Insecticidal activity of teak (*Tectona grandis* L.f.) leaves extracts against diamondback moth (*Plutella xylostella* L.) and mealybug (*Phenacoccus manihoti* Matile–Ferrero)

J. Pumnuan[1,*], A. Insung[1] and N. Montri[2]

[1] Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand
[2] Program in Horticulture, Division of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Prince of Chumphon Campus, Chumphon 86160, Thailand
* Corresponding author: jarongsak.pu@kmitl.ac.th
Submission: 19 April 2020 Revised: 10 February 2021 Accepted: 4 April 2021

ABSTRACT

The efficacy examination of ethanolic and methanolic extracts from three varieties of teak (*Tectona grandis* L.f.) leaves, i.e., Sak-Syamindra (SS), Sak-Mahesak (SM), and Sak-Thong (ST) against larvae of diamondback moth (*Plutella xylostella* L., DBM) and nymph of cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero, CMB) were investigated by using leaf dipping method. Various concentrations of extracts were applied and compared with the control (Tween-20 in water) and chemical insecticide groups. The insecticidal properties of teak extracts were studied in terms of oral toxicity test to both insects, anti-feedant activity, and inhibition of insect development assay employed to DBM and repellent test to CMB. The results presented that teak leave extract treatments gave a rather high effect in killing the larva of DBM with the median lethal concentration (LC_{50}) at 24 and 48 hr as 7.2–8.8 and 4.9–6.4%, respectively. The methanolic extracts obtained from SM and SS at 6% concentration could inhibit the development of DBM from larva to adult, lower than 10%. Both ethanolic and methanolic extracts from ST showed the highly effective result in anti-feedant rate, at 4% concentration presented completely anti-feedant activity. For the test to CMB, the results revealed that all extracts from SM were highly effective in killing the nymph of CMB with the LC_{50} at 24 and 48 hr as 3.9–4.2 and 2.3–2.4%, respectively. All extracts presented a rather low effect in repellency to the nymph. The performance of insecticides against both insects showed no significant difference compared with SM extracts ($p < 0.05$). The results of this study implied SM extract might be another alternative way for the development of botanical insecticides.

Keywords: Anti-feedant, inhibition of insect development, insecticidal property, oral toxicity, repellent

Thai J. Agric. Sci. (2021) Vol. 54(1): 1−13

INTRODUCTION

Diamondback moth (*Plutella xylostella* L., DBM) is an important insect pest of crops specifically the cabbage family throughout the world. Yi *et al.* (2011) reported that the cultivation of cruciferous vegetable in Asian counties is challenged by an infestation of DBM, which result in great loss of the yield. The vegetable damage is caused by larval feeding. They can be quite numerous, resulting in the complete removal of foliar tissue except for the leaf veins. The presence of larvae in florets can result in the complete rejection of produce (Capinera, 2000). Whereas, cassava mealybug (*Phenacoccus manihoti* Matile–Ferrero, CMB) appears specific to cassava. The CMB is the most economically important insect pests causing severe yield reduction in cassava fields (Bellotti, 2002), particularly, is one of the most severe pests of cassava in Asia. It has spread aggressively throughout Thailand's cassava–growing region since 2008 (Winotai *et al.*, 2010), and began causing yield losses as high as 50%, estimated at more than US$ 30 million, within two years of first detection (Bellotti *et al.*, 2012).

The chemical insecticide is widely accepted for its considerably high performance. Farmers prefer to use chemical pesticides for controlling these pests because chemicals have an immediate knockdown effect and are readily available in local markets (Jompong *et al.*, 2015). However, the application of these chemical insecticides has recently been questioned for their impacts on the farmer, customer and environment, and induces resistance to insecticides. Many reports explained that the insects have developed resistance to insecticides applied in the field (Wakgari and Giliomee, 2003; Sarfraz and Keddie, 2005; Sarfraz *et al.*, 2005). Remarkably, GPAC (2017) reported that DBM has a high propensity to develop resistance and there are more than 82 insecticide compounds recorded globally to which DBM has developed resistance. Likewise, the mealybug tends to have the potential to develop resistance to most chemical classes of insecticides (Saddiq *et al.*, 2014). Pesticide poisoning is then an important health problem in Thailand because of the intensive use of hazardous pesticides (Tawatsin *et al.*, 2015). Duangchinda *et al.* (2014) reported the interesting result of the examination of acetylcholinesterase (AChE) levels in the blood of farmers who applied organophosphate and carbamate insecticides. The samples were taken from 236 farmers in Sam Chuk district, Suphan Buri province, Thailand. Obtained results showed that 37.3% of farmers had AChE levels lower than standard (< 87.5 units/mL) indicating a risk to health (28.8%) and an unsafe condition (8.5%). The use of chemical insecticides was related to the AChE levels of farmers. These limitations of chemical pesticides have wildly appeared, therefore, drawn interest in the application of plant extract is a new alternative method. The plant extracts which are known as bio-pesticide or green-pesticides can be an appropriate good source besides chemical pesticides due to their safe, eco-friendly, and more compatible properties (Rahman *et al.*, 2016). Many researches point out that the plant extracts could control DBM including neem (*Azadirachta indica*), syringe (*Syringa vulgaris*), mesquite (*Prosopis juliflora*), kariyat (*Andrographis paniculata*), sesban (*Sesbania grandiflora*), bastard cedar (*Melia azedarach*), cloth of gold (*Lantana camara*), sweet wormwood (*Artemisia annua*) and hemp (*Cannabis sativa*) (Charleston *et al.*, 2001; Kumar *et al.*, 2009; Sangavi and Johnson Thangaraj Edward, 2017). While the extracts of peach (*Prunus persica*), eucalyptus (*Eucalyptus globulus*), ashok (*Polyalthia longifolia*), milk thistle (*Silybum marianum*), neem (*A. indica*), sour orange (*Citrus aurantium*), sweet orange (*Citrus sinensis*), clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum bejolghota*) and lemon grass (*Cymbopogon citratus*) (Roonjho *et al.*, 2013; Pumnuan and Insung, 2016; Majeed *et al.*, 2018) presented high efficacy against CMB. However, there are still many other plant extracts with high potential that could be applied to control various insect pests. For the utmost benefit of utilizing plenty of plants in controlling insect pests of a vegetable crop, the wild plants seem to be an interesting source and may result to be economic plants in the future.

