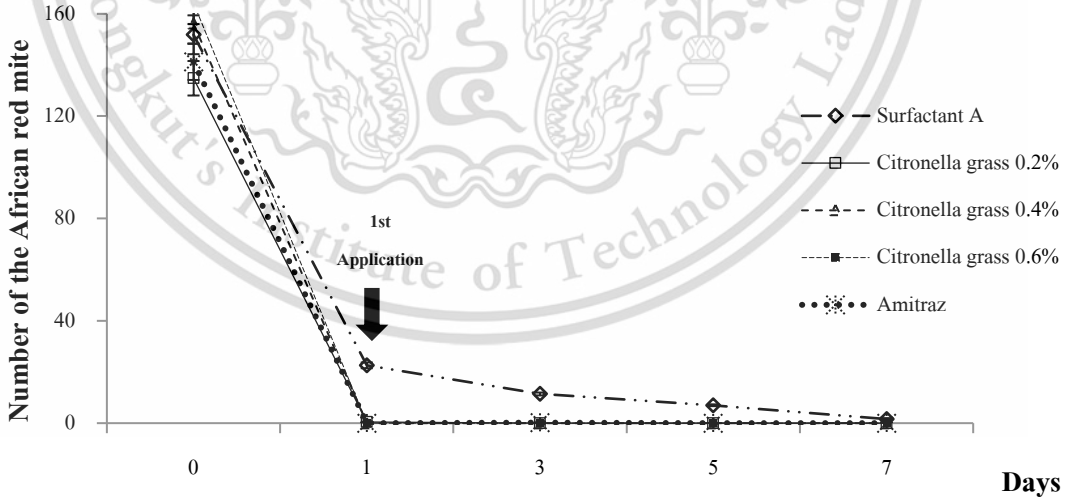

Figure 4.3. Number of the African red mite caused by various concentrations of essential oil nanoemulsions under greenhouse conditions by direct spray method.

Based on the phytotoxic characterization test which may affect by nEOs formula of citronella grass at concentrations of 0.2, 0.4 and 0.6% and compared to the insecticide (Amitraz) at recommendation rate. Those were observed after spraying at 0, 1, 3, 5 and 7 days on the mulberry leaves. It was found that the citronella grass nEOs at all concentrations. Showed the toxicity level on plants with 0-1 as normal leaves and 1-2 with slightly brown stripes. When the control (Surfactant A) and the insecticide (Amitraz) at recommendation rate was not found any toxic effect to plants (Figure 4.4)

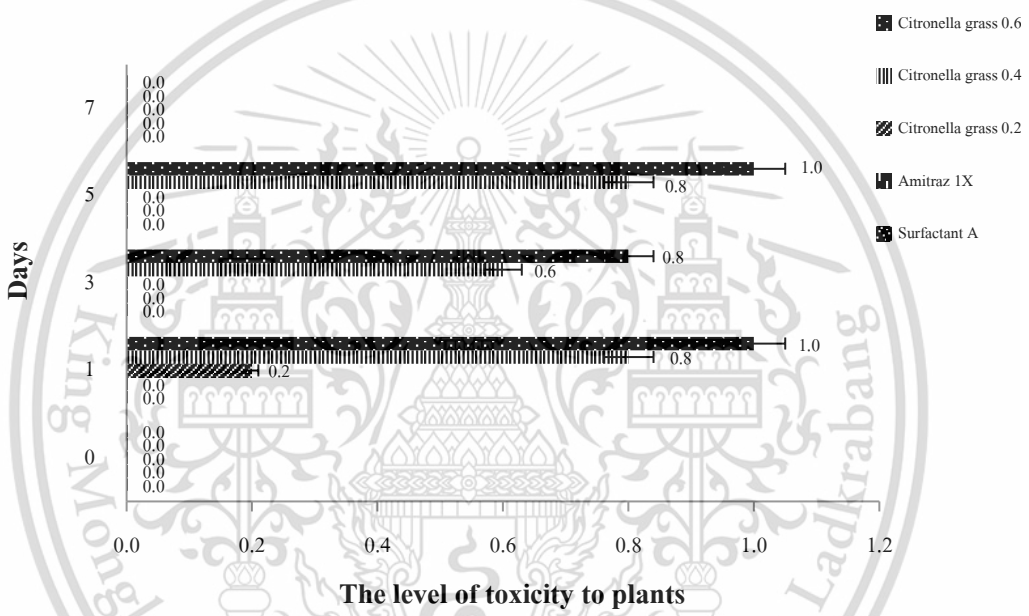

Figure 4.4. The level of phytotoxic to the mulberry leave caused by essential oil nanoemulsion at concentrations of 0.2, 0.4, and 0.6% at 0, 1, 3, 5 and 7 days under greenhouse conditions by direct spray method.

4.3 The phytotoxicity examination of essential oil nanoemulsion

The phytotoxicity test of nEOs formula of citronella grass compared with surfactant A at concentrations of 0.2, 0.4 and 0.6% was performed on the mulberry trees age of 2 months. After spraying for 0, 1, 3, 5 and 7 days then the phytotoxicity effect on the mulberry leaf was examined. It was found that both the nEOs formula from citronella grass and surfactant A of all concentrations presented the toxicity level to plants was 0-1 as normal leaves and 1-2 with slightly brown stripes. Compared with the control (water) which demonstrated of no plant toxicity characteristics (Figure 4.5 and 4.6).

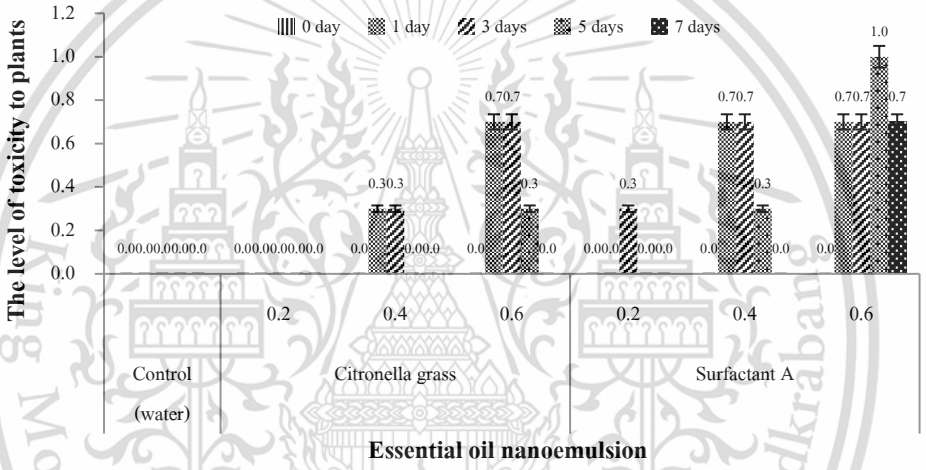

Figure 4.5. The level of phytotoxic effect to the mulberry leave caused by essential oil nanoemulsion at concentrations of 0.2, 0.4, and 0.6% at 0, 1, 3, 5 and 7 days after direct spraying under greenhouse conditions by direct spray method.

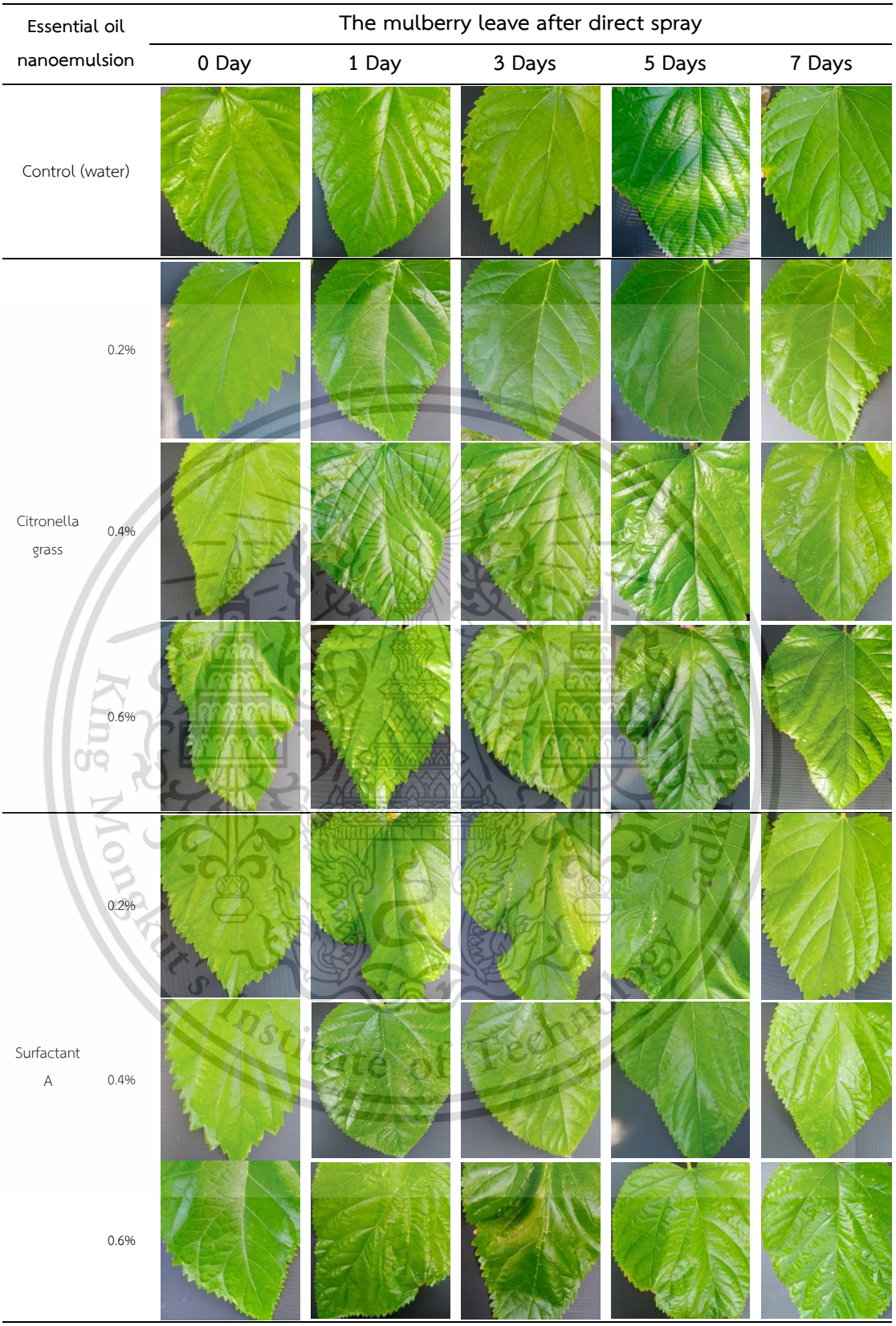

Figure 4.6. The phytotoxic effect to the mulberry leave caused by essential oil nanoemulsion at concentrations of 0.2, 0.4, and 0.6% at 0, 1, 3, 5 and 7 days after direct spraying under greenhouse conditions by direct spray method.

4.4 Toxicity of essential oil nanoemulsion against other mite pests

4.4.1 The orchid flat mite

The effectiveness of the nEOs formula of citronella grass at selected concentration of 0.4% compared with the insecticide (pyridaben) at recommendation rate by direct spray method. Found that, after 24 hrs the citronella grass nEOs was extremely effective at killing larvae and adults of the orchid flat mite. When the survival orchid flat mite was not found. Comparing with the insecticide (pyridaben), the survival of both larvae and adults of orchid flat mite were about 29-32 mites (Figure 4.7 and 4.8).

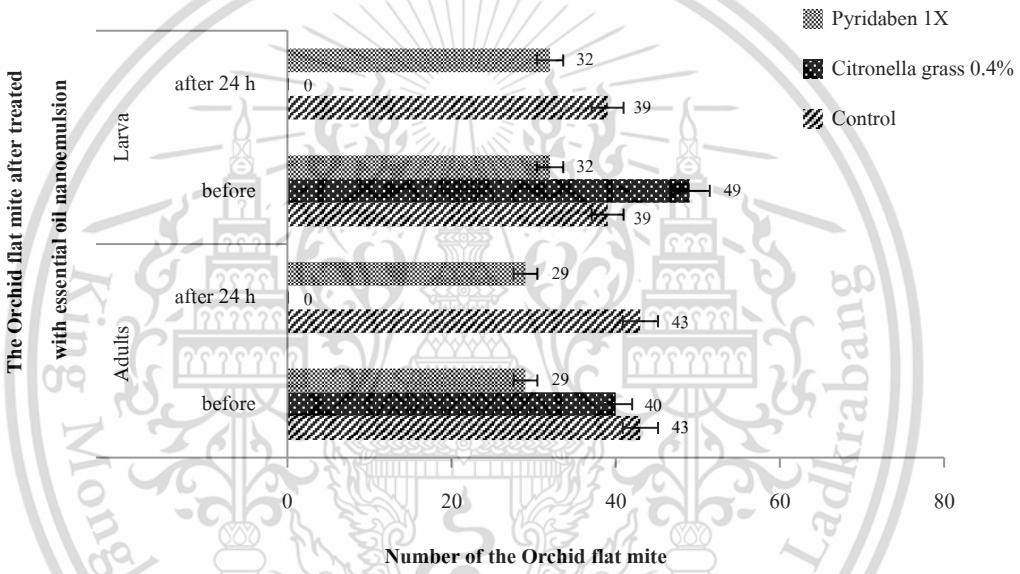

Figure 4.7. Number of the orchid flat mite caused by citronella grass essential oil nanoemulsions at 0.4% concentration under laboratory conditions by direct spray method.

