Essential Oils as Fumigants for Bed Bugs (Hemiptera: Cimicidae)[1]

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Abstract Certain plant-derived essential oils are classified as 'minimum risk' pesticides that require no registration with the Environmental Protection Agency and, therefore, have become attractive in formulations of pest-control products. In Petri dish assays, fumigation of a pyrethroid-susceptible strain of bed bugs, *Cimex lectularius* L. (Hemiptera: Cimicidae), with various essential oils results in mortality that approaches or equals 100% after 5 d. However, when bed bugs were exposed to the same essential oils in sealed, commercial trash bags for 5 d, only rosemary oil killed greater than 99% of the bed bugs. These results are compared with a commercial product that contains cold-pressed neem oil that killed 100% of the exposed bed bugs in both the Petri dish and trash bag studies.

Key Words bed bug, *Cimex lectularius*, essential oils, fumigation

The bed bug, *Cimex lectularius* L., is a blood-sucking pest of humans that has proven difficult to control (Pinto et al. 2007). A variety of chemical and nonchemical means have been employed to control bed bugs (Doggett et al. 2012). Of the nonchemical methods, heat has been used successfully and has the advantage of being able to treat articles such as books and other household items that may not be amenable to a liquid spray (Kells and Goblirsch 2011, Peirera et al. 2009). Fumigants such as sulfuryl fluoride (Vikane®, Dow AgroSciences, Indianapolis, IN) are also effective against bed bugs (Miller and Fisher 2008, Phillips et al. 2014). However, the fumigant is a restricted-use product that is not available to the general public. Rather, sulfuryl fluoride must be obtained by a certified (licensed) pesticide applicator and applied by either a certified pesticide applicator or someone under the direct supervision of one. A relatively recent addition to the bed bug treatment market is Cirkil®, a neem-based liquid product (Terramera; Vancouver, BC) that can be sprayed (Grossman 2014) and also used as a fumigant in sealed trash bags to treat potentially infested items that are not amenable to liquid treatments (see http://terramera.com/rag). Because Cirkil is a neem-based product, it is available to the general public over-the-counter. In an unpublished study we found Cirkil, as well as

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several other chemicals including 70% ethanol:water, to be quite efficacious against bed bugs when used as fumigants in Petri dish assays. Others (Black et al. 2014) have shown that solvents vapors can be effective as fumigants to kill bed bugs, indicating this insect may be easier to control by fumigation, particularly in smaller spaces, than by other means. This led us to examine compounds, including essential oils, for their fumigation action on bed bugs. Many essential plant oils appear on the list of compounds that require no registration by the U.S. Environmental Protection Agency (USEPA 2014). Because these 'minimum risk pesticides' are not subject to federal registration requirements, they have become attractive as pest-control agents, though efficacy has oftentimes remained unproven (Hinson et al. 2014). In this current study, we examine the fumigation activity of several essential oils, as well as other chemicals, for their ability to kill bed bugs in confined spaces and compare their activity to a neem oil-based, commercially available product.

Materials and Methods

Insects. A colony of *C. lectularius* was established from bugs originally obtained from Harold Harlan (Crownsville, MD). The colony was kept at ambient conditions (25 $\pm$ 2°C and 40 $\pm$ 15% room humidity [RH]) and fed weekly on expired, human red blood cells and plasma using an artificial (*in vitro*) feeding system (Feldlaufer et al. 2010). These bed bugs, often referred to as the "Harlan strain," are considered susceptible to pyrethroid insecticides (Moore and Miller 2006). For the experiments described below, nymphs (3^{rd}–5^{th} instar) and adults of both sexes were used and had not been fed for 8 d.

Chemicals. Essential oils were obtained from Mountain Rose Herbs (Eugene, OR). Neem seed oil was obtained from Organix South (Bowling Green, FL). Cirkil RTU was obtained from an online source (http://www.ePestSolutions.com) and acetophenone (1-phenylethanone) was obtained from Sigma-Aldrich (St. Louis, MO). Acetophenone was included in the study because it has an odor similar to Cirkil RTU.

