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Repellency and contact toxicity of crude extracts from three Thai plants (Zingiberaceae) against maize grain weevil, *Sitophilus zeamais* (Motschlusky) (Coleoptera: Curculionidae)

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ABSTRACT

In the present study, plant crude extracts extracted from the rhizomes of three medicinal plants, *Curcuma longa* L. (turmeric), *Zingiber cassumunar* Roxb. (cassumunar ginger), and *Kaempferia pulchra* (Ridl.) Ridl. (peacock ginger) were investigated for their biological activities, repellent and contact toxicity, against maize weevils, *Sitophilus zeamais* Motsch. The repellent activity was evaluated using the area preferences method whereas the contact toxicity was determined using the micro-applicator to drop onto the thorax of tested insects. For repellency test, cassumunar ginger with solvent hexane scored the highest repellency up to 99% at concentration 1,415 µg/cm^2 at 8 hours after application. The methanol extract of turmeric plant exhibited the highest repellency of 87% at 1,415 µg/cm^2 7 hours after exposure compared to other two solvents, hexane and methylene chloride with same plant. Peacock ginger plant with solvent hexane revealed the maximum repellency of 79% at 1,415 µg/cm^2 5 hours after exposure. On the other hand, for contact toxicity, turmeric plant was found to be the most effective in inducing mortality after one week of treatments. The turmeric crude extract with solvent hexane achieved 13% of mortality of adults *S. zeamais* at 45 µg/insect. The highest mortality that caused by plant cassumunar ginger was observed by this crude extract with solvent methylene chloride (8%) at 45 µg/insect at seven days after application. Methanol extract of peacock ginger has the lowest mortality (6%) compared to 2 other plants mentioned above. Thus, these results demonstrated that hexane extracts of *C. longa* and *Z. cassumunar* were one of the alternative extracts that were possible to use as insecticidal for *S. zeamais* control.

Key words: contact toxicity, crude extracts, medicinal plants, repellency, *Sitophilus zeamais*

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INTRODUCTION

The losses of grain in storage due to insects are the final components of the struggle to limit insect losses in agricultural production and it is not only the direct consumption of kernels, but also include accumulations of frass, exuviae, webbing, and insect cadavers. High levels of this insect detritus may result in grain that is unfit for human consumption (Weaver and Petroff, 2005; Udo, 2005; Alleoni and Ferreira, 2006). Stored products pests are important problem almost around the world by reducing both quantity and quality of grains. Grain loss caused by storage pests such as maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), which could be influenced by the storage time and population of insects involved in infestation, undermines food security (Abebe *et al*., 2009; Tefera *et al*., 2011). Their damages to stored grains and grain products are around 5-10% in the temperate zone and 20-30% in the tropical zone (Talukder, 2006; Rajendran and Sriranjini, 2008). Such damage may reach up to 40% in countries where modern storage technologies have not been introduced and climate conditions are favourable (Shaaya *et al.*, 1997; Abbasipour *et al.*, 2011).

Synthetic chemical insecticides have been used worldwide to control the pests of stored grain, particularly *S. zeamais* (Cherry *et al*., 2005). Unfortunately, sole reliance on chemical control leads to problems of pest resistance and resurgence, pesticide residues, destruction of beneficial fauna and environmental pollution, problem in crop pollination due to honey bee losses, domestic animal poisoning, contamination of livestock products, fish and wild life losses and contamination of underground water and rivers (Adilakshmi *et al*., 2008). Novel and environmentally compatible stored-product control agents are urgently needed to replace synthetic pesticides that are either not available for economic or regulatory reasons, or are ineffective, due to the increasing difficulty of managing pesticide resistance (Duke *et al*., 2003).

