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Toxicity property of clove oil (*Syzygium aromaticum* L.) and rice grain protection against *Rhyzopertha dominica* (Fabricius) and *Sitophilus zeamais* Motschulsky

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Abstract *Rhyzopertha dominica* and *Sitophilus zeamais* caused considerable damage on rice stored grains. These stored insects are normally controlled during storage mainly by synthetic insecticides for grain protection and fumigation. Plant extract was highly conducted with effectiveness against stored grain insects and can be an alternative application which is safety to natural environment and consumers. This study investigated the application of clove oil (*Syzygium aromaticum* L.) by grain treatment as well as effects to their insecticide activity, inhibition progeny production, and grain damage (weight loss) as compared with chlorpyrifos under laboratory. The results showed that clove oil coating at 200 μL/L after 7 days of application had performed the mortality percentage of *R. dominica* and *S. zeamais* at 99.33% and 95.33%, whereas chlorpyrifos was showed mortality percentage at 66.00% and 100%, respectively. The study indicated that this insecticide was lower effective to kill *R. dominica* than clove oil, but all of them were still effective to kill *S. zeamais.* For F1 inhibition emergence, the clove oil inhibited adult emergence both *R. dominica* and *S. zeamais*, whereas chlorpyrifos was highly inhibited to the emerged adults of *R. dominica* than *S. zeamais* adults. Weight loss and frass production of grain coated with clove oil showed the greater in *S. zeamais* than that in *R. dominica*. This attributed to more susceptible of *R. dominica* to clove oil than *S. zeamais*. Hence, grain coating with clove oil is an alternative method for promising as seed protectants from *R. dominica* and *S. zeamais* in warehouse in the future. Clove oil is safety, environmental-friendly, and reduces the use of synthetic insecticide. However, this method should be further studied in terms of reasonable application such as economic-value consideration.

Keywords: *Syzygium aromaticum* L., *Rhyzopertha dominica*, *Sitophilus zeamais*, grain coating, F1 inhibition emergence

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Introduction

Rice is the most important crop as it constitutes a principal food and mostly consumed by humans and animals in the worldwide. Rice production is the main agricultural crop practiced in Asia, especially in Thailand. However, during storage of rice grains are destroyed during the storage period by many stored insect pests that are responsible loss up to 10-40% annually in the world (Ahman *et al*., 2013). The stored product insects are considered to be a serious problem and will cause qualitative and quantitative losses including loss of grain weight, the percentage of seed germination capacity decrease, and devaluation of nutrition and product. The overall damages caused by the stored product insects in worldwide were estimated to be 10-40% annually (Mohan and Fields, 2002). The aforementioned reasons ensure that it is necessary to maintain stored seed quality with appropriate management.

R. dominica and *S. zeamais* are major pests of stored rice commodities. Both are regarded as the most destructive pests on rice grains owing to their high population fecundity with the ability to consume and to develop inside the grain kernel that will cause the great difficulty avoidance of the grain losses (Hayashi *et al*., 2004). Currently, the controlling of these pests is primarily dependent upon the related application of synthetic insecticides, including coating and fumigation (Hasan and Reichmuth, 2004). The grain coating is the application of organophosphate or pyrethroid groups and fumigation with phosphine. These are remarkable as the main control applications. However, intensive use of synthetic insecticides for controlling insect pests have serious results such as the development of insecticide resistance, contamination to environmental, unacceptable pesticide residues in food, lethal effects on non-target organisms, and harmful effect on workers (Michaelraj and Sharma, 2006). The several stored insect pests have developed resistance to phosphine and chlorpyrifos such as *R. dominica*, *S. zeamais*, *Triborium cataneum*, and *Trogoderma granarium* (Zeng, 1999).

