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Brown planthopper vector–virus transmission in rice and inhibitory effects of plant essential oils

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Abstract The plant essential oils (plant-EOs) are increasingly interested for the global trend of the sustainable agricultural productions and managements. In the present study, the anti-viral biological activity to inhibit transmission abilities of *Rice ragged stunt virus* (RRSV) and mortality of the brown planthopper (BPH, *Nilaparvata lugens* Stål) in different 10 plant-EOs from Thai herbal plants is reported. Of these, those concentrations of plant-EOs between 0.002 to 0.1% from lemongrass, star anise, black pepper, kaffir lime, and kaempfer were highly efficient (10-70%), when compared with the lime, galangal, holy basil, sweet basil, and betelvine which were slightly efficient (10-30%), respectively. In addition, the plant-EOs from lemongrass and star anise were showed 100% BPH mortality at 3% and 5% in all insects' growth stages. The eggs and instar-nymph stages of BPH vector showed the abnormal shapes and structures that were completely destroyed in 3 days after treatment, whereas the adult stage showed the malformed morphology and died in 5 DAT. The plant-EOs of star anise and lemongrass expressed an effective anti-viral activity to inhibit RRSV-transmission abilities and BPH mortality, which are possible to be the potential candidates for the further development as new commercial anti-viral agents, concrete strategic planning for sustainable agricultural development and management to improve the farmers' quality of life and food security in Thailand.

Keywords: Plant essential oils (plant-EOs), Anti-viral biological activity, *Rice ragged stunt virus* (RRSV), Brown planthopper (BPH, *Nilaparvata lugens* Stål), Lemongrass, Star anise

Introduction

The rice (*Oryza sativa* L.) is an economically and culturally significant food crop for the Thai people. The rice production in Thailand is highly dependent on the monsoon rainfall and irrigated management, which are the most vulnerable to damage by the outbreak of the monophagous sap-sucking (phloem-feeder) insect vector, the brown planthopper (BPH, *Nilaparvata*

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lugens Stål) (*Hemiptera*: *Delphacidae*) in the irrigated rice-cultivation systems of the central plain and lower northern regions, and to transmit rice plant viruses in a persistent-propagative manner (Ling and Aguiero, 1977; Ling *et al.*, 1977), expecially *Rice ragged stunt virus* (RRSV) (Chetanachit *et al.*, 1978), that have caused about 10-100% yield losses, respectively.

RRSV was the first discovered and reported in Indonesia in 1976 (Chen *et al.*, 1997). It has become one of the most important rice plant viral diseases in East and Southeast Asian including, Philippines (Ling, 1977) and Malaysia (Hashim and Ang, 1984) in 1977; India (Naik, 1979), Sri Lanka (Heinrichs and Khush, 1978; Jayasena and Hibino, 1987), and Taiwan (Chen, 1984) in 1978; Japan (Senboku *et al.*, 1978; Hibino, 1996) in 1978-1979; China (Zhou and Ling, 1979) in 1979 and Vietnam (Suprihanto *et al.*, 2015) in 2005, respectively. For Thailand, RRSV in the local Thai name was caused rice viral disease and called Rok-Bai-Ngik-Khao, that was the first discovered in Bang-Nam-Priao district, Chachengsao province, in 1977 (Weerapat and Pongprasert, 1978), and was reported and described in 1978 (Chetanachit *et al.*, 1978). The high levels of viral infections were observed in the irrigated rice-cultivation system of the central plain (highest plantations) and lower northern regions (main cultivation area) in 1979; 1980-1982; 1988-1990; 1998-1999; and 2009-2011, respectively, and have since occurred sporadically between 2011 to the present (Na Phatthalung and Tangkananond, 2017).

RRSV is a member of the genus *Oryzavirus* of the family *Reoviridae* which the particle is a non-enveloped turreted reovirus, and double-shelled icosahedral capsid protein (~55-65 nm in diameter) (Chen *et al.*, 1997). Its genome comprises the multiple segments of 10 linear double-stranded RNAs (linear-dsRNA, numbered S1 to S10), which the segments are ranged in size from 1,162 to 3,849 bps with a total length of 26.164 Kb (MW 16.5×10^6) (NCBI database; Kawano *et al.*, 1984). They code for proteins, which are replicated in both plants and insect vectors. These genomes encode at least eight structural proteins (SPs), including P1, P2, P3, P4a, P5, P8a, P8b, and P9, and three non-structural proteins (Pns), including Pns6, Pns7, and Pns10, respectively (Kusuma *et al.*, 2018).

The concerning outbreak of rice plant viruses and insect vectors, which the interesting alternatives of various substances of natural compounds in plants have been used for eco-friendly pest controlling and managing are green pesticides, especially the active plant-essential oils (plant-EOs). Their applications in the integrated management strategy and policy can be combined with different biological methods due to a wide range of action against important agricultural insect vectors and plant viruses or other pathogens. Among current agricultural alternative strategies, the plant-EOs are used to

target pests of agricultural significance, which encompass a large number of modern innovative technologies. They are important for the potential development of global insecticide market in the future, including the production in the large global agro-industrial scale (Isman *et al.*, 2011), multiple modes and site of action due to broad spectrum of antimicrobial activity and broad range of organisms (Kishore *et al.*, 2007), low mammalian toxicity and environmental pollution (Regnault-Roger *et al.*, 2012), rapid degradation by UV radiation. They are no effect on plant growth and development, and quality of the rice yield, and in addition to that, they are inexpensive, basic and easy understanding for using, no legal registrations, not necessarily available season long, and interfere with insect vector feeding as it may cover piercing-sucking mouth parts preventing feeding or viral transmission (Munneke *et al.*, 2004).

