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Effectiveness of Nano Plant Essential Oils against Brown Planthopper, *Nilaparvata lugens* (Stål)

Anuwat Lakyat, Jarongsak Pumnuan and Ammorn Insung*

Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

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Brown planthopper (BPH), *Nilaparvata lugens* (Stål) is an economically important insect pest of rice. This research study aimed to compare the efficiency of essential oil (EO) and nano essential oils (nEO) of star anise, (*Illicium vercum* (Hook.f.)) and lemon glass (*Cymbopogon citratus* (Dc.exNees)) against BPH and compared to essential oil mixed with tween used as surfactant at doubling concentration (EO2T). Contact toxicity was performed by applying the EOs at 0.0, 0.02, 0.04, 0.06, 0.08 and 0.1% concentrations into the plate which released 20 BPH adult. Then the insect mortality was observed at 12 and 24 hours. As for repellent activity, choice test was used when EOs at 0.02, 0.06 and 0.1% concentrations were applied to 20 BPH released in rice cage and compared with control surfactant. The results revealed that at 12 hours, both EO2T and nEO of plants were highly effective in killing BPH adult. EO2T of star anise showed the highest effective caused 100% mortality at 0.1% concentration with LC50 at 0.028%, followed by nEO of star anise, EO2T of lemon glass and nEO of lemon glass, which gave LC_{50} at 0.032, 0.042 and 0.044%, respectively. For the repellent efficiency test, 0.02% nEO of lemon glass showed the most repellent effect to adult, with 43.83 %RI. As for the toxicity at 24 hours, all EOs and nEOs had extremely effective in killing BPH adults. Those EO2T and nEO of star anise presented LC50 values at 0.025 and 0.031%, respectively. The nEO of lemon glass with 0.02% concentration, showed the most repellent effect to BPH adults, gave their %RI at 32.27%.

Keywords: brown planthopper, nano essential oil, residual exposure method, repellent efficiency test

Introduction

Rice is cultivated extensively in the most diverse ecosystems of tropical and sub-tropical regions of the world. It is the staple food for people in 39 countries, which include 2.70 billion people in Asia alone. Among various biotic constraints of rice production, the insect pests are of prime importance

* **Coressponding Author:** Ammorn Insung; **E-mail address:** kiammorn@gmail.com

and warm humid environment of the crop is also conducive for their survival and proliferation. Among them, brown planthopper (BPH), Nilaparvata lugens (Stål) is a tropical phloem sap feeder and one of the most serious and destructive pests of rice throughout Asia. (Normile, 2008; Heong and Hardy, 2009) The BPH is the most serious rice insect pest worldwide with severe outbreaks in many countries of Asia (Dyck and Thomas, 1979; Pathak and Khan, 1994) including Thailand (Escalada *et al.*, 2012; Sriratanasak *et al.*, 2011) BPH feeds on rice stems directly and it can transmit viral diseases to rice including rice grassy stunt or rice ragged stunt disease (Dale, 1994). Symptoms of rice infestation by BPH include: 1) fewer panicles and grains; 2) lower percentages of ripened grains and gram weight; and 3) high-yield loss from "hopperburn" (Sogawa and Cheng, 1979) Controlling BPH by integrated pest management includes cultural control (Oka, 1979), biological control (Chiu, 1979), chemical control (Heinrichs, 1979) and varietal resistance (Khush, 1979; Pathak and Khush, 1979; Wei *et al*., 2009), which might be the best choice for BPH control (Plantwise Knowledge Bank, 2014) though an insecticidal control method has been most common. However, repeated application of insecticides can cause BPH to develop resistance to insecticides with additional outbreaks (Basanth *et al.*, 2013) consequently, an intensive effort has been made to find alternative methods of pest control. Botanical insecticides and microbial pesticides are highly effective, safe, and ecologically acceptable (Matthews, 1999). Also, botanical insecticides make existing integrated pest management programs (IPM) more effective and sustainable, while decreasing the reliance on synthetic insecticides (Ma *et al*., 2000; Zabel *et al*., 2002) The increasing amount of studies on plant-insect chemical interactions in the last few decades has unveiled the potential of utilising botanical insecticides in the form of secondary plant metabolites, or allelochemicals, as pest control agents. This interest in natural pesticides from the leaves or seed is providing an alternative in pest management programmes to the synthetic insecticides (Whitten, 1992; Martinez and van Emden, 2001).

