USING PLANT NANO ESSENTIAL OIL FORMULAS FOR CONTROLLING CHICKEN ECTOPARASITES

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หัวข้อวิทยานิพนธ์	การใช้น้ำมันหอมระเหยสูตรนาโนจากพืช เพื่อการป้องกันกำจัดปรสิตภายนอกของไก่
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บทคัดย่อ

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อพัฒนาและประเมินประสิทธิภาพของน้ำมันหอมระเหยสูตรนาโน (Nano Essential Oil Formulas: NEOFs) ที่สกัดจากพืชสมุนไพร ได้แก่ กานพลู (*Syzygium aromaticum*), อบเชย (*Cinnamomum zeylanicum*) และขมิ้นชัน (*Curcuma longa*) เพื่อใช้ในการควบคุมปรสิตภายนอกในไก่ โดยเฉพาะเหาไก่ (*Menopon gallinae*) และไรขนไก่ (*Megninia ginglymura*) ซึ่งเป็นปรสิตที่ส่งผลกระทบอย่างมีนัยสำคัญต่อสุขภาพของสัตว์ปีก โดยก่อให้เกิดความเครียด ส่งผลให้ประสิทธิภาพการเจริญเติบโตและการให้ผลผลิตไข่ลดลง อีกทั้งยังอาจทำหน้าที่เป็นพาหะนำโรค จึงเป็นปัญหาที่ต้องให้ความสำคัญในทั้งระบบการเลี้ยงไก่สวยงามและไก่ไข่เชิงพาณิชย์ ในระยะเริ่มต้นได้มีการสำรวจฟาร์มเลี้ยงไก่สวยงามและไก่ไข่ในพื้นที่ฝั่งตะวันออกของกรุงเทพมหานคร เพื่อศึกษาความหลากหลายและอัตราการระบาดของปรสิตภายนอก พบปรสิตภายนอกทั้งหมด 8 ชนิด โดยมี *M. cubitalis* เป็นชนิดที่พบมากที่สุด โดยเฉพาะในไก่ไข่ที่เลี้ยงในกรง ซึ่งบริเวณที่พบการระบาดมากที่สุด ได้แก่ ส่วนก้น ปีก และแผ่นหลังของไก่

สูตรน้ำมันหอมระเหยนาโนได้รับการเตรียมโดยใช้น้ำมันหอมระเหยจากกานพลู อบเชย และขมิ้นชัน ซึ่งมีสารออกฤทธิ์หลัก ได้แก่ ยูจีนอล (eugenol) และอาร์-ทูเมอโรน (ar-turmerone) นำมาผสมในอัตราส่วนที่แตกต่างกัน ได้แก่ 4:0:0, 2:2:0, 2:0:2 และ 2:1:1 ตามลำดับ จากนั้นทำการทดสอบคุณลักษณะทางกายภาพของสูตร ได้แก่ ขนาดอนุภาค ดัชนีการกระจายตัว (Polydispersity Index: PDI) และค่าต่างศักย์ไฟฟ้า (Zeta Potential) ผลการทดสอบพบว่าน้ำมันหอมระเหยสูตรนาโนทั้งหมดมีขนาดอนุภาคอยู่ในระดับนาโน (<100 นาโนเมตร) โดยน้ำมันหอมระเหยสูตรนาโนที่มีอัตราส่วน 2:1:1 แสดงค่าขนาดอนุภาคเฉลี่ยมากที่สุด ในการทดสอบในห้องปฏิบัติการได้ประเมินคุณสมบัติการฆ่าและการขับไล่ปรสิตของน้ำมันหอมระเหยสูตรนาโน โดยใช้วิธีการสัมผัสตายและการ

ทดสอบการไล่ พบว่าน้ำมันหอมระเหยสูตรนาโนที่มีอัตราส่วน 4:0:0 และ 2:2:0 มีประสิทธิภาพสูงสุด โดยสามารถกำจัด *M. gallinae* ได้ถึง 100% ภายใน 6 ชั่วโมงที่ความเข้มข้น 0.30% และกำจัด *M. ginglymura* ได้ถึง 64.97% ที่ความเข้มข้น 0.10% นอกจากนี้ยังมีประสิทธิภาพในการขับไล่ที่โดดเด่น โดยแสดงค่าดัชนีการไล่สูงสุดถึง 96.67% ภายใน 1 ชั่วโมงแรกของการทดสอบ เมื่อนำน้ำมันหอมระเหยสูตรนาโนที่มีประสิทธิภาพสูงสุดไปทดสอบในฟาร์มไก่ไข่เชิงพาณิชย์ พบว่าสามารถลดจำนวนประชากรปรสิตภายนอกได้อย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่มควบคุมที่ไม่ได้รับการใช้สารทดสอบ และกลุ่มที่ใช้สารเคมีกำจัดแมลงไซเพอร์เมทริน อีกทั้งไม่พบสารเคมีตกค้างในไข่ที่เก็บตัวอย่างหลังการรักษา และไม่พบผลกระทบต่อการผลิตไข่ เช่น น้ำหนักไข่ ร้อยละการให้ไข่ของแม่ไก่ต่อวัน มวลไข่รวม และอัตราส่วนการเปลี่ยนอาหารเป็นไข่ (Feed Conversion Ratio: FCR) ผลการศึกษานี้ชี้ให้เห็นว่าน้ำมันหอมระเหยสูตรนาโนมีศักยภาพในการใช้เป็นทางเลือกที่ปลอดภัย เป็นมิตรต่อสิ่งแวดล้อม และมีประสิทธิภาพในการควบคุมปรสิตภายนอกในไก่ อีกทั้งสามารถประยุกต์ใช้ในระบบการผลิตสัตว์ปีกแบบยั่งยืนและเกษตรอินทรีย์ เพื่อช่วยลดการพึ่งพาสารเคมีกำจัดแมลงสังเคราะห์และส่งเสริมความปลอดภัยด้านอาหาร

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ABSTRACT

This study aimed to develop and evaluate the efficacy of nano essential oil formulas (NEOFs) derived from plants—namely clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*), and turmeric (*Curcuma longa*)—for the control of ectoparasites in chickens, particularly shaft lice (*Menopon gallinae*) and feather mites (*Megninia ginglymura*). These ectoparasites are known to cause significant stress, reduce growth performance and egg production, and potentially act as vectors for disease transmission in poultry, posing a serious concern in both beautiful chicken and laying chicken farming systems. A field survey was initially conducted across beautiful chicken and laying chicken farms in the eastern area of Bangkok to identify the diversity and prevalence of ectoparasites. A total of eight species were detected, with *M. cubitalis* being the most dominant, particularly in caged laying hens, where infestations were highest in the buttocks, wings, and back regions.

In the preparation of NEOFs, essential oils from clove, cinnamon, and turmeric—containing the main active compounds eugenol and ar-turmerone—were used. Nanoemulsion formulations were created in various ratios: 4:0:0, 2:2:0, 2:0:2, and 2:1:1, respectively. These formulations were then tested for particle size, polydispersity index (PDI), and zeta potential. The results showed that all formulations had particle sizes in the nano range (<100 nm), with the 2:1:1 formulation exhibiting

the largest particle size. Laboratory experiments were conducted to assess the insecticidal and repellent properties of these NEOFs. Contact residue exposure and repellency assays revealed that the formulations 4:0:0 and 2:2:0 demonstrated the highest efficacy, achieving 100% mortality of *M. gallinae* within six hours at a concentration of 0.30%, and up to 64.97% mortality of *M. ginglymura* at 0.10%. Furthermore, these formulations exhibited strong repellent activity, with repellency indices reaching up to 96.67% within the first hour. Subsequent field trials on commercial layer farms confirmed that the top-performing NEOFs significantly reduced ectoparasite populations compared to untreated and cypermethrin-treated control groups. Importantly, no pesticide residues were detected in eggs collected post-treatment, and no adverse effects were observed on egg production parameters, including egg weight, hen-day egg production percentage, total egg mass, and feed conversion ratio (FCR). The findings suggest that plant-derived nano-essential oil formulations offer a promising, eco-friendly alternative for controlling poultry ectoparasites. These natural-based products hold potential for integration into sustainable and organic poultry production systems, thereby minimizing reliance on synthetic chemical insecticides and enhancing food safety.

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Lastly, I sincerely hope this research will prove meaningful and beneficial to future scholars and practitioners. For any inquiries or further discussion, I warmly welcome correspondence at email: anuwatraky@gmail.com.

Anuwat Lakyat

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CHAPTER 1

INTRODUCTION

1.1 Research motivation

Thailand has nearly 18.2 million households, but mostly smallholders and locate in rural areas. Normally they traditionally possess indigenous chickens (Choprakarn, 2007; United Nations, 2021). In 2020, Bangkok had 126,988 chickens were recorded based on total chicken data in Thailand, albeit a few numbers compared to the total number of chickens in the country. Most of the chickens raised in Bangkok were beautiful chickens, in which kept them for competitions or kept them as pets rather than industrial farming (Department of Livestock Development, 2020). A household kept one cock with three to five hens to form a throng, annually. This helps to maintain picking with the beak order and relieves fighting in the throng. In a year, such throngs can produce up to 90–150-day-old chicks, equivalent to 30–75 merchantable birds of 1.0-1.5 kg body weight at four to five months of age (Choprakarn, 1983; Choprakarn *et al.*, 1998; Laopaiboon and Jitpraneechai, 1999; Namdaeng, 1991). The number of chickens per household varies extremely relating to the time of the year and the ability of the farmers. From October to February (cold and dried), the number of day-old chicks running around is at its largest, and the chicks' growth rate is also uplifting. This is because of the upper hatching estimate, and the availability of plenty of natural feeds and plant by-products. However, the numbers tend to decrease from March to September (sizzling and moist) due to a down hatching rate, a famine of natural feeds, local diseases, and endo and ectoparasites (Choprakarn *et al.*, 1998; Klinhom *et al.*, 2005; Laopaiboon and Jitpraneechai, 1999).

Ectoparasites, even with their harmful effects, are often disregarded. Some of the ectoparasites ordinary in poultry are ticks, fleas, louse and mites (Amede *et al.*, 2011; Ikpeze *et al.*, 2008). The occurrence of decease and morbidity due to various ectoparasitic ailments in chicken demands serious attempts to keep down the ailments. However, notwithstanding their ravaging impacts, ectoparasites receive hardly more attention than endoparasites and infectious diseases in almost all the

production systems. Even though, it has been attempted by few researchers (Belihu *et al.*, 2010; Mekuria and Gezahegn, 2010; Amede *et al.*, 2011; Tolossa and Tafesse, 2013; Dabasa *et al.*, 2017 a, b). Ectoparasites may raise a clinical issue for humans, transmit several infectious ailments, and act as a transit/intermediate host for a range of helminthic parasites. Native fowl parasitic contagions, which can cause health and economic problems in poultry production, are considered a source of infection in industrial poultry, wild birds, and humans. Currently, there is a poorness of information considering the prevalence of ectoparasites in local chickens (Ebrahimi *et al.*, 2016). Many ectoparasites are known to suck blood thereby causing irritation and morbidity. They also contest for feed, serve as means of poultry ailments and germs, that can straight influence bird hygiene. Ectoparasites influence the productivity potential of indigenous chickens and helmeted guinea fowls thus ought to be given more heedfulness. Albeit helmeted guinea fowls are known to be more ailment resistant to ailments than chicken, ectoparasite infestation is still a significant concern (Bhat *et al.*, 2014; Okaeme, 1988). The fowl tick (*Argas persicus*) is known to affect pigeons, turkeys, geese, ducks and chickens in sub-tropical and tropical countries. the stick-tight flea (*Echidnophaga gallinacean*) is the only flea commonly affecting chicken (Mungube *et al.*, 2008). The louse species affecting chicken are yellow body louse (*Menacanthus stramineus*), shaft louse (*Menopon gallinae*), chicken head louse (*Cuclotogaster heterographus*), wing louse (*Lipeurus caponis*), large chicken louse (*Goniodes gigas*) and fluff louse (*Goniocoites gallinae*). Mites are among the most ordinary of all the ectoparasites watched in poultry. Some of the species found on the skin of most poultry birds consist of common red mite (*Dermanyssus gallinae*), northern fowl mite (*Ornithonyssus sylviarum*) and tropical fowl mite (*Ornithonyssus bursa*). Mites of the family Dermanyssidae are the most economically important of the numerous ectoparasites of poultry. Severe infestations of mites in chicken consequences abated reproductive potential in males and egg production in females. (Salam *et al.,* 2009; Ikpeze *et al.*, 2008).

Although there are now widespread methods for preventing and eliminating ectoparasites in poultry using pesticides, no substance can completely protect and eliminate ectoparasites in chickens. Occasionally chemical contamination was found in poultry egg production (Shanta *et al.*, 2008; Pumnuan *et al.*, 2020). A study by

Alaboudi *et al.* (2019) found that 96% of egg samples were from 200 households with laying hens in Jordan. These were contaminated with pesticides, with approximately 66.5% of these samples showing contamination levels exceeding the maximum residue limit (MRL) of less than 0.01 ppm. Cypermethrin, which is an insecticide with the highest residues in 52% of egg samples from Rio Grande do Sul in 2015 (Dallegrave *et al.*, 2018). For this reason, it is very appropriate to study alternative, environmentally friendly insecticides and no toxic residues in meat or eggs, such as botanical insecticides (crude extract, essential oils etc.) that have properties to prevent and eliminate pests, etc. (Pumnuan *et al.*, 2020)

1.2 Objectives of the study

1.2.1 To classify the ectoparasite species of farm chicken found in eastern Bangkok.

1.2.2 To evaluate the efficacy of plant essential oil formulations and essential oil nano emulsion for the prevention and elimination of chickens ectoparasites.

1.2.3 To determine the appropriate concentration rates and the efficacy of essential oil nano emulsion in controlling the ectoparasites of chicken.

1.2.4 To develop a formula of essential oil nano emulsion for prevention and elimination of ectoparasites of chickens.

1.3 Benefits of the study

1.3.1 Knowing the ectoparasite species attacking different types of chickens in the east of Bangkok.

1.3.2 Getting the formulated essential oil nano emulsion for controlling those ectoparasites of chickens and referred as prototype product.

1.3.3 Setting information and recommendation of using the product to control chicken ectoparasites in farm conditions.

CHAPTER 2

THEORY AND LITERATURE REVIEWS

2.1 Poultry economic importance

Animal husbandry across the world today has gone through a lot of changes and developments from the past. Because the farming system must be upgraded to meet the needs of the world population, and the rapid expansion of the city and the resulting economic growth as a result, demand for animal products has increased over the past decade (Thornton and Herrero, 2010). Globally, in industrial poultry production, chickens and turkeys are the most popular, followed by ducks, geese and other poultry. There were 98% meat and 92% eggs, representing a large proportion of poultry production (Mottet and Tempio, 2017). In 2021, the global production of poultry meat was approximately 137.8 million tons (FAO, 2022). As for the production rate of livestock in Thailand in 2022, it was found to have contracted by 3.0% compared to 2021 because of the situation of animal epidemics and rising production costs. The egg production decreased due to the implementation of standards to stabilize the price of chicken eggs by reducing the number of hens standing in cages, this contrasts with the production of broiler chickens that have increased productivity from production expansion to meet market demand that has continued both domestic consumption and increased exports. By 2023, egg production was expected to increase as consumer demand grows and farmers manage their farms with better efficiency. As for broiler chickens, due to the continued demand of both domestic and international markets coupled with rising production costs (Office of Agriculture Economics, 2022). According to the forecast of the Food and Agriculture Organization of the United Nations or FAO confirmed that the amount of meat demand of the world population in the since 2030 would increase by 14%. Due to the increasing population and income, the demand for poultry meat would be as high as 17.8%. Poultry protein had the highest demand rate among other meats, because it was relatively cheap, and it was popular among developing countries. In high-income countries, poultry was consumed because white meat was viewed as a healthier dietary choice than other red meat types (OECD/FAO, 2021).

2.2 Problems of poultry farming

Diseases and parasites pose a health hazard to humans and pets in tropical countries. Ectoparasites cause serious diseases to the health of animals such as It causes nuisance, irritation, skin infections, anemia, tick fever, as well as being a carrier of various types of diseases, that are important to livestock and ultimately lead to livestock mortality, could have a negative impact on global economies if mass outbreaks occur (Abbas *et al.*, 2014; Yadav *et al.*, 2017). Nowadays, animal farmers are focusing on maximizing profitability and biosecurity to prevent disease outbreaks in pets and the risks arising from the production process. This may cause the loss of the animals being raised as well as the cost of care and treatment in the event of an epidemic. Indications of rigorous biosecurity measures often include prophylactic vaccination or the use of antimicrobial agents as well as, good farm management practices to prevent disease outbreaks such as improving the nutritional standards of animal feed, take care of the hygiene of the farm area and improving the health of farm animals (Ryan, 2019). These efforts have managed to implement programs to control communicable diseases but face political and social problems. Access to consumer information would be about animal welfare, assurance about food safety and provenance. Issues concerning the environmental impact and the cost of feed raw materials continue to increase every year. There is also an unexpected emergence of new diseases, and many countries' legislation limits farm management to ensure that biosecurity standards are met (Hafez, 2005; Hafez and Attia, 2020). Many of the ectoparasites that infest domestic animals are controlled with synthetic insecticides, which are the most widely used methods in the world. Although there are several problems such as insect resistance and consumer concern in terms of chemical residues in food and the environment (Maxwell *et al.*, 2002; El -Seedi *et al.*, 2017; Showler, 2017).

2.3 Infestations of ectoparasites in poultry

Almost all poultry species raised in the livestock industry are susceptible to external parasites. Therefore, there should be guidelines for managing and paying attention to the type and severity of the epidemic, for example, raising broilers and

laying hens which are the same breed, but it was found that northern fowl mites spread more economically among laying hens. The main cause of the outbreak may be that broilers grow relatively quickly and are not kept for long periods of time like laying hens, poultry rearing time is also important for the spread of ectoparasites in other commercial poultry species as well. Therefore, it is important to bear in mind that when management changes in poultry farming systems, parasites may be more likely to be affected as well (Mullens and Murillo, 2018). Ectoparasites have also been found to spread rapidly, often resulting from overcrowding and soiling of rearing areas, which results in poor sanitation of poultry (Tolossa *et al.*, 2009). They can cause health problems and can spread a wide range of infectious diseases. It also acts as a vector for a variety of helminths or endoparasites (Arends, 2003; Marques, 2007; Mekuria and Gezahegn, 2010; Firaol *et al.*, 2014). Ticks and mites, for example, are parasitic vectors of avian diseases such as Pastuerellosis, fowl pox, Newcastle disease and avian chlamydiosis (Audi and Asmau, 2014; Moyo *et al.*, 2015). The most common ectoparasites in poultry are louse, mites, ticks and fleas (Bhowmik *et al.*, 1982).

2.3.1 Chicken louse

Louses are a type of blood-sucking insects without wings that are commonly found in pets, including livestock (Ikpeze *et al.*, 2008). There are two types of lice: sucking lice (Anoplura) and biting lice (Mallophaga). Sucking lice have a larger body size than biting lice, about 2-3 mm in size, with a sharp mouth for piercing and sucking blood from the host's skin. The body color is gray to red, which depends on the amount of blood absorbed. This type of lice needs warmth from the host's body to survive but will only attack the specific host species. For the life cycle of lice, they live on the host from the egg stage to the adult stage. Poultry lice that cause economic damage, including *Menopon* spp., *Gonocoites* spp., and *Lipeurus* spp., are the most serious ectoparasites in poultry causing disease and irritation. This leads to an economic impact on the poultry industry. A severe outbreak in broilers could result in a weight loss of up to 711 g/chicken, and an outbreak in laying hens could result in a reduction in egg production of up to 66 eggs/chicken per year (Sychra *et al.*, 2008). Serious infestations of Mallopphaga lice have also been found in caged poultry. This type of lice can reproduce quickly, causing a widespread. In one type of

poultry, 3-5 species of ectoparasites are found living in different areas of the feathers such as the head, neck, breast, body and buttocks, by the nature of lice bites, they have claw-like claws for attaching to the host's feather. It has a flat body that makes it easy to move around on the bird's body. The lice bite will breed during the poultry shedding, this allows hatched lice to spread more easily to other hosts. These physical and behavioral traits allow lice to attach without detachment from the host. Therefore, to control lice in general, insecticides such as cypermethrin and carbaryl are commonly used. In addition, ivermectin is applied to the back of poultry to help prevent head lice better (Ruff, 1999; James, 2013; Philips *et al.*, 1994).

2.3.2 Chicken mites

Chicken mites are Arachnids whose mouthparts are adapted for biting and sucking. They have four pairs of legs and a round body with no distinct proportions. Poultry mites are small ectoparasites that are important to poultry farming as they can infect many poultry species. Some species of mites live on the host throughout their life cycle, others attack during the night and hide in cracks or corners of the cage during the day. Poultry mites of economic importance are poultry red mites (*Dermanyssus gallinae*), northern fowl mites (*Ornithonyssus sylvarium*), tropical fowl mites (*Ornithonyssus bursa*) and scaly leg mites (*knemidocoptes mutants*). (Onyekachi, 2021). Poultry mites cause anemia, skin irritation and weight loss. Poor immunity is weakened if chicken lice and mites are severely infested and can eventually lead to the death of the domesticated chicken (Sparagano *et al.*, 2014).

2.4 Prevention of ectoparasites in poultry

Each insecticide is designed to be toxic to insect pests. The mechanism of action is normally affecting on the body and nervous system (Gibbons *et al.*, 2014; Zaller and Brühl, 2019). The specificity of the limited availability of insecticides that can affect non-target organisms is clearly visible. There has been research reporting also on the use of pesticides affecting non-target organisms (Kwon *et al.*, 2004; Rogers *et al.*, 2019). Although people are now aware of the impact on the severity of insecticide use (Bright *et al.*, 2008; Köhler and Triebskorn, 2013). However, for

insecticides in the neonicotinoid group, the mechanism of action mimics the neurotransmitter acetylcholine by binding to nicotinic acetylcholine receptors, resulting in overstimulation of nerve impulses. In addition, they affect the physiology, behavior, immune system, reproduction and migration of birds when exposed to these substances (Lopez-Antia *et al.*, 2015; Eng *et al.*, 2017, 2019). The same effect occures in carbamate and organophosphate insecticides that have a mechanism of action to inhibit the activity of acetylcholinesterase, a neurotransmitter derived from synapses, causes the congestion of acetylcholine. This causes the death of the target insect pests; however, such substances can affect non-target organisms upon contact (Burgess *et al.*, 1999; Bishop *et al.*, 2000; Mineau and Tucker, 2002). Therefore, the effects of the use of pesticides should be studied outside of the laboratory. Most often, the action of a substance is considered when it is toxic at high concentrations over a short period of time (about 30-90 days). At the same time, exposure to low concentrations under natural conditions is common (Mineau, 2005; Cox and Surgan, 2006). Because testing and studying the mechanism of action of a single chemical without regard to substances can enhance the action of many types, such as the use of multiple active substances in combination or the use of synergistic substances (surfactant), etc (Cedergreen, 2014; Hua and Relyea, 2014; Lebrun et al. 2020). Several reports have concluded that pesticides may do more harm than good for the foreseen effects of chemical use. which affects pathogens and parasites that invade. It was found that most of these parasites died when exposed to chemical insecticides, but a small percentage developed resistance to the chemicals. In addition, the spread of pathogens or parasites results in reduced host immunity as well (Marcogliese and Pietrock, 2011; Sures *et al.*, 2017; Coors *et al.*, 2008; *Marcogliese et al.*, 2009; Booton *et al.*, 2018; Gentes *et al.*, 2007).

2.5 A new approach to control ectoparasites of chickens

Nowadays, more people are turning to organic farming. Which is aware of health and the environment, therefore leads to research to develop a new alternative way to control and eliminate ectoparasites. Among the new alternatives, essential oils are concerned as special attention. Essential oils contain 20-80 active substances obtained by steam distillation. These substances are easily volatile and low molecular weight terpenoids typically contain two to three major components of

terpenes or terpenoids, up to 30% of the essential oil (Bakkalai *et al.*, 2008). Several studies have reported the killing efficacy of essential oils against insect pests and found that the main compound in essential oils has a killing effect. However, other compounds in essential oils are also effective enhancers. This is because some compounds aid in cell accumulation and absorption of other toxic substances as well (Yang *et al.*, 2003; Cal, 2006). However, the mode of action of essential oils and most of their constituents is unknown. For example, terpinen-4-ol, a monoterpenoid found in high concentrations of tea tree oil, has an inhibitory effect on acetylcholinesterase. This is an enzyme necessary for the transmission of nerve impulses in insects (Mills *et al.*, 2004; Lopez and Pascual-Villalobos, 2010). On the other hand, the separation of the essential oils from the water may affect the physical characteristics of the insects at the same time, for example, clogging the skin layer and breathing holes, resulting in molting, suffocation and death (Burgess, 2009).

2.5.1 Clove (*Syzygium aromaticum* (L.) Merr. and L.M.Perry)

Clove is a plant that belongs to the family Myrtaceae and is indigenous to the Maluku islands in Indonesia but has recently been farmed in different places worldwide. In Thailand, it is sometimes planted, but it is not widespread. It is grown mainly in the Chanthaburi and Chumphon provinces. It likes to grow in loose soil with good drainage, high humidity, and lots of rain. It grows well at height above the mean sea level of about 900 meters. The clove tree is a slow-growing, evergreen tree that ranges greatly in maximal height, from a relatively paltry shrubby treelet at 8m to a middle-sized tree of up to 20m. It has a dense conical crown when immature but becomes cylindrical or pyramidal when mature. Flowers: inflorescence in a cluster, branching off at the end of the branch, inflorescence in a cascading inflorescence. Each inflorescence has 3 flowers, the peduncle is very short. Triangular bracts, flowers white-green, pale yellow or greenish yellow. The thalamus is a narrow, cylindrical cup. It is a rather narrow, cup-shaped ridge at the base. The calyx is green and turns red as the flower blooms. The base is joined together into a tube with 4-lobed, oval-shaped ends, narrow, pointed tip and smooth edges. The petals have 4 parallel or rounded petals with smooth, clear edges. There are many oil glands, they fall off easily, many stamens, and they fall off easily. Anthers oval or oblong-ovate, the semi-orbital ovary has two compartments, each containing many ovules Cloves are mainly

composed of volatile oil, gallotannic acid and contain several terpenoids, the most important of which is eugenol. There is also α -caryophyllene, β -caryophyllene, acetyl eugenol, methyl amyl ketone and chavicol, etc. There are also current research reports from preclinical research that found that eugenol contained in clove oil has the effect of inhibiting the growth of bacteria. Both Gram-positive and Gram-negative types and fungi. It also has analgesic and numbing effects. Therefore, clove oil has been put to good use. In dentistry, it helps relieve toothache and treat socket inflammation after tooth extraction. It is also effective in preventing and eliminating insect pests (Cortés-Rojas *et al.*, 2014; Nparks, n.d.; Department of Thai Traditional and Alternative Medicine, n.d.), such as psyllids (Czarnobai De Jorge *et al.*, 2022; Mann *et al.*, 2012; Tian *et al.*, 2015), beetles and weevils (Silvi Ikawati *et al.*, 2021; Jairoce *et al.*, 2016; Kerdchoechuen *et al.*, 2010; Ho *et al.*, 1994), moths (Helaly *et al.*, 2022; Oparaeke and Mbonu, 2010; Birah *et al.*, 2010), fire ants (Appel *et al.*, 2004; Kafle and Shih, 2013), and aphids (El-Shourbagy *et al.*, 2023; Kareem, 2012; Usha Rani, 2005) etc. **(Figure 2.1)**.

