A THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

Insecticidal Activities of Plant Essential Oil Compounds Showing Juvenile Hormone Agonist or Antagonist Activity

식물체 정유 화합물의 곤충 유충호르몬 교란물질 탐색 및 살충활성 검정

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Dong Hwan Park

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By
Dong Hwan Park

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Department of Agricultural Biotechnology
Seoul National University
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APPROVED AS A QUALIFIED THESIS OF Dong Hwan Park
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BY THE COMMITTEE MEMBERS

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ABSTRACT

Pests cause huge economic damages not only by harming crops or livestock production but also transmitting vector borne diseases like malaria, yellow fever and dengue fever. To control pests, chemical insecticides like organophosphates, carbamates, pyrethroids have been commonly used. However, use of chemical insecticides faced several limit such as environmental threat or resistance. Insect growth regulators (IGRs) are attractive alternative pesticides due to their low environmental toxicity and high specificity. Plant essential oils have been reported to show repellent, insecticidal and growth-reducing effect on many insect herbivores.

Recently, it has been reported that plants synthesize secondary metabolites regulating insect juvenile hormone (JH) receptor complex as a part of their defense mechanisms. In this study, 195 plant essential oil compounds were tested for their JH agonist and antagonist activities using a yeast two-hybrid system transformed with the *Aedes aegypti* JH receptor as a reporter system. Among them, 17 compound that showing high JH agonist (JHA) or antagonist (JHAN) activities were identified. They were grouped into 4 groups by their structural similarity. Their insecticidal activity and nematicidal activity were tested against *Aedes albopictus*, *Plutella xylostella*, *Plodia interpunctella*, *Laodelphax striatellus* and *Bursaphelenchus xylophilus.* Against *A, albopictus* 3rd instar larvae, 5 Plant essential oil compounds showed over 70% mortality at 10 ppm. Against 3rd instar larvae of *P. xylostella,* undecyl aldehyde indicated over 80% mortality at 200ppm. Against *P. interpunctella,* nerolidol showed over 80% mortality against 2nd instar larvae and undecyl acetate showed reduced egg hatching rate. Also benzyl sulfide, benzyl disulfide, benzyl trisulde and dodecyl aldehyde showed nematicidal activity against *B. xylophilus*. These results could provide insights on the plant-insect coevolution and may be useful for the development of insect specific and safe pesticides.

Key words: Insect growth regulator, Plant essential oils, Juvenile hormone agonist, Juvenile hormone antagonist

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Insect pests are always being huge problem to human civilization. Agricultural pests cause serious economic damage. Despite the using of pesticides, it is estimated that more than 20% of world's all potential crops are lost to pre-harvest and postharvest pests (Phillips & Throne, 2010). Also medical pests like mosquitoes, stink bug and blood feeding flies act as vectors of human diseases by carrying deadly pathogens. Malaria is considered to be the most threated disease transmitted by *Anopheline* mosquitoes, 3.2 billion people are at danger for malaria and an estimated more than 1 million deaths caused by *Plasmodium falciparum* and *P. vivax* (Nauen, 2007).

Controlling of insects pests are accomplished by chemical insecticides. In 1940, discovery of synthetic organic pesticides seemed to great victory against pests. These chemicals are effective, cheap and capable of wide range of insects. Use of insecticides increase More then 10-fold in 1945 to 2000 (Pimentel, 2009). However, since DDT, environmental and health threats by their high toxicity and bioaccumulation became main problem. Moreover, development of pest resistance and cross resistance became more hard to develop novel pesticides (Casida & Quistad, 1998).

Risks using of synthetic insecticides have become led to the movement seeking alternatives in pest control. One of the alternatives, due to their low toxicity and high specificity, insect growth regulators (IGRs) are attractive pesticides that mimic the action of insect hormones to disrupt insect development (Beckage, Rechcigl, & Rechcigl, 2000). Commercially available IGRs have been divided into three classes,

juvenile hormone (JH) analogues, ecdysone agonists and chitin synthesis inhibitors (Pener & Dhadialla, 2012).

One such mechanism was discovered incidentally through a study involving linden bug, *Pyrrhocoris apterus*. Researchers noticed that "the paper factor" juvabione, a sesquiterpenoid which later became the first botanical JH agonist (JHA), prevented linden bugs from developing into normal adults (Sláma & Williams, 1966b). This phenomenon was suggestive of the fact that plants have developed mechanisms that influence insect hormones as a means of defense against herbivores (W. Bowers, Fales, Thompson, & Uebel, 1966). Following the discovery of juvabione, numerous plant derived sesquiterpenoids were screened for their JHA activities (William S Bowers & Bodenstein, 1971). Despite extensive endeavors however, only few JHAs have been identified (WILLIAM S Bowers, 2012).

On the other hand, Among the botanical insecticides, Plant essential oils and their constituents are major categories that began to be researched in the 1980s (Catherine Regnault-Roger, 1997). Plant essential oils are derived from aromatic plants that have evolved chemical defenses against herbivorous insects for their survival. These substances have been reported to display insecticidal as well as deterrent and repellent activities through a variety of mechanisms (Papachristos & Stamopoulos, 2002; Catherine Regnault-Roger & Hamraoui, 1995; Weaver, Dunkel, Ntezurubanza, Jackson, & Stock, 1991).

It has been reported that *methoprene-tolerant* (*Met*) is involved in JH action as a JH receptor (Shemshedini & Wilson, 1990; Wilson & Fabian, 1986). Met is a member of a protein family known as basic-helix-loop-helix (bHLH)-Per-Arnt-Sim (PAS) transcription factors. bHLH-PAS proteins typically function as heterodimer

with other bHLH-PAS protein (Kewley, Whitelaw, & Chapman-Smith, 2004) . Met of *Aedes aegypti* dimerize with other bHLH-PAS transcription factors such as *Ftz*-F1-interacting steroid receptor coactivator (FISC) or Cycle (CYC) in a JH dependent manner (Li, Mead, & Zhu, 2011; Shin, Zou, Saha, & Raikhel, 2012).

JH-mediated interaction of Met and its partner proteins has been replicated through *in vitro* IGR screening system with yeast two-hybrid β-galactosidase assays, screening of JHA and JH antagonist (JHAN) could be enabled (Shin et al., 2012). Previous studies have hypothesized that plant derived compounds interfering with JH receptor-ligand interactions might be more effective in plant defense mechanisms than JHAs. JHANs isolated from plants such as *Lindera erythrocarpa*, showed high levels of mosquitocidal activities and affected ovarian development of *Aedes aegypti* mosquitoes. This suggested that plants use JHANs as part of their defense mechanisms (Lee et al., 2015).

Based on previous reports, Plant essential oils could be sources of novel JHA and JHAN compounds. In this study, chemical known as plant essential oil compounds were tested for their JHA or JHAN activities using yeast-two hybrid system β-galactosidase assay and their insecticidal effects and biological characteristics of selected JHA and JHAN candidates were investigated. These results could provide insights on the plant-insect coevolution and may be useful for the development of insect specific and safe pesticides.

LITERATURE REVIEW

1. Plant essential oils as insecticide

Side effects of synthetic insecticides on environment or health have generated interest to seeking sustainable alternatives in pest control. Botanical pesticides have potential to be alternatives for pest control. Over the last 50 years, thousands of plants have been screened as potential sources of repellents and insecticides (Sukumar, Perich, & Boobar, 1991). Many plants species produce secondary metabolites to protect them by killing or repelling insects as a part of their defense system.

Among the botanical insecticides, since 1980s, plant essential oils are began to develop with research (Catherine Regnault-Roger, 1997). Plant essential oils are derived from aromatic plants by hydro distillation, steam distillation, dry distillation, or mechanical cold pressing of plants (C. Regnault-Roger, Vincent, & Arnason, 2012). These are produced in 17500 aromatic species of higher plants. These are complex natural compounds which contain 20-80 phytochemicals. These phytochemicals mainly have low-molecular weight volatile mainly of terpenoids and to a lesser extent, aromatic compounds (Bernards, 2010).

Plant essential oils and their constituents showed effective against a large variety of insects. Nineteen plant essential oils reported to show insecticidal activity against Hessian fly (Cecidomyiidae) adults and eggs (Lamiri, Lhaloui, Benjilali, & Berrada, 2001). Thirty four plant essential oils fumigation activity and larvicidal activity by topical application against *Spodoptera littoralis* were determined (Pavela, 2005).

Also plant essential oils deterrent or repellent activities against pea aphid, *Acrythosiphon pisum* has been reported (Zapata, Lognay, & Smagghe, 2010). Park et al, tested 40 plant essential oils fumigant activity against larvae of *L.ingenua* and horseradish, anise and garlic oils showed the highest fumigant activity. Analysis by GC-MS to identification of major compound from horseradish, anise and garlic oils and compounds fumigant activity were tested individually (Park et al., 2006).

Several monoterpenes contained in plant essential oils are neurotoxic to insects. Thymol binds to GABA receptors and disrupts GABA synapses (Priestley, Williamson, Wafford, & Sattelle, 2003). Eugenol acts as a octopamine receptor activator and reduce the production of cyclic AMP (cAMP) in cockroach *Periplaneta americana* (Enan, 2001). Some monoterpenes like Terpinen-4-ol and 1,8-cineole inhibit acetylcholinesterase (Mills, Cleary, Walsh, & Gilmer, 2004).

2. Juvenile hormone

JHs are a group of acyclic sesquiterpenoids that secreted from endocrine glands called corpora allata. Main role of JHs were first recognized and described by Wigglesworth in the blood sucking bug, *Rhodnius prolixus* (V. B. Wigglesworth, 1934). Last instar larvae of *R. prolixus* that implanted young corpus allatum molted to supernumerary larvae, or additional larval instars. Later, absence of JHs at critical period allows to metamorphosis was discovered. It is revealed that JH could regulate the development as status quo agents or metamorphosis inhibitors. In addition, JHs important multiple roles are discovered in insects. JH control metamorphosis, diapause, reproduction, caste determination, and metabolism. (Michel Cusson, Tobe,

& McNeil, 1994; Nijhout, 1998; Raikhel, Brown, & Belles, 2005; Riddiford, 2008)

Structure of JH was first discovered by Roller et al (1967) in lipid extracts from cecropia (Röller, Dahm, Sweely, & Trost, 1967) and showed the hormone to be a sesquiterpenoid, methyl (2E, 6E, 10-cis)-10, 11-epoxy-7-ethyl-3, 11-dimethyl-2,6-tridecadienoate. This compound is now termed as JH I. and second JH, JH II, was identified in smaller amounts in cecropia moth (Meyer, Schneiderman, Hanzmann, & Ko, 1968). JH II differed from JH I contained a methyl group at C7 instead of the ethyl group. The third JH was identified from the cultured corpora allata of *Manduca sexta* in vitro. JH III is differed from the others by the fact that C3, 7, and 11 have methyl groups. Later it is reported that JH III is most universal JH in insect (Judy et al., 1973). And JH I, JH II were identified only in the Lepidoptera (Bergot, Schooley, & de Kort, 1981). JH 0 and 4-methyl JH I were identified in developing embryos of *Manduca sexta*. Another JH, JH III bisepoxide, was found from in vitro cultures larval ring glands of *Drosophila melanogaster* (Richard, Applebaum, & Gilbert, 1989). JH III bisepoxide have a second epoxide group. And JH III skipped bisepoxide was reported recently from the stink bug, *Plautia stali* (Kotaki, Shinada, Kaihara, Ohfune, & Numata, 2009).

