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Potency of some plant extracts as insect attractants in paddy field

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Abstract The brown planthopper, *Nilaparvata lugens* (family Delphacidae), rice green leafhopper, *Nephotettix virescens,* and zigzag leafhopper, *Recilia dorsalis* (family Cicadellidae) were determined to be the highest percentage frequency of occurrence in each bottle trap containing each lure. The examined insect species in the rice field had increased the number of predictions toward the end of the study. Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$) was found to be a common compound at this time for all plant extracts, and it could be further developed and used as an attractant for insect pests of rice paddy.

Keywords: Bottle trap, Bread flowers, Fragrant pandanus leaves, Lure, Vegetable soybeans

Introduction

Rice (*Oryza sativa* L.) is an important economic crop and a vital food for humans, especially in Asia, the population of Thailand predominantly consumes rice, and rice is also a paramount export product for the country. Currently, rice production and prices have plummeted. This is partly due to the problem of rice pests that destroy or interfere throughout the production season, such as the brown planthopper, leafhopper and rice borer etc. (Pathak, 1968, 1970; Pathak and Khan, 1994; Way and Heong, 1994; Berry *et al*., 2020). However, there are natural enemies in rice fields, which include predators, parasites and pathogens of various types of insect pests, which help bring the balance of the rice field ecosystem to a level that does not cause economic damage. (Debach and Rosen, 1991) The efficiency of management is mainly determined by the degree of complexity of the natural control mechanisms that maintain the balance of the ecosystem (Heong and Sogawa, 1994). When farmers provide organisms with a

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mechanism for mutual biological regulation (natural control), it is considered one of the Integrated Pest Management System (IPM) strategies that have been used in the past. It helped to reduce the use of chemicals in the past (Dara, 2019). Reduce production costs while making the environment safer. In addition, additional forms or methods can be used in the IPM process to control the rice production system, such as using extracts of medicinal plants as repellents or attractants.

Insect attractants used in crop systems often have chemical compositions comparable to those of plants or insects. (Semiochemicals) such as methyl eugenol, a sex pheromone used in communicating with fruit flies. Many medicinal plants, such as holy basil, sweet basil, and lemon basil, naturally contain methyl eugenol—a compound that has been widely applied in agricultural systems for pest control (Plimmer *et al*., 1982; Jacobson, 1996; Mahmood *et al*., 2002; Vargas *et al*., 2010). Although methyl eugenol is commonly used by farmers across Thailand, it remains unclear whether the compound in use is synthetically produced or extracted from plant sources. To control or manage insects in crop production systems to maximize their utilization. Therefore, this chemical is often used on insects in different ways. Insect pests of various kinds that prey on plants. This could be due to stimulation or exposure to volatile organic compounds (VOCs) secreted by plants. Volatile organic compounds (VOCs) emitted by plants have been shown to either repel or attract species in the environment. As an example, consider the herbivorous (phytophagous) insect (Degenhardt *et al*., 2003; Degenhardt, 2009).

Rice is a plant that grows in three phases: 1) the stem and leaf growth phase, 2) the reproductive phase, and 3) the rice ripening phase. At each stage of rice development, different chemicals are produced that stimulate internal hormones. VOCs are both primary and secondary metabolites. The main component responsible for the characteristic smell of rice is 2-acetyl-1-pyrroline (2AP). This chemical consists of tiny molecules that are volatile and evaporate quickly (Hinge *et al*., 2016a, b). Consequently, it could be a chemical that attracts herbivorous insects to rice fields. Hinge *et al*. (2016a) also showed that 2AP is often detected in high abundance in rice during the seed development stage, especially during ripening. Despite research into volatile organic compounds (VOCs) from plants that have significant effects on insects. However, research on attracting insects using traps mixed with extracts from rice or plants with similar composition has never been recorded.

