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<p>(54) Title: INSECT REPELLING FOOD PACKAGING MATERIALS</p>		
<p>(57) Abstract</p> <p>An insect repelling food packaging material comprising a food packaging material treated with a non toxic to humans insect repelling substance or combination of substances and optionally containing in addition appropriate non toxic synergists and insect repellent promoters.</p>		

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## INSECT REPELLING FOOD PACKAGING MATERIALS

The present invention concerns improved packaging materials that are insect repellent, non-toxic to humans environmentally compatible and suitable for protecting food and the like from insect infestation.

## BACKGROUND OF THE INVENTION

During packaging, storage and distribution, food packages are often exposed to a variety of conditions that bring about insect infestation of the packaged food by penetration of insects through the packaging material. To prevent or repel such insect infestation, it would be preferable to render the packaging material impervious to insect penetration. This is preferable to treating the food itself, for a variety of reasons. If the packaging material itself is rendered impervious to insect penetration, less insect repellent material is required to create an effective barrier against insect penetration into the packaged food, when the insect repellents are concentrated in the packaging material, relative to when the food itself is treated directly to achieve insect repellency. Furthermore, it is clearly a distinct advantage to avoid treating the food itself directly with insect repellents and to minimize the amount of insect repellents that is likely to be absorbed by the food in order to achieve protection from infestation by insects.

It should be pointed out however that most of the common insecticides in use today, whether in agriculture or in domestic use, particularly the synthetic ones, are to a greater or lesser extent toxic to humans and animals, as well as hazardous or at least significantly detrimental to the environment. As a consequence, The search for naturally occurring substances has become an important approach in the development of ecologically sound and compatible strategies for plant protection. This strategy seems to be eminently suitable for adaptation in the food industry to facilitate the achievement of effective pest and insect control.

Many known plants produce various natural chemicals that protect them from insect attack. Extracts from such plants often are effective in controlling insects other than those that normally attack the said plant from which the extract was derived. The following is a list of various publications dealing with aspects of this subject.

Anonymous (1992). *Neem: A Tree for Solving Global Problems*. National Academy Press, National Research Council, pp.141, Washington, DC.

Anonymous (1989). JMP User's Guide. First Printing. SAS Institute Inc. pp.464, Cary N.C., USA.

Daniel, S. H. and Smith, R.H. (1990). The repellent effect of neem (*Azadirachta indica* A.Juss) oil and its residual efficacy against *Callosobruchus maculatus* (Coleoptera: Bruchidae) on cowpea. In *Proceedings of the 5th International Working Conference on Stored-Products Protection*, eds F. Fleurat-Lessard and P. Ducom, Vol. II, pp. 1589-1596. Bordeaux, France, 1992.

Highland, H.A. (1977). Chemical treatments and construction features used for insect resistance. *Package Development and Systems* 13(3)251-256.

Islam, B.N. (1986) Use of some extracts from Meliaceae and Annonaceae for control of Rice Hispa, *Diuraphis armigera*, and The Pulse Beetle, *Callosobruchus chinensis*. In *Proceedings of the 3th International Neem Conference*. eds H. Schmutterer and K.R.S. Ascher, pp.217-242. Nairobi, Kenya, 1986.

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- Schmutterer, H. (1988). Potential of azadirachtin-containing pesticides for integrated pest control in developing and industrialized countries. *Journal of Insect Physiology* **34**, 713-719.

Subramanyam, B. and D.W. Hagstrum. Resistance Measurement and Management.  
*In Integrated Management of Insects in Stored Products* ed.by Subramanyam  
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The relevant background information contained in these publications is incorporated herein, by reference.

Over the last 25 years, intensive and pioneering research has been conducted on various plant materials including neem and its derivatives, turmeric and the like, etc. As a consequence, the potential role of botanicals in the fields of antifeedants, repellents, toxicants and growth regulators has been established (Islam, 1986). Numerous plant substances have been isolated and tested on stored-product insects, and from among these, azadirachtin (extracted from neem) appears particularly promising as a potential stored-product protectant (Subramanyam and Hagstrum, 1996). Mixing neem extracts with other materials can boost their power. Among these so-called "promoters" are sesame oil, pyrethrins, and piperonyl butoxide (Anon., 1992).

