

# Effectiveness of insect-repellent food packaging film incorporating thymol against rice weevil, *Sitophilus oryzae*<sup>†</sup>

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**This study aimed at assessing the repellent efficacy of different levels of thymol (2%, 4% and 6% (w/w)) in rice packaging against rice weevil, *Sitophilus oryzae*. Insect repellent assays performed in the study showed 51.84%, 48.15% and 48.15% repellency effects for 2%, 4% and 6% thymol film respectively, within three days. Films containing over 2% thymol exhibited a mortality rate of *Sitophilus oryzae* ( $n > 40\%$ ), while the penetration of rice weevil specimens through the repellent film was prevented, demonstrating the potential for the usage of thymol in insect-repellent packaging for rice.**

**Keywords:** Essential oils, insect repellent, packaging film, rice weevil, thymol.

INSECT infestation is the main cause of food loss among packed foods, both in direct and indirect damage<sup>1</sup>. In spite of the expenses associated with repackaging food and covering the costs of returned products, the willingness of consumers to accept these changes, as well as the potential repercussions on the company's image and trustworthiness, could be quite significant. The usage of insect-repellent chemicals for food storage increases the risk of food contamination. Hence, safer insect-repellent methods are necessary to protect the food products.

Rice weevil, *Sitophilus oryzae*, is a major hazardous insect species found in cereal-based food. It could attack packaged food during production, distribution, transportation or storage<sup>2</sup>. Minimum inspection during production phase could cause *S. oryzae* eggs to remain in the raw grains and hatch and breed in the package. Adult *S. oryzae* are categorized as penetrator insects with well-developed mouthparts capable of damaging packaging materials. This not only allows more insects to penetrate inside but also exposes the packed food to the environment and causes food to become stale or spoiled. Therefore, it is necessary to repel and prevent the invasion of *S. oryzae* in packed food products.

Essential oils (EOs) are highly volatile compounds with lipophilic properties and strong odours. In addition to their antibacterial and antioxidant properties, EOs display insect-repellent properties<sup>2</sup>, which can be used as alternatives to synthetic pesticides. Thymol is an EO that displays insect-repellent properties against mosquitoes and is also commonly used for its antibacterial properties<sup>2</sup>. The inclusion of thymol plays a role in the advancement of active packaging.

Incorporating active compounds into a packaging material is known as active packaging<sup>3</sup>. An active packaging incorporating thymol was developed using low-density polyethylene (LDPE)-based film and ethylene vinyl acetate (EVA) as the binder. Since LDPE is inexpensive and widely available, it has been considered the raw material for incorporating thymol into insect-repellent active packaging films. LDPE is not only durable but also capable of incorporating additives to enhance the strength of the film.

Thus, to develop insect-repellent packaging films, we compounded LDPE-based films with varying concentrations of thymol to achieve the following: (i) evaluate the insect-repellency effect, (ii) determine the mortality rate and penetration ability of *S. oryzae*, and (iii) compare mechanical properties of the films.

## Materials and methods

### Insects

The adults of rice weevil with an average length of 3 mm were obtained from laboratory rearing at 27°C and 70% relative humidity on local rice<sup>4</sup>. The colonies were cultured on locally infested packed rice (brand Cap Rambutan).

### Materials

Thymol was selected as an insect repellent in food packaging based on a report by Chung *et al.*<sup>5</sup>. Thymol (>99%) was purchased from Merck, Darmstadt, Germany. LDPE having a 2 g/10 min melting index, was purchased from Lotte Chemical Titan, Malaysia. EVA was purchased from Dairen Chemical (M) Sdn Bhd, Malaysia.