Apart from VOCs from rice, there are many chemical compounds found include: ethanol (alcohols), hexanal (aldehydes), ethyl acetate and ethyl alcohol (ester), Limonene (terpenes) and Acetone (ketones) that could also found in other plants including bread flowers and green soybean pods (Yahya *et al*., 2011;

Wang *et al*., 2018). Use of plant extracts as insect lure has seldom been addressed. To find this knowledge gap, we were interested in exploring the chemical composition of all plant extracts to find out what VOCs and other chemicals they contained to confirm their usage to attract insects in paddy. The extracts, which were expected to contain VOCs and other chemicals, were then developed as attractants and tested with a bottle trap to determine the potential of attractants from extracts of these plants. The current study was designed to serve as a guideline for evaluating (monitoring) the insects that are effective in paddy fields, particularly rice pests, as well as controlling/reducing rice pest populations.

Materials and methods

Analysis of the phytochemical composition of plant extracts using the GC-MS technique

Preparation of plant extracts

Plant extracts were obtained from pandanus leaves (*Pandanus amaryllifolius*), green soybean pods (*Glycine max*), and bread flowers (*Vallaris glabra*) (Figure 1).

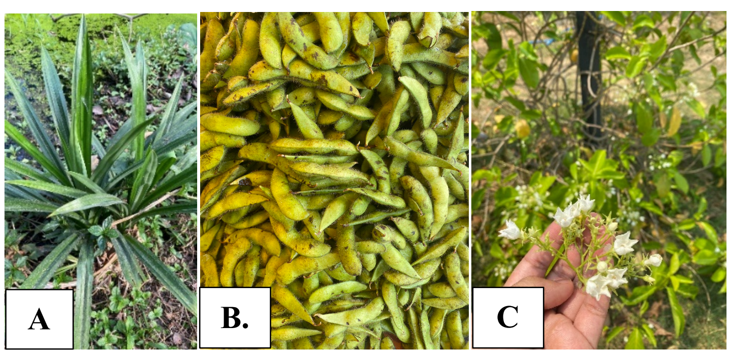

Figure 1. Three major plants used for extraction and chemical composition analysis i.e. pandanus leaves (a), green soybean pods (b) and bread flowers (c)

Water distillation

Green plant materials were chopped and placed in a glass distillation apparatus. A weight-to-volume (w/v) ratio of 2:1 (plant material to distilled water) was used. The apparatus was connected to a 2,000 mL heating mantle and maintained at 100°C for 1–2 hours. After distillation, the extracted essential oil was collected and stored in amber-colored vials for use in insect trapping experiments. A portion of each extract was also analyzed using gas chromatography-mass spectrometry (GC-MS) to identify secondary metabolites.

GC-MS analysis

Using a gas chromatography-mass spectrometry (GC-MS) system, extracts of pandanus leaves, chomanad flowers, and green soybean pods were tested to identify key chemical compounds. The conditions of the GC machine (brand Agilent Technology, model GC 7890A, USA) were used. The injection volume was 0.5 µl. An HP-5MS column with 30 m x 0.25 mm i.d. x 0.25 m film thickness was used. The flow rate of helium gas (He) into the column was set to 1.0 mL. per minute. The column temperature is programmed with an initial temperature of 50°C per minute until the temperature reaches 240°C. The analysis time is 40 minutes. MS (Agilent Technology brand, model MSD 5975C, USA) is an MS quadrupole that is directly connected to the GC via a transfer line and sets the temperature in an electron impact ionization (EI) to 150°C and the temperature of the ion source to 230°C sets. System. and in the scan mode system in the mass range of 30 to 500 AMU (Atomic Mass Unit). The obtained data is then compared with the databases of the National Institute of Standard and Technology (NIST) and the Wiley Libraries (Chikowe *et al*., 2024). A chromatogram was recorded in terms of time and signal size. A peak is the size of the signal found at a specific point in time. Both qualitative and quantitative analyzes were performed by comparing the time the substance elutes from the column to the detector (retention time, t_R) with the time of the standard material to identify the type of substance. (The area under the peak (peak area) or the peak height (peak height) of standard compounds at different concentrations. Let's establish a linear relationship to determine the substance content in the sample.

