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Green insecticide from herbal essential oils against house fly, *Musca domestica* L. (Muscidae: Diptera)

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Abstract The insecticidal effect of essential oils from *Cinnamomum verum* (cinnamon), *Myristica fragrans* (nutmeg) and *Syzygium aromaticum* (clove) against house fly (*Musca domestica* L.) using WHO susceptibility test. Each was applied in ethyl alcohol at concentrations of 1%, 5% and 10% (v/v). Ten percent concentrations of *M. fragrans* and *S. aromaticum* oils were found more effective than cypermethrin (positive control). They gave 100 % knockdown at 15 min, KT_{50} values of 5.94 and 3.80 min, respectively. In addition, 10% *M. fragrans* and *S. aromaticum* oils gave 98% and 100% mortality rate at 24 hrs and LC_{50} were 2.81 and 1.21%, respectively. While, cypermethrin showed 100% knockdown at 30 min, KT_{50} value of 7.28 min and LC_{50} value of 8.45%. The data demonstrated the potential of nutmeg and clove oils to be used as green insecticide against house fly.

Keywords: insecticidal, herbal essential oil, house fly, *Musca domestica* L.

Introduction

The house fly, *Musca domestica* L. (Diptera, Muscidae) is one of the most synanthropic insects in the world. It imposes itself on humans and eats all what is available, food and wastes. Therefore, housefly is considered mechanical vectors of pathogens (bacteria, protozoa and viruses) to human and causes economic problems to all farm animals (cattle, pig and poultry) (Olsen *et al*., 2001; Sangmaneedet *et al*., 2005; Jesikha, 2012). These pathogens may cause many contagious diseases such as food poisoning, diarrhea, cholera, typhoid and paratyphoid, shigellosis, and anthrax (Banjo *et al*., 2005; Fasanella *et al*., 2005; Yap *et al*., 2008). They are also vectors of a variety of eggs from worm parasites (Wattanachai *et al.*, 1996; Ugbogu *et al*., 2006). Moreover, myiases in humans have been reported (Dogra and Mahajan, 2010).

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House fly control is largely based upon the use of chemical insecticides. Majority of the chemical insecticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effect. The increased use of these insecticides may enter into the food chain, and thereby, the liver, kidney, etc., may be irreversibly damaged (Dhanasekaran *et al*., 2013). Currently, the use of eco-friendly, biodegradable insecticides from plants to control insects are gaining importance and botanicals have been found to be effective. Essential oils from plants have been reported to have potential insecticidal effects adult house flies, such as the oils from *Cymbopogon citratus*, *Mentha piperita* and *Lavandula angustifolia* showed 100% knockdown at 30 and 60 min., and LC_{50} values were 2.22, 2.62 and 3.26%, respectively (Sinthusiri and Soonwera, 2013). The essential oils from *Minthostachys verticillata*, *Hedeoma multiflora*, *Citrus sinensis*, *Citrus aurantium*, *Eucalyptus cinerea* and *Artemisia annua* showed LC_{50} of 0.5, 1.3, 3.9, 4.8, 5.5 and 6.5 mg/dm^3 in 30 min, respectively (Palacios *et al*, 2009a; Palacios *et al*, 2009b). Tarelli *et al*,. (2009) found KT_{50} at 2.3 and 19.0 min. for monoterpenes of eucalyptus oil (eucalyptol) and peppermint oil (menthone), respectively. Hence, essential oils from various plants may be potential alternatives to used house fly control. *Cinnamomum verum* J. Presl. *(Lauraceae) Myristica fragrans* Houtt. (Myristicaceae) and *Syzygium aromaticum* (L.) Merrill. & L.M. Perry (Myrtaceae) trees are the local plants in Asia, Southeast Asia and other parts of the tropics, their oils and volatile constituents are widely used in the prevention and treatment of human illnesses because they have the bioactivity of antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory and pesticidal activity (Latha *et al*., 2005; Bhamarapravati *et al*., 2006; Mayaud *et al*., 2008; Pandey and Singh, 2011; Djiani and Dicko, 2012).