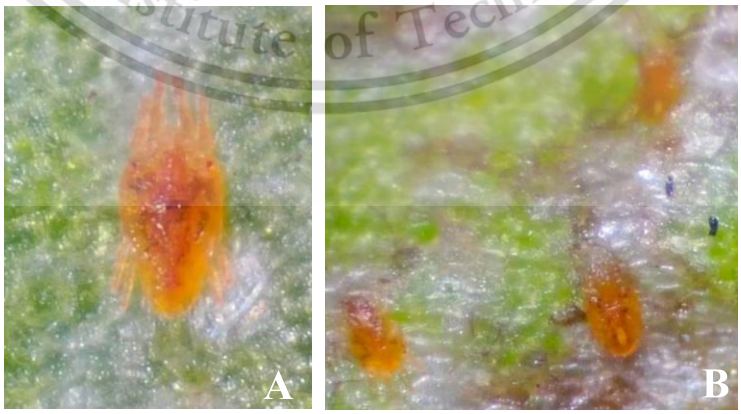

Figure 4.8. A**:** Alive adults of the orchid flat mite after direct spray in the control.
B**:** Death adult of the orchid flat mite after direct spray in the treatment of citronella grass essential oil nanoemulsions.

4.4.2 The broad mite

The nEOs formula of citronella grass at concentrations of 0.4% was tested against the broad mite and compared with the insecticide (Amitraz) at recommendation rate by direct spray method. The result informed that the nEOs formula of citronella grass was able to kill 55.4% and 73.5% at 12 and 24 hrs. Whereas insecticide (Amitraz) was not different from the control (water) (Figure 4.9).

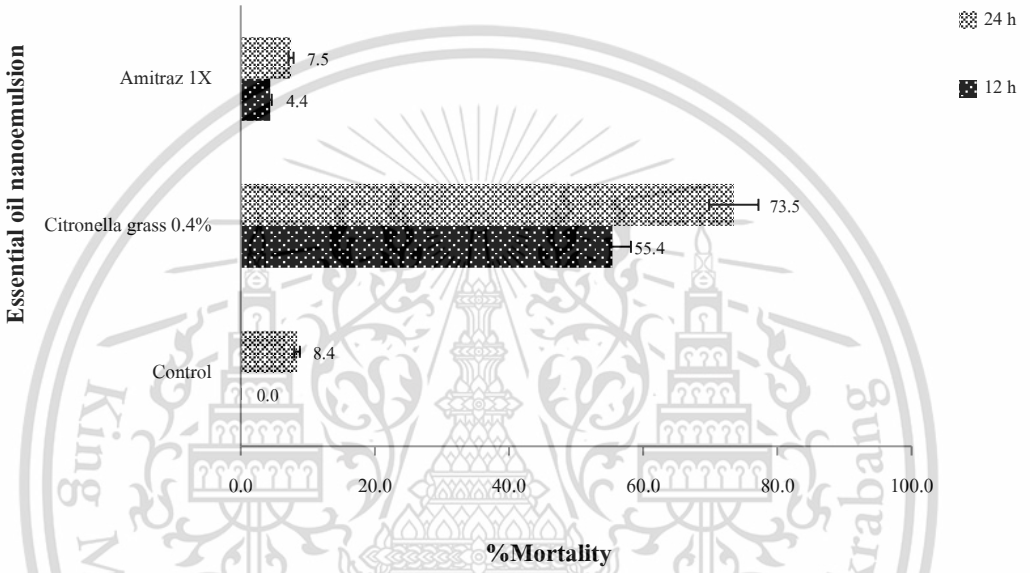

Figure 4.9. Mortality percentage of the broad mite (*Polyphagotarsonemus latus* (Banks)) caused by various concentrations of essential oil nanoemulsions at 12 and 24 hrs under laboratory conditions by direct spray method.

4.5 Field conditions

Field conditions experiment of using 0.4% concentration of citronella grass essential oil nanoemulsion to suppress the African red mite in durian orchard and compared to chemical acaricide, Amitraz at recommendation rate was performed by direct spray method. Twenty four hours after treatment observation revealed that the nanoemulsion presented the maximum control percentage of 82.31%, whereas Amitraz acaricide showed only 55.09%. And the control group (water) could a little bit decrease the mite 20.76% with significant different. (Figure 4.10).

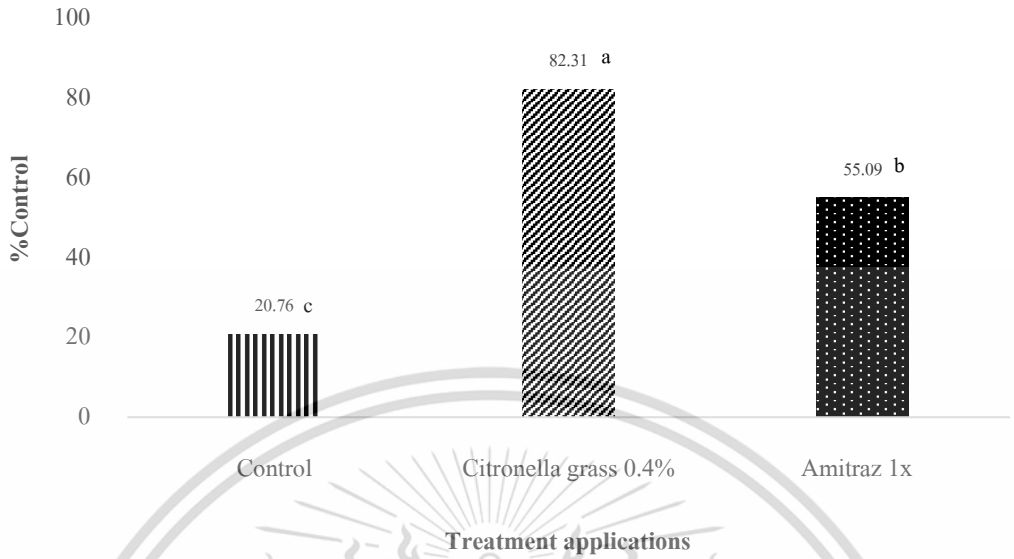

Figure 4.10. Control percentage in the African red mite, *Eutetranychus africanus* (Tucker) after treated with essential oil nanoemulsion under field conditions by direct spray method.

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CHAPTER 5
DISCUSSION

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

Out of 13 selected essential oils obtained from medicinal plants in this study, only 4 essential oil citronella grass, lemongrass, cinnamon and clove in forms of their emulsions demonstrated highly acaricidal activities against adults of the African red mite. Those essential oil emulsions (EOs) presented the acaricidal properties as toxicity, anti-hatchability, growth regulation and repellent similar to the reports of Isman (2000) Prakash and Rao (1997) and Papachristos and Stamopoulos (2002). Many reports have been demonstrated the insecticidal and acaricidal properties of various plant extracts or essential oils, for example, the essential oils of citronella grass, clove, cinnamon and lemongrass were extremely toxic against the European house dust mite, *Dermatophagoides pteronyssinus* (Insung et al. 2016). Pumnuan et al. (2009) also reported that lemon grass and citronella grass essential oils at the concentration of 75 µg/cm^3 were the most effective in inhibiting hatching of the mushroom mite, *Luciaphorus perniciosus* Rack, and resulted in 97.3 and 95.8% mortality. Danarun et al. (2015) reported that essential oil from lemon grass (*Cymbopogon citratus* Stapf) were effective to adult of the African red mite with EC_{50} at 2.43%, respectively. The essential oil of lemon grass showed insecticidal property to larger grain borer (*Prostephanus truncatus* (Horn)), rice weevil (*S. oryzae*) and house fly (*M. domestica*) (Masamba et al., 2003; Ahmed and Salam, 2010; Pinto et al., 2015).The essential oils of cinnamon, clove and lemon grass also expressed highly insecticidal activity against adults of thrips (*Frankliniella schultzei*) mealybug (*Pseudococcus jackbeardsleyi*) and stored product mites (*Suidasia pontifica* Oudemans) (Pumnuan and Insung, 2011; Pumnuan and Insung, 2016). In addition, the essential oil of cinnamon showed toxicity against cowpea weevil (*C. maculatus*), rice weevil (*S. oryzae*) and maize weevil (*S. zeamais*) (Ahmed and Salam, 2010; Pumnuan et al., 2012)

The four selected essential oils as citronella grass, lemon grass, cinnamon and clove which showed high efficiency in killing the mite were analyzed by GC-MS to find out their chemical compounds. It was found that essential oils of citronella grass

contained 37.97% citronella, followed by geraniol 18.60%. While the lemongrass essential oils had the highest amount of geranyl acetal at 29.03%, followed by citral 20.21%. In addition, the essential oils of clove and cinnamon had the similar main active ingredient which was eugenol at 81.55% and 98.45%, respectively. Sruthi et al. (2014) and Uddin et al. (2017) informed that the essential oils of clove and cinnamon contained about 80%-90% eugenol analyzed by GC and GC-MS. Silva et al. (2011) and Hamzah et al. (2013) presented that citronella grass essential oil contained the most important substances including citronellal, citronellol, geraniol, geranial and limonene. There were more than 80 components (Silva et al., 2011) appearing in its essential oil, where the essential oil of lemon grass had the most important substances mainly citral, limonene, citronellal, β-myrcene, geranial (α-citral) and neral (β-citral).

The emulsions of four most effective essential oils against the African mite then were made to be nanoemulsion by using surfactants and co-surfactants at appropriate various ratios. The use of surfactants than co-surfactants caused the better stability of emulsions. Normally, using less amount of surfactant than co-surfactant resulted in unsteadiness emulsions. The selected surfactants must be able to decrease the interfacial tension to be a very small amount to encourage the disperse process during the nanoemulsion preparation to obtain the desired nanoemulsion type (i.e., oil/water, water/oil, or bicontinuous) (McClements, 2011; Solans and Solé, 2012; Kwon et al., 2015; Komaiko and McClements, 2015; Jintapattanakit, 2018). The particle size analysis by using dynamic light scattering (DLS) technique, informed that the particle size of the citronella grass essential oils, clove and cinnamon ranged from 11.80-18.70 nm diameter, with polydispersity index of 0.04-0.15 Mn (M_n number-average molecular weight) and zeta potential at (-6.23)-(-7.92) mV. While the bigger particles size of lemongrass essential oil was 188.77 nm. Then, definitely this particle was reduced by high pressure homogenizer at 30000 psi, to be 34.57 nm diameter, with polydispersity index of 0.19 Mn and zeta potential at -4.39 mV. The particles size of the main chemical compositions as geraniol, citral and eugenol ranged from 11.07-18.00 nm diameter, had polydispersity index of 0.02-0.19 Mn and zeta potential of -10.30-3.38 mV. The polydispersity index is an indication of their quality with respect of size distribution (Danaei et al., 2018). The zeta potential values above 30 mV indicate physical stability (Marsalek, 2014). Unfortunately, poor zeta potential values were detected in this study. It was strongly mentioned that the emulsion with smaller particles of plant essential oils

could work better than larger particles. Additionally, use of co-surfactant was very important technique in order to prepare essential oil nanoemulsions (nEOs) and their mixing may have some promotion effect on the mite.

The higher concentration of all nEOs caused the more higher mortality rate of the African red mite. Citronella grass nEOs at 0.4% could kill the mite up to 97.2%, followed by geraniol (main chemical compound nanoemulsions (nMCs)) of 96.7% mortality but with nonsignificant difference. All surfactants prepared by mixing main surfactants and co-surfactants with different ratios (A-D), while other nEOs and nMCs showed 66.5%-90.0% and 55.1%-88.3% mortalities, respectively. The satisfactory result was found when the nEOs of citronella grass at 0.6%-1% could completely kill the mite with nonsignificant difference among mentioned concentrations. Obtained result indicated that normally many compounds existing in essential oil could work together better than chemical compound alone as well as there were some synergistic effects according to mixing between the precursor and surfactant. This was maybe due to the influence of surfactant and co-surfactant. Mixing of plant essential oils normally presented more toxicity to the mite tested. There were very few reports regarding the efficiency of citronella grass essential oil in controlling mite pest. Pumnuan and Insung. (2016) demonstrated that lemon grass and citronella grass essential oil at 3µg/L air caused 100% and 82.5% mortality, respectively of mushroom mite, *Dolichocybe indica* by using the fumigation method. Moreover, Saad et al. (2017) proved that the citronella essential oil composed widely insecticidal properties as contact-toxicity, repellency, and ovipositional deterrence to *Bemisia tabaci*. It also caused high mortality at the concentration of 6.66 lL/L air. Mwandila et al. (2013) found that the Syringa (*Melia azedarach*) seed extracts (SSE) performed acaricidal properties against adults, egg and nymphs of the red spider mite (*Tetranychus* spp.). Unfortunately, any reports regarding the interference of surfactants to mite egg-laid was not found. Pumnuan et al. (2009) also reported that lemon grass and citronella grass essential oils were the most effective in inhibiting hatching of the mushroom mite, *Luciaphorus perniciosus* Rack. Few studies about the repellent test of the selected essential oils against other mite pests. Kheradmand et al. (2015) informed that clove essential oil performed the most potent repellency effect to two-spotted spider mite, *Tetranychus urticae*. But in this study, showed relatively low repellent to the mite tested, especially compared with citronella grass nEOs. Thein et al. (2013) also emphasized that crude extract of

citronella was the most repellent to the maize weevil, *Sitophilus* spp. According to Lakyat et al. (2017) the repellent activity of lemongrass nEOs at very low concentration of 0.02% had the most repellent effect to adult of brown planthopper, *Nilaparvata lugens* with 43.83% RI. Monoterpenes in essential oils are cytotoxic to animal tissue, causing a radical reduction in mitochondria and golgi body numbers, damaging the respiratory system (Lee et al., 1997). However, there is few information about physiological actions of essential oils on insects or mites. Various essential oils and their constituents seem to cause systems of neurotoxic mode of action (Kostyukovsky et al., 2002). In addition, these essential oils were also highly toxic against many other insects and mites (Kim et al., 2003; Masamba et al., 2003; Kim et al., 2006; Akhtar et al., 2008; Hanifah et al., 2011; Pinto et al., 2015).