The *Methoprene-tolerant* (*Met*) gene was discovered from an ethyl methanesulfonate mutagenesis screen about lethal effect of the JH agonist methoprene against *Drosophila melanogaster*. *Met* mutation caused 100-fold-increased resistance to methoprene then wild type flies. Wilson& Fabian proposed, a role for Met in "JH reception" (Wilson & Fabian, 1986). Later, a high-affinity (Kd – 4.5 nM) JH 3-binding protein was found in the *Drosophila*, and decrease of binding of JH in *Met* mutants was the first evidence that MET might be involved in JH

reception (Shemshedini & Wilson, 1990).

It is identified that Met encodes basic-helix-loop-helix (bHLH) protein containing Per-Arnt-Sim (PAS) transcription regulator family member (Ashok, Turner, & Wilson, 1998). To form active transcription factors bHLH-PAS proteins need to pair with a partner of their family. The first discovered protein partner was Met itself and its paralog Gce (Godlewski, Wang, & Wilson, 2006). But in the presence of JH or JHA MET-MET and MET-GCE formation was reduced (Godlewski et al., 2006). This indicated that Met operates in a ligand-dependent manner (Jindra, Palli, & Riddiford, 2013). In fact, recent studies of *A. aegypti* Met forms a heterodimer with other bHLH-PAS factors such as the steroid receptor coactivator (SRC/FISC) or Cycle (CYC) in a JH-dependent manner (Jindra et al., 2013; Li et al., 2011; Shin et al., 2012).

3. Insect growth regulators (IGR)

Carol Williams suggested using of insects own hormone to pest control, and he termed as “third-generation insecticides” (C. M. Williams, 1967). Schneiderman used the term Insect growth regulators that regulate insect growth and development (Schneiderman, 1972). Now Insect growth regulators are termed as chemicals that interfere with insect specific development, normal growth and reproduction. The first IGRs used for pest control were JH mimics or JH agonist. And chitin synthesis inhibitors and ecdysteroid agonists have been added later.

These insecticides possess relatively low environmental toxicity, such as low toxicity to off-target like man, wildlife, and environment. Furthermore, IGRs high

specificity cause effect against only targeted specific taxa (Dhadialla, 2012).

A. Juvenile hormone agonists

JH regulates molting, metamorphosis and reproduction in insects. Due to its importance JH has long been considered as novel pesticides (M. Cusson, Sen, & Shinoda, 2013). The first JH active compounds were sesquiterpenoid farnesol and farnesal (V. Wigglesworth, 1961). Later these compounds were announced as JH precursors. Later, chemical structures of JHs were discovered. But chemical properties of natural JHs are unstable and have vulnerable sites for degradation caused by lights, water, and temperature to use them into pest management (Judy et al., 1973; Meyer et al., 1968; Röller et al., 1967; Sláma, 1999).

The first botanical JH agonist "The paper factor" contained Canadian balsam fir was first identified by Slama and Williams (Sláma & Williams, 1966b). *Pyrrhocoris apterus* reared on the paper towels made from Canadian balsam fir cause abnormal development like metamorphosis failure and became nymphal-adult intermediate creatures or extra instar nymphs. Also eggs from adults that normally developed showed reduced hatch rate. Eventually, it was discovered that the balsam fir contain juvabione acts as a JHA (Sláma & Williams, 1966a, 1966c; Slama, 1971).

After juvabione, numerous plant derived sesquiterpenoids were screened for their JHA activities (Bowers and Bodenstein 1971). Despite extensive endeavors , only few JHAs have been identified as of now (WILLIAM S Bowers,

2012). but large number of synthetic sesquiterpenoid JHA revealed and in 1972, Zoecon Corporation registered hydroprene and methoprene (isopropyl 11-methoxy 3,7,11 trimethyldodeca-2,4-dienoate), which became first JHA commercialized IGR insecticide (Henrick, 1982). They have been successfully used against mosquitoes, ants and flies and they are still favored as the least toxic, environmentally safe insecticides.

In 1981, Hoffmann-LaRoche laboratories reported that juvenoid contain 4-phenoxyphenyl group showed high JH activity. The most active molecule in 4-phenoxyphenyl series was fenoxycarb (Masner, Dorn, Vogel, Kalin, & Graf, 1981). As the one of the most successful JHA, pyriproxyfen has been commercialized in 1986. It is also fenoxycarb derivatives in which side chain has been replaced by pyridyl structure (HATAKOSHI, AGUI, & NAKAYAMA, 1986).

B. Juvenile hormone antagonists

Since JHA discovered, inspired thoughts that the reverse principle, anti-juvenile hormone agent could be explored to complement the use of JHA (Stall, 1986). And could offer more attractive method of control because accelerate metamorphosis would shorten the larval lifetime (Quistad, Cerf, Schooley, & Staal, 1981).

Fluoromevalonate (FMev), tetrahydro-4-fluoromethyl-4-hydroxy-2H-pyran-2-one, was previously known for its hypocholesteremic activity in mammalian systems, showed anti JH activity in Lepidoptera (Quistad et al., 1981). FMev

induced precocious metamorphosis in several Lepidoptera larvae. And later it was discovered that FMev act as a reversible inhibitor in JH biosynthesis (Quistad et al., 1981).Imidazole caused precocious metamorphosis in *Bombyx mori*. Later substituted imidazoles act as methyl farnesoate inhibitor in JH synthesis (Asano, Kuwano, & Eto, 1986; Unnithan, Andersen, Hisano, Kuwano, & Feyereisen, 1995).

Bowers discovered prococene 1 and prococene 2 that showed anti JH activity in the extract of *Ageratum houstonianum* (W. Bowers, 1976; WILLIAM S Bowers, 1977). These compounds induce precocious metamorphosis, inhibition of vitellogenic development in oocytes. These compounds were shown allactocidal activity by forming highly reactive epoxides in the corpora allata (Barovsky & Brooker, 1979; WILLIAM S Bowers, 1977; William S. Bowers, 1981; Hamnett, Ottridge, Pratt, Jennings, & Stott, 1981).

Recent studies have identified a JH antagonist (JHAN) from plants *Lindera erythrocarpa* and *Solidago serotine*. These compounds were found by yeast-two hybrid system and their JHAN activity and insecticidal activity to *Aedes aegypti* larvae were characterized. Also topical application of these compounds caused a retardation of follicle development in female mosquito ovaries. The discovery of JHANs, along with plant derived JHAs like juvabione, indicate that plants produce IGRs, and that they use these substances as a part of their defense system against herbivores (Lee et al., 2015).

C. Ecdysone agonist

The molting hormone Ecdysteroid is secreted from a pair of insect prothoracic glands as ecdysone and converted to 20-hydroxyecdysone the more active form that initiates a molting process (Smagghe, 2009).

Many investigations have been studied to find insecticides that target ecdysteroid receptors as a target site for insecticides with novel modes of action. The first reports on a non-steroidal ecdysone agonist by Rohm and Haas Co (Aller & Ramsay, 1988). This compounds belonging to bisacylhydrazine class and having ecdysone agonist activity against *Manduca sexta* via interaction with ecdysone receptor (Wing, Slawecki, & Carlson, 1988). It induced rapid inhibition of feeding and a premature molting by interfering with normal cuticle formation. However, RH-5849 was not commercialized due to their low insecticidal activity. But with high potency, Rohm and Hass Co discover additional compounds with the same mode of action. These compounds are tebufenozide (Heller, Klein, Mattioda, & Sagenmüller, 1992), methoxyfenozide (Le, Thirugnanam, Lidert, Carlson, & Ryan, 1996) and halofenozide (RohMid, 1996). These EA BAH classes of insecticides have been shown to displace ponasterone A in competitive radioligand-binding assays. Ponasterone A is a phytoecdysteroid that is about 100 times more potent than 20E. These were shown to selectively bind to EcRs from lepidopteran.

To date, methoxyfenozide is the most widely registered and used BAH insecticide, with registrations in more than 50 countries for use on a variety of crops ranging from vegetables to specialty uses (Smagghe, Gomez, & Dhadialla, 2012).

D. Chitin synthesis inhibitor

Chitin is natural aminopolysaccharide that found in insects, not in human. Chitin is a polymer of N- acetylglucosamine linked by beta-1-4 bonds and forms the exoskeleton of all arthropods and replaced during molt and metamorphosis. Chitin synthesis is an essential process for insect development and reproduction. The processes of its synthesis are considered as effective target site for insecticides because of their high selectivity and environmental safety (Merzendorfer, 2013).

Chitin synthesis inhibitors (CSIs) consist of diverse compounds acting by inhibiting chitin formation to varying degrees (Ishaaya & Casida, 1974). CSIs affect reproduction and development of chitin synthesize in various steps. The application of CSIs induces malformation of the cuticle and a reduction of chitin amounts in cuticle. Also CSIs negatively affect egg development and thus overall fecundity (Acheuk, Cusson, & Doumandji-Mitiche, 2012).

CSIs include pyrimidine-nucleoside peptides, benzoylurea, oxazolines, thiazolidines, tetrazines, thiadiazines, thiophthalimides, and certain chromo- and fluorophores (Merzendorfer, 2013). But the mode of action has been known only for some CSIs.

The pyrimidine-nucleoside peptides like nikkomycins and polyoxins have partial structural similarity with UDP-GlcNAc. These chemicals act as competitive inhibitors interfering with the nucleotide binding site of insect chitin synthases (Cohen, 1987). Polyoxins were isolated from Streptomyces species (Isono, Nagatsu, Kawashima, & Suzuki, 1965). Polyoxins block chitin

synthesis but only a few have been commercialized as fungicides not in insecticides. Overall however, nikkomycins and polyoxins have been shown poor insecticidal activity due to their low bioavailability because of inefficient absorption and efficient detoxification and clearance.

Benzoylphenyl ureas including diflubenzuron, triflumuron, chlorafluazuron, teflubenzuron, hexaflumuron, flufenoxuron, lufenuron, and novaluron have been shown to disrupt cuticle formation causing abortive molting and defects in egg hatching (Grosscurt, 1978; Mulder & Gijswijt, 1973). However, the mode of action of benzoylureas has remained elusive.

METERIAL AND METHODS

1. Plant essential oil compounds

The one hundred ninety five chemicals known as plant essential oil compounds were provided by Prof. Il Kwon Park from forest protection laboratory, Seoul national university (Table. 1). Plant essential oil compounds were dissolved in ethanol.