It has also been an age-old practice in rural India to mix dried neem leaves and turmeric powder with stored grain or to place them among warm clothes to keep away insects (Jorwani and Srivastava, 1984; Koul, 1992). In some traditional storage practices, neem leaves are mixed with grain in storage for 3-6 months (Anon., 1992). Azadirachtin, alcoholic and aqueous extracts of neem seeds, and enriched formulations have revealed virtually no oral or dermal toxicity to mammals

according to all tests carried out so far. Neem flowers and leaves are even eaten as a vegetable in India, Burma and Thailand (Schmutterer, 1988). Certain neem products may even benefit human health. The seeds and leaves contain compounds with demonstrated antiseptic, anti viral, and anti fungal activity. There are also hints that neem has anti inflammatory, hypotensive, and anti-ulcer effects. Also, tests in Germany have proven that neem extracts prevent tooth decay, and neem is now used as the active ingredient in certain popular toothpastes in Germany and India (Anon., 1992).

Therefore, the lack of acute toxicity in laboratory animals (oral LD<sub>50</sub> in rats >5000 mg/kg) and lack of evidence for chronic effects in animals, combined with a long historical use of neem preparations in traditional medicine in India, should make any neem-based formulation highly acceptable as an alternative to the widely prevailing synthetic neurotoxin-type insecticides (Isman et al., 1990).

Turmeric, *Curcuma longa* L., is a tropical herb of the Zingiberaceae family indigenous to southern Asia. The aromatic yellow powder from its mature rhizomes was used in Asian countries for many centuries as a yellow vegetable dye for silks and cottons. It is still used in foods as a condiment, particularly as an essential ingredient of curry powder, in medicine as a stomachic, carminative, anthelmintic, laxative, and cure for liver ailment, and also as an ant repellent in India (Su et al., 1982). Jilani et al., (1988) have stated that turmeric oil not only repels *Tribolium castaneum* but also interfere with its normal reproduction and development.

It is an object of certain aspects of the present invention to provide improved insect repelling food packaging materials.



It is a further object of certain aspects of the present invention to provide insect repelling food packaging materials that are non toxic to humans and animals.

It is a further object of certain aspects of the present invention to provide improved insect repelling food packaging materials treated with insect repelling substances or combination of substances and optionally containing in addition appropriate non toxic synergists and insect repellent promoters.

It is a further object of certain aspects of the present invention to provide improved insect repelling food packaging materials treated with natural non-toxic insect repelling substances or combination of substances and optionally containing in addition appropriate non toxic synergists and insect repellent promoters.

It is yet a further object of certain aspects of the present invention to provide an improved method for protecting food from insect infestation by providing insect repelling food packaging materials treated with natural insect repelling substances or combination of such substances and optionally containing in addition appropriate non toxic synergists and insect repellent promoters.

#### SUMMARY OF THE INVENTION

Thus there is provided in accordance with a preferred embodiment of the present invention an insect repelling food packaging material comprising a food packaging material treated with a non toxic to humans insect repelling substance or combination of substances and optionally containing in addition appropriate non toxic synergists and insect repellent promoters.

Neem seed extract can be mentioned as one example of such a non toxic to humans insect repelling substance. Optimum range for treatment of packaging

material with neem seed extracts has been found to be between 0.3% to 7.0%, preferably between 5% to 6%.

Turmeric extract can be mentioned as another example of such a non toxic to humans insect repelling substance. Optimum range for treatment of packaging material with turmeric extracts has been found to be between 5% to 40%, preferably between 15% to 25%.

Other suitable natural substances and combinations of substances can be considered as additional examples of such non toxic to humans insect repelling substances in accordance with additional preferred embodiments of the present invention.

#### DETAILED DESCRIPTION OF THE INVENTION

Fuller details and aspects of the present invention will now be presented in the following experimental procedures and examples.

#### MATERIALS AND METHODS

##### Repellents

The investigated Neem extracts were: NeemAzal T/S (1% azadirachtin AI) obtained from Eid Parry (India) Ltd.; azadirachtin (30% purity obtained from Trifolio-M GmbH as a NeemAzal powder)(Germany); cold pressed neem oil from seeds obtained from The House of Mistry Co. (England).

**Extraction of turmeric:** Powdered turmeric rhizomes was extracted in a Soxhlet extractor with petroleum ether (boiling point 40 to 60 °C) for 4 h.

Piperonyl butoxide (PBO)(90% technical grade) from laboratory stock was used to determine possible synergistic effect on repellency. In addition, natural pyrethrum extracts (50%) obtained from Yavnin Yave Industrial (Israel) Ltd. were tested for comparison.

### **Insects**

The test insects were adults of the lesser grain borer, *Rhyzopertha dominica* F. and the red flour beetle, *Tribolium castaneum* (Herbst.). Both species were reared on a mixture of broken wheat and 5% yeast (by weight). Cultures were kept at 27°C and 65% R.H. For the penetration test, only *R. dominica* was used. Adults that emerged at five day intervals were separated from rearing jars and were then placed in pre-treatment jars containing approximately 100 g of media until they were 10-15 d old. For repellency tests, emerging adults of both species were separated from rearing cultures at two-week intervals and were then held in pre-treatment jars as above until they were 7-21 d old.