The plant-EO characteristics for plant-virus-insect interactions, and viral-transmission inhibition activity, which many researches could be used successfully to control plant viruses, and may lead to additive or synergistic effects (Chouhan *et al.*, 2017). Helal (2019) reported that the plant-EOs of *Mentha piperita* (peppermint) and *Thymus vulgaris* (thyme) had the greatest transmission inhibition effects of the *Tobacco necrosis virus* (TNV) at 4,000 ppm, and *Cucumber mosaic virus* (CMV) at 3,000 ppm, respectively. Lu *et al.* (2013) reported that the plant-EOs of Chinese indigenous aromatic plants were effected the transmission inhibition of *Tobacco mosaic virus* (TMV) at 100 µg/mL. These research results have been suggested that the plant-EOs had directly inactivated plant viruses and interfered with viral-coat proteins, and inhibited the formation of viral-capsid proteins, which were necessary for the adsorption or entry into the host cells. Thus, all the advantages of plant-EOs and the inhibition effects play a significant important role in future crop protection and successfully pest management.

The purposes of study were to assess the *in vivo* anti-viral biological activity to the rice plant virus and the insect vector transmission inhibition efficiency. It may possible to be an important method for the prototype of sustainable agricultural managements in the agro-industrial products of Thailand.

Materials and methods

Viral materials

The samples of RRSV-infected rice plants were obtained from an irrigated rice field in Nong-Suea district, Pathumthani province, Thailand, in 2018-2019, which were kindly provided by the Division of Rice Research and

Development, Rice Department, Bangkok. The diseased rice plant samples were multiplied for routine stock cultures in the greenhouse condition at 26±1 ℃, under relative humidity of 70-90% and photoperiod of 8/16 hrs. (light/dark), and used as the viral-plant sources of inoculum to the vectors, respectively.

Insect vector materials

The expected viruliferous and non-viruliferous BPH populations (*N. lugens* Stål) were collected from the light trap in the same rice field of viral-rice plant materials, and maintained in the insect rearing cages (16″×16″×24″), which were reared on the rice plant seedlings, *Oryza sativa* L. variety RD-7 [7-10 cm height, 6-9 days after germination (DAG)] for routine stock cultures in the greenhouse condition. To obtain experimental BPH populations, the females generally mature were transferred for oviposition process. After 48 hrs., the rice RD-7 seedlings were replaced to ensure an sufficient nutrition and the infested rice plant seedlings with the BPH eggs were cultured in the insect-free cages for continuous producing the stock culture of non-viruliferous BPH populations, which RRSV cannot be transmitted via the BPH eggs, and their BPH offspring (the third generation, F_3) were used in this study.

RRSV transmission

The non-viruliferous of third instar-nymph BPH populations after moulting used to RRSV-inoculating, the 50 instar-nymphs were fed on infected rice plant samples for 24 hrs. of acquisition feeding period (AFP) after 3 hrs. of fasting period (FP), and the transmitted BPH populations were transferred and reared on the viral-free *O. sativa* L. var. RD-7 rice plant seedlings for 7 days of latent period (LP) and incubation period (IP) in the vectors. Next, the standard susceptible variety Taichung Native 1 (TN1) rice plant seedlings were inoculated with the viruliferous-BPH status populations for 24 hrs. of inoculation access period (IAP), and the inoculated TN1 rice plant seedlings were treated and grown under the greenhouse condition.

The anti-viral biological activity of plant-EOs

The source of plant-EOs and preparation

The USDA certified organic ingredients (100%) and pure plant-EOs in this research were purchased from Health Food Thailand Co. Ltd. (Botanicessence) as shown in Table 1 and the plants shown in Figure 1. The obtained plant-EOs were stored at 4 ℃ in dark glass vials, and the high

effectiveness of active plant-EOs were selected from 10 herbal plants by testing the anti-viral biological activity and viral-transmission inhibition efficiencies. The bioassay conditions were done under the greenhouse. The plant-EOs were diluted with distilled water to get 0.002 to 0.1% v/v solutions which Tween-20 was added as emulsifying agent, and control solutions were distilled water (DW), and mixed of distilled water and Tween-20 (DWT), respectively.