2. Insects

The *Aedes albopictus* mosquitoes provided by the Korea National Institute of Health were reared at 28°C and 70% relative humidity with a 12 h light :12 h dark cycle in aged tap water (Zheng, Zhang, Damiens, Lees, & Gilles, 2015). Larvae were fed Tetramin fish flakes, and adults were maintained on a 10% sucrose solution. The diamond back moth, *Plutella xylostella*, was reared on plastic cages at 25 ± 1 °C and 60% relative humidity (RH) under a 16 h light: 8 h dark light: dark cycle. Larvae were fed rape leaf, and adults were maintained on a 10% sucrose solution. The Indian meal moth, *Plodia interpunctella*, were reared described as Silhacek et al (Silhacek & Miller, 1972). At 25 ± 1 °C and 60% relative humidity (RH) under a 16 h light: 8 h dark cycle with artificial diet. The non-viruliferous small brown planthoppers, *Laodelphax striatellus*, were reared on rice in plastic cages at 25 ± 1 °C and 60% relative humidity (RH) under a 16 h light: 8 h dark cycle. Pine wood nematodes, *Bursaphelenchus xylophilus*, were provided by Prof. Il Kwon Park from forest protection laboratory, Seoul national university.

Table 1. List of plant essential oil compounds

No	Compound name	No	Compound name
1	Acetophenone	36	Cineole, 1,4
2	Acetyl eugenol	37	Cineole, 1,8(=Eucalyptol)
3	Acetyl isoeugenol	38	Cinnamaldehyde, trans-
4	Allocymene	39	Cinnamyl acetate
5	Angelic acid isoamyl ester	40	Cinnamic acid benzyl ester
6	Angelic acid isobutyl ester	41	Cinnamyl alcohol
7	Anethole, trans-	42	trans-Cinnamic acid
8	Anisaldehyde, m-	43	Citral mixture of ci and trans
9	Anisaldehyde, ρ-	44	Citronellal, (±)-
10	Anisaldehyde, σ-	45	Citronellal, (R)(+)
11	Anisole	46	Citronellal, (S)(-)
12	Aromadendrene, (+)-	47	Citronellol, β-
13	Asarone, α-	48	Copaene, (-)-α-
14	Asarone, β-	49	Cuparene, (+)-
15	Benzaldehyde Reagent Plus	50	Cymene, m-
16	Benzyl benzoate	51	Cymene, ρ-
17	Benzyl salicylate	52	Dipentene, mixture(±Limonene)
18	Bisabolol, α-	53	Dodecyl acetate
19	Borneol, contain ca 20% Isoborneol	54	Estragole
20	Bornyl acetate	55	Eucarvone
21	Butyric acid	56	Eugenol
22	Camphene, (+)-	57	Farnesol
23	Camphene, (-)-	58	Farnesyl acetate, trans-
24	Camphor, (±)-	59	Farnesyl acetate
25	Camphor, (1S)-(-)-	60	Fenchone, (-)-
26	Carene, 3-	61	Fenchone, (+)-
27	Carvacrol	62	Geranyl acetate
28	Carveol, (-)- mixture of isomers	63	Guaiol, (-)-
29	Carvone, (R)-(-)-	64	Globulol, (-)-
30	Carvone, (S)-(+)-	65	Hinokitiol
31	Carvone, dihydro-(+)- mixture of isomers	66	Humulene, α-
32	Caryophyllene oxide	67	3-Phenyl-1-propanol
33	Caryophyllene, β-	68	Isobornyl acetate
34	Cedrene, (-)-α-	69	Isoeugenol, mixture of cis and trans
35	Cedrol, (+)-	70	Isopulegol, (-)-

71	Isopulegol, (+)-	108	Sabinene hydrate
72	Isosafrole, mixture of cis and trans	109	Terpinen-4-ol, (+)-
73	Limonene, (-)-	110	Terpinen-4-ol, (±)-
74	Limonene, (R)(+)-	111	Terpinene, α-
75	Linalool oxide, mixture of isomers	112	Terpinene, γ-
76	Linalyl acetate	113	Terpinolene
77	Linolenic acid methyl ester	114	Thujopsene, (-)-
78	Menthol, (-)-	115	Thymol
79	Menthone,(-)- contains ca, 5% isomenthone	116	Neral
80	Methyl acetate	117	Nerol
81	Methyl eugenol	118	Geranial
82	Methyl isoeugenol	119	Geraniol
83	Methyl salicylate	120	trans-2-Decen-1-ol
84	Myrcene	121	trans-2-Heptenal
85	Myristicin	122	trans-2-Hexenal
86	Myrtenal, (1R)-(-)-	123	trans-2-Nonenal
87	Myrtenol, (1R)-(-)-	124	trans-2-Octenal
88	Nerolidol, cis-	125	Acetic acid cis-3-hexenyl ester
89	Nerolidol, cis amd trans isomers	126	cis-2-hexen-1-ol
90	Nonanal	127	Decyl aldehyde
91	Nonyl acetate	128	Dodecyl aldehyde
92	Nopinone, (1R)-(+)	129	Heptanal(=n-Heptaldehyde)
93	Ocimene	130	Hexanal
94	Octyl acetate	131	n-Hexyl aldehyde
95	Perillaldehyde, (-)-	132	Nonyl aldehyde
96	Perillyl alcohol, (S)-(-)-	133	Octyl aldehyde(=Octanal)
97	Phellandrene, d-	134	Tridecanal
98	Phellandrene, α-	135	Undecyl aldehyde
99	Phenyl ether	136	(Z)-3-hexen-1-ol
100	Phenylethanol, 2-	137	Allyl benzyl ether
101	Pinene, (-)-α-	138	Allyl methyl sulfide
102	Pinene, (1R)-(+)-α-	139	Benzyl sulfide
103	Pinene, (-)-β-	140	Benzyl disulfide
104	Pinocarveol, (-)-trans	141	Benzyl trisulfide
105	Piperitone	142	Diallyl disulfide
106	Pulegone, (R)(+)-	143	Diallyl sulfide
107	Pulegone, (S)(-)-	144	Isopropyl disulfide

145	Isopropyl sulfide	171	pentyl isovalerate(C5 IV)
146	Methyl propyl disulfide	172	pentyl 2-methylbutanoate
147	Propyl disulfide	173	pentyl 3-methyl-2-butenoate
148	Propyl sulfide	174	Pinocarvyl acetate
149	Sodium sulfide	175	1-phenyl-1-ethanol
150	Dipropyl trisulphide	176	2-methylbutyl angelate
151	Methyl propyl sulphide	177	2-methylbutyl isobutyrate
152	Methyl propyl trisulphide	178	2-methylbutyl isovalerate
153	Hexadecanyl acetate C16-Ac	179	2-methylbutyl 2-methylbutanoate
154	butyl isobutanoate	180	2-methylbutyl 3-methyl-2-butenoate
155	butyl isovalerate(C4 IV)	181	2-phenylethyl acetate
156	butyl 2-methylbutanoate(C4 2MB)	182	2-phenylethyl benzoate
157	butyl 3-methyl-2-butenoate(C4 IP)	183	3-methylbutyl isobutyrate
158	Cabreuva wood E:H 10%Fr (E)Nerolidol	184	3-methylbutyl isovalerate
159	Citromethyl acetate	185	3-methylbutyl 2-methylbutanoate
160	isoamyl anglate	186	3-methylbutyl 3-methyl-2-butenoate
161	isoamyl tiglate	187	3-methyl-2-butenyl isobutanoate
162	isobutyl isovalerate	188	3-methyl-2-butenyl isovalerate
163	isobutyl 2-methylbutanate	189	3-methyl-2-butenyl 2-methylbutanoate
164	isobutyl 3-methyl-2-butenoate	190	3-methyl-2-butenyl 3-methyl-2-butnoate
165	methyl cinnamate	191	3-phenyl-1-propan-1-ol
166	methyl N-methylanthranilate	192	Decyl acetate
167	myrtenyl acetate	193	Nonyl acetate
168	neryl acetate	194	Undecyl acetate
169	Patchouil E:H 10%Fr (Pat choulol)	195	Tetradecyl acetate
170	pentyl isobutanoate		

3. Yeast two-hybrid binding test using the β-galactosidase assays

The cDNA fragment encoding the full ORF of *A. aegypti* Met was synthesized (Bioneer, Korea) and inserted into the GAL4 DNA binding domain of the pGBKT7 (Clontech, USA) to make the bait plasmid. To construct the prey plasmid, a partial ORF (M1-V510) of *A. aegypti* FISC was introduced into the pGADT7 vector (Clontech, USA).

Both bait and prey plasmids were then transformed together into Y-187 yeast strains for the yeast two-hybrid binding test using quantitative *β*-galactosidase assays. The transformed Y187 cells were incubated at 30°C in SD -Leu/-Trp (DDO) media.

When yeast cell count reached 2.0×10^6 cells / ml and 100 μl of the cultured cells were distributed into wells of a 96-well plate. In order to estimate JHA activity, plant essential oil compounds (0.01, 0.1, 1 and 10 ppm) and 0.033 ppm of pyriproxyfen was added into each well. And the cells were incubated 3 h and subjected to the *β*-galactosidase assays using the *β*-galactosidase Assay Kit (Thermo Scientific, USA). The assay reaction mixtures in the 96-well plates were incubated at 30°C for 5 h and the OD_{420} was measured using iMark^{TM} microplate reader (BIO-RAD, USA). The obtained OD_{420} values were converted to an arbitrary unit representing JHA activity.

$$\text{JHA activity} = \frac{\text{OD}_{420} \text{ of sample}}{\text{OD}_{420} \text{ of pyriproxyfen (0.033ppm)}}$$

To determine JHAN activity, each well was treated with 0.033 ppm pyriproxyfen and each plant essential oil compound (0.01, 0.1, 1 and 10 ppm). A negative control

treated with 0.033 ppm pyriproxyfen and control solvent (ethanol, DMSO) was placed in each plate for JHAN tests. The cells were incubated for 3 h at 30°C and were subjected to the quantitative β-galactosidase assays. The obtained OD_{420} values for each plant essential oil compounds were converted into an arbitrary unit representing JHA or JHAN activity. The formulae used to convert absorbance values to JHA or JHAN activity are as follows:

$$\text{JHAN activity} = \frac{\text{OD}_{420} \text{ of pyriproxyfen (0.033ppm)} - \text{OD}_{420} \text{ of sample}}{\text{OD}_{420} \text{ of pyriproxyfen (0.033ppm)}}$$

4. Yeast growth inhibition test

The transformed Y187 yeast cells with *A. aegypti* Met-FISC were incubated at 30°C in DDO (SD -Leu/-Trp) media until OD_{600} values reached 0.3-0.4. After harvest, the cells were suspended in the fresh media at a concentration of 2.0×10^6 cells / ml and 200 μl of the cells was treated with 10 ppm of plant essential oil compound in 96-well plates. The treated cells were incubated at 30°C with shaking, and the OD_{600} of each sample was measured at every 3 h for 1 day.

5. Insect dipping bioassay

Ten 3rd instar *A. albopictus* larvae in 5 ml tap water with food mixtures were treated with 10 ppm of each plant essential oil compound. Positive control was treated with 10 ppm of JHA pyriproxyfen and negative control was treated with solvent (ethanol). The number of dead larvae for each compound was counted at 24

h after treatment until 3 days. All experiments were performed as three replicates and the average mortality was calculated.

The insecticidal activity of plant essential oil compounds were determined against 3rd instar larvae of *P. xylostella*, 2nd instar larvae of *P. interpunctella* and 3rd nymph of *L. striatellus*. Dipping solutions of 200 ppm plant essential oil compounds were prepared, mixed with DDW and 0.05% polyvinyl alcohol; insects were dipped 30 seconds. After dipping, water was removed with paper towel and insects moved to petri dishes for observation. Mortality was measured at 24 hour intervals for 3 days. All trials were replicated 3 times.

