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Toxicity of Malaysian Medicinal Plant Extracts Against *Sitophilus oryzae* and *Rhyzopertha dominica*

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ABSTRACT

The insecticidal activities of extracts from 22 Malaysian medicinal plant extracts from 8 botanical families were tested against rice weevil: *Sitophilus oryzae* (L.) and lesser grain borer: *Rhyzopertha dominica* (F.). The extracts were obtained using hexane, methanol, and dichloromethane to extract potential biopesticides from dried leaves. The toxicity levels were examined periodically based on antifeedant activity and contact toxicity assays using treated grain assay. Hexane extracts of *Alpinia conchigera*, *Alpinia scabra*, *Curcuma mangga*, *Curcuma purpurascens*, *Goniothalamus tapisoides*, *Piper sarmentosum*, and methanol extracts of *Curcuma aeruginosa*, *C. mangga*, and *Mitragyna speciosa* were the most potent extracts against *S. oryzae and R. dominica* with lethal concentration (LC_{50}) values of ≤ 0.42 mg/mL and ≤ 0.49 mg/mL, respectively. The contact toxicity test results showed that methanol extracts of *C. aeruginosa* and *C. mangga*, dichloromethane extracts of *Cryptocarya nigra*, and hexane extracts of *C. mangga*, and *C. purpurascens* resulted in 100% mortality of both pests within 28 days exposure of 5 mg/cm^2 concentration.

Keywords: Antifeedant, insecticidal activity, lesser grain borer, Malaysian medicinal plant, rice weevil

INTRODUCTION

The quality of stored grains and related food products can be affected severely due to insect and pest invasions. Even with proper measures, the damage caused to stored grains and food products by insects can be as high as 10%, reaching 30% in tropical countries (Nakakita, 1998, as cited in Rajashekar & Shivanandappa, 2010, pg. 910). Insect pests were partly responsible for losses of grains (wheat, maize, rice, and soybean) in pre-harvest, post-harvest, and storage, which total up to 1.741 mt (Mesterházy et al., 2020). For example, a report revealed that up to 0.557 million mt rice losses in Malaysia from the post-harvest losses (PHL) activities (Hamzah, 2017, as cited in Nodin et al., 2021, pg. 43). Since their introduction in the 1940s, synthetic insecticides are widely used to protect grains from insect infestation (MacIntyre, 1897). Some commonly used insecticides to control storage pests include organophosphates (e.g., diazinon and chlorpyrifos) and pyrethroids (e.g., deltamethrin and resmethrin). In addition, malathion and pirimiphos-methyl are often present as residual insecticides in stored products (Andrić & Pražić-Golić, 2014; Obeng-Ofori, 2010; World Health Organization [WHO], 1997). However, the lack of proper measures and indiscriminate use of synthetic insecticides has led to the widespread development of resistance, environmental pollution, and negative impact on natural enemies and health hazards to humans (Akhtar et al., 2007; Gill & Garg, 2014; Subramanyam & Hagstrum, 2020). For example, *S. oryzae* (L.) and *R. dominica* (F.), which are considered as main pests of stored food (Majeed et al., 2015), have developed levels of resistance to organophosphorus class pesticides, leaving with few options for their control (Sadeghi & Ebadollahi, 2015; Talukder, 2009). Therefore, it is a dire need to develop safer alternatives as well as insecticides with a target-selective and novel mode of action to reduce the adverse effects on non-targeted insects and natural enemies of insect pests.

Plants have long been considered a potential source of bioactive phytochemicals that can be exploited for the discovery of newer agents for insect control (Adeniyi et al., 2010; Wink, 1993). Through many studies, plant-derived secondary metabolites have been proven suitable for integrated pest management (IPM) use. Based on an IPM ecosystem-oriented strategy, the main target is the long-term prevention of the specific insects' pests, preferably by using a botanical pesticide. The superiority of biopesticides over commonly available ones are due to their several advantages, including biodegradability, selective toxicity to targeted insects, and less toxic to mammals, suggesting the need for developing a new class of biopesticides for insect control (Joseph et al., 2012; Salunke et al., 2009). Despite worldwide efforts to find alternatives for synthetic insecticides, there is still no botanical insecticide developed that is potent enough to replace synthetic insecticides to protect stored grains. With a rich floral diversity of about 15,000 species, Malaysian plants represent a potential source for the exploration of botanical insecticides (Ministry of Natural Resources and Environment [KeTSA], 2006; Sivapragasam, 2009). Mousa et al. (2011) showed that extracts of botanical plants, *Piper nigrum* and *Jatropha curcas*, contained compounds of piperine, oleic acid, linoleic acid, caryophyllene, and palmitic acid, were able to protect stored rice against *S. oryzae*.

Therefore, this study investigated the potential insecticidal activities of several Malaysian plants used in traditional medicine or bioactive properties. Insecticidal activity of hexane, dichloromethane, and methanol extracts of 22 Malaysian plants from the families Annonaceae, Apocynaceae, Calophyllaceae, Lauraceae, Meliaceae, Piperaceae, Rubiaceae, and Zingiberaceae were assessed by a range of bioassays against adult rice weevil (*Sitophilus oryzae*) (Coleoptera: Curculionidae) and lesser grain borer (*Rhyzopertha dominica*) (Coleoptera: Bostrychidae).

MATERIALS AND METHODS

Insects

Mixed-sex adults of *S. oryzae* and *R. dominica* (1- to 2-week-old) were used in this study. They were kept in the laboratory at a temperature and relative humidity of 26 ± 1ºC and 65 ± 5%, respectively, for 12 hours daylight and 12 hours dark and fed with hard rice supplemented with 5% yeast (Saf-instant, France). These insects have been reared in the laboratory for two generations.