Attractancy test

Two transparent plastic water bottles with a capacity of 1.5 liters were made. Start by cutting off a third of the base and assembling it. The extracts were dropped on cotton (10 drops) and inserted into the seams of the assembled bottles (Figure 2). Each plant extract was divided into a treatment using a bottle trap with extract-soaked cotton ball and an empty bottle trap (control group). Each test set was designed as a three-replication randomized complete block design (RCRD) experiment in which insect traps were randomly placed in paddy fields of a non-glutinous rice variety (RD 85) at the stem growth stage in Khlong Khlung District, Kamphaeng Phet Province. The insect traps were attached to one-meter-tall bamboo, and the bottle traps were stored for three consecutive days by checking in every day, including morning and evening, and collecting insect samples for categorization and analysis in the biology laboratory of the

Faculty of Science and Technology, Pibulsongkram Rajabhat University, and the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, respectively.

Figure 2. shows the plastic bottle trap used to attract insects in paddy field and an insect sample (zigzag leafhopper, *Recilia dorsalis*) caught from a trap.

Data analysis

External morphology was classified mainly at the order, family and species levels using the classification manual of Pathak and Khan (1994) mainly counted the number of insect samples collected from each species to calculate the mean and standard deviation ($\bar{x} \pm$ S.D.) as well as the percentage abundance of each captured insect species (% relative abundance; RA) using the SPSS package version 25.0 (IBM Crop., 2017). Then, using the Ecosim 7.72 application, evaluate the prediction or prediction of the number of insect species via the species accumulation curve (Gotelli and Entsminger, 2004).

Results

In total, we sampled 310 insects from paddy field, representing 13 insect species from 11 families and 5 orders. Bottle traps with pandanus leaf extract as bait yielded 8 species from 6 families in 4 orders, bottle traps with green soybean pod extract as bait yielded 10 species from 8 families in 5 orders while bottle traps with bread flower extract as bait yielded 12 species from 11 families in 5 orders, respectively as shown in Table 1.

Table 1. Insects captured in the rice field using bottle traps containing pandanus leaf, green soybean pod, and bread flower extracts (n = 3)

Insect fauna			Insect captured (x̄ ± S.D.)		
Order	**Family**	**Species**	**Pandanus leaf**	**Green soybean pods**	**Bread flowers**
Coleoptera	Chrysomelidae	*Dicladispa armigera*	0	1.33±1.15	5.00±2.65
	Coccinellidae	*Micraspis discolor*	1.00±1.00	3.00±2.00	0
Diptera	Cecidomyiidae	*Orseolia oryzae*	10 3.33±1.53	0	2.00±1.00
	Ephydridae	*Hydrellia philippina*	1.00±1.73	0.67±1.15	1.00±1.00
	Sciaridae	*Lycoriella ingenua*	1.67±0.58	0.67±1.15	0.33±0.58
	Tachinidae	*Argyrophylax nigrotibialis*	0	0.67±0.58	1.00±1.00
	Ulidiidae	*Poecilotraphera taeniata*	0	0.33±0.58	0.67±1.15)
Hemiptera	Cicadellidae	*Nephotettix virescens*	27 9.00±6.56	3.67±0.58	16.00±1.73
		Recilia dorsalis	6.00±6.25	8.33±4.73	10.33±8.14
	Delphacidae	*Nilaparvata lugens*	3.00±3.61	5.67±4.62	37 12.33±4.93
	Miridae	*Cyrtorhinus lividipennis*	0	0	1.67±0.58
Hymenoptera	Braconidae	*Tropobracon schoenobii*	0.33±0.58	0	1.33±0.58
Lepidoptera	Pyralidae	*Chilo suppressalis*	0	0.33±0.58	0.67±1.15