Teak (*Tectona grandis* L.f.) is a wild plant that has completely become an economic plant. It belongs to the family Verbenaceae and is an important timber plant in the world, commonly found in India and many Southeast Asian countries, especially Thailand, Indochina, and Myanmar (Kushwah *et al.*, 2013; Lanka and Parimala, 2017). Teak is well known for durability and insect resistance (Lanka and Parimala, 2017), it is also considered as a major constituent in many traditional medicines (Khare, 2007). Teak contains various chemical compounds in different parts of the plant body. The quality of the timber is mainly attributed to the accumulation of certain quinones and quinone derivatives (Kokutse *et al.*, 2006). The Bis (2-ethylhexyl) phthalate is a chemical compound extractable from teak wood and it could repel the termite (Alabi and Oyeku, 2017). The leaves of teak extract contain a greater number of secondary metabolites namely steroids, tannin, anthocyanin, flavonoids, diterpenes, physterol, and phlobatannin. (Godghate and Sawant, 2014). The earlier report revealed that the chemical compound in the teak affected pathogenic fungi and insect pests (Guerrero-Vásquez *et al.*, 2013; Alabi and Oyeku, 2017; Hamad *et al.*, 2019). The important issue of teak extract application to control insect pests of crop plants is the safety of such substance to customer. Hamdin *et al.* (2019) reported that the leave extract of teak did not cause any apparent *in vivo* toxicity in an animal model. No death or signs of acute toxicity were observed in Wistar rats treated with this extract. Therefore, it would be confident that we can provide teak extracts for agriculture.

To minimize the use of synthetic insecticides in plant protection and to avoid this chemical pollution in the environment, there is an urgent need to develop the safety alternative way which is an eco–friendly approach. With this regard, the most important consideration is the potential for controlling insect pests. With the outstanding potential of the chemical compound in the teak extract performed the effect on crop pests as mentioned above, it is interesting that it can be used to prevent insect pests. Besides, various varieties of teak may present different efficiencies to control insect pests. The undeniable fact that the diamondback moth is a major pest of cabbage crops in the tropical region, while the mealybug is a polyphagous insect pest. Therefore, both insects were selected and used for the test as the representative of chewing and piercing-sucking insect mouthparts, respectively. This research aimed to investigate the insecticidal properties (oral toxicity, repellent, and anti-feedant testes) of ethanolic and methanolic crude extracts from three varieties of teak (*T. grandis*) leaves Sak–Syamindra, Sak-Mahesak, and Sak-Thong against larvae of diamondback moth (*P. xylostella*) and nymph of cassava mealybug (*P. manihoti*). The examinations were designed by using leaf dipping method and compared with some chemical insecticides.

MATERIALS AND METHODS

Insect Preparation

The two tested insects namely, the diamondback moth (*Plutella xylostella* L., DBM) was reared on False Pak Choi leaves (*Brassica rapa* var. *parachinensis* (Bailey) Hanelt) and the mealybug (*Phenacoccus manihoti* Matile–Ferrero, CMB) was cultured on the pumpkin (*Cucurbita moschata* Decne.) in the laboratory of Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Thailand. The larva of DBM colony was originally collected from the field in Nakhon Pathom province located in central Thailand. While the adults of CMB colonies were gathered from cassava plantations in Kanchanaburi province. For the test of CMB, the nymph was initially transferred to okra fruit (*Abelmoschus esculentus* (L.) Moench) before the experiments. Both original insect locations did not have any previous exposure to pesticides. Insects were reared under laboratory conditions at room temperature. In case of DBM, prior to use F2 generation larvae in the various bioassay then, the 3rd stage of DBM larvae were starved for 2 hr. The 1st nymphal stage of the F2 generation of CMB reared on okra fruit was used for further experiments.

Teak Extracts and Insecticides Preparations

The leaves from three varieties of teak (*Tectona grandis* L.f.), i.e., Sak-Syamindra (SS), Sak-Mahesak (SM), and Sak–Thong (ST), were collected from Chumphon province located in southern Thailand. The fresh old leaf samples of the selected teak tree were air–dried for 5–7 d. After completely drying the plant part were crushed into fine powder. Extraction was made when these plant materials were undertaken by placing 1 kg of powdered plant material into a 20 L glass jar (top covered with fine cloth) and soaked in 4 L ethanol or methanol solvents kept at room temperature for 3 d. The filtrate was obtained via filtering using Whatman filter paper No.1 incorporation assistant by the vacuum pump. The ethanol or methanol solutions were then concentrated by rotary evaporation to obtain the ethanolic or methanolic crude extracts. The various concentrations of extracts, 0 (10%v/v Tween–20 in water) and 2, 4, 6, 8 and 10%w/v were prepared and then used for treating DBM, while the concentrations of extracts, 0 (6%v/v Tween-20 in water) and 1, 2, 3, 4, 5 and 6%w/v used for CMB.

The study of insecticidal activities of teak leave extracts against DBM was compared with fipronil insecticide, while of CMB experiment was compared with imidacloprid insecticide at both recommendation and double rates. The details of their active ingredients (different recommendation rates) were as follows; fipronil 5% SC (100 ppm) and imidacloprid 70% WG (100 ppm).