Plant Materials and Extraction

More than 20 Malaysian plant species with medicinal properties or traditional uses were selected for extraction (Table 1). Botanical identification of the collected plant samples was performed by Prof. Dr. Halijah Ibrahim and Mr. Teo Leong Eng from the Faculty of Science, Universiti Malaya, and Prof. Dr. Kamarudin Mat Salleh from the Faculty of Science and Technology, Universiti Kebangsaan Malaysia. The voucher specimens were deposited at the Herbarium of the Department of Chemistry, Universiti Malaya.

All the dried plant materials (1 kg) were macerated with hexane (17 L) (R&M Chemical, Malaysia) for 3 days. Then, the hexane extracts were evaporated to dryness under reduced pressure using a rotary evaporator. The hexane extraction process was repeated twice to maximize the extraction yield. First, the same extraction process was repeated by using dichloromethane (DCM) (R&M Chemical, Malaysia) (ethyl acetate for *Mitragyna speciose*, *Mesua elegans*, and *Mesua kunstleri*) followed by methanol (MeOH) (R&M Chemical, Malaysia).

Table 1

Selected plants for insecticidal activity investigation with their traditional uses, biological/pharmacological properties, types of chemical constituents, and major compounds contained in the plants

Plant families/species	Voucher specimen	Part used	Traditional uses/ Biological/ Pharmacological properties	Types of chemical constituents	Major compounds	References
Annonaceae						
Goniothalamus tapisoides	HUMS 000108	Bark	- Used as abortifacient and to cure poisonous animal bites, such as snake, scorpion sting, or insect bites, and to relieve stomachache	Styryl lactones, alkaloids, and flavonoids	Goniothalamin	Ahmad et al. (2010); Kim et al. (2012)
Apocynaceae						
Alstonia angustifolia	KL 5705	Leaves	- Used for treatment of remittent fever by applying to the spleen area	Indole alkaloids	Alstogustine and 19-epialstogustine	Naeem et al. (2017); Perry and Metzger (1980); Raja et al. (2013); S. Aziz et al. (2016)
Catharanthus roseus	KL 5763	Leaves	- Used for treatment of wasp stings, menorrhagia, and rheumatism - Anti-cancer, antioxidant, and antidiabetic activities	Indole alkaloids	Vindoline I, vindolidine II, vindolicine III, and vindolinine IV	Tiong et al. (2013)
Calophyllaceae						
Mesua elegans	KL 5232	Bark	- Acetylcholinesterase inhibitory activity	Coumarins	Mesuagenin A and Mesuagenin D	Awang, Chan, et al. (2010b)
Mesua kunstleri	KL 4485	Bark	- Neuroprotective activity	Coumarins	5,7-dihydroxy-6-(3-methylbutanoyl)-8-[*E*-3,7-dimethylocta-2,6-dienyl]-4-phenyl-2*H*-chromen-2-one	Chan et al. (2012)

Table 1 *(Continue)*

Plant families/ species	Voucher specimen	Part used	Traditional uses/ Biological/ Pharmacological properties	Types of chemical constituents	Major compounds	References
Lauraceae						
Alseodaphne corneri	KL 5641	Bark	- Antiplasmodial and antioxidant activities	Bisbenzylisoquinoline, aporphine, and benzylisoquinoline alkaloids	Gyrolidine and isocorydine	Zahari (2016)
Dehaasia longipedicellata	KL 5634	Bark	- Antiplasmodial and antioxidant activities	Morphinandienone, aporphine, and benzylisoquinoline alkaloids	Sebiferine and milonine	Zahari et al. (2014)
Cryptocarya nigra	KL 5272	Bark	- Antiplasmodial and antioxidant activities	Benzylisoquinoline and phenanthrene alkaloids	*N*-methylisococlaurine	Nasrullah et al. (2013)
Phoebe grandis	KL 5532	Leaves	- Cytotoxic and antibacterial activities	Aporphine, proaporphine, and proaporphine-tryptamine dimers		Amna et al. (2015); Mukhtar et al. (1997)
Meliaceae						
Walsura pinnata	KL 4571	Bark	- Antiproliferative and anti-colonogenic properties	Triterpenes	3-oxoolean-1-en-28-oic acid and betulonic acid	Leong et al. (2017); Mohamad et al. (2009)
Piperaceae						
Piper sarmentosum	KU 0110	Root	- Used for treatment of toothache, fungoid dermatitis on the feet, coughing, asthma, and pleurisy - Larvicidal, cytotoxicity, anti-amoebic, antiplasmodial, antimycobacterial, and antifungal activities	Phenylpropanoids and pyrrole amide	Asaricin, isoasarone, and *trans*-asarone	Hematpoor et al. (2016, 2018); Sawangjaroen et al. (2004); Tuntiwachwuttikul et al. (2006)

Table 1 *(Continue)*

Plant families/ species	Voucher specimen	Part used	Traditional uses/ Biological/ Pharmacological properties	Types of chemical constituents	Major compounds	References
Rubiaceae						
Nauclea officinalis	KL 5655	Bark	- Vasorelaxant and cholinesterase inhibitory activities	Indole alkaloids and quinoline alkaloid glycoside	Angustine and naucletine	Liew et al. (2012, 2015)
Nauclea subdita	KL 5254	Bark	- Cytotoxic activity	Indole alkaloids	Angustine and angustoline	Liew et al. (2014)
Mitragyna speciosa	KL 5321	Leaves	- Used as an antidepressant, cure for fever, treatment for diarrhea, and diabetes, improve blood circulation, and as a morphine substitute - Anti-inflammatory activity	Indole alkaloids	Mitragynine, speciociliatine, 7-hydroxymitragynine, palmitic acid, sitosterol, stigmasterol, campesterol, and tocopherol	Burkill (1935); Idayu et al. (2011); Tohar et al. (2019)
Zingiberaceae						
Alpinia conchigera	KL 5049	Rhizomes	- Used as a condiment and post-partum medicine - Cytotoxic and antimicrobial activities	Phenylpropanoid and monoterpenes	Acetoxychavicol acetate	A. N. Aziz et al. (2013); Awang, Azmi, et al. (2010a); Ibrahim et al. (2009); Sok et al. (2017)
Alpinia murdochii	HI 1420	Rhizomes	- Used for antimicrobial and anti-inflammatory actions	Glycoside, limonoids, and terpenoids	γ-selinene	Burkill (1935); Mohamad (2009)
Alpinia pahangensis	KU 001	Rhizomes	- Used to relieve flatulence	Monoterpenes and sesquiterpenes (Essential oil)	(E)-labda-8(17),12-diene-15,16-dial	Awang et al. (2011); Phang et al. (2013); Sivasothy et al. (2013)
Alpinia scabra	HI 1419	Rhizomes	- Used to cure gastric diseases and insect bites - Trematocidal activity	Alkaloids, limonoids, and terpenoids	-	Burkill (1935)