Figure 2.1 Clove (*Syzygium aromaticum* (L.) Merr. and L.M.Perry).

2.5.2 Cinnamon (*Cinnamomum zeylanicum* Blume)

The Cinnamon popularly known as Dalchini, belongs to the Family Lauracae. The main part of its tree which is used for spice purposes is its bark. Cinnamon is found widely in Sri Lanka but also grows in Malabar, Cochin-China, Sumatra and Eastern Islands. Besides India, it is also cultivated in Brazil, Mauritius, India, Jamaica and Thailand (Rawat *et al.*, n.d.). The tree is medium to large, 15 - 20 meters tall. It does not shed leaves. The canopy is round or dense and low in the shape of a pagoda. The outer bark is grey-brown, smooth or cracked into square flakes. There are air pockets scattered throughout. Pink inner bark, white sapwood, bark and leaves have a cinnamon-like aroma. Leaf characteristics are single leaves arranged opposite or almost opposite, parallelogram-shaped, rounded base, smooth leaf margins, pointed leaf tip, thick, hard and crisp leaf texture. Three leaf veins extend from the base of the leaf to the tip. The lower leaves have white stains. Young shoots are red. Their flower characteristic are inflorescence branched out at the end of the branch. The flowers are light yellow or light green, small size, 6 sepals, arranged in 2 layers, 3 petals per layer, each petal oval-shaped, pointed tip, with dense soft hairs, no petals, flowers have a foul odor. Fruit characteristics: present a single, hard seed, oval-shaped, small, about 1 centimeter long. The surface is smooth and shiny with white stains. The base is covered with calyx. The young fruit is green and white. When ripe, they have black, oval seeds (Association for the Development of Environmental Quality (Thailand), 2020). The barks of Ceylon cinnamon contain essential oils that have various antimicrobial properties. The active ingredients include eugenol, cinnamaldehyde, beta caryophyllene, linalool and methyl chavicol (Lertsatitthanakorn *et al.*, 2012; Anand *et al.*, 2016; Ghosh *et al.*, 2005; Wu *et al.*, 2017). The essential oils isolated from different cinnamon species are another important extract with potential insecticidal and pharmacological properties. The *C. camphora* essential oil was found to be effective against bacterial forms and house dust mites. In addition, it was found that there are other properties such as counting antifungal antimicrobial antidiabetic antioxidant anti-inflammatory antitermitic antimycotic nematicidal insecticidal mosquito larvicidal and additionally anticancer

activities etc. (Yu *et al.*, 2021; Wang *et al.*, 2005; Chang *et al.*, 2001; Mathew and Abraham, 2006; Tung *et al.*, 2010; Cheng *et al.*, 2009; Kong *et al.*, 2007; Cheng *et al.*, 2004; Kwon *et al.*, 2004; Lu *et al.*, 2011) **(Figure 2.2)**.

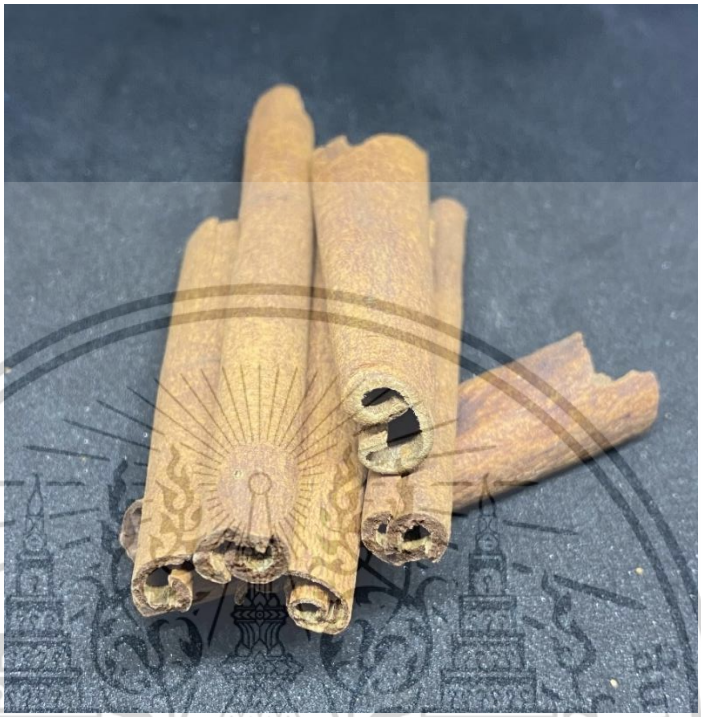

Figure 2.2 Cinnamon (*Cinnamomum zeylanicum* Blume).

2.5.3 Turmeric (*Curcuma longa* L.)

Turmeric is an herbal plant that belongs to the Zingiberaceae family. It is an herbaceous plant that is classified in the ginger family. The part of the turmeric plant that is above the ground is an artificial trunk. The trunk is wrapped with a sheath of mud and leaves surrounding it. It is greenish brown, about 50-70 cm tall, and has an underground stem called a rhizome. It consists of a main rhizome that is oval in shape, growing vertically. On the side of the rhizome, there are cylindrical-shaped sub-branches that split off on two sides, resembling fingers. It is called the rhizome. The flesh in the rhizome and stem is yellowish orange, has a unique fragrance. The native to countries in South Asia and Southeast Asia, it can grow well in tropical to subtropical areas. It was found that the species is distributed in India, Thailand, Malaysia and Sri Lanka, etc. Study and research of biological activities of extracts obtained from turmeric. Contains two main groups of substances: essential

oil and curcuminoids. These substances will be used to produce medicines. Can be used as an alternative to medicines available on the market. As for curcuminoids whose biological activity has been studied, they consist of 3 substances 10, namely curcumin, demethoxycurcumin, and bisdemethoxycurcumin. This substance is insoluble in water but is very soluble in alcohol and acetic acid (Horticulture research institute, n.d.; Dada Khalandar *et al.*, 2018) It was found that turmeric contains substances that have properties to prevent and eliminate many species of insect pests, such as beetles and weevils (Sulhath *et al.*, 2024; Vineesh *et al.*, 2023; Tripathi *et al.*, 2002), moths (De Souza Tavares *et al.*, 2016; Govindaraddi, 2005) and flies (Laurie *et al.*, 2024; Suckow and Suckow, 2006; Chen *et al.*, 2018; Shen *et al.*, 2012; Rawal *et al.*, 2014) etc. **(Figure 2.3)**.

Figure 2.3 Turmeric (*Curcuma longa* L.).

2.6 Innovative use of nano essential oils formulas

Essential oil particles when mixed with water are hydrophobic. Therefore, bio-polymers suitable for each type of essential oil should be used to help make the particles better dispersed in the water. and increase the efficiency of

the essential oil to spread evenly on the surface for microbial control. better (Salvia-Trujillo *et al.*, 2015; Das *et al.*, 2020; Lugani *et al.*, 2021). For example, various nanoencapsulation systems such as nano emulsion, solid lipid nanoparticles, nanofibers, liposomes, and edible films are available with practical utility in food preservation (Aswathanarayan and Vittal, 2019). As mentioned above, the nano emulsion form was found to have the best efficiency (Anwer *et al.*, 2014). Usually, the nano emulsion particle size was determined to be less than 100 nm (Hasan *et al.*, 2020; Amiri *et al.*, 2021). Nanoencapsulation therefore has advantages over other encapsulation systems for certain applications such as improved stability of encapsulated active compounds, large surface area to volume ratio, higher bioavailability, mass transfer behavior (Zhang *et al.*, 2021), enhanced biological efficiency (Donsì and Ferrari, 2016) and improved diffusion to the target system (McClements *et al.*, 2021). For this reason, nanoemulsions have various applications in several fields, such as pharmaceuticals, cosmetics, agriculture, etc (Chime *et al.*, 2014; Echeverría and De Albuquerque, 2019) At present, the innovation of using essential oils in the form of nanoemulsions has received great attention as a substitute for chemical pesticides. This is due to their effective performance without adverse effects on non-target organisms. For example, essential oils in the form of nanoemulsions have shown effectiveness against insects and mites (Ibrahim, 2020; Doungnapa *et al.*, 2021), such as insect cabbage pests (Tia *et al.*, 2023) stored product insect pests (Draz *et al.*, 2022; Sabbour and El-Aziz, 2019), mosquitoes (Mahran, 2022; Vivekanandhan *et al.*, 2023), poultry mites (Ismail *et al.*, 2020), and mite pests (Mossa *et al.*, 2023; Sarapothong *et al.*, 2017; Doungnapa *et al.*, 2017; Doungnapa *et al.*, 2021) However, there is currently a lack of knowledge on the utilization of essential oil nano emulsion innovation against microbial contamination, deterioration and the action to remain in the environment for a longer time.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Survey of ectoparasite found species in chickens.

3.1.1 Places to survey.

The process of surveying ectoparasites of beautiful chicken was done in farm at King Mongkut's Institute of Technology Ladkrabang and private beautiful chicken farms located in eastern Bangkok as showed in **Table 3.1** and **Figure 3.1**. The survey was made based on Al - Saffar and Al-Mawla (2008). The most infectious species were object to be used for further testing in the laboratory. There were two body areas of surveys: on feathers and skin as mentioned in **2.1.2** and **2.1.3**.

Table 3.1. Location (district) of farm, chicken variety and number of surveyed chickens.

Location (districts)[1/]	Chicken variety/number[2/]										
	LH	HR	SK	JB	PO	RI	BR	PB	SB	SE	Total
Ladkrabang	10	10	6	-	-	-	-	-	-	-	**26**
Khan na yao	-	-	2	2	2	2	2	-	-	-	**10**
Khlong sam wa	-	-	1	5	-	-	-	2	2	2	**12**
Total	**10**	**10**	**9**	**7**	**2**	**2**	**2**	**2**	**2**	**2**	**48**

[1/]Farms in the eastern area of Bangkok [2/] LH= Laying hens in a cage, HR= Hens reared in free cage, SK= Silkie, JB= Japan bantam, PO= Polish, RI= Rhode Island red, BR= Brahma, PB= Phu Phan black bone, SB= Serama bantam, SE= Sebright

Figure 3.1 A: Laying hens in a cage; B: Hens reared in free cage; C: Silkie; D: Japan bantam; E: Polish; F: Rhode Island red; G: Brahma; H: Phu Phan black bone; I: Serama bantam; J: Sebright.

3.1.2 Observation of ectoparasites on the chicken feather.

The ectoparasites were taken randomly from 5 points of each chicken body as neck, wing, breast, back and buttocks. Amount of 20 feathers were randomly collected from the object areas with about 5 cm^2 as follows: hackles are the feathers around the neck along the front of the neck and surrounding necklaces, wings are the area of soft and hard feathers both on the outside and inside of the chicken, the breast is the area where the feather in the front of the breast is located, the back is the part between the cape and saddle and the buttocks are the soft hairs around the anus (cloaca) **(Figure 3.2)**.

3.1.3 Suction application.

The suction was made randomly from each chicken at head, neck, body and buttocks, by using a vacuum aspirator connected to a test tube sizing 20*150 mm as specified areas with 5 minutes suction was performed in 5 cm^2. Where, the head is the cockscomb area down to the eyes and neck below. The neck is the area from the hackle to the front neck plumage or around the necklaces. The body is part of the breast to the insides of the point where there are soft feathers. Additionally, buttocks are the area as mentioned above **(Figure 3.2)**.

Figure 3.2 A: Pulling feathers method to observe the number of ectoparasites in various areas on the chicken's body; B: Using aspirator toward the skin of chickens.

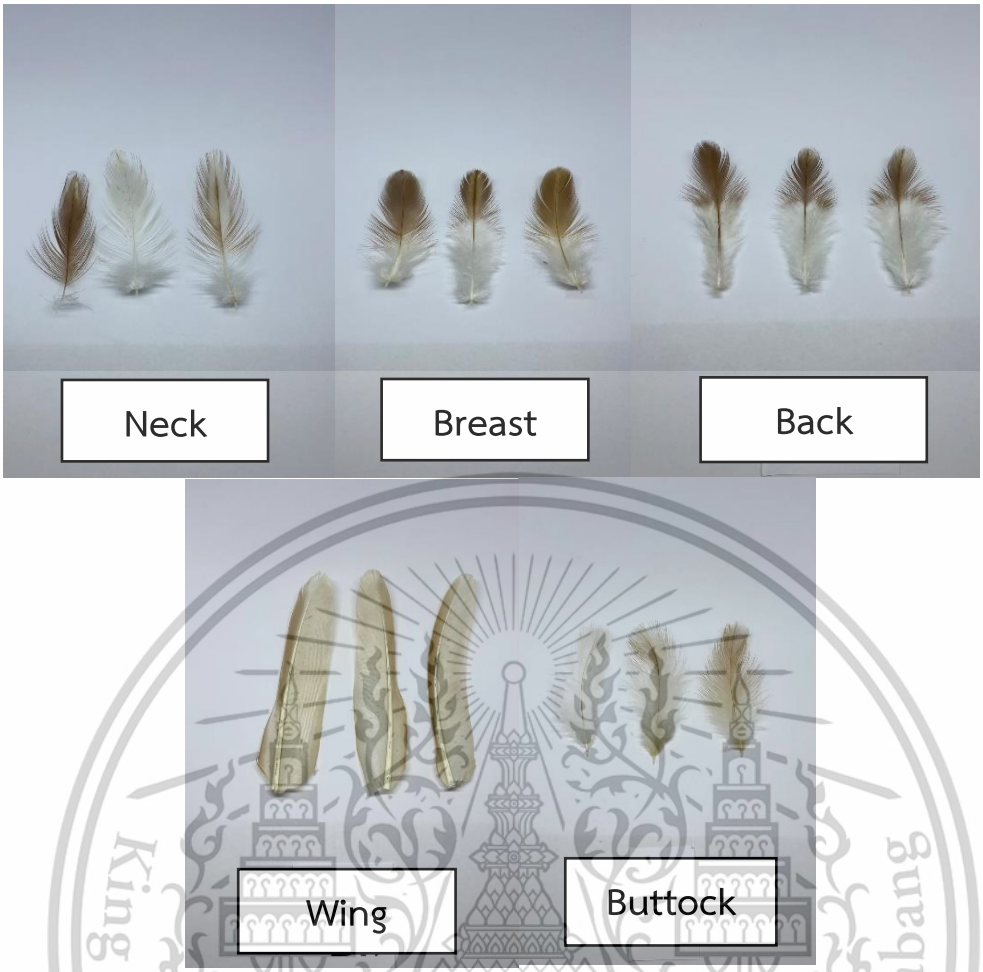

Figure 3.3 Feathers in different areas.

3.2 Preparation of essential oils (EOs).

3.2.1 Preparation of nano essential oils; NEOs.

Essential oils from medicinal plants used in the experiment were clove, cinnamon, and turmeric. The essential oil selection guideline was based on research results and academic papers that used medicinal plants to test their efficacy against ectoparasites by Pumnuan *et al.* (2020) and then nano essential oils (NEOs) were prepared as follows:

1. Nano essential oil with clove buds (*Syzygium aromaticum*, CL) and cinnamon leaves (*Cinnamomum zeylanicum*, CI) used as the main ingredients in the form of an emulsion with appropriate ratio of essential oil: surfactant: co-surfactant were prepared. NEO-CL and NEO-CI were defined as nano plants essential oil containing clove and cinnamon as the main constituents, respectively.

2. Nano essential oil with turmeric rhizomes (*Curcuma longa*, TU) as the main ingredient. in the form of an emulsion with the ratio of Essential oil: Surfactant: Co-surfactant. According to the recommended ratio from the research of Doungnapa *et al.* (2017).

Afterwards, the nano essential oils were analyzed for particle size (diameter), and polydispersity index and zeta potential by nano plus zeta/nano particle analyzer (Micromeritics Instrument Corporation; Japan).

Table 3.2 The balance of the hydrophilic and lipophilic of the surfactant used.

Surfactant	Hydrophilic–lipophilic balance (HLB)
PEG 400 (polyethylene glycol 400)	13.0
Span20	8.6
Span80	4.3
Span83	3.7
Span85	1.8
NP9 (Nonylphenol Ethoxylate)	12.9
Tween20	16.7
Tween40	15.6
Tween60	14.9
Tween80	15.0

3.2.2 Analysis of the chemical composition of nano essential oils (NEOs).

The essential oils (EOs) extracted from clove, cinnamon and turmeric, were analyzed using Gas Chromatography Mass Spectrometer (GC-MS) (Agilent Technologies Inc., USA). The GC-MS was equipped with an HP5MS capillary column (30 m length £ 0.25 mm I.D. £ 0.25 mm film thickness). Analysis parameters included direct injection of a 0.4 ml volume, a split mode with a split ratio of 50:1 v/v, and an injection

temperature of 250°C. Helium served as a carrier gas with a flow rate of 1 mL/min and an ionization voltage of 70 eV. Mass range detection was set to 50 to 500 m/z. The oven temperature, started at 50°C, held for 3 min, then increased by 10°C/min until reaching 200°C, after which it was raised by 15°C/min until it reached 260°C. The detector was maintained at 270°C. The results obtained were compared with those in Wiley's library (Wiley7n), accepting a quality match of over 85%. Nano-emulsions of the main compound standard (NCS) were prepared using clove, cinnamon and turmeric EOs. Eugenol (E) was identified as the primary constituent of clove and cinnamon EOs, while turmerone (T) was present in turmeric EO. NCS-E, containing eugenol (Fluka Analytical) was prepared in the same ratio as NEO-CL and NEO-CI. Similarly, NCS-T, consisting of turmerone (MedChemExpress), was prepared in the same ratio as NEO-TU. The particle size of the NCS at 1.0% in water was measured, along with their polydispersity index (PDI) and zeta potential by using a nano plus zeta/nano particle analyzer as done in NEO experiments.

3.2.3 Preparation of nano essential oil formulas (NEOFs) and nano-emulsions of the main compound standard (NCS).

When tested the efficiency of NEOs. Therefore, a nano essential oil from clove (NEO-CL) will be prepared as the main component, whereas nano essential oil from cinnamon (NEO-CI) and turmeric (NEO-TU) as secondary components. Then, nano essential oil formulas (NEOFs), different ratios of mixture were prepared as NEO-CL: CI: TU, namely 4:0:0, 2:2:0, 2:0:2 and 2:1:1, respectively.

Subsequently, the NEOFs at 1.0% in water would be analyzed for their particle size (diameter), polydispersity index and zeta Potential by using Nano plus Zeta/Nano Particle Analyzer as same as NEOF and NCS.

3.3 The efficacy of nano essential oil formulas (NEOFs) against chickens ectoparasites in the laboratory conditions.

3.3.1 Toxicity test in the form of insecticide by contact residue exposure method.

The lethal contact residue exposure test was be performed according to the method of Pumnuan *et al.* (2020). Initially, 1 ml of NEOFs was dropped onto filter paper, 9 cm diameter (Whatman™; No.1) and placed in a glass petri dish. Drying at room temperature for 5 min, and then 10 adult shaft louse (*Menopon gallinae*) and chicken mite (*Megninia ginglymura*) per dish were released. The Petri dish was tightly closed and wrapped with parafilm, kept at room temperature (25±3°C). Their percentage mortality was compared with happening in the control group. Then, two of NEOFs with the highest efficiency in killing *M. gallinae* and *M. ginglymura* were selected for toxicity testing at various concentrations. The actual mortality rate was compared with that of the control group **(Figure 3.4)**. The toxicity level was calculated to obtain LC_{50} and LC_{90} to determine the appropriate concentration of the test NEOFs for further use on the farm. Abbott's formula was used to calculate the actual death rates (Abbott, 1987). The experiment was designed in fifth completely randomized replicates, and the lethal concentration (LC_{50} and LC_{90}) of the different NEOFs were calculated using SPSS statistic package (version 11.0).

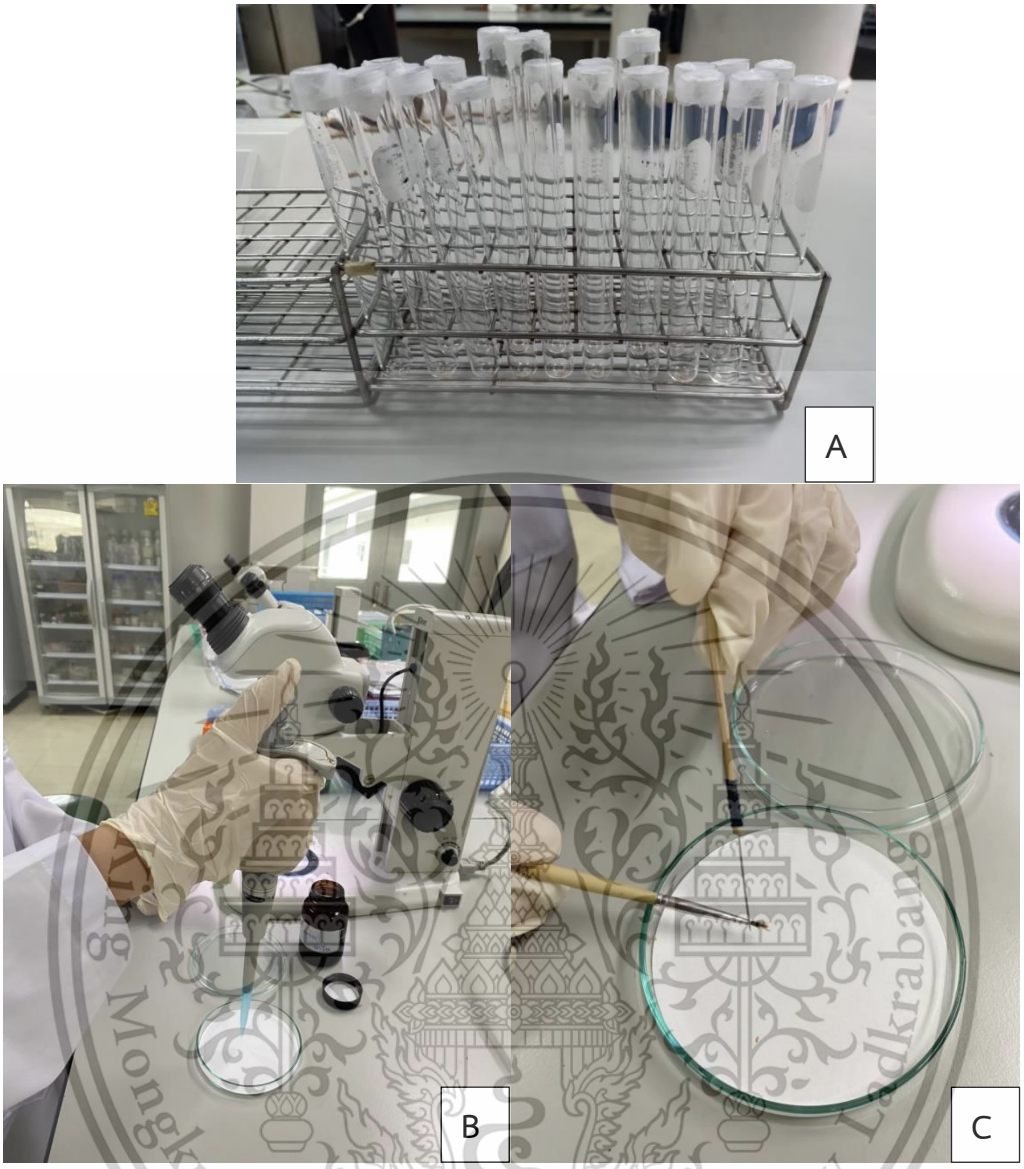

Figure 3.4 A: The chicken ectoparasites from the farm were kept in the test tubes. B: The test substance dropped onto a filter paper with a volume of 1 ml. C: Ectoparasites released onto a petri dish plate with amount of 10 adults/plate.

3.3.2 Repellent test.

Toxicity as a repellant was tested using NEOFs that were selected from the previous contact residue exposure data. They were treated against 2 species of ectoparasites namely, chicken, shaft louse (*M. gallinae*) and chicken mites (*M. ginglymura*) with the concentrations of 0.15 and 0.25%.

Repellent test of shaft louse (*M. gallinae*) was performed by dipping feathers in each concentration of each test solution for 1 minute, dried at room temperature (25±3°C) for 5 min, and placed in a petri dish lined with filter paper, 9 cm diameter **(Figure 3.5)**. The shaft louse adult was ripped onto chicken feathers with amount of 10 nymphs/ feather, then the petri dish was covered tightly and wrapped with parafilm, placed at room temperature. The number of shaft louse found on the feathers and on the filter paper at 3 and 6 hours was count and then the rate of repelling was calculated. As for the repellent test of chicken mites (*M. ginglymura*), it was performed by using a glass test tube (0.5 cm diameter, 8 cm long), one end side attached with a filter paper that was moistened with each concentration of each test NEOFs, while the other end side was moistened with the control group. At the beginning, those papers were moistened for 1 min, dried at room temperature for 5 minutes. In each test, the adults of mite with 10 mites/tube were used and placed at room temperature (25±3°C). The number of mites on each side of the test tube were count at 3 and 6 hours. The rate of the repellent index (%RI) was calculated by the following formula: %RI = [(C-T)/C+T)] × 100 (Pascual-Villalobos and Robledo, 1998). Positive and negative values indicate repellent and attractant effects, respectively.

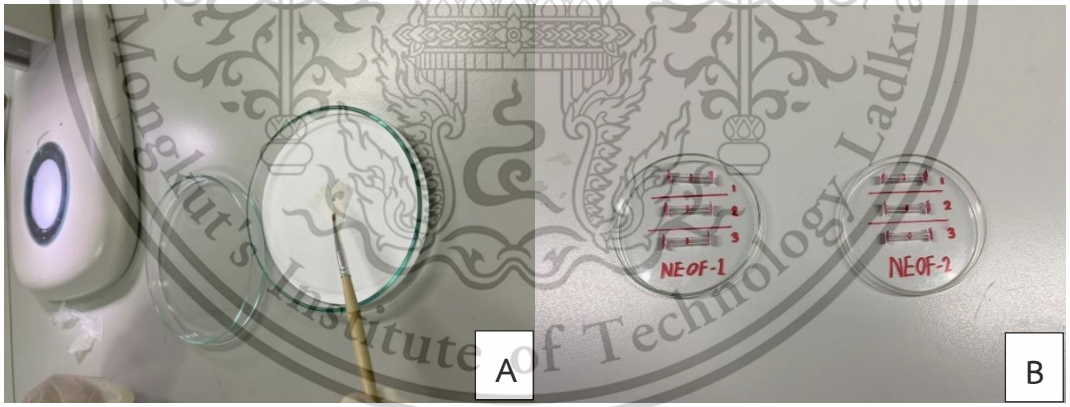

Figure 3.5 Repellent test, A: shaft louse (*Menopon gallinae*); B: chicken mites (*Megninia ginglymura*).