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### Preparation of LDPE-based insect repellent packaging film

The preliminary test was conducted for repellent effectiveness in the bioassays described in the preference area assays. Repellent films were prepared as reported by Cran *et al.*<sup>6</sup> and Mistry<sup>7</sup>, with slight modification. Table 1 shows the formulations used to prepare the film. EVA was mixed with thymol and ground using a ball miller (4 l high-energy planetary ball mill, Deco, China) to obtain a uniform mixed powder. After drying in an oven to remove moisture, LDPE was mixed homogeneously before compounding using a twin-screw extruder (Werner & Pfleiderer, Cleveland, USA) with a screw diameter measuring 25 mm, screw die gap of 3 mm and screw speed at 60 rpm. The procedure was continued with air-dried cooling and pelletized at 300 rpm using a pelletizer machine (Scheer, Switzerland). The mixed batch was converted into films using a blown film mould machine (TK/HD/40M, Tai King Machinery Factory, Taiwan). The temperature of the extruder was set to 120°C, 125°C, 130°C, 140°C, 130°C and 120°C in six different heating zones respectively, with a motor speed of 600 rpm (ref. 8). The films produced were stored and wrapped tightly in aluminium foil to reduce the loss of volatile properties.

### Mechanical properties of the developed film

The tensile strength developed of the film was based on the method of Sung *et al.*<sup>9</sup> with slight modification. The film strip's dimensions for sizing are 12.7 mm in width and 100 mm in gage length, based on the ASTM standard method D882-02 (ASTM D882, 2002) and probe TA-DGA was used to clamp the film. The lower clamp was held static with respect to the machine, and the film was pulled apart by the upper clamp. The grip separation was set at 50 mm, with 500 mm/min crosshead speed and trigger load of 0.1 N. The tensile strength (TS) at break was estimated as follows:

$$TS \text{ (N/mm}^2\text{)} = \frac{\text{Force at break}}{\text{Initial cross-sectional area (}w \times t\text{)}},$$

where  $w$  is the width of the gauge and  $t$  is the thickness of the film.

**Table 1.** Formulations used to develop the insect-repellent packaging film

Formulation	LDPE	EVA	Thymol
0% Thymol	90	10	0
2% Thymol	88	10	2
4% Thymol	86	10	4
6% Thymol	84	10	6

Note: Values shown are % (w/w).

### Repellency and penetration assays

**Preference area assays:** The preliminary test was conducted to determine the range of thymol concentrations against *S. oryzae* to develop insect-repellent packaging film. The method was based on those of Licciardello *et al.*<sup>3</sup> and Tabari *et al.*<sup>10</sup>. Whatman filter paper (8.2 cm diameter) was divided into two parts: one half was treated with 0.3 ml of thymol dilution in dimethylsulfoxide (DMSO) 1%, and the other half was impregnated with 0.3 ml of DMSO 1% alone (control). The solvent was vaporized for 15 min, and the treated filter paper was placed in a glass petri plate (8.2 cm diameter) by coupling one half of the filter paper treated with thymol and one half of the control paper. Ten adult *S. oryzae* (1.0–4.0 mm length) were placed at the centre of each plate with five replicates for each concentration (2%, 4% and 6%). Insects were enumerated after 15, 30, 60 and 120 min, and the results were expressed as a percentage of repellency ( $R\%$ ), calculated as follows:

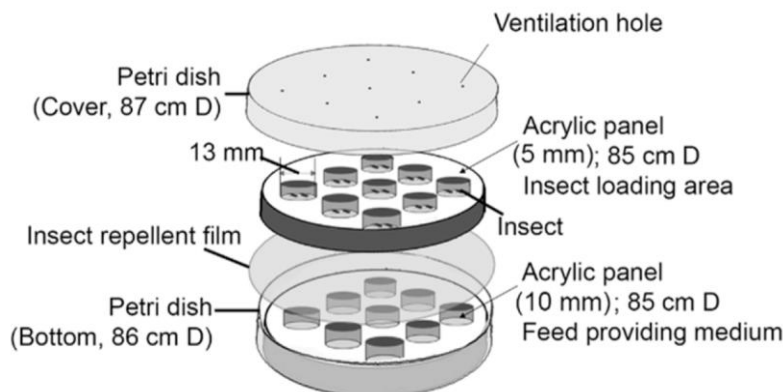
$$R\% = \frac{(C - T)}{(C + T)} \times 100,$$

where  $C$  is the number of *S. oryzae* adults on the control half and  $T$  is the number of *S. oryzae* adults on the treated half.