Table 1 *(Continue)*

Plant families/ species	Voucher specimen	Part used	Traditional uses/ Biological/ Pharmacological properties	Types of chemical constituents	Major compounds	References
Zingiberaceae						
Curcuma aeruginosa	HI 1349	Rhizomes	- Used in postpartum care: uterine involution, treatment of uterine pain, and uterine inflammation	Alkaloids and cucumenoids	Zedoalactone A, zedoalactone B, curcumin, and β-pinene	Perry and Metzger (1980); Thaina et al. (2009)
Curcuma mangga	HI 1350	Rhizomes	- Used in the treatment of stomachic, chest pain, fever, and general debility, postpartum, and to aid womb healing	Terpenoids, flavonoids, and cucumenoids	Zerumin B and curcumin	Abas et al. (2005)
Curcuma purpurascens	KL 5793	Rhizomes	- Traditionally used to treat boils, cough, fever, itch, scabies, and wounds	Monoterpenes and sesquiterpenes (Essential oil)	Turmerone, germacrone, and germacrene-B	Hong et al. (2014); Koller (2009)
Curcuma zedoaria	KL 5764	Rhizomes	- Used as an aromatic stomachic and to stimulate the blood flow	Flavonoids, cucumenoids, and sesquiterpenoids	Curzerene, dehydrocurdione, curcumenone, comosone II, curcumenol, and procurcumenol	Ahmed Hamdi et al. (2014); Lobo et al. (2009); Wilson et al. (2005)

Insecticidal Activity

Previous studies showed that *S. oryzae* was more susceptible to insecticides than *R. dominica,* and thus *S. oryzae* was chosen for the preliminary test in the present study (Athanassiou et al., 2016; El-Masry, 2008). Initially, 1 mg/mL solution of each plant extract was used, and based on the observed insecticidal activity, the extracts were classified as strong (mortality ≥ 80%), moderate (mortality 50–79%), weak (mortality 30–49%), and little or no activity (mortality below 30%). Extracts with strong or moderate activity (≥ 50%) were chosen for further investigation to determine the lethal concentration, effect at various exposure intervals, contact toxicity, and antifeedant activities.

Relative Dose Value on Treated Grain Assay

In determining the working concentration level, 20 adults (mixed sex) of *S. oryzae* and *R. dominica* were introduced separately in Petri dishes containing 5 g rice grain treated with different active extracts. The Petri dishes were covered and sealed with parafilm. Mortality was recorded at 24, 48, and 72 hours after the exposure, and the percentage of mortality was calculated (Abbott, 1987). The untreated group was considered the control and all the experiments had four replicates.

Effect of Exposure Interval on Pests' Survival

Two-week-old adults of *S. oryzae* and *R. dominica* (*n* = 20) were separately exposed for 21 days in vials containing rice grain treated with each active extract at the concentration of their LC_{50} values. The mortality was recorded after exposure on the 7th, 14th, and 21st days. The experimental protocol followed the same pattern as the treated grain assay. Upon completion of the exposure time, dead insects were recorded and discarded. Results were analyzed using the analysis of variance (ANOVA) of the Statistical Analysis System (SAS) (SAS Institute, 1987) to determine the significance of exposure interval (7, 14, and 21 days) (Ferizli et al., 2011).

Contact Toxicity

The assessment of chronic toxicity was obtained by applying 425 mg of each plant extract dissolved in 1 mL of methanol on filter papers (Whatman No. 1, diameter 10 cm) to achieve a concentration of 5.41 mg/cm^2 (Kim et al., 2003). Filter paper for the control group was treated with methanol only. After drying in a fume hood for 15 minutes, each filter paper was placed at the bottom of a Petri dish (10 cm diameter x 1.5 cm), and 20 adults of *S. oryzae* and *R. dominica* were separately placed in each Petri dish. All the Petri dishes were covered and sealed with parafilm for 28 days, with checking for mortality on the 7th, 14th, 21st, and 28th days.

Antifeedant Assay

The potential of antifeedant effects of the active plant extracts was studied as described by Arivoli and Tenny with slight modifications (Arivoli & Tennyson, 2013).

Briefly, 5 g of untreated organic rice was used as a test food. The rice was treated with ethanol (control group) or 5.0, 2.5, and 1.5 mg/mL solution of each extract and allowed to air-dry for 5 hours. Batches of 10 adults (2 weeks old) of *S. oryzae* and *R. dominica* were placed in each Petri dish and wrapped with parafilm. The antifeedant activity was recorded on the 28th day after treatment (DAT). The antifeedant activity was determined using the following formula for weight loss percentage (Saad & Abdelgaleil, 2018):

$$\% \text{ weight lose} = [(Wu–Wi)/Wu] \times 100$$

where Wu = weight of uninfected rice and Wi = the final weight. Plant extracts with $\geq$ 70% feeding inhibition at 5.0 mg/mL were considered highly active, and further assays were carried out at lower concentrations (2.5 and 1.5 mg/mL).

Statistical Analysis

All data were subjected to statistical analysis using ANOVA followed by Tukey's test using SAS software (version 9.1.3), and the results were considered significant when $p \leq 0.05$. The LC_{50} values were calculated by probit analysis (Finney, 1971) using POLO Plus (Ahn et al., 1998; Robertson et al., 1980).