Table 1. The plant-EO lists for anti-viral activity screening

No.	Thai Names	Local Names	Scientific Names	Parts
1	Dta-krai-baan	Lemon grass	*Cymbopogon citratus* DC. ex Nees	Leaves
2	Jan-bpaet-gleep	Star anise	*Illicium verum* Hook.f.	Fruits
3	Ma-groot	Kaffir lime	*Citrus hystrix* DC.	Leaves
4	Ma-naao	Lime	*Citrus aurantifolia* (Christm.) Swingle	Fruit peel
5	Ga-prao	Holy basil	*Ocimum tenuiflorum* L.	Leaves
6	Hoh-ra-paa	Sweet basil	*Ocimum basilicum* L.	leaves
7	Gra-chaai	Kaempfer	*Boesenbergia rotunda* (L.) Mansf.	Rhizome
8	Kaa	Galanga	*Alpinia galangal* (L.) Sw.	Rhizome
9	Prik-tai-dam	Black pepper	*Piper nigrum* L.	Seed
10	Ploo	Betelvine	*Piper betle* L.	Leaves

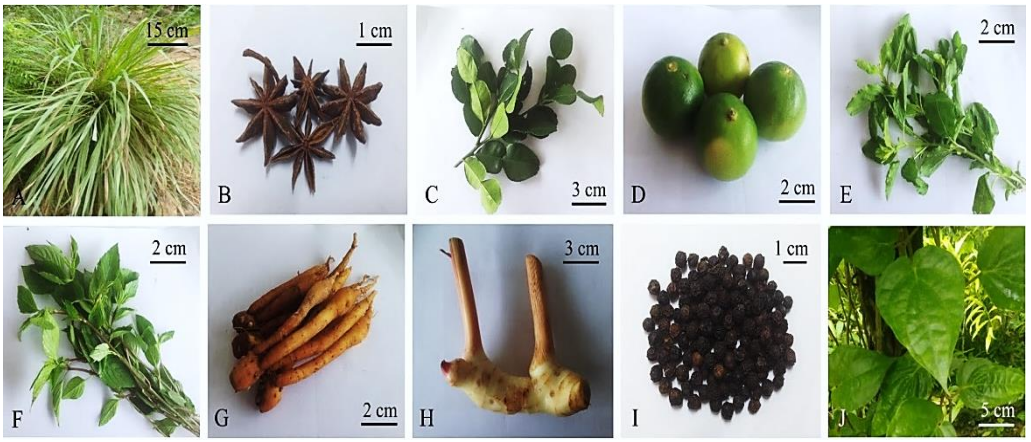

Figure 1. The list of herbal plants for anti-viral activity screening
Notes: (A) Lemon grass (*Cymbopogon citratus* DC. ex Nees), (B) Star anise (*Illicium verum* Hook.f.), (C) Kaffir lime (*Citrus hystrix* DC.), (D) Lime (*Citrus aurantifolia* (Christm.) Swingle), (E) Holy basil (*Ocimum tenuiflorum* L.), (F) Sweet basil (*O. basilicum* L.), (G) Kaempfer (*Boesenbergia rotunda* (L.) Mansf.), (H) Galanga (*Alpinia galangal* (L.) Sw.), (I) Black pepper (*Piper nigrum* L.), and (J) Betelvine (*P. betle* L.), respectively. (Photographed by one of the authors)

The anti-viral biological assays

The different durability period of active plant-EOs were detected after being sprayed by Direct Spray Method onto the insect vector and rice plant treatments, which was modified from Singh *et al.* (2014). All the tested treatments were collected and detected by Dot-Immunobinding Assay (DIBA), which the results were analyzed and compared with the control treatments, respectively.

The inhibitory efficiency of treated-plant

The infected rice plant samples were collected, and sprayed with distilled water for pre-cleaning. Afterward, the air drying step was 1 hr., and the rice plant samples were sprayed with 50 mL of various dilutions of plant-EOs. After drying step, the non-viruliferous BPH samples (N=10) were released to feed onto the sprayed rice plants (1 BPH/plant) for 1 day of AFP after being starved for 3 hrs. of FP. Next, the vectors were collected and detected by DIBA after being transferred, and reared on the virus-free rice plant seedlings, *O. sativa* L. variety RD-7 for 7 days of allowing the LP and IP in the vectors, which act as the active viral transmitters.

The inhibitory efficiency of treated-BPH vector

The viruliferous BPH populations were collected (N=10) after being sprayed with 5 mL of various dilutions of plant-EOs. Then the sprayed vector samples were released and fed on the virus-free rice plant seedling for 1 day of IAP after being starved for 3 hrs. of FP. Next, the inoculated rice plant samples were collected and detected by DIBA after being transferred into the observation cage under the greenhouse conditions for 14 days of allowing the LP and IP in rice plants, which acted as the active plant hosts.

The viral transmission inhibition rates

The ability of plant-EOs to protect against rice viral-infection to the vectors and rice plants were evaluated by viral transmission inhibition rate (%), which were calculated according to the following formula: viral transmission inhibition rate (%) = $[(C - T)/C] \times 100$, where C is the number of control sample, and T is the number of the treated sample, respectively.

Dot-immunobinding assay (DIBA)

DIBA was described by Hibi and Saito (1985), and modified from Na Phatthalung *et al.* (2015) and Na Phatthalung *et al.* (2017) for this research as follows, a nitrocellulose membrane [NCM, pore size 0.45 μm (Bio-Rad)] was