Insecticidal Properties Bioassay

Teak extract against DBM

The bioassay was adapted from Charoensak *et al.* (2009), the oral toxicity of the crude extracts against DBM larvae was investigated through no-choice bioassay using leaf discs (30 mm in diameter) prepared from False Pak Choi leaves cut by a cork borer. Onto each leaf disc was dipped in various concentrations of the extract solution for 1 min. Dipping with fipronil insecticide at both recommendation and double rates was also performed. All dipped False Pak Choi leaf discs were left at room temperature to air-dry, where after the leaf was placed in a plastic Petri dish (10 cm diameter, the top was cut and covered with fine cloth) and padded with moist filter paper marked on one side. Ten 3rd instar DBM larvae (previously starved for 2 hr) were then introduced in each plastic Petri dish containing a leaf disc. Three replications were applied for each treatment. Then, the mortality percentage of the larvae was observed at 24 and 48 hr for oral toxicity property. Data for the bioassay were corrected for mortality in the control using Abbott's formula (Abbott, 1987). In addition, the effects of continuous exposure of the plant extracts on larval development to pupae and adults were also recorded. The inhibition of insect development rate was calculated according to survival percentages of DBM pupae and adults. In case of anti–feedant activity, the same concentrations of extract solutions as referred to in the oral toxicity test were performed. Three replications were made for each treatment. The percentage of leaf damage area after 24 hr was observed and compared with the leaf area before treated. This percentage of anti-feedant activity in no-choice test was calculated according to the percentage of leaf damage using equation 1 (Abbaszadeh *et al.*, 2014):

$$\%\text{Anti-feedant activity} = \frac{\text{Leaf damage in treatment} - \%\text{Leaf damage in control}}{100 - \%\text{Leaf damage in control}} \times 100 \quad \text{----- (1)}$$

Teak extract against CMB

Insecticidal property of teak leave extracts against CMB nymph was investigated through no–choice bioassays using leaf dipping method prepared from okra fruit. The fruit was dipped with the various extract solutions and imidacloprid insecticide at both recommendation and double rates, for 1 min. All fruits were left at room temperature to air–dry, whereafter the fruit was placed in a plastic Petri dish (13 cm diameter, the top was cut and covered with fine cloth). Twenty CMB nymphs were then introduced into each plastic Petri dish containing a treated okra fruit. The mortality percentage of the nymph was observed at 24 and 48 hr. Data for the bioassay were corrected for mortality in the control using Abbott's formula (Abbott, 1987). For the repellent activity, the experiments of various concentrations of extract solutions were performed through choice tests. The treatment (T) and control (C) fruit groups were prepared by dipping each side of fruit and placed in a plastic Petri dish. After 24 hr, the number of insects was counted in the T and C. The percentage of repellent index (%RI) was calculated using equation 2 (Pascual-Villalobos and Robledo, 1998).

$$\%\text{RI} = \frac{C - T}{C + T} \times 100 \quad \text{----- (2)}$$

Statistical Analysis

The experiment was designed in three completely randomized replicates. The comparisons between each concentration of teak extract, stage of insect, and time observed from insecticidal activity examination were analyzed by applying analysis of variance (ANOVA). Multiple comparisons between factor levels were done by Duncan's multiple range tests (DMRT) at α = 0.05. The insecticidal activity was shown in the form of median lethal concentration (LC_{50}), which was calculated by the probit method.

RESULTS AND DISCUSSION

The tests of ethanolic and methanolic extracts from three varieties of teak leaves: Sak–Syamindra (SS), Sak–Mahesak (SM) and Sak–Thong (ST) to the 3rd instar larvae of DBM revealed that all teak leave extracts gave a rather high effect in killing the larva of DBM, at 10% concentration of extract caused 65.6–82.6 and >96.3% mortality at 24 and 48 hr, respectively. All three varieties of teak extracted by both solvents showed the larval mortality of DBM with no significant difference ($p > 0.05$) when the LC_{50} values at 24 and 48 hr were 7.2–8.8 and 4.9–6.4%, respectively (Table 1).

Table 1 Mortality percentage of diamondblack moth (*Plutella xylostella* L.) larvae after feeding with host plants treated with teak (*Tectona grandis* L.f.) leaf extracts

Varieties of teak	Solvents	%Mortality						LC_{50} (%)
		Concentrations of extracts						
		0%	2%	4%	6%	8%	10%	
24 hr								
SS	Ethanol	0.0^{D}	3.7^{Dcd}	6.7^{Da}	28.2^{Ca}	44.8^{Ba}	67.6^{Aa}	8.4
	Methanol	0.0^{CD}	14.8^{Cab}	13.7^{Ca}	20.0^{Ca}	47.7^{Ba}	80.0^{Aa}	7.8
ST	Ethanol	0.0^{C}	0.0^{Cd}	6.7^{Ca}	11.7^{Ca}	41.1^{Ba}	65.6^{Aa}	8.8
	Methanol	0.0^{D}	17.8^{Ca}	17.8^{Ca}	28.5^{Ca}	53.7^{Ba}	79.3^{Aa}	7.4
SM	Ethanol	0.0^{D}	7.0^{Dcd}	14.1^{CDa}	28.1^{Ca}	43.0^{Ba}	71.9^{Aa}	8.2
	Methanol	0.0^{E}	13.3^{Dabc}	17.8^{Da}	31.5^{Ca}	57.4^{Ba}	82.6^{Aa}	7.2
48 hr								
SS	Ethanol	0.0^{E}	14.4^{Ea}	33.3^{Da}	52.8^{Ca}	70.6^{Ba}	100.0^{Aa}	5.6
	Methanol	0.0^{E}	25.9^{Da}	44.8^{Ca}	56.7^{Ca}	75.9^{Ba}	100.0^{Aa}	4.9
ST	Ethanol	0.0^{E}	13.7^{Da}	23.3^{CDa}	32.5^{Ca}	65.6^{Ba}	96.3^{Aa}	6.4
	Methanol	0.0^{E}	25.2^{Da}	28.5^{Da}	46.3^{Ca}	71.1^{Ba}	100.0^{Aa}	5.6
SM	Ethanol	0.0^{D}	17.4^{CDa}	21.5^{Ca}	56.3^{Ba}	67.8^{Ba}	100.0^{Aa}	5.7
	Methanol	0.0^{E}	23.3^{Da}	35.6^{Da}	58.9^{Ca}	75.2^{Ba}	100.0^{Aa}	5.1