RESULTS

Screening for Insecticidal Activity

The preliminary test showed that the most active extracts against *S. oryzae* were from the hexane and MeOH extracts of the plant samples (Table 2). Insecticidal activity of the active plant extracts was observed when exposed to extract with a concentration of 1 mg/mL. Insecticidal activity for *Piper sarmentosum* extracts has been reported previously (Hematpoor et al., 2017). The results showed that the hexane (100%) and MeOH extracts (76.8%) exhibited strong and moderate activity after exposure to 0.5 mg/mL of extracts for 72 hrs. Based on the percentage of mortality, plants with insecticidal activity $\geq$ 50% after 72 hrs of exposure (18 extracts from 14 plants including hexane and MeOH extracts of *P. sarmentosum*) were selected for further studies to determine the lethal concentration, effect of time interval, antifeedant, and contact toxicity against *S. oryzae* and *R. dominica*.

Toxicity

The toxicity of the extracts towards the adults of *S. oryzae* and *R. dominica* were investigated by evaluating their LC_{50} values (concentration in mg/mL that killed 50% of the insects). The extracts were not equitoxic to both insects under investigation. MeOH extracts of *C. aeruginosa*, *C. mangga*, and *M. speciosa*, as well as hexane extracts of *A. conchigera*, *A. scabra*, *C. mangga*, *C. purpurascens*, *G. tapisoides*, and *P. sarmentosum*, were the most active extracts against both *S. oryzae* and *R. dominica* (Table 3).

Table 2

Insecticidal activity of plant extracts 1 mg/mL against S. oryzae *adults from treated grain assay*

Extract	Plant[x]	Mortality (mean ± SE, %)[y,z]		
		24 hrs	48 hrs	72 hrs
Control	-	00.0 ± 0.0^{f}	00.0 ± 0.0^{g}	00.0 ± 00.0^{d}
MeOH	*Alstonia angustifolia*	4.5 ± 2.7^{e}	19.1 ± 3.4^{f}	79.1 ± 2.3^{b,c}
	Curcuma aeruginosa	13.2 ± 2.1^{d}	43.2 ± 3.1^{d,e}	67.2 ± 1.7^{d}
	Curcuma mangga	15.0 ± 2.3^{c,d}	45.2 ± 4.8^{d}	86.7 ± 2.1^{b}
	Dehaasia longipedicellata	16.4 ± 4.8^{c}	75.1 ± 3.4^{b}	100.0 ± 0.0^{a}
	Mitragyna speciosa	20.6 ± 1.2^{c}	51.6 ± 1.3^{d}	75.0 ± 3.3^{c}
DCM	*Cryptocarya nigra*	3.1 ± 0.4^{e}	25.1 ± 1.7^{f}	86.3 ± 3.9^{b}
	Curcuma purpurascens	10.1 ± 1.0^{d}	61.1 ± 2.0^{c}	87.2 ± 3.7^{b}
	Phoebe grandis	10.0 ± 1.5^{d}	39.5 ± 3.2^{e}	74.5 ± 2.3^{c}
Hexane	*Alpinia conchigera*	35.2 ± 2.3^{a,b}	75.0 ± 2.9^{b}	81.5 ± 1.1^{b,c}
	Alpinia scabra	21.8 ± 3.5^{c}	71.3 ± 5.5^{b}	100.0 ± 0.0^{a}
	Curcuma aeruginosa	7.0 ± 4.9^{e}	19.5 ± 1.3^{g}	83.3 ± 2.9^{b}
	Curcuma mangga	42.0 ± 1.5^{a}	94.5 ± 1.3^{a}	100.0 ± 0.0^{a}
	Curcuma purpurascens	10.1 ± 1.0^{d}	61.1 ± 2.0^{c}	88.5 ± 3.6^{b}
	Curcuma zedoaria	21.2 ± 2.1^{c}	49.2 ± 2.4^{d}	85.0 ± 2.4^{b}
	Goniothalamus tapisoides	41.1 ± 1.5^{a}	98.8 ± 0.6^{a}	100.0 ± 0.0^{a}
	Mesua elegans	13.1 ± 0.9^{d}	21.5 ± 1.3^{f}	80.0 ± 1.8^{b}

Note.

[x] Plant extracts showed 50% mortality or lower after 72 hrs of treatment were not included. Hexane and MeOH extracts of *P. sarmentosum* caused 100.0% and 76.8% mortality, respectively, against *S. oryzae* after exposure to 0.5 mg/mL of extracts for 72 hrs (Hematpoor et al., 2017)

[y] Each column represented the mean of four replicates, each set up with 20 adults ($n = 80$)

[z] Mean within a column followed by the same letter are not significantly different ($p \leq 0.05$)

Table 3

Toxicity of 18 most active plant extracts against S. oryzae *and* R. dominica

Extract	Plant	LC$_{50}$ (mg/mL) (95 % confidence interval)[x,y,z]	
		Sitophilus oryzae	*Rhyzopertha dominica*
MeOH	*Alstonia angustifolia*	0.44 (0.37 to 0.50)	≥ 0.50
	Curcuma aeruginosa	0.42 (0.34 to 0.50)	0.31 (0.19 to 0.43)
	Curcuma mangga	0.21 (0.15 to 0.26)	0.34 (0.31 to 0.38)
	Dehaasia longipedicellata	0.34 (0.24 to 0.43)	≥ 0.50
	Mitragyna speciosa	0.30 (0.21 to 0.40)	0.49 (0.35 to 0.65)
	Piper sarmentosum	0.30 (0.28 to 0.35)	≥ 0.50