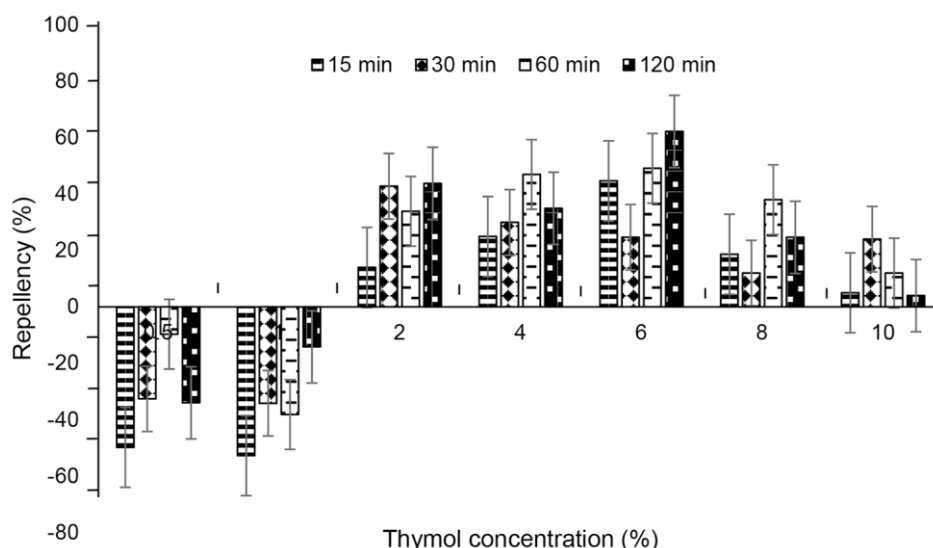
**Insect repellency and penetration assays:** The repellence test was conducted according to the method of Lee *et al.*<sup>11</sup> with some modifications. The testing process involved utilizing two stages of plastic petri dish (high-quality polystyrene material; Brandon, Malaysia) consisting of two round acrylic plates (10 and 5 mm thickness) with an 85 mm diameter dish. Nine pinholes were made in the cover for ventilation. Each acrylic plate had 9 wells, each 13 mm diameter (Figure 1).

The nine holes in the upper cyclic plate act as the insect-loading zone, the other nine in lower acrylic plate act as the feed-supplying zone, and the insect-repellent film was positioned between them. Rice (1 g; Cap Rambutan, Malaysia), a model food attractant, was placed in the holes of the lower acrylic plate. Five adult *S. oryzae* were inserted into each upper-plate hole for observation. All the Petri dishes were attached by transparent tape after placing a testing film between the acrylic plates in the petri dish. The penetration test system was then incubated at 29°C for three days. The number of insects that repelled and penetrated was recorded for each film every 12 h for 36 h. The penetration rate was calculated using the following equation.

$$\text{Penetration rate (\%)} = \frac{\text{Number of adults that penetrated into the feed supplying layer}}{\text{Initial number of larvae}} \times 100.$$



**Figure 1.** Schematic of the device for measuring penetration and repellent activities of *Sitophilus oryzae*. All the plates were attached using an adhesive tape after the insect-repellent film was inserted into the device.



**Figure 2.** Percentage repellency of *S. oryzae* at different concentrations of thymol for 15, 30, 60 and 120 min.

The penetration rate was calculated in triplicate for each concentration (2%, 4% and 6%).

### Statistical analysis

Data were analysed using analysis of variance (ANOVA) to determine the significant differences among the treatments. The means were compared using Tukey's comparison tests at a 5% confidence level of  $P < 0.05$ . IBM SPSS (version 21.0) was used for the statistical analysis.

## Results and discussion

### Preference area assays

Preliminary bioassays were carried out to determine the suitable range of thymol to be used in LDPE/EVA/thymol

insect-repellent packaging film. After 2 h of exposure to thymol on filter paper, most of the *S. oryzae* adults were found to group closely in one place, either in a controlled or treated area. Figure 2 shows the percentage of repellency within 120 min, revealing a close-response effect from 0.5% to 10% thymol concentration.