drawn into a square of 1×1 cm and immersed in the dilution buffer [0.01 M phosphate buffered saline (PBS), pH 7.4] for 5 min., and placed on a dried filter paper for 3-5 min. to get rid of the excessive buffer solution. The washing step was washed three times with washing buffer solution [PBS-T: 0.01 M PBS (pH 7.4), 0.5% Tween-20] by laboratory shaker for 15 min. each wash. The 5 μL of infected crude sap samples after being extracted by extraction buffer (EB) of plant tissues [Plant-EB: 0.01 M PBS (pH 7.4)] and insect vectors [BPH-EB: 0.01 M PBS (pH 7.4), 2% polyvinylpyrrolidone (PVP)] were dotted onto each square and allowed to dry for 5-10 min. at room temperature. Then, the dotted-membrane was put in a blocking buffer solution [PBS-T-SK: 0.01 M PBS (pH 7.4), 0.5% Tween-20, 5% skimmed milk (SK)], and shaken at room temperature for 30 min. After the washing step, an antiserum buffer solution (anti-RRSV IgG diluted 1:1,000 in PBS-T-SK) was added and incubated for overnight at 4℃. Following by the washing step, the membranes were incubated in an enzyme buffer solution [goat anti-rabbit serum conjugate alkaline phosphatase (GAR-AP, ZyMax™) diluted 1:5,000 in PBS-T] for 3 hrs. at 4℃. The washing step was performed before the reactions were visualized with substrate solution [BCIP/NBT alkaline phosphatase substrate (SIGMAFAST™) (pH 9.5)] after incubation for 1 hr. Subsequently, a positive signal was the appearance of blue-purple color in the region of the blot, which the reaction was terminated by putting the membrane into a stop solution (deionized water) for 5-10 min. Finally, the positive result was detected on the membrane by being observed the development of either purple-blue color on the blot in 1 hr.

Results

The RRSV-infected rice plant symptoms

The symptoms were developed in the susceptible rice variety TN1 after inoculated by the viruliferous BPH vector. It showed that the variation in their morphologies on the three general growth phases of rice plants after germination, which were preliminary appeared approximately 7 days after inoculation (DAI). For the vegetative phase (germination to panicle initiation) of rice plants showed the stunted rice plants with increasing excessive number of tillers, malformed leaves with dark-green, shorted-narrow, ragged and twisted or spiral shaped, and vein-swellings or galls on the underside of leaf blades and outer surface of the sheaths, respectively. Next, the rice-reproduction phase (panicle initiation to flowering) showed that the proportion per plant of ragged and twisted leaves was decreased and developed to the tightly rolls up, and sometimes increased the leaf yellowing (chlorosis).

Then the rice-ripening phase (flowering to mature grain) showed that delayed flowering, malformed spikelets and short clusters of excessive panicles, empty and dark-brown to black spots discoloration of unfilled grains, respectively (Figure 2).

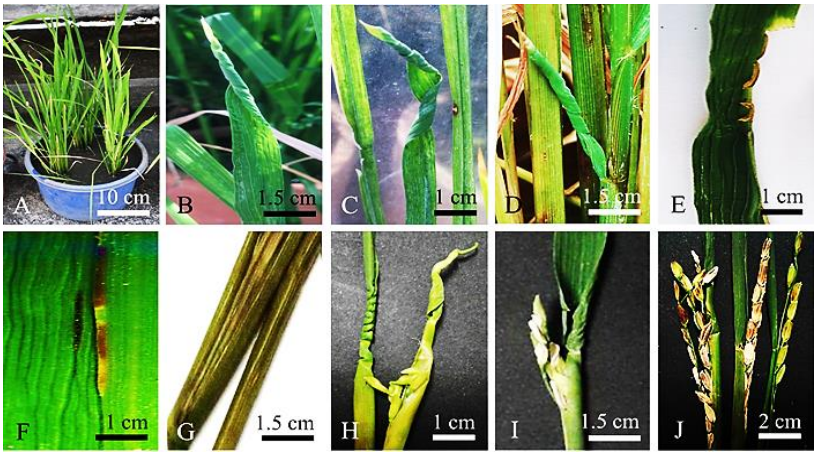

Figure 2. The symptoms of RRSV-infected cultivar TN1 rice plants
Notes: (A) Stunted rice plant growth, (B-D) Dark-green wave-malformed leaf veins and twisted-leaf tip, (E) Ragged leaf blade, (F and G) Discoloration development of vein-swelling or gall dark brown on the outer surface of the leaf blades and stem, (H and I) Dark-green flag leaf twisted, malformed and narrows-roll up, which morphogenic abnormalities in young rice seed, and (J) Incomplete panicles with yellow-brown to dark-brown unfilled grains, respectively.

Effect of plant-EOs on RRSV transmission by BPH vector

The evaluation of plant-EOs against potential of RRSV-transmission inhibition effects indicated that all treatments of the active plant-EO showed varying degrees of success which depending upon the used concentrations. The inhibitory effect was enhanced with increasing concentrations, and the range of active plant-EO concentrations were summarized in Table 2. These study results showed that all the tested plant-EOs at 10 different concentrations were effective in various degrees in the RRSV-transmission inhibition from the infected rice plants to non-viruliferous BPH status (Figure 3 and Table 2A) and the viruliferous-BPH status to the viral-free rice plant **(**Figure 4 and Table 2B). Range of the active plant-EO concentrations between 0.002 to 0.1% for transmission inhibition of lemongrass, star anise, black pepper, kaffir lime, and kaempfer were highly efficient (10-70%), when compared with the lime, galangal, holy basil, sweet basil, and betelvine were slightly efficient (10-30%), respectively.