Materials and methods

Insect cultures

An adult colony of brown planthopper (BPH) (Nilaparvata lugens) was obtained from Pathumtani Rice Research Center, Pathumtani province The insects were cultured on Khao Dawk Mali 105 rice in lnsectary at Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand.

Essential oil and nano essential oil preparation

Essential oils (EOs) of star anise and lemongrass used in the experiments were purchased from Thailand – China Flavours and Fragrances Industry Co., Ltd. The essential oils were diluted with distilled water to get 0.0, 0.02, 0.04, 0.06, 0.08 and 0.1% solutions which Tween-20 at doubling concentrations was added as an emulsifier.

To prepare nano plant essential oils, each EO was diluted in water when primary surfactant (surfactant) was tween20 (HLB = 16.7), and co-surfactant was ethylene glycol 400 (PEG400) (HLB = 13). The previous surfactant matches with the ingredients called Smix. Each couple would have ratios of 1: 1, 1: 1.5 to 1: 2, 1: 2.5, 1: 3, 1: 3.5, 1: 4 and 1: 4.5 and after that filled with distilled water for 10 ml, then essential oils of star anise and lemon grass, mixed with Smix ratios of 1: 1, 1: 1.5 to 1: 2, 1. 2.5, 1: 3, 1: 3.5, 1: 4 and 1: 4.5, set aside at room temperature. Reduction of particle size of plant essential oil was done by using High Pressure Homogenizer. Beside the satiability refers as zeta Potential Charge of nano plant essential oils was measured by Nano plus Zeta / Nano Particle Analyzer.

Residual exposure method

The efficiency of essential oils from star anise and lemon glass, diluted in water mixed with tween at doubling time and nano essential oils on BPH was evaluated using the residual exposure method. Amount of 1 ml of each treatment was poured onto filter paper, air dried for 10 min and placed within Petri dishes (5 cm diameter, 1.2 cm height). Then 20 BPH adults were transferred to each Petri dishes and BPH mortality was checked after 12 and 24 hrs. Abbott's formula was used to calculate the actual death rates (Abbott, 1925). The experiment was designed in three completely randomized replicates, and the median lethal concentration (LC50) of the different plant essential oils were calculated using SPSS statistic package (version 11.0).

Repellent efficiency test

The bioassay as choice test was performed when two clumps of rice were sprayed with various essential oils concentrations (T) and other two clumps of rice sprayed with water mixed with tween-20 used as surfactant at doubling concentration as a control (C). Twenty adults of BPH were released in the center of pot. Treated rice was covered with nylon net cloth. Observation was conducted at 12 and 24 hours after treatment. 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

Statistical analysis

The experiment was a completely randomized design with three replicates. The actual death rates were calculated using Abbot's formula (Abbott, 1987). The data was processed using the applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT) in SAS. Then, the statistical outputs were presented. The median lethal concentration (LC_{50}) was calculated by the probit analysis in SPSS.

Results

The residual exposure method

The results of residual exposure method showns in Table 1 that how essential oils with tween used at doubling rate (EO2T) and nano essential oils (nEO) effect to the BPH at 12 hours, EO2T of star anise showed the highest effective caused 100% mortality at 0.1% concentration with value of LC50 at 0.028%, followed by nEO of star anise, EO2Ts of lemon glass and nEOs of lemon glass, which gave LC50 at 0.032, 0.042 and 0.044, respectively. As for the toxicity at 24 hours, all EO2Ts and nEOs were extremely effective in killing BPH adults. Those EO2Ts of star anise, nEOs of star anise, EO2Ts of lemon glass and nEOs of lemon glass, presented LC_{50} values at 0.025, 0.031, 0.037 and 0.041, respectively. Everytimes, all EO2Ts and nEOs at 0.08 and 0.1% concentrations were not significantly different at 0.02, 0.04 and 0.06% concentrations.