Table 3 *(Continue)*

Extract	Plant	LC_{50} (mg/mL) (95 % confidence interval) ^{x,y,z}	
		Sitophilus oryzae	*Rhyzopertha dominica*
DCM	*Cryptocarya nigra*	0.46 (0.40 to 0.52)	≥ 0.50
	Curcuma purpurascens	0.40 (0.25 to 0.55)	≥ 0.50
	Phoebe grandis	≥ 0.50	≥ 0.50
Hexane	*Alpinia conchigera*	0.11 (0.07 to 0.15)	0.20 (0.14 to 0.27)
	Alpinia scabra	0.29 (0.20 to 0.38)	0.49 (0.43 to 0.62)
	Curcuma aeruginosa	≥ 0.50	≥ 0.50
	Curcuma mangga	0.19 (0.13 to 0.26)	0.28 (0.21 to 0.38)
	Curcuma purpurascens	0.33 (0.28 to 0.38)	0.35 (0.31 to 0.40)
	Curcuma zedoaria	0.38 (0.34 to 0.42)	≥ 0.50
	Goniothalamus tapisoides	0.21 (0.11 to 0.28)	0.35 (0.21 to 0.47)
	Mesua elegans	≥ 0.50	≥ 0.50
	Piper sarmentosum	0.28 (0.26 to 0.31)	0.38 (0.27 to 0.49)

Note.

^{x}Values were based on five concentrations, four replicates of 20 insects each

^{y}Significant values determined by comparing the confidential interval of each LC_{50} value

^{z}LC_{50} value ≥ 0.50 mg/mL was considered low insecticidal activity and was not shown

Effect of Exposure Interval on Survival

Plant extracts with LC_{50} < 0.50 mg/mL (Table 3) were investigated further for the effect of exposure interval due to the toxicity of the extract (Table 4) by evaluating the mortality of adults of both pests upon exposure to the extracts. Results showed that the level of toxicity of the extracts increased with a longer duration of exposure. Based on the relative LC_{50} values, extracts were identified with significant residual toxicity after 21 days of exposure. For MeOH extract of *P. sarmentosum*, DCM extracts of *C. nigra*, hexane extracts of *A. conchigera*, *A. scabra*, *C. mangga*, *Goniothalamus tapiosides*, and *P. sarmentosum*, the mortality of *S. oryzae* increased up to 90–100% within 21 days with *A. conchigera* and *G. tapiosides* being the most potent extracts (100% mortality). In the case of *R. dominica*, MeOH extracts of *C. aeruginosa*, *C. mangga*, *P. sarmentosum*, DCM extract of *C. nigra*, hexane extracts of *A. conchigera*, *G. tapiosides*, and *P. sarmentosum* showed the highest mortality (80–100%) within the 21-days of the experimental period. MeOH extract of *C. mangga* and hexane extract of *P. sarmentosum* were the most potent, with 100% mortality at the end of the 21-day exposure.

Table 4

Mortality of S. oryzae *and* R. dominica *adults exposed to rice grain treated with LC_{50} concentration of each plant extract for 21 days*[*]

Extract	Plant	Mean mortality (%) of *S. oryzae* in days after exposure			Mean mortality (%) of *R. dominica* in days after exposure		
		7th day	14th day	21st day	7th day	14th day	21st day
Control	No	00.0 ± 0.0^{d}	00.0 ± 0.0^{e}	0.8 ± 0.03^{f}	00.0 ± 0.0^{e}	00.0 ± 0.0^{f}	00.0 ± 0.0^{f}
MeOH	*Alstonia angustifolia*	58.7 ± 2.3^{b}	59.7 ± 4.2^{d}	59.7 ± 4.2^{d}	49.2 ± 2.5^{c}	50.5 ± 2.3^{d}	50.5 ± 2.3^{d}
	Curcuma aeruginosa	53.5 ± 2.4^{c}	$63.0 \pm 3.0^{c,d}$	66.3 ± 4.7^{c}	$66.7 \pm 2.4^{a,b}$	83.0 ± 2.3^{b}	83.0 ± 2.3^{b}
	Curcuma mangga	62.7 ± 3.6^{b}	65.5 ± 3.1^{c}	$65.5 \pm 3.1^{c,d}$	75.2 ± 3.7^{a}	93.5 ± 5.2^{a}	100.0 ± 0.0^{a}
	Dehaasia longipedicellata	67.2 ± 2.7^{a}	68.5 ± 4.3^{c}	69.1 ± 3.2^{c}	50.0 ± 2.8^{c}	53.7 ± 2.4^{d}	53.7 ± 2.4^{d}
	Mitragyna speciosa	69.2 ± 2.5^{a}	69.2 ± 2.5^{c}	69.2 ± 2.5^{c}	42.0 ± 3.6^{c}	41.2 ± 4.5^{e}	42.0 ± 2.2^{e}
	Piper sarmentosum	62.5 ± 3.5^{b}	66.5 ± 3.2^{c}	94.7 ± 3.8^{a}	63.5 ± 2.3^{b}	82.0 ± 1.9^{b}	84.2 ± 2.7^{b}
DCM	*Chisocheton erythrocarpus*	$65.8 \pm 4.2^{a,b}$	65.0 ± 1.0^{c}	83.5 ± 1.5^{b}	36.7 ± 2.3^{d}	37.7 ± 5.2^{e}	37.7 ± 2.3^{e}
	Cryptocarya nigra	$56.5 \pm 5.1^{b,c}$	97.2 ± 2.2^{a}	97.2 ± 4.1^{a}	60.2 ± 2.6^{b}	$84.7 \pm 2.0^{a,b}$	84.7 ± 2.0^{b}
	Curcuma purpurascens	57.6 ± 3.3^{b}	57.6 ± 4.3^{d}	57.6 ± 1.5^{e}	42.0 ± 0.9^{c}	62.7 ± 2.7^{c}	69.7 ± 2.8^{c}
Hexane	*Alpinia conchigera*	66.8 ± 2.8^{a}	100.0 ± 0.0^{a}		57.6 ± 3.9^{b}	65.7 ± 1.5^{c}	98.0 ± 1.4^{a}
	Alpinia scabra	74.2 ± 2.3^{a}	$96.8 \pm 4.7^{a,b}$	96.0 ± 3.1^{a}	63.0 ± 1.3^{b}	64.7 ± 2.8^{c}	67.5 ± 3.1^{c}
	Curcuma mangga	76.5 ± 3.3^{a}	92.7 ± 3.3^{b}	92.7 ± 4.7^{a}	53.2 ± 2.6^{c}	53.2 ± 2.6^{d}	53.2 ± 2.6^{d}
	Curcuma purpurascens	70.2 ± 2.6^{a}	75.2 ± 2.1^{c}	75.2 ± 2.1^{c}	58.7 ± 4.2^{b}	62.0 ± 3.3^{c}	62.0 ± 3.3^{c}
	Curcuma zedoaria	53.7 ± 3.5^{c}	64.2 ± 2.1^{c}	$65.7 \pm 0.9^{c,d}$	$55.9 \pm 3.2^{b,c}$	62.2 ± 2.9^{c}	64.3 ± 4.1^{c}
	Goniothalamus tapisoides	73.0 ± 4.3^{a}	100.0 ± 0.0^{a}		71.5 ± 2.2^{a}	87.2 ± 3.7^{a}	96.2 ± 3.6^{a}
	Piper sarmentosum	60.5 ± 2.3^{b}	69.0 ± 4.5^{c}	98.3 ± 1.3^{a}	69.7 ± 2.4^{a}	79.0 ± 2.0^{b}	100.0 ± 0.0^{a}

