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Toxicity of Essential Oils to Stored Product Pest and Application to Extrusion Coating Film for Extend Rice Storage Life

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Abstract: The toxicity of essential oils and wood vinegar was tested on stored-product insects that infest rice. 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*, were extracted by stream distillation. Raw wood vinegar was purified by filtering. Toxicity to red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) was evaluated under laboratory conditions using topical application method (contact toxicity) and residual exposure method (fumigant toxicity). Overall, exposure to residual volatiles achieved higher mortality than droplet application. Kaffir lime peel achieved the highest mortality; 60% by topical application and 100% by residual exposure. A lower toxicity was found for lemon grass, citronella grass, eucalyptus and wood vinegar. Thereafter, kaffir lime peel oil extrusion coated onto plastic film that was used for storing packaged rice. Rice was stored in the bags for one month and pest infestations were quantified. The treatments showed no infestation of red flour beetle *T. castaneum Herbst*. Additional, the treatments showed significant protection against the 2 other stored product insects, including saw-toothed grain beetle, *Oryzaephilus surinamensis* L and rice weevil *Sitophilus oryzae* Linn. The results suggest that kaffir lime oil may be useful as a grain protectant against rice-infesting insects when used as a coating for bags made of extrusion film.

Keywords: Essential Oils, Extrusion Coating, Stored Product Insects, Rice Storage Life

1. Introduction

Red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), distributed worldwide, is the most destructive pest of stored products. It can readily infest grains damaged in the harvesting operation [1] and has a high reproductive potential [2]. Control relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, pest resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to users. 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 [3]. According to consumers demand more natural processed products with long shelf-life but without chemical preservatives [4]. Essential oils are used in the foodstuff packaging material [5] or as plant and crop protectants [6]. Edible films or coatings have provided an interesting and often essential complementary means for controlling the quality, stability of food products and extend shelf-life [7]; [8]. Many essential oils are known to have fumigant activity against eggs, larvae and adults of *Tribolium castaneum*, and

the toxicity progressively increased with increased exposure times and concentrations [9].

Lemon grass *Cymbopogon citratus* Stapf, Family Poaceae, is a widely used herb in tropical countries, especially in Southeast Asia. The essential oil of the plant is used in aromatherapy. It is claimed to have antidepressant, antioxidant, antiseptic, astringent, bactericidal, fungicidal, nervine and sedative properties. Some of the reported phyto-constituents are essential oils that contain citral α, citral β, nerol geraniol, citronellal, etc. [10, 11].

Citronella grass (*Cymbopogon nardus* Linn), Family Gramineae, is the source of commercial citronella oil. Citronella oil is very widely used as an aromatic constituent of perfumery and cosmetic products. The complete oil is used as an insect repellent for humans and pets and can be applied in soap, detergents, household insecticides and technical products. 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 (EPA 1993) [12].

Eucalyptus *Eucalyptus globulus* Labill, Family Myrtaceae, is native to Australia and has been introduced in the past few centuries to other parts of the world including India, Europe and South Africa. Eucalyptus essential oil extracted from eucalyptus leaves has most of the properties of a typical volatile oil, also having powerful medicinal properties. Eucalyptus' effectiveness respiratory applications overshadows its other uses. It is antibacterial, anti-viral, anti-fungal, and anti-inflammatory, which means it is useful for many ailments [13].

Pomelo *Citrus maxima* (Burm) Merr, Family Rutaceae, is an indigenous plant in tropical parts of Asia. The pulp is stated to possess a number of therapeutic properties as reported in ancient and medieval literature: an appetizer, antitoxic, cardiac stimulant, and stomach tonic [14].

Kaffir lime *Citrus hystrix*, family: 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 [15]. The essential oils have very complex natural mixtures that can contain 20 to 60 components. They have been used widely as bactericides, virucides, fungicides, antiparasites, and insecticides in various applications [16].

Wood vinegar is a byproduct from charcoal production. Raw wood vinegar has approximately 200 chemicals compounds, such as acetic acid, formaldehyde, ethyl-valerate, phenol, methanol, tar, and etc. [17]. It has been used for organic farming and showed high efficiency as an insect repellent and had the highest efficacy in reducing pest infestation on soybean [18].

This study was carried out to investigate the toxicity properties of essential oils from these species and wood vinegar against red flour beetle *T. castaneum* Herbst. The high efficiency of the essential oils was selected and applied to extrusion coating film for rice storage life.

2. Material and Method

2.1. Insect Preparation

Red flour beetles *Tribolium castaneum* Herbst were collected from rice storage silos in Phitsanulok province, Thailand, and were laboratory-reared with eggs laid on grain rice and hatched at room temperature. Two-day old adults of red flour beetle were selected and prepared for bioassay tests.