3.4 The efficacy of using a nano essential oil formulas against chicken ectoparasites in laying hens farm conditions.

The efficacy test was conducted using 2 NEOFs in which those formulas were most effective in controlling chicken ectoparasites in the laboratory test. The

appreciate rate was determined based on LC_{90} value for use in hen farm conditions and compared to the insecticide (cypermethrin) at the recommended rate as well as compared with control group and non-treat group. The substance of each group was prepared in water supply, with a volume of 30 litres before immersing in this substance, the ectoparasites of the chickens were randomly counted before dipping the chickens in the test substance. The laying hens were immersed in the test substance of each experimental group for 1 minute, then the hens were picked up from the test substance and placed in cages according to the experimental plan. The same test was carried out twice: the first time when the laying hens were 35 weeks old and the second time when the laying hens were 40 weeks old. The chicken's ectoparasites were randomly observed before dipping the chickens in the test substance and after the chickens were dipped in the test substance on days 1, 3, 5, 7, and 14. Each chicken was randomly observed for ectoparasites found on each part of the chicken's feathers in all 5 areas, including the head, breast, wings, back, and buttocks. The observation of chicken ectoparasites were conducted by pulling 2 chicken feathers per area, placing them in a plastic petri dish and covering them with parafilm. Species identification and quantity of all ectoparasites found in each experiment was made. Record the images of ectoparasites found in the laboratory and the results were recorded as outbreak levels according to the method of Al-Saffar and Al-Mawla (2008) **(Figure 3.6)**.

Figure 3.6 A: Dipping the laying hens into each type of test substance.; B: Counting the number and species of chicken ectoparasites found in each test substance.

3.5 Study on the quality of eggs and laying an egg after using NEOFs to control the chickens ectoparasites in laying hens farm conditions.

The effect of using 2 NEOFs to control chicken ectoparasites in laying hens farm conditions. Was also evaluated by observing on the egg production performance as well. This includes the percentage of egg production per live hen (%hen day), average egg weight, total egg mass and efficiency of feed use per total egg mass (FCR/Egg mass) by monitoring randomly the quality of eggs every week after NEOFs treatment in farm conditions compared to the use of insecticides (cypermethrin) at the recommended rate **(Figure 3.7)**.

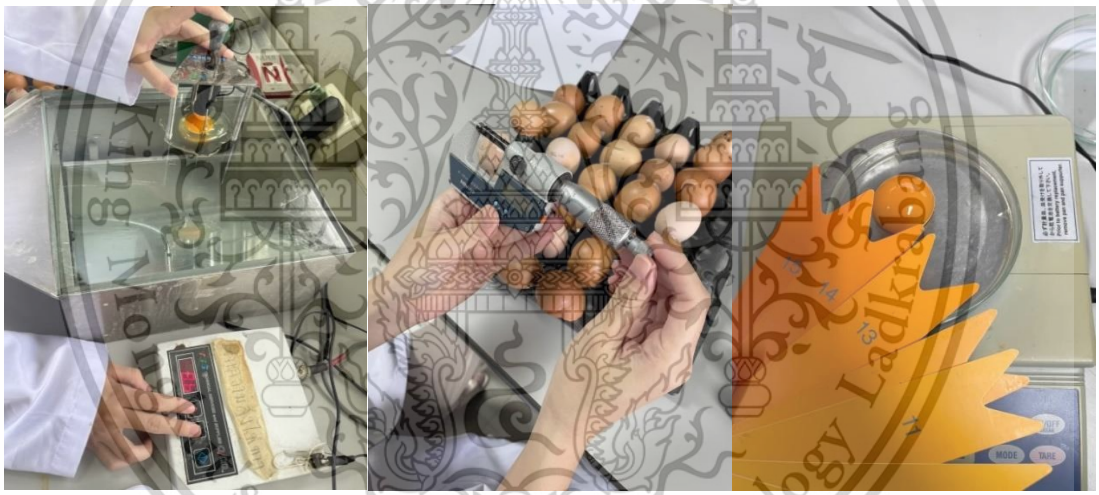

Figure 3.7 Method study on the quality of chicken eggs and laying hens after using the NEOFs to control the chicken ectoparasites in farm conditions.

3.6 Study on insecticide (cypermethrin) residued in eggs after treatments.

The analysis of insecticide (cypermethrin) residues in eggs. After using this chemical and other test substances to control the chicken ectoparasites in laying hen farms, was carried out on 1, 3, 5, 7 and 14 days after the first and second dips of the experiment. Ten eggs were randomly collected from each experiment and the insecticide (cypermethrin) residued in chicken eggs was analyzed immediately using the QuEChERS methods of AOAC (2007) and Weerawut and Thanit (2018). The analysis was performed to determine the number of toxic residues using gas chromatograph machine (GC) **(Figure 3.8)**.

Figure 3.8 Analysis preparation of insecticide (cypermethrin) residued in eggs after treatments to control the chicken ectoparasites in the farm conditions.

3.7 Statistical analysis.

In this study, the laboratory condition test was designed using a completely randomized design (CRD), and the experimental farm condition was designed using a randomized complete block design (RCBD). All data obtained were then calculated as the actual percentage of death using Abbott's formula. The results were analyzed using analysis of variance (ANOVA) and compared to determine the mean using Duncan's multiple range test at a 95% confidence level ($p < 0.05$) with the Statistical Analysis System (SAS) program. The lethal concentrations LC_{50} and LC_{90} were calculated, and the Chi-square value was determined using the Statistical Package for the Social Sciences (SPSS) program.

CHAPTER 4

MAIN RESULTS AND DISCUSSION

4.1 Results of a survey on the species and populations of ectoparasites in chickens

The obtained results indicated that 8 species of ectoparasites were found attacking beautiful chickens, namely: *Megninia cubitalis*, *Pterolichus obtusus*, *Megninia ortari*, *Cuclotogaster heterographus*, *Menopon gallinae*, *Lipeurus caponis*, *Goniocotes gallinae*, and *Menacanthus stramineus* **(Figure 4.1)**. The most prevalent ectoparasite found in the feathers of laying hens in a cage was *Megninia cubitalis*, with an average of 103.88 mites per chicken. These mites were found on the neck, wings, chest, back, and buttocks, with amounts of 52.1, 5.7, 119.8, 136.9, and 204.9 mites per chicken, respectively. Further findings showed that *Megninia cubitalis* also attacked Rhode Island red chickens, with an average of 32.40 mites per chicken, predominantly on the buttocks with an average of 85.00 mites per chicken. *Pterolichus obtusus* was found in only two types of chickens, Japan bantam and Polish, with averages of 0.12 and 7.80 mites per chicken, respectively. Additionally, *Megninia ortari* was exclusively found in silkie chickens, observed on the neck, wings, and buttocks with averages of 0.8, 0.7, and 0.3 mites per chicken, respectively. The louse *Lipeurus caponis* was the most common parasite in Polish chickens' backs, averaging 100.5 insects per chicken, and 35.90 insects per chicken. It also appeared on Rhode Island red chickens' chests and wings, with averages of 70.0 and 50.5 insects, respectively, and an average of 28.90 insects per chicken. *Menopon gallinae* was commonly found in hens reared in free-range cages, typically occurring on the buttocks with 21.1 insects per chicken and averaging 7.58 insects per chicken. *Cuclotogaster heterographus* was found in laying hens in cages, averaging 4.36 insects per chicken. *Menacanthus stramineus* was most common in Japan bantam chickens, with a total average of 0.52 insects. Moreover, *Goniocotes gallinae*, found in Silkie chickens, had an average of only 0.52 insects per chicken **(Table 4.1)**.

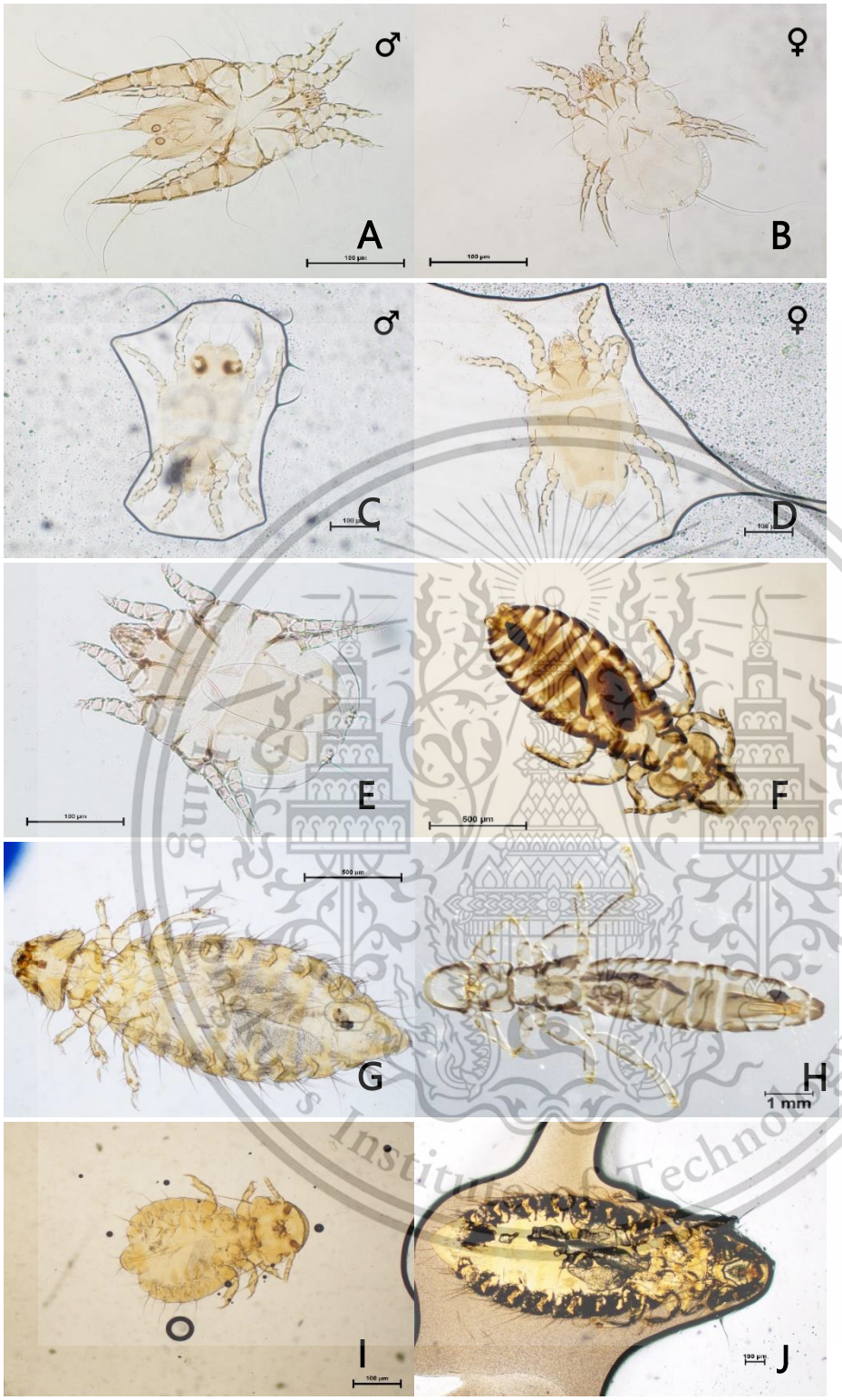

Figure 4.1 The surveyed species of chicken ectoparasite, namely, A: *Megninia cubitalis* (Male); B: *Megninia cubitalis* (Female); C: *Pterolichus obtusus* (Male); D: *Pterolichus obtusus* (Female); E: *Megninia ortari*; F: *Cuclotogaster heterographus*; G: *Menopon gallinae*; H: *Lipeurus caponis*; I: *Goniocotes gallinae*; J: *Menacanthus stramineus*.

Table 4.1 The ectoparasite species found on feathers in different parts of various chicken in eastern area of Bangkok.

Ectoparasite	Part of chicken	Mean of ectoparasite[1/]									
		Chicken variety[2/]									
		LH	HR	SK	JB	PO	RI	BR	PB	SB	SE
Mite											
Megninia cubitalis	Neck	52.1	-	1.7	11.2	12.0	5.0	17.5	-	5.0	-
	Wings	5.7	-	0.9	8.8	5.0	19.5	-	-	-	4.0
	Chest	119.8	-	1.7	13.8	3.5	36.0	28.0	3.0	4.5	1.5
	Back	136.9	-	1.8	16.9	0.5	16.5	15.5	-	3.0	-
	Buttocks	204.9	1.8	2.6	2.5	24.0	85.0	25.0	-	19.0	-
	Mean per chicken	**103.88**	**0.36**	**1.74**	**10.64**	**9.00**	**32.40**	**17.20**	**0.60**	**6.30**	**1.10**
Megninia ortari	Neck	-	-	0.8	-	-	-	-	-	-	-
	Wings	-	-	0.7	-	-	-	-	-	-	-
	Chest	-	-	-	-	-	-	-	-	-	-
	Back	-	-	-	-	-	-	-	-	-	-
	Buttocks	-	-	0.3	-	-	-	-	-	-	-
	Mean per chicken	**-**	**-**	**0.36**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Pterolichus obtusus	Neck	-	-	-	-	17.0	-	-	-	-	-
	Wings	-	-	-	-	22.0	-	-	-	-	-
	Chest	-	-	-	-	-	-	-	-	-	-
	Back	-	-	-	0.3	-	-	-	-	-	-
	Buttocks	-	-	-	0.3	-	-	-	-	-	-
	Mean per chicken	**-**	**-**	**-**	**0.12**	**7.80**	**-**	**-**	**-**	**-**	**-**
Louse											
Cuclotogaster heterographus	Neck	3.3	-	-	4.3	-	2.0	-	-	-	-
	Wings	3.5	-	-	-	-	-	-	-	-	-
	Chest	4.7	-	-	-	-	-	-	-	-	-
	Back	7.7	-	-	-	-	-	-	-	-	-
	Buttocks	2.6	-	-	-	-	-	-	-	-	-
	Mean per chicken	**4.36**	**-**	**-**	**0.86**	**-**	**0.40**	**-**	**-**	**-**	**-**
Menopon gallinae	Neck	-	1.7	0.8	1.7	2.0	-	-	-	-	-
	Wings	-	10.6	1.1	4.7	1.0	2.0	-	-	-	-
	Chest	-	2.5	0.7	1.6	3.0	5.0	1.5	-	-	-
	Back	-	2.0	0.4	0.6	5.5	-	2.0	-	-	-
	Buttocks	-	21.1	1.0	1.6	-	-	-	-	5.0	-
	Mean per chicken	**-**	**7.58**	**0.80**	**2.04**	**2.30**	**1.40**	**0.80**	**-**	**1.00**	**-**
Lipeurus caponis	Neck	-	-	9.8	24.0	3.5	6.0	1.5	-	7.5	-
	Wings	-	-	2.8	18.0	6.0	50.5	-	-	1.5	-
	Chest	-	-	0.1	2.6	12.5	70.0	0.5	-	2.5	-
	Back	-	-	0.6	21.1	100.5	12.0	1.5	-	-	-
	Buttocks	-	-	1.3	5.1	57.0	6.0	1.0	-	1.0	-
	Mean per chicken	**-**	**-**	**2.92**	**14.16**	**35.90**	**28.90**	**0.90**	**-**	**2.50**	**-**
Goniocotes gallinae	Neck	-	-	2.4	1.6	-	-	-	-	-	-
	Wings	-	-	-	0.9	-	-	-	-	-	-
	Chest	-	-	0.1	-	-	-	-	-	-	-
	Back	-	-	0.1	-	-	-	-	-	-	-
	Buttocks	-	-	-	-	-	-	-	-	-	-
	Mean per chicken	**-**	**-**	**0.52**	**0.50**	**-**	**-**	**-**	**-**	**-**	**-**
Menacanthus stramineus	Neck	-	-	-	-	1.0	-	-	-	-	-
	Wings	-	-	-	0.6	-	-	-	-	-	-
	Chest	-	-	-	0.7	-	-	-	-	-	-
	Back	-	-	-	1.1	-	-	-	-	-	-
	Buttocks	-	-	-	1.0	-	-	-	-	-	-
	Mean per chicken	**-**	**-**	**-**	**0.68**	**0.20**	**-**	**-**	**-**	**-**	**-**

[1/] Means of ectoparasite found on 10 feathers per part of chicken. [2/] LH= Laying hens in a cage, HR= Hens reared in free cage, SK= Silkie, JB= Japan bantam, PO= Polish, RI= Rhode Island red, BR= Brahma, PB= Phu Phan black bone, SB= Serama bantam, SE= Sebright, - Not found

The study of ectoparasites found on different parts of chicken skin indicated that the most common mite was *Megninia cubitalis*, which abundantly attacked laying hens in cages and could be found in all parts of the chicken. The most preferred area was the buttocks, with an average of 168.3 mites, followed by the body, neck, and head, with averages of 163.6, 99.4, and 71.4 mites, respectively. This mite species appeared in all chickens except for hens reared in free range. *Megninia ortari* was predominantly found in Silkie chickens, with an average of 2.40 mites per chicken, followed by Serama bantam chickens, which had an average of 0.88 mites per chicken. *Pterolichus obtusus* was found in only one type of chicken, Polish chickens, with an average of 1.0 mite per chicken. In the case of louse ectoparasites, *Cuclotogaster heterographus* was most commonly found on laying hens in cages on the body, neck, and buttocks, with averages of 43.8, 8.2, and 4.0 insects, respectively (but not found on the head), resulting in an average of 14.0 insects per chicken. A few were found in Silkie chickens, averaging 0.15 insects per chicken. *Menopon gallinae* was primarily found in hens reared in free-range conditions, with an average of 8.87 insects per chicken. It was found in almost all chicken species except for laying hens in cages and Phu Phan black-boned chickens. As for lice, *Lipeurus caponis* was found in Japan bantam chickens with an average of 2.35 insects per chicken and was also discovered in four other chicken varieties: Brahma, Rhode Island red, Polish, and Silkie, with averages of 0.88, 0.38, 0.25, and 0.03 insects per chicken, respectively. *Menacanthus stramineus* lice were found in only two varieties of chicken, Japan bantam and Polish, with averages of 2.18 and 0.75 insects per chicken, respectively. Finally, *Goniocotes gallinae* could be detected in Silkie chickens with a total average of 0.05 insects per chicken **(Table 4.2)**.

Table 4.2 The ectoparasite species found on different skin parts of various chicken varieties in eastern area of Bangkok.

Ectoparasite	Part of chicken	Mean of ectoparasite[1/]									
		Chicken variety[2/]									
		LH	HR	SK	JB	PO	RI	BR	PB	SB	SE
Mite											
Megninia cubitalis	Head	71.4	-	6.6	17.0	1.5	13.5	-	6.0	-	22.5
	Neck	99.4	-	4.6	5.7	0.5	8.5	18.5	4.0	-	1.5
	Body	163.6	-	22.5	7.0	0.5	18.5	18.0	1.5	10.0	17.0
	Buttocks	168.3	-	12.6	17.3	4.5	42.6	30.5	-	2.0	0.5
	Mean per chicken	**125.68**	**-**	**11.58**	**11.75**	**1.75**	**20.78**	**16.75**	**2.88**	**3.00**	**10.38**
Megninia ortari	Head	-	-	0.4	-	-	-	-	-	-	-
	Neck	-	-	0.3	-	-	-	-	-	3.5	-
	Body	-	-	-	-	-	-	-	-	-	-
	Buttocks	-	-	8.9	-	-	-	-	-	-	-
	Mean per chicken	**-**	**-**	**2.40**	**-**	**-**	**-**	**-**	**-**	**0.88**	**-**
Pterolichus obtusus	Head	-	-	-	-	1.5	-	-	-	-	-
	Neck	-	-	-	-	-	-	-	-	-	-
	Body	-	-	-	-	2.5	-	-	-	-	-
	Buttocks	-	-	-	-	-	-	-	-	-	-
	Mean per chicken	**-**	**-**	**-**	**-**	**1.00**	**-**	**-**	**-**	**-**	**-**
Louse											
Cuclotogaster heterographus	Head	-	-	-	-	-	-	-	-	-	-
	Neck	8.2	-	0.6	-	-	-	-	-	-	-
	Body	43.8	-	-	-	-	-	-	-	-	-
	Buttocks	4.0	-	-	-	-	-	-	-	-	-
	Mean per chicken	**14.0**	**-**	**0.15**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Menopon gallinae	Head	-	0.7	-	0.3	1.5	-	-	-	-	-
	Neck	-	22.0	8.2	0.9	-	1.5	1.0	-	-	-
	Body	-	9.6	1.8	-	11.0	2.5	1.5	-	-	0.5
	Buttock	-	2.8	1.8	1.3	22.5	-	-	-	23.0	-
	Mean per chicken	**-**	**8.78**	**2.95**	**0.63**	**8.75**	**1.00**	**0.63**	**-**	**5.75**	**0.13**
Lipeurus caponis	Head	-	-	-	0.9	1.0	1.0	1.5	-	-	-
	Neck	-	-	-	5.6	-	-	-	-	-	-
	Body	-	-	-	2.9	-	-	-	-	-	-
	Buttock	-	-	0.1	-	-	0.5	2	-	-	-
	Mean per chicken	**-**	**-**	**0.03**	**2.35**	**0.25**	**0.38**	**0.88**	**-**	**-**	**-**
Goniocotes gallinae	Head	-	-	-	-	-	-	-	-	-	-
	Neck	-	-	-	-	-	-	-	-	-	-
	Body	-	-	-	-	-	-	-	-	-	-
	Buttock	-	-	0.2	-	-	-	-	-	-	-
	Mean per chicken	**-**	**-**	**0.05**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Menacanthus stramineus	Head	-	-	-	0.3	-	-	-	-	-	-
	Neck	-	-	-	-	-	-	-	-	-	-
	Body	-	-	-	0.4	-	-	-	-	-	-
	Buttock	-	-	-	8.0	3.0	-	-	-	-	-
	Mean per chicken	**-**	**-**	**-**	**2.18**	**0.75**	**-**	**-**	**-**	**-**	**-**

1/ Means of ectoparasite found on 5 cm2 per part of chicken. 2/ LH= Laying hens in a cage, HR= Hens reared in free cage, SK= Silkie, JB= Japan bantam, PO= Polish, RI= Rhode Island red, BR= Brahma, PB= Phu Phan black bone, SB= Serama bantam, SE= Sebright, - Not found

The analysis of prevalent factors regarding ectoparasites between the feather and skin areas of chickens indicated that the prevalence of *Megninia cubitalis* found on feathers and skin was 77.08% and 66.66%, respectively (X^2 = 0.2110; P = 0.6460). Meanwhile, *Megninia ortari* was observed on the feather and skin areas with prevalences of 14.58% and 10.41%, respectively (X^2 = 0.2964; P = 0.5861). *Pterolichus obtusus* was present on feathers and skin with prevalences of 6.25% and 2.08%, respectively (X^2 = 0.9604; P = 0.3271). The prevalence of these three mite species showed no significant differences at a confidence level of 0.05. As for the lice, *Cuclotogaster heterographus* was abundant on both feathers and skin at 29.16% and 25.00%, respectively (X^2 = 0.1211; P = 0.7278). For *Menopon gallinae*, the prevalences were 60.41% and 64.58%, respectively (X^2 = 0.0410; P = 0.8394). The prevalences of *Goniocotes gallinae* were 12.50% and 2.08%, respectively (X^2 = 3.3366; P = 0.6775), and for *Menacanthus stramineus*, the prevalences were both 8.33% (X^2 = 0; P = 1.0000). All of the above lice species showed no significant differences at a confidence level of 0.05. Finally, for *Lipeurus caponis*, the prevalences were 47.91% and 16.66%, respectively (X^2 = 5.5640; P = 0.0183), making it the only ectoparasite with a statistically significant difference at the 0.05 level **(Table 4.3)**.

A study conducted on ectoparasitic infestations in chickens from poultry farms in the eastern area of Bangkok revealed that the feather mite *Megninia cubitalis* was the most frequently observed species, infesting all types of chickens. Similar outbreaks of *M. cubitalis* have been reported in laying hens in the State of Minas Gerais, Brazil (Rezende *et al.*, 2015), a region with a tropical climate comparable to that of Thailand. This finding aligns with the report of Sangvaranond (2003), who noted the presence of *M. cubitalis* in domesticated chickens in central Thailand, with mites predominantly inhabiting the body and wings. Outbreaks have also been reported on private farms in Chachoengsao province and across several other provinces in Thailand. Severe infestations can lead to pyodermatitis, which may ultimately result in the death of affected chickens (Sangvaranond, 2009). Another species observed was *Megninia ortari*, predominantly found in specific chicken breeds such as Silkie and Sebright chickens. Mites of the genus *Megninia* are particularly significant in the laying hen industry, where they are known to decrease productivity by inhibiting egg production. The saliva of these mites induces severe itching and can lead to secondary infections such as pyodermatitis. Furthermore, they also pose a threat to other avian species,

including parrots, turkeys, and pigeons (Guimarães *et al.*, 2001; Tucci *et al.*, 2005; Rezende *et al.*, 2013). The prevalence of *Megninia spp.* varies significantly depending on climatic and geographical conditions (Mumcuoglu and Lutsky, 1990; Hernandez *et al.*, 2006; Rezende *et al.*, 2015). Additionally, infestations of *Pterolichus obtusus* were noted in certain chicken breeds, including Silkie and Polish chickens. Sangvaranond (1993) identified *P. obtusus* as the most commonly occurring natural mite species in native chickens, accounting for approximately 70% of the ectoparasite population. Feather mites like *P. obtusus* specialize in residing within the microhabitats provided by feathers and typically pose minimal issues unless present in large numbers (Dabert and Mironov, 1999; Jankovska *et al.*, 2012). The shaft louse, *Menopon gallinae* was commonly found among domestic chickens, with prevalence rates of 60.41% on feathers and 64.58% on skin. These findings corroborate with Sangvaranond (2009), who reported that amblyceran lice—biting lice—are significant ectoparasites of both domestic and native chickens across various Thai provinces. These lice are among the most widespread and consequential. Prevalence rates reported from Bulgaria, the Kashmir Valley, and Malawi were 35.9%, 34.4%, and 33.3%, respectively (Prelezov *et al.*, 2006; Salam *et al.*, 2009; Banda, 2011). The wing louse *Lipeurus caponis* was also found to infest chicken feathers, with a significant difference in distribution compared to skin, as it predominantly resides on feather shafts. Pumnuan *et al.* (2020) similarly reported high numbers of *L. caponis* in native chickens at the Urban Livestock Learning Center, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Thailand. Sangvaranond (2003) also noted that *L. caponis* was less prevalent than *M. gallinae*. Both lice species are commonly found in chickens throughout Thailand. Rahman and Haziqah (2015) reported similar findings in Penang, Malaysia, where *M. gallinae* was more prevalent than *L. caponis*. These two lice species are considered critical vectors for the spread of ectoparasites among poultry. Additional outbreaks have been reported in Libya, the United States, Algeria, Bangladesh, and Ethiopia (Mansur *et al.*, 2019). The louse *Cuclotogaster heterographus* was identified with a comparable prevalence to findings by Shanta *et al.* (2006) and Belihu *et al.* (2009), who reported prevalence rates around 25%, with common infestation sites being the head and neck. Most surveyed chickens were found to harbor at least one louse species, such as *M. gallinae*, *M. pallidulus*, *L. caponis*, *G. gallinae*, and *G. dissimilis*, which typically reside in the downy feathers of the neck, back, abdomen, and

wings (Rahman and Haziqah, 2015). In Libya, an ectoparasitic outbreak affecting over 70% of the chicken population involved species such as *M. gallinae*, *M. stramineus*, and *L. caponis*. Occasional outbreaks have also been documented in Algeria, the United States, and Bangladesh (Mansur *et al.,* 2019). *Goniocotes gallinae* was also found to be widely distributed in several Thailand provinces, including Roi Et, Surin, and Buriram, particularly residing in the feathers at the top of the shanks (Nopwinyoowong and Sukolapong, 1994). This study also recorded a prevalence of approximately 8% for *Menacanthus stramineus*, aligning with Jassim and Hadi (2019), who observed its preference for residing on down feathers around the tail, chest, and thighs. Similarly, Dik and Halajian (2013) reported this louse species in skin and feather samples from the body feathers, wings, chest, and body, with a prevalence of approximately 2% in Iran. Sangvaranond (2009) noted a 4% prevalence rate of *M. stramineus* in native chickens in Thailand. The prevalence of ectoparasites in beautiful and domestic chickens varies across regions due to differing climatic conditions, underlining the importance of tailored ectoparasite management strategies in poultry farming.