The nematicidal activity of plant essential oil compounds was determined against *B. xylophilus*. Test solutions were introduced into in wells of 96-well plates. In each well, the concentration of nematodes was between 50 and 150 nematodes (mixtures of juvenile and adult nematodes), per 100 μl of water. Plant essential oil compounds were treated with 200 ppm concentration. Mortality of nematodes was recorded after 72 h under a microscope.

6. Mosquito fumigation bioassay

To demonstrate fumigation activities, fumigation glass cylinder (diameter, 5 cm; height 10 cm) with a wire sieve to separate upper side and lower side were prepared. The *A. albopictus* adults, which eclosed within one day, were placed on the sieve and sealed with para-film (Pechiney Plastic Packaging Company, Chicago, IL, USA). Each glass vial was contained 6 adult mosquitoes (3 male, 3 female) fed on a diet of 10% sucrose solution. In lower side, paper disc (8mm, Advantec, Tokyo, Japan) which applied with 1mg plant essential oil compounds was placed in the bottom of

a glass cylinder. Then lower side of glass vials were sealed with para-film. And glass cylinder was kept at 25°C. Dead mosquitoes were counted at 24 h after treatment until 3 days. All treatment was replicated three times.

7. Indian meal moth egg bioassay

The Indian meal moth eggs laid in black paper within 16 hours were collected. A dipping solution of 200 ppm plant essential oil compounds was prepared, dissolved with acetone. Papers were cut and dipped into plant essential oil compounds solution 10 seconds and then moved into distilled water 30 seconds to cleanse. Total number of eggs was counted after treatment. At 120 h, hatching eggs was checked. (Dyby & Silhacek, 1997)

RESULTS

1. JHA and JHAN activities of plant essential oil compounds

To determine their ability to simulate or interfere with pyriproxyfen-mediated binding of *A. aegypti* MET-FISC, 195 compounds derived from plant essential oils were tested using *in vitro* yeast two-hybrid β-galactosidase assay. Among these 195 plant essential oil compounds, 9 compounds including farnesyl acetate, nerolidol, benzyl trisulfide, nonyl acetate, decyl acetate, undecyl acetate, and dodecyl acetate simulate the binding of *A. aegypti* Met-FISC, demonstrating that these compounds have high JHA activity (Fig. 1). On the contrary, another 21 compounds including farnesol, benzyl benzoate, benzyl salicylate, cinnamic acid benzyl ester, nonyl aldehyde, decyl aldehyde, undecyl aldehyde, and dodecyl aldehyde highly interfere with the binding, suggesting that these compounds have relatively high JHAN activity (Fig. 2). Yeast growth inhibition tests were conducted to investigate the possibility of false signals originating from anti-yeast activities of the plant essential oil compounds (Fig. 3). Among 21 plant essential oil compounds showing high JHAN activity, the addition of 19 compounds resulted in the normal growth of Y187 yeast cells transformed with Met and FISC in non-selective double dropout minimal (DDO, -Leu/-Trp) media, indicating that these compounds directly disrupt the JH receptor complex and exhibit JHAN activity.

Based on their high JHA/JHAN activities and chemical structures, 17 plant essential oil compounds were selected, and they could be classified into 4 groups according to structural relationship between them (Fig. 4).

Table 2. Screening of JHA activity of plant essential oil compounds.

JHA activity	
0.6 ≤ JHA activity	4
0.5 ≤ JHA activity < 0.6	3
0.4 ≤ JHA activity < 0.5	2
0.3 ≤ JHA activity < 0.4	2
0.2 ≤ JHA activity < 0.3	13
0.1 ≤ JHA activity < 0.2	16
JHA activity < 0.1	155

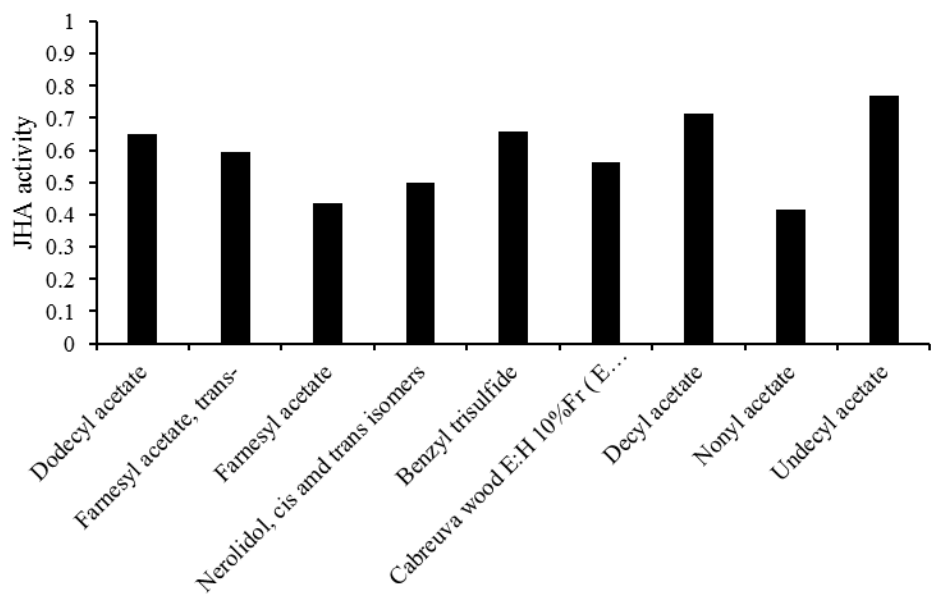

Figure 1. Plant essential oil compounds that showed JHA activity over 0.4.

Table 3. Screening of JHAN activity of plant essential oil compounds.

JHAN activity	
0.6 ≤ JHAN activity	8
0.5 ≤ JHAN activity < 0.6	8
0.4 ≤ JHAN activity < 0.5	5
0.3 ≤ JHAN activity < 0.4	15
0.2 ≤ JHAN activity < 0.3	30
0.1 ≤ JHAN activity < 0.2	58
JHAN activity < 0.1	71

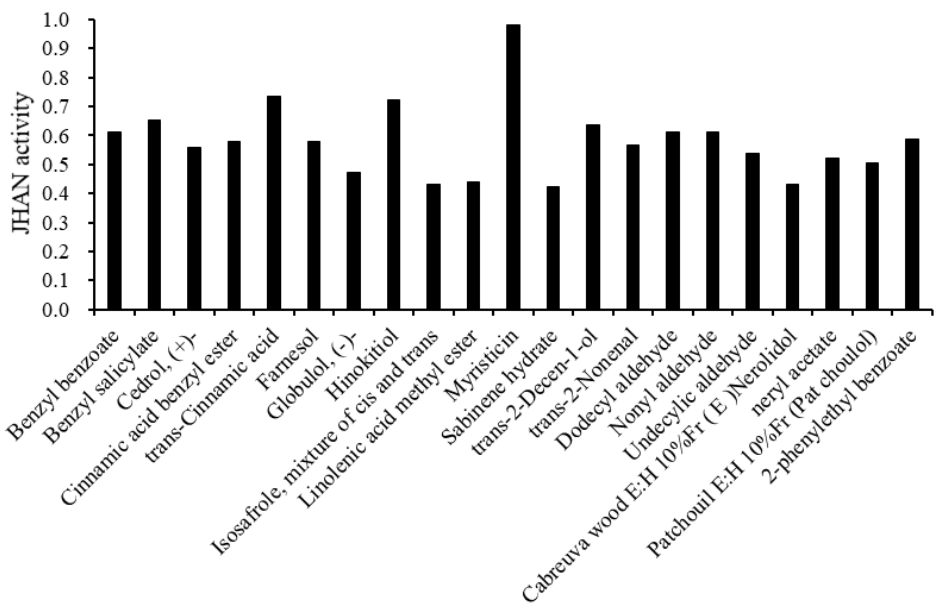

Figure 2. Plant essential oil compounds that showed JHAN activity over 0.4.

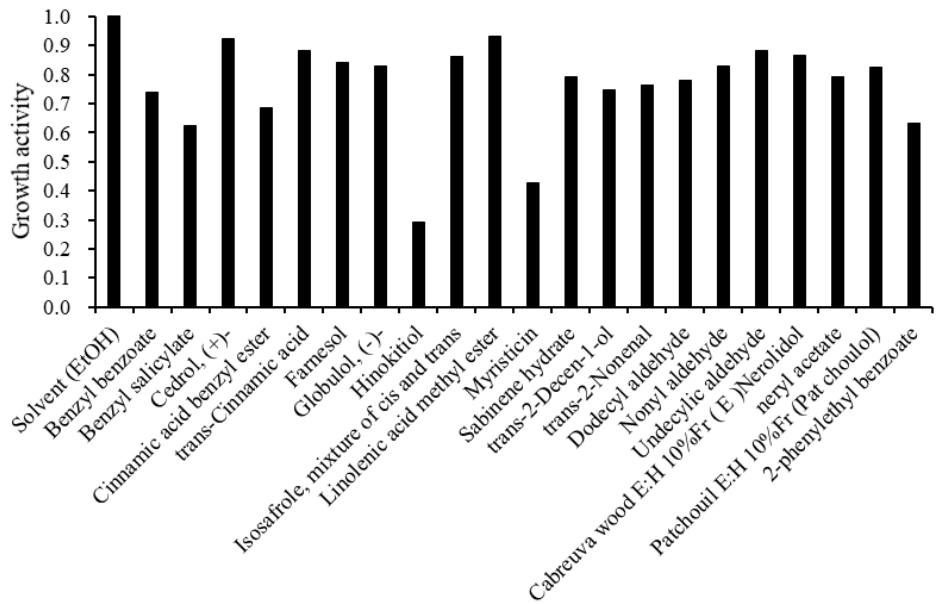

Figure 3. Yeast growth inhibition test of plant essential oil compounds showing high level of JHAN activity.