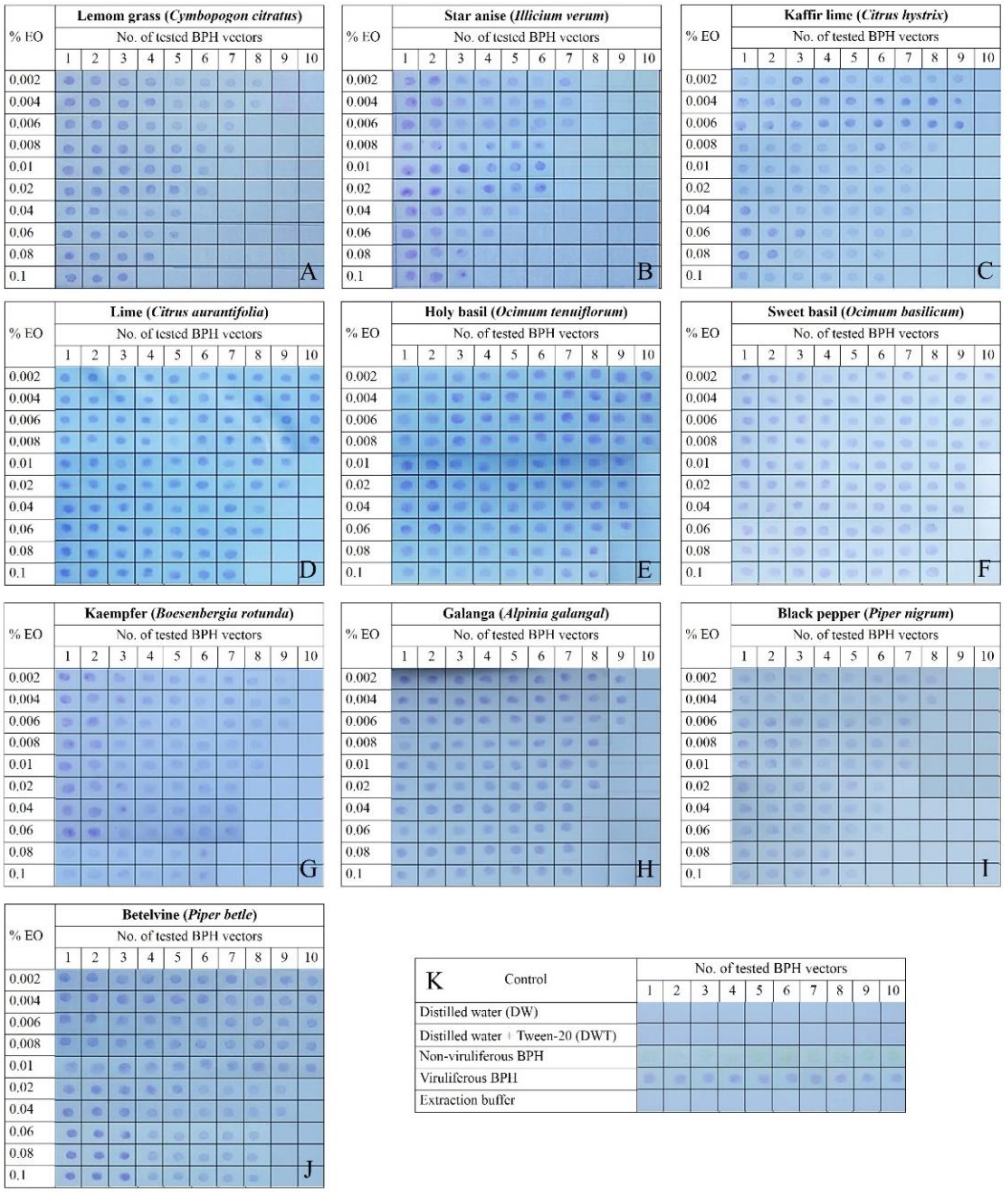

Figure 3. The DIBA of the RRSV transmission inhibition from infected rice plants to non-viruliferous BPH by plant-EOs

Notes: (A) Lemongrass, (B) Star anise, (C) Kaffir lime, (D) Lime, (E) Holy basil, (F) Sweet basil, (G) Kaempfer, (H) Galanga, (I) Black pepper, (J) Betelvine, and (K) Control treatments, respectively. The positive results were shown the blue-purple colored spot of insoluble substrate product onto the nitrocellulose membrane (NCM), whereas the negative results were shown the yellow-brown colored spot of the BPH crude sap.

