

Acaricidal activity of two plant essential oils on the adult stage of the European house dust mite, *Dermatophagoides pteronyssinus* Trouessart (Acari: Pyroglyphidae)

Hussien A. Rezk and Gadelhak G. Gadelhak

Economic Entomology Dept. Faculty of Agriculture (Elshatby) Alexandria University,
Alexandria, Egypt.

ABSTRACT

The plant essential oils, thymol and eugenol, were tested for their acaricidal activity on the adult stage of the European house dust mite, *Dermatophagoides pteronyssinus* Trouessart (Acari: Pyroglyphidae). Five concentrations (25, 50, 100, 250 and 500 ppm) of each of the two essential plant oils were applied on 0.5 g house dust. Mortality was recorded five time intervals (5, 15, 30, 60 minutes and 12 hrs). Thymol was found to be the least effective after five minutes where the LC_{50} was about 168.3 ppm (confidence limits at $p = 0.05$ ranging 122.4 – 232.7 ppm). The highest mortality was reached after one hour with an LC_{50} as low as 19.4 ppm (11.01 – 33.6 ppm at $p = 0.05$). At five min interval, eugenol was found to be less effective with an LC_{50} about 294.5 ppm (confidence limits at $p = 0.05$ ranging 206.1 – 422.7 ppm). The highest mortality was reached after one hour where the LC_{50} was as low as 32.8 ppm (24.5 – 43.7 ppm at $p = 0.05$). A reduction in the LC_{50} values over time was noticed for the two compounds. The optimum concentration for thymol was found to be 100 ppm where it reached 96 % mortality after one hr and 100 % mortality after 12 hr. Whereas, the optimum concentration for eugenol was found to be 250 ppm where 96 % mortality after one hr and 100 % mortality after 12 hr were reached. For eugenol, the LC_{50} values seem to get a little higher indicating a less efficacy compared to thymol.

INTRODUCTION

House dust appears to be a complex mixture of organic and inorganic materials. It contains allergens causing asthmatic symptoms. Analysis of dust did not come about until 1964 when a group of investigators led by Voorhorst suggested that dust mites may be responsible for the dust allergen

(Voorhorst *et al.* 1964). Although many species of mites occur in the household, *Dermatophagoides spp.* (Pyroglyphidae) constitutes 70 – 90 % of the mite population in house dust (Bronswijk and Sinha 1971; Bronswijk 1973, 1978; Wharton 1976 and Rezk *et al.* 1996). The organic component of house dust is important to the mites that use these materials as a food resource (Tovey 1993; Phanuvich *et al.*, 1997). House dust mites are common inhabitants of homes all over the world (Bronswijk 1978). Abundance of house dust mites in homes in temperate regions exhibits a seasonal fluctuation, with its peak density occurring during the summer (June, July, August and September) and the lowest density occurring during winter and late heating season (February, March and April) (Lang and Mulla 1978). Many species of mites from family Pyroglyphidae are found in house dust. European house dust mite, *Dermatophagoides pteronyssinus* Trouessart, is considered as a major cause to respiratory allergy diseases (Wraith and Cunningham 1975; Cookson and Makoni 1978; Tovey *et al.* 1981). These mites live in bedding, carpets, stuffed furniture, old clothing and stuffed toys and feed on human skin scales. The bedroom is the most important, because the bedroom is where people usually spend 1/3 to 1/2 of their time. Dust mites are most common in humid climates. They don't survive when the humidity is below 50 %. If droppings of dust mites are inhaled or come in contact with the skin, they may cause allergy, asthma and/or eczema symptoms. The allergenic effects of dust mites can be reduced when populations are maintained below the level of 1000 mites per gram of house dust (Robinson 1996). If the density of *Dermatophagoides spp.* is retained below 100 / gram of dust, asthmatic attacks will be prevented (Peat 1995 and Stewart 1995). European house dust mite, *D. pteronyssinus*, can be reared in a wide variety of media, usually a mixture consisting of a protein source and yeast products (Wharton 1976).