It was revealed that the green leafhopper, *Nephotettix virescens* (35.63%) and the zigzag leafhopper, *Recilia dorsalis* (23.68%) in the family Cicadellidae and the brown leafhopper *Nilaparvata lugens* (11.84%) (family Delphacidae) discovered from the pandanus leaf extract were found to have the highest abundance. Meanwhile, the top three insects found in bottle traps containing extracts from green soybean pods were zigzag leafhopper, *R. dorsalis* (33.78%), brown planthopper, *N. lugens* (22.97%), and green leafhopper, *Ne. virescens* (14.86%), respectively, and the three dominant insects found from bottle traps containing bread flower extracts were the brown planthopper, *N. lugens* (31.88%), the zigzag leafhopper, *R. dorsalis* (23.13%), and the green leafhopper, *Ne. virescens* (19.38%). In addition, after the survey was completed, it was found that the graph showed an increase in the number of insect species that could be caught in rice fields using bottle traps containing pandanus leaf extract, green soybean pods and bread flowers as baits raised futher (species accumulation curve) as depicted in Figure 3.

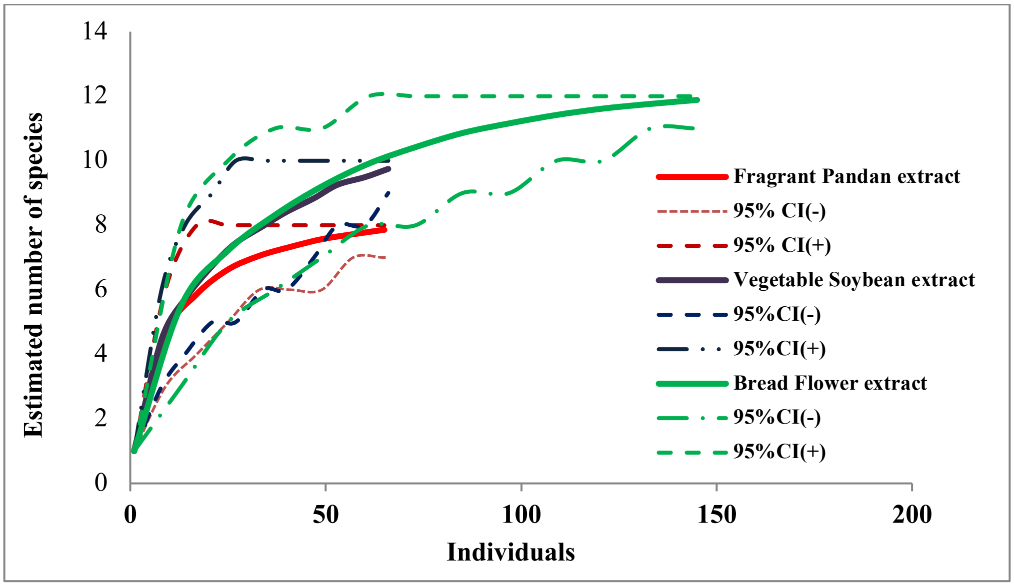

Figure 3. shows the species accumulation curve or the relationship between the number of insect species captured in the paddy field from bottle traps with pandanus leaf, green soybean pod and bread flower extracts as insect attractants

Chemical composition analysis from the extract of pandanus leaves, green soybean pods, and bread flowers using the GC-MS technique revealed that the chemical components required 3 to 42 minutes for passing through the column. The extracts of pandanus leaves, green soybean pods and bread flowers each contained 14, 8 and 10 chemical components, respectively, that met the standard values recorded in the NIST Library. In addition, it was found that hexadecanoic acid methyl ester was a commond chemical found in all three plant extracts in the retention time (t_R) area of about 31.6 as exhibited in Table 2.