Note: Data were based on 3rd instars larvae, n = 10 larvae/ replicate of 3 replications.
Means in the same column followed by the same common letter and means in the same row followed by the same capital letters are not significantly different at $p < 0.05$ as determined by DMRT. Three varieties of teak: SS = Sak–Syamindra, SM = Sak–Mahesak and ST = Sak–Thong.
LC_{50} = median lethal concentration

The promising result of the inhibition of insect development test was also found when the extracts of both solvents obtained from SM and SS caused inhibiting the development of DBM from larva to adult with only 3.3–11.6% at 6% concentration. Remarkably, the SM and SS extracts of both solvents could completely inhibit the development of DBM at 8% concentration. However, each concentration of all three varieties of teak extracted by both solvents was effective in inhibiting development from larva to adult of DBM with no significant difference among them ($p > 0.05$), but the ethanolic extracts obtained from ST was low effective (Table 2).

Table 2 Survival percentage of diamondblack moth (*Plutella xylostella* L.) after feeding with host plants treated with teak (*Tectona grandis* L.f.) leaf extracts

Varieties of teak	Solvents	%Survival					
		Concentrations of extracts					
		0%	2%	4%	6%	8%	10%
Pupae							
SS	Ethanol	100.0^{A}	74.8^{Ba}	53.3^{Ca}	31.0^{Da}	18.3^{DEab}	0.0^{E}
	Methanol	100.0^{A}	59.3^{Ba}	40.3^{Ca}	26.7^{CDa}	13.4^{DEbc}	0.0^{E}
ST	Ethanol	100.0^{A}	75.6^{Ba}	60.0^{Ca}	45.8^{Da}	24.1^{Ea}	0.0^{E}
	Methanol	100.0^{A}	64.1^{Ba}	53.7^{BCa}	42.2^{Ca}	21.5^{Dab}	0.0^{E}
SM	Ethanol	100.0^{A}	71.5^{Ba}	60.7^{Ca}	21.5^{Da}	12.5^{Dbc}	0.0^{E}
	Methanol	100.0^{A}	66.7^{Ba}	46.3^{Ca}	17.0^{Da}	7.4^{Dec}	0.0^{E}
Adult							
SS	Ethanol	100.0^{A}	60.4^{Ba}	30.0^{Ca}	11.6^{Da}	0.0^{Db}	0.0^{D}
	Methanol	100.0^{A}	44.4^{Ba}	21.6^{Ca}	10.0^{Da}	0.0^{Db}	0.0^{D}
ST	Ethanol	100.0^{A}	61.9^{Ba}	43.3^{Ca}	24.2^{Da}	10.4^{Ea}	0.0^{E}
	Methanol	100.0^{A}	46.3^{Ba}	35.9^{Ba}	14.1^{Ca}	0.0^{Db}	0.0^{D}
SM	Ethanol	100.0^{A}	53.7^{Ba}	32.2^{Ca}	10.7^{Da}	0.0^{Db}	0.0^{D}
	Methanol	100.0^{A}	46.7^{Ba}	25.2^{Ca}	3.3^{Da}	0.0^{Db}	0.0^{D}

Note: Data were based on 10 larvae/ replicate of 3 replications.
Means in the same column followed by the same common letter and means in the same row followed by the same capital letters are not significantly different at $p < 0.05$ as determined by DMRT. Three varieties of teak: SS = Sak-Syamindra, SM = Sak-Mahesak and ST = Sak-Thong

For the anti-feedant property test, ST extracts of both solvents showed the rather highly effective result in anti-feedant activity when 4% concentration presented 100% anti–feedant activity. While methanolic extract of SS at concentration of 2% performed effective result in anti–feedant activity as 89.9% which was more than that of the other varieties of teak extracts (Figure 1).

The insecticidal activity of fipronil insecticide against DBM at 48 hr showed that it could kill DBM for 58.0% and 74.0% at recommendation and double rates, respectively with no significant difference ($p > 0.05$) compared to methanolic extracts obtained from SM as 58.9, 75.2 and 100.0% at 6, 8 and 10% concentrations, respectively. Whereas, the insecticide presented with low inhibited development of DBM developed from larva to adult for about 28.0 and 8.0% at recommendation and double rates, respectively. The methanolic extracts obtained from SM performed 3.3% at 6% concentrations but the extract at 8% concentration completely inhibited the development of DBM (Table 3). It implied that ethanolic or methanolic extracts of SM gave a satisfactory performance for controlling larva of DBM equivalent to the action of fipronil insecticide. However, the extracts presented higher effective inhibition of growth of DBM developed from larva to adult more than that of insecticide.