### **Repellency test**

The propensity of the tested extracts to repel insects (repellency test) was determined against *T. castaneum* and *R. dominica* adults using the method described by Laudani et al. (1955) and McDonald et al. (1970). Filter papers (Filtrak 3 HW)(10x20 cm) were treated with 4 ml of acetone solutions of the extracts at dosages of Turmeric at 800 µg/cm<sup>2</sup>, NeemAzal T:S at 50 µg/cm<sup>2</sup> (containing 1% A.I. of azadirachtin),

azadirachtin at 30% purity, neem oil at 800  $\mu\text{g}/\text{cm}^2$ , pyrethrum extract at 5  $\mu\text{g}/\text{cm}^2$  natural (50% A.I.), and the mixtures of all the above with piperonyl butoxide (PBO) at the dosage of 50  $\mu\text{g}/\text{cm}^2$ . Treated papers cut into 10x20 cm strips were kept for 4 days in insect rearing room. Each treated strip was attached lengthwise, edge to edge, to two untreated 5x20 cm strips, to which acetone alone had been similarly applied, by Scotch tape on the reverse side. Two glass rings, 2.5 cm high and 6.4 cm i.d., were then placed over the two matched papers so that the joined edges of the papers bisected the rings. Ten adults of each species, were then exposed separately on the test arenas inside each glass ring, and their numbers on the treated and untreated halves were recorded after one hour and after eight hours exposure on the first day, and then at 9 a.m and 4 p.m. each day for 5 consecutive days. All tests were run at 27°C and 65% R.H. Each test was repeated four times. The average of counts over each 5-day period was converted to percent repellency, as described by Laudani et al. (1955). The mean repellency was then assigned a repellency class using the following scale:

Percent repellency	Repellency class
<0.1	0
0.1-20	I
20.1-40	II
40.1-60	III
60.1-80	IV
80.1-100	V

### Penetration test

Office paper (80 g/m<sup>2</sup>)(110 µm thick) was chosen as the test material after preliminary tests revealed its low resistance to penetration by *R. dominica* adults. The paper was cut into 28 mm diameter discs which were treated with 100 µl acetone solution of NeemAzal T/S, and azadirachtin, at dosages of 31.25, 62.5, 125, 250 and 500 µg/cm<sup>2</sup> (A.I. azadirachtin), cold pressed neem oil from seeds at dosages of 160, 320, 640, 1280, and 2,560 µg/cm<sup>2</sup>, turmeric extract at dosages of 160, 320, 640, 1280, and 2,560 µg/cm<sup>2</sup>, and natural pyrethrum extract (50% purity) at 2.5, 5, 10, 20, 40, 80, and 160 µg/cm<sup>2</sup> (A.I.). Paper discs treated only with 100 µl acetone served as control. After the solutions were applied to the paper discs, the acetone solvent was allowed to evaporate in the fumigation hood and the discs were then held at 27°C until used for bioassay. Penetration tests were carried out at delays of 1, 15, 30, 45, 60, and 75 days after treatment, or for as long as the treatment remained effective.

The device used for the penetration test consisted of two identical open-ended glass cylinders (24 mm i.d., 28 mm o.d., 26 mm height) each with four notches spaced at equal distances around the outer rim. The impregnated office paper, and a piece of wire-mesh (US standard No. 25) were cut into 28 mm diameter discs and were placed together on top of one cylinder. Then the second cylinder was placed over the wire-mesh. The two cylinders were then pressed together and held in place with two rubber bands, secured by the notches in the cylinder rims, (Navarro *et al.*, 1998). Ten 10-15 d old *R. dominica* adults were placed inside the top cylinder and

were kept in the test devices for 24 and 48 h. All tests were run at 27 °C and 65% R.H., and each test was repeated five times. At the end of each exposure period, the discs were examined on a black surface under a binocular microscope at 15x magnification. The number of perforations appearing as black circles were counted, and a comparative analysis was performed using the Student's t test for residual effect and the differences between control and dosages applied were determined using Dunnet's test (Anon., 1989).

## RESULTS

### Repellency

Table 1 shows the percent repellency and repellency class assigned to the substances tested. Against *T.castaneum*, the most effective substances were NeemAzal T/S (repellency class IV) and turmeric extract (repellency class III and IV), while the least effective was azadirachtin (classes II and III).

Table 1. Average repellency of several neem extracts and pyrethrum alone or in combination with piperonyl butoxide (PBO) to *Rhyzopertha dominica* and *Tribolium castaneum* adults during 5 days exposure (Roman numbers in brackets indicate repellency class values).