The mentioned reasons inspire that there showed be need of the study for the alternative development in integrated pest control strategy against stored insect pests especially for controlling *R. dominica* and *S. zeamais* (Naima *et al*., 2013). Using oil extracted from plants is considered for controlling stored-product insects as the replacement of the synthetic insecticide. Oil extracted oils from plants contained secondary metabolites revealed to be toxic to stored insects pests, whereas some others had shown feeding disruption and grain protection effects (Rajendran and Sriranjini, 2008). Singh *et al*. (2012) reported that some plant derivations as disrupt reproduction or inhibition of insect growth. Moreover, the extract of the plant has been described as eco-friendly, less harmful to the consumers and can reduce cross-resistance. The following of the experimental result was showed that clove oil was the most mortality effectiveness against *R. dominica* and *S. zeamais* population more than some other plants (turmeric, black pepper, citronella, thaim, and wide spider flower) in contact, fumigation, and feeding deterrence methods. Clove oil constitutes monoterpenoids which causes insect mortality by inhibiting acetylcholinesterase enzyme activity (Houghton *et al*., 2006). The toxicity of clove oil was confirmed by Paranhos *et al*. (2006) who revealed high *Zabrotes subfasciatus* mortality and oviposition decrease in grain protection. However, the study of seed coating of clove oil for the insecticide effects and disruption of population progeny against *R. dominica* and *S. zeamais* is lacking in reports.

In this study, we have investigated the effect of insecticide toxicity, inhibition of F1 emergence, and weight loss of insect damage. Coating of clove oil for an alternative insecticide on rice grain was studied against *R. dominica* and *S. zeamais*.

Materials and methods

Insect culture

The adults of *R. dominica* and *S. zeamais* were reared separately in cylindrical plastic containers which contained paddy rice with moisture content at 15% t and mixed with barley in the ratio of 2:1. Prior to use for insect rearing, the un-infested rice seeds were sterilized by freezing at a temperature of 4–6 ºC for 14 days to killing other insects contaminated in the grains. Hundred unsexual adults of *R. dominica* and *S. zeamais* were released in each plastic container and kept under condition at 30±3 °C and 76 ± 5 % RH. All insect adults remained confined for 7 days to induce oviposition and were sieved out of the container. Egg-infested grains were kept in the dark until insect's emergence of the next generation. This procedure was performed for five generations to ensure the number of insect populations necessary to experiment. Insects of both species in experiments used were 0–14 days old adults after emergence.

Plant oil extraction

The clove oil was used and selected in this experiment based on safe of mammalian toxicity. Dried bud flowers of clove were purchased from an herbal shop in Hat Yai, Songkhla, Thailand. The dried cloves were extracted by water distillation apparatus. One-hundred grams (100 g) of dried samples were placed in the flask containing (1 L) and mixed with 500 ml of sterile water. The distillation for clove oil process was undertaken for 12 hours, and water was eliminated by using sodium sulfate anhydrous. Clove oil was stored in a refrigerator at 10–12 ºC until being used for the experiments.

Assessment toxicity of clove oil to R. dominica and S. zeamais

Clove oil was diluted in distilled water mixed with red food coloring (2 g/L) at different concentrations (10, 30, 50, 100, 150, and 200 μl/L) and added with emulsifier of Tween-80, simultaneously. The un-infested rice grains about 100 g were placed into 250 ml conical flask. The solutions of clove oil at different concentrations in amounts of 3 ml were pipetted into each flask. The flask was then shaken vigorously for 15–20 min to ensure that grains were thoroughly coated. The grains were air-dried for 1 hour before introducing fifty unsexed adults of *R. dominica* and *S. zeamais* and were placed in a separate plastic cup (400 ml) with a lid and stored in the laboratory. The same procedure was applied for a 0.4% chlorpyrifos solution and distilled water which served as positive and negative control, respectively. The top of each plastic cup was covered with a piece of muslin cloth and a rubber band was tied around. All plastic cups were then kept in the dark under the room temperature. A completely randomized design (CRD) with three replicates per treatment was used. The numbers of dead both insect species were counted and recorded on days 1, 3, 5, 7, 14, and 21 after application. The corrected mortality percentage was calculated by using Abbott's formula (Abbott 1925) as %Corrected mortality = [(%mortality of treatment–%mortality of control)/100–%mortality of control)]*100.