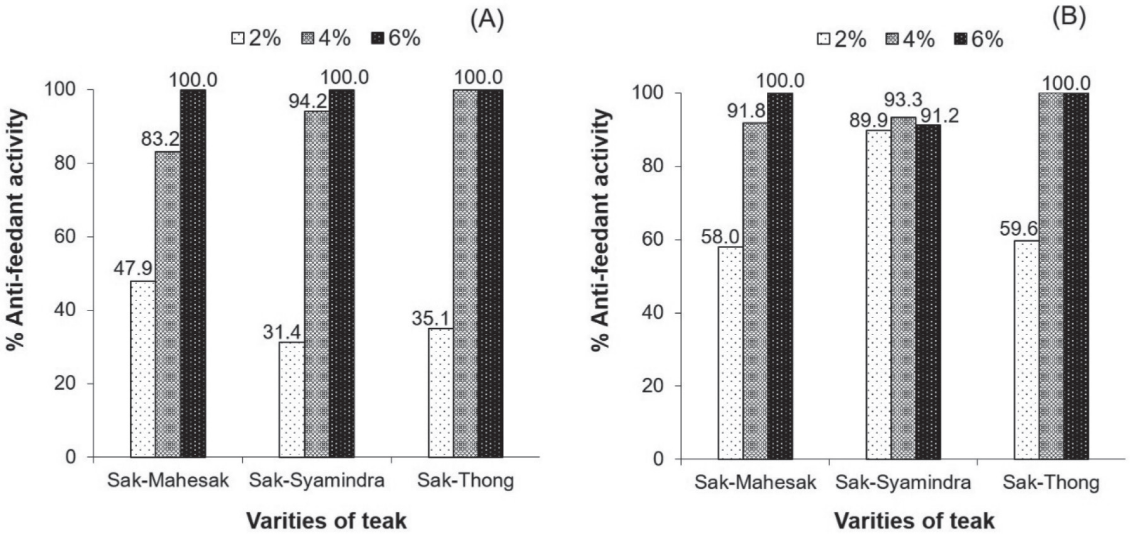

Figure 1 Percentage of anti-feedant activity of ethanolic (A) and methanolic (B) extracts from three varieties of teak (*Tectona grandis* L.f.) leaf against the diamondblack moth (*Plutella xylostella* L.) larvae

Table 3 Mortality and survival percentages of diamondblack moth (*Plutella xylostella* L.) after feeding with host plants treated with chemical insecticides and teak (*Tectona grandis* L.f.) leaf extracts

Insecticides and teak leaf extracts	%Larval mortality		%Pupal and adult survival	
	24 hr	48 hr	Pupae	Adult
Control	0.0^{E}	0.0^{C}	100.0^{A}	100.0^{A}
Fipronil (recommended rate 100 ppm)	40.0^{CD}	58.0^{B}	32.0^{B}	28.0^{B}
Fipronil (double recommended rate 200 ppm)	54.0^{BC}	74.0^{B}	10.0^{C}	8.0^{C}
6% SM leaves extracted by methanol	31.5^{D}	58.9^{B}	17.0^{C}	3.3^{C}
8% SM leaves extracted by methanol	57.4^{B}	75.2^{B}	7.4^{C}	0.0^{C}
10% SM leaves extracted by methanol	82.6^{A}	100.0^{B}	0.0^{C}	0.0^{C}

Note: Data were based on 10 larvae/ replicate of 3 replications.
Means in the same column followed by the same capital letter are not significantly different at $p < 0.05$ as determined by DMRT. SM = teak variety Sak–Mahesak

The insecticidal activity test to CMB showed that both extracts of SM gave most effective in killing the nymph of CMB when the concentration of 3% caused 45.5% and 71.3–74.9% mortality at 24 and 48 hr observation, respectively, with a higher number than the other extracts. The extracts of SM showed the LC_{50} with 3.9 and 4.2% at 24 hr and 2.3 and 2.4% at 48 hr. However, all 6% extracts of the teak were effective to CMB which caused about 79.9–100% mortality at 48 hr with no significant difference ($p > 0.05$) (Table 4).

In addition, the ST and SM extracts at 6% concentration showed higher repellent property (33.4–43.3%RI) more than SS extract (2.0–24.9%RI) (Figure 2), while the imidacloprid insecticide presented %RI as 50.8 that was higher than SM extract at 6% concentration (Table 5). The toxicity of imidacloprid insecticide against CMB presented with lower activity in killing CMB (88.3%) at the recommended concentration when SM extracts at the concentration of 6% gave higher mortality (91.9 and 100.0%) at 48 hr, with no statistically significant difference ($p > 0.05$) (Table 5). The study showed that the ethanolic and methanolic extracts of SM could kill CMB nymph better than imidacloprid used at recommendation rate. Surprising result obtained when double recommendation rates of insecticide had equivalent effect comparing to SM extracts.

Table 4 Mortality percentage of mealybug (*Phenacoccus manihoti* Matile-Ferrero) nymph after feeding with host plants treated with teak (*Tectona grandis* L.f.) leaf extracts

Varieties of teak	Solvents	%Mortality							LC_{50} (%)
		Concentrations of extracts							
		0%	1%	2%	3%	4%	5%	6%	
24 hr									
SS	Ethanol	0.0^{D}	4.0^{CDb}	7.1^{CDb}	10.2^{CDc}	21.9^{BCc}	32.7^{Ba}	63.8^{Aa}	5.6
	Methanol	0.0^{C}	1.7^{Cb}	8.6^{Cb}	13.3^{Cc}	32.1^{Bbc}	55.8^{Aa}	64.7^{Aa}	5.0
ST	Ethanol	0.0^{D}	5.1^{Db}	15.5^{CDab}	28.5^{BCb}	33.4^{BCbc}	44.3^{Ba}	69.6^{Aa}	4.9
	Methanol	0.0^{C}	13.4^{Cab}	16.5^{Cab}	38.9^{Bab}	43.5^{Bab}	44.8^{Ba}	64.2^{Aa}	4.8
SM	Ethanol	0.0^{D}	26.9^{Ca}	30.8^{Ca}	45.5^{Ba}	47.3^{Bab}	51.8^{Ba}	67.8^{Aa}	4.2
	Methanol	0.0^{D}	9.3^{Db}	30.4^{Ca}	45.5^{BCa}	57.6^{ABa}	63.3^{Aa}	72.6^{Aa}	3.9
48 hr									
SS	Ethanol	0.0^{D}	11.0^{CDb}	20.1^{CDc}	23.3^{Cb}	46.6^{Bd}	64.1^{Bc}	83.9^{Aa}	4.1
	Methanol	0.0^{D}	20.7^{Cb}	24.9^{Cc}	34.7^{Cb}	52.6^{Bcd}	77.6^{Aabc}	79.9^{Aa}	3.7
ST	Ethanol	0.0^{E}	18.6^{Db}	35.5^{Cbc}	40.1^{Cb}	65.5^{Bbc}	72.4^{Bbc}	96.2^{Aa}	3.2
	Methanol	0.0^{E}	18.2^{Db}	55.6^{Ca}	59.7^{Ca}	74.6^{Bab}	86.1^{ABab}	95.3^{Aa}	2.6
SM	Ethanol	0.0^{E}	38.6^{Da}	52.1^{Cab}	71.3^{Ba}	79.9^{ABab}	86.3^{Aab}	91.9^{Aa}	2.3
	Methanol	0.0^{F}	26.0^{Eab}	38.7^{Dabc}	74.9^{Ca}	84.0^{BCa}	90.7^{ABa}	100.0^{Aa}	2.4