Therefore, the present study aimed to investigate *Cinnamomum verum* (cinnamon), *Myristica fragrans* (nutmeg) and *Syzygium aromaticum* (clove) essential oils as an insecticidal against adult house fly.

Materials and methods

Rearing of houseflies

House fly adults were collected from Udomsuk market, Huatakae, Ladkrabang, Bangkok, Thailand. They were reared in gauzier cotton cages (30x30x30 cm) at 30-35°C and 70-80% relative humidity in the laboratory of Entomology and Environmental Programme, Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. They were fed with 10% syrup soaked in cotton wool and powder milk 30 g. Then, 300 g of

Mackerel fish were placed on a plastic tray (18x25x9 cm) lined with sterile coconut husks for house flies to feed and lay their eggs. Four days-old of adult house flies were used for the study.

Essential oils

The essential oils from *Cinnamomum verum* J. Presl. (cinnamon), *Myristica fragrans* Houtt. (nutmeg) and *Syzygium aromaticum* (L.) Merrill. & L.M. Perry (clove) were tested in this study. They were extracted by water distillation at the laboratory of KMITL and prepared as 1%, 5%, and 10% solutions in ethyl alcohol (v/v). All formulations were kept at room temperature before testing.

Chemical Insecticide

Cypermethrin (Kumakai®) was purchased from M.D Manufacturing Co. Ltd., Pranakornsri Ayutaya province, Thailand and used as standard.

Insecticide susceptibility test

The insecticide susceptibility test adapted from WHO guidelines (2006) used a susceptibility test kit. Each tube was marked with either a red spot or a green spot. Ten house flies were collected and released into the green spot tubes lined with clean filter paper (Whatman®No.1, 12x 15 cm) with a movable slide attached. The same size filter paper was cut for the bioassay. The filter paper was impregnated with 2 ml of each essential oil. After the filter paper dried for 20 minutes, it was inserted into the red spot tube for exposure to the house flies. The house flies were exposed to the treated paper for 1 hour in each tube. Knockdown rates were recorded at 5, 10, 30 and 60 minutes. At the end of the exposure period, house flies were released back into the green spot tube and provided with 10% syrup. Mortality was observed after 24 hours. Each test was performed in 5 replicated with simultaneous control sets; the positive controls were impregnated with cypermethrin and the negative controls were ethyl alcohols.

Statistical analysis

The data was pooled and analyzed by standard probit analysis to obtain KT_{50} and LC_{50}. The knockdown and mortality data were statistically analyzed using one-way ANOVA and the data means were compared by Duncan's multiple range test. Significant difference was considered at $P<0.05$. The susceptibility results were determined for each insecticide using WHO criteria (WHO, 1998): 98-100% mortality indicated susceptibility, 80-97% mortality suggested possible resistance needing confirmation and mortality <80% suggested resistance. If mortality exceeded 20% in the control, the whole test should be rejected and repeated. If mortality in the controls were between 5-20%, results with the treated samples should be corrected using Abbott's formula.

Results

Percent knockdown and KT_{50} values of three essential oils at 1%, 5% and 10% concentrations against house flies were shown in Table 1. At 1% concentration, the essential oil from *S. aromaticum* had high knockdown against house flies, there were 90% knockdown at 60 min, KT_{50} was 16 min. While cypermethrin (positive control) gave KT_{50} at 21.62 min. At 5% concentration, *M. fragrans*, *S. aromaticum* and *C. verum* showed 96% knockdown at 60 min, KT_{50} were 12.87, 12.91 and 18.17 min, respectively. Cypermethrin showed 94% knockdown at 60 min, KT_{50} was 15.90 min. At 10% concentration, *S. aromaticum* and *M. fragrans* had 100% knockdown at 15 min, KT_{50} were 3.80 and 5.94 min, respectively. Meanwhile, cypermethrin showed 100% knockdown at 30 min, KT_{50} was 7.28 min. Ethyl alcohols (negative control) percent knockdown could not be found.