F1 progeny deterrent of clove oil and weight loss

After the 21–day mortality count, all remaining adults of both species were removed from treated and untreated (control) rice grains. The number of *R. dominica* and *S. zeamais* adults that successfully emerged was recorded daily until the emergence of insects was no longer present. Then the adults were removed from the plastic cups to prevent breeding and egg-laying in the future. The number of F1 emerged adults or reproduction inhibition rate (%IR) was computed according to Tapondjou *et al*. (2002) as (IR%) = [(Cn-Tn)/Cn]*100, where C is the number of emerged adults in control, and T is the number of emerged adults in treatment. All samples had been weighed for the weights of contents which included whole grain, damaged grain, and frass.

Statistical analysis

The data obtained including the mortality percentages, reproduction inhibition rate (%IR), and weight loss and frass percentages were subjected to the analysis of variance (ANOVA). Means of treatments were compared by using Tukey's multiple range tests. The lethal concentration (LC_{50} and LC_{90}) was calculated using probit analysis (Finney, 1971).

Results

Insecticidal activity of grain protectant effects of clove oil

Results of accumulative mortality percentage of both insects to each of concentrations on paddy rice using grain coating as shown in Table 1 which revealed that all clove oil concentrations were toxic to *R. dominica* and *S. zeamais*. Their mortalities were mostly significantly different ($P<0.01$) among treatments. Results of adult mortality percentage of *R. dominica* and *S. zeamais* were mostly significantly different ($P<0.01$) among treatments. Morality percentage of these insects increased with a rise in concentration and exposure time and shown clearly toxic that application provided suitable grains protection to rice against those both insect species. (Table 1). *R. dominica* was more susceptible to clove oil than *S. zeamais* due to lower LC_{50} and LC_{90} (Table 2) and had higher mortality of *R. dominica* (Table 1).

Furthermore, clove oil exhibited significantly higher *R. dominica* mortality at 100 μl/L of treatment as compared to chlorpyrifos during the assessed period of time (Table 1). The study indicates that clove oil at 100 μl/L was more effective to control *R. dominica* than chlorpyrifos. In opposite to the synthetic insecticide chlorpyrifos, *S. zeamais* had significantly higher mortality in paddy rice grains coated with chlorpyrifos as compared with clove oil at all concentrations and times after treatment (Table 1). These were consistent with the result of this study that *R. domoinica* was more tolerant to chlorpyrifos than *S. zeamais* (Table 1).

Table 1. Accumulative mortality percentages of *Rhyzopertha dominica* and *Sitophilus zeamais* after grain coating with different concentrations of *Syzygium aromaticum* oil for 1, 3, 5, 7, 14, and 21 days

Insect species	Concentrations (µL/L)	% Mortality (Mean±SE)[1/]					
		1d	3d	5d	7d	14d	21d
R. dominica	10	5.33±0.67c[2/]	12.67±0.67cd	18.00±3.06de	24.00±5.03cd	36.67±6.70e	40.00±6.11d
	30	8.00±1.15c	18.67±0.6c	23.33±2.40d	36.67±6.96c	64.00±6.43d	67.33±5.70c
	50	11.33±0.67c	23.33±1.76c	46.67±5.46c	64.67±8.11b	77.33±6.36cd	78.67±6.36bc
	100	33.33±1.76b	56.00±5.29b	76.67±5.33ab	89.33±2.91a	95.33±2.91abc	97.33±2.67ab
	150	50.00±2.31a	70.67±1.33a	84.67±2.91a	92.00±1.15a	97.33±0.67ab	99.33±0.67a
	200	57.33±3.06a	81.33±3.33a	92.67±2.91a	99.33±0.67a	100.00±0.00a	100.00±0.00a
	chlorpyrifos	28.00±6.43a	56.67±4.37b	63.33±5.70bc	66.00±5.03b	80.00±2.31bcd	84.00±3.06abc
	water (control)	0.00±0.00c	0.00±0.00d	2.00±1.15e	3.33±0.67d	6.00±0.67f	6.67±0.67e
	F-test	*	**	**	**	**	**
S. zeamais	10	2.00±1.15d	5.33±1.33e	10.67±1.76ef	14.67±0.67ef	22.67±1.33e	25.33±2.40e
	30	6.67±2.40d	14.00±4.00de	20.00±2.31de	20.00±2.31de	26.00±2.31e	30.67±3.71e
	50	10.00±1.15d	20.67±3.33d	28.67±3.53d	30.67±3.53d	45.33±1.76d	51.33±1.76d
	100	32.67±4.37c	47.33±2.91c	61.33±4.06c	65.33±5.21c	70.67±4.06c	72.00±3.46c
	150	49.33±1.76b	62.00±1.15b	75.33±2.40b	80.67±4.81b	84.00±4.16b	84.67±3.53c
	200	49.33±2.40b	72.67±4.37b	90.00±4.00a	95.33±2.91ab	97.00±2.67a	97.33±2.67ab
	chlorpyrifos	96.00±3.06a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00	100.00±0.00a
	0 (control)	1.33±1.33d	3.33±0.67e	3.33±0.67f	3.33±0.67f	4.67±0.67	5.33±0.67f
	F-test	**	**	**	**	**	**