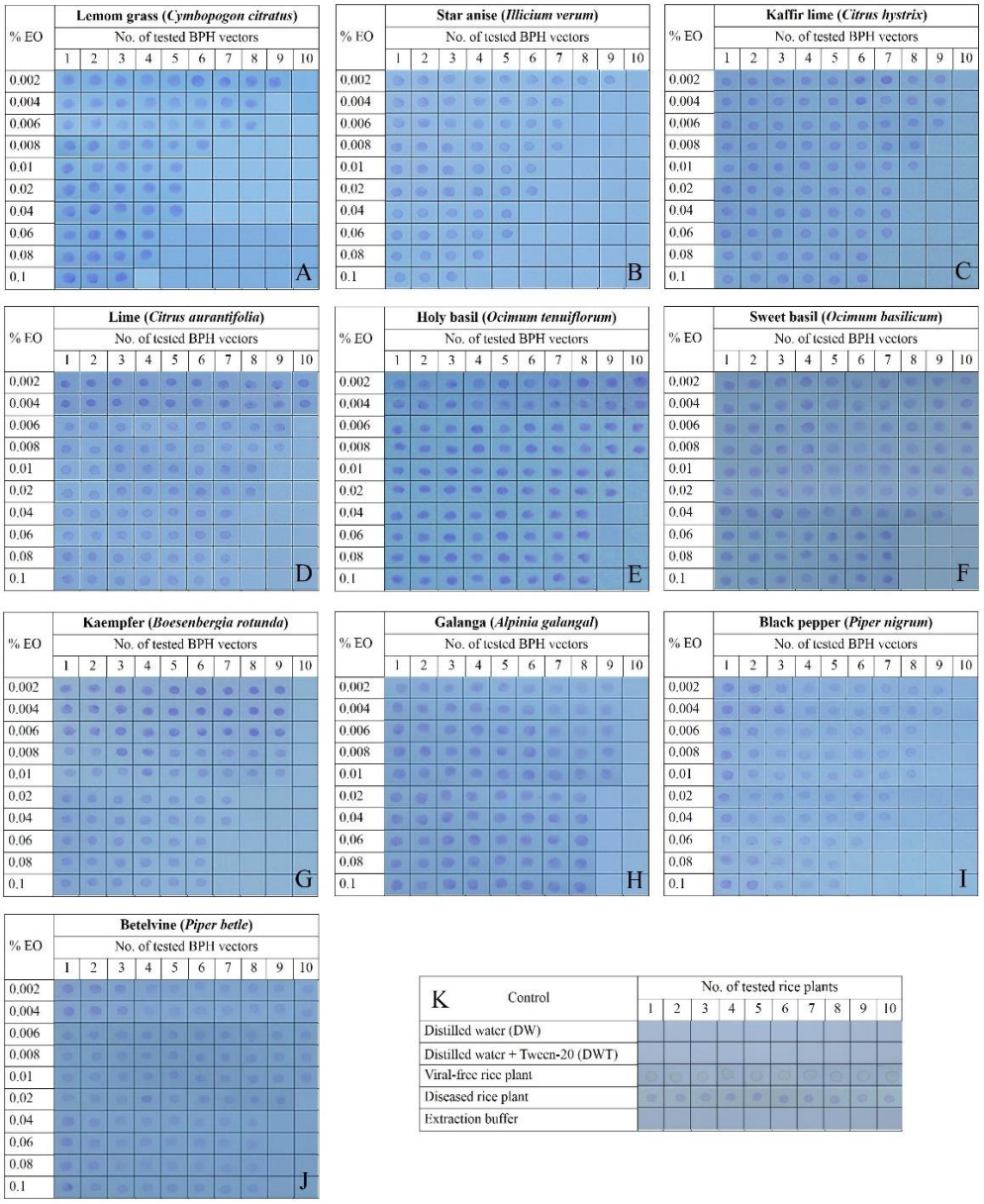

Figure 4. The DIBA of the RRSV transmission inhibition from viruliferous-BPH to viral-free rice plants by plant-EOs

Notes: (A) Lemongrass, (B) Star anise, (C) Kaffir lime, (D) Lime, (E) Holy basil, (F) Sweet basil, (G) Kaempfer, (H) Galanga, (I) Black pepper, (J) Betelvine, and (K) Control traetments, respectively. The positive results were shown the blue-purple colored spot of insoluble substrate product onto the nitrocellulose membrane (NCM), whereas the negative results were shown the green colored spot of the rice plant crude sap.

Table 2. The summary of the RRSV-transmission inhibitory effect of the plant-EOs

Plant-EOs	(A) Transmission from Infected Rice Plant to Non-Viruliferous BPH		(B) Transmission from Viruliferous BPH to Viral-Free Rice Plant	
	Active Conc. Range	**%RRSV-TIE**	**Active Conc. Range**	**%RRSV-TIE**
Lemongrass*	0.002-0.1%	20-70	0.002-0.1%	10-70
Star anise*	0.002-0.1%	30-70	0.002-0.1%	10-70
Kaffir lime *	0.002-0.1%	10-40	0.002-0.1%	10-40
Lime	0.01-0.1%	10-30	0.008-0.1%	10-30
Holy basil	0.01-0.1%	10-20	0.006-0.1%	10-30
Sweet basil	0.01-0.1%	10-20	0.004-0.1%	10-20
Kaempfer *	0.002-0.1%	10-40	0.002-0.1%	10-40
Galangal	0.002-0.1%	10-30	0.002-0.1%	10-20
Black pepper *	0.002-0.1%	20-50	0.002-0.1%	10-50
Betelvine	0.02-0.1%	10-20	0.02-0.1%	10-20
DW (control)	–	0	–	0
DWT (control)	–	0	–	0

Notes:*The most active plant-EO for RRSV-transmission inhibition effects (RRSV-TIE); distilled water (DW); distilled water+Tween-20 (DWT)

Morphological effect of plant-EOs to BPH vector

The active plant-EO of star anise and lemongrass for testing the effect on the mortality of BPH at the concentrations of 3% and 5% were applied. The highest doses of selected plant-EOs caused mortality and deformities on the BPH resulted to occur irreversible damage to the physiological processes essential to the survival and development stages of BPH.