Based on the bar chart in Figure 2, 0.5% and 1% thymol showed a negative repellency effect to *S. oryzae* and the repellency effect was only observed after 30 min. In all specimens, there was no observable significance in the control film (with 0% thymol concentration) and the films with 0.5% and 1.0% thymol concentrations, whether in treated or non-treated areas. However, for plates containing thymol concentrations ranging from 2% to 6%, distinct preferences emerged across various time intervals. The treatment using 6% thymol concentration showed the highest repellency ( $P < 0.05$ ) compared to other concentrations. One hour after the trials, most of the insects in the thymol-added

**Table 2.** Mechanical strength of LDPE/EVA/thymol insect-repellent films incorporated with different concentrations of thymol (mean  $\pm$  SD,  $n = 4$ )

Type of film	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	Young's Modulus (MPa)
Plain LDPE	0.081 $\pm$ 0.001	15.23 $\pm$ 0.87	65.24 $\pm$ 0.02	21.21 $\pm$ 0.13
Control @ LDPE/EVA/0% thymol	0.088 $\pm$ 0.001	16.99 $\pm$ 1.47	70.31 $\pm$ 0.05	23.03 $\pm$ 2.06
LDPE/EVA/2% thymol	0.088 $\pm$ 0.001	14.72 $\pm$ 0.42	70.43 $\pm$ 0.82	20.97 $\pm$ 0.40
LDPE/EVA/4% thymol	0.087 $\pm$ 0.001	16.56 $\pm$ 0.78	70.90 $\pm$ 0.50	23.36 $\pm$ 1.10
LDPE/EVA/6% thymol	0.088 $\pm$ 0.001	13.12 $\pm$ 0.19	70.52 $\pm$ 1.04	18.61 $\pm$ 0.33

filter paper were found to group close to the sidewalls of the petri dish and under the filter paper. This result matches with the findings reported by Kim *et al.*<sup>12</sup> which indicated that *S. oryzae*, belonging to the Tenebrionidae family, exhibited a tendency to display a repellent effect by seeking refuge under storage mediums.

In a similar study, Youssefi *et al.*<sup>13</sup> reported that the usage of 0.25–5% thymol showed more than a 90% repellency effect on *Ixodes ricinus* larvae within 60 min. However, since repellency is towards the larvae and not the adult insects, the results are debatable. In another study, LDPE film incorporated with thymol was found to repel *Plodia interpunctella* larvae emerge from their hatches and breed within the test environment in a span of three weeks timeframe<sup>14</sup>.

Furthermore, our observations revealed that a greater thymol concentration proved lethal to adult *S. oryzae* when exposed to this condition for 60 min. The result agrees with Pandey *et al.*<sup>15</sup>, which reported that using thymol could not only repel but also kill *Anopheles phasehensi* specimens. In their study, higher concentrations of thymol were able to prevent the survival of the mosquito species by reducing the number of hatching eggs<sup>15</sup>.

### Mechanical properties

Table 2 shows the mechanical properties of the developed repellent films. The thickness of the control film was 0.088 mm; it was significantly ( $P < 0.05$ ) thinner than that of the repellent film. The tensile strength of the control film and the 2%, 4% and 6% thymol films were not significantly ( $P < 0.05$ ) different from each other. The addition of thymol did not influence the tensile strength of the films.

Table 2 shows the mechanical strength of LDPE/EVA/thymol insect-repellent packaging films with different percentages of thymol. The tensile strength of the LDPE film changes slightly with the addition of EVA. An increase in EVA is known to reduce the tensile strength of LDPE films since EVA has high interfacial adhesion incorporation with the LDPE polymer<sup>16</sup>. However, the slight tensile strength increment in this study could be due to the aggregation of the compatibilizer added into the LDPE polymer, which increases the tensile strength of the film. However, the tensile strength of the LDPE film did not change much with the incorporation of EVA when thymol was added. This could be due to