[1]/averaged from 3 replications.

[2/] means within a column followed by the same letters are significantly different (P<0.05) according to Turkey's comparison tests, * significantly at P<0.05, ** significantly at P<0.01.

Table 2. LC_{50} and LC_{90} of *Syzygium aromaticum* oil against of *Rhyzopertha dominica* and *Sitophilus zeamais* after rice grain coating for 1, 3, 5, 7, 14, and 21 days

Time after seed coating (Days)	Insect species	LC_{50} (μL/L)	95% confident limit		LC_{90} (μL/L)	95% confident limit	
			Lower	Upper		Lower	Upper
1	*R. dominica*	171.31	114.88	372.01	993.25	430.52	1959.20
	S. zeamais	181.20	154.53	223.05	739.27	518.07	1250.91
	T-test	*[1/]			ns		
3	*R. dominica*	80.42	49.44	143.27	447.42	216.68	834.51
	S. zeamais	113.66	100.09	130.93	445.51	343.75	799.91
	T-test	ns			**		
5	*R. dominica*	48.51	29.85	72.45	214.17	128.26	632.01
	S. zeamais	73.57	50.95	113.08	286.59	175.34	633.86
	T-test	ns			ns		
7	*R. dominica*	31.96	19.06	46.41	124.43	80.53	282.53
	S. zeamais	63.62	34.26	106.8	246.25	131.83	576.03
	T-test	ns			ns		
14	*R. dominica*	19.03	15.65	22.4	78.29	65.67	97.19
	S. zeamais	48.91	24.02	85.31	225.46	118.04	442.06
	T-test	ns			ns		
21	*R. dominica*	17.39	10.66	24.06	67.82	48.37	85.64
	S. zeamais	43.86	22.48	72.12	219.77	118.80	364.87
	T-test	ns			ns		

[1/] means within a column followed by the same letters are significantly different ($P<0.05$) according to Turkey's comparison tests,
* significantly at $P<0.05$, ** significantly at $P<0.01$ and ns non signigicantly.

Inhibition F1 progeny of clove oil and weight loss

The progeny emergence rate of *R. dominica* and *S. zeamais* in F_1 generation on rough rice grains treated with different concentrations of *S. aromaticum* oil and chlorpyrifos after 21 days of treatment was presented in Figure 1. The results showed that emerged adults were significantly different ($p<0.05$) among the treatments. Clove oil inhibited adult emergence both *R. dominica* and *S. zeamais*. The result indicated the effect of followed concentration-dependent pattern with the low number of emerged progenies. In particular, *R. dominica* was markedly inhibited rather than *S. zeamais*. In contrast to chlorpyrifos, the adult emergence of *S. zeamais* was highly inhibited, whereas that of *R. dominica* was less effective (Figure 1). The lowest tested concentrations (10 µL/L) inhibited the emergence of the F_1 progeny of *R. dominica* by 30.51%, whereas F_1 progeny of *S. zeamais* by 25.76%. A 96.30 % reduction in the F_1 progeny of *R. dominica*, and 93.64% reduction in the F_1 progeny of *S. zeamais*, were observed at a concentration of 200 µL/L. In case of the insecticide grain treatment, chlorpyrifos (40% w/v) completely prevented the emergence of *S. zeamais* adults, whereas chlorpyrifos reduced the progeny of *R. dominica* by 24.44% (Figure1).