The mortality rate, susceptibility and LC_{50} values of house flies for three essential oils were shown in Table 2. At 1% concentration, house flies were resistant to all essential oils and cypermethrin with the mortality rates ranging from 16 to 40%. At 5% concentration, house flies were susceptible (S) to *S. aromaticum* with 100% mortality rates. They were resistance susceptible (RS) to *M. fragrans* and *C. verum*, with the mortality rates of 92 and 82%, respectively. Cypermethrin gave 28% mortality rate which showed resistance to the house flies. At 10% concentration *M. fragrans* increased the mortality rates to 98%. Thus, house flies were susceptible (S) to *S. aromaticum* and *M. fragrans*, resistance susceptible (RS) to *C. verum*, and resistance (R) to cypermethrin. There were significant differences of mortality rates which calculated by using one-way ANOVA. All essential oils gave LC_{50} values at 24 hours after exposure less than cypermetrin. LC_{50} values of *S. aromaticum*, *M. fragrans* and *C. verum* were 1.21, 2.81 and 3.07%, respectively while cypermethrin was 8.45%.

Table 1. KT_{50} values and percent knockdown of house flies by 1, 5 and 10 % concentration of three essential oils after exposure at 5, 15, 30 and 60 minutes

Herbal essential oils	1% Concentration				KT_{50}	5% Concentration				KT_{50}	10% Concentration				KT_{50}
	5 min	15 min	30 min	60 min	min	5 min	15 min	30 min	60 min	min	5 min	15 min	30 min	60 min	min
C. verum	0	0^{C}	20 ± 10.00^{B}	38 ± 25.90^{B}	65.73	0^{A}	58 ± 11.00^{B}	92 ± 8.40^{A}	96 ± 8.90^{A}	18.17	6 ± 8.90^{C}	86 ± 7.10^{B}	94 ± 5.50^{B}	96 ± 5.50^{B}	14.53
M. fragrans	0	0^{C}	16 ± 20.70^{B}	52 ± 17.90^{B}	57.21	0^{A}	90 ± 12.20^{A}	96 ± 5.50^{A}	96 ± 8.90^{A}	12.87	32 ± 30.30^{B}	100^{A}	100^{A}	100^{A}	5.94
S. aromaticum	0	78 ± 4.50^{A}	82 ± 4.50^{A}	90 ± 7.10^{A}	16.00	0^{A}	84 ± 8.90^{A}	84 ± 8.90^{AB}	96 ± 5.50^{A}	12.91	90 ± 10.00^{A}	100^{A}	100^{A}	100^{A}	3.80
cypermethrin	0	54 ± 11.40^{B}	84 ± 11.40^{A}	90 ± 10.00^{A}	21.62	2 ± 4.50^{A}	70 ± 18.70^{AB}	92 ± 13.00^{A}	94 ± 8.90^{A}	15.90	18 ± 21.70^{BC}	96 ± 5.50^{A}	100^{A}	100^{A}	7.28
ethyl alcohol	0	0^{C}	0^{C}	0^{C}	0	0^{A}	0^{D}	0^{D}	0^{B}	0	0^{C}	0^{C}	0^{C}	0^{C}	0
CV%	NA	30.37	32.46	30.64		547.72	20.59	12.9	8.35		68.51	4.96	2.71	2.7	

KT_{50}, 50% knockdown time: NA, not available
Mean % knockdown in each column followed by the same letter are not significantly different (one-way ANOVA and Duncan's multiple range test)

Table 2. The mortality rates, susceptibility and LC_{50} values of house flies to three essential oils