Substance	Dosage ( $\mu\text{g}/\text{cm}^2$ )	<i>Tribolium castaneum</i>				<i>Rhyzopertha dominica</i>			
		without PBO	with PBO	without PBO	with PBO	without PBO	with PBO	without PBO	with PBO
Turmeric	800	51.6 (III)	62.4 (IV)	59.2 (III)	70.1 (IV)				
Neem Oil	800	50.1 (III)	57.8 (III)	55.2 (III)	51.4 (III)				
NeemAzal T/S	50	63.8 (IV)	68.1 (IV)	57.2 (III)	60.5 (IV)				
Azadirachtin	50	33.6 (II)	41.2 (III)	29.7 (II)	39.3 (II)				
Pyrethrum	5	51.2 (III)	67.3 (IV)	*	*				
Piperonyl butoxide	50		25.3 (II)		22.6 (II)				
Control	16.2	10.4 (I)		5.8 (I)					

\* Insects were moribund

Against *R. dominica*, NeemAzal T/S and turmeric extract provided the most effective repellency (class III and IV), but exposure to the pyrethrum extract caused insects to die. It can be seen from Table 1 that for both insect species, there was no synergistic effect of PBO on the repellency values of the treatments.

### Penetration

Tables 2 and 3 show a protective effect of up to 75 days provided by NeemAzal T/S at different dosages, against penetration by *R. dominica* over the two exposure periods of 24 and 48 h, respectively. Penetration through the paper discs was greatly reduced by all the dosages and for all the time delays after treatment. The residual effect of NeemAzal T/S extract for the confined exposure of 24 h period lasted until the end of the 75 day experimental period except for the dosage of 31.25  $\mu\text{g}/\text{cm}^2$ .

The residual effect for the confined exposure of 48 h showed that the higher dosages of the extracts gave a long lasting effect against penetration by insects (Table 3). Except for the 75 day time delay, all the dosages of NeemAzal T/S extract resulted in significantly lower penetrations than those of the control treatment for both exposure periods.



Table 2. Average number of perforations made by 10 *Rhyzopertha dominica* adults confined for 24 h on *Curcuma longa* petroleum ether extract treated and untreated papers .

Dosage ( $\mu\text{g}/\text{cm}^2$ )	Time delay after exposure (days)						
	1	15	30	45	60	75	
160	2.20 a a	0.80 a a	1.40 a a	2.00 a a	2.00 a a	1.60 a a	a a
320	0.20 b b	0.40 b b	1.80 a a	2.20 a a	1.80 a a	1.60 a a	a a
640	0.40 a b	1.00 a a	2.00 a a	1.40 a ab	1.20 a ab	2.40 a a	a a
1280	0.00 b a	0.00 b a	0.20 b a	0.40 a a	1.20 a a	1.40 a a	a a
2560	0.00 b a	0.00 b a	0.00 b a	0.00 b a	0.20 a ab	0.80 a b	a b
Control	1.30 a a	1.50 a a	2.10 a a	1.80 a a	1.90 a a	2.00 a a	a a

\* Values followed by the same letter within a column are not significantly different at the 5% level

\*\* Values followed by the same bold letter within a row are not significantly different at the 5% l

Table 3. Average number of perforations made by 10 *Rhyzopertha dominica* adults confined for 48 h on Neem Azal T/S treated, and untreated papers .

Dosage ( $\mu\text{g}/\text{cm}^2$ )	Time delay after exposure (days)						
	1	15	30	45	60	75	
31	0.0 <b>b*</b>	0.0 <b>b**</b>	0.2 <b>b</b>	0.4 <b>b</b>	1.0 <b>b</b>	3.6 <b>a</b>	<b>a</b>
63	0.2 <b>b</b>	0.0 <b>b</b>	0.0 <b>b</b>	0.4 <b>b</b>	0.6 <b>b</b>	1.2 <b>b</b>	<b>a</b>
125	0.0 <b>b</b>	0.2 <b>b</b>	0.0 <b>b</b>	0.2 <b>b</b>	0.8 <b>b</b>	1.4 <b>b</b>	<b>a</b>
250	0.0 <b>b</b>	0.0 <b>b</b>	0.0 <b>b</b>	0.4 <b>b</b>	0.2 <b>b</b>	0.6 <b>b</b>	<b>a</b>
500	0.0 <b>b</b>	0.0 <b>b</b>	0.0 <b>b</b>	0.0 <b>b</b>	0.0 <b>b</b>	0.2 <b>b</b>	<b>a</b>
Control	3.3 <b>a</b>	3.9 <b>a</b>	3.7 <b>a</b>	4.1 <b>a</b>	3.5 <b>a</b>	4.0 <b>a</b>	<b>a</b>