2.2. 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 using steam distillation. 500 g dried sample of each plant were placed in a distillation flask with water and heated to boiling point. 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 and was diluted for testing on the red flour beetle. Tween 80 was used for emulsion to stabilize the essential oils before testing.

2.3. 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 at 120-430°C. The outside air when passing through the chimney to produce pyroligneous liquor cooled the smoke from carbonization. The hot steam condensed into liquid known as raw wood vinegar was collected and was purified by filtering before use.

2.4. Insects Bioassay Test

Two methods were used in the experiment: 1) Topical Application assesses contact toxicity; a 0.5 µl droplet of the diluted essential oil at a ratio of 1:9 for each treatment was placed on the head area of the red flour beetle with a micro-applicator. The treated insects were moved to a cup with grain rice (10 insects /cup) for observation of mortality. 2) Residual Exposure assesses fumigant potential; the diluted essential oil at a ratio of 1:9 was sprayed on filter paper and placed on a petri dish. Red flour beetles were added after the treatment evaporated (10 insects / petri dish). Each treatment was carried out in 4 replicates. Mortality was observed 12, 24, 36, 48 and 72 hours after adding the beetles. When control mortality occurred on the experimental test, the mortality was corrected by Abbott's formula [19]:

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

2.5. 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 film. This film, at high temperature, is drawn down and coated by the selected essential oils (Figure 1). In this study, the high toxicity: kaffir lime oil, lemon grass oil, and citronella grass oil were selected for extrusion coating (water as control treatment) with 1% concentration. Only the kaffir lime oil was diluted in various concentration at 1%, 2%, 3% and 4%. The selected essential oils were 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 form bags (10 x 15 cm). One hundred grams of rice purchased from a supermarket was placed in each unsealed bag. Each concentration treatment was replicated three times. Infestation of stored products insects was observed after storage at room temperature for 1 month.

2.6. 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 $P< 0.05$.

3. Results and Discussion

3.1. Toxicity of Essential Oils on Red Flour Beetle Tribolium Castaneum Herbst

Table 1. *Toxicities of essential oils and wood vinegar on red flour beetle Tribolium castaneum Herbst by topical application.*

Treatment	% Mortality					
	12h	24h	36h	48h	72h	df
Control (water)	0	0	0	0	0^{c}	ns
Lemon grass	6.67	16.67	23.34	26.67	26.67^{ab}	ns
Citronella grass	0	16.67	20.0	20.0	20.0^{ab}	ns
Pomelo peel	0	3.33	6.66	6.66	6.66^{bc}	ns
Eucalyptus	3.33	6.66	13.33	16.66	16.66^{ab}	ns
Kaffir lime peel	16.67	26.67	43.34	60.01	60.01^{a}	*
Wood vinegar	0	6.67	6.67	10.0	10.0^{b}	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 red flour beetle Tribolium castaneum Herbst by residual exposure.*

Treatment	% Mortality					
	12h	24h	36h	48h	72h	df
Control (water)	0	0	0	0	0^{c}	ns
Lemon grass	30.0	46.67	56.67	56.57	56.67^{ab}	*
Citronella grass	23.33	33.33	33.33	33.33	33.33^{b}	ns
Pomelo peel	0	0	0	0	0^{c}	ns
Eucalyptus	3.33	3.33	3.33	3.33	3.33^{c}	ns
Kaffir lime peel	50.0	86.67	100	100	100^{a}	*
Wood vinegar	0	0	0	0	0^{c}	ns

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

In this study, five essential oils were tested against the adult red flour beetle *Tribolium castaneum* Herbst in the laboratory using two methods, topical application to test contact toxicity and residual exposure to test fumigant toxicity. In general, toxicity was higher when the essential oils were used as fumigants. The three test substances that showed the highest contact toxicity (oils of kaffir lime, lemon grass and citronella grass) also showed the highest fumigant toxicity. Kaffir lime essential oil stood out as the most effective fumigant, achieving 100% mortality after 36 hours. Table 1 and 2 showed the tested essential oils, kaffir lime oil produced the highest toxicity in red flour beetle, followed by lemon grass and citronella grass. Topical application of kaffir lime essential oil led to 60.0% mortality after 48 hours and residual exposure achieved 100% mortality within 36 hours. Additional, the wood vinegar and pomelo peel oil treatments were least effective in the topical application experiments and had no effect on mortality in the residual exposure experiments. Therefore, essential oils have been shown to have higher fumigation toxicity than contact toxicity against the red flour beetle, *T. castaneum* [20]. Fumigation is a successful method of eradicating stored product pests present in food products. A number of studies have shown the fumigation effects of volatile oils against *T. castaneum* [21]; [22]. A direct comparison of the potency of contact toxicities of the essential oils could not be made because different experimental methods were employed.

