



## Efficacy of Essential Oils against *Oryzaephilus Surinamensis* L. (Coleoptera: Silvanidae) and Application to Packaging Film by Extrusion Coating

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### ABSTRACT

This study was carried out to investigate the toxicity properties of essential oils against saw-toothed grain beetle *Oryzaephilus surinamensis* L. The highest efficiency of the essential oils from 5 plants: lemon grass *Cymbopogon citratus* Stapf, citronella grass *Cymbopogon nardus* Linn, pomelo peel *Citrus maxima* (Burm) Merr, eucalyptus *Eucalyptus globulus* Labill, and kaffir lime peel *Citrus hystrix* was selected and applied on extrusion coating film for rice storage life. The toxicity test was evaluated under laboratory conditions by using two methods, namely Topical Application Method (contact poison) and Residual Exposure Method (inhalation poison or fumigant toxicity). Mortality of the adult saw-toothed grain beetle *Oryzaephilus surinamensis* was observed and recorded every 12 hours until 72 hours. The results revealed that lemon grass and kaffir lime peel achieved a high mortality of saw-toothed grain beetle at 86.66 % and 76.67% by topical application and at 100% and 93.33% by residual exposure respectively. A lower toxicity of treatments was found for pomelo and wood vinegar. Hence, lemon grass oil, kaffir lime peel oil and citronella grass oil were selected and applied to extrusion coating film formed into bags to test for its ability to extend the storage life of rice. One month after observing the infestation of store product pests, the 1% of lemon grass oil, kaffir lime oil and citronella grass oil show significant protection against the saw-toothed grain beetle *Oryzaephilus surinamensis* L., throughout the experiment when compared to the control (uncoated). Additionally, the essential oils may be useful as a grain protectant with extrusion coating film against the other stored product insects infested rice in the coated bags including red flour beetle *Tribolium castaneum* (Herbst) and rice weevils *Sitophilus oryzae* Linn.

**Keywords:** Essential Oils, Extrusion Coating, Saw-Toothed Grain Beetle *Oryzaephilus Surinamensis* L., Mortality

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### INTRODUCTION

Saw-toothed grain beetle, *Oryzaephilus Surinamensis* L. (Coleoptera: Silvanidae) is an important and widespread pest of different food materials. It is one of the common pests of grain products occurring in Thailand, and is usually found as a secondary pest on grain damaged by other insects, such as the grain weevil, *Sitophilus granarius* L., because of its inability to damage whole cereal grains [1], [2]. It is difficult for insecticides to be applied effectively because the insect has developed a strong resistance to several insecticides [3], [4]. An alternative to synthetic pesticides is the use of essential oils; the

toxicity of a large number of essential oils and their constituents has been evaluated against a number of stored-product insects [5].

The use of essential oils is extremely diverse depending on the source, quality, extraction procedure, etc. Essential oils are aromatic and volatile liquids obtained from plant material, including flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plants [6]. Lemon grass *Cymbopogon citratus* Stapf: Poaceae, is a widely used herb in tropical countries, especially in Southeast Asia. The essential oil of the plant is used in aromatherapy. It holds antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties. [7], [8]. Citronella grass *Cymbopogon nardus* Linn: Gramineae, is the source of the commercial citronella oil. In Thailand, a preparation of crude citronella oil mixed with leaves of neem

(*Azadirachta indica* A. Juss) and rhizomes of *Alpinia galanga* (L.) is applied as a biopesticide in agriculture. The United States Environmental Protection Agency considers citronella oil a biopesticide with a non-toxic mode of action [9]. *Eucalyptus globulus* Labill: Myrtaceae, the essential oil extracted from eucalyptus leaves has most of the properties of a typical volatile oil, also having powerful medicinal properties [10]. Pomelo *Citrus maxima* (Burm) Merr: Rutaceae, is an indigenous plant in tropical parts of Asia. The pulp is stated to possess the following properties as reported in ancient and medieval literature: appetizer, antitoxic, cardiac stimulant, and stomach tonic [11]. Kaffir lime *Citrus hystrix*: Rutaceae is a citrus fruit native to tropical Asia, including Thailand. Its fruit and leaves are used in Southeast Asian cuisine and its essential oil is used in perfumery [12]. Wood vinegar is a byproduct from charcoal production. Components include acetic acid, formaldehyde, ethyl-valerate, phenol, methanol, tar, etc. [13]. It has been used in organic farming, showing efficiency as an insect repellent reducing pest infestation on soybean [14].