5.2. The efficacy test of essential oil nanoemulsion to other mites.

The nEOs formula of citronella grass at concentration of 0.4% was used to test to the orchid flat mite and the broad mite by direct spray method. The obtained result explained that the citronella grass nEOs was extremely effective in controlling the orchid flat mite and the broad mite. However, there were few documents regarding acaricidal activity of plant essential oil against the both mite pests. Venzona et al. (2008) also tested the acaricidal effect of neem, *Azadirachta indica* extract on *Polyphagotarsonemus latus*. at concentrations of 0.13 g a.i/l and found to have an effect population growth rate becoming negative, when mites were contact to plants treated with neem. Whereas, hundreds of regarding the plant extract or plant essential oil performing high toxic property to the other mite pests, especially to Tetranychidae and Acarid mites. Aslan et al. (2004) reported that investigated the toxicity of essential oil vapours from *Thymus vulgaris* L., *Ocimum basilicum* L., and *Satureja hortensis* to *T. urticae* and those gave satisfactory result. Choi et al. (2004) tested 53 essential plant oils for their toxicity against the eggs and adults of *T. urticae* as well as the adults of *Phytoseiulus persimilis* Athias-Henriot obtained result presented that citronella java, lemon eucalyptus, peppermint oils gave > 90% mortality against adult *T. urticae*. Miresmaili et al. (2006) compared the toxicity of *Rosemarinus officinalis* L. essential oil and a blend of its major constituents against *T. urticae* on two different host plants. It was found that some constituents accounted for most of the toxicity but some were relatively inactive. According to this study, the nEOs tends to be used to control the

mites in field condition. When, Mead (2012) found that the lemongrass oil was more toxic against adults of *T. urticae* used the spraying method than the leaf dipping method. An importantly reduced hatchability percentage of *T. urticae* eggs than control that recorded 85.33,77.33, 57.33 and 33.33 % for concentration 0.25, 0.5, 1.0 and 2.0 %, respectively. In addition, Elhalawany and Dewidar. (2017) reported that the seven essential oils namely; lemon grass, spearmint, rosemary, marjoram herbs, fennel and coriander seeds and flower of chamomile tested for their toxicity against eggs and adults of *Tetranychus urticae, Phytoseiulus persimilis* and *Neoseiulus californicus*, in five concentrations 4, 3, 2, 1 and 0.5%. Then, the LC_{50} values for the adult females after 72h of *T. urticae* were 1.28, 0.85, 0.53, 1.61, 0.44, 3.11 and 0.46%, respectively. The LC_{50} values of the seven oils ranged between 7.09 and 9.63% for *P. persimilis*, ranged from 4.94 to 9.63% for *N. californicus* at 48h. Miresmailli and Isman (2006) found that rosemary oil can affect the oviposition behavior the two spotted spider mite, *T. urticae* and *Phytoseiulus persimilis*. Sornlek. (2001) reported that essential oil of *P. nigrum* showed acaricidal effect on the spider mite, *Eutetranychus cendanai* Rimando with LC_{50} at 23.6 ml/l. Pumnuan et al. (2009) revealed that lemon grass (*Cymbopogon citratus*) and citronella grass essential oils at the concentration of 75 µg/cm^3 were extremely effective in inhibiting egg hatching of the mushroom mite, *Luciaphorus perniciosus* Rack, and resulted in 97.3 and 95.8% mortality. Pumnuan et al. (2010) also demonstrated that the essential oil of *Litsea cubeba* showed to be the most toxic to *Luciaphorus perniciosus* by both contact and fumigation methods with LD_{50} values equaling to 0.932 and 0.166 µg/cm^3, respectively, followed by essential oil of *Litsea salicifolia* and *Melaleuca cajuputi*.

5.3. The efficacy of essential oil nanoemulsion in controlling the African red mite.

According to research results explained the high toxicity of essential oil nanoemulsions in controlling and mite pests. And importantly, they would playmore powerful than that of the essential oils. This could be an alternative tool for mite management to meet the needs of organic or safety farms. They have deferitely the advantage of being non-toxic to mammals and readily biodegradable on plants and in the soil (Kleeberg, 1992; Isman, 2006) For example, the essential oils of citronella grass, clove, cinnamon and lemongrass were extremely toxic against the European house

dust mite, *Dermatophagoides pteronyssinus*. This essential oil is an ethanol based (with would be nanoparticle) and could be commercialized in order to control the house dust mites (Insung et al. 2016). As for the control of the African red mite, Insung et al. (2008) reported that extract from *piper retrofractum* at the concentration of 1% could completely control the African red mite. However, there are few studies regarding using of nanotechnology available in insect or mite pest controls. Sarapothong et al. (2017) presented that nano-essential oil of black pepper and surfactants showed high effectiveness against African red mite at concentration of 1% caused the mite mortalities of 96 and 92% and gave LC_{50} at 0.34 and 0.54%, respectively. Pumnuan et al. (2009) reported the effect of essential oils obtained from seed coat and seed kernel of black pepper, (*Piper nigrum* Linn.) against the African red mite and found that the essential oil obtained from seed kernel of black pepper performed an effect in killing the African red mite. While the essential oil obtained from seed coat was effectual in repellency. In addition, essential oil from lemon grass was highly effective to adult of the African red mite (Danarun et al., 2015). The increasing amount of studies on plant-insect chemical interactions in the last few decades has unveiled the potential of utilising botanical insecticides in the form of secondary plant metabolites, or allelochemicals, as pest control agents. This interest in natural pesticides from the leaves or seed is providing an alternative in pest management programmes to the synthetic insecticides (Whitten, 1992; Martinez and van Emden, 2001)

5.4. Phytotoxicity effect of essential oil nanoemulsion.

In the primary test, the nEOs formula of citronella grass exhibited was no significant phytotoxicity effect on mulberry trees when compared to the control. Especially, citronella grass (nEOs) at 0.2-0.4% showed no significant color changes. The changes can be a result of biological or other influencing factors, such as use timing, plant species, temperature and essential oil type. Different ratios and concentrations of even the same EOs can result in different effects. It is possible that mixtures of EOs might reduce the concentration of phytotoxic constituents in the mixtures, while at the same time generating synergy effects against insects or mites (Miresmailli et al., 2006; Tripathi et al., 2009; Choi et al., 2010; Kim et al., 2012). However, Plooya et al. (2009) discussed that with the proper application, individual terpenoids which was the

major compound in essential oil can provide selected barriers as coatings against the gas exchange, respiration and moisture loss on plants. Solgi and Ghorbanpour (2014) showed a potential coating property of gum Arabic which was biopolymer obtained from stems and branches of Acacia tree. Maqbool et al. (2011) reported synergistic effects of the combination between gum Arabic and EOs of cinnamon and lemon grass against antracnose on bananas and papayas. In addition, higher EO concentrations tend to exhibit the more physiological changes in plants (Meyer et al., 2008). Phytotoxic effects are normally dependent on factors such as plant species, oil concentrations, the volume of EO applied, and the size of plants (Bainard et al., 2006; Boyd and Brennan, 2006). As mentioned above implied that more concerned with the phytotoxic effect is needed when using the application of essential oil emulsion to control the mite or insect pests.

5.5. Advantage and limitation of essential oil nanoemulsion products.

Nanoemulsion has gained a lot of attention these past three to four years especially in pharmaceutical preparations, because drugs generally become less effective due to the instability and poor solubility of the delivery agents. This nanoemulsion technique plays a huge role in the cosmetic and pharmaceutical industries for the skin. Due to doing nanoemulsion will increase stability during the storage of essential substances. It may also be used as a precursor to making products nanoencapsulation as well (Saleengam and Chamchong., 2012). There are several techniques for nanoemulsion such as aqueous titration, high pressure homogenization, solvent evaporation, precipitation, supercritical fluid extraction, etc. (Bravo-Osuna et al., 2006; Chang et al., 2010; Chaudhuri and Paria, 2010; Chavanpatil. et al.,2007; Mai Hoa et al.,2009; Wang et al., 2009). In this study, we made essential oil nanoemulsion by the way of using appropriate proportion of surfactant and co-surfactant as well as via high pressure homogenization equipment. Each technique was its advantages and disadvantages for example, the choice of high pressure homogenization method. It requires high capital because the tools are expensive, while the aqueous titration method is easy to do and does not require high capital. Therefore, nanotechnology and nanomaterials are successfully and safely used in fields such as medicine, environmental science and food processing. However, the use of nanomaterials in agriculture especially for plant protection and production. There was still relatively

little research, they were mainly used as conductors and semi-conductors, medical devices, sensors, coatings, catalysts and also as pesticides (Sahayaraj, 2017). In the end, the global view of the study strongly demonstrated that the citronella grass essential oil nanoemulsion could be alternatively applied for mite pest control and may be performed as commercial product.

CHAPTER 6
CONCLUSION AND SUGGESTION

6.1. Conclusion

The efficiency test in terms of acaricidal property, egg laying inhibition and repellent properties of plant essential oil emulsions (EOs) and nanoemulsions (nEOs) as well as their main chemical compound nanoemulsions against the African red mite (*Eutetranychus africanus*) demonstrated that citronella grass essential oil nanoemulsion at 0.6% concentration was extremely toxic with 100% mite mortality. Besides, all surfactants used at high concentration also presented high toxic effect to the mite. Nanoemulsion of geraniol, one of the main chemical compounds of citronella grass essential oil and citronella grass essential oil nanoemulsion at 0.2% showed potent egg inhibition to the African red mite with the average number of eggs laid per female as 0.1 and 0.3 egg, respectively, whereas, 4.7 eggs were obtained in the control. In addition, citronella grass and lemon grass essential oil nanoemulsions at 0.1% showed high repellent to the African red mite, with 95% RI. In consequence, citronella grass essential oil nanoemulsion is an appropriate material to be used to control the African red mite.

The formulation study the nEOs of citronella grass exhibited was no significant phytotoxicity effect on plants when compared to the control. Especially, citronella grass nEOs at 0.2-0.4% presented no significant differences in color changes and physiological changes. While the concentration at 0.4% of citronella grass expressed toxic effect against the orchid flat mite and the broad mite and able to control pest mites well. In addition, when this nanoemulsion tested at a concentration of 0.4% by direct spray method against the African red mite in field conditions, it was seen that citronella grass nanoemulsion essential oil gave the maximum control percentage of 82.31% compared to the control and the acaricide (Amitraz) had the control percentage of only 20.76-55.09%. Therefor, the use of plant essential oils, especially, in the form of nanoparticle to control insect and mite pests to replace the use of synthetic chemicals, or reducing the imported chemicals from foreign countries is promising work tended to be possibly. Essential oils from plants also decay faster,

non toxic residue in the environment and very low toxic to worm blood animals as well.

6.2. Suggestion

The main objective of this research study was to produce and develop of plant essential oils for controlling the African red mite (*Eutetranychus africanus* (Tucker)) and other mite pests based on nanotechnology with be interested in their toxicity, repellent and egg-laying inhibition. Remarkably, citronella grass essential oil nanoemulsion above was efficient in controlling mite pests and maybe an effect to control other insect pest species. This research report provides the basis for decision-making for the development and utilization of essential oils from these plants, to be used in real conditions to reduce the amount of pesticide residues in both agricultural products and environment. It would give more benefits for producers, consumers and also environmentally friendly. However, for the preparation of plant essential oil nanoemulsion, it should be concerned about the use of surfactants and co-surfactants types as well as the price of developed products.

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Effectiveness of Nano Turmeric Essential Oil against the African Red Mite [*Eutetranychus africanus* (Tucker)]

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Thanaporn Doungnapa, Jarongsak Pumnuan and Ammorn Insung (2017). Effectiveness of Nano Turmeric Essential Oil against the African Red Mite [*Eutetranychus africanus* (Tucker)]. International Journal of Agricultural Technology 13(7.2): 1659-1667.

The study aimed to proof the efficiency in terms of toxicity and repellent properties of nano essential oil of turmeric (*Curcuma longa* Linn.) against the African red mite (*Eutetranychus africanus* (Tucker)) by using leaf dipping method. The mulberry leaf was cut into circle, 2.7 cm in diameter and dipped in various concentrations of nano essential oil at 0.0 (water), 0.2, 0.4, 0.6, 0.8 and 1.0% for 1 min. Then mite mortality was observed at 24 h and compared to the treatments of various concentrations of surfactant, Tween80 and ethylene glycol 400 (PEG400). As for repellent test, the choice test was performed by dipping an half cut leaf into the nano essential oil at 0.02, 0.06 and 0.1% concentrations, whereas the other half leaf was dipped in surfactant. The repellent rate was checked at 24 h. The result showed that nano turmeric essential oil at concentration 1% had a high toxic property, showed 95.5% mortality and showed LC_{50} and LC_{90} at 0.39 and 0.74%, respectively, whereas different concentrations of surfactant also showed high toxic effect to the mite with LC_{50} and LC_{90} at 0.49 and 0.98%, respectively. Anyhow, there was no significant difference among them. In addition, nano turmeric essential oil at 0.1% showed extremely the repellent property to the African red mite, presented 90%RI at 24 h.