The effects of plant-EOs of star anise and lemongrass were investigated on eggs and adult stages of BPH after being sprayed, and the morphologies were observed under stereoscopic microscope. Results showed the malformed structures compared with the control. After being treated, the egg and instar-nymph of BPH showed the abnormal shapes and structures, and completely destroyed at 3 days after treatment (DAT) (Figure 5), whereas the adult stage showed the malformed morphology after 5 DAT, with appearing the deformities of the structures of body segments at abdomen, legs, wings, and mouth. In addition, the outer cuticle color of body was changed to a brownish black (Figure 6).

Figure 5. The morphological and physiological effects of plant-EOs on the BPH-eggs

Notes: (A-C) Non-treatment (control), malformed BPH-egg after treatment (3 DAT) with 3% (D) and 5% (E and F) of star anise oil and 3% (G) and 5% (H and I) of lemongrass oil, respectively.

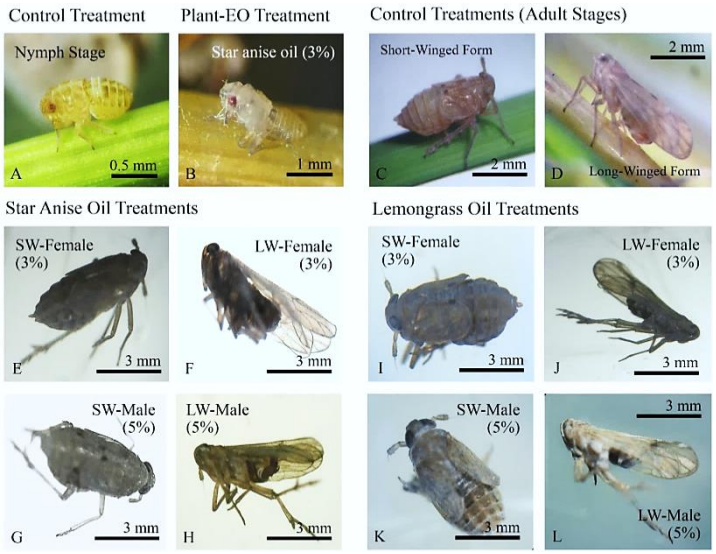

Figure 6. The morphological and physiological effects of plant-EOs on the nymph and adult of BPH

Notes: (A) Non-treatment (control) of the BPH-nymph stage and (B) malformed morphology after treatment with star anise oil (3%, 3 DAT), respectively. (C and D) Non-treatment (control) of the adult stage of short-winged (SW) brachypterous form and long-winged (LW) macropterous form and malformed morphology after treatment with star anise oil at 3% (adult BPH female, E and F) and 5% (adult BPH male, G and H), respectively, and lemongrass oil at 3% (adult BPH female, I and J) and 5% (adult BPH male, K and L), respectively.

Discussion

The RRSV-infected rice plant symptoms were identified to the typical symptoms which observed in paddy field. However, Bailiss and Senananyake (1984) and Onasanya *et al.* (2004) reported that the reduced characteristics of infected rice plants were attributed to induce abnormal plant growth and to develop after initial infection. In addition, the plant height was one of the essential factors and was the same as the leaf length, leaf wide, leaf number, and tiller number, which were used for the observation of symptoms and evaluation of viral-disease in plants. The evaluation of plant-EOs against the potential of RRSV-transmission inhibition effects, and morphological effect of plant-EOs to the BPH vector were indicated that the plant-EOs of star anise and lemongrass possessed effective anti-viral activity to inhibit RRSV-transmission abilities and BPH mortality, which possible to be potential candidates for the further development as anti-viral agents. Moreover, the anti-viral activity of the botanical pesticides is direct-virucidal effects during an initial period of viral replication, which is the important key in achieving action (Bhanuprakash *et al.*, 2008). In addition, Reichling *et al.* (2009) reported that the anti-viral activity research is specifically focused, and relevant clinically directed toward an enveloped virus with limited to non-enveloped virus when considered to infection period and intracellular efficacy (Búfalo *et al.*, 2009).

The inhibition mechanisms of active-EO included as follows: (1) reduction the activities of viral infecting host cells by the viral capsid or spikes degradation (Gilling *et al.*, 2014), (2) viral nucleic acid denaturation (Helal, 2019), (3) uncoating of viral particles by interfering with endosome-lysosome fusion, and leading to non-specific binding to host cells (indiscriminate) and cell activity inhibition (Abonyi *et al.*, 2009) and (4) inhibiting viral replication, entry into host cells, and cell-to-cell movement. Sharifi-Rad *et al.* (2017) reported that the functions of active-EO were allelopathy, adaptation to abiotic stresses, intra- and inter-plant signaling, direct defense against herbivores and pathogens, and indirect defense. The results of the morphological effect of plant-EOs to the BPH vector were the same as the report of Nathan *et al.* (2008) who studied the effect of azadirachtin (AZA) from neem (*Azadirachta indica* A. Juss) in both of the ovary and mature stages, and showed the follicle epithelial cells (FEC) of egg were disrupted and completely destroyed after treated, whereas the mortality was associated mainly with failure to hatch and moult, and deformities of the morphological structures of nymph and adult stages, respectively. Therefore, the plant-EOs of star anise and lemongrass in this study had a considerable potential for BPH-insect control and applying for the commercial anti viral agents according to the report of Chantawee *et al.* (2012) and Lakyat *et al.* (2017).