Natural product such as botanical insecticides may provide potential alternatives and offer the possibility as a solution to control pests due to their bioactive chemicals that displayed strong biological activities (Cosimi *et al.*, 2009; Franz *et al*., 2011; Lu *et al.*, 2012). Many researchers are trying to find out the natural products that can replace the chemicals. They are generally less expensive, easily processed, and used by farmers and small industries. It may be safer for the environments compared to synthetic insecticides (Kim *et al.*, 2005; Abbasipour *et al.*, 2011). In addition, the large number of plant products and their constituents has been screened against a number of stored-product insects and most of them are non-pollutant, less toxic, biodegradable in nature, increased food safety, improved the production profitability, and reduced pesticide resistance and degradation in environment (Pugazhvendan *et al*., 2009; Erdogan *et al*., 2012). Plant crude extracts and essential oils have potential for use in crop and stored-product protection. They contain monoterpenoids, diterpenoids, sesquiterpenoids and other compounds that exhibited ovicidal, larvicidal, repellent, oviposition deterrent, feeding deterrency, emergence inhibition, poisons, antifeedant and toxic effects in a wide range of insects (Isman, 2006; Rozman *et al.*, 2007; Odeyemi *et al.*, 2008; Ukeh *et al.*, 2009; Ishii *et al.*, 2010; Kedia *et al*., 2015).

Curcuma longa L. (turmeric), *Zingiber cassumunar* Roxb. (cassumunar ginger) and *Kaempferia pulchra* (Ridl.) Ridl. (peacock ginger) belong to the ginger family, Zingiberaceae. *Curcuma longa* is originally from India but widely cultivated in tropical Asia and in southern parts of Russian Federation (Teuscher, 2006). *Curcuma longa* is a perennial plant widely used as a spice, a colorant and also as a major ingredient of curry powder. This plant species has a long history of use as a traditional medicine in China and India, reflecting its diverse and beneficial health effects (Hayakawa *et al.*, 2011). In addition, the curcuma species contains phenolic compounds found in the plant's rhizomes. The anti-rheumatic activity of curcumin isolated from *C. longa* has been clinically demonstrated in case of rheumatoid arthritis, abdominal pain, bruises, chest pains, fever, also for arsenic poisoning, post-partum haemorrhage, and primary syphilis, aperient, astringent, carminative, cordial, detergent, maturant, stimulant and tonic (Duke, 2003; Sabu, 2006). *Curcuma longa* has some phytochemical and medicinal properties, but its use for the control of crop pests and the information on its efficacy against stored grain pest are still scanty. So, the insecticidal and repellent activity tests of *C. longa* extracts have been attempted and the investigation has been designed to evaluate the efficacy of the test plant as a possible source of potential secondary metabolites to be used as environment friendly pest control agents. Abida *et al*. (2010) revealed that this plant was promising. The LD_{50} values for rhizome extract against *Tribolium castaneum* (Herbst) were 0.337 and 0.201 mg cm^{-2} for 24 and 48 hours exposure respectively through surface film assay.

Zingiber cassumunar is the major herb used to alleviate pain or inflammation, the treatment of asthma, as well as for muscle and joint pain (Suksaeree *et al.*, 2013). Rhizome is used in diabetes and also as a substitute for *C. longa.* Bussaman *et al.* (2012) found that the rhizome of *Z. cassumunar* has strong acaricidal activity that caused 100% mortality to mushroom mite, *Luciaphorus* sp, while Talukder and Khanam (2011) revealed that all parts of *Z. cassumunar* were toxic to adults of *Sitophilus oryzae* (L.) and *Callosobruchus chinensis* (L.) and 13 days old *T. castaneum* larvae under fumigant test. This plant also showed repellent activity against *Leptotrombidium imphalum* (Vercammen-Grandjean&Langston) at concentration ranged between 40-100% and insecticidal activity to *Culex quinquefasciatus* at LC_{50} equaled 202.3 mg/L (Mann and Kauffman, 2012). *Kaempferia pulchra* is distributed widely in many provinces in Thailand (Sirirugsa, 1999). Rhizome extracts of *K. pulchra* and *K. parviflora* were reported to be toxic to mushroom mite *Luciaphorus* sp. by causing high mortality at 72.22 and 88.89%, respectively (Bussaman *et al*., 2012). Regarding, *C. longa*, *Z. cassumunar* and *K. pulchra* are known for their insecticidal property against other insects including some stored product insect pests. Therefore, this study aimed to evaluate these medicinal plants for their nature as grain protectant, repellent and contact toxicity against *S. zeamais*, a major pest of stored maize, wheat and rice in tropical and sub-tropical countries under laboratory conditions.

MATERIALS AND METHODS

Insects Rearing

Sitophilus zeamais obtained from Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand was maintained at the laboratory in Department of Entomology, Kasetsart University, Bangkhen Campus, Bangkok, Thailand. A total of 500 adult weevils were currently reared on 100 gram of brown rice, *Oryza sativa* L. at 25°C and 70% RH. All insects were placed in glass bottles and the rearing jars were maintained in the laboratory at 29 ± 5°C and 75 ± 5% RH.