Repellent efficiency test

The results of the choice test are shown in Figure 1. The repellent index (%RI) showed that at the 12 hours, nEO of lemon glass with 0.02% concentration showed the most repellent effect to adult, with 43.8 %RI, followed by nEO of star anise 0.02%, EO2Ts of lemon glass 0.1% and EO2Ts of star anise 0.1%, which gave % RI at 23.0, 21.9 and 20.8%, respectively. Later on, 24 hours. The nEO of lemongrass with 0.02% concentration showed the most repellent effect to BPH adult, gave their % RI at 34.3%, followed by nEO of lemon glass 0.1%, nEO of star anise 0.02% and nEO of star anise 0.1%, which gave % RI at 17.1, 15.7 and 14.2%, respectively.

Discussion

The results showed that the essential oil with tween at doubling concentrations (EO2T) and nano essential oils (nEO) of star anise and lemon glass were extremely effective in killing BPH adult, whereas EO2T of star anise showed the highest effectiveness caused 100% mortality at 0.1% concentration, followed by nEO of star anise, EO2T of lemon glass and nEO of lemon glass. Likewise, Insung *et al.* (2014) reported lemon glass, star anise and dill EOs (0.5%) mixed with petroleum oil (0.5%) in water showed high insecticidal properties against BPH by direct spray method in insectary. Chantawee *et al.* (2012) reported Insecticidal property of some plants essential oils against adult of BPH by using direct spray method. The essential oils of lemon grass and star anise at 0.2% concentration in corporate with petroleum oil were the most toxic to the BPH, caused more than 68% mortality. The comparative study presented that the insecticide (imidacloprid) at recommendation rate and double rate caused only about 50% mortality.

For the repellent efficiency test, 0.02% nano essential oils of lemon glass showed the most repellent effect to BPH adult at the time, 12 and 24 hours. Likewise, Musa *et al.* (2015) reported that lemongrass was a safe and natural insect repellants that was just as effective as the commercial chemical product. Olivero-Verbel *et al*. (2010) reported these compounds could be responsible for higher bio activity of lemon glass essential oil against Tribolium castaneum. The, results presented that the essential oils of lemon glass was good candidate for use as repellent against *Tribolium castaneum*.

Table 1. Mortality percentage of brown planthopper, *Nilaparvata lugens* (Stål) after exposured to essential oils with tween and nano essential oils

Time	Essential oils	Mortality (%) (Mean ±SD) Concentration (%) 0.0	0.02	0.04	0.06	0.08	0.1	Pr > F	%CV	LC_{50}	Slope	Standard Error
12 hours	EO2T of Star anise	1.9 ±3.2Ac	14.2 ±10.3Ab	91.3 ±12.3Aa	100 ±0.0Aa	100 ±0.0Aa	100 ±0.0Aa	<.0001	9.88	0.028	102.30	9.31
	EO2T of Lemon glass	2.2 ±3.8Ac	8.8 ±4.3Ac	45.0 ±22.3Bb	94.2 ±6.3Aa	96.7 ±5.8Aa	97.5 ±4.3Aa	<.0001	17.74	0.042	51.00	3.43
	nEO of Star anise	0.0 ±0.0Ad	9.8 ±4.1Ac	78.5 ±8.7Ab	100 ±0.0Aa	100 ±0.0Aa	100 ±0.0Aa	<.0001	6.08	0.032	106.36	10.21
	nEO of Lemon glass	1.1 ±1.9Ae	20.5 ±14.5Ad	35.2 ±10.2Bc	70.6 ±7.4Bb	100 ±0.0Aa	100 ±0.0Aa	<.0001	14.44	0.044	47.45	3.28
	%CV	207.28	70.49	23.06	5.32	2.91	2.14					
24 hours	EO2T Star anise	8.0 ±2.6Ac	22.4 ±4.5Ab	91.2 ±12.3Aa	100 ±0.0Aa	100 ±0.0Aa	100 ±0.0Aa	<.0001	7.79	0.025	74.24	6.08
	EO2T of Lemon glass	4.3 ±5.2ABc	16.5 ±6.3Ac	48.6 ±20.2Bb	95.8 ±7.2Aa	96.6 ±5.8Aa	100 ±0.0Aa	<.0001	16.03	0.037	51.89	3.63
	nEO of Star anise	1.1 ±1.9Bd	12.9 ±3.7Ac	82.1 ±6.2Ab	100 ±0.0Aa	100 ±0.0Aa	100 ±0.0Aa	<.0001	4.60	0.031	95.04	8.64
	nEO of Lemon glass	8.8 ±3.1Ad	20.5 ±14.5Ad	37.6 ±10.2Bc	73.2 ±8Bb	100 ±0.0Aa	100 ±0.0Aa	<.0001	14.15	0.041	41.20	2.78
	%CV	61.14	46.55	20.44	5.83	2.91	0.0					

EO2T = tween used as surfactant at doubling concentration., nEO = nano essential oils.