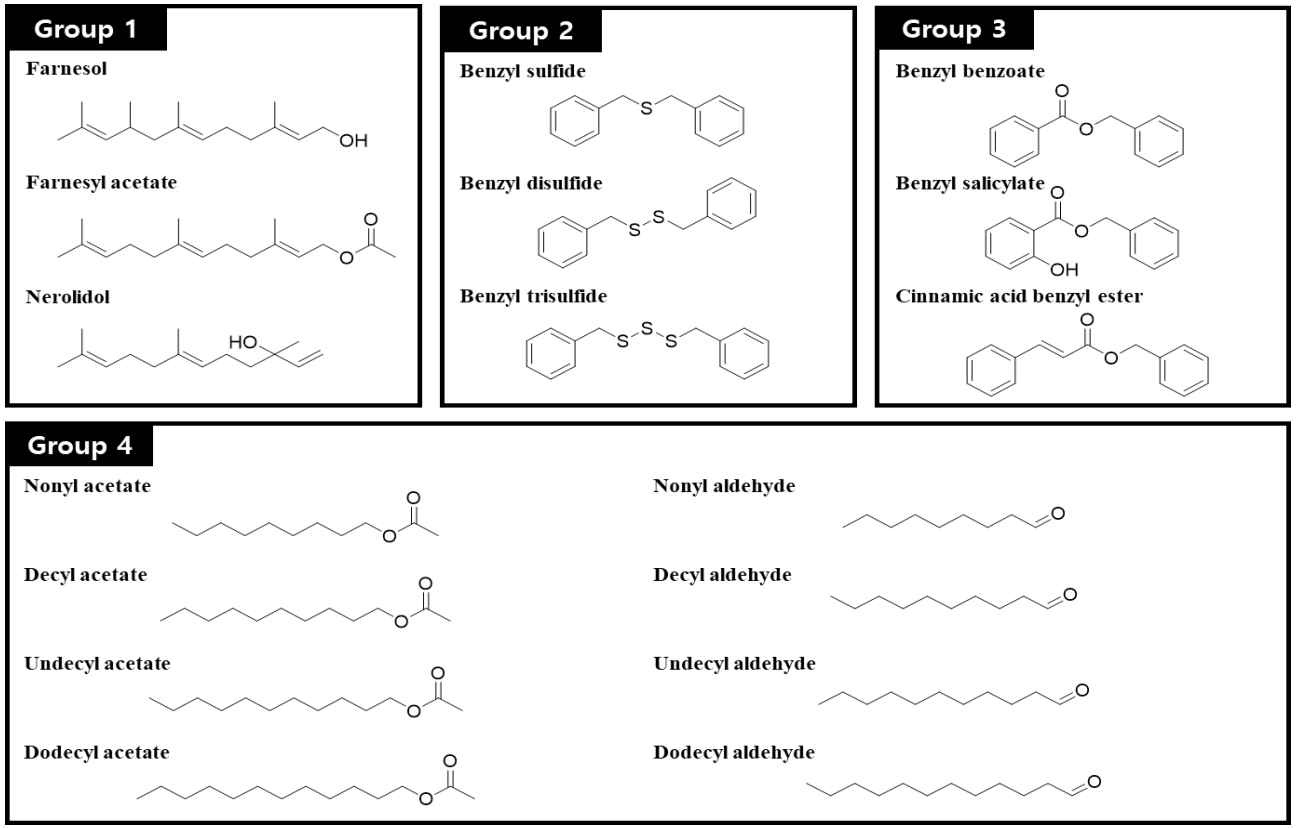

Figure 4. Chemical structure of plant essential oil compounds showing high level of JHA or JHAN activities.

Group 1 consists of sesquiterpenoids, farnesol, farnesyl acetate, and nerolidol. Whereas farnesyl acetate and nerolidol displayed high JHA activity, farnesol which is known as JH precursor showed high JHAN activity. Group 2 are benzyl compounds with two benzene rings connected by sulfide bonds. While JHA activity of group 2 compounds increased with increasing number of sulfide bonds, JHAN activity of them decreased with increasing number of sulfide bonds. Group 3 compounds are benzyl esters with aromatic acids, which showed high JHAN activity. Group 4 compounds are either acetate esters or aldehydes containing alkyl side chains with a backbone of 9-12 carbons. Whereas compounds with an acetate ester functional group showed JHA activity, compounds with an aldehyde group showed JHAN activity.

Selected compounds were subjected to a concentration-dependent β-galactosidase assay to test the effects of increasing concentration on their JHA and JHAN activities, respectively. Among group 1 compounds, the absorbance of farnesyl acetate and nerolidol increased with increasing concentration in the JHA tests, while farnesol showed no JHA activity even at high concentrations (Fig. 5A). However, the absorbance of farnesol decreased with increasing concentration in the JHAN tests (Fig. 6A). The absorbance of group 2 compounds increased with increasing concentration in the JHA tests, and benzyl trisulfide showed the highest JHA activity (Fig. 5B). In the JHAN tests, group 2 compounds displayed a pattern of decreasing absorbance with regards to increasing concentration and benzyl sulfide showed the highest JHAN activity (Fig. 6B). While no noticeable differences between the concentrations of group 3 compounds were observed in the JHA tests (Fig. 5C), the absorbance of these compounds decreased with increasing concentration in the

JHAN tests (Fig. 6C).

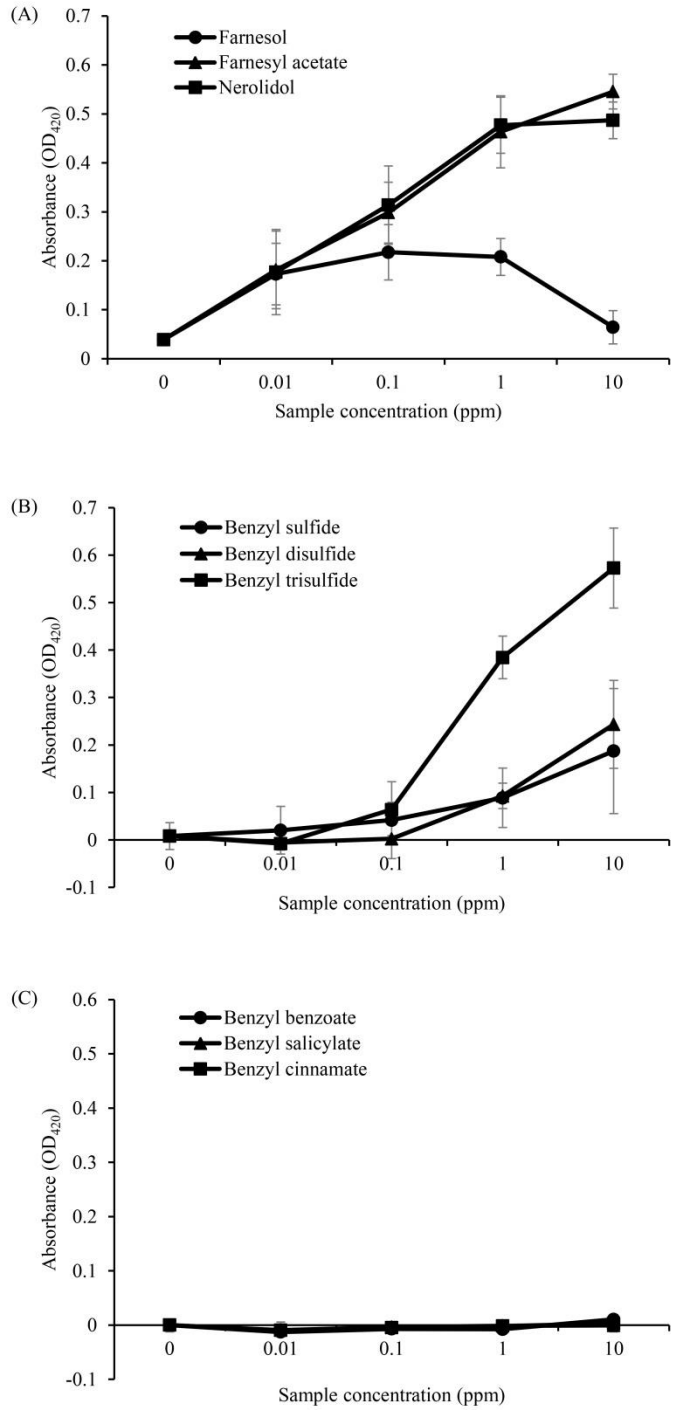

Figure 5. Concentration-dependent stimulation of Met-FISC binding by plant essential oil compounds belong to group 1 (A), group 2 (B), and group 3 (C).

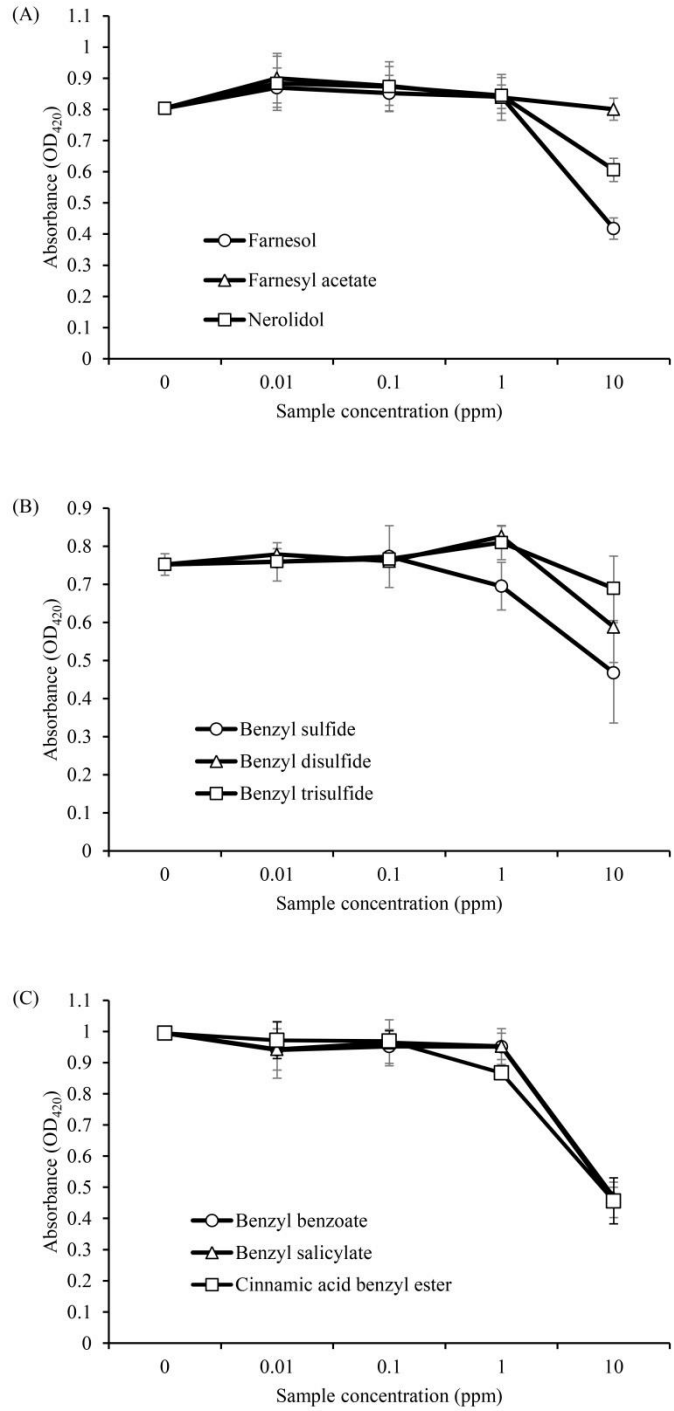

Figure 6. Concentration-dependent inhibition of pyriproxyfen-mediated Met-FISC binding of the plant essential oil compounds belong to group 1 (A), group 2 (B), and group 3 (C). To initiating the binding, 0.033 ppm of pyriproxyfen was applied into each reaction.

2. Insecticidal activity of plant essential oil compounds

The insecticidal activities of plant essential oil compounds with JHA or JHAN activity were determined against to 3rd instar larvae of *A. albopictus* (Fig. 7). When *A. albopictus* larvae were treated with 10 ppm of each plant essential oil compounds, 7 compounds showed high levels of mosquitocidal activities with mortalities above 50% at 72 h. Among them, larvicidal activities of benzyl sulfide, undecyl acetate, dodecyl acetate, nonyl aldehyde, and dodecyl aldehyde were higher than that of pyriproxyfen.