Note: Data were based on nymph, n = 20 nymph/ replicate of 3 replications
Means in the same column followed by the same common letter and means in the same row followed by the same capital letters are not significantly different at $p < 0.05$ as determined by DMRT. Three varieties of teak: SS = Sak–Syamindra, SM = Sak–Mahesak and ST = Sak–Thong.
LC_{50} = median lethal concentration

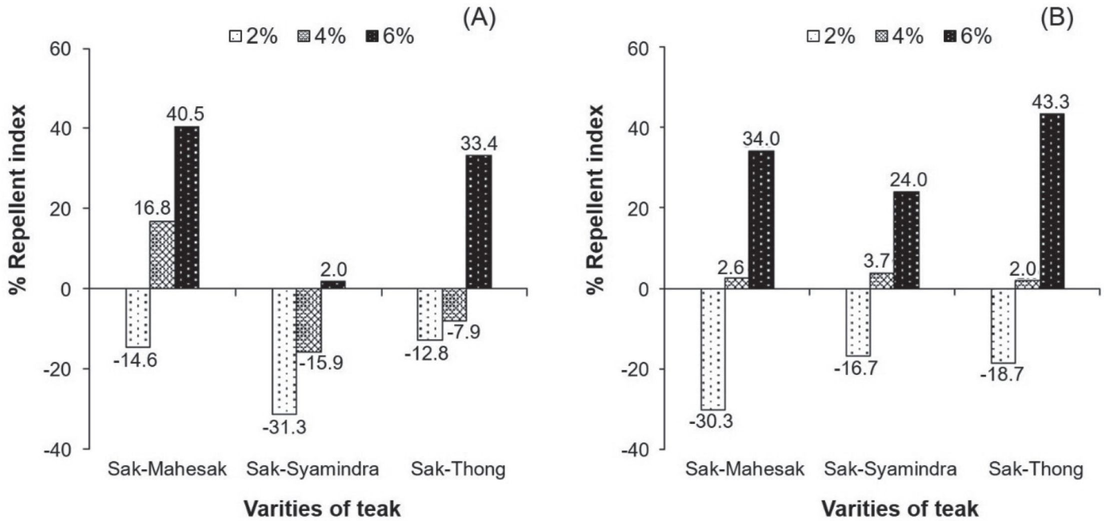

Figure 2 Percentage of repellent index (%RI) of ethanolic (A) and methanolic (B) extracts from three varieties of teak (*Tectona grandis* L.f.) leaf at various concentrations against mealybug (*Phenacoccus manihoti* Matile-Ferrero) nymph by dipping method

Table 5 Mortality percentage of mealybug (*Phenacoccus manihoti* Matile-Ferrero) nymph and repellent index (%RI) caused by chemical insecticides and teak (*Tectona grandis* L.f.) leaf extracts

Insecticides and teak leaf extracts	%Mortality		%Repellent index
	24 hr	48 hr	
Control	0.0^{D}	0.0^{B}	–
Imidacloprid (recommended rate 100 ppm)	39.7^{C}	88.3^{A}	50.8^{A}
Imidacloprid (double recommended rate 200 ppm)	68.1^{AB}	100.0^{A}	–
5% SM leaves extracted by ethanol	51.8^{BC}	86.3^{A}	–
5% SM leaves extracted by methanol	63.3^{AB}	90.7^{A}	–
6% SM leaves extracted by ethanol	67.8^{AB}	91.9^{A}	40.5^{A}
6% SM leaves extracted by methanol	72.6^{A}	100.0^{A}	34.0^{A}

Note: Data were based on nymph, n = 20 nymph/ replicate of 3 replications
Means in the same column followed by the same capital letter are not significantly different at $p < 0.05$ as determined by DMRT. SM = teak variety Sak–Mahesak

Many factors influencing the effectiveness of plant extracts to control insect pests are different plant species, part of plant, tested insects, methods of extraction, type of solvents harvesting, season of plants, and method of application. Dhale (2013) found that Ocimum americanum played as insecticide while Ocimum gratissimum and Ocimum tenuiflorum had insect repellent property. Charoensak et al. (2009) reported that the hexane extract of Anethum graveolens and Polygonum odoratum were highly effective in killing tobacco cutworm (Spodoptera litura) and inhibiting the growth of the cutworm during the larval stage but could not inhibit the growth of pupae developing to adult. However, these extracts had low anti-feedant efficiencies. Our results found that the extracts of three varieties of teak leaves extracted by using the same solvent affected each insect congruous. Various varieties of the same teak species (T. grandis) may contain similar chemical compounds and express their efficiency against insects relatively with no significant difference. The report of Avetisyan et al. (2017) remarkably proved that different varieties of basil (Ocimum basilicum var. purpureum and O. basilicum var. thyrsiflora) had the same sets of chemical compounds, but there were different quantities. However, ST ethanolic and methanolic extracts showed rather highly effective resulted in anti–feedant activity on DBM. The SM and SS methanolic extracts caused inhibiting the development of DBM developed from larva to adult, and SM ethanolic and methanolic extracts were highly effective in killing the CMB nymph.