Means in column followed by different capital letter indicate significant differences between the substances.

Means in row followed by different common letter indicate significant differences between the substances at (P □ 0.05) (one-way ANOVA).

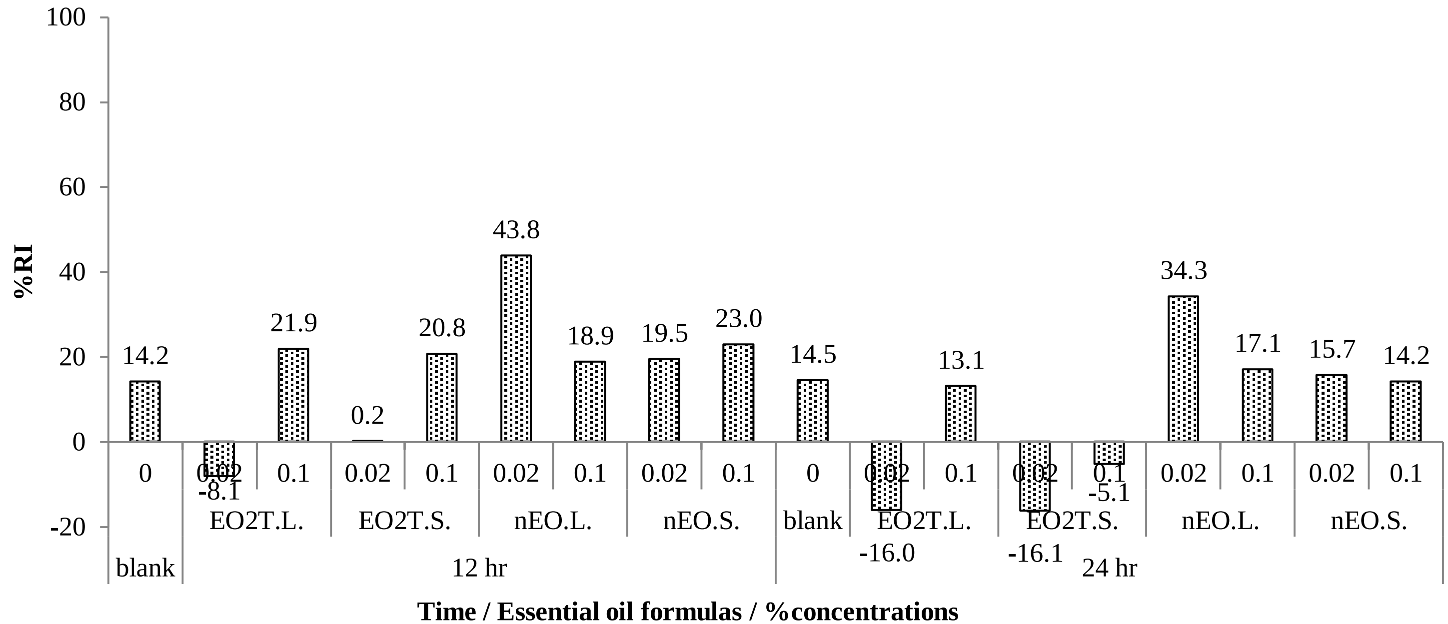

Figure 1. Repellent index (%RI) of essential oils with tween and nano essential oils to adult stages of *Nilaparvata lugens* (Stål) as determined in a choice test bioassay after 12 and 24 hours.

Conclusion

The results revealed that at 12 and 24 hours, all tween used as surfactant at doubling concentration and nano essential oils at 0.08 and 0.1% concentrations were highly effective in killing BPH adult. For the repellent efficiency test, 0.02% nano essential oils of lemon glass showed the most repellent effect to BPH adult with 43.83, 32.27 %RI at 12 and 24 hours, respectively.

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