Fumigation of bed bugs in Petri dishes. Glass Petri dishes (94 $\times$ 20 mm), in which the bottom was separated into equal quarters by two perpendicular ridges (Felsen and Weil 1951), were used initially to determine the fumigation activity of test compounds against bed bugs. Pie-shaped pieces of filter paper (Whatman No. 1) were cut to quadrant size and placed in 3 of the 4 quadrants. Bed bug adults and nymphs ($n = 33$–77 per dish) were placed on the middle of the 3 quadrants while a cotton ball containing 0.3 ml of test compound was placed on the glass bottom of the opposite quadrant (Fig. 1). The Petri dish cover was put in place and sealed with Parafilm®M (Bemis Company, Inc., Oshkosh, WI). Dishes were kept at ambient conditions (25 $\pm$2°C and 40 $\pm$ 15% RH) and mortality was assessed at 120 h (5 d) posttreatment. Bed bugs were scored as dead, alive, or morbid–moribund (Feldlaufer et al. 2013). Two replicates were conducted. Because bed bugs would not come into direct contact with the treated cotton ball, we were able to use these Petri dishes to conduct an initial screening of compounds for fumigation activity.

Fumigation of bed bugs in trash bags. Commercial plastic trash bags (813 $\times$ 762 mm; 26.7-μm thickness; The Glad Co., Oakland, CA) designed for up to a 114-

Fig. 1. Petri dish experiments. Mock-up showing divided Petri dish used for initial screening of compounds for fumigation activity. Bed bugs were placed in one quadrant of the dish and test compounds were applied to cotton ball. The Petri dish was then sealed with parafilm. Mortality was assessed 5 d posttreatment.

L trash can were used in our larger fumigation study, similar to the methodology suggested for the use of Cirkil RTU (http://www.terramera.com/doc/RAG-IN-A-BAG-WhitePaper.pdf). Inside each bag were placed materials that represented items that could not be sprayed; specifically 2 boxes of Kimwipes® (121 × 121 mm each; Kimberly-Clark, Roswell, GA), a degaussed computer hard drive, a telephone handset, a CD "jewel" case, a newspaper, and a federal laboratory book (286 × 222 mm). Containers containing bed bugs were also placed within the bag (Fig. 2). These containers consisted of glass, wide-mouth, 240-mL canning jars (Kerr®, Jarden Home Brands, Daleville, IN) which had a circular piece of filter paper (55-mm diameter Whatman No. 1) on the bottom and contained three sheets of fan-folded filter paper (40 × 140 mm; Whatman No. 1) that acted as a harborage. To

Fig. 2. Trash bag experiments. Cutaway of the contents of a trash bag used in fumigation experiments. Mixed stages and sexes of bed bugs were placed in a wide-mouth canning jar with fan-folded filter paper as a harborage. The jar had a fine nylon (100-mesh) cover and was surrounded with representative items that could not receive a liquid treatment (see Materials and Methods for actual contents). Test compounds were applied to the top cloth and the bag was sealed with tape. The bag was opened 5 d posttreatment, at which time bed bug mortality was assessed.

each jar was added several hundred bed bugs ($n = 190$– 277) of mixed stages, and the jar was secured with a top made of fine (100-mesh) nylon attached to the inside of a cap band (Feldlaufer et al. 2014). By confining bed bugs to this container, we were able to account for all bed bugs in any given bag.

Test compounds consisted of essential oils (cinnamon, clove, geranium, lemongrass, peppermint, rosemary, and thyme) that exhibited greater than 95% mortality in the Petri dish experiments. Cirkil RTU and acetophenone were also included in the current experiment as they also exhibited high mortality in the Petri dish experiments. Neem oil was tested because it is listed as an ingredient of Cirkil RTU. Each test compound (30 mL) was poured onto a folded cotton towel (172 $\times$ 102 mm) that was on the flat surface provided by the laboratory notebook and separated by a WYPALL® X60 (Kimberly-Clark, Roswell, GA) wiper (180 $\times$ 165 mm). To prevent the treated compounds from contacting the notebook directly, a small sheet of plastic (356 $\times$ 280 mm) was placed between the notebook and the wiper. The arrangement of items in the bag was such that the jar containing the bed bugs was not in direct contact with the treatment (Fig. 2). A control bag contained all items, though the cotton towel was not treated (bagged control). Each bag was sealed with tape and kept for 5 d at ambient temperature and RH (25 $\pm$ 2°C and 40

Table 1. Bed bug mortality* when exposed to various compounds by fumigation in Petri dishes.