Control of house dust mite may be achieved either physically by excluding the mites or the use of proper acaricides. Robinson (1996) suggested that a successful house dust mites program has several components: Suppression of dust mite populations, Removal and denaturalization of the allergens (dead mites and mite feces) in the environment and reduction in the household relative humidity. Physical control plays an important role in the control of house dust mites. Nishioka *et al.* (1998) found that the bedding encasement with the mite blocking fibers was effective for preventing atopic infants from being sensitized to house dust mites. Also, Adilah *et al.* (1997) found that frequent vacuum

cleaning over a short time significantly reduces house dust mite allergen levels in carpets. Washing by hot, cold water or dry clean and heating has been shown to kill most of mites (Anderson 1984; Bischoff *et al.* 1998; Furimizo and Natuhara 1989 and Wharton 1976). Biological control of house dust mites using predatory mites of the genus *Cheyletus* showed limited application, since these mites are large and easily seen, and they are also allergenic (Morris and Rimmer 2001). Chemical control using acaricides might not be recommended to be used inside the homes for their toxicity to humans (Mitchell *et al.* 1985). Green *et al.* (1989) reported that a chemical spray containing tannic acid with an acaricide was shown to be somewhat successful in reducing mites and allergens. Using benzyl benzoate and essential plant oil (eucalyptus oil) when washing fabrics reduced mites and allergens (McDonald and Tovey 1993; Tovey and McDonald 1997). In China, Ho *et al.*, 1998 studied the suppression of *Dermatophagoides farinae* by the wood of six trees and they found that essential oils of China fir and Japanese fir killed approximately half of tested mites. Plant products and extracts are currently receiving greater attention for acaricidal activity which may be useful for the control of mite populations (Calderon *et al.*, 1997 and Zaitoon 2001).

Therefore, the present work was planned to study the acaricidal activity of two natural products (Thymol and Eugenol) on the adult stage of the European house dust mite, *Dermatophagoides pteronyssinus*, under laboratory conditions.

MATERIALS AND METHODS

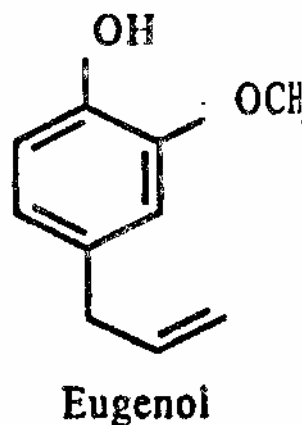
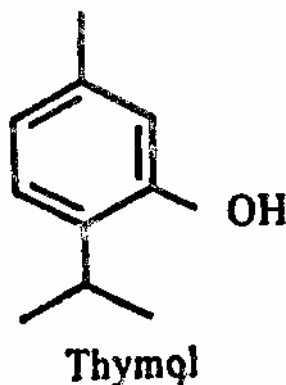
House dust mite rearing: European house dust mite, *Dermatophagoides pteronyssinus*, was isolated from mattress dust and reared on a finely-ground mixture of dust, dried yeast and dried milk (1 : 1 : 0.5) under complete darkness. Clean dried jar was used as a breeding container. One hundred newly emerged tritonymphal stages (males and females) of dust mites were introduced into a jar, filled with feeding media. The stock jars were kept in an incubator at an average constant temperature of 25 ± 2 °C and 80 ± 5 % RH. After five months (during a 22- wk period) huge numbers of different stages were available for the different experiments (Saint Georges 1987 and Andersen 1988 and 1991).

Plant essential oils: Plant essential oils were supplied by Ecosmart Technologies CO. (Protecting people and the plant with natural insecticides project) 318 Seaboard lane, Suite 202, Franklin, TN 37067, USA. These compounds are approved as common food and beverage additives and are generally recognized as safe for widespread use in the flavor and fragrance industries. These compounds are:

1-Thymol (98%): The scientific chemical name of this compound is (5-Methyl-2-isopropyl phenol). Extracted from Thyme, *Thymus vulgaris* L.

2-Eugenol (99%): The scientific chemical name of this compound is (2-Methoxy-4-(2-propenyl) phenol). Extracted from clove, *Eugenia aromatica*.