Table 4.3 Distribution of various ectoparasite found on feather and skin of different chickens in eastern area of Bangkok.

Ectoparasite		Part of chicken	Number examined	Number positive	Prevalence (%)	χ^2	P value*
Mite	*Megninia cubitalis*	Feather	48	37	77.08	0.2110	0.6460^{ns}
		Skin	48	32	66.66		
	Megninia ortari	Feather	48	7	14.58	0.2964	0.5861^{ns}
		Skin	48	5	10.41		
	Pterolichus obtusus	Feather	48	3	6.25	0.9604	0.3271^{ns}
		Skin	48	1	2.08		
Louse	*Cuclotogaster heterographus*	Feather	48	14	29.16	0.1211	0.7278^{ns}
		Skin	48	12	25.00		
	Menopon gallinae	Feather	48	29	60.41	0.0410	0.8394^{ns}
		Skin	48	31	64.58		
	Lipeurus caponis	Feather	48	23	47.91	5.5640	0.0183*
		Skin	48	8	16.66		
	Goniocotes gallinae	Feather	48	6	12.50	3.3366	0.6775^{ns}
		Skin	48	1	2.08		
	Menacanthus stramineus	Feather	48	4	8.33	0	1.0000^{ns}
		Skin	48	4	8.33		

*The P values were calculated by Chi-square (X2) test. ns= non-significant (typically ≤ 0.05), *= A p-value less than 0.05 is statistically significant difference.

4.2 Development of plant nano essential oil formulas (NEOFs)

The major chemical compounds of EOs from clove, cinnamon and turmeric which analyzed by GC-MS demonstrated the eugenol was a major compound in clove and cinnamon EOs with 84.60 and 72.00%, respectively. While ar-turmerone was a major compound in turmeric EO with 53.38%. The amount minor chemical compounds of clove were as follows; caryophyllene (10.06%), *alpha*-humulene (2.09%), caryophyllene oxide (0.69%) and *delta*-cadinene (0.52%), etc. **(Table 4.4)**.

Table 4.4 Chemical composition of clove (*Syzygium aromaticum*) essential oil.

No.	Compounds	Retention Time	% Area	% Match
1	p-Hydroxyallylbenzene	10.97	0.10	96
2	Eugenol	12.59	84.60	98
3	Alpha.-Copaene	12.8	0.32	94
4	Caryophyllene	13.39	10.06	99
5	Alpha.-Humulene	13.81	2.09	99
6	Isoledene	14.02	0.14	96
7	Delta.-Cadinene	14.59	0.52	99
8	Alpha.-Caryophyllene alcohol	15.24	0.10	64
9	Caryophyllene oxide	15.39	0.69	96
10	Adamantane	15.98	0.29	89
11	Beta.-Elemene	16.21	0.25	80
12	Caryophyllenol-II	16.35	0.13	90

The amount minor chemical compounds of cinnamon were as follows; benzyl benzoate (4.02%), caryophyllene (3.66%), eugenol acetate (3.19%), linalool L (2.23%), *trans*-cinnamyl acetate (2.18%), o-cymene (1.77%), cinnamaldehyde (1.71%), safrole (1.50%), *alpha*-pinene (1.21%), copaene (0.91%), *beta*-thujene (0.74%), *alpha*-humulene (0.70%), caryophyllene oxide (0.60%), anethol (0.52%) and rubicene (0.51%), etc. **(Table4.5)**.

Table 4.5 Chemical composition of cinnamon (*Cinnamomum zeylanicum*) essential oil.

No.	Compounds	Retention Time	% Area	% Match
1	Alpha.-Pinene	5.86	1.21	96
2	Camphene	6.12	0.40	97
3	Benzaldehyde	6.32	0.15	96
4	Beta.-Pinene	6.61	0.31	97
5	Alpha.-Phellandrene	7.06	0.31	95
6	Delta.3-Carene	7.16	0.09	97
7	Alpha.-Terpinene	7.27	0.09	98
8	o-Cymene	7.4	1.77	97
9	Beta.-Thujene	7.49	0.74	94
10	Linalool L	8.62	2.23	97
11	4-Terpinenol	9.87	0.12	98
12	Alpha. Terpineol	10.06	0.27	91
13	Gamma.-Phenylpropanol	10.64	0.08	96
14	p-Hydroxyallylbenzene	10.97	0.10	97
15	Cinnamaldehyde	11.3	1.71	97
16	Safrole	11.5	1.50	97
17	Styrone	11.84	0.12	98
18	Eugenol	12.58	72.00	98
19	Copaene	12.78	0.91	99
20	Vanillin	13.04	0.38	97
21	Caryophyllene	13.37	3.66	99
22	Trans-Cinnamyl acetate	13.57	2.18	99
23	Alpha.-Humulene	13.8	0.70	99
24	Ledene	14.3	0.13	97
25	Eugenol acetate	14.58	3.19	99
26	Caryophyllene oxide	15.4	0.60	96
27	Benzoic acid, benzyl ester	17.39	4.02	98
28	Anethol	25.75	0.52	83
29	Rubicene	26.08	0.51	78

In addition, the following minor chemical compounds of turmeric were; alpha-tumerone (13.36%), beta-sesquiphellandrene (5.14%), 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [s-(r*,s*)]- (4.97%), cucalyptol (4.53%), l-phellandrene (3.17%), benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (3.11%), 4-sec-butyl-ethylbenzene (2.90%), 4,7,10-cycloundecatriene, 1,1,4,8-tetramethyl-, cis, cis, cis- (1.29%), *trans*-caryophyllene (1.22%), 1,8-cineole (1.18%) and beta.-bisabolene (1.07%), etc. **(Table 4.6)**.

Table 4.6 Chemical composition of turmeric (*Curcuma longa*) essential oil.

No.	Compounds	Retention Time	% Area	% Match
1	l-Phellandrene	7.51	3.17	95
2	Benzene, 1-methyl-4-(1-methylethyl)-	7.87	0.67	97
3	1,8-Cineole	8.00	1.18	98
4	Cucalyptol	9.00	4.53	98
5	Trans-Caryophyllene	13.94	1.22	99
6	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	14.67	3.11	99
7	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [s-(R*,S*)]-	14.83	4.97	95
8	Beta.-Bisabolene	14.99	1.07	99
9	Beta.-Sesquiphellandrene	15.19	5.14	98
10	2,4-DINITRO-6-(2-BUTYL)-PHENYL-.beta.,.beta.-DIMETHYLACRYLSAEUREESTER	16.03	0.52	53
11	4-sec-Butyl-ethylbenzene	16.15	2.90	53
12	4,7,10-Cycloundecatriene, 1,1,4,8-tetramethyl-, cis, cis, cis-	16.50	1.29	35
13	Benzene, 1,3,5-trimethyl- (CAS)	16.78	0.71	38
14	AR-TUMERONE	16.95	53.38	91
15	ALPHA.-TUMERONE	17.29	13.36	91
16	(6S,1'R)-6-(1',5'-DIMETHYLHEX-4'-ENYL)-3-METHYLCYCLOHEX-2-ENONE	17.74	0.63	93
17	4-Pentenoic acid, 2,4-dimethyl-, methyl ester	17.87	0.58	14
18	2,4-DINITRO-6-(2-BUTYL)-PHENYL-.beta.,.beta.-DIMETHYLACRYLSAEUREESTER	17.95	0.84	59
19	(+)-.alpha.-Atlantone	18.04	0.73	58

Upon studying the particle size of the plant nano essential oil at 1% concentration with water, in the form of an essential oil emulsion, it was found that nano essential oils

from clove, cinnamon, and turmeric, which have not yet been formulated, as well as NCSs derived from eugenol and turmerone chemical standards, ranged from 17.15 to 20.80 nm. Meanwhile, the NEOF-1, NEOF-2, and NEOF-3 with a mixture ratio of plant nano essential oil, NEO-CL : NEO-CI : NEO-TU equal to 4:0:0, 2:2:0, and 2:0:2 respectively, exhibit particle sizes less than 100.0 nm (20.76 nm, 20.66 nm, and 78.22 nm, respectively). The plant nano essential oil formulas with a mixing ratio of NEO-CL : NEO-CI : NEO-TU as 2:1:1 (NEOF-4) has a particle size exceeding 100 nm **(Table 4.7)**.

Table 4.7 Particle size, polydispersity index (PDI), and zeta-potential of various nano essential oils, chemical standards, and nano essential oil formulas at 1.0% with water.

Nanoemulsions (1.0% in water)	Particle size (nm)	PDI	Zeta Potential (mV)
Nano essential oils (NEOs)			
NEO-CL (clove : Tween60 : PEG400 = 2:9:2)	20.72±0.43	0.28±0.01	-0.28±1.53
NEO-CI (cinnamon : Tween60 : PEG400 = 2:9:2)	20.80±0.24	0.23±0.01	-3.03±0.53
NEO-TU (turmeric : Tween 80 : PEG400 = 2:6:3)	18.18±1.87	0.30±0.01	-4.59±0.37
Nanoemulsions of main compound standards (NCSs)			
NCS-E (eugenol : Tween60 : PEG400 = 2:9:2)	20.44±0.30	0.24±0.01	-3.28±0.87
NCS-T (turmerone : Tween 80 : PEG400 = 2:6:3)	17.15±1.76	0.32±0.02	-3.43±0.91
Nano essential oil formulas (NEOFs)			
NEOF-1 (NEO-CL : NEO-CI : NEO-TU = 4:0:0)	20.76±0.44	0.34±0.02	-4.89±0.92
NEOF-2 (NEO-CL : NEO-CI : NEO-TU = 2:2:0)	20.66±0.47	0.30±0.03	-3.06±0.71
NEOF-3 (NEO-CL : NEO-CI : NEO-TU = 2:0:2)	78.22±0.36	0.22±0.01	-2.59±0.92
NEOF-4 (NEO-CL : NEO-CI : NEO-TU = 2:1:1)	166.02±2.50	0.34±0.01	-0.87±0.59

Essential oils (EOs) are secondary metabolites produced by plants, serving a variety of biological and ecological functions. These oils are complex mixtures of naturally occurring compounds and can consist of multiple constituents at varying concentrations. Typically, two to three major compounds are responsible for the specific biological properties of each essential oil (Bakkali *et al.*, 2008). Previous reports have identified eugenol as the principal component of clove and cinnamon essential oils (Jumbo et al. 2014; Pumnuan and Insung, 2016; Jumbo *et al.*, 2018; Shahina *et al.*, 2022). In contrast, turmeric essential oil is primarily composed of turmerone derivatives, including ar-turmerone, α-turmerone, and β-turmerone (Liju *et al.*, 2011; Jaiswal and Naik, 2021; Jayaprakasha *et al.*, 2005). The chemical composition

of essential oils may vary significantly due to geographic origin, seasonal changes, plant growth stage, and anatomical part of the plant used for extraction. Such factors contribute to the diversity and variability in the bioactivity and formulation behavior of essential oils. In this study, most of the tested emulsified formulations—namely NEOs, NCSs, and NEOFs, excluding NEOF-4—demonstrated average particle sizes of less than 100 nm when dispersed in water at a concentration of 1.0%. These systems are thus classified as nanoemulsions, which are isotropic dispersions of two immiscible liquids, typically oil and water. When the average droplet size is within the range of 100–400 nm, the system may alternatively be described as a microemulsion (Cimino *et al.*, 2021). Nanoemulsions, particularly those with an oil-in-water (O/W) structure, often appear translucent or slightly opaque. Their stability is primarily achieved through emulsification processes that lead to the formation of stable colloidal systems (Perumal *et al.*, 2021). The efficiency of active compound delivery in such formulations relies heavily on the use of surfactants and co-surfactants to reduce interfacial tension and stabilize the dispersed phase (Cimino *et al.*, 2021). Surfactants and co-surfactants employed in oil-in-water nanoemulsions containing essential oils include poloxamer 188, lecithin, Pluronic® F68, Tween 60, Tween 80, decanoyl/octanoyl glycerides, glycerin monostearate, Span 8, and polyethylene glycol 400 (PEG400) (Shi *et al.*, 2016; Singh *et al.*, 2021; Cimino *et al.*, 2021; Doungnapa *et al.*, 2021; Yahya *et al.*, 2022). In the present study, Tween 60 and Tween 80 were used as surfactants, while PEG400 served as a co-surfactant. These components were combined in different ratios as recommended by Doungnapa *et al.* (2021). The resulting formulations exhibited polydispersity index (PDI) values ranging from 0.02 to 0.34. A lower PDI (typically < 0.3) indicates a more uniform particle size distribution, which correlates with enhanced physical stability of the nanoemulsion (Perumal *et al.*, 2021). Furthermore, the zeta potential measurements of the formulations revealed negative values ranging from -4.59 mV to -0.28 mV. These values suggest the presence of non-ionic surfactants and may also be attributed to the surface adsorption of negatively charged essential oil molecules or functional groups naturally present in the oil components (Perumal *et al.*, 2021).

4.3 The effectiveness of nano essential oil formulas against chicken ectoparasites in the laboratory condition

4.3.1 Toxicity test in the form of insecticide by contact residue exposure method

The concentration starting from 0.25% for 1 hour showed that all four nano essential oil formulas (NEOFs) had an efficacy in killing chicken lice greater than 50%. It was found that NEOFs 4:0:0 and 2:2:0 had the best killing efficacy at 77.02% and 78.94%, respectively, with LC_{50} and LC_{90} values of 0.246-0.348 and 0.230-0.332, respectively. After 2 hours, all four essential oil formulas could kill over 70% of the chicken lice. It was observed that nano essential oil formulas 4:0:0 and 2:2:0 had the highest efficacy in killing chicken lice without significant statistical difference ($P<0.05$), with mortality rates of 86.01% and 90.03%, respectively, and LC_{50} and LC_{90} values of 0.215-0.307 and 0.186-0.280, respectively. Following this, the killing efficacy of the NEOFs 2:1:1 and 2:0:2 was noted. After 3 hours, it was found that NEOFs 4:0:0 and 2:2:0 had the best efficacy in killing chicken lice, with mortality rates exceeding 90%. When comparing the killing rates of these two essential oil formulas, there was no significant statistical difference ($P<0.05$), with LC_{50} and LC_{90} values of 0.174-0.267 and 0.159-0.254, respectively. Finally, after 6 hours, at concentrations of 0.30% and above, the nano essential oil formulas 4:0:0 and 2:2:0 had the best efficacy in killing chicken lice, achieving a mortality rate of 100%, followed by nano essential oil formulas 2:1:1 and 2:0:2, which showed mortality rates of 94.34% and 84.53%, respectively **(Table 4.8 and 4.9)**.

Table 4.8 Effectiveness of the nano essential oil formulas against the shaft louse (*menopon gallinae*) by contact residue exposure method.

Time (hours)	Nano essential oil formulas[1] (CL:CI:TU)	Percentages of mortality (Mean±SD) [2]						%CV
		Concentrations (%)						
		0	0.15	0.20	0.25	0.30	0.35	
1	4:0:0	0±0.0^{Ca}	5.81±5.04^{Ca}	20.45±3.94^{Bc}	77.02±5.58^{Aa}	78.68±6.69^{Aa}	79.80±12.25^{Aa}	15.285
	2:2:0	0±0.0^{Da}	5.59±4.90^{Da}	41.65±7.21^{Ca}	78.94±3.53^{Ba}	74.45±2.79^{Bab}	89.10±5.01^{Aa}	9.309
	2:0:2	0±0.0^{Ea}	5.34±4.64^{Ea}	22.69±2.52^{Dbc}	51.77±11.09^{Cc}	64.96±7.29^{Bb}	80.63±2.44^{Aa}	15.749
	2:1:1	0±0.0^{Da}	0±0.0^{Da}	34.60±10.29^{Cab}	64.16±2.57^{Bb}	70.60±2.37^{Bab}	84.75±5.59^{Aa}	11.774
	%CV	0.000	100.582	22.449	9.681	7.310	8.717	
2	4:0:0	0±0.0^{Da}	15.51±6.28^{Ca}	35.10±7.28^{Bb}	85.86±4.37^{Aa}	86.01±6.16^{Aa}	93.27±5.92^{Aa}	10.540
	2:2:0	0±0.0^{Da}	25.84±6.72^{Ca}	64.20±7.56^{Ba}	88.08±4.14^{Aa}	90.03±2.22^{Aa}	97.22±4.81^{Aa}	8.143
	2:0:2	0±0.0^{Ea}	13.80±8.07^{Da}	31.92±7.57^{Cb}	74.24±1.31^{Bb}	76.75±3.34^{Bb}	92.62±0.86^{Aa}	9.875
	2:1:1	0±0.0^{Fa}	16.03±8.67^{Ea}	39.90±3.06^{Db}	71.67±5.13^{Cb}	82.42±4.79^{Bab}	93.89±41.64^{Aa}	10.277
	%CV	0.000	42.161	15.541	5.014	5.235	4.964	
3	4:0:0	5.16±4.51^{Ea}	30.19±8.25^{Da}	50.47±2.77^{Cbc}	90.95±0.46^{Ba}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	6.378
	2:2:0	5.16±4.51^{Ea}	36.11±6.17^{Da}	67.88±3.21^{Ca}	90.36±0.88^{Ba}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	5.105
	2:0:2	5.16±4.51^{Ea}	22.81±13.08^{Da}	40.30±6.28^{Cc}	75.77±5.89^{Bb}	80.17±5.25^{Bc}	97.66±4.06^{Aa}	8.776
	2:1:1	5.16±4.51^{Fa}	24.97±3.51^{Ea}	52.07±8.30^{Db}	75.57±1.88^{Cb}	89.38±4.86^{Bb}	100.00±0.00^{Aa}	8.004
	%CV	87.367	29.831	10.667	3.766	3.871	2.041	
6	4:0:0	9.39±3.43^{Ca}	56.91±8.89^{Ba}	57.64±6.96^{Ba}	93.87±5.31^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	7.587
	2:2:0	9.39±3.43^{Da}	58.62±9.36^{Ca}	72.28±2.32^{Ba}	96.93±5.31^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	6.462
	2:0:2	9.39±3.43^{Ca}	37.79±27.23^{Ba}	56.42±11.08^{Ba}	81.05±0.97^{Ab}	84.53±1.31^{Ac}	100.00±0.00^{Aa}	19.666
	2:1:1	9.39±3.43^{Da}	41.75±6.80^{Ca}	75.20±13.59^{Ba}	91.48±0.66^{Aa}	94.34±4.90^{Ab}	100.00±0.00^{Aa}	9.715
	%CV	36.499	31.674	14.538	4.185	2.677	0.000	

[1/] Clove (CL), Cinnamon (CI), Turmeric (TU), [2/]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

Table 4.9 The lethal concentration percentage (LC) of the shaft louse (*menopon gallinae*) from nano essential oil formulas (NEOFs).

Time (hours)	Nano essential oil formulas[1] (CL:CI:TU)	Regression equation	Chi-Square	LC_{50} (Lower-Upper)	LC_{90} (Lower-Upper)	SE
1	4:0:0	Y=-3.087+12.569x	45.033	0.246 (0.172-0.309)	0.348 (0.292-0.610)	0.251
	2:2:0	Y=-2.887+12.574x	30.672	0.230 (0.173-0.273)	0.332 (0.285-0.476)	0.243
	2:0:2	Y=-3.122+11.775x	5.712	0.265 (0.255-0.276)	0.374 (0.355-0.399)	0.255
	2:1:1	Y=-3.194+12.800x	26.462	0.250 (0.205-0.292)	0.350 (0.304-0.485)	0.262
2	4:0:0	Y=-3.009+13.979x	21.393	0.215 (0.174-0.247)	0.307 (0.271-0.390)	0.253
	2:2:0	Y=-2.523+13.587x	11.631	0.186 (0.153-0.210)	0.280 (0.252-0.329)	0.237
	2:0:2	Y=-2.937+12.834x	10.832	0.229 (0.203-252)	0.329 (0.298-0.385)	0.244
	2:1:1	Y=-2.848+12.913x	3.425	0.221 (0.210-0.230)	0.320 (0.305-0.338)	0.241
3	4:0:0	Y=-2.380+13.708x	39.473	0.174 (0.090-0.222)	0.267 (0.219-0.421)	0.216
	2:2:0	Y=-2.131+13.421x	15.147	0.159 (0.117-0.188)	0.254 (0.222-0.313)	0.205
	2:0:2	Y=-2.077+10.215x	18.881	0.203 (0.157-0.241)	0.329 (0.283-0.429)	0.178
	2:1:1	Y=-2.147+11.332x	14.744	0.189 (0.150-0.220)	0.303 (0.266-0.372)	0.188
6	4:0:0	Y=-1.559+11.212x	25.760	0.139 (0.068-0.183)	0.253 (0.207-0.353)	0.161
	2:2:0	Y=-1.518+12.006x	11.379	0.126 (0.087-0.156)	0.233 (0.202-0.282)	0.164
	2:0:2	Y=-1.564+9.253x	11.799	0.169 (0.128-0.201)	0.308 (0.268-0.376)	0.151
	2:1:1	Y=-1.557+10.883x	10.265	0.143 (0.106-0.171)	0.261 (0.229-0.310)	0.158

[1/]Clove (CL), Cinnamon (CI), Turmeric (TU)

1 and 2 hours, at a concentration of 0.1%, it was found that nano essential oil formula 4:0:0 had a mortality rate for chicken mites of 50.74%, with LC_{50} and LC_{90} values of 0.099 and 0.132, respectively. The formula 2:2:0 had a lower mortality rate of 10.00%. After 3 hours, nano essential oil formula 4:0:0 showed increased efficacy, with a chicken mite mortality rate of 64.97%. The formula 2:2:0 also exhibited an increased mortality rate for chicken mites, but it remained lower than that of formula 4:0:0, at 27.41%. Finally, at 6 hours, nano essential oil formula 4:0:0 maintained the same mortality rate of 64.97%, whereas nano essential oil formula 2:2:0 showed an increased mortality rate of 31.11%, but still lower than the first formula. It can be observed that as time increased to 2, 3, and 6 hours, the efficacy of the nano essential oil formulas at each concentration varied. For example, at 6 hours, formula 4:0:0 had a Chi-Square value of 22.305, which is quite high, indicating that the results differed significantly from what was expected (**Table 4.10 and 4.11**).

Table 4.10 Effectiveness of the nano essential oil formulas against the chicken mite (*Megninia ginglymura*) by contact residue exposure method.

Time (hours)	Nano essential oil formulas[1] (CL:CI: TU)		Percentage of mortality (Mean±SD)[2] Concentrations (%)				%CV
			0.000	0.025	0.050	0.1	
1	4:0:0		0.0±0.0^{Ba}	0.0±0.0^{Ba}	3.03±5.25^{Ba}	50.74±4.49^{Aa}	25.691
	2:2:0		0.0±0.0^{Aa}	0.0±0.0^{Aa}	2.08±3.61^{Aa}	10.00±10.00^{Ab}	175.963
		%CV	0.000	0.000	176.148	25.522	
2	4:0:0		0.0±0.0^{Ba}	12.22±10.72^{Ba}	15.82±18.63^{Ba}	50.74±4.49^{Aa}	55.739
	2:2:0		0.0±0.0^{Aa}	0.0±0.0^{Aa}	4.17±7.22^{Aa}	10.00±10.00^{Ab}	174.101
		%CV	0.000	123.980	141.360	25.522	
3	4:0:0		0.0±0.0^{Ba}	12.22±10.72^{Ba}	15.82±18.63^{Ba}	64.97±11.18^{Aa}	52.094
	2:2:0		0.0±0.0^{Ba}	16.67±16.67^{ABa}	23.01±12.78^{Aa}	27.41±4.49^{Ab}	64.046
		%CV	0.000	96.987	82.310	18.448	
6	4:0:0		0.0±0.0^{Ca}	32.59±12.24^{Ba}	38.30±11.53^{Ba}	64.97±11.18^{Aa}	29.722
	2:2:0		0.0±0.0^{Ba}	16.67±16.67^{ABa}	23.81±15.71^{Aa}	31.11±1.92^{Ab}	64.194
		%CV	0.000	59.360	44.353	16.700	

[1/] Clove (CL), Cinnamon (CI), Turmeric (TU), [2/]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

Table 4.11 The lethal concentration percentage (LC) of the chicken mite (*Megninia ginglymura*) from nano essential oil formulas (NEOFs).