Keywords: nano turmeric essential oil, African red mite, *Eutetranychus africanus*, toxicity, repellent

Introduction

The African red mite (*Eutetranychus africanus* (Tucker)) is an economic important pest of many economic crops, such as tangerines, grapefruit, lime, bergamot, papaya and durian. The larvae, nymphs and adults of the African red mite suck the fluid on the front or top of the leaves, particularly in durian plant, it causes many white spots on the leaves. If a severe outbreak can cause leaf and fruit loss eventually. The use of chemicals is the way that farmers often widely apply to control the insect or mite pests. But the use of chemicals directly affect to the farmers, consumers and the environment. It also results in

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increased resistance of mite to acaricide and increased environmental risks. The search for replacing the use of synthetic chemicals by other control methods is increasing nowadays, especially natural products because of their low toxicity, and consumer approval. Moreover, they are considered to be environment friendly due to their biodegradable characteristics (Katz *et al.,* 2008; Lertsutthiwong and Rojsitthisak, 2011; Chung *et al.,* 2013). Botanical pesticide is one of the main components of safety control strategies as well as nanotechnology is helpfully used to improve its efficiency. There are several methods to produce polymeric micro or nanocapsules, such as: spray-drying, complex coacervation (or phase separation), atomization and liposomes. One of them, the emulsion cross-linking method, is based on an oil-in-water emulsion of the polymer aqueous solution in the oily core material. Micelles undergo a hardening process with the addition of a crosslink agent. Nevertheless, the system variables are difficult to control since the size of the microcapsules depends on the extent of cross-linking agent and by stirring speed (Streck *et al.*, 2014; Agnihotri *et al*., 2004).

Therefore, the use of plant essential oils, especially, in the form of nanoparticle to control insect and mite pests to replace the use of synthetic chemicals, or reducing the import of chemicals from foreign countries is promising work. Essential oils from plants also decay faster, non toxic residue in the environment and very low toxic to worm blood animals as well. Insung *et al.* (2008) reported that extract from *Piper retrofractum* at the concentration of 1% could completely control the African red mite. When, Pumnuan et al. (2009) found that lemon grass and citronella grass essential oils at the concentration of 75 μg/cm^3 were the most effective in inhibiting hatching of the mushroom mite, *Luciaphorus perniciosus* Rack, and resulted in 97.3 and 95.8% mortality. Pumnuan *et al.* (2010) also reported that the essential oil of *Litsea cubeba* showed to be the most toxic to *Luciaphorus perniciosus* by both contact and fumigation methods with LD_{50} values equaling to 0.932 and 0.166 μg/cm^3, respectively, followed by essential oil of *Litsea salicifolia* and *Melaleuca cajuputi*. Previous study by Danarun *et al.* (2015) reported that essential oil from turmeric (*Curcuma longa* Linn.) and lemon grass (*Cymbopogon citratus* Stapf) were highly effective to adult of the African red mite with EC_{50} at 1.66 and 2.43%, respectively.

The purpose of this study was to investigate the efficiency in terms of toxicity and repellent properties of nano essential oil from turmeric (*Curcuma longa* Linn.) against the African red mite (*Eutetranychus africanus* (Tucker)) by using leaf dipping method.

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Materials and methods

The African red mite cultures

The African red mite (Eutetranychus africanus (Tucker)) was cultured in the laboratory by putting mulberry leaves on a cotton swab soaked in a tray to serve as a food source. Plenty of female and male adults of the African red mite were released on the leaves. The dried leaf was changed every 4-5 days and kept at 25±2 ℃, 85±2% relative humidity.

Essential oil preparation

Based on a study of Danarun *et al.* (2015) and other scientific papers revealed that turmeric (*Curcuma longa* Linn.) essential oil was evaluated as high toxicity to the African red mite. This essential oil was arranged by procurement from Thai-China Flavours and Fragrances Industry Co., Ltd., Thailand, HACCP (Hazard Analysis and Critical Control Point).

Preparation for nano essential oil

The essential oil of turmeric was diluted in water by using emulsifiers or surfactants. Then the essential oil particles were resized to be nanoparticle by the dilution of surfactant and co-surfactant at different concentration. In this experiment, Tween80 (HLB = 15) was used as primary surfactant and ethylene glycol 400 (PEG400) (HLB = 13) as co surfactant. Then, the surfactant and co-surfactant, were mixed each other with a mix ratio called Smix as follows 1: 1, 1: 1.5, 1: 2, 1: 2.5, 1: 3, 1: 3.5, 1: 4 and 1: 4.5 (1:1 means 100 µl:100 µl). After that distilled water was filled up to obtain 10 ml. Then turmeric essential oil was mixed with Smix at the ratio 1: 1, 1: 1.5, 1: 2, 1: 2.5, 1: 3, 1: 3.5, 1: 4 and 1: 4.5 at room temperature for further experiment. Reduction of particle size of plant essential oil was done by High Pressure Homogenizer. Beside, the stability refers as zeta Potential Charge of nano plant essential oils was measured by Nano plus Zeta / Nano Particle Analyzer.

Bioassay

Toxicity properties by leaf dipping method.

In this study, toxicity property of nano essential oil of turmeric against the African red mite was investigated by using leaf dipping method. The mulberry leaf was cut into circle, 2.7 cm in diameter and dipped for 1 min with various concentration of nano essential oil at 0.0 (water), 0.2, 0.4, 0.6, 0.8 and

1.0%. Discharged to air-dry at room temperature 15-20 min, then amout 10-15 adults of the African red mite were introduced. The mite mortality was observed at 24 h and compared to the surfactant at different concentration. The data was analyzed after Abbott's formula.(Abbott, 1987)

Repellent properties by leaf dipping method.

In this study, repellent property of nano essential oil of turmeric against the African red mite was performed by using leaf dipping method. The mulberry leaf was cut into circle, 2.7 cm in diameter. Then the cut leaf was separated into 2 equal parts and dipped one side with essential oil at 0.02, 0.06, and 0.1%, the other one was dipped with surfactant (control). Both were discharged to air-dry at room temperature 15-20 min. About 10-15 adults of African red mite, were released. Then the repellent activity was observed at 24 h to gain repellent index: %RI; according to Pascual-Villalobos & Robledo. (1998) as the equation below.

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

by C = control

T = treatment

Statistical analysis

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The experiment was designed in three completely randomized design with five replicates. The data obtained was statistically analyzed by analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT). The values of LC_{50} (50% lethal concentration) and LC_{90} (90% lethal concentration) were calculated by the probit method.

Results and Discussion

Toxicity property of nano essential oil of turmeric against the African red mite by using leaf dipping method revealed that nano turmeric essential oil at concentration 1% had a high toxic property, showed 95.5% mortality and showed LC50 and LC90 at 0.39 and 0.74%, respectively. Whereas, the surfactant gave the LC50 and LC90 values of 0.49 and 0.98%, respectively. (Table 1) and (Figure 1). Normally, there were no significant diffrance compared to surfactant, but significantly different appeared at 0.6% concentration ($P<0.05$).

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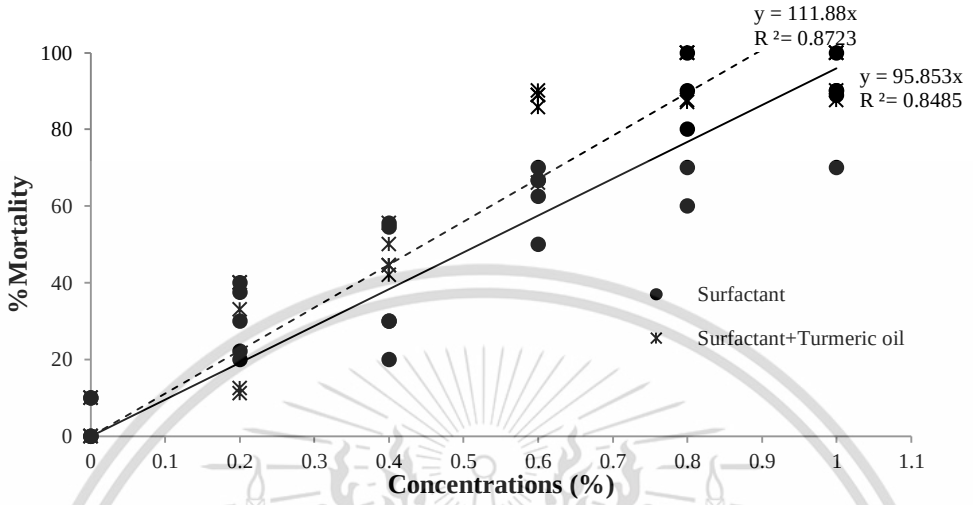

Figure 1. Percentage of mortality of the African red mite (*Eutetranychus africanus* (Tucker)) after treated with nano turmeric essential oil and surfactant (Tween80+PEG400) at various concentrations at 24 h by leaf dipping method.

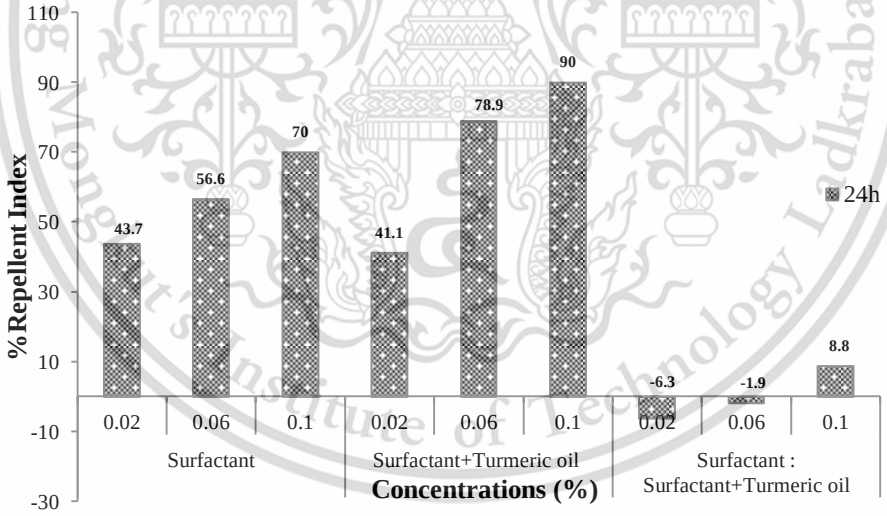

Figure 2. Percentage of repellent index of against nano turmeric essential oil and surfactant (Tween80+PEG400) at various concentrations the African red mite (*Eutetranychus africanus* (Tucker)) at 24 h by leaf dipping method.

Table 1. Percentage of mortality of the African red mite (*Eutetranychus africanus* (Tucker)) caused by nano turmeric essential oil and surfactant (Tween80+PEG400) at various concentrations at 24 h by leaf dipping method.

Nano-Essential oils	% Mortality [1/]						LC_{50}	LC_{90}	Slope ±SE
	Concentration (%)								
	0.0	**0.2**	**0.4**	**0.6**	**0.8**	**1**			
Surfactant	5.00 ±5.48^{Da}	29.9 ±8.9^{Ca}	38.0 ±16.1^{Ca}	63.2 ±7.8^{Bb}	80.0 ±15.8^{Aa}	87.8 ±10.9^{Aa}	0.49	0.98	2.584 ±0.192
Surfactant+Turmeric oil	5.00 ±5.48^{Da}	27.3 ±14.5^{Ca}	47.3 ±5.5^{Ba}	83.9 ±10.1^{Aa}	94.9 ±7.0^{Aa}	95.5 ±6.2^{Aa}	0.39	0.74	3.632 ±0.244
%CV	91.28	41.92	28.15	12.31	13.97	9.69			

[1] Means ± SD in column followed by the same common letter and means in row followed by capital letter are not significantly different (P<0.05) according to DMRT

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The repellent property of nano essential oil of turmeric against the African red mite revealed that the nano essential oil of turmeric at 0.06-0.1% concentration showed mostly effective %RI (repellent index) of 78.9 to 90%, followed of surfactant which presented %RI of 56.6 to 70%, respectively. (Figure 2) Turmeric essential oil with surfactant at various concentrations caused very low repellent activity to the African red mite.