	Trial No. 1		Trial No. 2		
Compound**	No. Dead	No. Alive†	No. Dead	No. Alive	% Mortality ± SEM
Cirkil® RTU	59	0	37	0	100
Acetophenone	50	0	66	0	100
Cedarwood	0	48	0	61	0
Cinnamon	51	0	34	0	100
Citronella	41	10	32	1	86.9 ± 3.7
Clove	68	0	60	2	98.7 ± 1.0
Geranium	42	2	49	0	97.8 ± 1.5
Lemongrass	42	0	52	0	100
Neem	2	75	1	51	2.4 ± 1.4
Peppermint	40	0	48	0	100
Rosemary	42	0	36	0	100
Thyme	62	0	71	0	100
Control	0	67	0	53	0

* Mortality was assessed 5 d posttreatment.
** 0.3 mL of each compound was applied to cotton ball.
† All bed bugs scored alive were 'healthy' except the 5 bed bugs exposed to acetophenone; these were considered 'morbid' (see Feldlaufer et al. 2013).

± 15%, respectively). Because we did not know the permeability of the bags, and the room where the bags were kept had a faint odor of essential oils, we kept an additional jar of bed bugs in the room to see if they experienced any mortality (room control).

As in the Petri dish experiments, mortality in bed bugs used in 'trash bag' fumigation experiments was assessed 5 d posttreatment. Mortality was assessed as previously described (see Feldlaufer et al. 2013); any bed bugs that had not died were recorded as either "healthy" (i.e., ran quickly away when probed) or morbid–moribund.

Data analysis. In the Petri dish and trash bag studies, standard errors of the mean (SEM) were calculated and converted to percentages in those instances where neither 0% nor 100% mortality was observed. In these instances (0% and 100%) there is no variance.

Results

Fumigation of bed bugs in Petri dishes. The results of the Petri dish fumigation experiments are given in Table 1. Five essential oils (cinnamon,

Table 2. Bed bug mortality* (%) when exposed to various compounds by fumigation in trash bags.

Compound**	No. Dead	No. Alive†	Total	% Mortality ± SEM
Acetophenone	249	5	254	98.0 ± 0.9
Cirkil® RTU	218	0	218	100
Cinnamon oil	1	267	268	0.4 ± 0.4
Clove oil	0	219	219	0.0
Geranium oil	15	175	190	7.9 ± 2.0
Lemongrass oil	32	263	295	10.8 ± 1.8
Neem oil	2	253	255	0.8 ± 0.6
Peppermint oil	43	185	228	18.8 ± 2.6
Rosemary oil	218	1	219	99.5 ± 0.5
Thyme oil	85	192	277	30.7 ± 2.8
Bagged control	1	207	208	0.5 ± 0.5
Room control	0	225	225	0.0

* Mortality was assessed 5 d posttreatment.
** 30 mL of each compound was applied to a cotton cloth in each bag.
† All bed bugs scored alive were 'healthy' except the 5 bed bugs exposed to acetophenone; these were considered 'morbid' (see Feldlaufer et al. 2013).

lemongrass, peppermint, rosemary, and thyme), as well as acetophenone and Cirkil RTU, caused 100% mortality of bed bugs in both replicates after 5 d. Bed bugs exhibited greater than 97% mortality after 5 d when exposed to two other essential oils (clove oil and geranium oil). We observed less than 90% mortality of bed bugs exposed to citronella oil, and no mortality was observed in bed bugs exposed to the cedarwood oil we used in our study. Based on these results, citronella and cedarwood oils were not included in further testing. Bed bugs exposed to the neem oil we used in this study averaged only 2.4% mortality; no mortality was observed in the bed bugs confined to control dishes. In all dishes that contained living bed bugs, there were no signs of morbidity or moribundity in the bed bugs after the 5 d treatment; if alive, they were deemed "healthy" because they ran away from any probing stimulus provided.