This study was carried out to investigate the toxicity properties of essential oils from lemon grass *C. citratus* Stapf, citronella grass *C. nardus* Linn, pomelo peel *C. maxima* (Burm) Merr, eucalyptus *E. globulus* Labill, kaffir lime peel *C. hystrix* and wood vinegar against saw-toothed grain beetle *O. surinamensis* L. as well as the application of the essential oils to extrusion coating film for rice storage life.

## MATERIAL AND METHODS

### Insect preparation

Saw-toothed grain beetle *Oryzaephilus surinamensis* L. were collected from rice storage silos in Phitsanulok province, Thailand, and were laboratory-reared with laid eggs on grain rice and hatched at room temperature. Two-day old adults of saw-toothed grain beetle were identified and prepared for bioassay tests.

### Plants extract preparation

Fresh lemon grass, citronella grass, pomelo peel, eucalyptus leaves and kaffir lime peel were collected, cut into small pieces and air dried at 25 °C for sample extraction with the steam distillation method. 500 g dried sample of each plant were placed in a distillation flask with approximately much water and heated on a heating mantle at about 100°C. The flask was allowed to boil for 5 hours until the distillation was completed. The distillate was collected in a separating funnel in which the aqueous portion was separated from the essential oils. The essential

oils were collected and kept in a stoppered cylinder at 4°C prior to use. And were then diluted for testing on saw-toothed grain beetle *Oryzaephilus surinamensis* L. Tween 80 was used for emulsion to stabilize the essential oils before testing.

### Preparation and purification method of raw wood vinegar

Wood vinegar is made from burning fresh wood in a charcoal kiln (or Iwate kiln). The wood was burnt in 120-430°C. The smoke from carbonization was cooled by the outside air when passing through the chimney to produce pyroligneous liquor. The hot steam condensed into liquid was known as raw wood vinegar and must be purified before using.

### Insects bioassay test

Two methods were used in the experiment: 1. Topical Application Method is contact poison; 0.5 µl droplet of the diluted essential oil at a ratio of 1:9 of each treatment was dropped on the head area of the red flour beetle with a micro applicator, and then the tested insects were moved to the cup with grain rice (10 insects /cup) for observation of mortality. 2. Residual Exposure Method is inhalation poison; the diluted essential oil at a ratio of 1:9 of each treatment was sprayed on filter paper and placed on the petri dish, and then the red flour beetle was placed on the petri dish after the treatment evaporated (10 insects / petri dish). Each treatment was carried out in 4 replicates. The mortality was observed for 12, 24, 36, 48 and 72 hours. When control mortality occurred on the experimental test, the mortality was corrected by Abbott's formula [15]:

$$\% \text{Mortality} = \frac{\% \text{test mortality} - \% \text{control mortality}}{100 - \% \text{control mortality}} \times 100$$

### Extrusion coating film with the essential oils

In the extrusion coating process, low-density polyethylene (LDPE) is melted under heat and pressure in an extruder and the molten polymer is extruded through a slit die as a thin web. This web, at high temperature, is drawn down and coated by the selected essential oils. In this study, the high efficiency of treatments: lemon grass oil, kaffir lime oil and citronella grass oil was selected for extrusion coating (water as control treatment). The selected essential oils to be coated was fed continuously over the rubber pressure and was rapidly cooled by the chill roll and was then taken up by a wind-up mechanism. The extrusion coated film was cut to be forming bags (4x6 square inch) for packing rice storage life. One hundred grams of rice from supermar-

ket was placed in each different concentration of leech lime coated bags. Each treatment was carried out in 3 replicates and infestation of stored products insects was observed under temperature room for 1 month.