Toxicity and repellent properties of nano essential oil of turmeric, against the African red mite was recorded. Danarun *et al*. (2015) reported that essential oil from turmeric and lemon grass were highly effective to adult of the African red mite with LC_{50} at 1.66 and 2.43%, respectively. There was much difference when LC_{50} at 0.39% was obtained in this study. Beside, higher concentration of nano essential oil caused higher mortality of mite. Previous research on the indigenous medicinal plants to check the invasion of red flour beetle against wheat grains. Results painted that percent mortality was directly proportional to increasing concentration of extracts. Both *Allium sativum* and *C. longa* significantly reduced the larval, pupal and adult emergence. The botanicals could be used as effective tool against *Tribolium castaneum* (Herbst) along-with other IPM tactics. (Ali *et al.,* 2014) Singkhornart *et al.* (2007) revealed the toxicity towards the maize weevil, that turmeric at concentration greater than 15 µl.cm-2, were highly toxic produced 100% mortality within 1 day after treated fumigation. Acaricidal effects of some plants against two spotted spider mites, *Tetranychus urticae* Koch were also performed (Aslan *et al.*, 2004; Choi *et al.*, 2004; Miresmaili *et al*., 2006). Aslan *et al.* (2004) investigated the toxicity of essential oil vapours from *Thymus vulgaris* L., *Ocimum basilicum* L., and *Satureja hortensis* to *T. urticae* and those gave satisfactory result. Choi et al. (2004) tested 53 essential plant oils for their toxicity against the eggs and adults of *T. urticae* as well as the adults of Phytoseiulus persimilis Athias-Henriot obtained result presented that citronella java, lemon eucalyptus, peppermint oils gave > 90% mortality against adult *T. urticae*. Miresmaili *et al*. (2006) compared the toxicity of *Rosemarinus officinalis* L. essential oil and a blend of its major constituents against T. urticae on two different host plants. It was found that some constituents accounted for most of the toxicity but some were relatively inactive. Insung *et al*. (2008) reported that extract from piper retrofractum at the concentration of 1% could completely control the African red mite. As for the other reports dealing with using essential oil to control mite, Pumnuan *et al*. (2009) found that lemon grass and citronella grass essential oils at the concentration of 75 µg/cm3 were the most effective in inhibiting hatching of Luciaphorus perniciosus Rack, and resulted in 97.3 and 95.8% mortality. Pumnuan *et al*. (2010) also reported that essential oil of *Litsea cubeba* found to

be the most toxic to *Luciaphorus perniciosus* by both contact and fumigation methods with LD_{50} values equaling to 0.932 and 0.166 μg/cm^3, respectively.

Conclusion

The study of effectiveness in terms of toxicity and repellent properties of nano essential oil of turmeric (*Curcuma longa* Linn.) against the African red mite (*Eutetranychus africanus* (Tucker)) showed that nano turmeric essential oil at concentration 1% had a high toxic property, showed 95.5% mortality and it showed LC_{50} and LC_{90} values at 0.39 and 0.74%, respectively, whereas different concentrations of surfactant also showed high toxic effect to the mite with LC_{50} and LC_{90} values at 0.49 and 0.98%, respectively. Anyhow, there was no significant difference among them. In addition, nano turmeric essential oil at 0.1% showed extremely the repellent property to the African red mite, presented 90%RI at 24 h.

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Synergistic Effect between Nano Plant Essential Oils and Surfactants on the African Red Mite (*Eutetranychus Africanus* (Tucker))

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Abstract

Nano particle formula of plant essential oils from citronella grass (Cymbopogon nardus Rendle.), turmeric (Curcuma longa Linn.), lemon grass (Cymbopogon citratus (Dc.exNees).), clove (Syzygium aromaticum (L.) Merr.&L.M.Perry.) and cinnamon (Cinnamomum bejolghotha (Buch-Ham.)Sweet.) and their surfactants were tested against the African red mite (Eutetranychus africanus (Tucker)) to evaluate the toxicity and egg laying effect on mite. Nano essential oils were prepared by using different surfactants and concentrations via High Pressure Homogenizer. Then essential oil particles were measured by using Nano plus Zeta / Nano Particle Analyzer. The mulberry leave used as its host was cut into a 2.7 diameter circle and then dipping method was employed. Cut leave was dipped in nano essential oil and surfactant and air dried for 15 minutes, ten female mites were introduced to the cut leave placed on soaked cotton kept in Petri disc. The mortality of mite as well as number of eggs found were checked at 24 hours. The result revealed that nano essential oil of citronella grass showed extremely toxic effect to the mite that only 0.6% concentration gave 100% mortality, followed by nano essential oil of turmeric and cinnamon presented 94.4 and 74.2% mortality, respectively. The mortality caused by nano essential oil of cinnamon was significantly different from those others. According to their LC50 values, nano essential oil of lemon grass was the third place instead of cinnamon, then, their LC50 values were 0.13, 0.33 and 0.41 respectively. The surfactants, especially Tween20 : ethylene glycol 400 (PEG400) (4:3.5) was also highly toxic to the mite when 0.6% concentration showed 98.2% mortality with LC50 at 0.17. Similar results with lower effect were obtained from other surfactants. Both nano plant essential oils and surfactants also presented highly egg laying effect on mite. Average number of eggs as 0.3, 0.4 and 0.7 egg were found of nano essential oil from citronella grass (at 0.2%), turmeric and cinnamon (at 0.4%), respectively. For the effect of surfactant, Tween20 : PEG400 (4:3.5) at 0.2% concentration gave average number of egg only 0.1 egg, whereas, 4.7 eggs were found on the control (water) treatment. Obtained result may conclude that nano plant essential oils and their surfactants presented some synergistic acaricidal properties to the African red mite.

Keywords: synergistic effect, nano essential oil, surfactant, African red mite, toxicity

1. Introduction

Plant mites are small and with the naked eyes they are hardly visible as small reddish or brown dots on the leave surface. The small size of mites makes it difficult for farmers or field workers to recognize them. Correct identification is also important because not all mites are pests. Several predatory mites can be found on the leaves these are beneficial as natural enemies of the plant mites (Mite and Spider Research Section, 2013; Jeppson et al., 1975). The African red mite (Eutetranychus africanus (Tucker)) (Actinedida; Tetranychidae) is relatively the most important mite pest species attacking the greatest of plants particularly citrus, apple, peach, grape, guava, papaya, cotton, eggplant, jackfruit, breadfruit, horse-radish, cassava, soybean, cowpea, castor bean, watermelon, garden pea, a variety of ornamentals. It is remarkably an important pest of durian in Thailand, especially during the cool season (late October to early March). The larvae, nymphs and adults of the African red mite suck the fluid on the front or top of the leaves, particularly in durian plant, it causes many white spots on the leaves. If a severe outbreak can cause leaf and fruit loss eventually (Jeppson et al., 1975; Meyer, 1974; Meyer, 1987).

The chemical method is often widely applied to control the insect or mite pests because of convenient application and high efficacy. But it brought harm on users, consumers, and the environment. It also results in increased resistance of mite to acaricide and increased environmental risks (Kulpiyawat and Chandrapatya. 1989). With this reason, the search for replacing the use of synthetic chemicals by other control methods is increasing nowadays, especially the use of natural products which shows of their low toxicity, and consumer approval. Moreover, they are considered to be environment friendly due to their biodegradable characteristics (Katz et al., 2008; Lertsutthiwong and Rojsitthisak, 2011; Chung et al., 2013). Insung and Pumnuan (2008) stated that the essential oil of citronella grass (Cymbopogon nardus) was remarkably toxic to D. pteronyssinus with LD50 value at 0.935 µg/cm3 24 hrs after fumigation. When, Insung et al. (2008) reported that extract from Piper retrofractum at the concentration of 1% could completely control the African red mite. Moreover, essential oil of P. nigrum showed acaricidal effect on the spider mite, Eutetranychus cendanai Rimando with LC50 at 23.6 ml/l (Sornlek, 2001). There are few studies regarding using of nanotechnology available in insect or mite pest controls. This research focused on if nanoparticles of essential oils with high potential for use in mite pest management. Plant extracts or essential oils would be one of the main components of safety control strategies. Nanotechnology is helpfully used to improve the essential oil efficiency. The nanoemulsions are oil-in-water dispersions having in different variety of droplet size. The nanoemulsions are stable for a long time. Furthermore, the stability depending on the individual system or mixture of ingredients, low viscosity and droplet size make them better looking system for many industrial applications (Solans et al., 2003;

Bouchernal et al., 2004: Ehrlich et al., 2008). Sarapothong et al. (2017) presented that nano-essential oil of black pepper and surfactants showed high effectiveness against African red mite at concentration of 1% caused the mite mortalities of 96 and 92% and gave LC50 at 0.34 and 0.54%, respectively. Nano-essential oil of black pepper presented moderately repellency effect, with 42.39-51.67 %RI at 24 hrs. Lakyat et al. (2017) revealed the repellent activity of nano essential oils from lemon glass at 0.02% concentration showed the most repellent effect to adult brown planthopper, Nilaparvata lugens, with 43.83 %RI.

The purpose of this study was to investigate the efficiency in terms of toxicity and egg inhibition caused by nano plant essential oils (nEOs) and surfactants as well as the synergistic effect between them against the African red mite (Eutetranychus africanus (Tucker)) by using leaf dipping method.

2. Materials and methods

2.1. The African red mite cultures

The African red mite (Eutetranychus africanus (Tucker)) was cultured in the laboratory of Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. Mulberry leaves were put on a cotton swab soaked in a tray to serve as its food source. Plenty of female and male adults of the African red mite were released on the leaves kept at 25±2℃, 85±2% relative humidity. The damaged or dried leaf was changed every 4-5 days.

2.2. Essential oil preparation

According to previous study by Danarun et al., 2015 and Doungnapa et al., 2017 revealed that medicinal plants of 5 species namely, citronella grass (Cymbopogon nardus) lemon grass (Cymbopogon citratus) turmeric (Curcuma longa) clove (Syzygium aromaticum) and cinnamon (Cinnamomum bejolghota) were relatively highly toxic to the African red mite. Those essential oils were arranged by procurement from private company as shown in Table 1.

Table 1. Essential oils from various medicinal plants used to test against the African red mite (Eutetranychus africanus (Tucker)).

Family/Scientific name	Common name	Plant part
MYRTACEAE		
1. Syzygium aromaticum (L.) Merr.&L.M. Perry	Clove	Dried flower bud
LAURACEAE		
2. Cinnamomum bejolghota Sweet.	Cinnamon	Fresh leaf
ZINGIBERACEAE		

3. Curcuma longa Linn.	Turmeric	Fresh rhizome
GRAMINEAE		
4. Cymbopogon nardus Rendle.	Citronella grass	Fresh leaf
5. Cymbopogon citratus (DC. Ex Nees) Stapf.	Lemon grass	Fresh leaf

2.3. Preparation of nano essential oil

Those 5 essential oils were diluted in water by using emulsifiers or surfactants. Then the essential oil particles were resized to be nanoparticle by the dilution with surfactant and co-surfactant at different concentrations when Tween20 (HLB=16.7), Tween60 (HLB=14.9), or Tween80 (HLB = 15) were used as primary surfactants whereas, ethylene glycol 400 (PEG400) (HLB = 13) used as co-surfactant mentioned as following steps. Firstly, each essential oil was merged with the surfactant, Tween20, Tween60 or Tween80 as follows 1: 1, 1: 1.5, 1: 2, 1: 2.5, 1: 3, 1: 3.5, 1: 4 and 1: 4.5 (1: 1 means 100 µl:100 µl) for 10 ml. Consequently, each solution was added with co-surfactant (PEG400) called Smix as followed 1: 1, 1: 1.5, 1: 2, 1: 2.5, 1: 3, 1: 3.5, 1: 4 and 1: 4.5 at room temperature. The prepared essential oil solutions were then put in to High Pressure Homogenizer to minimize the particle and then to Nano plus Zeta/Nano Particle Analyzer to measure the particle size.

2.4. Bioassay

- Toxicity test

In this experiment, toxicity property of nano essential oil (nEOs) of medicinal plant against the African red mite was evaluated by using leaf dipping method. The mulberry leave used as its host was cut into a 2.7 diameter circle and then cut leave was dipped in nano essential oil with various concentrations at 0.0 (water), 0.0 (surfactants), 0.2, 0.4, 0.6, 0.8 and 1.0% and compared with imidacloprid insecticide at 1X and 2X recommendation rates. All treatments were allowed to air dried for 15 minutes. Ten female mites were introduced to the cut leave placed on soaked cotton kept in Petri disc. Then, mite mortality was observed at 24 h.

- Test of egg inhibition

Egg inhibition caused by nano essential oil (nEOs) was performed by using leaf dipping method. Various concentrations at 0.0 (water), 0.0 (surfactant and co-surfactant or Blank), 0.02, 0.04, 0.06, 0.08, 0.1 0.2 and 0.4%, as well as imidacloprid at (1X) and (2X) were dipped for 1 minute. Treated leaves mulberry were left at room temperature to air-dry. Amount of 10 adult females, were released to treated leaves. And the number of egg laid by female mite was observed at 24 h.

2.5. Statistical analysis

In this study, Abbott's formula (Abbott, 1987) was applied to obtain the mite actual death rate. The experiment was designed in completely randomized design with five replicates. The data obtained were statistically analyzed by analysis of variance (ANOVA) and Duncan's

multiple range tests (DMRT). The values of LC50 (50% lethal concentration) and LC90 (90% lethal concentration) were calculated by the probits method.