\* Values followed by the same letter within a column are not significantly different at the 5% level

\*\* Values followed by the same bold letter within a row are not significantly different at the 5% level

Table 4 shows the protective effect of Neem oil at different dosages against penetration by *R. dominica* for time delays of up to 30 days for the two exposure periods. The table shows that perforations through the paper discs were greatly reduced by the application of the neem oil at all dosage levels. The complete residual (no penetration by insects) effect of Neem oil for both the 24 h and 48 h exposure periods was only obtained at the highest dosage of 2,560  $\mu\text{g}/\text{cm}^2$  after a one day time-delay. However, the tests showed that for both exposure periods the two higher dosages gave significant protection against penetration, until the maximum time-delay examined in this experiment of 30 days. At the 24 h exposure period all the dosages of neem oil resulted in significantly lower penetration than that of control at 30 days after treatment.

Table 4. Average number of perforations made by 10 *Rhyzopertha dominica* adults confined for 24 and 48 h on Neem Oil treated and untreated papers .

Dosage ( $\mu\text{g}/\text{cm}^2$ )	Confined exposure for 24 h after time delay (days)			Confined exposure for 48 h after time delay (days)		
	1	15	30	1	15	30
160	1.0 a* a**	1.8 a a	1.0 b a	3.0 a b	6.0 a a	3.4 a b
320	0.6 a a	1.4 a a	0.8 b a	2.0 a a	2.4 a a	2.8 a a
640	0.8 a a	1.8 a a	1.2 b a	1.4 a a	3.2 a a	2.0 a a
1280	0.0 b a	0.6 a a	0.4 b a	0.8 b a	1.2 a a	1.0 b a
2560	0.0 b a	0.2 a a	0.2 b a	0.0 b a	0.4 b a	0.2 b a
Control	1.3 a b	1.5 a a	2.1 a a	3.3 a a	3.9 a a	3.7 a a

\* At each exposure time, values followed by the same letter within a column are not significantly different the 5% level

\*\* Values followed by the same bold letter within a row are not significantly different at the 5% level

Table 5 shows the effect of azadirachtin at different dosages against penetration by *R. dominica* over the two exposure times of 24 and 48 h. Although penetration was significantly reduced by azadirachtin especially at the confined exposure of 48 h, penetration was still apparent (Table 5).

Table 5. Average number of perforations made by 10 *Rhyzopertha dominica* adults confined on azadirachtin treated and untreated papers for 24 and 48 h, after 1 day of time delay.

Dosage ( $\mu\text{g}/\text{cm}^2$ )	24 h		48 h	
31	0.8	a*	1.2	b
63	0.2	b	0.8	b
125	0.4	a	1.0	b
250	0.2	b	1.2	b
500	0.0	b	0.8	b
Control	1.3	a	3.3	a

\*At each exposure time, values followed by the same letter within a column are not significantly different at the 5% level

Table 6 shows the protective effect of pyrethrum extract at different dosages against penetration by *R. dominica* after a one and fifteen day time delay, at the two exposure times. Results showed that pyrethrum reduced penetration at all the dosages except 2.5 and 5  $\mu\text{g}/\text{cm}^2$ , though high dosages caused insect mortality

Table 6. Average number of perforations made by 10 *Rhyzopertha dominica* adults confined on pyrethrum extract treated and untreated papers for 24 and 48 h.

Dosage ( $\mu\text{g}/\text{cm}^2$ )	Confined exposure for 24 h after time delay (days)			Confined exposure for 48 h after time delay (days)		
	1	15		1	15	
2.5	0.8 a*	<b>a**</b>	0.6 b a	2.4 a a	1.2 b a	a
5.0	0.6 b a		1.2 a a	1.0 b b	2.8 a a	
10.0	0.4 b a		0.6 b a	0.6 b a	0.4 b a	
20.0	0.4 b a		0.2 b a	1.6 a a	1.8 b a	
40.0	0.2 b a		0.2 b a	0.0 <sup>x</sup> b a	0.0 <sup>x</sup> b a	
80.0	0.0 <sup>x</sup> b a		0.0 <sup>x</sup> b a	0.0 <sup>x</sup> b a	0.0 <sup>x</sup> b a	
160.0	0.0 <sup>x</sup> b a		0.0 <sup>x</sup> b a	0.0 <sup>x</sup> b a	0.0 <sup>x</sup> b a	
Control	1.3 a a		1.5 a a	3.3 a a	3.9 a a	

\* At each exposure time, values followed by the same letter within a column are not significantly different at the 5% level

\*\* Values followed by the same bold letter within a row are not significantly different at the 5% level

<sup>x</sup> Insects were moribund

Table 7 shows a protective effect of up to 75 days provided by turmeric extract at different dosages, against penetration by *R. dominica* over the exposuperiod of 24 h. Penetration through the paper discs was reduced by higher dosages (1280  $\mu\text{g}/\text{cm}^2$  and 2560  $\mu\text{g}/\text{cm}^2$ ) for all the time delays after treatment. The residual effect of turmeric extract for the confined exposure of 24 h period was clear until the end of the 75 day experimental period at the highest dosage.