Table 2. Chemical composition of extracts obtained from pandanus leaves, green soybean pods and bread flowers by GC-MS as per NIST library

Peak No.	Chemical component	Formula	Retention Time (t_R, min)
Pandanus leaves, *Pandanus amaryllifolius*			
1	Glutanic acid, monomethyl ester	($C_6H_{10}O_4$)	11.902
2	Coumaran	(C_8H_8O)	14.251
3	3-Hydroxyl-N-methylphenethylamine	($C_9H_{13}NO$)	20.522
4	N'-Isopropylureidoacetic acid	($C_6H_{12}N_2O_3$)	22.250
5	Quinic acid ($C_7H_{12}O_6$), Saccharin methylated	($C_8H_7NO_3S$)	24.468
6	5-Methyl-1R, 3-trans-cyclohexanediol	($C_7H_{14}O_2$)	25.002
7	Neophytadiene	($C_{20}H_{38}$)	25.611
8	Butanoic acid, 2-hexylimino-,	($C_{10}H_{19}NO_2$)	29.814
9	Butanoic acid, 2-hexylimino-	($C_{10}H_{19}NO_2$)	30.334
10	3-Methylene-7, 11-dimethyl-1-dodecene	($C_{15}H_{28}$)	30.655
11	Hexadecanoic acid, methyl ester	($C_{17}H_{34}O_2$)	31.602
12	n-Hexadecanoic acid	($C_{16}H_{32}O_2$)	35.356
13	Phytol	($C_{20}H_{40}O$)	35.451
14	9,12,15-Octadecatrienal	($C_{18}H_{30}O$)	35.872
Green soybean pods, *Glycine max*			
1	Maltol	($C_6H_6O_3$)	10.009
2	Phenol, 2,6-dimethoxy-	($C_6H_{10}O_3$)	18.091
3	Sucrose	($C_{12}H_{22}O_{11}$)	20.912
4	3-O-Methyl-d-glucose	($C_7H_{14}O_6$)	26.530
5	Hexadecanoic acid, methyl ester	($C_{17}H_{34}O_2$)	31.621
6	n-Hexadecanoic acid	($C_{16}H_{32}O_2$)	32.350
7	Linoleic acid, methyl ester	($C_{19}H_{34}O_2$)	35.020
8	Linoleic acid	($C_{18}H_{32}O_2$)	35.704
Bread flowers, *Vallaris glabra*			
1	Catechol	($C_6H_6O_2$)	13.671
2	N-(Piperidin-3-yl) acetamide	($C_7H_{14}O_{20}$)	14.556
3	2-Methoxyl-4-vinylphenol	($C_9H_{10}O_2$)	16.908
4	Indoline, hexahydro-5-methoxy-, cris-	($C_9H_{17}NO$)	19.978
5	2-Deoxy-D-galactose	($C_6H_{12}O_5$)	22.101
6	2-Methyl-1-ethylpyrrolidine	($C_7H_{15}N$)	23.545
7	Piperidine,3-isopropyl-	($C_8H_{17}N$)	23.899
8	5-Hydroxylcyclooctane-1,2-dione	($C_8H_{12}O_3$)	24.442
9	Hexadecanoic acid, methyl ester	($C_{17}H_{34}O_2$)	31.610
10	α-D-Mannopyranoside, methyl 3,6-anhydro-	($C_{18}H_{30}O$)	32.267