3. Results and Discussion

The toxicity property test of nano essential oil of medicinal plant against the African red mite (E. Africanus) revealed that 5 nano essential oils and the surfactants at higher concentration caused the higher percentage of the African red mite mortality. The nano essential oil of citronella grass was the most effective in killing the mite. Followed by nano essential oil of turmeric, lemon grass, cinnamon and clove, with their LC50 values were 0.13, 0.33, 0.48 0.42 and 0.41% concentration, respectively (Table 2). This was according to the report of Insung and Pumnuan (2008) stated that the essential oil of citronella grass (Cymbopogon nardus) was remarkably toxic to the house dust mite, Dermatophagoides pteronyssinus when LD50 value at 0.935 µg/cm3 at 24 hrs after fumigation was obtained. Pumnuan et al. (2009) found that lemon grass (Cymbopogon citratus) and citronella grass essential oils at the concentration of 75 µg/cm3 were extremely effective in inhibiting egg hatching of the mushroom mite, Luciaphorus perniciosus Rack, and resulted in 97.3 and 95.8% mortality. Moore et al. (2007) presented that high repellent effects of essential oils of Cymbopogon spp. On the adults of the sandfly, Phlebotomus. Duboscqi. Those grasses have been traditionally used for repelling mosquitoes in jungle regions such as the Bolivian Amazon. In addition, essential oil of citronella grass (C. winterianus) at 1% (w v-1) caused mortality in Frankliniella schultzei and Myzus persicae at 34.3 and 96.9%, respectively, with the LC50 and LC90 values for M. persicae were 0.36 and 0.66%, respectively. Thus, citronella grass essential oil at 1% (w v-1) was more toxic to M. persicae than F. schultzei (Patrícia et al. 2013). In this study 0.2% concentration of surfactant (Tween20:PEG400 as ratio 4:3.5 used for citronella grass oil) had the highest toxicity to the African red mite with 74% mortality. The surfactant (Tween80:PEG400 ; 3:1.5 for turmeric oil) had the least statistically different effect to the African red mite with 32.3% mortality. The observation at 0.4% concentration showed that surfactant (of citronella grass oil) presented the highest effects when 90% mites mortality was found with statistical difference. Surfactants of 5 nano essential oils at different concentrations from 0.6 to 1% resulted in the mortality rate of 66.9 to 99.0%. When, surfactant of clove and cinnamon oils showed very much effect to the African red mite with statistical difference from others. Normally, the lower concentration of surfactant as 0.2% presented higher mortality than that of nEOs (excepted for citronella grass). On the other hand, higher concentrations of nEOs presented the higher effect to the mites over than surfactants. Then, above result referred that both nEOs and surfactant performed the mortality effect on the mites. When they were used together some synergistic effect was appeared. Interesting result was found on chemical insecticides (imidacloprid) which showed very low toxicity to the mite with only 5% mite mortality at recommendation rate used.

Table 2 Mortality percentage of the African red mite (Eutetranychus africanus (Tucker)) caused by nano essential oils and surfactants with various concentrations at 24 h by leaf

dipping method.

Nano-essential oil/Surfactant	% Mortality								
	% Concentration						LC50	LC90	Slope±SE
	0 (water)	0.2	0.4	0.6	0.8	1			
Citronella grass	2.7±4.4Ad	87.5±2 0.1Ab	97.2±6. 5Aa	100.0±0 .0Aa	100.0±0 .0Aa	100.0± 0.0Aa	0. 13	0. 29	8.16 ±0.7 8
Surfactant (citronella grass oil)	2.7±4 .4Ac	74.0±2 3.0Ab	90.0±10 .0Aa	98.2±4. 1Aa	98.2±4. 1Aa	99.0±3. 2Aa	0. 17	0. 43	4.99 ±0.3 9
Turmeric	2.7±4 .4Ad	24.1±2 2.9Bc	77.9±18 .2Abb	94.4±6. 2Aba	97.1±4. 8Aba	90.0±1 4.9Aab	0. 33	0. 69	3.57 ±0.2 3
Surfactant (turmeric oil)	2.7±4 .4Ac	32.3±2 1.9Bc	57.1±19 .8BCb	66.9±27 .9Cb	94.0±8. 9Aba	95.3±1 1.6Aa	0. 40	0. 79	3.28 ±0.2 2
Lemon grass	2.7±4 .4Ad	41.5±3 0.2Bc	54.5±34 .0BCbc	72.3±23 .2Bcab	84.6±17 .4Ba	86.8±2 8.4Aa	0. 41	0. 92	2.49 ±0.1 9
Surfactant (lemon grass oil)	2.7±4 .4Ac	47.8±2 4.7Bb	50.7±14 .1Cb	79.1±20 .3ABCa	88.0±8. 4Aba	82.6±2 3.2Aba	0. 38	0. 92	2.36 ±0.1 8
Clove	2.7±4 .4Ad	23.4±2 1.0Bc	49.4±26 .3Cb	60.3±22 .6Cb	86.6±13 .5Aba	87.6±2 1.9Aa	0. 48	0. 93	2.83 ±0.2 0
Cinnamon	2.7±4 .4Ae	21.4±1 6.8Bd	57.3±28 .5BCc	74.2±25 .7BCb	87.8±14 .9Abab	96.2±6. 3Aa	0. 42	0. 79	3.46 ±0.2 3
Surfactant (clove and cinnamon oil)	2.7±4 .4Ac	40.9±1 4.3Bb	62.7±8. 0Bca	76.0±11. 4Bca	71.3±5. 1Ca	67.7±2 5.3Ba	0. 44	1. 20	1.68 ±0.1 6
%CV	161.6 7	52.83	33.44	23.19	12.16	19.96			

Chemical	Concentration

	Recommendation rate	Double rate
Imidacloprid	5.0±12.7	5.9±6.9

1 Means ± SD in column followed by capital letter and means in row followed by the same common letter are not significantly different (P<0.05) according to DMRT

In this study, egg inhibition of mite caused by nano essential oils and surfactants at various concentrations within 24 h was also investigated by leaf dipping method. The nEOs from various medicinal plants with higher concentration showed relatively lower egg laying of mite. Because of very little different concentrations of nEOs, number of egg laying was then fluctuate. It was happened, particularly on nEOs of citronella grass, cinnamon and clove. As for 0.02%, clove had the most effect on the number of eggs laid when 2.7 eggs was found, followed by cinnamon and citronella grass oils with the number of egg found of 3.3 and 3.4 eggs, respectively, which was not statistically different. In addition, the nEOs at 0.4% concentration revealed that nEOs of turmeric gave the highest egg inhibition laying effect, when only 0.4 egg was obtained, followed by nEOs of cinnamon, clove and citronella grass, which gave 0.7, 0.8 and 0.8 eggs, respectively but with no statistical difference. About blanks, when the concentration was getting higher the more effect on eggs laid was found. Control (water) resulted in egg laying of mite about 2 times bigger than that of blanks 0.1% concentration (Table 3). And definitely it caused higher number of egg laid when compared with nEOs. However, the used nEOs at low concentration could inhibit the number of eggs laid less than the blank at 0.2-0.4% concentrations. Surprisingly, imidacloprid showed no effect on egg inhibition of the mite. Pumnuan et al. (2009) also found that lemon grass and citronella grass essential oils at the concentration of 75 µg/cm3 were the most effective in inhibiting hatching of Luciaphorus perniciosus Rack, and resulted in 97.3 and 95.8% mortality, respectively by fumigation method. In addition, Danarun et al. (2015) reported that essential oil from turmeric and lemon grass were highly effective to adult of the African red mite with LC50 at 1.66 and 2.43%, respectively which was bigger number than this study (0.33 and 0.41%). Previous research on toxicity test of the indigenous medicinal plants to red flour beetle, Tribolium castaneum was performed and pointed that percent mortality was directly proportional to increasing concentration of extracts. Both Allium sativum and Curcuma longa significantly reduced the larval, pupal and adult emergence. Hence, those botanicals can be used as effective tool against Tribolium castaneum along-with other IPM tactics (Ali et al., 2014). Singkhornart et al. (2007) had made toxicity test towards the maize weevil, Sitophilus zeamais and the results showed that turmeric oil at concentration greater than 15 µl.cm-2 were highly toxic produced 100% mortality within 1 day after treated fumigation. Few studies dealing with Eos effected on mites. Aslan et al. (2004) found the acaricidal property of essential oil vapours from Thymus vulgaris L., Ocimum

basilicum L., and Satureja hortensis on Tetranychus urticae. Previous research referred that there were high acaricidal effects of caraway seed, citronella java, lemon eucalyptus, pennyroyal, and peppermint oils against the two spotted spider mites, T. urticae Koch and Phytoseiulus persimilis (Aslan et al., 2004; Choi et al., 2004; Miresmaili et al., 2006). According to this study, the nEOs tends to be used to control the mites in field condition.

Table 3 Number of egg of the African red mite (Eutetranychus africanus (Tucker)) caused by nano essential oils and surfactants at various concentrations at 24 h by leaf dipping method.

Nano-Essential oils	Number of egg(s)										
	% Concentration										
	0 (water)	Blank 0.1	Blank 0.2	Blank 0.4	0.02	0.04	0.06	0.08	0.1	0.2	0.4
Citronella grass	4.7±1.3Aa	1.9±1.3Abcde	0.1±0.1Bf	0.3±0.6Aef	3.4±1.7Ab	2.2±1.0Bbcde	1.7±0.8Cbcde	2.2±1.7Abcd	1.8±0.6Abcd	0.3±0.4Bdef	0.8±1.1Acdef
Turmeric	4.7±1.3Aa	2.9±1.0Abc	1.3±1.1Abdc	0.3±0.7Ae	3.9±1.0Aab	2.7±1.2Abbc	2.8±1.3Abbc	2.7±1.4Abc	2.7±1.3Abc	2.1±1.3Acd	0.4±0.8Ae
Cinnamon	4.7±1.3Aa	2.3±1.0Abc	2.0±1.1Ac	0.7±0.7Ad	3.3±1.0Ab	3.4±1.0Abb	2.8±1.0Cbc	1.9±0.7Ac	2.3±0.9Abc	1.9±1.7Ac	0.7±1.1Ad
Clove	4.7±1.3Aa	2.3±1.0Ab	2.0±1.1Ab	0.7±0.7Ac	2.7±1.5Ab	2.6±1.3Ab	2.1±1.1Abb	1.8±0.8Abc	2.2±1.1Ab	2.2±1.4Ab	0.8±0.9Ac
Lemon grass	4.7±1.3Aa	2.8±1.2Abcd	1.3±1.5Abef	0.9±0.8Af	3.8±1.3Aab	2.5±0.8Abcde	3.3±1.1Abc	2.2±1.4Acdef	1.9±1.0Adef	1.8±1.0Adef	1.0±1.3Af
%CV	26.92	44.96	78.16	109.78	39.09	40.50	41.26	58.33	46.15	72.12	143.05

Chemical	Concentration	
	Recommendation rate	Double rate
Imidacloprid	4.0±1.2	4.9±0.9

1 Means ± SD in column followed by capital letter and means in row followed by the same common letter are not significantly different (P<0.05) according to DMRT

4. Conclusion

The test of toxicity and egg inhibition caused by nano plant essential oils and surfactants as well as the synergistic effect between them against the African red mite (Eutetranychus africanus (Tucker)) presented that nano essential oil of citronella grass at 0.6% concentration had the highest toxicity, showed 100% mite mortality with LC50 value at 0.13%, whereas different concentrations of its surfactant also showed high toxic effect to the mite with LC50 at 0.17%. Nano essential oil of citronella grass at low concentration as 0.2% showed rather low egg inhibition to the African red mite, presented the average number of eggs as 0.3 at 24 h and when compared to its surfactant which gave only 0.1 egg. As a result, there were some expressions of synergistic effect of nEOs and surfactants on the mortality and egg inhibition of mite.

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RESEARCH

Acaricidal activity of essential oil nanoemulsion against the African red mite (*Eutetranychus africanus*)

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ABSTRACT

The African red mite (*Eutetranychus africanus* [Tucker]) is an important pest species of some economic plants. The objective of the present study was to evaluate the effectiveness of nanoemulsions consisting of citronella grass, lemongrass, clove, and cinnamon essential oils and their main chemical compounds against the African red mite; their toxicity, egg-laying inhibition, and repellent effect on the mite were determined. Essential oil nanoemulsions (nEOs) were prepared by mixing the essential oils with different surfactants and co-surfactants. Approximately 10 to 15 female mites were transferred to treated leaves placed on soaked cotton and kept in a Petri dish. Mite mortality and number of eggs laid were checked after 24 h. As for the repellency test, the selected test consisted of dipping a half leaf into the nEOs, while the other half leaf was dipped in a control (water). The repellency rate was evaluated after 24 h. Results revealed that citronella grass nEOs exhibited a highly toxic effect on the mite and only a 0.6% concentration caused 100% mite mortality. Geraniol, one of the main chemical compounds of citronella grass, represented the highest egg-laying inhibition at a 0.2% concentration with a mean of 0.1 egg compared with 4.7 eggs in the control. Furthermore, nEOs from citronella grass at a 0.1% concentration were extremely repellent against the mite with 95% repellency after 24 h. Therefore, the citronella grass essential oil nanoemulsion is appropriate for use to effectively control the African red mite.

Key words: Egg inhibition, essential oil nanoemulsion, *Eutetranychus africanus*, repellent, toxicity.

INTRODUCTION

The African red mite (*Eutetranychus africanus* [Tucker]) (Actinedida: Tetranychidae) is one of the most significant pest species for some plants (Attia et al., 2013). A wide range of damage caused by this mite pest in various plants, fruits, and a variety of ornamentals has been reported (Liburd and Rhodes, 2019). It is a very important pest in Thailand. The larvae, nymphs, and adults of the African red mite suck the fluid on the adaxial or upper side of the plant leaves and can produce many white spots on the leaves. A severe outbreak can eventually cause leaf and fruit loss (Beyzavi et al., 2013; Khanjani et al., 2017).

A chemical control is often applied to insect or mite pests because of its ease of application and high effectiveness. However, it is harmful to users, consumers, and the environment; it also results in increased mite resistance to acaricides and greater environmental risks. The search to replace the use of synthetic chemicals by other control methods is now increasing, particularly natural products which show low toxicity to non-target organisms and are approved by the consumer. In addition, they are classified as environmentally friendly due to their biodegradable characteristics (Lertsutthiwong and Rojsitthisak, 2011; Tirello et al., 2012; Chung et al., 2013).