Preparation of Crude Extracts

Rhizomes of turmeric (*C. longa*), cassumunar ginger (*Z. cassumunar*) and peacock ginger (*K. pulchra*) were collected from Mahasarakham province, Thailand in October 2013. All plants have been air-dried and ground into powder. The powder was soaked in hexane for 3 days before the plant materials filtrated using Whatman filter paper No.1, the mask then extracted again with methylene chloride and methanol as mentioned above. All solutions were evaporated using vacuum evaporator having only dry crude extracts which kept in refrigerator at 4-10°C for further study.

Repellency test

The repellent activity was evaluated using the area preferences method. Petri-dishes of 9 cm in diameter were used to confine insects during experiment. The crude extracts of *C. longa*, *Z. cassumunar* and *K. pulchra* were diluted in absolute ethanol to different concentrations (1, 3, 5, 7, and 9% equivalent to 157, 472, 786, 1,101 and 1,415 µg/cm^2) and absolute ethanol was used as control. The Whatman filter papers (9 cm in diameter) were cut in half where 500 µL of each crude extract applied separately to one half of the filter paper in each petri-dish as uniformly as possible with a micropipette. The other half (control) was treated with 500 µL of absolute ethanol. Both treated half and control sides were placed at room temperature until completely dried. Full disc was carefully remade by attaching the tested half to control halves with cello-tape. Each remade filter paper was placed in a petri-dish with different directions to avoid any stimuli affecting the distribution of insects. Ten unsexed adult insects were released at the centre of each filter-paper disc and a cover with an open hole of 5 cm in diameter covered with fine nylon mesh that placed on the petri-dish. Number of the insects present on each side of the filter paper has been observed at hourly interval up to the eighth hour. There are 10 replications per treatment and they will be assigned in completely randomized design (CRD). The percent of repellency of plant crude extract is calculated using formula:

PR(%) = Nc/(Nc+Nt)×100 where Nc was the number of insect presenting in the control half and Nt was the number of insect presenting in the treated half.

Direct contact toxicity

This experiment uses micro-applicator (Burkard Manufacturing Company Ltd., England®) to drop 0.5 µL onto the thorax of each insect with different concentrations: 1, 3, 5, 7 and 9% that equivalent to 5, 15, 25, 35 and 45 µg/insect. Each ten treated insects then were put in a small plastic cup which has 4 cm in diameter and 2.5 cm in height. Controls were prepared using ethanol. Culture media were added to each treatment after 24 h. This experiment has 10 replications and they will be assigned in completely randomized design (CRD). Mortality of insect was observed daily until end-point mortality was reached 1 week after treatment. .

Data analysis

Data from ten independent replications were subjected to statistical analysis using SPSS Statistical Package where significant differences existed means were separated using Duncan's Multiple Range Tests (DMRT). Data involving counts and percentages were square root and arcsine transformed, respectively before analyses using Analysis of variance at 0.05 probability level.

RESULTS

Repellency

Results given in tables (Tables 1-3) describe the repellent activity of different solvents and concentrations of plant crude extracts. The results revealed that repellent activity did not happen significantly and tend to be attractants. However, eventhough almost all the plant crude extracts did not show the repellent potential almost at the first 3 hours, but as time progressed, the level of repellent activity was increased. Table 1 indicated the repellent effect of *C. longa* (turmeric) plant extracts with 3 different solvents such as hexane, methylene chloride and methanol. Methanol extract of plant *C. longa* exhibited strong repellent effect to the maize weevil. The highest dose (1,415 µg/cm^2) strongly repelled the weevil until 8-hrs after exposure. It reached up to 87% respectively at 7-hrs after treatment. However, the tested concentrations of methylene chloride extract did not even show the potential of repellent while hexane extract showed less repellency. Table 2 described the repellent effect of *Z. cassumunar* (cassumunar ginger) crude extracts to *S. zeamais*. The most significant repellent activity showed by plant extract of *Z. cassumunar* with solvent hexane. This hexane extract of *Z. cassumunar* repelled *S. zeamais* (PR>50%) at all hours. The repellent activity increased significantly to 92, 96 and 99% respectively at concentration 1,415 µg/cm^2 at 6, 7, and 8 hours of exposure. The same plant with two other solvents, methylene dichloride and methanol, showed weak repellent activity. The repellent effect of *K. pulchra* extract have shown to *S. zeamais* in Table 3. Percentage repellency over 50% was found all tested concentrations of hexane extract at 4, 5, 6, 7 and 8 hours after exposure. The highest repellent activity revealed at the highest dose at 5 hrs after exposure, however no significant effect of concentrations. On fifth hours, the methylene chloride extract of this plant showed significant repellence activity at concentration 786 µg/cm^2 compared to concentration 157 µg/cm^2 and 472 µg/cm^2, but when the concentration increased, the result was insignificant. The highest repellency with methanol solvent was observed at 1 and 8 hours of exposure at 472 and 1,415 µg/cm^2 of concentration, respectively. Nevertheless, percentage repellency over 50% was found all tested concentrations of methanol extract at 1-7 hrs after exposure.