Time (hours)	Nano essential oil formulas[1] (CL:CI:TU)	Regression equation	Chi-Square	LC_{50} (Lower-Upper)	LC_{90} (Lower-Upper)	SE
1	4:0:0	Y=-3.830+38.683x	0.246	0.099 (0.093-0.106)	0.132 (0.122-0.148)	0.456
	2:2:0	Y=-2.921+16.120x	0.870	0.181 (0.142-0.314)	0.261 (0.194-0.495)	0.393
2	4:0:0	Y=-1.941+19.799x	6.192	0.098 (0.067-0.375)	0.163 (0.110-0.898)	0.163
	2:2:0	Y=-2.801+15.609x	3.008	0.179 (0.141-0.297)	0.262 (0.196-0.469)	0.348
3	4:0:0	Y=-2.085+24.583x	6.835	0.085 (0.058-0.181)	0.137 (0.098-0.401)	0.169
	2:2:0	Y=-1.491+10.408x	14.916	0.143 (-)	0.266 (-)	0.139
6	4:0:0	Y=-1.294+18.157x	22.305	0.071 (-)	0.142 (-)	0.124
	2:2:0	Y=-1.512+11.585x	13.975	0.130 (-)	0.241 (-)	0.139

[1/] Clove (CL), Cinnamon (CI), Turmeric (TU)

The efficacy of nano essential oil formulas (NEOFs) in controlling ectoparasites in poultry is largely dependent on their chemical composition. In the present study, among the various NEOFs tested, only two—NEOF-1 and NEOF-2, demonstrated significantly high efficacy at a concentration of 0.25% under laboratory conditions. These two formulations were primarily composed of clove and cinnamon essential oils, which are known for their potent bioactive properties. These findings agree with previous work by Pumnuan *et al.* (2020), who reported that clove essential oil, either alone or in combination with cinnamon or turmeric oils, may serve as an effective botanical alternative for controlling poultry lice infestations on farms. Specifically, clove oil and its combination with cinnamon oil proved more effective in the control of *Menacanthus ginglymura* and *Menopon gallinae* than combinations involving turmeric oil. This suggests that the synergistic effects of specific essential oil components may enhance antiparasitic activity. The inferior performance of single-source essential oils compared to blended formulations indicates that combinations of multiple essential oils may yield more potent insecticidal effects. Although

clove essential oil has demonstrated higher insecticidal activity than cinnamon essential oil against several insect pests, including the bean weevil (*Acanthoscelides obtectus*) (Jumbo *et al.*, 2014), cowpea weevil (*Callosobruchus maculatus*) (Jumbo *et al.*, 2018), thrips, mealybugs (Pumnuan and Insung, 2016), and maize weevil (*Sitophilus zeamais*) (Gonzales Correa *et al.*, 2015), its pesticidal potential appears to diminish under combination conditions. Numerous studies support this observation, highlighting the superior pesticidal and antimicrobial efficacy of essential oil blends over individual oils. For instance, synergistic interactions among essential oil components have been shown to enhance antimicrobial activity (Sukatta *et al.*, 2008; Goñi *et al.*, 2009) and insecticidal potency (Benelli *et al.*, 2017; Ríos *et al.*, 2017). These synergistic effects are often attributed to the complex interactions among the constituent compounds, which may affect multiple physiological targets in the parasites, thereby improving overall effectiveness and reducing the likelihood of resistance development.

4.3.2 Repellent test

In the test of the efficacy of nano essential oil formulas in repelling shaft lice (*Menopon gallinae*), it was found that formula 4:0:0 at a concentration of 0.25% had the highest efficacy at 1 hour, with a repellent index percentage of 96.67% (X^2=16.7045, P=0.00004). However, as time increased, its efficacy in repelling decreased to 83.33% (X^2=7.5000, P=0.00617) and 80.00% (X^2=5.9341, P=0.01485), respectively. There were statistically significant differences when comparing the repelling and attracting efficacy of the nano essential oil formulas at all concentrations. Following this, nano essential oil formula 2:2:0 at a concentration of 0.25% had a repelling efficacy of 93.33% (X^2=13.8714, P=0.00019). At 3 and 6 hours, the repellent indices were 80.00% (X^2=5.9341, P=0.01485) and 86.67% (X^2=9.3196, P=0.00226), respectively. There were also statistically significant differences when comparing the repelling and attracting efficacy of the nano essential oil formulas at all concentrations **(Table 4.12)**.

In the efficacy test of nano essential oil formulas for repelling chicken mites (*Megninia ginglymura*), it was found that at a concentration of 0.025%, the nano-emulsion formula 4:0:0 showed the best efficacy at 1 hour, with a repellency index percentage of 83.33% (X^2=7.5000, P=0.00617) and a statistically significant difference at a confidence level of 0.01 when comparing repellency and attraction efficacy. At 3 and 6 hours, the repellency index

percentages were 70.00% and 76.67%, respectively. The next best formula was 2:2:0 at the same concentration, with a repellency index percentage of 63.33 (X^2=1.0860, P=0.29736) and no statistically significant difference when comparing repellency and attraction efficacy. Following this, at 3 and 6 hours, the repellency index percentages were 70.00% and 63.33%, respectively. Furthermore, at a concentration level of 0.15% at 1, 3, and 6 hours, the best formula was 2:2:0, with repellency index percentages of 76.67%, 60.00%, and 73.33%, respectively. The next best was formula 4:0:0, with repellency index percentages of 46.67%, 70.00%, and 73.33%, respectively. Finally, at a concentration level of 0.25% at 1, 3, and 6 hours, the best formula was 4:0:0, with repellency index percentages of 70.00%, 70.00%, and 66.67%, respectively, followed by formula 2:2:0, with repellency index percentages of 66.67%, 66.67%, and 73.33%, respectively **(Table 4.13)**.

The present study demonstrated that the nano-formulated essential oil (NEOF) treatments exhibited higher efficacy against *Megninia ginglymura* compared to *Menopon gallinae*. This differential effectiveness may be attributed to differences in their morphological characteristics; *M. ginglymura* is a smaller mite species (approximately 0.5 mm in length), whereas *M. gallinae* is a larger louse species (approximately 2.0 mm in length). Nonetheless, the NEOFs, while more potent against *M. ginglymura*, were also capable of effectively killing *M. gallinae*, suggesting a broad-spectrum insecticidal potential. Conversely, the repellent efficacy of the tested formulations was found to be greater against *M. gallinae* than *M. ginglymura*. This could be due to behavioral differences between the two ectoparasites; *M. gallinae* exhibits higher mobility, which may increase its sensitivity to repellent stimuli. Among the tested formulations, NEOF-1 and NEOF-2 demonstrated superior repellent performance. These formulations primarily contained clove and cinnamon essential oils, both of which have been reported to possess eugenol as a major bioactive constituent. Eugenol is a well-documented bioactive compound known for its insect-repellent properties. De Jorge *et al.* (2022) confirmed its effectiveness as a repellent against pear psyllids, while Abenaim *et al.* (2022) highlighted its potential as a viable repellent agent for managing a wide range of insect pests. Prior studies have supported eugenol's repellency against pests such as the maize weevil (*Sitophilus zeamais*), red flour beetle (*Tribolium castaneum*), saw-toothed grain beetle (*Oryzaephilus surinamensis*), and various seed-boring insects (Ogendo *et al.*, 2008; Al-Harbi *et al.*, 2021). In summary, the differential response of *M. ginglymura* and *M. gallinae* to NEOFs underscores the importance of considering both the biological and behavioral characteristics of target ectoparasites. The

presence of eugenol-rich essential oils in NEOF-1 and NEOF-2 may play a crucial role in their dual insecticidal and repellent activity, making these formulations promising candidates for integrated ectoparasite management in poultry.

Table 4.12 Percentage response (repellency and attraction) of the shaft louse (*Menopon gallinae*) to different nano essential oil formulas (NEOFs) by contact method after various exposure times.

Time (hours)	Nano essential oil formulas[1] (CL:CI: TU)	Concentration	Response		Chi-Square(X^2)	P value
			Repellency	Attraction		
			%	%		
1	4:0:0	0.025	83.33	16.67	7.5000	0.00617**
		0.15	60.00	40.00	0.6061	0.43627ns
		0.25	96.67	3.33	16.7045	0.00004**
	2:2:0	0.025	26.67	73.33	3.4548	0.06307ns
		0.15	90.00	10.00	11.4286	0.00072**
		0.25	93.33	6.677	13.8714	0.00019**
3	4:0:0	0.025	80.00	20.00	5.9341	0.01485*
		0.15	63.33	36.67	1.0860	0.29736ns
		0.25	83.33	16.67	7.5000	0.00617**
	2:2:0	0.025	23.33	76.67	4.5933	0.03209*
		0.15	83.33	16.67	7.5000	0.00617**
		0.25	80.00	20.00	5.9341	0.01485*
6	4:0:0	0.025	83.33	16.67	7.5000	0.00617**
		0.15	60.00	40.00	0.6061	0.43627ns
		0.25	80.00	20.00	5.9341	0.01485*
	2:2:0	0.025	26.67	73.33	3.4548	0.06307ns
		0.15	86.67	13.33	9.3196	0.00226**
		0.25	86.67	13.33	9.3196	0.00226**

[1/] Clove (CL), Cinnamon (CI), Turmeric (TU)*, ** Significant difference at P< 0.05 and P< 0.01, respectively ; ns = nonsignificant difference.

Table 4.13 Percentage response (repellency and attraction) of the chicken mite (*Megninia ginglymura*) to different nano essential oil formulas (NEOFs) by contact method after various exposure times.

Time	Nano essential oil formulas[1] (CL:CI: TU)	Concentration	Response		Chi-Square(X^2)	P value
			Repellency	Attraction		
			%	%		
1	4:0:0	0.025	83.33	16.67	7.5000	0.00617**
		0.15	46.67	53.33	0.0667	0.796143ns
		0.25	70.00	30.00	2.5000	0.113846ns
	2:2:0	0.025	63.33	36.67	1.0860	0.297365ns
		0.15	76.67	23.33	4.5933	0.032097*
		0.25	66.67	33.33	1.7143	0.19043ns
3	4:0:0	0.025	70.00	30.00	2.5000	0.113846ns
		0.15	70.00	30.00	2.5000	0.113846ns
		0.25	70.00	30.00	2.5000	0.113846ns
	2:2:0	0.025	70.00	30.00	2.5000	0.113846ns
		0.15	60.00	40.00	0.6061	0.436275ns
		0.25	66.67	33.33	1.7143	0.19043ns
6	4:0:0	0.025	76.67	23.33	4.5933	0.032097*
		0.15	73.33	26.67	3.4548	0.06307ns
		0.25	66.67	33.33	1.7143	0.19043ns
	2:2:0	0.025	63.33	36.67	1.0860	0.297365ns
		0.15	73.33	26.67	3.4548	0.06307ns
		0.25	73.33	26.67	3.4548	0.06307ns

[1/] Clove (CL), Cinnamon (CI), Turmeric (TU)*, ** Significant difference at P< 0.05 and P< 0.01, respectively ; ns = nonsignificant difference.

4.4 The effectiveness of nano essential oil formulas against chicken ectoparasites in a laying hens farm conditions.

In the efficacy test of nano essential oil formulas (NEOFs) against ectoparasites of chickens under experimental farm conditions, it was found that all types of nano essential oil formulas were effective in controlling ectoparasites of chickens in both laying hens in a cage and hens reared in cage. In the first trial, both of NEOFs was able to control up to 73.5-86.0% of parasites in the farm setting. After the first trial, only 14.0-26.5% of parasites remained. The second trial showed an efficacy of up to 95.6-98.9% in eliminating ectoparasites of chickens, there was no statistically significant difference. In the blank group (plain water), the number of parasites continuously increased by as much as 3-5 times compared to the initial count before the trial. In the control group (cypermethrin), the number of ectoparasites significantly decreased on the 3rd day after the first trial. Finally, in the surfactant test group, 85.6% control of ectoparasites was observed in caged laying hens, even though the number of ectoparasites increased after the application of the test substance **(Table 4.14)**. Furthermore, when surveying the percentage prevalence of ectoparasites on laying hens both before and after treatment with NEOFs, as shown in **Figures 4.2 and 4.3**, the survey results revealed the detection of 11 species of ectoparasites infesting the chickens before treatment. The most prevalent species were the mites *Megninia ginglymura* and *Pterolichus obtusus*, with counts of 326.7 and 113.5 mites per sampling unit, respectively. The louse, *Lipeurus caponis* and *Menopon gallinae* were also found in high numbers, with 1,418.3 and 323.4 mites per sampling unit, respectively. NEOFs (NEOF-1 (4:0:0) and NEOF-2 (2:2:0)) demonstrated effective prevention and elimination of ectoparasites on chickens, completely eradicating *M. ginglymura*, *Megninia ortari, Ormithonyssus bursa*, *Ornithonyssus sylviarum, Cuclotogaster heterogoraphus, Goniocotes gallinae, Menacanthus stramineus, and Menacanthus pallidulus.* Additionally, they were found to control the parasites *P. obtusus, L. caponis, and M. gallinae* by more than 98% 14 days after application.

The application of medicinal plants in the control of insect and mite pests has demonstrated considerable potential, particularly using essential oils (EOs) derived from clove and cinnamon. Numerous studies have confirmed the broad-spectrum efficacy of these EOs against pests affecting agricultural crops (Pumnuan and Insung, 2016), stored products (Jumbo *et al.*, 2014), public health vectors (Saad *et al.*, 2006; Sanga *et al.*, 2023), companion animals (Ellse and Wall, 2014), and livestock (Pumnuan *et al.*, 2020; Abbas *et al.*, 2018; Lee *et al.*, 2019). Moreover, these EOs have exhibited parasiticidal activity against poultry ectoparasites when formulated as nano essential oil formulas (NEOFs) under controlled laboratory conditions (Lakyat *et al.*, 2024). The present study builds upon this body of evidence by validating their efficacy in farm conditions. Our findings indicate that NEOFs are effective in managing ectoparasites in laying hen farms, although their efficacy is somewhat lower than that of conventional chemical pesticides. Following the initial application, NEOFs achieved over 75% ectoparasite reduction by day 3, whereas cypermethrin—a commonly used synthetic pesticide—provided over 95% control within the same period. This disparity highlights a limitation associated with EO-based treatments, primarily due to the high volatility of EOs, which contributes to their relatively short duration of parasiticidal activity (Ellse and Wall, 2014). However, the inclusion of surfactants in the formulation appears to address this limitation to some extent by reducing the evaporation rate of EOs and enhancing their bioavailability and dispersion through improved surface wetting properties (Cimino *et al.*, 2021). The present study supports this assertion, demonstrating that surfactants modestly improved the parasiticidal efficacy of NEOFs, in agreement with previous research indicating that certain surfactants possess intrinsic insecticidal activity (Liu and Stansly, 2000).

Table 4.14 The ectoparasites found on laying hens in KMITL farm.

Treatment	The percentage of all ectoparasites found in chicken parts was randomly surveyed before and after using the substance											
	Weeks after using the substance											
					1	2	6				7	8
	Before using the substance (1st trial)	Days after using the substance					Before using the substance (2nd trial)	Days after using the substance				
	0	1	3	5	7	14	0	1	3	5	7	14
Laying hens in a cage												
NEOF-1 (4:0:0)	100.0	59.7	22.8	19.7	25.2	17.6	52.7	13.0	3.6	3.6	1.1	2.8
NEOF-2 (2:2:0)	100.0	32.3	22.8	29.0	19.5	26.5	47.0	27.8	4.2	7.1	4.8	4.4
Cypermethrin	100.0	19.9	3.9	3.3	1.5	0.8	3.5	0.7	0.5	0.6	0.0	0.0
Surfactant (Control)	100.0	57.3	35.5	46.1	32.0	41.1	88.5	59.2	37.7	16.3	16.8	14.4
Blank	100.0	101.3	121.5	95.7	121.7	137.6	1174.8	588.0	684.7	604.5	696.5	505.0
Hens reared in cage												
NEOF-1 (4:0:0)	100.0	54.6	19.1	14.8	17.6	14.2	44.0	20.3	1.8	2.3	1.5	2.5
NEOF-2 (2:2:0)	100.0	20.7	14.5	18.6	12.8	14.0	30.0	14.7	1.1	2.3	0.5	1.1
Cypermethrin	100.0	15.1	3.3	2.2	6.3	0.5	6.0	0.4	0.2	0.2	0.0	0.2
Surfactant (Control)	100.0	45.2	33.6	41.9	29.1	37.5	97.8	98.1	53.7	145.7	185.9	142.3
Blank	100.0	84.6	87.8	87.7	105.3	127.1	545.4	447.9	227.9	241.1	141.5	331.8

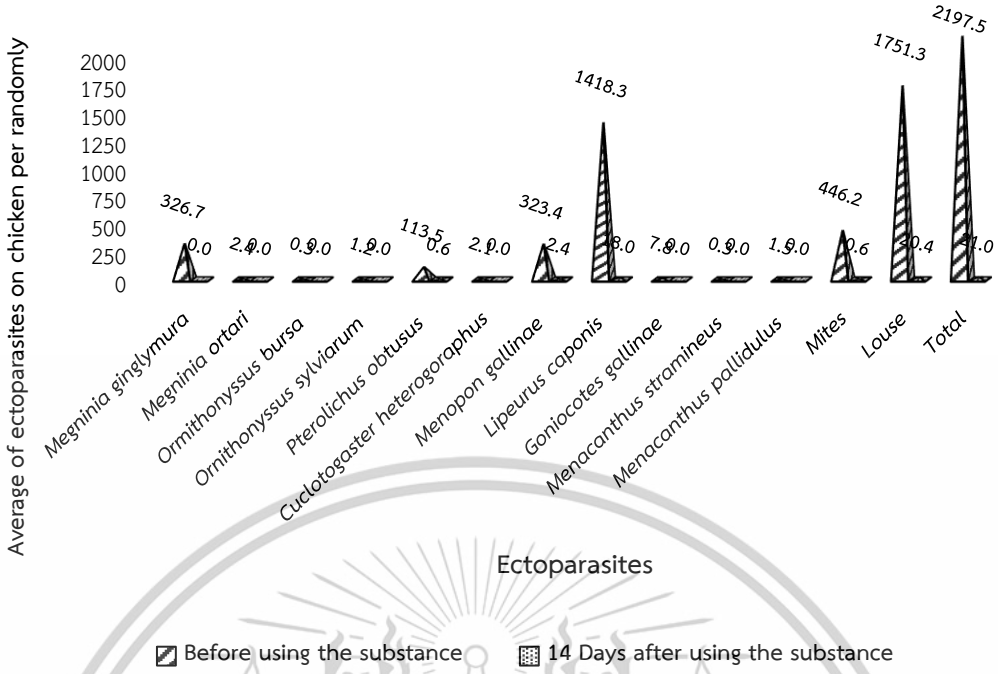

Figure 4.2 The prevalence of chicken ectoparasites (mites and lice) per random set on chicken body before treatment and 14 days after the second treatment with nano essential oil formulas (NEOFs) in egg-laying chicken farm conditions.

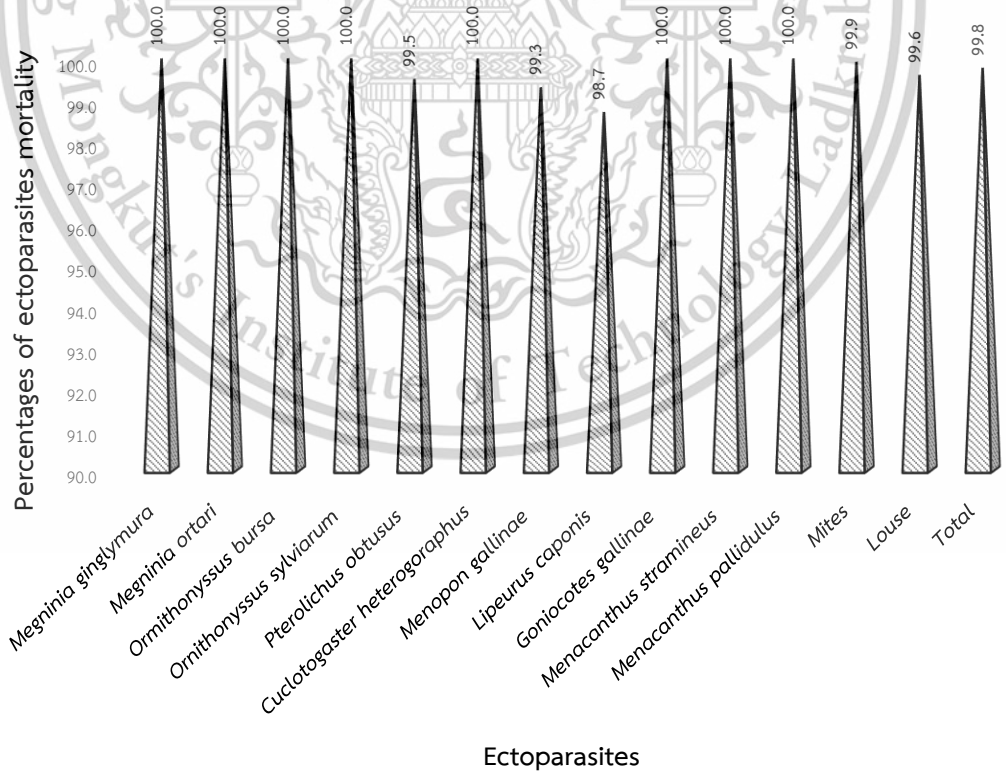

Figure 4.3 Percentages of chicken ectoparasite mortality with nano essential oil formulas (NEOFs) in egg-laying chicken farm conditions.

4.5 The results regarding the quality of eggs were obtained after testing the effectiveness of nano essential oil formulas against chicken ectoparasites in a laying hens farm conditions.

This experiment demonstrated that both nano essential oil formulas (NEOFs), namely NEOF-1 (4:0:0) and NEOF-2 (2:2:0), are effective in controlling ectoparasites in laying hens. Furthermore, their impact on egg production and egg quality was either beneficial or comparable to the group treated with the chemical agent cypermethrin. Specifically, the percentage of hen-day production and egg mass, laying hens in a cage treated with NEOF-2 exhibited the highest percentage of hen-day production at various measurement points, such as 68.71% in the second week of the first cycle. This value was superior to both the control group and the cypermethrin-treated group. NEOF-1 also demonstrated positive effects, particularly with egg mass values that were similar to or even exceeded those of the cypermethrin group. Conversely, the blank group showed a significant decline in %hen-day production (e.g., 52.14% in the second week of the first cycle and 47.41% in the second cycle). This highlights the detrimental impact of ectoparasite infestation on the productivity of hens reared in cages. The average %hen-day production in the NEOFs-treated and cypermethrin-treated groups was comparable and significantly better than that of the blank group. In the second cycle, the NEOF-2 group achieved a %hen-day production as high as 53.17%, while the blank group declined to only 43.23%, indicating the potential of NEOFs to mitigate the adverse effects of parasites. Regarding egg quality (Egg Weight and Egg Mass), egg weight tended to remain stable across all experimental groups, with no statistically significant differences observed. This suggests that NEOFs do not directly affect egg size. The egg mass of the NEOF-2 group showed a tendency to be high at various experimental stages, such as 33.18 g in the second week of the first cycle in laying hens in a cage. This value was similar to that of the chemically treated group and higher than the control group. The Feed conversion ratio (FCR) for the NEOFs-treated groups was favorable, particularly for NEOF-1, which had an FCR of 3.00 in the first cycle (week 2), lower than that of cypermethrin (3.12). This indicates efficient feed utilization. In contrast, the blank group exhibited the highest FCR values

(e.g., 4.18 in the second week of the first cycle and 7.20 in the second cycle hens reared in cage) **(Table 4.15 and 4.16)**, demonstrating inefficient feed utilization in the absence of effective parasite control. The comparison between NEOFs and cypermethrin revealed that both NEOFs effectively competed with cypermethrin in terms of efficacy, yielding comparable results across all key parameters and, in some instances, demonstrating superior outcomes. A key advantage of NEOFs is their natural origin, ensuring safety for consumers and animals and eliminating the risk of chemical residues.

The presence of ectoparasites in egg-laying chicken farms has a significant impact on egg production and overall farm productivity. Our study demonstrated that a 3 - 5-fold increase in ectoparasite populations was associated with a notable decline in the percentage of Hen-day egg production (%Hen-day) and a significant rise in the feed conversion ratio (FCR) compared to treated groups. Extremely ectoparasite infestations can lead to various health issues in hens, including reduced egg production, increased mortality rates, and the onset of anemia, all of which negatively affect the economic productivity of the egg industry (Flochlay *et al.*, 2017; Bennett *et al.*, 2011). Higher ectoparasite burdens are also known to induce considerable stress in poultry, resulting in pain, skin irritation, feather-pecking, and cannibalistic behaviors. These stress-related responses further contribute to increased feed and water consumption, a decline in egg production and egg quality, and overall deterioration in animal health and welfare (Flochlay *et al.*, 2017; Hiluf *et al.*, 2018; Tucci *et al.*, 2014). The findings of this study reaffirm that ectoparasite infestations in laying hens have a critical impact on both health and productivity. Although chronic infestations may not cause immediate mortality, the cumulative economic losses incurred from reduced performance are substantial over time (Sangvaranond, 2003). Previous studies have estimated that ectoparasite infestations can lead to a 20–30% reduction in poultry productivity (Tucci *et al.*, 2014; Tucci *et al.,* 2005; Soares *et al.*, 2016), highlighting the necessity of effective ectoparasite control measures in poultry farming operations.

Table 4.15 Egg-laying performance of chickens and quality of eggs after using the nano essential oil formulas (NEOFs) to controlling poultry ectoparasites in farm conditions (laying hens in a cage and hens reared in a cages) compared with cypermethrin insecticide application. (1st trial).

Treatments[1]	After treated 1st within 1 weeks				After treated 1st within 2 weeks			
	%Hen day	Egg weight (g)	Egg mass (g)	FCR / Egg mass	%Hen day	Egg weight (g)	Egg mass (g)	FCR / Egg mass
Laying hens in a cage								
NEOF-1 (4:0:0)	61.95[a]	50.68[a]	31.12[a]	3.23[a]	67.43[a]	49.91[a]	33.71[a]	3.00[b]
NEOF-2 (2:2:0)	63.71[a]	49.13[a]	31.28[a]	3.12[a]	68.71[a]	48.51[a]	33.18[a]	3.15[b]
Cypermethrin	61.71[a]	48.06[a]	29.70[a]	3.31[a]	69.14[a]	48.40[a]	33.46[a]	3.12[b]
Control	64.81[a]	48.17[a]	31.21[a]	3.28[a]	64.14[a]	50.79[a]	32.55[a]	3.32[b]
Blank	60.00[a]	50.20[a]	30.09[a]	3.44[a]	52.14[b]	49.00[a]	25.62[a]	4.18[a]
Hens reared in cage								
NEOF-1 (4:0:0)	53.57[a]	51.15[a]	27.41[a]	5.86[a]	54.28[a]	48.22[a]	26.20[a]	5.59[a]
NEOF-2 (2:2:0)	57.14[a]	50.49[a]	28.94[a]	5.85[a]	52.29[a]	49.04[a]	25.67[a]	5.49[a]
Cypermethrin	52.86[a]	47.22[a]	24.95[a]	6.00[a]	52.14[a]	48.68[a]	25.39[a]	5.22[a]
Control	55.00[a]	49.90[a]	27.33[a]	6.96[a]	50.00[a]	47.18[a]	23.52[a]	6.63[a]
Blank	51.07[a]	46.23[a]	23.74[a]	7.40[a]	47.86[a]	44.81[a]	21.64[a]	7.20[a]

[a,b] Significantly different at $p < 0.05$.