Discussion

From the study on the feasibility of using extracts of pandanus leaf, green soybean pods and bread flowers with plastic bottle traps in the rice paddy ecosystem of RD 85 at the tillering stage. During the survey, a total of 13 insects were caught. The most common of the first three groups are zigzag leafhopper (family Cicadellidae) and green leafhopper (family Cicadellidae) and brown leafhopper (family Delphacidae) in the order Hemiptera. In addition, other pest species that are considered a potentially important group of rice pests have also been found, such as: the rice leaf whorl maggot, *Hydrellia philippina* (Diptera: Ephydridae), which often suck rice juice as food and rice gall midge, *Orseolia oryzae* (Diptera: Cecidomyiidae), which could generally feed at the base of the growing shoot (Pathak and Khan, 1994; Kutuk *et al*., 2008; Berry *et al*., 2020). Besides, some beneficial insects including ladybird beetle, *Micraspis discolor* (Coleoptera: Coccinellidae), hairy ground flies, *Argyrophylax nigrotibialis* (Diptera: Tachinidae), predaceous mirid bug, *Cyrtorhinus lividipennis* (Hemiptera: Miridae), braconid wasp*, Tropobracon schoenobii* (Hymenoptera: Braconidae), respectively. Yet these insects become insect pest predators and parasitoids in rice fields for a short time during rice development (Hendrichs *et al*., 1995), and surveys only capture a small number (less than 10%). The species accumulation curves for all insect visitor taxa observed in the rice field showed an increasing number of predictions towards the end of the survey. It was possible that screening or deploying insect-attracting traps in rice fields from planting to harvest would be beneficial. Insect species have also increased in the rice fields which was cosistent with Berry *et al*. (2020).

Extracts from pandanus leaves, green soybean pods, and bread flowers each contained distinct chemical components; however, hexadecanoic acid, methyl ester was consistently found in all three. This compound ($C_{17}H_{34}O_2$), also known as methyl palmitate, belongs to the class of fatty acid methyl esters (FAMEs), which are formed by the esterification of fatty acids with methanol (National Center for Biotechnology Information, 2024). This chemical can also be isolated from rice (Sirilertpanich *et al*., 2024). Previous researchs have confirmed that hexadecanoic acid, methyl ester, can attract insects to plants, where they can deposit eggs, consume food, and mate. It has been reported from many insects, including the beet armyworm (*Spodoptera exigua*), navel orangeworm (*Amyelois transitella*) (Xia, 2020), cotton bollworm (*Helicoverpa armigera*) (Pande *et al*., 2019), and groundnut borer (*Caryedon serratus*) (Tewari *et al*., 2015). Additionally, Feng *et al*. (2022) speculated that plant volatile organic compounds can attract plant bug, *Lygus pratensis*, an agricultural pest known to harm several crops. these findings demonstrated the high attractant impact of heptadecane, dodecane, decanal, and β-pinene on *L. pratensis* adults, suggesting their potential use as effective attractants for the ecological control of *L. pratensis.* On the other hand, as previously reported (Chinachanta *et al*., 2021; Sirilertpanich *et al*., 2024), there may be other aromatic substances that resemble rice, but this could be due to the preparation of substances that might affect the system in the analysis, the compounds in the NIST library are not available, therefore they may not be detected or those substances were volatile oils, resulting in less substances being extracted. Alternatively, the substance's stability is inadequate for this investigation. Therefore, headspace volatile should be further detected. However, this study represents only a single aspect of the more general concept of compounds that are intended to attract insects into the system. The cultivation of plants with aromas to attract insects into the ecosystem may be promoted as an aspect of an ecological engineering system to increase the biodiversity of both harmful and beneficial insects to control each other as natural control, potentially reducing losses from outbreaks of various types of rice field pests.

In brief, this study evaluated the possibility of employing extracts from different plants, including pandanus leaves, green soybean pods and bread flowers. It was discovered that more than 80% of rice insect pests were planthoppers and leafhoppers in the order Hemiptera. In this regard, chemicals acquired during the verification are also included. GC-MS can be found in all plants, particularly compounds with the hexadecanoic acid, methyl ester group, which may attract a variety of insects, as documented in this study. These extracts could be used as alternatives to cultivating the plants themselves to attract and trap insect pests. Additionally, they may promote ecological interactions among insect communities, fostering a balance between pest populations and their natural enemies. This approach aligns well with the principles of Integrated Pest Management (IPM) in sustainable agriculture.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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