Because plant essential oil compounds are highly volatile, fumigation bioassays against *A. albopictus* adults were conducted with undecyl acetate, dodecyl acetate, undecyl aldehyde, and dodecyl aldehyde which showed high larvicidal activity (Fig. 8). Whereas dodecyl acetate and dodecyl aldehyde showed very low mosquitocidal activity, undecyl acetate and undecyl aldehyde showed 100% mortality at 72 h. In addition, undecyl aldehyde showed more rapid toxicity against adult mosquitoes.

The Insecticidal activities of plant essential oil compounds against lepidopteran pests were investigated by dipping methods at 200 ppm concentration. Against 3rd instar larvae of *P. xylostella*, undecyl aldehyde showed the highest insecticidal activity with 81% larval mortality (Fig. 9). In contrast, nerolidol showed the highest insecticidal activity against 2nd instar larvae of *P. interpunctella* with 81% mortality (Fig. 10). When *P. interpunctella* eggs were exposed to the plant essential oil compounds, undecyl acetate showed noticeable toxicity with 24% of hatching rate (Fig. 11).

For sap sucking *L. striatellus*, farnesyl acetate and decyl acetate showed higher

insecticidal activities than that of pyriproxyfen with 55% and 48% mortalities, respectively (Fig. 12).

The nematicidal activities of plant essential oil compounds with JHA or JHAN activity were determined against *B. xylophilus* (Fig. 13). Among them, Benzyl sulfide, benzyl disulfide, benzyl trisulfide, and dodecyl aldehyde showed over 80% mortality at 72 h.

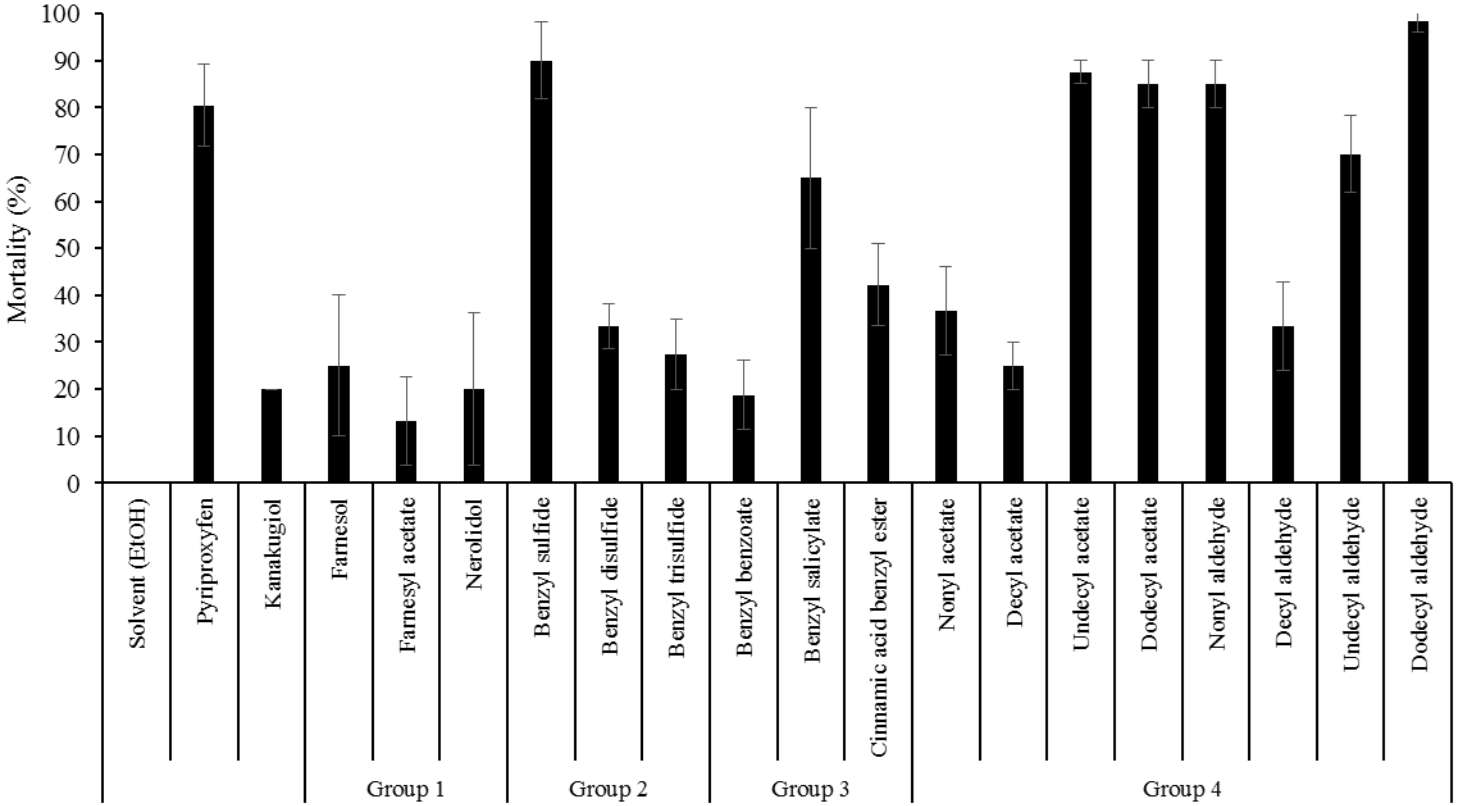

Figure 7. Insecticidal activity of plant essential oil compounds against 3rd instar larvae of *Aedes albopictus*.

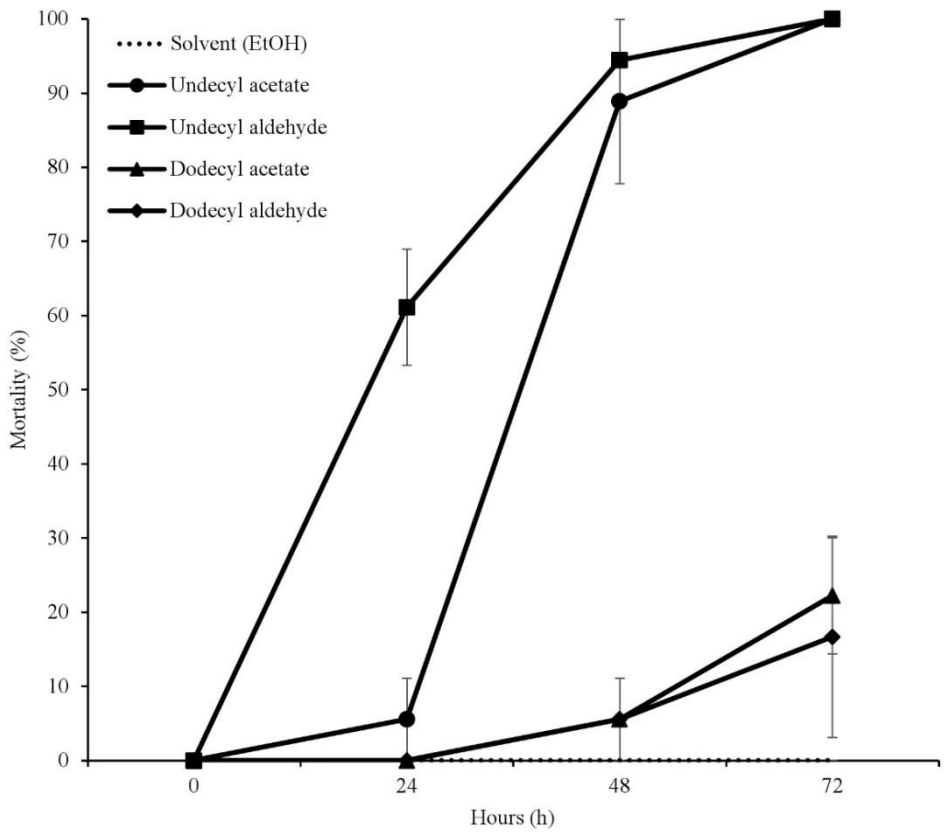

Figure 8. Insecticidal activity of plant essential oil compounds against *Aedes albopictus* adults.

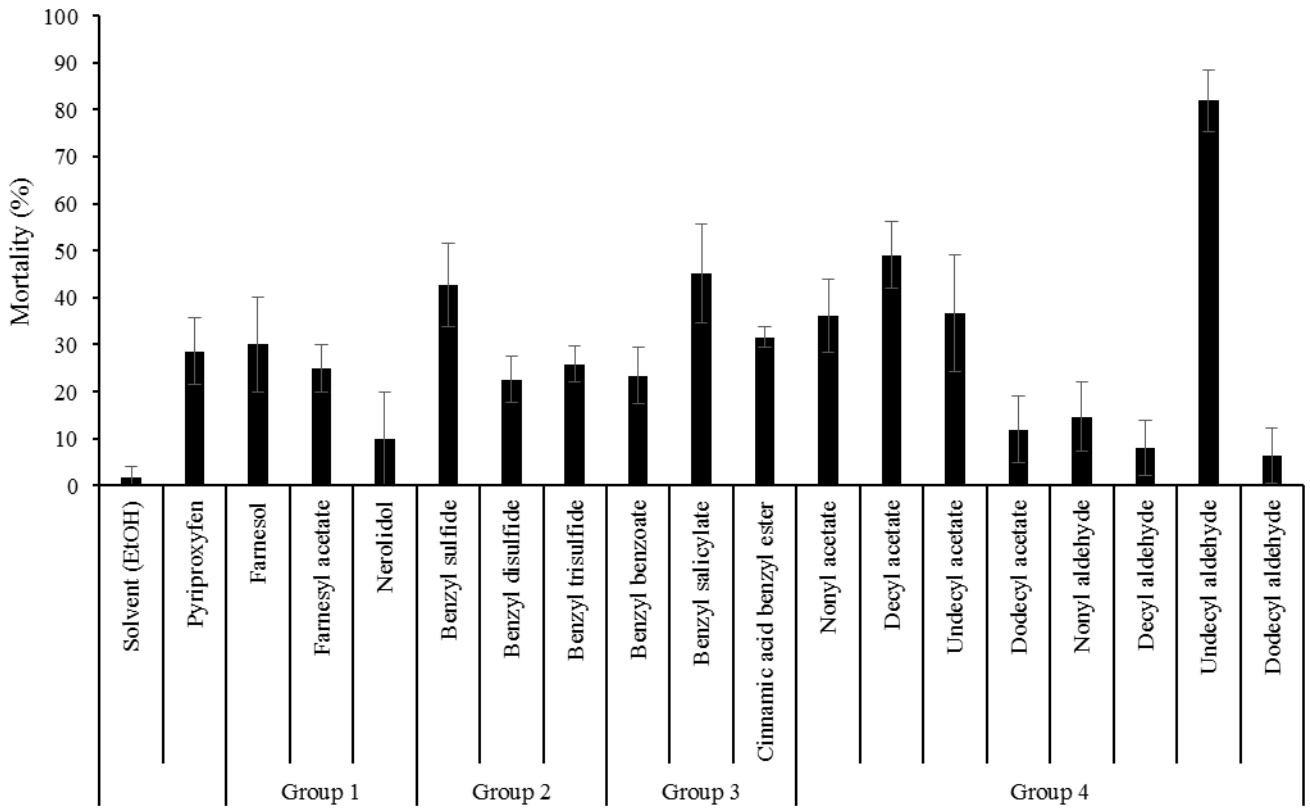

Figure 9. Insecticidal activity of plant essential oil compounds against 3rd instar larvae of *Plutella xylostella*.