The plant-EO effects have already been assayed and accepted on the various rice insect pests, such as the green rice leafhopper (GRL: *Nephotettix virescens* Distant; *Homoptera*: *Cicadellidae*) (Saxena *et al.*, 1987), white-backed planthopper (WBPH: *Sogatella furcifera* Horvath; *Homoptera*: *Delphacidae*) (Heyde *et al.*, 1984), rice leaffolder (RLF: *Cnaphalocrocis medinalis* Guenee; *Lepidoptera*: *Crambidae*) (Saxena *et al.*, 1981), small rice stink bug (*Oebalus poecilus* Dallas; *Hemiptera*: *Pentatomidae*) (Sutherland *et al.*, 2002), zig-zagged winged leafhopper (ZLH: *Recilia dorsalis* Motsuchulsky; *Hemiptera*: *Cicadellidae*) (Insung *et al.*, 2017), and rice thrips (*Stenchaetothrips biformis* Bagnall; *Thysanoptera*: *Thripidae*) (Pillai and Pooniah, 1988).

The plants and their essential oils (EOs) that over 17,500 plant species (Svoboda and Greenaway, 2003) are potentially and significantly useful sources for viral transmission inhibition by insect vectors, especially the piercing-sucking mouthpart vector species. The outbreaks of BPH in Thailand and the other Asia rice-growing countries have caused the yield loss in rice crop, which the value economic damages estimated as more than $300 million annually (9,000 billion Bahts), and the BPH has evolved and developed high levels of the two-major types of insecticide resistance (behavioural and physiological resistance) (Wang *et al.*, 2008). In addition, Mahmood *et al.* (2016) reported that the classes of insecticide were toxic to humans that caused the serious acute and chronic effects, and accumulate in the environment. Therefore, the active plant-EOs played an important role as great, green and safe alternatives for the eco-friendly management strategy.

The plant-EOs for insect pest control, which typically forms in the volatile liquids, or semi-liquid complex mixtures, and were extracted mainly from the all plant organs, aerial parts (71.88%) and leaves (28.51%) (Campolo *et al.*, 2018) in the content about 1-15% (Butnariu and Sarac, 2018). Furthermore, Ouis and Hariri (2018) and Akram *et al.* (2017) supported that the extraction methods of plant-EO were an expensive, delicate, thermally unstable, and use the large amount of botanical materials to extract a tiny amount of oil in milliliters. However, more than 3,000 of plant-EOs were manufactured on an industrial scale (Sarma *et al.*, 2019), which Carrubba *et al.* (2006) reported that many studies around the world to improve plant-EO productivity for agricultural using under the integrated pest management (IPM or pest control service).

Oraby and El-Borollosy (2013) stated that the active plant-EO can manage the insect vector damaging effects on crops and also reducing their plant viral-transmission ability, resulting to increase in quality and quantity of crop parameters. The obtained results in the present study was initiated to

evaluate the plant-EO for transmission inhibition effects of plant virus and indicated that there could be useful as potential control agents. In addition, Singh (1981) reported that the mechanism of viral-transmission inhibition on the EO sprayed-insect vectors or dipped-plants may be modified the plant physiology or disturb the metabolism of the inoculated cells. The hypothesizes of mechanical surface adhesion and mechanical lubricating effects were supported that the plant-EO had optimal surface tension for covering with the general surface structure of probing stylet or body-cuticle (extracellular layer) of insect vectors to inhibit transmission and possibility for coating around the viral-particles before penetrating into the plant cells with the semi or persistent pattern. Whereas the inactivating of the stylet tip and penetrating into the cuticle layer of insect vector made to prevent from the infection-starting or development process after the introducing and landing on the site of infection on the plant tissues by insect vector, which were found in the non-persistent pattern (Simons and Beasley, 1977). Therefore, the inhibition of viral transmission by plant-EO occurs at the virus-vector or virus-vector host-plant relationship.

Generally, the many active compounds of plant-EO tended to be more effective on the soft-bodied insects than the hard-bodied insects which environmental friendly persistence and strongly bioactivity of plant viral-transmission inhibition. This observation revealed that plant viral-transmission inhibition effects of plant-EO, which applied to RRSV were found to be the high inhibition rate (%) of star anise and lemongrass in a range of efficient concentration of 10-70% at 0.002-0.1%. Although a definite inhibition transmission mechanism of plant-EO against RRSV remained unknown, this result suggested that the finding of the present work will be available to enhance plant defenses against RRSV infection.

Verifying of the transmission inhibition effects of plant viruses by active plant-EO were extremely important to consider the bio-active compounds in plants as a promising agent for applying and creating the new commercial anti viral agents and bioinsecticides which expanded range of alternative methods to increase and promote the development of sustainable crop production systems. They enhanced crop yields, reduced ecological damage, protected and improved the quality of life for producers and consumers. However, the above results presented several directions for the future research finding should be focused on the transmission inhibition mechanisms of plant-EOs from infected-hosts for determining and understanding the role active compounds and integrated in pest management program and prevention of viral plant diseases in Thailand and worldwide.

Our findings demonstrated that the plant-EOs of star anise and lemongrass expressed the effective anti-viral activity to inhibit RRSV-transmission abilities and BPH mortality at the initial stage through the adult stage of the insect's growth. The results indicated that both plant-EOs were able to interfere after host infection, and potential candidates for the further development for anti-viral agents.

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