Table 4.16 Egg-laying performance of chickens and quality of eggs after using the nano essential oil formulas (NEOFs) to controlling poultry ectoparasites in farm conditions (laying hens in a cage and hens reared in a cages) compared with cypermethrin insecticide application. (2nd trial).

Treatments[1]	After treated 2nd within 1 weeks				After treated 2nd within 2 weeks			
	%Hen day	Egg weight (g)	Egg mass (g)	FCR / Egg mass	%Hen day	Egg weight (g)	Egg mass (g)	FCR / Egg mass
Laying hens in a cage								
NEOF-1 (4:0:0)	56.00[a]	47.88[a]	26.81[ab]	2.67[b]	51.81[a]	49.94[a]	25.75[a]	3.85[a]
NEOF-2 (2:2:0)	57.74[a]	49.67[a]	28.67[a]	2.69[b]	59.17[a]	47.90[a]	28.24[a]	3.50[a]
Cypermethrin	59.52[a]	49.68[a]	29.48[a]	2.63[b]	64.52[a]	49.10[a]	31.68[a]	2.85[a]
Control	56.41[a]	49.91[a]	28.17[a]	3.16[ab]	61.19[a]	50.76[a]	31.18[a]	3.28[a]
Blank	47.41[b]	48.44[a]	22.96[b]	3.60[a]	68.14[a]	47.79[a]	32.50[a]	3.30[a]
Hens reared in cage								
NEOF-1 (4:0:0)	49.57[a]	48.50[a]	24.06[a]	3.48[b]	29.30[a]	50.79[a]	14.95[a]	7.64[a]
NEOF-2 (2:2:0)	49.93[a]	47.83[a]	23.89[a]	3.60[b]	53.17[a]	46.58[ab]	24.82[a]	4.47[a]
Cypermethrin	47.95[a]	49.23[a]	23.61[a]	3.65[b]	52.38[a]	45.03[b]	23.60[a]	4.32[a]
Control	47.13[a]	48.65[a]	22.93[a]	3.86[b]	37.93[a]	43.63[b]	16.42[a]	6.73[a]
Blank	36.67[b]	45.17[a]	16.60[b]	4.66[a]	43.23[a]	45.13[b]	19.62[a]	5.69[a]

[a,b] Significantly different at $p < 0.05$.

4.6 The results obtained regarding toxic residues in eggs after conducting the study comparing nano essential oil formulas and cypermethrin insecticides in controlling chicken ectoparasites.

The experimental results indicated that the cypermethrin residue was non-detectable (ND <0.002 ppm) in all samples from the nano essential oil formulas (NEOFs) treated groups, encompassing both laying hens in a cage and hens reared in cage treated with NEOF-1 and NEOF-2. This finding underscores the safety of consuming eggs following short-term and long-term application of NEOFs. In contrast, the cypermethrin-treated group exhibited detectable residues in chicken eggs from day 3 to day 14 post-application. Laying hens in a cage, the highest residue level detected was 0.191 ppm (day 7 post-application in the second cycle), while hens reared in cage, the maximum residue detected was 0.117 ppm. Finally, the control and blank groups showed no detectable residues (ND) across all measurement time points **(Table 4.17)**.

Consumer safety and product quality are fundamental priorities in agricultural practices. Despite the growing emphasis on organic farming, the presence of chemical pesticide residues in agricultural products remains a significant concern (Marangi *et al.*, 2012; Hildmann *et al.*, 2015; Hamasalim *et al.*, 2023; Túri *et al.*, 2000; Abdelfatah and Abu-Zeid, 2016; Hamid *et al.*, 2017). Numerous studies have demonstrated that residues from livestock chemicals can persist and ultimately reach consumers. For instance, Dallegrave *et al.* (2018) investigated pesticide residues in meat, milk, and eggs, finding that cypermethrin was the most prevalent insecticide detected. It was present in all samples of beef and fish, 98% of chicken samples, 92% of milk samples, and 52% of egg samples. This high incidence is alarming, as cypermethrin exposure has been associated with liver and kidney damage in consumers and has also been reported to induce genetic alterations in rabbits following chronic low-dose exposure (Lestremau *et al.*, 2014). Our study further demonstrated that the use of chemical pesticides in egg production can result in residues persisting for over eight weeks

post-treatment. Although the detected residue levels were relatively low, continuous exposure may lead to bioaccumulation in human tissues, potentially causing cytotoxic disorders, immunotoxicity, hormonal imbalances, and even carcinogenesis. Additionally, pesticide exposure poses considerable health risks to farm workers who are involved in the handling and application of these chemicals (Pedroso *et al.*, 2022).

Table 4.17 The insecticide residue in egg after using the nano essential oil formulas (NEOFs) to controlling chicken ectoparasites in farm conditions (laying hens in a cage and hens reared in a cages) compared with cypermethrin insecticide application.

Treatments[1]	The percentage of all ectoparasites found in chicken parts was randomly surveyed before and after using the substance											
	Weeks after using the substance											
					1	2					7	8
	Before using the substance (1^{st} time)	Days after using the substance					Before using the substance (2^{nd} time)	Days after using the substance				
	0	1	3	5	7	14	0	1	3	5	7	14
Laying hens in a cage												
NEOF-1 (4:0:0)	ND[2]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
NEOF-2 (2:2:0)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cypermethrin	ND	ND	0.048	0.101	0.123	0.104	0.029	0.030	0.102	0.173	0.191	0.153
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Hens reared in cage												
NEOF-1 (4:0:0)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
NEOF-2 (2:2:0)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cypermethrin	ND	ND	0.060	0.085	0.106	0.061	0.026	0.022	0.062	0.101	0.117	0.073
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[1] Control (Tween60 : PEG400 = 9:2), Cypermethrin insecticide 35%EC (recommended dose; 0.1%), NEOF-1 (NEO-CL : NEO-CI = 4:0), NEOF-2 (NEO-CL : NEO-CI = 2:2), NEO-CL (clove : Tween60 : PEG400 = 2:9:2), NEO-CI (cinnamon : Tween60 : PEG400 = 2:9:2).

[2] ND = Not detected (<0.002 ppm)

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions

The survey identified eight ectoparasite species infesting chickens. The feather mite, *Megninia cubitalis* was most common in caged laying hens. Other mites and louse showed varying prevalence across different chicken breeds and body locations. Statistical analysis of prevalence on feathers versus skin showed no significant differences for most species, except for the louse *Lipeurus caponis*, which was more common on feathers.

The study analyzed the chemical composition of essential oils (EOs) from clove, cinnamon, and turmeric using GC-MS. Eugenol was the dominant compound in clove (84.60%) and cinnamon (72.00%) EOs, while ar-turmerone was the major component in turmeric EO (53.38%). Minor compounds were also identified for each EO. Unformulated nano-emulsions of clove, cinnamon, and turmeric EOs, as well as nanoemulsions of main compound standards (NCSs) of eugenol and turmerone, exhibited particle sizes ranging from 17.15 to 20.80 nm. Three specific nano essential oil formulas (NEOF-1, NEOF-2, and NEOF-3) with varying ratios of clove (CL), cinnamon (CI), and turmeric (TU) nano-emulsions showed particle sizes below 100.0 nm (20.76 nm, 20.66 nm, and 78.22 nm, respectively). However, NEOF-4 (NEO-CL : NEO-CI : NEO-TU at 2:1:1) had a particle size exceeding 100 nm.

Nano essential oil formulas, 4:0:0 and 2:2:0 demonstrated optimal insecticidal efficacy across all evaluated time intervals, with no statistically significant difference observed in their killing efficacy ($P < 0.05$). Consequently, the data suggests that nano essential oil formulas are efficacious in the management of *Menopon gallinae* infestations in poultry, particularly under conditions of elevated concentration and prolonged exposure duration.

In the evaluation of the efficacy of the two nano essential oil formulas against *Megninia gingglymura*, NEOF-1 (4:0:0) exhibited superior acaricidal activity compared to NEOF-2 (2:2:0), notably with increasing concentrations of the active substance and extended experimental periods. This finding has implications for the control of mite infestations in poultry, a factor of considerable importance for poultry health and current egg production.

The evaluation of the repellent efficacy of the two nano essential oil formulas against shaft louse (*Menopon gallinae*) revealed that NEOF-1 (4:0:0) at a concentration of 0.25% demonstrated the highest repellent activity at the 1 hour time point. However, this repellent efficacy diminished with increasing time. This observation contrasts with NEOF-2 (2:0:0) at a concentration of 0.25%, which displayed an inverse relationship in repellent efficacy across the experimental time course.

Based on the assessment of the repellent efficacy of the two nano essential oil formulas to chicken mite (*Megninia gingglymura)*, NEOF-1 (4:0:0) at a concentration of 0.025% exhibited the most pronounced repellent efficacy across all evaluated time intervals.

The nano essential oil formulas, NEOF-1 (4:0:0) and NEOF-2 (2:2:0) demonstrated efficacy in controlling ectoparasites of laying hens without exhibiting adverse effects on egg quality and production when compared to the chemical agent cypermethrin. These findings suggest their suitability for development as alternative products in laying hen farms to promote future sustainability.

The nano essential oil formulas, NEOF-1 (4:0:0) and NEOF-2 (2:2:0) can be utilized for the control of ectoparasites in laying hens without inducing detectable residues in eggs. Conversely, the application of cypermethrin continues to pose a risk of residues at detectable levels. Consequently, NEOFs represent a safe and efficacious approach that can be confidently implemented in organic or chemical-free farming systems.

5.2 Suggestions

1. Selection of nano essential oil formulas: It is recommended to utilize nano essential oil formulas (NEOFs) with high efficacy against multiple ectoparasite species, such as NEOF-1 and NEOF-2, to ensure comprehensive parasitic control.

2. Formulation optimization based on composition: Formulas with high concentrations of eugenol (e.g., NEOF-1 (4:0:0) and NEOF-2 (2:2:0)) are particularly suitable for ectoparasite management due to their potent bioactivity. In contrast, formulations incorporating turmeric essential oil should be optimized to reduce particle size, as NEOF-3 (2:0:2) and NEOF-4 (2:1:1) exhibited significantly larger particles, which may affect bioavailability and stability.

3. Contact residue exposure testing recommendations: Based on the results of the contact residue exposure assays, NEOF-1 (4:0:0) and NEOF-2 (2:2:0) are recommended for controlling external parasites in chickens. Application should be at the minimum effective concentration to ensure both efficacy and safety.

4. Repellency testing recommendations: For repellency efficacy, a minimum concentration of 0.15–0.25% is advised to effectively repel chicken ectoparasites, particularly within the initial hours post-application.

5. Field application in poultry Farms: Under commercial laying hen farm conditions, the use of NEOF-1 (4:0:0) or NEOF-2 (2:2:0) is recommended as a substitute for cypermethrin. Spraying intervals of every 7–14 days are suggested to maintain parasite control while reducing the risk of toxic residues in eggs.

6. Impact on egg quality: The use of NEOFs has shown no adverse effects on egg quality parameters, indicating that these formulations are suitable for commercial applications without compromising productivity.

7. Egg residue analysis: Given the absence of detectable residues in eggs treated with NEOFs, these formulations are strongly recommended over chemical insecticides such as cypermethrin to minimize the risk of toxic residues entering the food chain.

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APPENDIX

APPENDIX A

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Ectoparasite species attacking chicken in eastern area of Bangkok, Thailand

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Abstract Ectoparasites of chickens are important for poultry farming and other types of poultry rearing by ways of causing nuisance and being as transmitter of various diseases to poultry resulting in poor-quality and quantity products. From the directly survey result, it was found that *Megninia cubitalis* mostly attacked laying hens reared in a cage at neck, wing, chest, back and buttock with the average number of 52.1- 204.9 mites per chicken, and followed by injured on Rhode Island red with 85.0 mites, abundantly at buttock. And the louse, *Lipeurus caponis* was found in Polish chicken with 100.5 insects that mostly appeared at back of chicken, and followed by the appearance in Rhode Island red with 70.0 insects, abundantly at chest. This insect preferred to live at wing and chest. Whereas, result from suction method showed that *Megninia cubitalis* was also found in laying hen reared in a cage with the average number of 71.4-168.3 mites per chicken. It lives everywhere on the body as neck, head and buttock. Besides, *Cuclotogaster heterographus* was observed on the body area equal to 43.8 insects, where the louse, *Menopon gallinae* was monitored in Polish chickens with totally, 22.5 insects, plenty at the buttock area.

Keywords: Ectoparasites, Chicken, Mites, Louse

Introduction

Thailand has situated in mainland Southeast Asia, the climate is subtropical with relatively high temperatures (24–36 °C) and high humidity (66–83 %) and nearly 18.2 million households, mostly smallholders, are in rural areas. Most of them traditionally possess indigenous chickens (Choprakarn, 2007, United Nations, 2021). In 2020, Bangkok had 126.988 chickens based on total chicken data in Thailand, albeit a few numbers compared to the total number of chickens in the country. Most of the chickens raised in Bangkok were beautiful chickens, in which kept them for competitions or kept them as pets rather than industrial farming (Department of Livestock Development, 2020). A household kept one cock with three to five hens to form a throng,

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annually. This helps to maintain picking with the beak order and relieves fighting in the throng. In a year, such throngs can produce up to 90–150-day-old chicks, equivalent to 30–75 merchantable birds of 1.0-1.5 kg body weight at four to five months of age (Choprakarn, 1983; Choprakarn *et al.,* 1998; Laopaiboon and Jitpraneechai, 1999; Namdaeng, 1991). The number of chickens per household varies extremely relating to the time of the year and the ability of the farmers. From October to February (cold and dried), the number of day-old chicks running around is at its largest, and the chicks' growth rate is also uplifting. This is because of the upper hatching estimate, and the availability of plenty of natural feeds and plant by-products. However, the numbers tend to decrease from March to September (sizzling and moist) due to a down hatching rate, a famine of natural feeds, local diseases, and endo and ectoparasites (Choprakarn *et al.,* 1998; Klinhom *et al.,* 2005; Laopaiboon and Jitpraneechai, 1999).

Ectoparasites, even with their harmful effects, are often disregarded. Some of the ectoparasites ordinary in poultry are ticks, fleas, louse and mites (Amede *et al.,* 2011; Ikpeze *et al.,* 2008). The occurrence of decease and morbidity due to various ectoparasitic ailments in chicken demands serious attempts to keep down the ailments. However, notwithstanding their ravaging impacts, ectoparasites receive hardly more attention than endoparasites and infectious diseases in almost all the production systems. Even though, it has been attempted by few researchers (Belihu *et al.,* 2010; Mekuria and Gezahegn, 2010; Amede *et al.,* 2011; Tolossa and Tafesse, 2013; Dabasa *et al.,* 2017a, b). Ectoparasites may raise a clinical issue for humans, transmit several infectious ailments, and act as a transit/intermediate host for a range of helminthic parasites. Native fowl parasitic contagions, which can cause health and economic problems in poultry production, are considered a source of infection in industrial poultry, wild birds, and humans. Currently, there is a poorness of information considering the prevalence of ectoparasites in local chickens (Ebrahimi *et al.,* 2016). Many ectoparasites are known to suck blood thereby causing irritation and morbidity. They also contest for feed, serve as means of poultry ailments and germs, that can straight influence bird hygiene. Ectoparasites influence the productivity potential of indigenous chickens and helmeted guinea fowls thus ought to be given more heedfulness. Albeit helmeted guinea fowls are known to be more ailment resistant to ailments than chicken, ectoparasite infestation is still a significant concern (Bhat *et al.,* 2014; Okaeme, 1988). The fowl tick (*Argas persicus*) is known to affect pigeons, turkeys, geese, ducks and chickens in sub-tropical and tropical countries. the stick-tight flea (*Echidnophaga gallinacean*) is the only flea commonly affecting chicken (Mungube *et al.,* 2008). The louse species affecting chicken are yellow

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body louse (*Menacanthus stramineus*), shaft louse (*Menopon gallinae*), chicken head louse (*Cuclotogaster heterographus*), wing louse (*Lipeurus caponis*), large chicken louse (*Goniodes gigas*) and fluff louse (*Goniocoites gallinae*). Mites are among the most ordinary of all the ectoparasites watched in poultry. Some of the species found on the skin of most poultry birds consist of common red mite (*Dermanyssus gallinae*), northern fowl mite (*Ornithonyssus sylviarum*) and tropical fowl mite (*Ornithonyssus bursa*). Mites of the family Dermanyssidae are the most economically important of the numerous ectoparasites of poultry. Severe infestations of mites in chicken consequences abated reproductive potential in males and egg production in females. (Salam *et al.,* 2009; Ikpeze *et al.,* 2008). From the foregoing, therefore, the infestation of ectoparasites attacking beautiful chickens in the eastern area of Bangkok was studied. This study was designed to examine the prevalence of ectoparasites in chickens that invaded each beautiful chicken species by surveying different parts of the chicken both on the skin and feathers as well as to identify the specific habitats of each species of ectoparasites.

Materials and methods

Survey area

The process of surveying ectoparasites of beautiful chicken reared in various farms in the east of Bangkok area, including Minburi, Nong Chok and Lat Krabang districts from November 2020 to May 2021. Survey methods were adapted from Al-Saffar and Al-Mawla, (2008) by collecting samples from various beautiful chickens raised on breeding (Table 1).

Table 1. Name of farm, chicken variety chicken number of surveying

Name of Farm[1/]	Chicken variety[2/]										
	LH	HR	SK	JB	PO	RI	BR	PB	SB	SE	Total
KMITL	10	10	6	-	-	-	-	-	-	-	**26**
Bang-Zeek	-	-	2	2	2	2	2	-	-	-	**10**
Pongsak	-	-	1	-	-	-	-	2	2	2	**7**
Bang-Keang	-	-	-	5	-	-	-	-	-	-	**5**
Total	**10**	**10**	**9**	**7**	**2**	**2**	**2**	**2**	**2**	**2**	**48**

[1/]Farms in the eastern area of Bangkok [2/] LH= Laying hens in a cage, HR= Hens reared in free cage, SK= Silkie, JB= Japan bantam, PO= Polish, RI= Rhode Island red, BR= Brahma, PB= Phu Phan black bone, SB= Serama bantam, SE= Sebright

Survey on the chicken feather

The ectoparasites were taken randomly from 5 points of each chicken body as neck, wing, breast, back and buttocks. Amount of 20 feathers were

randomly collected from the object areas with about 5 cm^2 as follows: hackles are the feathers around the neck along the front of the neck and surrounding necklaces, wings are the area of soft and hard feathers both on the outside and inside of the chicken, the breast is the area where the feather in the front of the breast is located, the back is the part between the cape and saddle and the buttocks are the soft hairs around the anus (cloaca).

Suction on the skin by using aspirator

The suction was made randomly from each chicken at head, neck, body and buttocks, by using a vacuum aspirator connected to a test tube sizing 20*150 mm. As specified areas, 5 minutes suction was performed in 5 cm^2. Where, the head is the cockscomb area down to the eyes and neck below. The neck is the area from the hackle to the front neck plumage or around the necklaces. The body is part of the breast to the insides of the point where there are soft feathers. Buttocks are the same area as mentioned above.

Sample retention and classification

Totally, 48 beautiful chickens were randomly selected to collect the ectoparasites according to the regularities by experimental samples that specimen preparation of ectoparasite should not exceed 72 hours after sample collection (Paliy *et al.*, 2018) and the samples must be cleaned of dirt debris skin of the body with a paintbrush. The louse and mite got the same method of preserving specimens. Innitially, the sample was placed in a 20*150 mm test tube with approximately 2 ml of 70% ethanol to kill the ectoparasite and wrapped by the parafilm tightly to prevent alcohol evaporation and then, labeling was made. The specimen preparation was made by positioning those ectoparasites on slides, dropped previously with Hoyer's medium. In the case of louse, maybe pierced on the abdomen with a small needle to allow Hoyer's medium to infiltrate. This makes the body of the louse with more transparent. If louse stomach was full of blood, it could be pierced and cleaned with a solution of potassium hydroxide at a concentration of 10%. This method would be appropriate for large body mite as well. After that sample was rinsed with distilled water to remove potassium hydroxide (Krantz, 1970). The position of the ectoparasite placed on the slide was arranged by using a sterile needle and put with a coverslip, and information labeling should be placed. The sample slides were classified under a stereomicroscope according to their morphological characteristics after entomological keys as described such as

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Furman and Catts, 1970; Soulsby, 1982; Lapage, 1968; Baker and Wharton, 1964.

Data and statistical analysis

All collected data were entered into Microsoft Excel 365 program. Analyzed Pearson's chi-square(X^2) logistic regression was applied to assess the association of different variables and statistical analysis, a confidence level of 95% and P-values less than 5% were judged as significant by using SPSS statistics V.20 package. The prevalence was calculated as a percent of infected animals from the total number of animals examined.

Results

The obtained result informed that 8 species of ectoparasites were found attacking beautiful chicken namely, *Megninia cubitalis, Megninia ortari, Pterolichus obtusus, Cuclotogaster heterographus, Menopon gallinae, Lipeurus caponis, Goniocotes gallinae* and *Menacanthus stramineus*. The most prevalent ectoparasite found in feather of laying hens in a cage was *Megninia cubitalis*, with the average amount 103.88 mites, in which found on the neck, wing, chest, back and buttocks, with 52.1, 5.7, 119.8, 136.9 and 204.9 mites, respectively, followed by the attacking on Rhode Island red chicken, where on feather presented an average number of 32.40 mites per chicken, with the greatest number found on the buttocks with the average of 85.00 mites. *Pterolichus obtusus* was also found in only two types of chickens, Japan bantam and Polish, with an average of 0.12 and 7.80 mites, respectively. Remarkably *Megninia ortari*, found only in silkie chickens which was observed in the neck, wings and buttocks, with an average of 0.8, 0.7 and 0.3 mites, respectively. The louse *Lipeurus caponis* was the most common parasite found in back Polish chickens, with an average of 100.5 insects and 35.90 insects per chicken, followed by appeared in Rhode Island red chickens on the chest and wings, with 70.0 and 50.5 insects, respectively with an average of 28.90 insects per chicken. The other louse, *Menopon gallinae*, most commonly found in hens reared in free cage, and normally occured in the buttocks for 21.1 insects, with an average number of 21.1 insects per chicken, followed by *Cuclotogaster heterographus* found in laying hens in a cage, with an average of 4.36 insects per chicken. For *Menacanthus stramineus* was most common in Japan bantam chickens with a total average of 0.52 insects. Whereas, *Goniocotes gallinae*, found in Silkie chickens had an average of only 0.52 insects (Table 2).