Direct contact toxicity

The results given in the figures 1-3 described the toxicological effect of plant extract from *C. longa, Z. cassumunar* and *K. pulchra* with solvent hexane, methylene chloride and methanol. Although they belonged to the same level of activity, their average varied with the concentrations. Overall results showed that the concentrations were less

Table 1. Repellency (%) of *Curcuma longa* rhizome extracts to *Sitophilus zeamais* adults, using treated filter paper. The values are Mean±SE.

Crude extracts	Dose of extracts (µg/cm²)	Duration of exposure (hours)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Hexane	157	27±4.7b	27±5.8bc	28±8.0b	28 ±8.7b	22 ±6.6b	20±6.8b	17±8.3b	18±10.4c
	472	36±8.1ab	21±5.9c	20±7.8b	27±8.3b	17±6.9b	17 ±8.6b	11 ± 6.9b	21±8.4bc
	786	46±7.0ab	46±8.3ab	41±8.8ab	46 ±8.1ab	54±8.5a	60±10.5a	48± 12.3a	50±11.6ab
	1,101	56 ±8.1a	53±7.9a	57±7.9a	68±7.9a	68±6.5a	60±6.3a	63±6.2a	58±5.1a
	1,415	37±7.3ab	33±8.3abc	39±9.6ab	53±10.7ab	56±10.5a	69±7.8a	60±13.3a	48±12.9abc
	$F_{(4,45)}$	2.379	3.278	2.782	3.921	8.100	9.100	6.143	3.293
	P	0.066	0.019	0.038	0.008	<0.001	<0.001	<0.001	0.019
Methylene chloride	157	32±9.9ab	33±10.0ab	34±9.9a	25±8.3a	20±7.8ab	13±5.9b	17±4.9bc	15±4.3c
	472	10±3.3b	9±2.8b	11±5.3a	10±5.2a	10±2.9b	8±2.9b	10±2.9c	8±2.9c
	786	40±10.4a	27±9.4ab	22±9.5a	28±9.0a	30±8.3ab	27±7.8ab	25±7.3bc	24±5.4bc
	1,101	33±9.0ab	39±10.4a	33±11.7a	36±11.6a	40±11.8a	45±12.0a	49±10.2a	50±10.8a
	1,415	39±7.8a	38±9.75ab	25±6.7a	21±7.7a	30±8.4ab	32±10.5ab	39±11.0ab	42±10.3ab
	$F_{(4,45)}$	2.048	1.593	1.036	1.292	1.852	2.370	3.884	5.689
	P	0.104	0.193	0.399	0.288	0.135	0.067	0.009	0.001
Methanol	157	52±6.5a	48±4.7a	44±5.8b	50±7.3b	47±5.9b	32±7.4c	40±6.3b	44±7.0b
	472	50±8.2a	48±8.7a	46±7.8b	43±7.2b	42±7.7b	45±8.9bc	50±8.4b	51±12.3b
	786	70±5.8a	67±7.6a	65±7.5ab	71±6.6a	65±4.3a	66±7.3ab	72±6.6a	79±7.3a
	1,101	60±8.9a	60±8.2a	58±6.5ab	62±6.6ab	78±5.5a	77±8.0a	78±4.4a	77±5.6a
	1,415	72±7.4a	66±6.9a	71±7.1a	77±3.7a	82±4.7a	85±3.7a	87±3.9a	86±3.4a
	$F_{(4,45)}$	1.827	1.624	2.845	4.880	9.690	8.670	10.187	5.816
	P	0.140	0.185	0.035	0.002	<0.001	<0.001	<0.001	0.001

For each crude extract, means in the same column followed by the same letters do not differ significantly (P = 0.05) as determined by Duncan's multiple range test.