The chemical structures of these compounds are:



Experimental treatments: After preliminary experiments, five concentrations of 25, 50, 100, 250 and 500 ppm / 0.5g dust and a control were selected. Each concentration was mixed with 0.5 gram of house dust and placed on a microcell (3 cm. in diameter and 1.5 cm. in depth). Ten mites were introduced on the top of the microcell with the aid a fin brush. Experiments were replicated five times for each concentration. Mortality counts were done, 5 min, 15 min, 30 min, 60 min and 12 hr after exposure. The LC₅₀ values and their confidence limits (C.L.) were calculated according to Finney (1971).

RESULTS AND DISCUSSION

The effect of two natural essential plant oils, (Thymol and Eugenol) on the mortality of the European house dust mite, *Dermatophagoides pteronyssinus* Trouessart, was studied at five concentrations over different time intervals under laboratory conditions. After five minutes, thymol was found to be least effective at 25 ppm with a mortality reaching 12 %, where at 500 ppm the mortality reached 72 %. The LC_{50} for this time interval was about 168.3ppm (with confidence limits at $p = 0.05$ ranging 122.4 – 232.7 ppm). The highest mortality was reached after one hour where the 25 ppm gave 79 % and both 250 and 500 ppm concentrations reached 100 % with an LC_{50} as low as 19.4 ppm (11.01 – 33.6 ppm at $p = 0.05$) as indicated in Table (1) and Figure (1). Meanwhile, the best concentration was found to be 100 ppm where it reaches 96 % mortality after one hr and 100 % mortality after 12 hr. A considerable reduction in the LC_{50} values was noticed over time as shown in Figure (3).

As for eugenol, the values seem to get a little higher indicating a lesser effect compared to thymol. At five min. interval eugenol was found to be least effective at 25 ppm with a mortality reaching 4 %, where at 500 ppm the mortality reached 58 %. The LC_{50} for this time interval was about 294.5.ppm (with confidence limits at $p = 0.05$ ranging 206.1 – 422.7ppm). The highest mortality was reached after one hour where the 25 ppm gave 66 % and only the 500 ppm concentration reached 100 % with an LC_{50} as low as 32.8 ppm (24.5 – 43.7 ppm at $p = 0.05$) as shown in Table (1) and Figure (2). A similar reduction in the LC_{50} values over time was noticed as illustrated in Figure (3). The optimum concentration was found to be the 250 ppm where it reached 96 % mortality after one hr and 100 % mortality after 12 hrs. Compared to eugenol, the acaricidal activity of thymol was higher in the early time intervals up to one hour exposure. However after 12 hrs, both compounds seem to equilibrate (Table 1). The reduction of the eugenol activity when compared to thymol could be attributed to its chemical structure. The presence of a double bond in the aliphatic chain and a methoxy group on the phenol ring may have increased its hydrophilicity leading to a reduction in lipid penetration through the integument. Since the *D. pteronyssinus* belongs to suborder acaridida or astigmatid mites (Krantz,

Table (1): The LC₅₀ values and their confidence limits for two plant essential oils on the adult stag of the European house dust mites, *D. pteronyssinus*, at five time intervals.

Natural products	Time	Regression of N.E.D response (y), Log dose (x)	LC ₅₀	95 % C.L		Slope	P
				Lower	Upper		
Thymol	5 min.	y= -2.81+1.264x	168.26	122.36	232.66	1.26	0.48
	15 min.	y= -2.01+1.648x	83.077	57.52	119.68	1.05	0.86
	30 min.	y= -1.68+1.005x	47.335	29.79	74.52	1.61	0.85
	one hour	y= -1.73+1.341x	19.388	11.02	33.62	1.34	0.79
	12 hours	y= -2.31+1.909x	16.309	9.79	26.88	1.91	0.66
Eugenol	5 min.	y= -3.42+1.387x	294.44	206.08	422.69	1.39	0.50
	15 min.	y= -2.68+1.225x	155.64	112.79	215.27	1.23	0.85
	30 min.	y= -2.59+1.407x	62.729	46.46	84.45	1.41	0.49
	one hour	y= -2.98+1.963x	32.808	24.53	43.72	1.96	0.64
	12 hours	y= -3.29+2.414x	22.982	16.61	31.63	1.41	0.54