Note. *Results expressed as the mean of four replicates, each set with 20 adults ($n = 80$). Mean values within the same column annotated with the same letter in the superscript are not significantly different ($p \leq 0.05$)

Contact Toxicity

Contact toxicity was performed by direct contact application on both insects with plant extracts. MeOH extracts of *C. aeruginosa* and *C. mangga*, DCM extract of *C. nigra*, and hexane extracts of *A. conchigera*, *C. mangga*, *C. purpurascens*, and *C. zedoaria* showed 100% mortality against *S. oryzae* adults within 28 days (Table 5). Results of the plant extracts against *R. dominica* adults followed the same trend except for hexane extracts of *A. conchigera* and *C. zedoaria*, which showed a maximum of 80% mortality within the experimental period. Similar to the previous test where *R. dominica* was more resistant to extracts as compared to *S. oryzae* ($x^2 = 22.81$, $p \leq 0.0001$).

Antifeedant Activity

The antifeedant activity was carried out to investigate the plant extracts' ability to inhibit the insects' normal feeding behavior. In the antifeedant activity study, extracts with more than 70% feeding inhibition for both insects at a concentration of 5.0 mg/mL (MeOH extracts of *A. angustifolia* and *C. aeruginosa*, DCM extract of *C. nigra*, hexane extracts of *A. conchigera*, *A. scabra*, *C. manga*, and *C. purpurascens*) were further chosen to be examined for their inhibitory effect at the concentrations of 2.5 and 1.5 mg/mL. At the concentration of 2.5 mg/mL, methanol extracts of *C. aeruginosa* and hexane extracts of *A. conchigera* and *C. mangga* showed more than 90% feeding inhibition on both insects. Besides, the results indicated that the MeOH extracts of *C. aeruginosa*, hexane extracts of *A. conchigera*, *C. manga*, and *C. purpurascens* had higher antifeedant activity on both insects, although *R. dominica* is more resistant at the concentration of 1.5 mg/mL ($x^2 = 14.4$, $p \leq 0.001$) (Table 6).

DISCUSSION

Investigation of plant-derived extracts and phytochemicals possessing biological activities and medicinal properties for pest control has significantly increased in recent years due to their selective toxicity on insect pests with little or no such effects on non-targeted organisms, the environment, and human health. It is now well established that the complex mixture of secondary metabolites contributes to plant extracts' activity even against some resistant strains of insect pests (Ahn et al., 1998; Akhtar et al., 2007; Anuradha et al., 2010; Zoubiri & Baaliouamer, 2014). In many cases, plant products have successfully combat various stored-product insect pests (Rajendran & Sriranjini, 2008; Shaaya et al., 1997). However, a study by Kim et al. (2003) showed that the susceptibility of a particular storage insect pest could differ from different plant extracts. Besides, a study revealed that *S. oryzae* adults were more susceptible to certain plant extracts as compared to *R. dominica* and the period of exposure interval is more important compared to the dosage of the extract (El-Nahal et al., 1989). In search for potent biopesticides, Jacobson (1989) identified several families, including Annonaceae, Asteraceae, Canellaceae, Lamiaceae, Meliaceae, and Rutaceae, with promising insecticidal activity, while

Table 5

Insecticidal activities of the plant extract against S. oryzae *and* R. dominica *adults, using the filter paper diffusion method, exposed to 5.41 mg/cm²*