Table 7. Average number of perforations made by 10 *Rhyzopertha dominica* adults confined for 24 h on *Curcuma longa* petroleum ether extract treated and untreated papers.

Dosage ( $\mu\text{g}/\text{cm}^2$ )	Time delay after exposure (days)									
	1	15	30	45	60	75				
160	2.20	a a	0.80	a a	1.40	a a	2.00	a a	1.60	a
320	0.20	b b	0.40	b b	1.80	a a	2.20	a a	1.80	a a
640	0.40	a b	1.00	a	2.00	a a	1.40	a ab	1.20	a ab
1280	0.00	b a	0.00	b a	0.20	b a	0.40	a a	1.20	a a
2560	0.00	b a	0.00	b a	0.00	b a	0.00	b a	0.20	a ab
Control	1.30	a a	1.50	a a	2.10	a a	1.80	a a	1.90	a a

\* Values followed by the same letter within a column are not significantly different at the 5% level

\*\* Values followed by the same bold letter within a row are not significantly different at the 5% level

Table 8 shows a protective effect of up to 75 days provided by Turmeric extract at different dosages, against penetration by *R. dominica* over the exposure period of 48 h. At higher dosages (1280 µg/cm<sup>2</sup> and 2560 µg/cm<sup>2</sup>), penetration through the paper discs was reduced for all the time delays after treatment. The residual effect of turmeric extract for the confined exposure of 48 h period was clear until the end of the 75 day experimental period at the highest dosage.

Table 8. Average number of perforations made by 10 *Rhyzopertha dominica* adults confined for 48 h on *Curcuma longa* petrolium ether extract treated and untreated papers.

Dosage (µg/cm <sup>2</sup> )	Time delay after exposure (days)						
	1	15	30	45	60	75	
160	2.80 a b	2.40 a b	4.40 a b	2.40 a a	4.00 a b	4.80 a	a
320	3.20 a a	2.40 a a	2.80 a a	3.20 a a	3.40 a a	3.00 a	a
640	1.00 b b	1.40 b b	2.60 a a	3.20 a a	2.20 a a	4.20 a	a
1280	0.00 b b	0.00 b b	0.60 b b	2.00 b a	1.80 a a	2.40 a	a
2560	0.00 b a	0.00 b a	0.00 b a	0.00 b a	0.80 b a	0.80 b	b
Control	3.30 a a	3.90 a a	3.70 a a	4.10 a a	3.50 a a	4.00 a	a

\* Values followed by the same letter within a column are not significantly different at the 5% level

\*\* Values followed by the same bold letter within a row are not significantly different at the 5% level



## DISCUSSION

### Repellency

Of the substances tested turmeric extract and NeemAzal T/S gave the highest levels of repellency, while azadirachtin had a low repellent effect on both insect species. Also, in no case did the addition of the synergist PBO have a significant influence on the repellency effect. McDonald et al., 1970 reported that pyrethrins 5  $\mu\text{g}/\text{cm}^2$  gave 26.5 %, piperonyl butoxide (50  $\mu\text{g}/\text{cm}^2$ ) gave 27.4 %, and the mixture of them (as a Standard) gave 57.3 repellency for the first week of repellency tests. Jilani and Su (1983) reported that most of the extracts that they investigated had a lower value than repellency class III (40.1-60%) which is considered as the standard for a promising repellent. They found that neem produced a maximum average repellency of only class III and turmeric extract (with petroleum ether) produced a 92.6% repellency (class V) at a dosage of 680  $\mu\text{g}/\text{cm}^2$ . Neem seed petroleum-ether extract at a dosage of 680  $\mu\text{g}/\text{cm}^2$  gave a 81.5% repellency (class V), for the first week and at a dosage of 170  $\mu\text{g}/\text{cm}^2$  gave a 63% repellency (class IV) against *T. castaneum*. Jilani et al., (1988) reported that Turmeric oil extracted with n-hexane produced 93% repellency (class V) at a dosage of 800  $\mu\text{g}/\text{cm}^2$ . Where neem leaves are mixed with grain in traditional practice, the grain is usually held in storage for 3-6 months. Although the ingredients responsible for keeping out the stored-grain pests have not yet been identified, they seem to work well. In this context, repellency seems to be of primary importance (Anon, 1992). Jilani and Saxena (1990) reported that the repellency effect of neem oil (at 800  $\mu\text{g}/\text{cm}^2$ ) and Margosan (Commercial derivatives of neem at 200  $\mu\text{g}/\text{cm}^2$ ) which were the minimum dosages that the authors applied against *R. dominica*, were 77% and 64% respectively (repellency class IV for both). However, in our experiment, Neem oil at the same dosage gave