N.E.D. = Normal equivalent deviate

C.L. = Confidence limits

P. = Probability

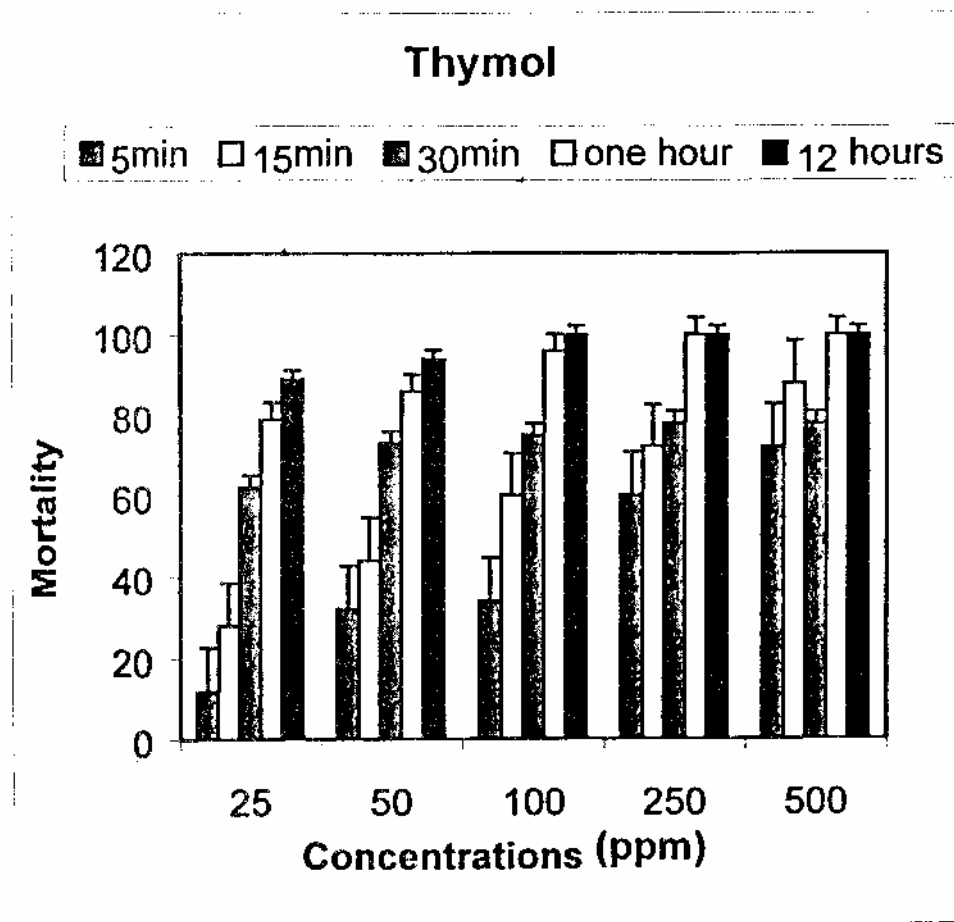


Figure (1) Percent mortality of the European house dust mite, *D. pteronyssinus*, when treated with different concentrations of the plant essential oil (Thymol) at five time intervals.