### Statistical Analysis

The significance of treatments was calculated by one-way Analysis of Variance (ANOVA) and effective treatment was separated by the Duncan's New Multiple Ranges Test (DMRT). Differences between means were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Efficiency of toxicity properties of essential oils against saw-toothed grain beetle (*Oryzaephilus surinamensis* L)

The efficacy test of essential oils and wood vinegar by topical application is shown in Table 1. The result showed that treatments of lemon grass showed high efficiency to saw-toothed grain beetle with mortality at 86.66 %, followed by kaffir lime and citronella grass with mortality at 76.67 % and 56.67% respectively. Particularly, lemon grass showed a highly significant difference ( $P < 0.05$ ) of mortality at 100.0% within 12 hours by residual exposure as shown on Table 2. Therefore, 72 hours after application by residual exposure, the treatment of lemon grass, kaffir lime and citronella grass showed efficiency with mortality at 100.0%, 93.33% and 86.66% respectively. Treatments of wood vinegar and pomelo peel showed lowest efficiency, only 23.33% to 33.33% of mortality by topical application. Particularly, after 12 hours, the saw-toothed grain beetle had not affected the toxicities of the wood vinegar treatment with mortality at 0% by residual exposure (Table 2).

**Table 1.** Toxicities of essential oils and wood vinegar on saw-toothed grain beetle (*Oryzaephilus surinamensis* L.) by topical application

| Treatment        | % Mortality |       |       |       |                     | df |
|------------------|-------------|-------|-------|-------|---------------------|----|
|                  | 12h         | 24h   | 36h   | 48h   | 72h                 |    |
| Control (water)  | 0           | 0     | 0     | 0     | 0 <sup>d</sup>      | ns |
| Lemon grass      | 63.33       | 66.66 | 76.66 | 86.66 | 86.66 <sup>a</sup>  | *  |
| Citronella grass | 33.33       | 40.0  | 50.0  | 56.67 | 56.67 <sup>bc</sup> | *  |
| Pomelo peel      | 3.33        | 10.0  | 23.33 | 23.33 | 23.33 <sup>cd</sup> | ns |
| Eucalyptus       | 6.67        | 6.67  | 10.0  | 23.33 | 23.33 <sup>cd</sup> | ns |
| Kaffir lime peel | 60.0        | 60.0  | 66.67 | 76.67 | 76.67 <sup>ab</sup> | *  |
| Wood vinegar     | 6.67        | 10.0  | 20.0  | 33.33 | 33.33 <sup>c</sup>  | ns |

ns = non-significant; \* = significant difference, means (followed by the same letter) are not significantly different at 5% level by DMRT

**Table 2.** Toxicities of essential oils and wood vinegar on saw-toothed grain beetle (*Oryzaephilus surinamensis* L.) by residual exposure

| Treatment        | % Mortality |       |       |       |                     | df |
|------------------|-------------|-------|-------|-------|---------------------|----|
|                  | 12h         | 24h   | 36h   | 48h   | 72h                 |    |
| Control (water)  | 0           | 0     | 0     | 0     | 0 <sup>c</sup>      | ns |
| Lemon grass      | 100.0       | 100.0 | 100.0 | 100.0 | 100.0 <sup>a</sup>  | ** |
| Citronella grass | 53.33       | 56.66 | 86.66 | 86.66 | 86.66 <sup>ab</sup> | *  |
| Pomelo peel      | 6.67        | 10.0  | 13.33 | 13.33 | 13.33 <sup>b</sup>  | ns |
| Eucalyptus       | 73.33       | 80.0  | 80.0  | 80.0  | 80.0 <sup>ab</sup>  | *  |
| Kaffir lime peel | 66.67       | 80.0  | 93.33 | 93.33 | 93.33 <sup>a</sup>  | ** |
| Wood vinegar     | 0           | 3.33  | 3.33  | 3.33  | 3.33 <sup>bc</sup>  | ns |

ns = non-significant; \* = significant difference, means (followed by the same letter) are not significantly different at 5% level by DMRT