Table 2. Repellency (%) of *Zingiber cassumunar* rhizome extracts to *Sitophilus zeamais* adults, using treated filter paper. The values are Mean±SE.

Crude extracts	Dose of extracts (µg/cm²)	Duration of exposure (hours)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Hexane	157	63±9.1a	50±10.8b	51±10.4a	54±12.6a	61±9.4a	63±10.9a	56±12.0b	56±11.1b
	472	81±6.2a	84±5.2a	86±4.3a	79±5.3a	79±6.7a	77±5.9a	75±8.9ab	73±8.4b
	786	70±8.4a	67±9.4ab	61±11.6a	66±12.3a	65±11.95a	66±11.9a	62±13.7b	59±13.6b
	1,101	70±11.4a	72±10.3ab	77±9.9a	72±9.5a	72±9.2a	71±9.5a	71±11.3ab	68±11.8b
	1,415	80±6.3a	85±5.82a	80±7.5a	87±5.4a	87±6.1a	92±2.9a	96±2.2a	99±1.0a
	$F_{(4,45)}$	0.799	2.288	1.770	1.235	1.308	1.449	2.242	3.476
	P	0.532	0.075	0.151	0.310	0.282	0.233	0.079	0.015
Methylene chloride	157	65±7.5a	77±7.31a	79±6.1a	75±6.5a	75±8.9a	72±8.8a	67±9.20a	63±9.6a
	472	35±9.92b	36±10.9b	31±11.4b	42±10.4b	44±11.8b	48±10.2a	48±9.9a	50±8.7a
	786	55±9.2ab	57±34.0ab	58±8.5a	64±8.8ab	64±7.8ab	72±5.9a	70±8.1a	70±7.8a
	1,101	60±9.7ab	56±10.8ab	66±10.7a	70±9.1a	66±11.8ab	63±12.0a	61±10.7a	65±10.5a
	1,415	66±9.3a	66±11.9ab	73±8.3a	75±7.5a	75±7.0a	74±7.6a	73±7.5a	70±6.8a
	$F_{(4, 45)}$	1.902	1.584	4.149	2.565	1.729	1.398	1.062	0.879
	P	0.127	0.195	0.006	0.051	0.160	0.250	0.387	0.484
Methanol	157	44± 4.8a	50±5.6a	43± 12.7a	60± 5.8a	59± 4.6a	55± 7.5a	55± 7.3a	55± 8.6a
	472	53± 7.9a	53± 6.7a	56± 5.4a	56± 8.5a	49± 6.7a	52± 8.7a	51± 7.2a	46± 7.2a
	786	50±7.2a	50± 8.0a	49± 7.9a	43± 7.2a	52± 8.8a	51± 7.4a	60± 8.4a	58± 6.8a
	1,101	53± 7.3a	56± 7.0a	50± 9.2a	49± 7.9a	38± 5.5a	43± 8.2a	42± 9.6a	38± 8.0a
	1,415	56± 11.7a	56± 10.8a	56± 12.8a	50± 11.8a	48± 12.0a	55± 10.7a	45± 9.3a	49± 8.4a
	$F_{(4,45)}$	0.318	0.147	0.295	0.603	0.906	0.329	0.743	1.010
	P	0.865	0.963	0.880	0.663	0.469	0.857	0.568	0.413

For each crude extract, means in the same column followed by the same letters do not differ significantly (P = 0.05) as determined by Duncan's multiple range test.

Table 3. Repellency (%) of *Kaempferia pulchra* rhizome extracts to *Sitophilus zeamais* adults, using treated filter paper. The values are Mean±SE.