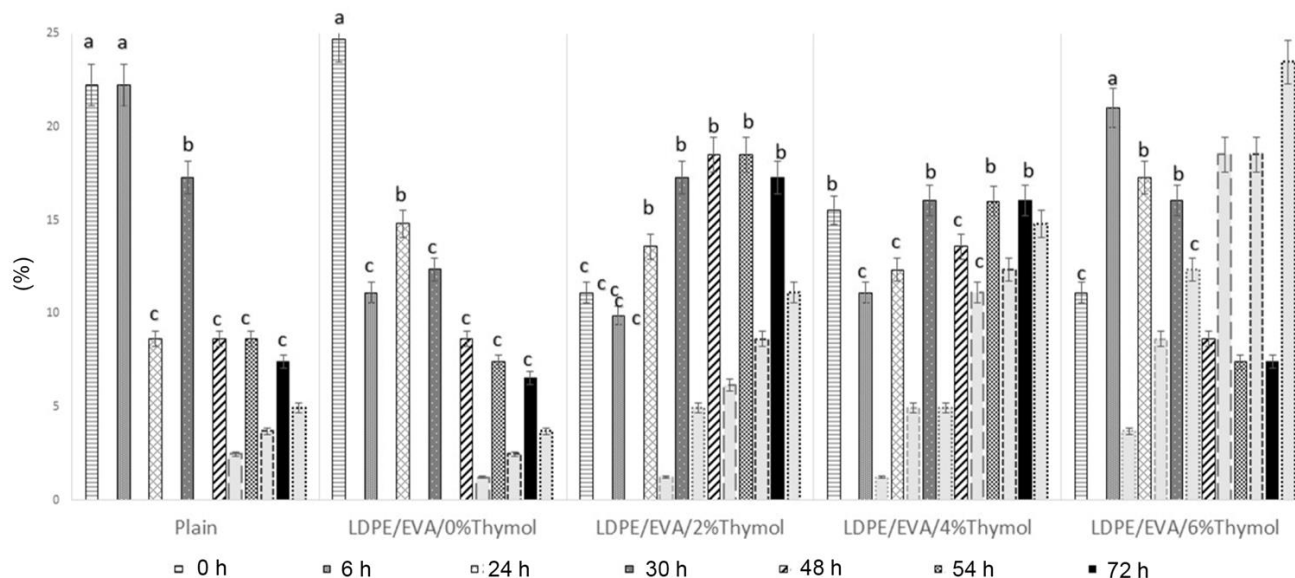
thymol's hygroscopicity effect, which reduces the aggregation between the EVA compatibilizer and LDPE polymer.

### Repellency and penetration assays

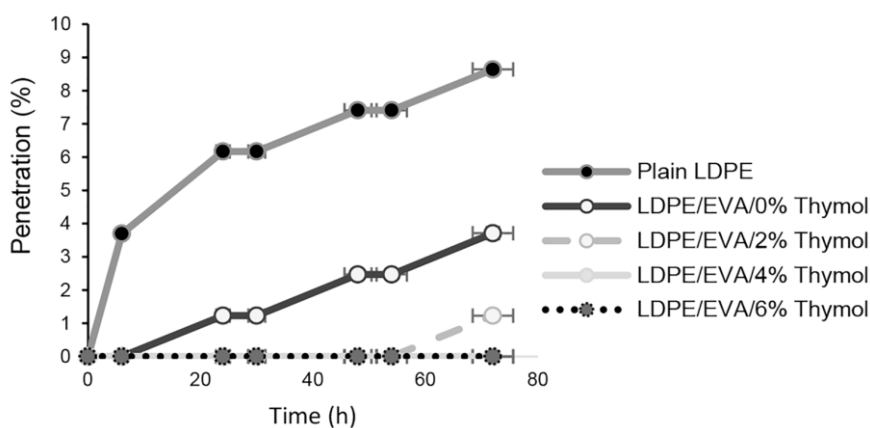
In general, the repelling effect increases from 0 to 48 h of treatment for LDPE/EVA/2% thymol film and subsequently decreases at 72 h storage (Figure 3). The decrease in the repellent percentage does not signify a loss of the insect-repelling capability. However, the percentage repellence reduces as the number of dead *S. oryzae* increases. Similarly, the effect of using LDPE/EVA/4% thymol film shows an increased repelling effect from 0 up to 30 h (15.52–16.05%), which is maintained until 72 h of storage. The percentage of dead *S. oryzae* using LDPE/EVA/4% thymol film shows a higher value (14.81) than LDPE/EVA/2% thymol film (11.11) on 72 h of storage. A different result was obtained using LDPE/EVA/6% thymol film, where the repelling effect decreased from 6 to 72 h of storage (20.99–7.41%). This result stands in contrast to the percentage of mortality rate of *S. oryzae*, which increases from 3.7% of dead *S. oryzae* at 6 h to 23.46% at 72 h of storage. The test indicates that using 6% thymol in the film could kill *S. oryzae* as early as 6 h of storage with no supplies, implying that a higher concentration of thymol used in a film could have a lethal effect on insects in a short time.