Herbal essential oils	1% concentration		5% concentration		10% concentration		LC_{50} at 24 hrs
	%Mortality	Susceptibility	%Mortality	Susceptibility	%Mortality	Susceptibility	
C. verum	24 ± 19.50^{AB}	R	82 ± 11.00^{B}	RS	88 ± 8.37^{B}	RS	3.07
M. fragrans	16 ± 26.10^{BC}	R	92 ± 8.40^{AB}	RS	98 ± 4.47^{A}	S	2.81
S. aromaticum	40 ± 14.10^{A}	R	100 ± 0^{A}	S	100 ± 0^{A}	S	1.21
cypermethrin	24 ± 0.89^{AB}	R	28 ± 1.92^{C}	R	60 ± 1.22^{C}	R	8.45
ethyl alcohol	0^{C}	R	0^{D}	R	0^{D}	R	NA
CV%	79.29		14.41		8.5		

LC_{50}, 50% lethal concentration: Mean % mortality in each column followed by the same letter are not significantly different (one-way ANOVA and Duncan's multiple range test).
NA, not available: S, Susceptible is defined as 98-100% mortality; RS, Resistance susceptible is defined as 80-97% mortality; R, Resistance is defined as <80%mortality

Discussions

Insecticidal effect of *C. verum*, *M. fragrans* and *S. aromaticum* oils against four days old adult house flies. The results showed that 10% *M. fragrans* and *S. aromaticum* oils had high knockdown rates and susceptibility to houseflies more than cypermetrin. Similarly, Palacios *et al*. (2009a) reported *M. fragrans* oil had effect to house flies, with LC_{50} value was 8.8 mg/dm^3. Jung *et al.* (2007) reported *M. fragrans* seed compound which (1R)-(+)-camphor, (1S)-

(-)-camphor, dipentene, (1R)-(+)-β-pinene, and (+)-α-terpineol were more toxic to adult female german cockroach than propoxur. Phuakbuakhao and Soonwera (2010) found *S. aromaticum* oil had highly effected to american cockroach adult which 100% mortality at 5 min and LT_{50} was 0.59 min. Omara *et al*. (2013) found *S. aromaticum* oil gave highly significant effect against american cockroach nymphs and adult by fumigant test, with LC_{50} value of 1.06 μl and 8.20 μl and 100% mortality at a concentration of 7.5 and 17.5 μl/L of air, respectively. This oil was also most effective to adult mosquitoes and cowpea weevil. Makhaik *et al.* (2005) found *S. aromaticum* oil gave LC_{50} and LC_{95} values at 0.5 and 0.9%, respectively for *Culex Quinquefasciatus* and 2.7 and 5.3%, respectively for *Aedes aegypti*. Mahfuz and Khalequzzaman (2007) found *S. aromaticum* oil was highly toxic against *Callosobruchus maculates*, with LD_{50} of 92.81 and 69.62 μg/cm^2 after expose at 24 and 48 hrs., respectively.

Moreover, *M. fragrans* and *S. aromaticum* has been used in medicine, flavoring food, dental products, perfumes and lotions. In East Asia, the seed of *M. fragrans* were used in medical properties for digestive and kidney problems. In the British herbal Pharmacopoeia, Myristica seed oil is indicated as specific for flatulent dyspepsia, nausea, diarrhea and dysentery (Jung *et al.*, 2007). Dried flower of *S. aromaticum* has long been considered to use in folk medicine as analgesic, toothache, sore throat, respiratory disorder, digestive system ailment, dyspepsia, gastritis, diarrhea, diuretic and carminative (Pandey and Singh, 2011; Debjit *et al*, 2012). Both essential oils have been found inhibited against food borne pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus anthracis*, *B. mycoides*, *B. pumilus*, *B. subtili*, *Saccharomyces cervisiae*, and *Shigella* spp. (Latha *et al*., 2005; Pandey and Singh, 2011).

In conclusion, *M. fragrans* and *S. aromaticum* oils, act not only toxic to adult house flies but also many useful to human. Thus, essential oils from *M. fragrans* and *S. aromaticum* were interesting alternative insecticidal to control house flies. Because, they safe to human, animal and environmental friendly.

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