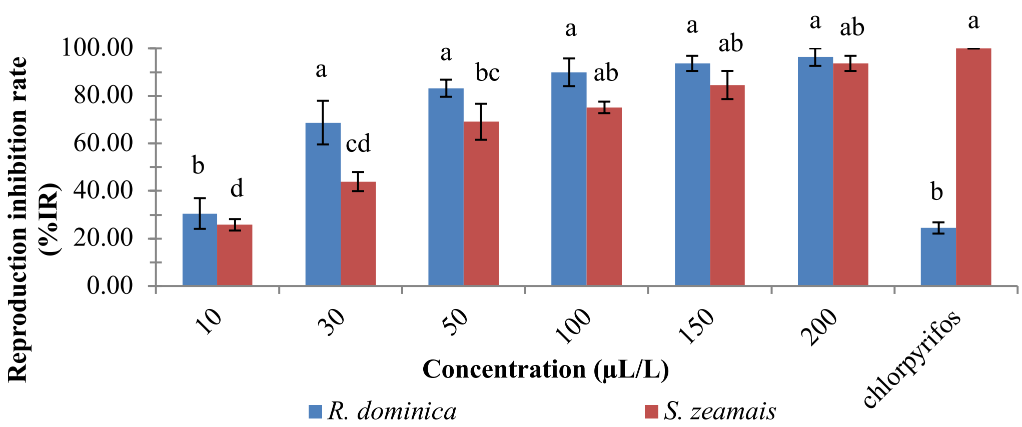

Figure 1. Reproduction inhibition rate (%IR) of the F1 progeny of *Rhyzopertha dominica* and *Sitophilus zeamais* after treatment with different concentrations of clove oil and chlorpyrifos

Feeding deterrence expressed as weight loss and frass arising from an adult of *R. dominica* and *S. zeamais* feeding on rough rice grains coated with different concentrations of *S. aromaticum* oil in a comparison with chlorpyrifos and control are presented in Figure 2. Weight loss and frass production were significantly (p<0.05) higher in control than in both *S. aromaticum* oil and chlorpyrifos after 21 days of treatment. These losses of grains coated with clove oil were greater in *S. zeamais* than in *R. dominica*. These losses of grains coated with clove oil were greater in *S. zeamais* than in *R. dominica*. This was attributed to more susceptible of *R. dominica* to clove oil than *S. zeamais.* This was opposite to the result of seeds coated with chlorpyrifos (Figure 2).

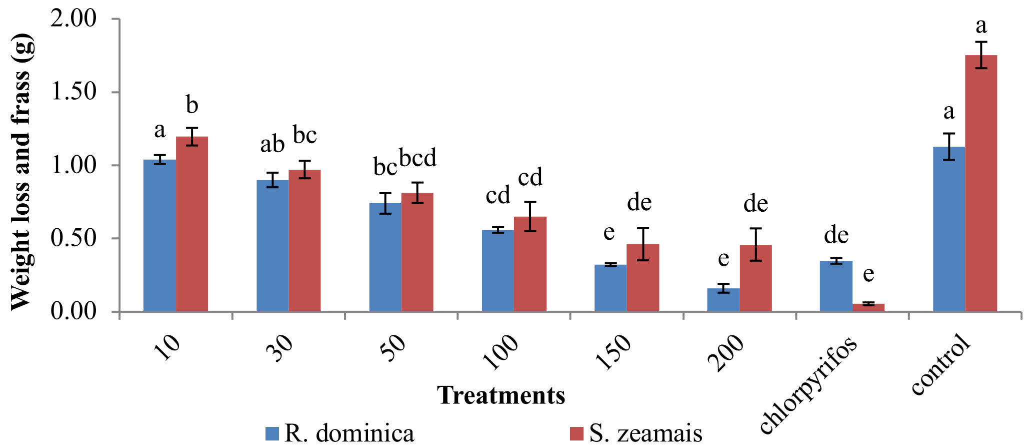

Figure 2. Weight loss and frass production by *Rhyzopertha dominica* and *Sitophilus zeamais* after grain coating with different concentrations of clove oil as compared to chlorpyrifos and control (distilled water)