There are few studies regarding the use of available nanotechnology for insect or mite pest control. Plant extracts or essential oils are one of the main components of safe control strategies. Nanotechnology helps to improve the efficiency of essential oils that are oil-in-water dispersions with a variety of very small droplet sizes; moreover, they are stable over time. However, stability depends on the individual system or ingredient mixture, and low viscosity and droplet size make them a more suitable system for applications (Gao et al., 2011; Solans and Solé, 2012; Rice et al., 2013; Kwon et al., 2015; Jintapattanakit, 2018). Therefore, essential oil nanoemulsions (nEOs) from plants are very promising for use as botanical acaricides. Sarapothong et al. (2017) showed that black pepper (*Piper nigrum* L.) nEOs at a 1% concentration exhibited high effectiveness against the African red mite; mite mortality was 96% and median lethal concentration (LC_{50}) was 0.34%. These black pepper nEOs showed a moderate repellency effect; the repellency index (RI) was 42.39% to 51.67% after 24 h. Lakyat et al. (2017) reported repellent activity of lemongrass (*Cymbopogon citratus* [DC.] Stapf) nEOs with at very low concentration of 0.02%, and the most repellent activity was against adult brown planthoppers (*Nilaparvata lugens* Stal; Hemiptera: Delphacidae) with 43.83% RI.

The present study focused on evaluating high-potential nEOs for use in mite pest management. Therefore, the objective was to evaluate the effectiveness of some nEOs in terms of acaricidal, egg-laying inhibition, and repellent effects against the African red mite using the leaf dipping method.

MATERIALS AND METHODS

African red mite rearing

The African red mite (*Eutetranychus africanus* [Tucker]) adults were randomly collected from an organic durian (*Durio zibethinus* Murray) orchard in Chanthaburi province, Thailand. They were initially reared on mulberry (*Morus alba* L.) leaves placed on a cotton swab soaked with water on a tray to serve as their food source. Thousands of African red mite adults were reared on mulberry leaves and maintained at room temperature (25 ± 2 °C) and 85 ± 2% relative humidity (RH). Each leaf was removed every 4 to 5 d and a fresh leaf was provided. Mite rearing and all the bioassay experiments were conducted 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.

Plant essential oils and main chemical compounds

The essential oils (EOs) were obtained from 13 species of medicinal plants and preliminary use allowed evaluating their effectiveness against the African red mite. The EOs were obtained from the Thai-China Flavors and Fragrances Industry Co. Ltd. in Thailand and prepared according to principles of hazard analysis and critical control point (HACCP). The EOs were from citronella grass (*Cymbopogon nardus* [L.] Rendle), lemongrass (*C. citratus* [DC.] Stapf), cinnamon (*Cinnamomum bejolghota* [Buch.-Ham.] Sweet), clove (*Syzygium aromaticum* [L.] Merr. & L.M. Perry), black pepper (*Piper nigrum* L.), eucalyptus (*Eucalyptus globulus* Labill.), star anise (*Illicium verum* Hook. f.), ginger (*Zingiber officinale* Roscoe), sweet fennel (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *dulce* [Mill.] Batt.), kaffir lime (*Citrus hystrix* DC), cassumunar ginger (*Z. cassumunar* Roxb.), Siam cardamom (*Amomum cardamomum* L.), and sweet basil (*Ocimum basilicum* L.)

Chemical compounds found in effective EOs from different medicinal plants were verified by column chromatography. The silica gel G-60 was used for chromatography as the stationary phase, while the petroleum and ethyl alcohol solvent mixture was selected as the mobile phase. The aliquot of each fraction was analyzed by thin layer chromatography (TLC) (Saenmanot et al., 2018). The pure fraction examples of EOs were analyzed for their major components by gas chromatography-mass spectrometry (GC-MS) according to Pumnuan and Insung (2016b). The main chemical compounds appearing in effective EOs, geraniol, citral, and eugenol, were ordered from Sigma-Aldrich (St. Louis, Missouri, USA).

Preparation of essential oil emulsions and main chemical compound nanoemulsions

The 13 EOs were diluted in water with Tween-20 as an emulsifier or surfactant. The EO emulsions contained different surfactant concentrations when properly dissolved that were used for further bioassay experiments.

Selected effective EOs and their main chemical compounds such as geraniol, citral, and eugenol were diluted in water with surfactants. The EO particles appearing in the emulsions were resized into nanoparticles by adding the co-surfactant at different concentrations. In the present experiment, Tween-20 (hydrophilic-lipophilic balance (HLB) = 16.7) and Tween-60 (HLB = 14.9) were used as primary surfactants, whereas polyethylene glycol 400 (PEG400) (HLB = 13) was used as co-surfactant as follows. Firstly, each EO or the main chemical compound was merged with the primary surfactant, Tween-20 or Tween-60 at 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, and 1:4.5 (1:1 is 100 µL:100 µL) for 10 mL. Co-surfactant polyethylene glycol (PEG400) (Smix) was added to each solution at 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, and 1:4.5. The prepared EOs were then placed into a nanoparticle size analyzer (NanoPlus Zeta, Otsuka Electronic Co., Ltd., Osaka, Japan) to measure particle size. When EO particles in the emulsion were larger than 100 nm, the emulsion was passed through a high pressure homogenizer to minimize particle size, which was then measured again. These prepared essential oil nanoemulsions (nEOs) and their main chemical compound nanoemulsions (nMC) were used for further toxicity assessments.

Toxicity tests

The effectiveness of 13 EOs against the African red mite was primarily assessed by the leaf dipping method. The mulberry leaf was cut into 2.7 cm diameter circles and dipped for 1 min with each of these EOs at 2% and removed for air-drying at room temperature (25 ± 2 °C, 85 ± 2% RH) for 15 min. Approximately 10 to 15 adult female mites were selected under a microscope and introduced to the treated leaf. Mite mortality was observed after 24 h. Mites were determined to be dead when they did not move even if prodded with a small hairbrush. The EOs were highly effective in killing mites with more than 70% mortality; these EOs were tested in new experiments as nEOs with different concentrations. In addition, the main chemical compounds contained in the effective EOs were also verified by column chromatography as nanoemulsions.

The toxicity properties of nEOs and nMCs against the African red mite were also evaluated by the leaf dipping method. The mulberry leaf was cut into 2.7 diameter circles and dipped for 1 min in each nanoemulsion at different concentrations: 0.0% (water as control) and surfactants at 0.0%, 0.2%, 0.4%, 0.6%, 0.8%, and 1.0%. All treatments were air-dried for 15 min. Approximately 10 to 15 adult female mites were transferred to the treated leaves on soaked cotton and kept in a Petri dish. Mite mortality was observed after 24 h. In the case that mites died in the control, Abbott's formula (Abbott, 1987) was applied according to the actual death rate as

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

where T is test mortality (%) with nEOs and C is control mortality (%).

Egg-laying inhibition test

Egg-laying inhibition caused by nEOs was determined by the abovementioned method. The mulberry leaves were dipped in different concentrations of nEOs at 0.0 (water) and surfactant and co-surfactant or blank at 0.0, 0.02, 0.04, 0.06, 0.08, 0.10, and 0.20 for 1 min. Treated leaves were air-dried at room temperature for 15 min. Later, 10 to 15 adult females were released to treated leaves. The number of eggs laid by each female mite was observed under a microscope after 24 h.

Repellency and attraction test

The response of the African red mite to seven nEOs was evaluated for repellency and attraction activity using a similar method, and the selected test was performed. The leaves cut in circles were equally divided, and the first half was dipped in nEOs at 0.02%, 0.06%, and 0.1%. Meanwhile, the other half was dipped in water or surfactant (control). Both halves were removed for air-drying at room temperature for 15 min. Approximately 10 to 15 adult females of the African red mite were transferred, and the percentages of repellency and attraction response were determined after 24 h.

Statistical analysis

In the present study, Abbott's formula was applied to obtain the actual mite death rate. The experiment was performed using a completely randomized design with five replicates per treatment. The data obtained were analyzed by ANOVA, and the differences among treatments were tested by Duncan's multiple range test with SAS software (SAS Institute, Cary, North Carolina, USA). As for the percentage response in terms of the repellency and attraction test, frequencies of mites in the selected test were analyzed by the χ^2 test with SPSS software (IBM, Armonk, New York, USA).

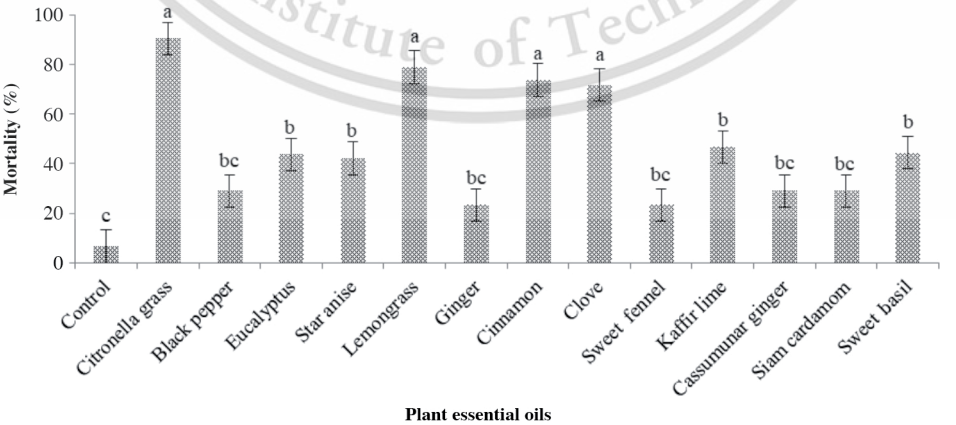
RESULTS AND DISCUSSION

The toxicity of EOs obtained from 13 selected medicinal plants, citronella grass, lemongrass, cinnamon, clove, black pepper, eucalyptus, star anise, ginger, sweet fennel, kaffir lime, cassumunar ginger, siam cardamom, and sweet basil in emulsions against adult African red mites revealed that EO emulsions of citronella grass, lemongrass, cinnamon, and clove at 2% were highly effective in killing the African red mite with more than 70% mite mortality (Figure 1). This concurred with the report by Insung et al. (2016), who found that EOs of citronella grass, clove, cinnamon, and lemongrass were extremely toxic against the European house dust mite (*Dermatophagoides pteronyssinus*). The clove EO also exhibited high insecticidal activity against adult thrips (*Frankliniella schultzei*) and mealybugs (*Pseudococcus jackbeardsleyi*) (Pumnuan and Insung, 2016b).

The four selected EOs, citronella grass, lemon grass, cinnamon, and clove, which were highly effective in killing the mite were analyzed by GC-MS to determine their chemical compounds. The citronella grass EO contained 37.97% citronella and 18.60% geraniol. Meanwhile, the lemongrass EO had the highest amount of geranyl acetal at 29.03% and 20.21% citral. In addition, the clove and cinnamon EOs had the same main active ingredient eugenol at 81.55% and 98.45%, respectively. Sruthi et al. (2014) and Uddin et al. (2017) reported that the clove and cinnamon EOs contained approximately 80% to 90% eugenol analyzed by GC and GC-MS. Silva et al. (2011) and Hamzah et al. (2013) indicated that citronella grass EO contained the most important substances, including citronellal, citronellol, geraniol, geranial, and limonene. There were more than 80 components (Silva et al., 2011) appearing in the EOs in which the lemon grass EO had the most important substances, mainly citral, limonene, citronellal, β-myrcene, geranial (α-citral), and neral (β-citral).

Nanoemulsions of the four most effective EOs against the African mite were made from emulsions using surfactants and co-surfactants at appropriate ratios. Surfactants provided better emulsion stability than co-surfactants. The use of less surfactant than co-surfactant resulted in unstable emulsions. The selected surfactants must be able to decrease interfacial tension to a very small amount to encourage the dispersion process during nanoemulsion preparation to obtain the desired type of nanoemulsion (i.e., oil/water, water/oil, or bicontinuous) (McClements, 2011; Solans and Solé, 2012; Kwon et al., 2015; Komaiko and McClements, 2015; Jintapattanakit, 2018). Particle size analysis by the dynamic light scattering technique indicated that the particle size of citronella grass, clove, and cinnamon EOs ranged from 11.80 to 18.70 nm diameter, polydispersity index from 0.04 to 0.15 M_n (M_n is the number average molecular weight), and zeta potential from -6.23 to -7.92 mV. Meanwhile, the largest particle size of lemongrass EO was 188.77 nm. Finally, this particle was reduced with a high pressure homogenizer at 30000 psi to a 34.57 nm diameter, polydispersity index of 0.19 M_n, and zeta potential of -4.39 mV. The particle size of the main chemical compounds geraniol, citral, and eugenol ranged from 11.07 to 18.00 nm diameter and polydispersity index from 0.02 to 0.19 M_n, and zeta potential from -10.30 to 3.38 mV. The polydispersity index is an indicator of their quality with respect to size distribution (Danaei et al., 2018). The zeta potential values greater than 30 mV indicate physical stability (Marsalek, 2014). Unfortunately, poor zeta potential values were detected in this study.

Figure 1. Percentage mortality of the African red mite caused by different plant essential oils at 2% concentration after 24 h by the leaf dipping method.



The same letter above each column indicates nonsignificant differences according to Duncan's multiple range test.

The acaricidal activity assessment reported that citronella grass nEOs at 1% and 2% could completely control the African red mite (4.0% to 28.8% and 71.7% to 90.4% mortality, respectively), followed by cinnamon, clove, and lemongrass nEOs. It would appear that the nEOs at 1% exhibited more than three times the activity of EOs, particularly citronella grass EO at 1% that caused 28.6% mite mortality, whereas nEOs produced 100% mite mortality. A similar result was observed for the other plant EOs. It should be noted that mite percentage mortality among nEOs at 1% and 2% was not significantly different (Table 1). It was previously mentioned that the emulsion with smaller particles of plant EOs could work better than larger particles. Additionally, using co-surfactant was a very important technique to prepare nEOs and their mixture could promote effectiveness on the mite.