Crude Extracts	Dose of extracts (µg/cm^2)	Duration of exposure (hours)							
		1	2	3	4	5	6	7	8
Hexane	157	58±9.9ab	58±12.0a	60±12.4a	61±13.2a	62±14.1a	58±11.0a	57±12.1a	52±9.5a
	472	39±8.1b	47±8.6a	49±9.9a	51±8.5a	59±8.8a	56±10.9a	51±8.4a	51±9.8a
	786	68±6.1a	72±6.1a	72±6.3a	77±6.7a	76±8.2a	75±9.1a	75±7.7a	70±7.6a
	1,101	58±11.4ab	63±10.8a	66±9.6a	66±9.8a	65±10.6a	56±13.4a	61±12.4a	58±12.7a
	1,415	52±8.4ab	70±8.0a	70±7.8a	74±7.5a	79±7.9a	74±8.1a	69±7.4a	67±7.0a
	$F_{(4,45)}$	1.400	1.167	0.909	1.209	0.753	0.847	0.615	0.866
	P	0.249	0.338	0.467	0.320	0.561	0.503	0.654	0.492
Methylene chloride	157	35±9.8b	38±8.7b	57±9.9a	50±10.7a	44±10.9b	49±10.3a	53±10.2a	52±9.8a
	472	45±7.3ab	51±8.9ab	50±11.6a	52±11.4a	41±12.2b	47±11.4a	48±12.5a	61±11.5a
	786	62±4.4a	71±4.3a	71±8.9a	71±9.4a	77±8.8a	63±10.1a	66±9.3a	67±9.3a
	1,101	53±6.2ab	57±8.4ab	58±10.7a	55±10.4a	52±9.6ab	60±9.9a	53±9.3a	50±9.8a
	1,415	50±5.4ab	56±6.4ab	44±9.8a	52±10.6a	47±9.8ab	48±8.5a	51±9.8a	50±11.6a
	$F_{(4,45)}$	2.105	2.485	0.983	0.640	1.960	0.555	0.450	0.537
	P	0.096	0.057	0.426	0.637	0.117	0.697	0.772	0.710
Methanol	157	69± 4.6a	59± 6.6a	62± 7.3a	65± 4.8a	56± 4.0a	60± 5.4a	54± 3.7ab	51± 3.1c
	472	76± 4.8a	75± 5.0a	75±5.0a	63± 5.4a	61± 3.8a	57± 3.4a	51±3.8b	46± 3.7c
	786	62±6.1a	63±6.3a	64±4.3a	63±5.4a	59±5.7a	63±4.7a	58±6.3ab	60± 5.6bc
	1,101	73± 6.2a	72± 8.1a	66± 7.2a	61± 4.8a	70± 6.2a	65± 7.8a	65± 6.0ab	68± 6.1ab
	1,415	73± 6 .7a	68± 8.7a	64± 7.5a	64± 6.9a	64± 6.0a	67± 8.2a	70± 7.3a	76± 5.0a
	$F_{(4,45)}$	0.896	0.847	0.643	0.073	1.046	0.416	1.951	6.376
	P	0.474	0.503	0.635	0.990	0.394	0.796	0.118	0.000

For each crude extract, means in the same column followed by the same letters do not differ significantly (P = 0.05) as determined by Duncan's multiple range test.

effective regarding to their toxicological activity against *S. zeamais.* The mortality of this pest sometimes increases as the concentration and time of exposure progressed, but sometimes the plant extracts did not affect at all to the pests.

Extract of *C. longa* with solvent hexane was the most effective compared to others (Fig. 1). The highest mortality was 13% at 45 µg/insect respectively at the interval of 7 day while the minimum mortality was 1% at 5 µg/insect. It had significant difference with its control (0%). Similarly, another bar indicated the effect of plant *C. longa* with solvent methylene chloride. It also showed the significant mortality (6%) at 45 µg/insect at interval of 7 day and the minimum mortality is 1% at 7 day of exposure. The toxicological effect of plant *C. longa* with solvent methanol revealed that the highest mortality was 6% at 45 µg/insect at interval of 7 day. It showed significant difference with its control. Figure 2 recorded that the plant *Z. cassumunar* at 25 µg/insect with solvent hexane has 7% as the highest mortality. This has significant different with the control but has no difference with other concentrations, 35 and 45 µg/insect.