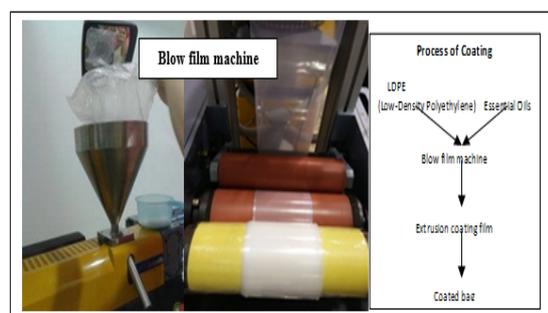
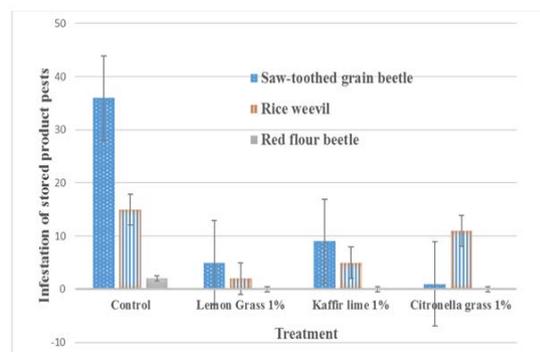
### Application of the selected essential oils on extrusion coating film for rice storage life

Given that lemon grass and kaffir lime extract produced high mortality both in the residual exposure and topical application trials, followed by citronella grass. They were applied to extrusion coating film and formed into rice storage bags to assess whether it could prevent infestation of stored product pests. After one month, it showed that all of the treatments are able to protect against the stored product pests. Particularly, no infestation by the red flour beetle *Tribolium castaneum* Herbst throughout the experiment. However, only a small number of infestations of other stored product insects such as saw-toothed grain beetle *Oryzaephilus surinamensis* L. and rice weevil *Sitophilus oryzae* Linn were found in the treatment. Citronella grass coated bags was the highest protectant to saw-toothed grain beetle *Oryzaephilus surinamensis* L., followed by lemon grass and kaffir lime and they were a significant difference ( $P < 0.05$ ) when compared to the uncoated bags (control). Meantime, coated bags of lemon grass and kaffir lime gave the high protectant with low infestation number of rice weevil *Sitophilus oryzae* Linn found when compared to control (Table 3).

**Table 3.** Efficiency of coated essential oils by extrusion coating film for rice storage life

| Treatment              | Infestation of stored product pests<br>(number of insects)                |  |   |
|------------------------|---|--|---|
|                        | Saw-toothed<br>grain beetle<br><i>Oryzaephilus<br/>surinamensis</i><br>L. | Rice weevil<br><i>Sitophilus<br/>oryzae</i> Linn | Red flour<br>beetle<br><i>Tribolium<br/>castaneum</i><br>Herbst |
| Control                | 36.0 <sup>a</sup>   | 15.0 <sup>a</sup>                                | 2   |
| Lemon<br>Grass 1%      | 5.0 <sup>b</sup>  | 2.0 <sup>c</sup>                                 | 0   |
| Kaffir lime<br>1%      | 9.0 <sup>b</sup>  | 5.0 <sup>b</sup>                                 | 0   |
| Citronella<br>grass 1% | 1.0 <sup>c</sup>  | 11.0 <sup>ab</sup>                               | 0   |
| F-test                 | *   | *  | ns  |

ns = non-significant; \* = significant difference, means (followed by the same letter) are not significantly different at 5% level by DMRT

**Fig.1.** Process of extrusion coating film with essential oils for rice storage life**Fig.2.** Efficiency of coated essential oils by extrusion coating film for rice storage life