class III repellency. This may be explained by the findings of Isman et al. (1990) who reported that azadirachtin content varied widely between different neem oil samples. This would lead us to conclude that the specific effectiveness of any particular neem oil sample should be tested and confirmed before being applied to practice in accordance with the present invention. In this respect it should be noted that two of the twelve oils that they investigated did not contain detectable levels of azadirachtin (detection limit=50ppm), while the remaining oils contained from 188 to 4,026 ppm.

### **Penetration**

With respect to the neem extracts and derivatives, most promising results were obtained with NeemAzal T/S, for which the residual effect lasted for up to 75 days at the dosage of 500  $\mu\text{g}/\text{cm}^2$ . The results with neem oil were less effective, while for azadirachtin, results showed that it had a protective effect, but to a limited extent. We found that azadirachtin at a dosage of 500  $\mu\text{g}/\text{cm}^2$  gave no penetration for the 24 h exposure, but at the same dosage at 48 h it gave 0.80 penetrations/ device. Malik and Naqvi (1984), using the device described by Highland et al. (1970), found that for azadirachtin, treated with 0.75 ml of 1% azadirachtin in acetone solution on 7 cm diameter Whatman No. 44 filter paper, (equal to 19.5  $\mu\text{g}/\text{cm}^2$ ) gave no penetration for 24 h exposure, but for 48 h gave 0.25 penetrations/device. They found that penetration through control filter paper was 1.25 holes/disc for 24 h and 2.25 holes/disc for 48 h exposure. Using our penetration device, we found that penetration through the control was 1.30 holes/disc for 24 h and 3.30 holes/disc for 48 h exposure. Jilani and Su (1983) reported that the insects tended to make punctures which were not large enough for them to escape through during their 72-h

observation period. During our tests, however, many times punctures made by insects were large enough to enable them to escape.

At the 2% concentration, the differences between the extracts were not significant, but all the treatments had significantly fewer punctures than the untreated, even though no treatment gave zero penetration (Jilani and Su, 1983). Same reported that at the 1 or 0.5% concentrations, the lowest number of punctures was observed in the neem extract treatment and these were significantly different from those treated with turmeric, fenugreek extracts and untreated control. Jilani and Saxena (1990) reported that *R. dominica* adults made significantly fewer punctures on filter paper treated with their test substances as compared with control. In their experiment, using filter paper treated with Margosan, they found fewer punctures than for papers treated with turmeric oil, sweetflag oil, and neem oil. At their highest concentration of 1000  $\mu\text{g}/\text{cm}^2$  neem oil gave no punctures for 24 h, but it caused 0.3 punctures for 48 h exposure. Margosan at the same concentration gave no punctures for both 24 h and 48 h. These results are in accordance with our results that relate to a one day time delay (Tables 2, 3 and 4). On the other hand, there are no records in the literature on the long term residual effectiveness using the penetration test. In this context our results showed that the most promising material tested was NeemAzal T/S which gave complete protection at the highest concentration for up to 75 days for 24 h and 60 days for 48 h exposure. The 30 days residual effect of neem oil was evident only at the highest dosage of 2560  $\mu\text{g}/\text{cm}^2$  for the 24 and 48 h exposure tests where penetration was significantly lower than that of the control.

Koul (1987) reported that neem oil as an antifeedant was effective against *Spodoptera litura* larvae only at very high concentrations. Isman *et al.*, (1990) reported that azadirachtin content varied widely between neem oil samples. They showed that there is a clear trend in which bioactivity of neem oils is related to azadirachtin content, and also that bioactivity of azadirachtin is enhanced by the presence of the oil as carried out by comparing bioactivity of pure azadirachtin to oil spiked with azadirachtin using the *Peridroma* chronic growth bioassay. They concluded that the presence of other constituents in these oils synergise or activate azadirachtin.

Isman *et al.*, (1990) reported that advantages of neem preparations over pure azadirachtin include the presence of other potentially active constituents and the possibility that a botanical preparation may enhance the stability of azadirachtin and other active ingredients. Mordue and Blackwell (1993) reported that limonoid mixtures may be more effective than azadirachtin alone; also that neem oil itself has insecticidal properties unrelated to its azadirachtin content and that crude formulations may contain volatile repellent components.