Table 2. The ectoparasite species found on feather at in different parts of various chicken in eastern area of Bangkok

Ectoparasite	Part of chicken	Mean of ectoparasite[1/] Chicken variety[2/]									
		LH	HR	SK	JB	PO	RI	BR	PB	SB	SE
Mite											
Megninia cubitalis	Neck	52.1	-	1.7	11.2	12.0	5.0	17.5	-	5.0	-
	Wings	5.7	-	0.9	8.8	5.0	19.5	-	-	-	4.0
	Chest	119.8	-	1.7	13.8	3.5	36.0	28.0	3.0	4.5	1.5
	Back	136.9	-	1.8	16.9	0.5	16.5	15.5	-	3.0	-
	Buttocks	204.9	1.8	2.6	2.5	24.0	85.0	25.0	-	19.0	-
	Mean per chicken	**103.88**	**0.36**	**1.74**	**10.64**	**9.00**	**32.40**	**17.20**	**0.60**	**6.30**	**1.10**
Megninia ortari	Neck	-	-	0.8	-	-	-	-	-	-	-
	Wings	-	-	0.7	-	-	-	-	-	-	-
	Chest	-	-	-	-	-	-	-	-	-	-
	Back	-	-	-	-	-	-	-	-	-	-
	Buttocks	-	-	0.3	-	-	-	-	-	-	-
	Mean per chicken	-	-	**0.36**	-	-	-	-	-	-	-
Pterolichus obtusus	Neck	-	-	-	-	17.0	-	-	-	-	-
	Wings	-	-	-	-	22.0	-	-	-	-	-
	Chest	-	-	-	-	-	-	-	-	-	-
	Back	-	-	-	0.3	-	-	-	-	-	-
	Buttocks	-	-	-	0.3	-	-	-	-	-	-
	Mean per chicken	-	-	-	**0.12**	**7.80**	-	-	-	-	-
Louse											
Cuclotogaster heterographius	Neck	3.3	-	-	**4.3**	-	2.0	-	-	-	-
	Wings	3.5	-	-	-	-	-	-	-	-	-
	Chest	4.7	-	-	-	-	-	-	-	-	-
	Back	7.7	-	-	-	-	-	-	-	-	-
	Buttocks	2.6	-	-	-	-	-	-	-	-	-
	Mean per chicken	**4.36**	-	-	**0.86**	-	**0.40**	-	-	-	-
Menopon gallinae	Neck	-	1.7	0.8	1.7	2.0	-	-	-	-	-
	Wings	-	10.6	1.1	4.7	1.0	2.0	-	-	-	-
	Chest	-	2.5	0.7	1.6	3.0	5.0	1.5	-	-	-
	Back	-	2.0	0.4	0.6	5.5	-	2.0	-	-	-
	Buttocks	-	21.1	1.0	1.6	-	-	-	-	5.0	-
	Mean per chicken	-	**7.58**	**0.80**	**2.04**	**2.30**	**1.40**	**0.80**	-	**1.00**	-
Lipeurus caponis	Neck	-	-	9.8	24.0	3.5	6.0	1.5	-	7.5	-
	Wings	-	-	2.8	18.0	6.0	50.5	-	-	1.5	-
	Chest	-	-	0.1	2.6	12.5	70.0	0.5	-	2.5	-
	Back	-	-	0.6	21.1	100.5	12.0	1.5	-	-	-
	Buttocks	-	-	1.3	5.1	57.0	6.0	1.0	-	1.0	-
	Mean per chicken	-	-	**2.92**	**14.16**	**35.90**	**28.90**	**0.90**	-	**2.50**	-
Goniocotes gallinae	Neck	-	-	2.4	1.6	-	-	-	-	-	-
	Wings	-	-	-	0.9	-	-	-	-	-	-
	Chest	-	-	0.1	-	-	-	-	-	-	-
	Back	-	-	0.1	-	-	-	-	-	-	-
	Buttocks	-	-	-	-	-	-	-	-	-	-
	Mean per chicken	-	-	**0.52**	**0.50**	-	-	-	-	-	-
Menacanthus stramineus	Neck	-	-	-	-	1.0	-	-	-	-	-
	Wings	-	-	-	0.6	-	-	-	-	-	-
	Chest	-	-	-	0.7	-	-	-	-	-	-
	Back	-	-	-	1.1	-	-	-	-	-	-
	Buttocks	-	-	-	1.0	-	-	-	-	-	-
	Mean per chicken	-	-	-	**0.68**	**0.20**	-	-	-	-	-

[1/] Means of ectoparasite found on 10 feathers per part of chicken. [2/] LH= Laying hens in a cage, HR= Hens reared in free cage, SK= Silkie, JB= Japan bantam, PO= Polish, RI= Rhode Island red, BR= Brahma, PB= Phu Phan black bone, SB= Serama bantam, SE= Sebright, - Not found

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Table 3. The ectoparasite species found on different skin parts of various chicken varieties in eastern area of Bangkok

Ectoparasite	Part of chicken	Mean of ectoparasite[1/] Chicken variety[2/]									
		LH	HR	SK	JB	PO	RI	BR	PB	SB	SE
Mite											
Megninia cubitalis	Head	71.4	-	6.6	17.0	1.5	13.5	-	6.0	-	22.5
	Neck	99.4	-	4.6	5.7	0.5	8.5	18.5	4.0	-	1.5
	Body	163.6	-	22.5	7.0	0.5	18.5	18.0	1.5	10.0	17.0
	Buttocks	168.3	-	12.6	17.3	4.5	42.6	30.5	-	2.0	0.5
Mean per chicken		**125.68**	-	**11.58**	**11.75**	**1.75**	**20.78**	**16.75**	**2.88**	**3.00**	**10.38**
Megninia ortari	Head	-	-	0.4	-	-	-	-	-	-	-
	Neck	-	-	0.3	-	-	-	-	-	3.5	-
	Body	-	-	-	-	-	-	-	-	-	-
	Buttocks	-	-	8.9	-	-	-	-	-	-	-
Mean per chicken		-	-	**2.40**	-	-	-	-	-	**0.88**	-
Pterolichus obtusus	Head	-	-	-	-	1.5	-	-	-	-	-
	Neck	-	-	-	-	-	-	-	-	-	-
	Body	-	-	-	-	2.5	-	-	-	-	-
	Buttocks	-	-	-	-	-	-	-	-	-	-
Mean per chicken		-	-	-	-	**1.00**	-	-	-	-	-
Louse											
Cuclotogaster heterographus	Head	-	-	-	-	-	-	-	-	-	-
	Neck	8.2	-	0.6	-	-	-	-	-	-	-
	Body	43.8	-	-	-	-	-	-	-	-	-
	Buttocks	4.0	-	-	-	-	-	-	-	-	-
Mean per chicken		**14.0**	-	**0.15**	-	-	-	-	-	-	-
Menopon gallinae	Head	-	0.7	-	0.3	1.5	-	-	-	-	-
	Neck	-	22.0	8.2	0.9	-	1.5	1.0	-	-	-
	Body	-	9.6	1.8	-	11.0	2.5	1.5	-	-	0.5
	Buttock	-	2.8	1.8	1.3	22.5	-	-	-	23.0	-
Mean per chicken		-	**8.78**	**2.95**	**0.63**	**8.75**	**1.00**	**0.63**	-	**5.75**	**0.13**
Lipeurus caponis	Head	-	-	-	0.9	1.0	1.0	1.5	-	-	-
	Neck	-	-	-	5.6	-	-	-	-	-	-
	Body	-	-	-	2.9	-	-	-	-	-	-
	Buttock	-	-	0.1	-	-	0.5	2	-	-	-
Mean per chicken		-	-	**0.03**	**2.35**	**0.25**	**0.38**	**0.88**	-	-	-
Goniocotes gallinae	Head	-	-	-	-	-	-	-	-	-	-
	Neck	-	-	-	-	-	-	-	-	-	-
	Body	-	-	-	-	-	-	-	-	-	-
	Buttock	-	-	0.2	-	-	-	-	-	-	-
Mean per chicken		-	-	**0.05**	-	-	-	-	-	-	-
Menacanthus stramineus	Head	-	-	-	0.3	-	-	-	-	-	-
	Neck	-	-	-	-	-	-	-	-	-	-
	Body	-	-	-	0.4	-	-	-	-	-	-
	Buttock	-	-	-	8.0	3.0	-	-	-	-	-
Mean per chicken		-	-	-	**2.18**	**0.75**	-	-	-	-	-

[1/] Means of ectoparasite found on 5 cm² per part of chicken. [2/] LH= Laying hens in a cage, HR= Hens reared in free cage, SK= Silkie, JB= Japan bantam, PO= Polish, RI= Rhode Island red, BR= Brahma, PB= Phu Phan black bone, SB= Serama bantam, SE= Sebright, - Not found

The study of ectoparasites found on different parts of chicken skin, indicated that the most common mite was *Megninia cubitalis*, abundantly attacking laying hens in a cage which could be found in all parts of the chicken. The most prefered area was the buttock with an average of 168.3 mites followed by attacking the body, neck and head, with an average of 163.6, 99.4

and 71.4 mites, respectively. This mite species appeared in all chickens except for hens reared in free cage. *Megninia ortari* was predominantly found in Silkie chickens with an average of 2.40 mites per chicken, followed by happening on Serama bantam chicken with an average of 0.88 mites per chicken. *Pterolichus obtusus* mite was found in only one type of chicken, Polish chicken with an average of 1.0 mites per chicken. In case of louse ectoparasites, *Cuclotogaster heterographus*, most commonly found on laying hens in a cage on the body, neck and the buttock, with averages of 43.8, 8.2 and 4.0 insects, respectively (but not found on the head) with an average of 14.0 insects per chicken. Few was found in Silkie chickens at the average of 0.15 insects per chicken. *Menopon gallinae*, found mainly in hen reared in free cage with an average of 8.87 insects per chicken. It was found in almost chicken species except for laying hen in a cage and Phu Phan black bone. As for lice, *Lipeurus caponis*, lived in Japan bantam chickens with an average of 2.35 insects per chicken and was also living with 4 chicken varieties as Brahma, Rhode Island red, Polish and Silkie, with averages of 0.880.38, 0.25 and 0.03 insects per chicken, respectively. *Menacanthus stramineus*, lice were found only two varieties of chicken, Japan bantam and Polish, with an average of 2.18 and 0.75 insects per chicken, respectively. Finally, *Goniocotes gallinae*, this louse species could be detected in a silkie chicken with a total average of 0.05 insects per chicken (Table 3).

The prevalent factors analysis of ectoparasites between the feather and skin areas of the chickens indicated that the prevalence of *Megninia cubitalis* found on the feather and skin had an abundance of 77.08 and 66.66%, respectively (X^2 = 0.2110; P = 0.6460). When, *Megninia ortari* was observed on the feather and skin area with 14.58 and 10.41%, respectively (X^2 = 0.2964; P = 0.5861). *Pterolichus obtusus*, mite was presented on feather and skin with prevalence of 6.25 and 2.08%, respectively (X^2 = 0.9604; P = 0.3271). Whereas, the prevalence of the three mites species were non-significant differences at a confidence level of 0.05. As for the lice, it was found that the *Cuclotogaster heterographus*, was abundant on both feather and skin at 29.16 and 25.00%, respectively (X^2 = 0.1211; P = 0.7278). For *Menopon gallinae*, the prevalences were 60.41 and 64.58%, respectively (X^2 = 0.0410; P = 0.8394). The prevalences of *Goniocotes gallinae* louse were 12.50 and 2.08%, respectively (X^2 = 3.3366; P = 0.6775), as well as *Menacanthus stramineus*, the prevalences were 8.33 and 8.33%, respectively (X^2 = 0; P = 1.0000). All the above lice were found with non-significant at a confidence level of 0.05. In the end, *Lipeurus caponis*, the prevalences were 47.91 and 16.66 %, respectively (X^2 = 5.5640; P = 0.0183), it was the only ectoparasite with a statistically significant difference at 0.05 (Table 4).

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Table 4. Distribution of various ectoparasite found on feather and skin of different of chickens in eastern area of Bangkok

Ectoparasite		Part of chicken	Number examined	Number positive	Prevalence (%)	χ^2	P value*
Mite	*Megninia cubitalis*	Feather	48	37	77.08	0.2110	0.6460^{ns}
		Skin	48	32	66.66		
	Megninia ortari	Feather	48	7	14.58	0.2964	0.5861^{ns}
		Skin	48	5	10.41		
	Pterolichus obtusus	Feather	48	3	6.25	0.9604	0.3271^{ns}
		Skin	48	1	2.08		
Louse	*Cuclotogaster heterographus*	Feather	48	14	29.16	0.1211	0.7278^{ns}
		Skin	48	12	25.00		
	Menopon gallinae	Feather	48	29	60.41	0.0410	0.8394^{ns}
		Skin	48	31	64.58		
	Lipeurus caponis	Feather	48	23	47.91	5.5640	0.0183*
		Skin	48	8	16.66		
	Goniocotes gallinae	Feather	48	6	12.50	3.3366	0.6775^{ns}
		Skin	48	1	2.08		
	Menacanthus stramineus	Feather	48	4	8.33	0	1.0000^{ns}
		Skin	48	4	8.33		

*The P values were calculated by Chi-square (X^2) test. ns= non-significant (typically > 0.05), *= A p-value less than 0.05 is statistically significant difference.

Discussion

The obtianed results regarding the infestation of ectoparasites of beautiful chickens in the eastern Bangkok area informed that *Megninia cubitalis* was the most common mite found in both the feather and skin areas with the prevalent rate of 77.08 and 66.66%, respectively, and were found abundantly all types of chickens. An outbreak of *M. cubitalis* was reported in laying flocks from the State of Minas Gerais, Brazil (Rezende *et al.*, 2015), where it has a climate similar to that of tropical Thailand. Consistent with Sangvaranond (2003) who reported that *M. cubitalis* was found in domesticated chickens in central Thailand. They lived on the body and wings of chickens. Its outbreak in chickens was also raised in private farms in Chachoengsao province and it appeared in many provinces of Thailand. If it infested in large numbers, may cause the host with pyodermatitis, and finally resulting in the death of chicken (Sangvaranond, 2009). Another mite species was *Megninia ortari* that could be found in some species of chicken, such as in silky chickens on the neck, wings and buttocks, and in Sebright chickens on the neck with the prevalent rates of 35.41 and 25.00%, respectively. In the laying hen industry, mite of the genus *Megninia* was very importance, by causing the birds cannot produce eggs or getting less productivity. The saliva of the mite causes itching and can cause pyodermatitis. Futhermore it can also damage other species of poultry such as parrots, turkeys and pigeons (Guimarães *et al.*, 2001; Tucci *et al.*, 2005;

Rezende *et al.*, 2013). The outbreak of *Megninia spp.* may vary according to climatic and geographical conditions, such as varying from 2.6% in Israel to 89.6% in Cuba. In Brazil, an occurrence of 18.09% of the Megninia genus was observed in chicken houses of poultry farms posture in the state of Minas Gerais (Mumcuoglu and Lutsky, 1990; Hernández *et al.*, 2006; Rezende *et al.*, 2015). Additionally, the infestation of *Pterolichus obtusus* was found in some chicken species surveyed, Silky and Polish chickens, on feather and skin with 6.25 and 2.08%, respectively. A study by Sangvaranond (1993) found that the *Pterolichus obtusus* mite was the most abundant naturally occurring mite in native chickens, accounting for up to 70% of the population. The ectoparasites play an important role in bird life. Feather mites are ectoparasites that specialize in living on plumage and skin, which adapted to inhabit the microhabitats on the bird's body (Dabert and Mironov, 1999). Feather mites *P. obtusus* were found in 2 dead black grouse feathers from the monitored area with 40 mites in 20 g of feathers and 13 mites in 1 g of feathers, respectively. This was in the plumage and rarely caused problems, unless it was presented in large numbers (Jankovska *et al.*, 2012).

The louse, *Menopon gallinae*, can be found commonly in domesticated chickens. In the study, its prevalent rates observed in the feather and skin areas were as high as 60.41 and 64.58%, respectively. This was consistent with report of Sangvaranond (2009), who stressed that amblyceran as louse bites were important ectoparasites of domestic and native chickens found in many provinces of Thailand. This insect species is the most important and widely distributed species, the prevalences of this louse were reported from many areas as its occurring 35.9% in Bulgaria, 34.4% in Kashmir valley and 33.3% in Malawi (Banda, 2011; Prelezov *et al.*, 2006; Salam *et al.*, 2009). *Lipeurus caponis* is an ectoparasite species that can be found also on feather, with a prevalence of 47.91%, but with few prevalence of 16.66% on the skin, with a statistically significant difference. It could be said that *L. caponis* lives mainly on the hairs and therefore are rarely found on the skin. Likewise, Pumnuan *et al.* (2020) mentioned that many chicken lice (*L. caponis*) were found from native chickens at Learning Center and Management System Integrated with Urban Livestock Farm Learning, School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand. Moreover, Sangvaranond (2003) reported that *L. caponis* was found inferior to Menopon gallinae. Both ectoparasite species are common in domesticated and native chickens in Thailand.Similar results were reported by Rahman and Haziqah (2015) who mentioned that in Penang Island, Malaysia, number of *M. gallinae* was found more than that of *L. caponis*, with the prevalent values of 76.7 and 63.3%, respectively. These 2 species were very important in the infestation of

chickens. In additional outbreaks were also observed in other countries such as Libya (14.29%), USA (20%), Algeria (41.6%), Bangladesh (48%) and Ethiopia (18.75%) (Mansur *et al.,* 2019). The next louse species was *Cuclotogaster heterographus* with its prevalent values on feather and skin were 29.16 and 25.00%, respectively, which coincided with the survey of Shanta *et al.* (2006) and Belihu *et al.* (2009), also reported prevalence of 25 and 25%, respectively, with predominantly found in the head and neck of chickens. Most of the chickens surveyed indicated that at least one species of lice, such as *M. gallinae, M. pallidulus, L. caponis, G. gallinae* and *G. dissimilis* was dicovered. These lice tend to live in the fluff of the body's feathers, especially the neck, back, abdomen and wings (Rahman and Haziqah, 2015). In Libya, there was an outbreak of chicken lice parasites. When the abundance of louse in the environment was happened for more than 70% composed of *M. gallinae, M. stramines* and *L. caponis*. There were occasionally outbreaks in other countries such as Algeria, USA and Bangladesh (Mansur *et al.,* 2019). Subsequently, *Goniocotes gallinae*, this louse was observed on feather and skin with 12.50 and 2.08% prevalence, respectively, similar to that reported by Shanta *et al.* (2006) with the prevalence on feather at 14%. This louse spcies distributed widly in many provices of Thailand such as Roi-Et, Surin and Buriram. It lives in the thighs of the chicken feathers (Nopwinyoowong and Sukolapong, 1994). Finally, louse species was *Menacanthus stramineus*, found in some species of chickens surveyed, with the same prevalence on feather and skin of 8.33% also, predominantly on the buttocks. This was in accordance with Jassim and Hadi, (2019) who reported that louse species often lives on the fluff around the tail, chest and thighs. Likewise, Dik and Halajian, (2013) showed of this species could be found on the skin, ventral feathers of the wings, chest and abdomen. The prevalence surveyed in Iran was 22.7%. Sangvaranond (2009) also reported this louse species found in Thailand with a 3.80% rate of lice in native chickens. From this study, it could be concluded those the prevalent rates of ectoparasites in beautiful chickens was vary depending on region with different climate conditions.

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APPENDIX B

Poultry Science

Nanoemulsion-based plant essential oil formulations: in vitro evaluation of pesticidal activity against ectoparasites in poultry

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ABSTRACT Ectoparasite infestations significantly impact the health and productivity of poultry. Chemical applications, although common for pest control, lead to pesticide residues and parasite resistance in poultry. Nanoemulsion-based plant essential oil formulations (**NEOFs**) provide a promising alternative for controlling poultry ectoparasites. This study aimed to assess the efficacy of NEOFs from clove, cinnamon, and turmeric essential oils (**EOs**) against ectoparasites, *Menopon gallinae* and *Megninia ginglymura*, under laboratory conditions. The toxicity and repellent properties of the NEOFs were examined, with the major chemical compounds of the EOs analyzed using chromatography mass spectrometer. Results identified eugenol as the dominant component in clove and cinnamon EOs (84.60 and 75.19%, respectively), while turmerone (68.46%) was the major compound in turmeric EO. NEOFs with clove:cinnamon:turmeric ratios of 4:0:0, 2:2:0, and 2:0:2 had particle size of 20.76 nm, 20.66 nm, and 89.56 nm, respectively, while those based on eugenol and turmerone standards had sizes <21.0 nm. In addition, NEOFs at 0.3% concentration with ratios of 4:0:0 and 2:2:0 achieved full control of both ectoparasites. These formulas demonstrated exceptional potency in exterminating ectoparasites, with LC_{50} and LC_{90} at <0.160 and <0.250%, respectively, 6 h after treatments. Furthermore, both NEOFs showed higher repellence responses in *M. gallinae* compared to *M. ginglymura*. The toxicities of these NEOFs were comparably effective against both parasites, showing no significant difference compared with chemical insecticide treatment. Therefore, further research will explore the practicality of using clove and cinnamon-derived NEOFs under farm conditions.

Key words: clove, cinnamon, nanoemulsion, ectoparasite, poultry

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INTRODUCTION

Poultry farming is vital to Thailand, being a major revenue source within the country's livestock sector. As one of the world's leading producers of agricultural commodities, Thailand's poultry industry is the second largest in Southeast Asia and is expected to expand further (Netherlands Embassy in Bangkok, 2016). Most of poultry population and production are located in central Thailand, with the integrated commercial farms accounting for 80 to 90% of national production (Rushton et al., 2007). Meanwhile, traditional poultry scattered across the country primarily cater to local consumption (Heft-Neal et al., 2009). A key challenge impacting the economic success of poultry farming is ectoparasitic infestation. These external parasites can cause significant health issues for poultry, including reduced growth, lower egg production, and in severe cases, death (McCrea et al., 2005; Onyekachi, 2021). Common poultry ectoparasites include lice, mites, fleas, and ticks, all of which negatively impact reproductive potential, reduced egg production and health of poultry. Their feeding behaviors can cause irritation, restlessness and debility, and severe infestations may result in fatal anemia (Salam et al., 2009; Mirzaei et al., 2016). In Bangkok, Thailand, several reports indicated that chicken lice, *Lipeurus caponis*, *Menopon gallinae*, and *Goniocotes gallinae*, are the most prevalent poultry ectoparasites, while *Megninia cubitalis* being the predominant species of chicken mite (Lakyat et al., 2022). Traditionally, pesticides such as pyrethroids, organophosphates, carbamates, and macrocyclic lactones have been widely used for ectoparasitic control in poultry. However, these agents have led to resistance among parasites (Coles and Dryden, 2014; Sparagano et al., 2022), and their use has resulted in harmful environmental

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residues and potential toxicity to humans and animals (Sharma et al., 2019). Despite the widespread use of these pesticides, none provide complete protection against poultry ectoparasites. This necessitates the exploration of alternative, eco-friendly pesticides, potentially derived from medicinal plants with pesticidal properties (Shanta et al., 2008; Pumnuan et al., 2020). Poultry treated with chemical pesticides often produce eggs contaminated with toxicity residues. A study conducted by Alaboudi et al. (2019) revealed that 96% of egg samples from a total of 200 households with laying hens in Jordan, were contaminated with pesticides. Concerningly, 66.5% of these samples showed contamination levels exceeding the maximum residue limit (**MRL**) of less than 0.01 ppm. In particular, cypermethrin, an insecticide, had the highest incidence being present in 52% of egg samples from Rio Grande do Sul in 2015 (Dallegrave et al., 2018). However, such applications provide inadequate protection against poultry ectoparasites. Consequently, research is now focusing on highly effective, human-safe, and environmentally friendly alternative green pesticides with no toxic residues in meat or eggs. These pesticides are derived from plant EOs with pesticidal properties (Pumnuan et al., 2020).

Several papers reported that various plant EOs showed high efficiency in controlling poultry ectoparasites. For instance, EOs of ginger and citronella eliminated the highest effectiveness against lice (*M. gallinae*) and mites (*Ornithonyssus bursa*) (Vigad et al., 2021). Especially, EOs of clove, cinnamon, and turmeric effectively killed lice (*Lipeurus caponis*) completely after 12 h of exposure (Pumnuan et al., 2020). Furthermore, the main chemicals in plant EOs, including botanical pesticides, are recognized for their potential in controlling a wide range of pests (Isman, 2006). Specifically, eugenol, a compound present in clove and cinnamon EOs, has been shown to have potent insecticidal and acaricidal properties (Fichi et al., 2007a,b; Abenaim et al., 2022). For example, eugenol has demonstrated significant ovicidal activities against parasitic mites (*Sarcoptes scabiei*) (Li et al., 2021). Studies have suggested that clove and cinnamon EOs, and their components, could be used to develop fumigants, repellents, and attractants to control poultry red mite, *Dermanyssus gallinae* (Sparagano et al., 2013; Lee et al., 2019).

Therefore, this research aimed to evaluate the effectiveness of EOs obtained from 3 plants; clove, cinnamon, and turmeric, and their major compounds. These would be incorporated into farming formulas, based on nanoemulsion, to control ectoparasites infesting laying hens under laboratory conditions.

MATERIALS AND METHODS

Preparation of Plant Essential Oil-Based Nanoemulsions

Essential oils (**EOs**) were obtained from 3 dried medicinal plants: clove buds (*Syzygium aromaticum*), cinnamon leaves (*Cinnamomum zeylanicum*), and turmeric rhizomes (*Curcuma longa*). The selection was based on previous research and academic papers about their efficacy against ectoparasites (Pumnuan et al., 2020). Nanoemulsions of these EOs (**NEOs**) were prepared according to the methodology described by Doungnapa et al. (2021). Specifically, nanoemulsion of clove (**NEO-CL**) and cinnamon (**NEO-CI**) were prepared with EO:Surfactant:Cosurfactant ratios of 2:9:2, while for turmeric (**NEO-TU**), the ratio was 2:6:3. All NEOs, at a 1.0% concentration in water, were then evaluated for particle size and zeta potential.

Ectoparasite Used

The ectoparasites *Menopon gallinae* (shaft louse) and *Megninia ginglymura* (chicken mite) were collected from the Smart Chicken Farm at the School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (**KMITL**), Thailand. Adult ectoparasites were screened from chicken feathers and skin using an aspirator, and tested in the laboratory within 2 h of collection.

Chemical Characterization of Plant Essential Oils

The EOs extracted from clove, cinnamon and turmeric, were analyzed using Gas Chromatography Mass Spectrometer (**GC-MS**) (Agilent Technologies Inc., USA). The GC-MS was equipped with an HP5MS capillary column (30 m length × 0.25 mm I.D. × 0.25 μm film thickness). Analysis parameters included direct injection of a 0.4 μl volume, a split mode with a split ratio of 50:1 v/v, and an injection temperature of 250°C. Helium served as a carrier gas with a flow rate of 1 mL/min and an ionization voltage of 70 eV. Mass range detection was set to 50 to 500 *m/z*. The oven temperature, started at 50°C, held for 3 min, then increased by 10°C/min until reaching 200°C, after which it was raised by 15°C/min until it reached 260°C. The detector was maintained at 270°C. The results obtained were compared with those in Wiley's library (**Wiley7n**), accepting a quality match of over 85%.

Nanoemulsions of the main compound standard (**NCS**) were prepared using clove, cinnamon and turmeric EOs. Eugenol (**E**) was identified as the primary constituent of clove and cinnamon EOs, while turmerone (**T**) was present in turmeric EO. NCS-E, containing eugenol (Fluka Analytical) was prepared in the same ratio as NEO-CL and NEO-CI. Similarly, NCS-T, consisting of turmerone (MedChemExpress), was prepared in the same ratio as NEO-TU. The particle size of the NCS at 1.0% in water were was measured, along with their polydispersity index (**PDI**) and zeta potential by using a Nano plus Zeta/Nano Particle Analyzer as done in NEO experiments.

Preparation of Nanoemulsion

Based on a report by Pumnuan et al. (2020), clove EO has demonstrated higher efficacy in killing chicken lice (*Liperus caponis*) compared to cinnamon and turmeric

EOs. Consequently, a formula called NEO-CL was prepared as the main component, while NEO-CI and NEO-TU were used as secondary components. These mixtures were referred to as nanoemulsion-based plant essential oil formulations (**NEOFs**). Four different ratios of mixture were prepared as NEO-CL:NEO-CI:NEO-TU, namely 4:0:0, 2:2:0, 2:0:2 and 2:1:1, respectively. Subsequently, the NEOFs at 1.0% in water were analyzed for the particle size and zeta potential.

Particle Size and Zeta Potential Assessment and Morphological Analysis

The particle size (diameter), PDI, and zeta potential of NEO, NCS, and NEOF at 1% in water were measured using a Nano plus Zeta/Nano Particle Analyzer (Micromeritics Instrument Corporation, Japan) with the manufacturer's software.

The morphological structure of all nanoemulsion-based plant EO formulations was observed using Field Emission Transmission Electron Microscopy (**FE-TEM**) (Thermo Fisher Scientific: Talos F200i, Czech Republic). A drop of the 1% nanoemulsion-based sample in water was placed on a copper grid for 1 min. The sample was then stained with 2% uranyl acetate for 10 min at room temperature. Subsequently, the copper grid with the sample was placed in vacuum chamber to absorb moisture overnight. Finally, the sample was observed under the FE-TEM at an acceleration voltage 200 kV.

Contact Toxicity Bioassays

The lethal contact residue exposure test was performed according to the method described by Pumnuan et al. (2020). A total of 1 mL of each NEOF at 3.0% was dropped onto a 9 cm diameter filter paper (Whatman; No.1) and placed in a glass petri dish. The dishes were dried at room temperature (25°C ± 3°C) for 5 min, and then 10 adults of each poultry ectoparasites (shaft louse, *M. gallinae* and chicken mites, *M. ginglymura*) were released. The petri dishes were tightly closed and wrapped with parafilm and kept at room temperature. The percentage mortality was measured at 3 and 6 h, and the actual mortality was calculated and compared with the control group (0.25% surfactant in water). The 2 formulas with the highest efficiency in killing ectoparasites were further tested for toxicity at concentrations of 0.15, 0.20, 0.25, 0.30, and 0.35%. The actual mortality rate was checked and compared with that of the control group. The toxicity level was calculated to determine the LC_{50} and LC_{90} values. Data comparisons were also made with the insecticide group (cypermethrin 35% EC, recommendation rate; 0.1%) and NCS. The experimental design was carried out using a completely randomized design (**CRD**), with 3 replicates.