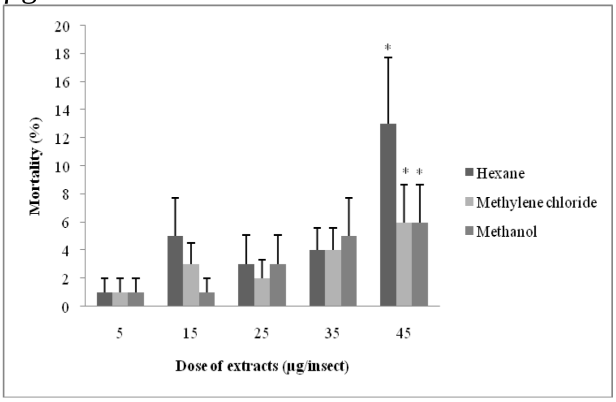

Fig. 1. Accumulated mortality (%) caused by *Curcuma longa* rhizome extracts to *Sitophilus zeamais* adults, using micro-applicator. The values are Mean±SE. *For each crude extracts, a treatment is significantly different from the control as determined by Duncan's multiple range test (*P* = 0.05, n=10)

It also happened to the methylene chloride and methanol extract of *Z. cassumunar*, eventhough the highest mortality (8 and 6%, respectively) at 45 µg/insect significantly different compared to its control, but they had no difference if compared to other lower concentrations.

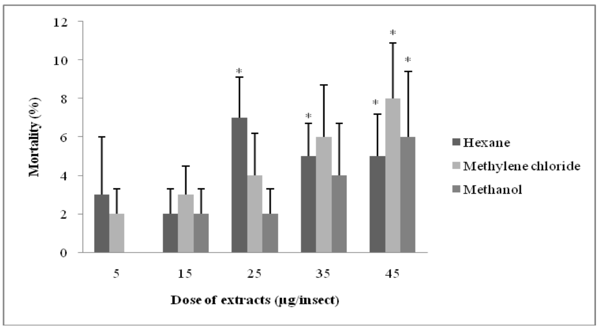

Fig. 2. Accumulated mortality (%) caused by *Zingiber cassumunar* rhizome extracts to *Sitophilus zeamais* adults, using micro-applicator. The values are Mean±SE. *For each crude extracts, a treatment is significantly different from the control as determined by Duncan's multiple range test (*P* = 0.05, n=10).

The experiments of the crude extract of *K. pulchra* with solvent hexane and methylene chloride showed no significant toxic effect against *S. zeamais* adults (Fig. 3) eventhough it showed mortality (4% at 15 µg/insect at interval of 7 day by applying solvent hexane and only shows 1% at 25 µg/insect with methylene dichloride solvent). However, the plant extract of *K. pulchra* with solvent methanol can reduce the insect until 6% at 45 µg/insect at interval of 7 day but still showed no significant difference compared to most other concentrations.

DISCUSSION

Study data from literature review stated a highly promising efficacy of *C. longa* as natural pesticides. Not only information about the use of *C. longa* as a spice and apart from its multiple medicinal uses, but also this plants are credited with interesting pesticidal properties against certain agricultural pests and with promising repellent properties against mosquito species (Roy *et al*., 2014).

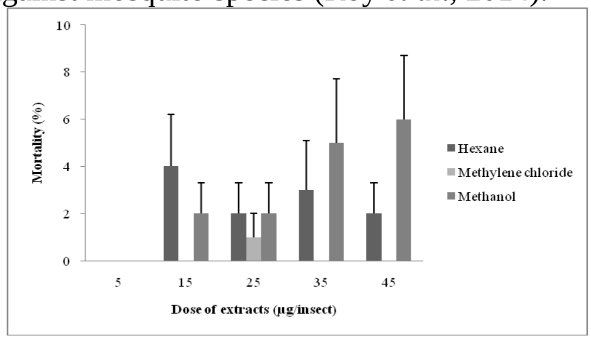

Fig. 3. Accumulated mortality (%) caused by *Kaempferia pulchra* rhizome extracts to *Sitophilus zeamais* adults, using micro-applicator. The values are Mean±SE. *For each crude extracts, a treatment is significantly different from the control as determined by Duncan's multiple range test (*P* = 0.05, n=10).