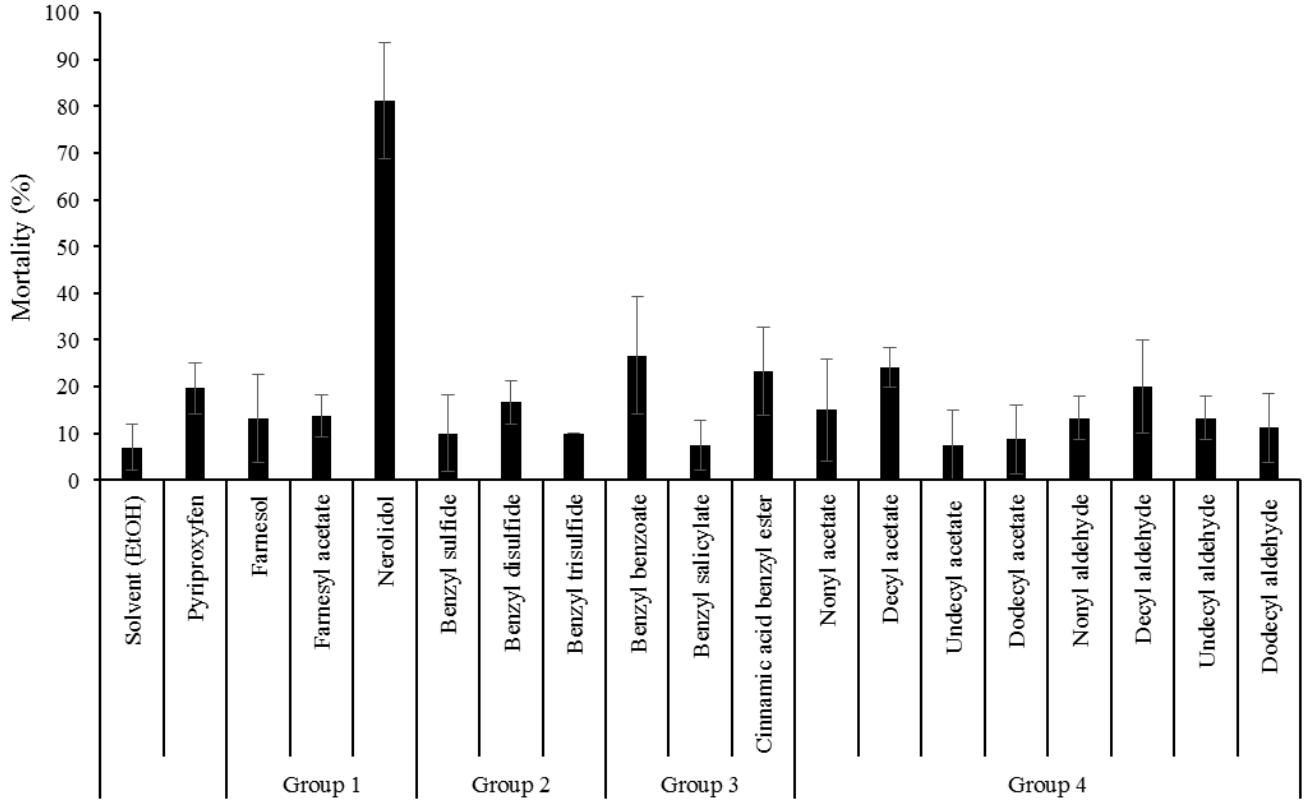

Figure 10. Insecticidal activity of plant essential oil compounds against 2nd instar larvae of *Plodia interpunctella*.

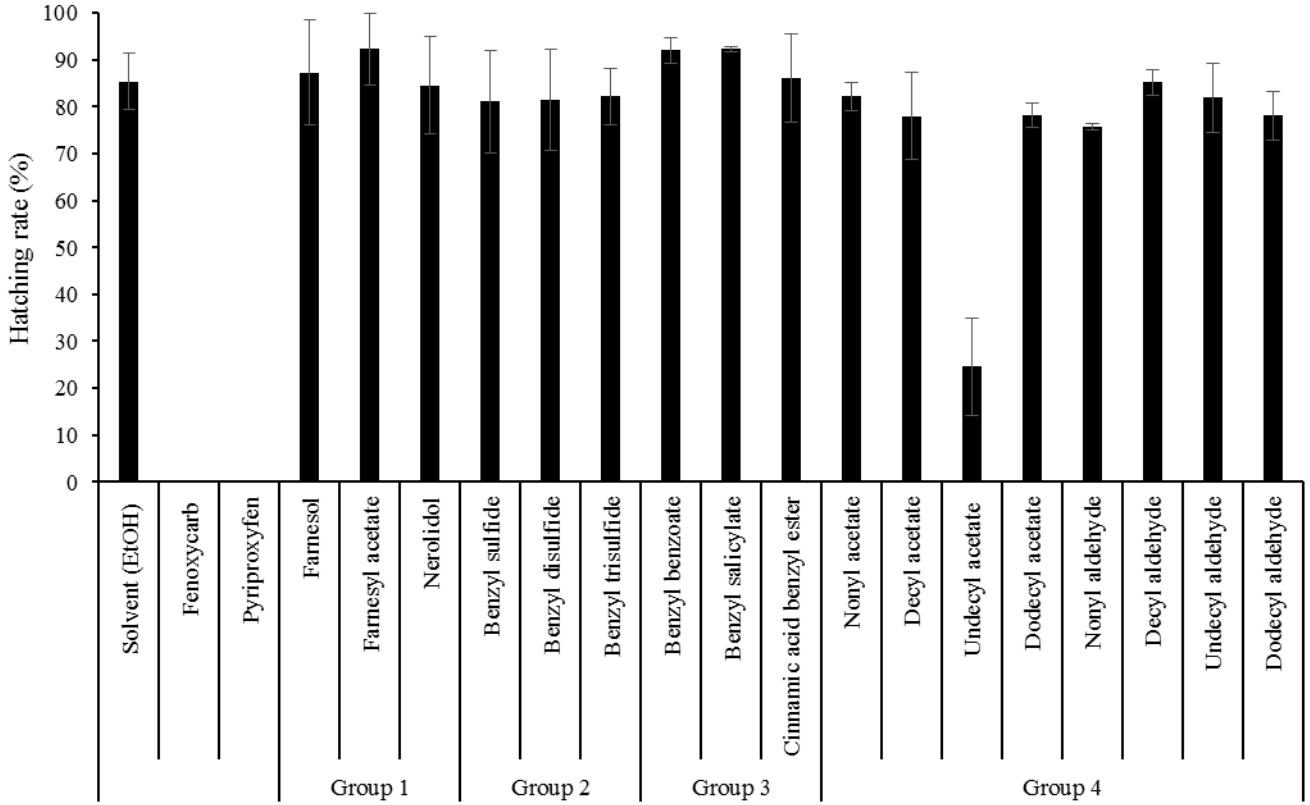

Figure 11. Ovicidal activity of plant essential oil compounds against *Plodia interpunctella* eggs.

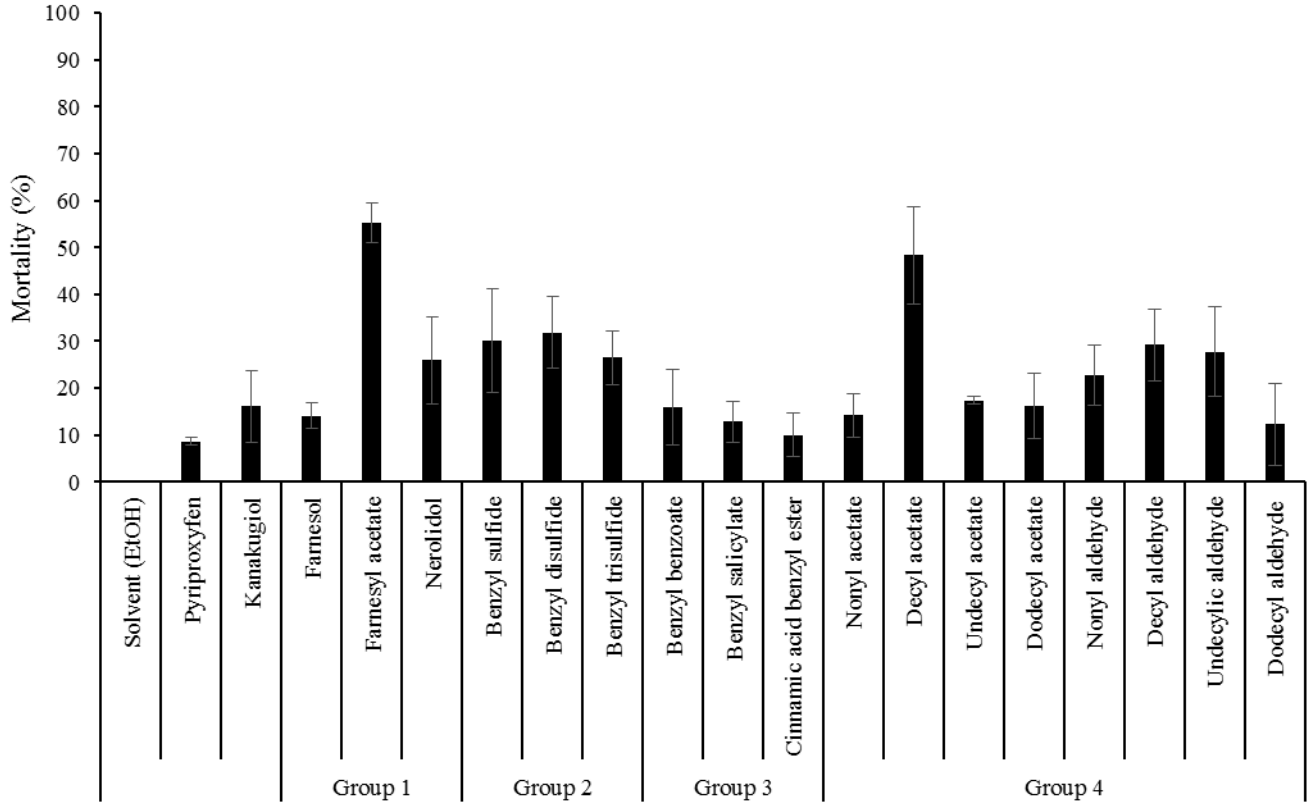

Figure 12. Insecticidal activity of plant essential oil compounds against 3rd instar nymph of *Laodelphax striatellus*.