The above data support our conclusion that NeemAzal T/S ( which contains azadirachtin, other related limonoids and neem oil) gives better results than both azadirachtin and neem oil in preventing penetration by insects. Our results showed that natural pyrethrum caused a reduction in penetration. At high dosages (40, 80 and 160  $\mu\text{g}/\text{cm}^2$ ) insects died, but at the lower dosages, penetration could be reduced for a short period.

We can therefore conclude that the residual effect of some neem extract can last for a long time at the high dosages in storage conditions where the degradation problem caused by sunlight is less of a concern. This is supported by the observations of other investigators. (Anon, 1992; Daniel and Smith, 1990; Makonjuola, 1989; Mordue and Blackwell, 1993). Although Malik and Naqvi (1984) regarded the activity of plant substances in preventing insect penetration as an antifeedant effect, the insects fail to penetrate because they are repelled by the substances, and not because of an antifeedant effect.

With turmeric extract, in the repellency tests, most promising results were obtained for which the repellency class were III and IV at the dosage of 800  $\mu\text{g}/\text{cm}^2$  for both insect species tested. The penetration results with turmeric extract showed that it had a protective effect in a dose dependent manner (Table 7 and 8). We found that turmeric extract at a dosage of 2560  $\mu\text{g}/\text{cm}^2$  gave no penetration for the 24 h and 48 h exposure up to 45 days. Jilani and Su (1983) reported that turmeric petroleum ether extract at all dosages applied reduced punctures significantly.

Jilani and Saxena (1990) reported that *R. dominica* adults made significantly fewer punctures on filter paper treated with their test substances as compared with control. In their experiment, using filter paper treated with turmeric oil at a dosage of 1000  $\mu\text{g}/\text{cm}^2$  they found 0.0 punctures for 24 h, but it caused 0.7 punctures for 48 h. These results are in accordance with our results that relate to a one day time delay (Tables 7 and 8). On the other hand, there are no records in the literature on the long term residual effectiveness using the penetration test. In this context our results showed that turmeric was a promising material which gave complete protection at

the high dosages (1280 and 2560  $\mu\text{g}/\text{cm}^2$ ) for up to 45 days for 24 h and 48 h exposure. At the same dosages after 60 and 75 days delay, extract reduced punctures significantly.

On the basis of the repellency and penetration test results, it has been shown that turmeric and neem extracts affect insect behaviour in that they caused insects not to penetrate barriers treated with them. This effect of repelling storage insects, has been utilized in accordance with the present invention to treat packaging materials so as to impart to them resistance to the penetration of insects. This in turn protects the packaged food from insect infestation in an effective, user safe and environmentally friendly manner.

While certain embodiments of the invention have been hereinbefore particularly described, it will be apparent to anyone skilled in the art that many modifications and variations may be made, that do not deviate from the main features or spirit of the invention. The invention is accordingly not to be construed as restricted to such embodiments, but rather to its concept, spirit and general scope.

**CLAIMS:**

1. An insect repelling food packaging material comprising a food packaging material treated with a non toxic to humans insect repelling substance or combination of substances and optionally containing in addition appropriate non toxic synergists and insect repellent promoters.
2. An insect repelling food packaging material as in claim 1 wherein the non-toxic to humans insect repelling substance is an extract of neem seeds.
3. An insect repelling food packaging material as in claim 2 wherein the extract of neem seeds is present in the food packaging material at a level of between 0.3% to 7.0% on a solvent free extract wt/wt basis.
4. An insect repelling food packaging material as in claim 2 wherein the extract of neem seeds is present in the food packaging material at a level of between 5% to 6% on a solvent free extract wt/wt basis.
5. An insect repelling food packaging material as in claim 1 wherein the non-toxic to humans insect repelling substance is an extract of turmeric.
6. An insect repelling food packaging material as in claim 5 wherein the extract of turmeric is present in the food packaging material at a level of between 5% to 40% on a solvent free extract wt/wt basis.
7. An insect repelling food packaging material as in claim 5 wherein the extract of turmeric is present in the food packaging material at a level of between 15% to 25% on a solvent free extract wt/wt basis.
8. A method of protecting food from insect infestation by wrapping it with insect repelling food packaging material as in claim 1.

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IL99/00354

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC(6) : A01N 25/00, 65/00; A61K 35/78, 39/385;  
 US CL : 424/195.1, 405  
 According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**  
 Minimum documentation searched (classification system followed by classification symbols):  
 U.S. : NONE

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
 NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 NONE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,556,562 A (LARSON) 03 December 1985, see entire document.	1-8

Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 04 OCTOBER 1999	Date of mailing of the international search report <b>17 NOV 1999</b>
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