Extract	Plant[x]	Mean mortality (%) against *S. oryzae* after days of exposure[y,z]				Mean mortality (%) against *R. dominica* after days of exposure[y,z]			
		7th	14th	21st	28th	7th	14th	21st	28th
Control	-	00.0 ± 0.0^{f}	00.0 ± 0.0^{e}	1.2 ± 0.5^{e}	1.4 ± 0.5^{d}	00.0 ± 0.0^{f}	00.0 ± 0.0^{f}	00.0 ± 0.0^{e}	1.8 ± 0.4^{e}
MeOH	*Curcuma aeruginosa*	100.0 ± 0.0^{a}				87.0 ± 4.4^{a}	99.7 ± 0.2^{a}	100.0 ± 0.0^{a}	
	Curcuma manga	87.0 ± 5.3^{b}	100.0 ± 0.0^{a}			68.2 ± 3.7^{b}	74.5 ± 3.4^{b}	78.5 ± 3.1^{b}	100.0 ± 0.0^{a}
	Mitragyna speciose	22.2 ± 5.4^{d}	22.2 ± 5.4^{d}	26.2 ± 4.1^{d}	27.7 ± 4.7^{c}	21.7 ± 4.9^{d}	32.7 ± 2.3^{d}	32.7 ± 2.3^{d}	33.5 ± 4.7^{d}
DCM	*Cryptocarya nigra*	84.5 ± 3.5^{b}	100.0 ± 0.0^{a}			80.5 ± 5.5^{a}	100.0 ± 0.0^{a}		
Hexane	*Alpinia conchigera*	93.5 ± 2.7^{b}	94.2 ± 2.3^{b}	94.2 ± 2.3^{b}	100.0 ± 0.0^{a}	35.2 ± 2.7^{c}	77.2 ± 4.2^{b}	79.2 ± 3.3^{b}	79.2 ± 3.3^{b}
	Alpinia scabra	39.5 ± 2.7^{c}	56.7 ± 4.3^{c}	56.7 ± 4.3^{c}	56.7 ± 4.3^{b}	46.0 ± 3.2^{c}	59.5 ± 3.3^{c}	59.5 ± 3.3^{c}	59.5 ± 3.3^{c}
	Curcuma manga	100.0 ± 0.0^{a}				82.7 ± 4.5^{a}	100 ± 0.0^{a}		
	Curcuma purpurascens	100.0 ± 0.0^{a}				88.7 ± 3.6^{a}	100 ± 0.0^{a}		
	Curcuma zedoaria	90.0 ± 4.6^{b}	90.7 ± 4.4^{b}	100.0 ± 0.0^{a}		62.7 ± 2.8^{b}	72 ± 3.3^{b}	80.2 ± 1.8^{b}	80.2 ± 1.8^{b}
	Goniothalamus tapisoides	9.5 ± 3.3^{e}	31.7 ± 2.0^{d}	32.8 ± 2.5^{d}	32.8 ± 2.5^{c}	5.7 ± 1.7^{e}	26 ± 3.9^{e}	33.7 ± 2.9^{d}	38.5 ± 3.8^{d}

Note.

[x] The plant extracts which did not show contact toxicity within 14 days were not included

[y] Each column represented the mean of four replicates, each set up with 20 adults ($n = 80$)

[z] Means within a column followed by the same letter are not significantly different ($p \leq 0.05$)

Table 6

Antifeedant activity of the active extracts against S. oryzae *and* R. dominica *adults after 28 days of exposure*

Extract	Plant[x]	Antifeedant index (%) mean ± SE[w] of *S. oryzae*[y,z]			Antifeedant index (%) mean ± SE[w] of *R. dominica*[y,z]		
		5.0 mg/mL	2.5 mg/mL	1.5 mg/mL	5.0 mg/mL	2.5 mg/mL	1.5 mg/mL
MeOH	*Alstonia angustifolia*	100.0 ± 0.0^{a}	$77.2 \pm 4.6^{b,c}$	31.6 ± 2.8^{d}	99.2 ± 0.4^{a}	86.7 ± 2.9^{b}	49.2 ± 5.9^{b}
	Curcuma aeruginosa	97.2 ± 1.5^{a}	94.7 ± 1.2^{a}	72.5 ± 3.5^{b}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	67.5 ± 3.6^{a}
	Curcuma mangga	59.2 ± 3.8^{e}			69.3 ± 4.0^{c}		
	Dehaasia longipedicellata	34.3 ± 3.3^{g}			20.5 ± 3.1^{f}		
	Mitragyna speciosa	47.7 ± 3.7^{f}			39.7 ± 4.8^{d}		
	Piper sarmentosum	12.0 ± 3.3^{i}			16.0 ± 3.8^{f}		
DCM	*Cryptocarya nigra*	88.5 ± 3.4^{c}	75.7 ± 3.3^{c}	55.9 ± 4.5^{c}	93.0 ± 2.5^{b}	88.7 ± 3.9^{b}	45.7 ± 3.9^{b}
	Curcuma purpurascens	30.0 ± 6.5^{g}			13.5 ± 3.7^{g}		
	Phoebe grandis	11.2 ± 3.2^{i}			$22.5 \pm 3.9^{e,f}$		
Hexane	*Alpinia conchigera*	100.0 ± 0.0^{a}	93.7 ± 2.4^{a}	82.5 ± 4.1^{a}	93.2 ± 2.2^{b}	$82.9 \pm 2.8^{b,c}$	33.7 ± 4.0^{c}
	Alpinia scabra	79.5 ± 4.1^{d}	42.1 ± 3.6^{d}	8.5 ± 2.2^{e}	71.0 ± 3.9^{c}	44.6 ± 2.8^{d}	11.4 ± 5.1^{d}
	Curcuma mangga	100.0 ± 0.0^{a}	93.0 ± 2.8^{a}	81.4 ± 4.8^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	47.5 ± 5.9^{b}
	Curcuma purpurascens	94.3 ± 4.6^{b}	80.7 ± 2.8^{b}	51.4 ± 3.7^{c}	100.0 ± 0.0^{a}	74.1 ± 4.9^{c}	9.2 ± 2.2^{d}
	Curcuma zedoaria	$51.5 \pm 5.9^{e,f}$			47.0 ± 4.6^{d}		
	Goniothalamus tapisoides	16.7 ± 5.4^{h}			19.0 ± 5.0^{f}		
	Mesua elegans	20.7 ± 2.1^{h}			27.0 ± 4.0^{e}		
	Piper sarmentosum	36.7 ± 5.2^{g}			41.2 ± 4.6^{d}		

Note.

[w] Standard error of *S. oryzae* and *R. dominica*

[x] Plant extracts with ≥ 70% feeding inhibition at 5.0 mg/mL were further evaluated at lower concentrations (2.5 and 1.5 mg/cm^2)

[y] Each column represented the mean of four replicates, each set up with 20 adults ($n = 80$)

[z] Mean within a column followed by the same letter are not significantly different ($p \leq 0.05$)

Chaubey (2012a, 2012b) and Tripathi et al. (2002) found that insecticidal activity was more common with the plants from the family Zingiberaceae with potent insecticidal activity against both the pests (Sok et al., 2017).