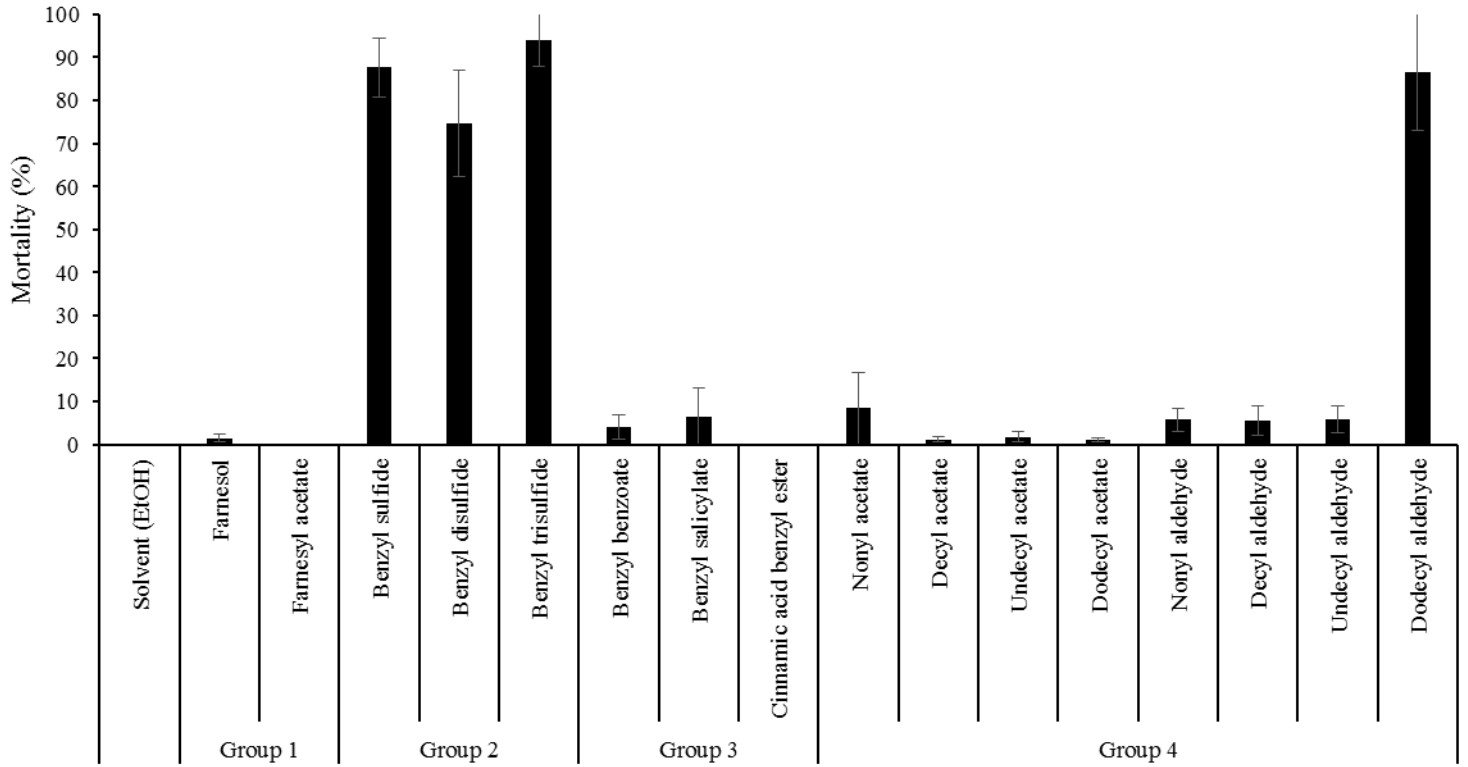

Figure 13. Nematicidal activity of plant essential oil compounds against *Bursaphelenchus xylophilus*.

DISCUSSION

Plants produce various secondary metabolites with insecticidal and repellent activities for defense against herbivores over the long evolutionary history of insect and plant interactions (D. H. Williams, Stone, Hauck, & Rahman, 1989). These plant-derived compounds have become the focus of research on the development of an eco-friendly insecticides due to the richness of their bioactive substances, target specificity and biodegradable nature (Liu et al., 2006). Among them, plant-derived IGRs have been regarded as ideal defensive tactic for plants because they are specific to insects and intrinsically nontoxic to plants (WILLIAM S Bowers, 2012). Furthermore, it may be more difficult for insects to acquire resistance to plant-derived IGRs because they act at the same site and in the same way as natural insect hormones (WILLIAM S Bowers, 2012). Taken together, it is reasonable to assume that plants might be useful sources of novel insecticidal compounds with JHA and JHAN activities. However, only a few JHAs have been reported from plants due to the absence of screening methods.

In this study, several plant-derived JHAs and JHANs were identified from plant essential oil compounds via *in vitro* screening using yeast cells transformed with the *A. aegypti* JH receptors, Met and FISC. In concentration-dependent activity test, nerolidol was shown to readily stimulate Met-FISC binding *in vitro*. Interestingly, nerolidol also showed JHAN activity at high concentration, suggesting that this compound may act as a JHA at low concentrations of JH *in vivo*, but at high concentrations of JH *in vivo*, it could act as an antagonist and compete with JH for binding of Met-FISC receptor complex.

Natural occurrence of farnesol, farnesyl acetate and nerolidol were reported in many plant species. It has been reported that farnesol and nerolidol were synthesized when plants were damaged by herbivores (Schnee, Köllner, Gershenzon, & Degenhardt, 2002). This might be a clue for high insecticidal activities of nerolidol against *P. interpunctella* and farnesyl acetate against *L. striatellus.*

Chemical structures of the group 4 compounds with JHA activity were similar to those of JHs in that they were esters with hydrocarbon side chains. Although the JHs of insects have been reported as a group of methyl ester sesquiterpene epoxides (Cheong, Huang, Bendena, Tobe, & Hui, 2015), neither the terpenoid structure nor epoxide group were essential for JHA activity because the group 4 compounds with JHA activity were not sesquiterpene epoxides. This was further supported by the previous reports that methyl farnesoate, a JH III lacking the epoxide group, acts as a JH in crustaceans and Drosophila (Nagaraju, 2011; Wen et al., 2015). Instead, it was shown that the ester group is critical for JHA activity because group 4 compounds with JHAN activity, in which the acetate ester group was replaced with aldehyde group, exhibited JHAN activities rather than JHA activities.

The group 4 compounds consisted of acyclic alkyl side chains whether they were acetate esters or aldehydes. While the acetate esters comprised of alkyls of 9-12 carbon numbers (i.e., nonyl, decyl, undecyl, and dodecyl acetate) showed high level of JHA activities, octyl acetate did not show JHA activity at all. These findings were consistent with previous reports that the distance between the electronegative end points of JHs dramatically affected their biological activities (Ramaseshadri, Farkaš, & Reddy Palli, 2012). Moreover, hexyl, heptyl, and octyl aldehyde comprised of alkyls of 6-8 carbon numbers were shown to have very low or no JHAN activities,

whereas the aldehydes with alkyl side chains of 9-12 carbon numbers (i.e., nonyl, decyl, undecyl, and dodecyl aldehyde) shown to have high levels of JHAN activities. These results suggested that the length of the alkyl side chain is crucial for not only JHA activities but also JHAN activities. Since both JHAs and JHANs interact with Met to stimulate or interfere with Met-FISC binding, it seems likely that the alkyl side chains of the 9-12 carbon atoms might play a critical role in the interaction with Met.

Insecticidal activities of plant essential oil compounds vary with insect species and life stage. Because of the fact that the screening system used *A. aegypti* MET-FISC, JHA and JHAN activities might be skewed towered mosquitoes. Such differences may also be caused by different susceptibilities of main JH. Corpora allata (CA) of lepidopteran larva secretes JH I and JH II while adult Lepidoptera secretes JH III. In hemipteran, on the other hand, their CA secretes JH III skipped bisepoxide (JHSB3). Structural differences of JH used by different species could be resulted from differences in Met. For example, JH of *Pyrrhocoris* is a JHSB3 and its PAS-B domain sequence has a tryptophan residue instead of tyrosine unlike *Plautia stali*, which produce JH II (Kotaki et al., 2009; Kotaki, Shinada, Kaihara, Ohfune, & Numata, 2011).

In conclusion, several plant essential oil compounds with JHA and JHAN activities were identified using *in vitro* yeast two hybrid system β-galactosidase assays. They showed insecticidal activities against mosquitoes, moths and plant hoppers. These results suggest that the JHAs and JHANs are likely to be deployed in plants as major constituents of defense mechanisms against insect herbivores. The plant-derived IGR compounds identified in this study are expected to provide better

understanding of plant-herbivore interactions and could be exploited for the development of novel insecticides, which are effective and environmentally safe.

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ABSTRACT ON KOREAN

식물체 정유 화합물의 곤충 유충호르몬 교란물질 탐색 및 살충활성 검정

서울대학교
농생명공학부 곤충학전공

박 동 환

초 록

해충은 농작물이나 인축에 큰 경제적 피해를 입힐 뿐만 아니라, 말라리아, 황열 및 댕기열 등의 질병을 매개하여 인류의 건강에 큰 위협을 끼치고 있다. 이러한 해충을 방제하기 위해 유기인계나 카바메이트계, 피레스로이드계 등의 합성살충제가 이용되고 있다. 그러나 합성 살충제는 저항성이나 환경에 대한 독성으로 사용이 점차 힘들어 지고 있다. 이에 대한 대체 살충제로 곤충생장조절제는 저독성이고 곤충 특이적으로 작용하여 매력적인 대안 살충제로 알려져 있다.

식물체 정유에서 식식성 곤충에 대한 살충활성과 기피 활성 그리고 성

장 방해 활성이 보고가 되었고 최근에 식물들이 곤충에 대한 방어 기작으로 생성하는 2차 대사산물 중 곤충 유충호르몬 수용체를 교란하는 물질을 만든다는 것이 알려졌다.

본 연구에서는 195개의 식물체 정유 화합물을 대상으로 그들의 유충호르몬 아고니스트 및 안타고니스트 활성을 *Aedes aegypti*의 유충호르몬 수용 복합체의 유전자를 도입한 yeast two hybrid system을 통해서 알아보았다. 이들 중 17개의 화합물에서 높은 유충호르몬 아고니스트 및 안타고니스트 활성이 확인되었다. 이들은 그 구조적 유사성에 따라서 4개의 그룹으로 분류하였고 그들의 살충활성 및 살선충활성을 흰줄숲모기, 배추좀나방, 화랑곡나방, 애멸구 및 소나무재선충을 상대로 실험해보았다. 흰줄숲모기 3령 유충을 대상으로 한 생물검정에서 5개의 식물체 정유 화합물이 10 ppm의 농도에서 70%이상의 살충활성을 나타내었다. 또한 배추좀나방 3령 유충에 대해서 undecyl aldehyde가 200 ppm의 농도에서 80%의 살충성을 나타내었고, 화랑곡나방 2령 유충을 대상으로 nerolidol이 200 ppm에서 80%정도의 살충력을 보였으며 undecyl acetate의 경우 화랑곡나방 알의 부화율을 감소시키는 것이 확인되었다. 그리고 benzyl sulfide, benzyl disulfide, benzyl trisulfide, dodecyl aldehyde에서 높은 수준의 살선충활성이 소나무 재선충을 대상으로 확인되었다. 본 결과를 바탕으로 식물과 곤충간의 상호작용에 대해서 단서가 될 수 있고 또한 곤충특이적이고 환경에 비교적 안전한 살충제 개발에 도움이 될 것으로 생각된다.

Key words: 유충 호르몬 아고니스트, 유충호르몬 안타고니스트, 식물체 정유, 곤충 성장조절물질

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