Repellency Bioassays

NEOFs selected from previous contact residue exposure tests were examined for their repellency and attractant activities against 2 species of ectoparasites: shaft louse and the feather mites. The experimental design was carried out using CRD, by performing at 0.15 and 0.25% NEOFs, with 3 replicates.

For the repellent and attractant tests on the shaft louse, feathers were dipped in each concentration of the test solution for 1 min, dried at room temperature (25°C ± 3°C) for 5 min, and then released with 10 adult shaft lice per feather. The petri dishes were tightly covered, wrapped with parafilm and placed at room temperature. The number of shaft lice on the feathers and on the filter paper (control) was counted at 3 and 6 h, and the repellent and attractant rates were calculated.

The repellent test on chicken mites was performed using a glass test tube (0.5 cm diameter, 8 cm length) with one end attached to a filter paper and moistened with each concentration of each NEOF, while the other end was moistened with the control group (0.25% surfactant in water). The treated papers were moistened for 1 min and dried at room temperature for 5 min, and then 10 adult mites per tube were inserted. The tubes were placed at room temperature, and the number of mites on each side of the tube was evaluated at 3 and 6 h to determine the repellent and attractant rate.

Data Analysis

The experiment was designed in 3 randomized replicates. The data obtained were statistically analyzed using analysis of variance (**ANOVA**), and the difference between treatments was tested by Duncan's multiple range test (**DMRT**). The actual death rates were calculated via Abbot's formula (Abbott, 1987). The toxicity test results, represented by LC_{50} and LC_{90} values (lethal concentration of NEOFs required to kill 50 and 90% of ectoparasites, respectively) were determined using probit analysis. The data from the repellency were analyzed by the χ^2 test.

RESULTS

Chemical Characterization of Plant Essential Oils

The GC-MS analysis of the EOs obtained from the clove, cinnamon, and turmeric revealed the major compounds present in each oil. Eugenol was found to be the predominant compound in clove and cinnamon EOs, constituting 84.60 and 75.19% of the oils, respectively. Turmerone emerged as a major compound in turmeric EO, comprising 68.46% of the oil. In addition to the major compounds, minor chemical compounds were also detected in the EOs (Table 1).

Table 1. The main components in clove, cinnamon, and turmeric essential oils.

Clove		Cinnamon		Turmeric	
Chemical components	%	Chemical components	%	Chemical components	%
Eugenol	84.60	Eugenol	75.19	Turmerone	68.46
Caryophyllene	10.06	Benzyl benzoate	4.20	Cyclohexane	6.37
Alpha-Humulene	2.09	Caryophyllene	3.66	Zingiberene	5.27
Caryophyllene oxide	0.69	Linalool L	2.23	*Alpha*-Terpinolene	3.71
Delta-Cadinene	0.52	*trans*-Cinnamyl acetate	2.18	Curcumene	3.17
Other compounds	2.04	o-Cymene	1.77	1-Phellandrene	2.45
		Cinnamaldehyde	1.71	Cyclododecene	1.19
		Safrole	1.50	1,8-Cineole	1.76
		Alpha-Pinene	1.21	*Trans*-Caryophyllene	1.26
		Copaene	0.91	*Beta*-Cymene	1.00
		Beta-Thujene	0.74	*Alpha*-Atlantone	0.62
		Alpha-Humulene	0.70	Bisabolone (6S, 7R)	0.56
		Caryophyllene oxide	0.60	Other compounds	4.18
		Anethol	0.52		
		Rubicene	0.51		
		Other compounds	2.37		

Particle Size and Zeta Potential Assessment and Morphological Analysis

The particle size, PDI, and zeta potential of the nanoemulsions containing NEOs, NCSs, and NEOFs at a concentration of 1.0% in water were determined. The particle size of all NEOs from clove, cinnamon, and turmeric, as well as NCSs derived from eugenol and turmerone chemical standards, ranged from 17.15 to 20.80 nm. The NEOF-1, NEOF-2, and NEOF-3 formulations, which were mixtures of NEO-CL:NEO-CI:NEO-TU with ratios of 4:0:0, 2:2:0, and 2:0:2, respectively, exhibited particle sizes of 20.76 nm, 20.66 nm, and 89.56 nm, respectively, all below 100.0 nm. On the other hand, the mixed NEOF-4 formulation with a ratio of 2:1:1 resulted in a particle size larger than 100 nm (Table 2).

TEM images of the nanoemulsion-based plant EO formulations revealed rough-shaped droplets with opaque and nonsmooth surfaces, exhibiting particle sizes ranging from 17.15 to 20.80 nm for NEOs, NCSs, and NEOF-1 and NEOF-2 formulations. Additionally, smooth-surfaced spherical droplets were observed with particle sizes of 89.56 nm and 106.02 nm for NEOF-3 and NEOF-4 formulations, respectively (Figure 1).

Contact Toxicity and Repellent Efficacy

The effectiveness of the NEOFs, obtained by mixing various NEOs with different ratios, was evaluated. NEOF-1 and NEOF-2, at a concentration of 0.3%, exhibited complete toxicity against both ectoparasites (*M. gallinae* and *M. ginglymura*) at 3 h after treatment. Similarly, NEOF-3 and NEOF-4, at the same concentration, showed high efficiency in killing *M. ginglymura* (100%), but their effectiveness against *M. gallinae* was less than 90% (Figure 2). NEOF-1 and NEOF-2, identified as the most effective formulations against both ectoparasites, were selected for further toxicity testing at concentrations ranging from 0.15 to 0.35%. The results showed that these formulas achieved LC_{50} values of 0.149 to 0.160% and LC_{90} values of 0.224 to 0.242% against *M. gallinae* at 6 h after treatment. Furthermore, they exhibited toxicity levels of 0.072 to 0.120% and 0.131 to 0.204% against *M. ginglymura* at 6 h after treatment, respectively (Table 3).

The response effects of NEOFs, specifically NEOF-1 and NEOF-2, were examined in vitro to assess their repellent and attractant properties against the ectoparasites *M. gallinae* and *M. ginglymura*. The results

Table 2. Particle size, polydispersity index (PDI), and zeta-potential of various nanoemulsions of essential oils, chemical standards, and essential oil formulas at 1.0% in water.

Nanoemulsions (1.0% in water)	Particle size (nm)	PDI	Zeta potential (mV)
Plant essential oil-based nanoemulsions (NEOs)			
NEO-CL (clove:Tween60:PEG400 = 2:9:2)	20.72 ± 0.43	0.28 ± 0.01	−0.28 ± 1.53
NEO-CI (cinnamon:Tween60:PEG400 = 2:9:2)	20.80 ± 0.24	0.23 ± 0.01	−3.03 ± 0.53
NEO-TU (turmeric:Tween 80:PEG400 = 2:6:3)	18.18 ± 1.87	0.30 ± 0.01	−4.59 ± 0.37
Nanoemulsions of main compound standards (NCSs)			
NCS-E (eugenol:Tween60:PEG400 = 2:9:2)	20.44 ± 0.30	0.24 ± 0.01	−3.28 ± 0.87
NCS-T (turmerone:Tween 80:PEG400 = 2:6:3)	17.15 ± 1.76	0.32 ± 0.02	−3.43 ± 0.91
Nanoemulsion-based plant essential oil formulations (NEOFs)			
NEOF-1 (NEO-CL:NEO-CI:NEO-TU = 4:0:0)	20.76 ± 0.44	0.34 ± 0.02	−4.89 ± 0.92
NEOF-2 (NEO-CL:NEO-CI:NEO-TU = 2:2:0)	20.66 ± 0.47	0.30 ± 0.03	−3.06 ± 0.71
NEOF-3 (NEO-CL:NEO-CI:NEO-TU = 2:0:2)	89.56 ± 0.36	0.52 ± 0.02	−1.96 ± 0.65
NEOF-4 (NEO-CL:NEO-CI:NEO-TU = 2:1:1)	103.05 ± 2.68	0.27 ± 0.03	−1.52 ± 0.94

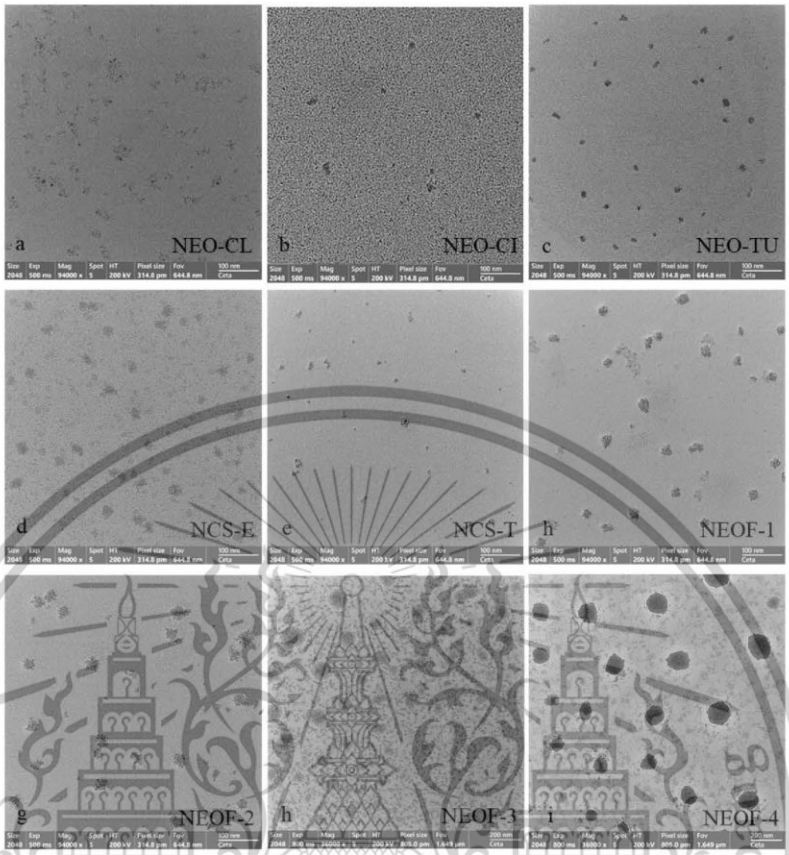

Figure 1. TEM morphological analysis of various nanoemulsions of essential oils, chemical standards and essential oil formulas at 1.0% in water, observed at a magnification of 94,000× (A–G) and 36,000× (H and I). NEOFs are the mixture of various NEOs with different ratio, NEOF-1 (NEO-CL:NEO-CI:NEO-TU — 4:0:0), NEOF-2 (NEO-CL:NEO-CI:NEO-TU — 2:2:0), NEO-CL (clove:Tween60:PEG400 — 2:9:2), NEO-CI (cinnamon: Tween60:PEG400 — 2:9:2), NEO-TU (turmeric:Tween 80:PEG400 — 2:6:3).

revealed that the NEOFs exhibited higher repellent responses toward *M. gallinae* compared to *M. ginglymura*. At a concentration of 0.25%, the NEOFs demonstrated repellency rates exceeding 80.0% for *M. gallinae*, with a significant difference observed. Interestingly, NEOF-2 displayed higher repellent percentages against *M. gallinae* compared to NEOF-1. NEOF-1 at a concentration of 0.25% exhibited repellency rates that were not significantly different from NEOF-2. Furthermore, the repellent response of NEOFs at concentrations of 0.15 and 0.25% against *M. ginglymura* ranged from 60.0 to 73.3%, which did not significantly differ from the attractant response rates of 26.7 to 40.0%. Overall, the response effects of NEOFs across all treatments remained consistent after 3 h and 6 h of treatment (Table 4).

The NEOFs demonstrated high effectiveness in terms of their toxicity properties and repellent efficiency against both poultry ectoparasites. At a concentration of 0.25%, the NEOFs achieved complete mortality of both ectoparasites at 6 h after treatment, with no significant difference compared to the cypermethrin insecticide and NCS-E group. However, the NEOFs exhibited lower activity with mortality rates of less than 90% against both ectoparasites at 3 h after treatment. Regarding repellent efficiency, the NEOFs and NCS-E at a concentration of 0.25% showed a higher repellent response effect against *M. gallinae* (>80%) compared to *M. ginglymura* (>66.7%). However, their repellency rates were lower than those observed in the cypermethrin insecticide group (100%) (Table 5).

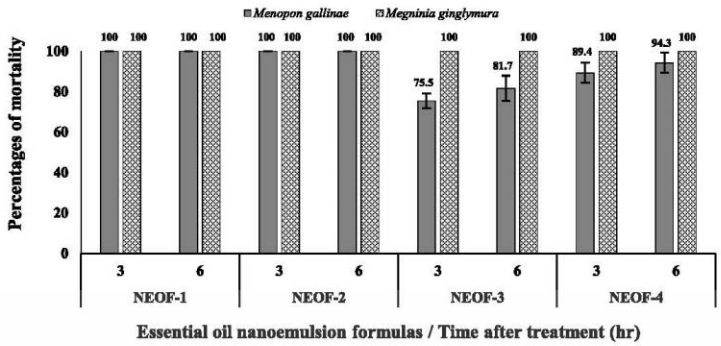

Figure 2. Percentages of mortality of the shaft louse (*Menopon gallinae*) and the feather mite (*Megninia ginglymura*) caused by different nanoemulsion-based plant essential oil formulations (NEOFs) at 0.3% concentration by contact method after various exposure times.

DISCUSSION

Plant EOs are complex mixtures of secondary metabolites produced by plants for various purposes. They can contain various compounds at different concentrations, with a few major components that determine their biological properties (Bakkali et al., 2008). In this study, eugenol was identified as the major chemical compound in clove and cinnamon EOs, accounting for over 75.00% of their composition (Table 1). This finding is consistent with previous studies by Jumbo et al. (2014), Pumnuan and Insung (2016), Jumbo et al. (2018), and Shahina et al. (2022). Turmerone was found to be the major chemical compound in turmeric EO, comprising 68.46% of its composition (Table 1). This is in line with other reports that have demonstrated turmerone as a major compound in turmeric EO, accounting for around 60% of its composition, possibly in the forms of *ar*-turmerone, α-turmerone, and β-turmerone (Jayaprakasha et al., 2005; Liju et al., 2011; Jaiswal and Naik, 2021). However, variations in EO composition can occur due to factors such as geographical location, season, growth stage, and plant parts used.

Most of the emulsion treatments (NEOs, NCSs, and NEOFs, except NEOF-4) at a concentration of 1.0% in water exhibited a nanoemulsion form, with mean droplet sizes below 100 nm (Table 2). Emulsions are isotropic dispersions of 2 nonmiscible liquids, oil, and water. If the mean droplet size is between 100 and 400 nm, they are referred to as microemulsions (Cimino et al., 2021).

Table 3. Toxicity values (LC_{50} and LC_{90}) of different nanoemulsion-based plant essential oil formulations (NEOFs) at various concentrations against the shaft louse (*Menopon gallinae*) and the feather mite (*Megninia ginglymura*) by contact method after various exposure times.

Treatments (NEOFs)[2]	Toxicity[1] Regression[3]	LC_{50} (%) (range)	LC_{90} (%) (range)	SE	χ^2	*P*
Menopon gallinae						
3 h after treatment						
NEOF-1	Y = −3.639 + 19.572x	0.186 (0.166–0.202)	0.251 (0.232–0.286)	1.759	8.148	0.086*
NEOF-2	Y = −3.153 + 18.282x	0.172 (0.162–0.181)	0.243 (0.232–0.257)	1.729	1.872	0.759^{ns}
6 h after treatment						
NEOF-1	Y = −2.474 + 15.507x	0.160 (0.095–0.192)	0.242 (0.208–0.331)	1.384	22.331	<0.001**
NEOF-2	Y = −2.531 + 17.010x	0.149 (0.116–0.169)	0.224 (0.203–0.261)	1.638	8.589	<0.001**
Megninia ginglymura						
3 h after treatment						
NEOF-1	Y = −2.136 + 25.049x	0.085 (0.068–0.103)	0.136 (0.117–0.173)	1.575	17.837	0.001**
NEOF-2	Y = −1.990 + 14.480x	0.137 (0.116–0.176)	0.226 (0.184–0.327)	1.360	11.161	0.025*
6 h after treatment						
NEOF-1	Y = −1.548 + 21.539x	0.072 (0.048–0.095)	0.131 (0.106–0.188)	1.391	25.564	<0.001**
NEOF-2	Y = −1.818 + 15.166x	0.120 (0.091–0.173)	0.204 (0.159–0.395)	1.490	12.782	<0.005**

[1]Data were determined based on $n = 10$ adults of ectoparasites/3 replications lethal concentrations of nanoemulsions of plant essential oil formulas (NEOFs) needed to kill 50 and 90% of the insects or mite (LC_{50} and LC_{90}, respectively) at 3 and 6 h after treatment.

[2]NEOFs are the mixture of various NEOs with different ratios, NEOF-1 (NEO-CL:NEO-CI:NEO-TU = 4:0:0), NEOF-2 (NEO-CL:NEO-CI:NEO-TU = 2:2:0), NEO-CL (clove:Tween60:PEG400 = 2:9:2), NEO-CI (cinnamon:Tween60:PEG400 = 2:9:2), NEO-TU (turmeric:Tween 80:PEG400 = 2:6:3).

[3]Probit (Y) = Intercept + Slope × (Concentration: x), *, **: Significant difference at $P < 0.05$ and $P < 0.01$, respectively, ns: nonsignificant difference. SE: standard error, χ^2: chi-square value.

Table 4. Percentage response (repellency and attraction) of the shaft louse (*Menopon gallinae*) and the feather mites (*Megninia ginglymura*) to different nanoemulsion-based plant essential oil formulations (**NEOFs**) by contact method after various exposure times.

		Menopon gallinae				*Megninia ginglymura*			
		Response[1]				Response[1]			
Treatments (NEOFs)[2]	Concentrations (%)	%R	%A	χ^2	*P*	%R	%A	χ^2	*P*
3 h after treatment									
NEOF-1	0.15	63.3	36.7	1.086	0.297^{ns}	70.0	30.0	2.500	0.113^{ns}
	0.25	83.3	16.7	7.500	0.006^{**}	70.0	30.0	2.500	0.113^{ns}
NEOF-2	0.15	83.3	16.7	7.500	0.006^{**}	60.0	40.0	0.606	0.436^{ns}
	0.25	80.0	20.0	5.934	0.014^{*}	66.7	33.3	1.714	0.190^{ns}
6 h after treatment									
NEOF-1	0.15	60.0	40.0	0.606	0.436^{ns}	73.3	26.7	3.455	0.063^{ns}
	0.25	80.0	20.0	5.934	0.014^{*}	66.7	33.3	1.714	0.190^{ns}
NEOF-2	0.15	86.7	13.3	9.319	0.002^{**}	73.3	26.7	3.455	0.063^{ns}
	0.25	86.7	13.3	9.319	0.002^{**}	73.3	26.7	3.455	0.063^{ns}
Control	0.00	50	50	-	-	Control	50	50	-

[1]Data were determined based on $n = 10$ adults of ectoparasites/3 replications, %R: indicates the percentage response to the treatment (repellency), %A: indicates the percentage response to the control (attraction) at 3 and 6 h after treatment, *, **: Significant difference at $P < 0.05$ and $P < 0.01$, respectively, ns: nonsignificant difference. SE: standard error, χ^2: chi-square value.

[2]NEOFs are the mixture of various NEOs with different ratios, NEOF-1 (NEO-CL:NEO-CI:NEO-TU = 4:0:0), NEOF-2 (NEO-CL:NEO-CI:NEO-TU = 2:2:0), NEO-CL (clove:Tween60:PEG400 = 2:9:2), NEO-CI (cinnamon:Tween60:PEG400 = 2:9:2), NEO-TU (turmeric:Tween 80:PEG400 = 2:6:3).

These nanoemulsions are translucent or cloudy and are created by emulsifying the oily and aqueous phases using an emulsifier, resulting in a stable colloidal system (Perumal et al., 2021). The selection of emulsifier type and oil-to-emulsifier ratios was carefully considered. Nanoemulsions of EOs in water have been shown to enhance the bioavailability and diffusion of EOs due to the wetting ability of surfactants. Cosurfactants are often required because their free energy is higher than that of separate oil and water phases (Cimino et al., 2021). Many studies have reported the use of surfactants and cosurfactants as emulsifiers in nanoemulsions of EOs in water, including poloxamer 188, lecithin, PluronicF68, Tween60, Tween80, decanoyl/octanoyl-glycerides, glycerin monostearate, span 8, and polyethylene glycol 400 (**PEG400**) (Shi et al., 2016; Cimino et al., 2021; Doungnapa et al., 2021; Singh et al., 2021; Yahya et al., 2022). In this study, Tween60 and Tween80 were used as surfactants, and PEG400 was used as a cosurfactant in the nanoemulsion-based formulations of different oils and their ratios, following the recommendations of Doungnapa et al. (2021). The PDI values obtained in this study ranged from 0.02 to 0.34, indicating homogeneity in the particle size distribution of the nanoemulsions (Table 2). A low PDI value (<0.3) indicates a highly stable nanoemulsion, while a higher PDI value (>0.7) suggests a lower degree of uniformity and extensive particle size distribution (Perumal et al., 2021).

The zeta potential values obtained in this study ranged from −4.59 to −0.28 mV, indicating negative zeta potentials (Table 2). This can be attributed to factors such as nonionic surfactants, the negative charge of EO droplets, the adsorption of negative ions on the surface of EO droplets, and the presence of functional groups in the chemical constituents of EO (Perumal et al., 2021). Morphological analysis of the

Table 5. Percentage of mortalities of the shaft louse (*Menopon gallinae*) and the feather mite (*Megninia ginglymura*) and repellent response of different nanoemulsion-based plant essential oil formulations (NEOFs) compared with the insecticide, NCS-E, and control groups.

		Toxicity[2]			
		Menopon gallinae		*Megninia ginglymura*	
Treatments[1]	After treatments (h)	%M	%R	%M	%R
Control (surfactant 0.25%)	3	0.0	-	0.0	-
	6	0.0	-	0.0	-
Cypermethrin insecticide (0.1%)	3	100.0	100.0^{a}	100.0	100.0^{a}
	6	100.0	100.0^{a}	100.0	100.0^{a}
NCS-E (0.25%)	3	90.3	80.0^{b}	100.0	70.0^{b}
	6	96.7	83.3^{b}	100.0	73.3^{b}
NEOF-1 (0.25%)	3	93.3	83.3^{b}	100.0	70.0^{b}
	6	100.0	80.0^{b}	100.0	66.7^{b}
NEOF-2 (0.25%)	3	96.7	80.0^{b}	100.0	66.7^{b}
	6	100.0	86.7^{b}	100.0	73.3^{b}
Sig.[3]		ns	*	ns	**

[1]Control (Tween60:PEG400 = 9:2), cypermethrin insecticide 35%EC (recommended dose; 0.1%), NCS-E (eugenol:Tween60:PEG400 = 2:9:2), NEOF-1 (NEO-CL:NEO-CI:NEO-TU = 4:0:0), NEOF-2 (NEO-CL:NEO-CI:NEO-TU = 2:2:0), NEO-CL (clove:Tween60:PEG400 = 2:9:2), NEO-CI (cinnamon:Tween60:PEG400 = 2:9:2), NEO-TU (turmeric:Tween 80:PEG400 = 2:6:3).

[2]Data were determined based on $n = 10$ adults of ectoparasites/3 replications, %M: percentages of actual mortality, %R: indicates the percentage response to the treatment (repellency).

[3]*, **: Significant difference at $P < 0.05$ and $P < 0.01$, respectively, ns: nonsignificant difference.

[a,b]Significantly different at $p < 0.05$.

nanoemulsion-based EO formulations revealed opaque and nonsmooth surfaces for particle sizes above 20 nm, while dark and spherical surfaces were observed for nanoparticle sizes larger than 78 nm. These morphological characteristics are similar to the findings reported by Perumal et al. (2021), where nanoemulsions from EOs with diameters ranging from 70 to 100 nm exhibited a dark and spherical morphology. Kumari et al. (2018) also observed bright and smooth-surfaced spherical structures in thymol nanoemulsions ranging from 90 to 180 nm. Interestingly, nanoemulsions with nanometric micelle diameters (<20 nm) exhibited nonsmooth surfaced micellar droplets, similar to the findings reported by Moradi and Barati (2019) of rough spherical shapes.

The efficacy of the NEOFs depended on the specific chemical compounds present in the oils. NEOF-1 contained only clove EO, while NEOF-2 integrated both clove and cinnamon EOs (Figure 2). This finding aligns with previous research by Pumnuan et al. (2020), which suggested that combining clove EO with cinnamon or turmeric EOs could be used as an alternative medicinal insecticide for controlling chicken lice on farms. In this study, the combination of clove and cinnamon EOs demonstrated higher activity against the ectoparasites *M. ginglymura* and *M. gallinae* compared to the combination with turmeric EO (Figure 2). Although clove EO has been shown to have higher insecticidal activity than cinnamon EO against bean weevils (Jumbo et al., 2014), cowpea weevils (Jumbo et al., 2018), thrips, mealybugs (Pumnuan and Insung, 2016), and maize weevils (Gonzales Correa et al., 2015), its potential is lower in combination conditions. Numerous studies have demonstrated that the combination of EOs exhibits higher pesticide potential than pure EOs, as observed in antimicrobial activity (Sukatta et al., 2008; Gorni et al., 2009) and insecticidal activity (Benelli et al., 2017; Ríos et al., 2017). The NEOFs in this study exhibited higher pesticidal activity against *M. ginglymura* than *M. gallinae*, additionally, it was observed LC_{50} and LC_{90} value of *M. ginglymura* lower than *M. gallinae* (Tables 3–5). This might be due to the smaller size of *M. ginglymura* (approximately 0.5 mm in length) compared to the shaft louse *M. gallinae* (approximately 2.0 mm in length). However, when applied as a pesticide to control *M. gallinae*, the NEOFs also exhibited effectiveness against *M. ginglymura*. Conversely, the repellent effect tests of NEOF-1 and NEOF-2 demonstrated higher repellent activity against *M. gallinae* than *M. ginglymura* (Tables 4 and 5), likely because *M. gallinae* is more mobility greater than *M. ginglymura*. The repellent effect of NEOF-1 and NEOF-2 might be attributed to eugenol, which serves as an active ingredient and is the major chemical compound in clove and cinnamon EOs. Also, several reports that eugenol has been shown to be an effective repellent against the other insect and mites (Ogendo et al., 2008; Al-Harbi et al., 2021; Abenaim et al., 2022; De Jorge et al., 2022).

To enhance the efficiency of botanical pesticides, the use of NEOFs is recommended, as nanoemulsions have been shown to significantly increase the stability compared to pure EO (Firooziyan et al., 2022). Future research should focus on applying NEOFs as nanoemulsion formulations based on clove and cinnamon EOs under poultry farm conditions to further explore their potential efficacy. Overall, the findings of this study contribute to our understanding of the specific chemical compounds in EOs, the formulation and stability of NEOFs, and their potential as botanical pesticides.

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DISCLOSURES

All authors declare no conflicts of interest.

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Academic Publication :

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