Fumigation of bed bugs in trash bags. Bed bugs exhibited generally lower mortalities in the larger-scale fumigation experiments utilizing trash bags (Table 2) compared to the Petri dish experiments. In fact, in no instance did the mortality seen in the trash bag experiment exceed the mortality initially observed in the Petri dish experiments. The commercial product Cirkil RTU killed 100% of the bed bugs while acetophenone killed 98.0%. Of the seven essential oils tested, only rosemary oil killed greater than 99% of the bed bugs exposed to this vapor. The mortality observed in the other six essential oils tested in the trash bag experiment ranged from 0–30% mortality. Neem seed oil, likewise, did not perform as well in the trash bag experiment, killing less than 1% of the bed bugs exposed to this oil's vapors.

Discussion

Natural products such as pyrethrum have been used as both insecticides and as models for more-potent, synthetic pesticides (Gerwick and Sparks 2014). Other natural products such as extracts of seeds from the Indian neem tree, *Azadirachta indica* A. Juss, contain numerous bioactive compounds (Biswas et al. 2002, Bramachari 2004), though azadirachtin is believed to be the main active compound against insects (Isman 2006). However, azadirachtin content in neem seed oils from different ecotypes of the neem tree can vary by as much as 25-fold (Kumar and Parmar 1996), making it necessary to concentrate the active ingredient for commercial use. While Cirkil RTU was active in both the Petri dish and trash bag experiments, exhibiting 100% mortality in both, the neem seed oil we used in our study was not. The differences in azadirachtin content of different neem seed oils is a likely explanation for the disparity in our results comparing an organic neem seed oil with a commercial product containing cold-pressed neem oil. The high mortality rates we observed in bed bugs exposed to acetophenone, however, tends to complicate this interpretation. Acetophenone is used as both a fragrance and a food-flavoring agent (USEPA 2013), and while Cirkil RTU does not specifically list acetophenone as an ingredient, the commercial product has the odor of this compound. Essential oils, though not essential for the plant's life, are generally volatile, aromatic extracts that impart the characteristic odor or flavor of the plant from which it was obtained (see Isman 2006, Lubbe and Verpoorte 2011). Most plant essential oils are complex mixtures of many substances, each having different odors and chemical properties. Because of their perceived pleasant odor, essential oils are widely used in the fragrance and food industries, and several essential oils are exempt from registration as insecticides (USEPA 2014). Several 'natural-based' commercial products, including some that contain essential oils as ingredients, have been evaluated for contact toxicity against bed bugs with mixed results (Hinson et al. 2014). Those deemed effective, however, contained other ingredients, so it is not possible to determine which individual compound or combination of compounds is responsible for bed bug mortality. While plant essential oils have also been evaluated as fumigants, primarily against stored-product insects (Shaaya et al. 1991, Isman 2000), Choe (as reported in Grossman 2014), demonstrated that methyl salicylate (a component of wintergreen oil) and eugenol (a component of clove oil) were effective in killing bed bugs by fumigation.

While several essential oils were effective fumigants against bed bugs in Petri dishes, only rosemary oil proved effective in our larger-scale (trash bag) study. Rosemary oil is obtained from the shrub *Rosmarinus officinalis* L. and contains a variety of monoterpenes and terpene alcohols including α-pinene, eucalyptol (= 1,8-cineole), borneol, and linalool (Angioni et al. 2004, Bousbia et al. 2009). While these individual components could be tested for activity against bed bugs (separately), they would ultimately require registration with the USEPA as a pesticide because only rosemary oil (and not the individual constituents) is exempt from registration.

While both Petri dish and trash bag assays are technically laboratory assays, the trash bag assay was designed to more-closely simulate the usage of fumigant compounds under field conditions. As such, the differences in our results indicate that an initial screening of putative bed bug fumigants using Petri dishes may not be particularly useful. Many compounds that were effective in the Petri dish assay produced less mortality in the trash bag assay. Our results further emphasize the need for caution when interpreting results based solely on laboratory findings.

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