The plants have evolved defense chemicals against various insects such as phenols, polyphenols, terpenoids, and alkaloids that could be isolated by using various extraction methods. Some plant extracts had active substances with high potential for development of the bio–insecticides (Pavela, 2016). Godghate and Sawant (2014) reported that the saponin compound could be found in teak leave methanolic extracts. The saponin compound had been implied in mechanisms of plant resistance against potential herbivores (Nielsen *et al.*, 2010). In this study, 6% concentration of ST and SM extracts showed higher repellent property (33.4% for ethanolic extract and 43.3% for methanolic extract), this low concentration tended to attract CMB nymph. Alabi and Oyeku (2017) informed that only extracts containing a high percentage of Bis (2-ethylhexyl) phthalate could repel the termite, whereas others appeared to attract this insect.

Currently, DBM has developed resistance to insecticides for more than 82 insecticide compounds recorded globally (GPAC, 2017). In this study, fipronil insecticidal activity against DBM showed the low performance to control DBM (<55%) at 24 hr and presented low inhibiting growth of DBM. It was interesting that ethanolic and methanolic SM leaves teak extracts presented their insecticidal properties against DBM and CMB non significantly different when compared to the insecticides. However, other information as suitable formulas for application with a lower concentration of substances and application technique appropriating for the area conditions.

Nowadays, teak is an economic plant of the most valuable hardwoods, which is paid premium prices at international markets and use of wood only. It seems to be nice if the teak leaves would be alternatively used as bio-insecticide for the protection of other crop plants. It may increase income for teak tree culture farmers, as well as minimize the use of chemical insecticides and promote agricultural sustainability.

CONCLUSIONS

The ethanolic and methanolic crude extracts from Sak-Mahesak teak leave showed a high effect in killing larvae of diamondback moth with the LC_{50} at 24 hr as 7.2 and 8.2%, respectively, and remarkably at the 8% concentration could completely inhibit the development from larva to adult. Those extracts also performed high potential in controlling cassava mealybug nymph when they gave the LC_{50} at 24 hr with 3.9% for methanolic extract and 4.2% for ethanolic extract. The insecticidal activities of these teak extracts against both insects showed no significant difference when compared to insecticides in laboratory conditions. The results implied those extracts would be developed as a botanical insecticide to be used as another alternative way for insect pest control. However, more information regarding the Sak-Mahesak teak leave extract development and suitable application should be further investigated.

ACKNOWLEDGEMENTS

The research project was conducted in Plant Genetics Conservation Project under The Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhron, Utilization of Teak Leaf for Agricultural and Medicinal Purposes. This work “Application of Teak Leaf (*Tectona grandis*) Extracts to Control Diamondback Moth (*Plutella xylostella*) and Mealybug (*Phenacoccus manihoti*)” was supported by 2017 Thailand Government Budget Grant #A118–0260–010.

REFERENCES

Abbaszadeh, G., C. Srivastava and S. Walia. 2014. Insecticidal and antifeedant activities of clerodane diterpenoids isolated from the Indian bhant tree, *Clerodendron infortunatum*, against the cotton bollworm, *Helicoverpa armigera*. J. Insect Sci. 14: 29.

Abbott, W.S. 1987. A method of computing the effectiveness of an insecticide. 1925. J. Am. Mosq. Control Assoc. 3: 302–303.

Alabi, K. and T. Oyeku. 2017. The chemical constituents extractable from teak tree (*Tectona grandis* Linn) obtained from Fountain University, Osogbo. Nig. J. Basic Appl. Sci. 25(1): 73–80.

Avetisyan, A., A. Markosian, M. Petrosyan, N. Sahakyan, A. Babayan, S. Aloyan and A. Trchounian. 2017. Chemical composition and some biological activities of the essential oils from basil *Ocimum* different cultivars. BMC Complement Altern. Med. 17: 60.

Bellotti, A.C. 2002. Arthropod pests, pp. 209–235. *In*: R.J. Hillocks, J.M. Thresh and A.C. Bellotti, (Eds), Cassava: Biology, Production and Utilization. CABI Publishing, Wallingford, UK.

Bellotti, A.C., B.V. Herrera Campo and G. Hyman. 2012. Cassava production and pest management: present and potential threats in a changing environment. Trop. Plant Biol. 5: 39–72.

Capinera, J.L. 2000. Diamondback moth, *Plutella xylostella* (Linnaeus) (Insecta: Lepidoptera: Plutellidae). EENY-119. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Charleston, D.S., M. Dicke, L.E.M. Vet and K. Rami. 2001. Integration of biological control and botanical pesticides–evaluation in a tritrophic context, pp. 207–216. *In*: Proceedings of the 4th International Workshop. Melbourne, Australia.

Charoensak, S., J. Pumnuan and A. Insung. 2009. Efficiency of extracts from indigenous herbs of northeastern Thailand in controlling the tobacco cutworm, *Spodoptera litula* (f.). As. J. Food Ag–Ind. Special issue: S234–S240.

Dhale, D.A. 2013. Plants used for insect and pest control in North Maharashtra, India. J. Ethno. Trad. Med. Photon. 118: 379–388.

Duangchinda, A., B. Anurugsa and N. Hungspreug. 2014. The use of organophosphate and carbamate pesticides on paddy fields and cholinesterase levels of farmers in Sam Chuk district, Suphan Buri province, Thailand. Thammasat Int. J. Sci. Technol. 19(1): 39–51.

Godghate, A.G. and R.S. Sawant. 2014. Phytochemical analysis of leaves of *Tectona grandis* Linn. Int. J. Pharm. Biol. Sci. 5(1): 355–359.

GPAC (Grains Pest Advisory Committee). 2017. Resistance Management Strategy for Diamondback Moth in Australian Canola. GRDC Factsheet. Grains Research and Development Corporation.