In fact, in the present study, hexane, DCM, and MeOH extracts of 22 Malaysian plants from 8 plant families with medicinal or pharmacological backgrounds were screened for insecticidal activity against adults of *S. oryzae* and *R. dominica*. The insect species directly affected the insecticidal activity of active plant extracts and was proportional to the exposure time. It was found that several plants from Annonaceae, Piperaceae, and Rubiaceae have shown strong insecticidal activity against both adults of *S. oryzae* and *R. dominica* together with Zingiberaceae with more than 67% of mortality after 72 hrs of treatment (Table 2). For some plants, several extracts were found to be active, indicating that the activity is not confined to compounds of any specific polarity. For example, hexane and MeOH extracts of *C. mangga* exhibited strong insecticidal activity against both insects, suggesting the active compounds are non-polar and polar. In the case of *P. sarmentosum*, hexane and MeOH extracts showed strong activity against *S. oryzae*. However, only hexane extract was active against *R. dominica*, thus suggesting that active compounds can be of different polarity.

The exposure interval-based bioassay implicates the importance of time duration for the observed activity of the extracts (El-Nahal et al., 1989). In the present study, the active plant extracts' toxicity levels rose over time. Thus, the mortality of *S. oryzae* caused by MeOH extract of *P. sarmentosum*, DCM extracts of *C. nigra*, hexane extracts of *A. conchigera*, *A. scabra*, *C. mangga*, *G. tapiosides*, and *P. sarmentosum* reached up to 90–100% after 21 days of exposure at their respective LC_{50} concentrations (Table 4). The mortality of *R. dominica* increased similarly when exposed to MeOH extracts of *C. aeruginosa*, *C. mangga*, *P. sarmentosum*, DCM extract of *C. nigra*, hexane extracts of *A. conchigera*, *G. tapiosides*, and *P. sarmentosum* over 21 days.

MeOH extracts of *C. aeruginosa*, hexane extracts of *C. mangga* and *C. purpurascens* had the strongest contact toxicity against *S. oryzae* within 7 days of exposure to 5.41 mg/cm^2. In addition, MeOH extracts of *C. mangga*, DCM extracts of *C. nigra*, and hexane extracts of *A. conchigera* and *C. zedoaria* could cause up to 100% mortality of *S. oryzae*; however, they needed longer exposure. For *R. dominica*, MeOH extracts of *C. aeruginosa* and *C. mangga*, DCM extract of *C. nigra*, and hexane extracts of *C. mangga* and *C. purpurascens* could also cause up to 100% mortality by exposure for 28 days. Furthermore, most of these plants are reported to possess cytotoxic or anti-androgenic compounds such as 1 'S-1'-acetoxychavicol acetate (*A. conchigera*) (Malek et al., 2011), germacrone (*C. aeruginosa*), (*E*)-labda-8(17),12-dien-15,16-dial (*C. mangga*) (Ahmed Hamdi et al., 2014), and curcumenone (*C. zedoaria*) (Pan et al., 2016), which could be related to the toxicity towards the insects.

The use of antifeedant agents for pest control is considered superior over insecticidal agents as they inhibit insect feeding but do not directly kill insects and allow them to be available to their natural enemies (Arivoli & Tennyson, 2013; Frazier, 1986). Thus, using plant extracts with antifeedant activity holds promise in controlling insect resistance in storage pest management and helping maintain natural balance (Arivoli & Tennyson, 2013). In this study, MeOH extracts of *A. angustifolia* and *C. aeruginosa*, DCM extract of *C. nigra*, hexane extracts of *A. conchigera*, *A. scabra*, *C. manga*, and *C. purpurascens* showed potent antifeedant activity (more than 70% of the antifeedant index) against both pests at a concentration of 5.0 and 2.5 mg/mL. Therefore, these plants hold considerable potential for controlling *S. oryzae* and *R. dominica* populations in stored products. Comparatively, MeOH extracts of *C. aeruginosa* and hexane extracts of *A. conchigera* and *C. mangga* were the most potent feeding deterrent against *S. oryzae*, with 70% inhibition even at the concentration of 1.5 mg/mL. The extracts mentioned above were relatively potent against *R. dominica*, although they were more resistant than *S. oryzae*. Previous studies have shown alkaloids and terpenoids are the most potent insect antifeedant (Arivoli & Tennyson, 2013; Perry & Metzger, 1980; Schoonhoven, 1982) where *A. angustifolia* (Nasrullah et al., 2013; Raja et al., 2013) and *C. nigra* (Sun et al., 2017) are rich in alkaloid content, while *Curcuma* species (Sun et al., 2017) contain many terpenoid types of compounds. Besides, Zingiberaceae species have been reported to possess antifeedant activity (Dadang et al., 1998).

Hexane extracts, particularly from the plants of the Zingiberaceae family, contain a high percentage of volatile compounds (Pan et al., 2016; Sukari et al., 2008), which are suitable for fumigation against stored-product insects. A recent study of High-performance Liquid Chromatography (HPLC) analysis performed by Iskandar et al. (2021) on goniothalamin (GTN) compound from *G. tapiosides* found that the intensity and peak area of GTN in hexane extract was found to be the highest compared to other solvents extracts. Previous studies have shown that some plant extracts are useful in managing coleopterous insects (for example, *S. oryzae* and *R. dominica*) because of their fumigation ability in addition to their probable slow toxic effect minimizing the rate and frequency of pesticide application (Ahn et al., 1998; Kim et al., 2012). In addition, plant extracts with a rapid or slow mode of action can also be very useful in protecting stored products through the successful harmonizing of their immediate and late effects (Schmutterer, 1992, as cited in Sharma et al., 2014, pg.100).

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CONFLICT OF INTEREST

The authors declare no conflict of interest in the preparation of this manuscript.

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