The response of *S. oryzae* was examined by applying different concentrations of thymol in LDPE-based films. To elucidate the insecticidal properties of food packaging films, cumulative penetration rates were measured for 72 h. Figure 4 shows the results. We found that plain LDPE and 0% thymol in LDPE films showed significant penetration, while additional thymol showed almost no penetration within 72 h of storage. In contrast, approximately 11% of *S. oryzae* had penetrated through the plain LDPE film after 54 h with no supplies, indicating a less inhibitory effect of the film. An average of 3% *S. oryzae* could penetrate through the LDPE/EVA/0% thymol film after 54 h of storage with no supplies.

The penetration decreased as EVA was added to the LDPE film. This could be because EVA contributes towards higher mechanical strength (16.99  $\pm$  1.47 MPa) compared to plain LDPE film (15.23  $\pm$  0.87 MPa) (Table 2). Hence, the penetration of *S. oryzae* through the film is reduced with higher tensile strength films. Compared to plain LDPE and



**Figure 3.** Comparison of insect-repelling effects of LDPE/EVA/thymol packaging films with different concentrations of thymol (0%, 2%, 4% and 6% w/w). The bold line bar (□) indicates the number of *S. oryzae* found on the wall of the test container or on the side of the acrylic plate surface, while the dashed line bar (▤) indicates the dead *S. oryzae* test for 72 h. Means labelled with different letters are significantly different in the number of *S. oryzae* found on the test film ( $P < 0.05$ ).



**Figure 4.** Comparison of insect penetration on LDPE/EVA/thymol insect-repellent packaging films with different concentrations of thymol (0%, 2%, 4% and 6% w/w). There is a significant difference in the average number of *S. oryzae* detected on the film for each hourly interval ( $P < 0.05$ ).

LDPE/EVA/0% thymol films, no remarkable insect penetration effect was seen in LDPE/EVA/2% thymol film until 72 h storage period (only 1.23% penetration of *S. oryzae*). Furthermore, a higher concentration of thymol, viz. LDPE/EVA/4% thymol film and LDPE/EVA/6% thymol film showed no penetration effect until 72 h of storage. This is expected, as a higher concentration of thymol has a lethal effect on *S. oryzae*, while the remaining specimens become passive until 72 h.

## Conclusion

LDPE/EVA/4% thymol film showed the highest tensile strength (16.56 MPa) compared to other concentrations of

thymol. Higher tensile strength increases the capability of the film to withstand physical forces, thus making them better at protecting food products during transportation. Based on the repelling and penetration study, we can conclude that even low amounts of thymol, as low as 2%, could kill *S. oryzae* (33.33%) with a high repellency percentage (51.84) in a period of up to 72 h. The penetration study was successfully performed using *S. oryzae* on plain LDPE and 0% thymol films, whereas only a penetration percentage of 1.23% managed to breach the LDPE/EVA/2% thymol film barrier. All the tests were carried out to determine the most suitable concentration of thymol in the films to form the desired insect-repellent food packaging. Based on the analysis, LDPE/EVA/4% thymol was determined

as the most suitable formulation in developing insect-repellent films for packaging in order to protect food products from repelling insects.

**Conflict of interest:** The authors declare that there is no conflict of interest.

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## Erratum

### Degradation behaviour, impact of post-harvest processing and dietary risk assessment of frequently detected pesticides in curry leaves

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[*Curr. Sci.*, 2023, **125**(4), 422–427]

Page 427, Reference 11 should read as Handa, S., Agnihotri, N. and Kulshrestha, G., Maximum residue limits of pesticides. In *Pesticide Residues Significance, Management and Analysis*, Research Periodicals and Book Publishing House, Houston, 1999, pp. 9–21.