Guerrero–Vásquez, G.A., Z.A. Carlos Kleber, M.G. Jose and A.M. Francisco. 2013. Practical first total synthesis of the potent phytotoxic (+)–naphthotectone, isolated from *Tectona grandis.* Eur. J. Org. Chem. 27: 6175–6180.

Hamad, Y.K., Y. Abobakr, M.Z.M. Salem, H.M. Ali, A.S. Al–Sarar and A.A. Al–Zabib. 2019. Activity of plant extracts/essential oils against three plant pathogenic fungi and mosquito larvae: GC/MS analysis of bioactive compounds. BioResources 14(2): 4489–4511.

Hamdin, C.D., E.S. Prasedya, S.W. Utami, D. Galanova, D.C. Saputro, A. Nurrochmad, R. Murwanti, A. Jupri and H. Sunarpi. 2019. Acute toxicity of Indonesian natural food colorant *Tectona grandis* leaf extract in Wistar rats. J. Med. Sci. 19(2): 69–74.

Jompong, U., J. Pumnuan and A. Insung. 2015. Insecticide application in mushroom farms: a survey study in Nongyaplong district, Phetchburi province, Thailand. KMITL. Sci. Tech. J. 15(2): 80–87.

Khare, C.P. 2007. Indian Medicinal Plants: An Illustrated Dictionary. Springer Verlag, Heidelberg.

Kokutse, A.D., A. Stokes, H. Bailléres, K. Kokou and C. Baudasse. 2006. Decay resistance of Togolese teak (*Tectona grandis* L.f) heartwood and relationship with colour. Trees 20: 219–223.

Kumar, R., K.C. Sharma and D. Kumar. 2009. Studies on ovicidal effects of some plant extracts against the diamondback moth, *Plutella xylostella* (L.) infesting cauliflower crop. Biol. Forum Int. J. 1(1): 47–50.

Kushwah, A.S., P. Kaur and R. Singh. 2013. *In–vitro* antioxidant potential and phytochemical screening of *Tectona grandis* Linn. leaves. Int. J. Pharm. Med. Res. 1: 33–38.

Lanka, S. and Parimala. 2017. Antimicrobial activities of *Tectona grandis* leaf and bark extracts. Eur. J. Pharm. Med. Res. 4(12): 245–248.

Majeed, M.Z., M.I. Nawaz, R.R. Khan, U. Farooq and C. Ma. 2018. Insecticidal effect of some plant extracts on citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae). Trop. Subtrop. Agroecosyst. 21: 421–430.

Nielsen, N.J., J. Nielsen and D. Staerk. 2010. New resistance–correlated saponins from the insect–resistant crucifer *Barbarea vulgaris*. J. Agric. Food Chem. 58: 5509–5514.

Pascual–Villalobos, M.J. and A. Robledo. 1998. Screening for anti–insect activity in Mediterranean plants. Ind. Crops Prod. 8: 183–194.

Pavela, R. 2016. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects – a review. Plant Protect. Sci. 52(4): 229–241.

Pumnuan, J. and A. Insung. 2016. Fumigant toxicity of plant essential oils in controlling thrips, *Frankliniella schultzei* (thysanoptera: thripidae) and mealybug, *Pseudococcus jackbeardsleyi* (Hemiptera: Pseudococcidae). J. Ent. Res. 40(1): 1–10.

Rahman, S., S.K. Biswas, N.C. Barman and T. Ferdous. 2016. Plant extract as selective pesticide for integrated pest management. Biotechnol. Res. J. 2(1): 6–10.

Roonjho, A.R., W.A. Gillani, A. Rasool, N. Akhtar, T. Mahmood, A. Arsalan, M. Afzal, I. Khan, M.A. Ranjha, M. Irfan and J. Khanhod. 2013. Repellency effects of different plant extracts to cotton mealy bug, *Phenococcus solenopsis* Tinsley (Hemiptera: Pseudococcidae). Pakistan J. Agric. Res. 26(3): 213–219.

Saddiq, B., S.A. Shad, H.A.A. Khan, M. Aslam, M. Ejaz and M.B.S. Afzal. 2014. Resistance in the mealybug *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae) in Pakistan to selected organophosphate and pyrethroid insecticides. Crop Protect. 66: 29–33.

Sangavi, R. and Y.S. Johnson Thangaraj Edward. 2017. Anti–insect activities of plant extracts on the diamondback moth, *Plutella xylostella* (L.). Int. J. Curr. Microbiol. App. Sci. 6(12): 28–39.

Sarfraz, M. and B.A. Keddie. 2005. Conserving the efficacy of insecticides against *Plutella xylostella* (L.) (Lep., Plutellidae). J. Appl. Entomol. 129: 149–157.

Sarfraz, M., L.M. Dosdall and B.A. Keddie. 2005. Spinosad: a promising tool for integrated pest management. Outlooks Pest Manag. 16: 78–84.

Tawatsin, A., U. Thavara and P. Siriyasatien. 2015. Pesticides used in Thailand and toxic effects to human health. Med. Res. Arch. 3: 1–10.

Wakgari, W.M. and J.H. Giliomee. 2003. Natural enemies of three mealybug species (Hemiptera: Pseudococcidae) found on citrus and effects of some insecticides on the mealybug parasitoid *Coccidoxenoides peregrines* (Hymenoptera: Encytridae) in South Africa. Bull. Entomol. Res. 93: 243–254.

Winotai, A., G. Goergen, M. Tamo and P. Neuenschwander. 2010. Cassava mealybug has reached Asia. Biocontrol News Inf. 31(2): 10N–11N.

Yi, D., L. Cui, Y. Liv, M. Zhauang, Y. Zhauang, Z. Fang and L. Yang. 2011. Transformation of cabbage (*Brassica oleracea* L. var. *capitata*) with *Bt cry1Ba3* gene for control of diamondback moth. Agr. Sci. China. 10(11): 1693–1700.