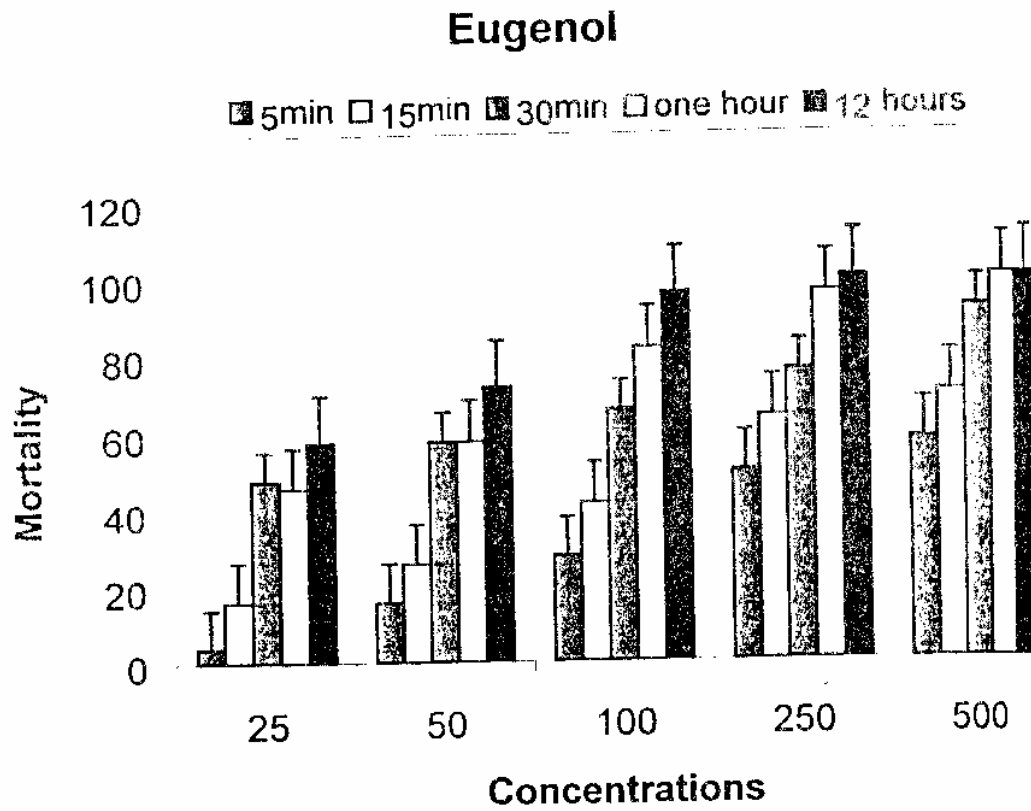


Figure (2) Percent mortality of the European house dust mite, *D. pteronyssinus*, when treated with different concentrations of plant essential oil (Eugenol) at five time intervals.

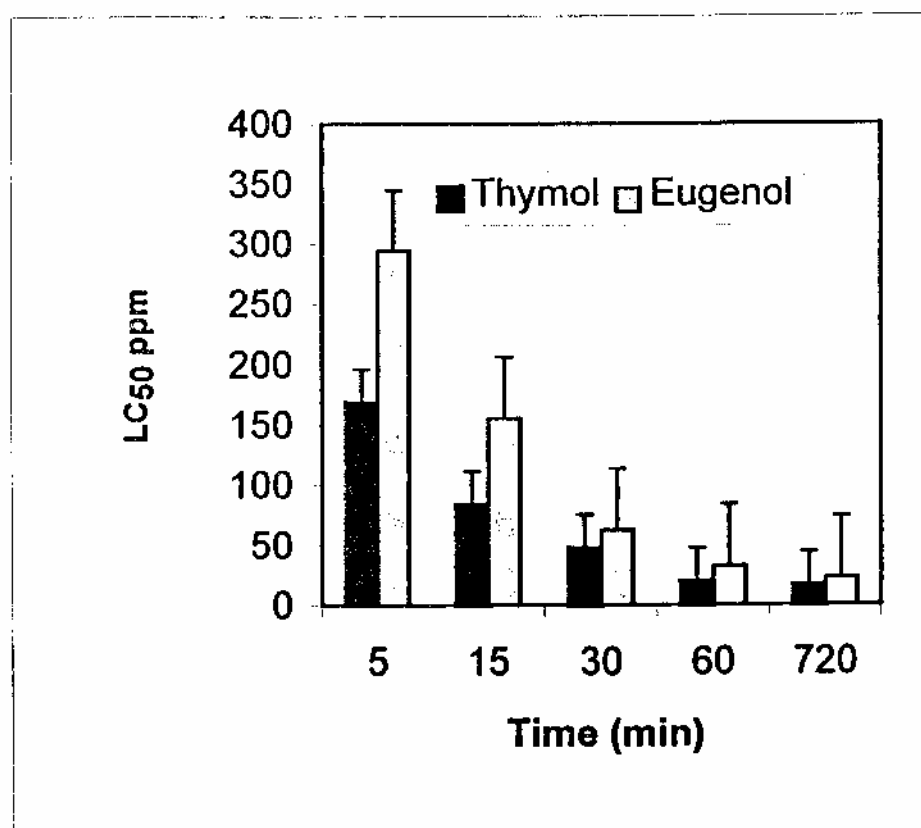


Figure (3) LC₅₀ values over time for two plant essential oils on the European house dust mites, *D. pteronyssinus*.