## DISCUSSION

It is difficult for insecticides to be applied effectively to control the saw-toothed grain beetle *O. surinamensis*. Several researchers have applied modified atmospheres, carbon dioxide, and fumigant toxicity of allyl acetate to prevent population growth of the saw-toothed grain beetle [16], [17], [18]. Essential oils were also reported that have potential activity for stored product pest control could be used as a multipurpose

pest controlling agent [19]. Hence, in this study, five essential oils were tested against the saw-toothed grain beetle *Oryzaephilus surinamensis* L in the laboratory based on two methods of testing topical application and residual exposure. Lemon grass *Cymbopogon citratus* achieved the highest mortality; 86.66 % by contact poison and 100% by fumigant poison. Fumigation is a successful method of eradicating stored product pests present in food products. Essential oils are used in a wide variety of consumer goods, they are used in the nutritional and agricultural fields for their reported antibacterial, antifungal, antiviral, nematocidal, insecticidal, and antioxidant properties [20].

For many years, different measures have been taken to reduce the number of pests attacking stored cereal grain. Essential oils are used as incorporated into the foodstuff packaging material [21] or as plant and crop protectants [22]. However, many of essential oils uses have been lost with time [6]; therefore, according to this study the applications of essential oils for packaged consumer food products requires extrusion coating process, including the appropriated formulation of the essential oils.

The saw-toothed grain beetle is an extremely destructive pest of packaged consumer food products. Previous studies have shown that the saw-toothed grain beetle infested consumer food packages with flaws than when packages had no flaws. Females laid more eggs into or near the hole in a plastic packaging film, when they were able to contact the food through the hole than when they could not contact the food. The study has shown the importance of sound packaging in preventing insect infestation [23]. The presence of stored-product insects in packaged food may differ, according to the type of commodity, the insect species and the type of the packaging material [5]. Various plastic films have been tested for their ability to protect products from insect infestation. The most important plastic materials in use for food packaging are polyethylene, polypropylene and polyester [24]. Shukla et al., [25] reported that adults of saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.); red flour beetle, *Tribolium castaneum* (Herbst); and the cowpea weevil, *Callosobruchus maculatus* (F.), were able to penetrate polyethylene with less than 0.08 mm in thickness. Generally, most of the stored-product insects enter packaged food through already existing holes or openings [26]. Adler [27] noted that saw-toothed grain beetle adults do not chew the plastic in order to create holes, but enter from already existing holes. Moreover, the majority of the insects that existed in packaged rice and not usually able to chew holes in plastic

films were *S. oryzae* and *O. surinamensis* [28]. There are many developing methods for preventing package infestations. For example: the elimination of holes in packages may help to drastically reduce the infestation levels by *S. oryzae*. Insect-resistant packaging with suitable materials may prevent infestation. Hou et al. [29] successfully tested insect repellents that were applied to packages, while application of low-risk insecticides may also be effective [26]. The use of an odour barrier as an over-wrap, especially for boxed-products, has helped in drastically reducing infestations of *P. interpunctella* larvae (cited by [26]).

Based on this work, polyethylene is extruded as a thin web and coated by the essential oils: kaffir lime peel oil, lemon grass oil and citronella grass oil to extrusion coating film and formed into storage bags by unsealed. It may explain the presence of insects in packaged products before it was packaged, infestation of raw commodities or cross-infestation during processing. Lucas and Riudavets [30] noted that packaging of already infested product is common with milled rice. Because although the weevil population has high mortality during the conventional polishing process but some insect eggs may survive in the final product of rice.

### CONCLUSION

According to most of food products being lost to various pests during post-harvest storage, consumers demand more natural processed products with long shelf-life but without chemical preservatives. Lemon grass and kaffir lime peel demonstrated the high contact and fumigant toxicity to saw-toothed grain beetle. Essential oils from kaffir lime peel and lemon grass have acceptance as food ingredients and environmentally-friendly. The applying of these essential oils may be useful as a grain protectant with extrusion coating film against the stored product insects. However, more development of packaging material may be worthy of further investigation.

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