The highest concentration of all the nEOs caused the highest mortality rate of the African red mite. Citronella grass nEOs at 0.4% killed up to 97.2% of the mites, followed by geraniol nMCs at 96.7% mortality; however, there was nonsignificant difference among them. All surfactants prepared by mixing main surfactants and co-surfactants at different ratios (A to D, Table 2) and 0.4% concentration exhibited mite mortality from 60.1% to 89.7%, while other nEOs and nMCs showed 66.5% to 90.0% and 55.1% to 88.3% mortality, respectively. However, there were significant differences among a group of treatments. The result was satisfactory when citronella grass nEO at 0.6% to 1.0% could completely kill the mites, and there were nonsignificant differences among the concentrations (Table 2). When comparing the effectiveness of citronella grass nEO and geraniol nMC with others at 0.4%, this implied that other treatments had a lower percentage mortality of the African red mite. All the surfactants also had a high toxic effect on the mite, particularly at 0.4% to 1.0%, which caused 60.1% to 100% mite mortality. Results indicated that many compounds existing in EOs could usually work better together than a chemical compound alone; there was also some synergistic effect depending on the mixture between the precursor and surfactant. Results were disappointing when the performance of nEO and nMC formulas showed generally lower mite mortality than citronella grass nEO alone. At 0.6%, they produced from 87.2% to 98.3% mortality compared with 100% by citronella grass nEO. This could be due to the influence of the surfactant and co-surfactant. Mixtures of plant EOs usually exhibited more toxicity to the tested mites. There are very few reports regarding the effectiveness of citronella grass EO to control the mite pest. Pumnuan and Insung (2016a) demonstrated that lemongrass and citronella grass EOs applied at 3 μg L^{-1} air caused 100% and 82.5% mortality, respectively, on mushroom mite (*Dolichocybe indica*) using the fumigation method. In addition, Saad et al. (2017) proved that citronella EO had extensive insecticidal properties such as contact toxicity, repellency, and ovipositional deterrence on *Bemisia tabaci*, causing high mortality when applied at 6.66 μL L^{-1} air.

Experimental results of egg-laying inhibition caused by nEOs, nMCs, and surfactants at 0.02% to 0.2% by the leaf dipping method revealed that the highest concentration greatly reduced the number of eggs laid. The clove nEO showed that the minimum number of eggs laid was 2.7 eggs per adult female at the 0.02% concentration, followed by eugenol, citral, cinnamon, and citronella grass EOs with 3.1, 3.2, 3.4, and 3.4 eggs, respectively; there was nonsignificant difference among them. Geraniol at 0.2% also produced a high egg-laying inhibition effect with 0.1 eggs, followed by citronella grass EO with 0.3 eggs, and there was nonsignificant difference between them. The highest surfactant concentration had more effect on the number of eggs laid. In the control (water), the number of eggs laid was 4.7 eggs, approximately six times more than for surfactant at 0.2% (Table 3). When it was compared to nEOs, a larger number of eggs laid was obtained. Additionally, nEOs at a low concentration could inhibit the number of eggs similar to the surfactant. The nMCs results generally tended to show more activity at higher concentrations, especially geraniol that had the best performance.

Table 1. Percentage mortality of the African red mite caused by plant essential oil emulsions and essential oil nanoemulsions at 1% and 2% concentrations by the leaf dipping method.

	Essential oil emulsion		Essential oil nanoemulsion	
	1%	2%	1%	2%
	%		%	
Citronella grass	28.6 ± 6.2Ac	90.4 ± 2.6Ab	100.0 ± 0.0Aa	100.0 ± 0.0Aa
Lemongrass	28.8 ± 6.8Ac	78.9 ± 8.4ABb	86.8 ± 28.4Aa	100.0 ± 0.0Aa
Cinnamon	10.7 ± 6.2Bc	73.7 ± 11.6Ab	96.2 ± 6.3Aa	100.0 ± 0.0Aa
Clove	4.0 ± 0.7Bc	71.7 ± 1.7Ab	87.6 ± 21.9Aa	100.0 ± 0.0Aa
CV, %	30.77	9.32	19.63	-

CV: Coefficient of variation.
Means in a column followed by the same uppercase letter and means in a row followed by the same lowercase letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 2. Percentage mortality of the African red mite caused by different essential oil and main chemical compound emulsions at different nanoemulsion concentrations by the leaf dipping method.

Emulsion formation	Nanoemulsion concentration (%)					
	0.0 (water)	0.2	0.4	0.6	0.8	1.0
	%					
Surfactant						
A = Tween-20(4.0):PEG400(3.5)	0.0a	73.3ab	89.7ab	98.1a	98.1ab	99.0ab
B = Tween-20(4.0):PEG400(1.0)	0.0a	84.8a	88.3abc	97.9a	98.1ab	100.0a
C = Tween-60(4.5):PEG400(2.5)	0.0a	48.2bcde	63.2bcde	80.9abcd	85.2cd	93.0abc
D = Tween-60(4.5):PEG400(1.0)	0.0a	42.6cde	60.1cde	67.1de	69.9e	76.6d
Essential oil nanoemulsion						
Citronella grass in A	0.0a	87.2a	97.2a	100.0a	100.0a	100.0a
Lemongrass in C	0.0a	39.9de	53.2de	71.6cde	84.2cd	86.4cd
Cinnamon in D	0.0a	19.3ef	56.1de	73.5bcde	87.5abcd	96.1abc
Clove in D	0.0a	21.3def	48.0e	59.2e	86.2bcd	87.3bcd
Main chemical compound nanoemulsion						
Geraniol in B	0.0a	81.1a	96.7a	97.0a	100.0a	100.0a
Citral in C	0.0a	69.8abc	81.8abcd	90.0abc	95.0abcd	100.0a
Eugenol in D	0.0a	45.0bcde	63.9bcde	81.7abcd	84.0d	90.9abc
Essential oil nanoemulsion formula						
Citronella grass:Lemongrass (3:1)	0.0a	44.2bcde	66.5bcde	87.2abc	93.3abcd	98.3abc
Citronella grass:Cinnamon (3:1)	0.0a	43.8cde	90.0ab	98.3a	100.0a	100.0a
Citronella grass:Clove (3:1)	0.0a	51.3bcd	85.0abc	94.6a	98.3ab	100.0a
Citronella grass:Lemongrass (2:2)	0.0a	39.1de	81.0abcd	88.7abc	90.0abcd	95.0abc
Citronella grass:Cinnamon (2:2)	0.0a	44.2bcde	85.9abc	95.0a	100.0a	100.0a
Citronella grass:Clove (2:2)	0.0a	38.1de	76.4abcd	91.7abc	93.3abcd	94.7abc
Main chemical compound nanoemulsion formula						
Geraniol:Citral (3:1)	0.0a	9.2f	65.2bcde	93.8ab	94.3abcd	100.0a
Geraniol:Eugenol (3:1)	0.0a	19.5ef	55.1de	88.3abc	93.3abcd	100.0a
Geraniol:Citral (2:2)	0.0a	69.7abc	88.3abc	91.8abc	96.7abc	98.3abc
Geraniol:Eugenol (2:2)	0.0a	33.9def	74.2abcde	86.3abcd	96.7abc	98.3abc
CV, %		45.79	27.84	17.40	9.82	9.32

CV: Coefficient of variation.
Means in a column followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 3. Number of eggs laid by the African red mite after treated with essential oil and main chemical compound nanoemulsions at different concentrations by the leaf dipping method.

Concentration (%)	Number of eggs per female							
		Essential oil nanoemulsion				Main chemical compound nanoemulsion		
	Surfactant A	Citronella grass	Lemongrass	Cinnamon	Clove	Geraniol	Citral	Eugenol
0.0 (water)	4.7Aa	4.7Aa	4.7Aa	4.7Aa	4.7Aa	4.7Aa	4.7Aa	4.7Aa
0.02	2.8Ab	3.4Ab	3.8Aab	3.4Ab	2.7Ab	3.8Aab	3.2Ab	3.1Ab
0.04	2.8Ab	2.2Ac	3.3Abc	3.3Abc	2.6Ab	3.0Abc	2.0Abc	3.0Ab
0.06	2.1Ab	2.2Ac	2.5Acd	2.8Abcd	2.1Ab	2.1Acd	1.9Abc	2.3Abc
0.08	1.8Abc	1.8Ac	2.2Ad	2.3Acd	2.2Ab	1.6Ade	1.7Abc	2.1Abc
0.10	1.6ABbc	1.7Ac	1.9Ad	1.9Ad	2.2Ab	1.5ABde	0.5Bc	2.1Abc
0.20	0.8ABCc	0.3Cd	1.8Ad	1.9Ad	1.8Ab	0.1Ce	0.5BCc	1.5ABc

A: Tween-20:PEG400(4.0:3.5).
Means in a column followed by the same uppercase letter and means in a row followed by the same lowercase letter are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

A comparative study on the use of plant-derived compounds to control different developmental stages of mite pests by Mwandila et al. (2013) found that syringa (*Melia azedarach*) seed extracts had acaricidal properties against adults, eggs, and nymphs of the red spider mite (*Tetranychus* spp.) Saad et al. (2017) also demonstrated that citronella EO induced ovipositional deterrence on *B. tabaci*. Unfortunately, no reports regarding the interference of surfactants on mite egg-laying were found.

For the repellency test, citronella grass and lemongrass nEOs at 0.06% to 0.10% had the highest repellent effectiveness of 85.0% to 95.0% repellence, and these values were not significantly different when compared to other treatments. However, it was apparent that at lower concentrations such as 0.02%, clove nEOs and citral nMCs both showed 66.67% and 71.67% repellency, respectively. They were significantly different to others at the 95% confidence level ($P < 0.05$) in which they responded as attractants to the African red mites up to 28.33% and 33.33%, respectively (Table 4). There are few studies related to testing the repellency of the selected EOs against other mite pests. Kheradmand et al. (2015) reported that clove EO had the most potent repellent effect on the two-spotted spider mite (*Tetranychus urticae*). However, in the present study, it showed relatively low repellency to the tested mite, especially compared with citronella grass nEOs. Thein et al. (2013) also emphasized that crude citronella extract was the most repellent on the maize weevil (*Sitophilus* spp.) According to Lakyat et al. (2017), the repellent activity of lemongrass nEO at the very low concentration of 0.02% had the most repellent effect on adult brown planthopper (*Nilaparvata lugens*) with a 43.83% repellency index. Monoterpenes in EOs are cytotoxic to animal tissue, radically decrease mitochondria and Golgi body numbers, and damage the respiratory system (Lee et al., 1997). However, there is limited information about the physiological actions of EOs on insects or mites. Different EOs and their components seem to have a neurotoxic mode of action (Kostyukovsky et al., 2002).

Table 4. Percentage response of the African red mite to different concentrations of essential oil and main chemical compound nanoemulsions.

		Response			
	Concentration	Repellency	Attraction		
Emulsion	%	%	%	$\chi 2$	P
Essential oil nanoemulsion					
Citronella grass	0.02	81.67	18.33	13.375	0.000**
	0.06	93.33	6.67	27.743	0.000**
	0.10	95.00	5.00	30.740	0.000**
Lemongrass	0.02	81.67	18.33	13.375	0.000**
	0.06	85.00	15.00	16.752	0.000**
	0.10	95.00	5.00	30.470	0.000**
Cinnamon	0.02	85.00	15.00	16.752	0.000**
	0.06	86.67	13.33	18.639	0.000**
	0.10	90.00	10.00	22.857	0.000**
Clove	0.02	66.67	33.33	3.429	0.641^{ns}
	0.06	80.00	20.00	11.868	0.001**
	0.10	81.67	18.33	13.375	0.000**
Main chemical compound nanoemulsion					
Geraniol	0.02	81.67	18.33	13.375	0.000**
	0.06	83.33	16.67	15.000	0.000**
	0.10	85.00	15.00	16.752	0.000**
Citral	0.02	71.67	28.33	5.911	0.150^{ns}
	0.06	73.33	26.67	6.910	0.009**
	0.10	83.33	16.67	15.000	0.000**
Eugenol	0.02	70.00	30.00	5.000	0.025*
	0.06	73.33	26.67	6.910	0.009**
	0.10	76.67	23.33	9.187	0.002**
Control		30.00	30.00		

*,** Significant difference at $P < 0.05$ and $P < 0.01$, respectively; ^{ns} nonsignificant difference.

CONCLUSIONS

Effectiveness testing in terms of acaricidal, egg-laying inhibition, and repellent properties of plant essential oil (EO) emulsions, nanoemulsions, and their main chemical compound nanoemulsions against the African red mite (*Eutetranychus africanus*) demonstrated that the citronella grass EO nanoemulsion at the 0.6% concentration was extremely toxic with 100% mite mortality. In addition, all the surfactants used at high concentrations also exhibited a high toxic effect on the mite. Geraniol, one of the main chemical compounds of citronella grass EO and citronella grass EO nanoemulsion at 0.2% showed potent egg-laying inhibition of the African red mite; the mean number of eggs laid by each female was 0.1 and 0.3 eggs, respectively, while 4.7 eggs were obtained in the control. Citronella grass and lemongrass EO nanoemulsions at 0.1% also showed a high repellency of 95% on the African red mite. Therefore, the citronella grass EO nanoemulsion is an appropriate substance to control the African red mite.

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