Discussion

The present study showed that the oil of clove exhibite toxicity against of both insect species after coating seed. This result might be attributed to their chemical constitutes containing in clove oil, particularly eugenol which was the key component in clove oil as probably caused the major factor in both died insects. Similar results bad been reported for the clove oil component which eugenol was highly toxic and showed antioviposition to *Zabrotes subfasciatus* (Paranhos *et al*., 2006). These results agreed with Samson and Parher (1990) reported that the *S. zeamais* was most susceptible to chlorpyrifos and fenitrothion, whereas resistance of *R. dominica*. Groor (2004) mentioned that chlorpyrifos was effective against a wide range of stored insect pests, except *R. dominica*. These were consistent with the result of this study that *R. domoinica* was more tolerant to chlorpyrifos than *S. zeamais*. Moreover, inhibition F1 progeny, the results agreed with the study of Sharma and Meshram (2006) finding that *S. aromaticum* oil at the concentration of 25-250 ppm inhibited F1 progeny from 50.42-72.50%. Ho *et al*. (1997) investigated grain coating with the oil from fresh garlic (*Allium sativum*) and found that *T. castaneum* and *S. zeamais* failed to lay their eggs at the concentrations of >2,000 ppm in rice, and F1 progeny production was inhibited at the concentration of >5,000 ppm in wheat, respectively. The emergence of *R. dominica* significantly decreased after treated with 1 ml/100 g rice of clove oil extracted by hexane and methanol (Ho *et al*., 1994). Although mechanism of clove oil protection grain did not know clearly to F1 progeny inhibition, the study shown effectiveness to prevent oviposition, suppression the egg hatching or larvae survival leading to those no presented insects and caused mortality of larva before penetrating into the grain, This study may being to the attributed that the female insects that able to lays egg on the grain surface but it egg cannot have hatching or developing normally. Also, wheat grains coated with 5.0% neem powder reduced grain damage by *R. domonica* 2.55%, 3.15%, and 7.13% as compared to control after 32, 64, and 96 days of treatment (Patel *et al*., 1993). Eucalyptus oil was also reported to be highly effective to reduce grain damage of 6.37% and weight loss of 3.48% as compared to control of those values of 28.58% and 18.39%, respectively in wheat after 120 days of grain coating (Singh *et al*., 2012). Clove oil as grain coating showed evidently more effectiveness to control *R. dominica* than *S. zeamais.* This may be attributed to different movement behavior of these two insect species. *R. dominica* moved slowly in a downward direction to the bottom of container leading to higher contact opportunity with oil more than *S. zeamais* which was rushed rapidly to the top surface of grains resulting in less contact to the oil. Even the mechanism of plant oil as seed coating against stored insect has not clearly clarified, the results of our study demonstrated that clove oil possessed actions of killing, antifeeding, and suppressing progeny production. A reduction of progeny production might attribute to a less extent of oviposition, egg hatchability, as well as a survival of larval and pupal stages.

The results obtained from this study clearly indicated that rice grains coated with chlorpyrifos remained highly effective to control S. *zeamais* by increasing mortality, reducing grain damage, and completely suppressing F1 progeny emergence. On the other hand, *R. dominica* showed more tolerant to chlorpyrifos than *S. zeamais* resulting in a low effectiveness to kill *R. dominica*, to protect grain damages, and to inhibit progeny emergence as compared to *S. zeamais*. However, clove oil exhibited highly effectiveness on grain rice protection against *R. dominica* due to low insect infestation, seed damage, and progeny production. Previously, clove oil extract on grain protection and insecticidal activity are lacking in reports. Hence, this study has presented an interesting of research toward of grain coating with clove oil as an alternative method for a rice grain protection from *R. dominica*. However, this method should be further studied in terms of reasonable application such as economic-value consideration.

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