3.2. Application of the Selected Essential Oils on Extrusion Coating Film for Rice Storage Life

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 [5] or as plant and crop protectants [23]. However, many of essential oils uses have been lost with time [24], therefore this study the applications of essential oils for packaged consumer food products requires extrusion coating process, including the appropriated formulation of the essential oils. According to kaffir lime given extract produced high mortality in the residual exposure trials followed by lemon grass and citronella grass. Consequently, essential oil of kaffir lime was diluted in various concentration from 1% to 4%, meantime essential oil of lemon grass and citronella grass were tested only 1%. All formulation of the essential oils was selected as a film coating that was used to produce plastic rice storage bags. The process of extrusion coating film with the three essential oils for rice storage life as shown on figure 1. Bags were filled with rice and were left unsealed for 1 month. Infestation with the target insect, *Tribolium castaneum* Herbst and other stored product pests was assessed after one months of storage. All of the treatments included control showed on infestation by *Tribolium castaneum* Herbst but showed a small infestation with had significantly protection *Oryzaephilus surinamensis* L. and *Sitophilus oryzae* Linn. Presume that use of extrusion coating films with essential oils shows potential to protect rice from infestation of the stored insects. After one month, all of treatments showed no infestation by red flour beetle as shown on Table 3. 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 all of the treatments. The number of the 2 other stored product insects found on the coated bags was significantly less than the control ($P < 0.05$). The degree of infestation by saw-toothed grain x to different from the essential oils treatment. Saw-toothed grain beetle response to the coated citronella grass oil, meantime rice weevil tend to respond to the coated citronella grass oil.

The essential oils may be a potential grain protectant by killing various life stages of *T. castaneum* through contact and fumigant actions [25]. Essential oils were also reported that have potential activity for stored product pest control could be used as a multipurpose pest controlling agent [26]. However, many of essential oils uses have been lost with time [24], hence this study the applications of essential oils for packaged consumer food products requires extrusion coating process. Although, the present work revealed that 1-month exposure of stored rice to volatile essential oils can reduce pest infestations. The present study extended the use of essential oils by using them as coatings applied to extruded film that was then used to form rice storage bags.

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 [27]. Shukla et al [28] reported that adults of red flour beetle, *Tribolium castaneum* (Herbst) was 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 [28].

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.

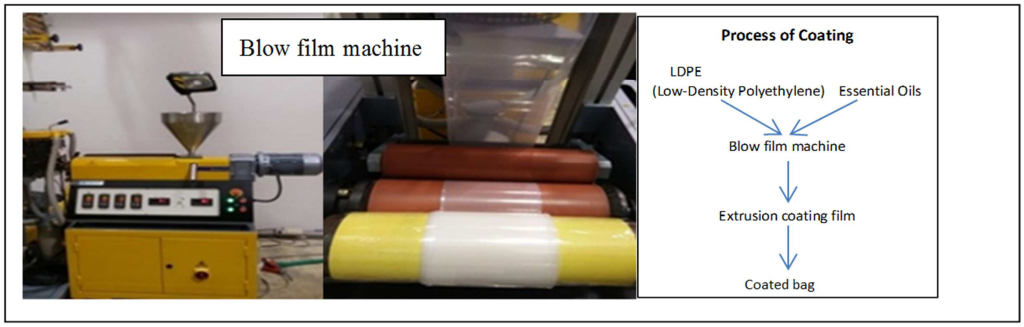

Figure 1. *Process of extrusion coating film with essential oils for rice storage life.*

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

Treatment	Infestation of stored product pests (number of insects)		
	***Tribolium castaneum* Herbst**	***Oryzaephilus surinamensis* L.**	***Sitophilus oryzae* Linn**
Control	2	36.0^{a}	15.0^{a}
Lemon Grass 1%	0	5.0^{b}	2.0^{b}
Citronella grass 1%	0	1.0	1.0
Kaffir lime 1%	0	9.0^{b}	5.0^{b}
Kaffir lime 2%	0	6.0^{b}	8.0^{b}
Kaffir lime 3%	0	5.0^{b}	5.0^{b}
Kaffir lime 4%	0	5.0^{b}	2.0^{b}
F-test	ns	*	*

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

4. Conclusion

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 as incorporated into the foodstuff packaging material and crop protectants. This work results that the kaffir lime, lemon grass and citronella grass may be useful as a grain protectant against these insects when used as a coating for bags made of extrusion film. This approach could ensure reduced pest levels in domestically-stored rice and extended shelf-life, using environmentally-friendly and aromatic essential oils such as kaffir lime peel oil and lemon grass oil that already have acceptance as food ingredients. The possibility of applying essential oils as plastic film coatings to control insects in domestically-stored products may be worthy of further investigation.

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