Their report can be confirmed by our work that *C. longa* along with 2 plants, *Z. cassumunar* and *K. Pulchra*, in Zingiberaceae showed potential for controlling *S. zeamais*, agricultural pests of stored products. Methanol extract of *C. longa* stated strong repellent effect to the maize weevil. These finding also has similarity with the research done by Ishii *et al*. (2010). They found that 20 mg/ml of ethanol crude extract of turmeric gave moderate repellence activity against *S. zeamais* (34.31%). Hexane extract of *Z. cassumunar* also showed strong repellent activity in this study. The result looks similar to those of Khanam *et al*. (2008) who reported that *Z. cassumunar* extracted by petroleum ether, non polar solvent as hexane, showed strong repellent effect against *T. castaneum* and *T. confusum*. Other studies have reported that an essential oil of *Z. officinale* at 20 mg/mL and 700 µL/mL repelled *S. zeamais* adults and *Prostephanus truncates* Horn adults, respectively (Ishii *et al*., 2010; Ogbonna *et al*., 2014). The repellent effect of *Z. cassumunar* hexane extract may involve with several factors. One of which was chemical compounds such as sabinene, y-terpinene, terpinen-4-ol, (E)-1-(3,4-dimethoxyphenyl) butadiene containing in the extract (Sukatta *et al*., 2009). The same plant with two other solvents, methylene dichloride and methanol, showed weak repellent activity. Khanam *et al*. (2008) reported that methanol extract of cassumunar ginger also repelled insect *T. confusum* at a lower rate.

Extract of *C. longa* with solvent hexane showed low contact toxicity in this study. These results were in agreement with the research obtained by Matter *et al*. (2008) who showed that 4% of petroleum ether extract of *C. longa* showed low effectiveness (20.4%) against *Sitophilus oryzae* (L.). Surprisingly, the same extract at 4% of *C. longa* scored the highest mortality (90.8%) against *Rhyzoperta dominica* (Fab.). In another experiment, Ali *et al*. (2014) observed that maximum mortality of *T. castaneum* caused by acetone extract of *C. longa* was 11% at concentration 20% with the exposure time of 2 days as the concentration increased. Moreover, Asawalam and Chukwuekezie (2012) evaluated the significant mortality effect (90%) of *S. zeamais* assessed by petroleum ether extract of *C. longa* after 42 days of treatment.

The insecticidal activity of *C. longa* extracts against *S. zeamais* could be attributed to the presence of monoterpenoids, sesquiterpenoids, and curcuminoids (Tang and Eisenbrand, 1992). Bhardwaj *et al*. (2011) found that *C. longa* contains pungent, odoriferous oils, and oleoresins that possess many kinds of biological activities. The main components of this plant which act as insect repellent are turmerone and *ar*-tumerone (Tripathi *et al.*, 2002). Tavares *et al*. (2013) recorded that the mortality of *S. zeamais* with *ar*-turmerone increased with the higher concentration of essential oils of turmeric. *Ar*-turmerone, a chemical constituent of *Curcuma* spp. such as *C. longa*, *C. amada*, *C. domestica*, and *C. xanthorrhiza* possesses repellent activity against *T. castaneum* (Su *et al*., 1982), but Lee *et al*. (2001) revealed that 2.1 mg cm^{-2} of *ar*-turmerone was ineffective (<10% mortality) against adult of *S. oryzae*, *Callosobruchus chinensis* L., *Lasioderma serricorne* Fabr., and also the larvae of *Plodia interpunctella* Hubner eventhough *ar*-turmerone caused 100 and 64% mortality at 1,000 and 500 ppm, respectively against *Nilaparvata lugens* (Stal) and 100 and 82% mortality at 1,000 and 500 ppm, respectively against *Plutella xylostella* L. larvae. It has opposite results which done by Franz *et al*. (2011). It showed that the oils of *Z. officinale* were the most efficient, causing 85% mortality of *S. oryzae* at low concentration (1%) after 48 h. In addition, the essential oil of *Z. zerumbet* was also toxic to *S. zeamai*s adults. The LD_{50} and LD_{90} values were 21 and 30 µg/mg, respectively (Suthisut *et al*., 2011). On the another study, Liu *et al*. (2014) revealed that essential oil of *K. galanga* exhibited contact toxicity against the booklouse, *Liposcelis bostrychopila* Badonnel with an LC_{50} value of 68.6 mu g/cm^2.

The results obtained from these studies have exposed good potential for the use of crude extract of *C. longa*, *Z. cassumunar* and *K. pulchra* for the control of *S. zeamais* in stored maize grains. The methanol extract of *C. longa,* hexane extract of *Z. cassumunar* and hexane and methanol extracts of *K. pulchra* exhibited significantly strong repellent activity against this *S. zeamais*. Hexane extract of *C. longa*, methylene chloride of *Z. cassumunar* and methanol extract of *K. pulchra* also could be effectively used to control this maize weevil due to its weak toxic effect which possessed the highest mortality of this insect.

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