1978), spiracles are not present which means that the only route for any acaricide would be primarily through the integument in addition to feeding.

The application of plant essential oils have been mainly tried for their insecticidal activity (Jacobson 1983 and Xie *et al.*, 1995). Few authors recommended the use of these oils as wood and floor treatments to reduce

the population growth of house dust mites in human dwellings (McDonald and Tovey, 1993; Ho *et al* 1998). Colin, (1990) revealed that essential oils of thyme and sage were effective against parasitic bee mite, *Varroa jacobsoni*. In China, the essential oils of china fir and Japanese fir caused 50 % mortality when applied on the American house dust mite, *D. farinae* (Ho *et al*, 1998). Thymol is known to be biotoxic against aquatic snails (Mraston and Hostettmann, 1985 and Radwan, 2001). Also, El-Zemity (2001) found that thymol was the most effective compound against either *T. pisana* or *H. aspersa* snails followed by eugenol and pulegone.

In conclusion, the use of the plant essential oils (Thymol and Eugenol) as acaricides proved to be effective against the adult stage of the European house dust mite, *Dermatophagoides pteronyssinus*. Thymol is higher in the early time intervals. But after 12 hrs, both compounds seem to equilibrate. Their use is simple, inexpensive and environmentally acceptable and safe as an alternative for effective control of the mites. Other Helpful Suggestions: Remove carpeting from the bedroom. Use wood, leather or vinyl furniture instead of upholstered furniture in the bedroom. The person with a dust mite allergy should not vacuum or be in a room while it is being vacuumed. Keep the indoor moisture low (The ideal humidity level is 30 – 40 %). Further studies on house dust mites control is needed especially under household environment.

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النشاط الأبداى لنوعين من الزيوت النباتيه كمبيدات أكاروسيه على الطور الكامل
لحلم تراب المنازل الأوروبى *Dermatophagoides pteronyssinus*
(أكارى: بيروجليفيدى)

د/ حسين عبدالله رزق - د/ جاد الحق جابر جاد الحق
قسم الحشرات الأقتصاديه - كلية الزراعة (الشاطبي) - جامعة الإسكندريه

اجريت دراسه معمليه لمعرفة كفاءة نوعين من الزيوت النباتيه كمبيدات أكاروسيه ضد الطور الكامل لحلم تراب المنازل الأوروبى *Dermatophagoides pteronyssinus* كيديل لاستخدام المبيدات داخل المنازل وهذه الزيوت هى الليمون و الليمونول Thymol and Eugenol . وقد تم استخدام عدد خمسة تركيزات مختلفه لكل من نوعى الزيت وهى ٢٥ - ٥٠ - ١٠٠ - ٢٥٠ - ٥٠٠ جزء فى المليون بعد خلطها بنصف جرام تراب منازل وتم حساب النسبه المئويه للموت بعد ٥ - ١٥ - ٣٠ - ٦٠ دقيقه و ١٢ ساعه. أظهرت النتائج أن الليمون يعطى نتائج أفضل من الليمونول وذلك بعد المعامله مباشره حيث كانت قيم LC_{50} بعد ٥ دقائق من المعامله حوالى ١٦٨,٣ - ٢٩٤,٥ جزء فى المليون للليمون والليمونول على الترتيب. كما أوضحت النتائج ايضا أن أحسن تركيز يمكن استخدامه بالنسبه للليمون هو ١٠٠ جزء فى المليون حيث أعطى ٩٦% نسبة موت بعد ساعه من المعامله و ١٠٠% بعد ١٢ ساعه فى حين أعطى الليمونول نفس نسبة الموت بعد ١٢ ساعه من المعامله ولكن بتركيز ٢٥٠ جزء فى المليون.

ومن هذه النتائج يتضح أمكانيه استخدام هذه المركبات داخل المنازل لمكافحة الطور الكامل لحلم تراب المنازل الأوروبى حيث تتميز بالأمان الكامل للإنسان والبيئه ولكن يفضل إجراء مزيد من التجارب التطبيقيه داخل المنازل تحت ظروف البيئه الطبيعيه للحلم