

Original Article

Evaluation of Some Plant Essential Oils against the Brown-Banded Cockroach, *Supella longipalpa* (Blattaria: Ectobiidae): A Mechanical Vector of Human Pathogens

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Abstract

Background: Essential oils, as secondary plant compounds, present a safer alternative to conventional insecticides in insect control programs. So five essential oils including eucalyptus, mint, yarrow, oregano and rosemary oils were evaluated against the brown-banded cockroach *Supella longipalpa*.

Methods: Evaluation was done against the 3rd and 4th instar nymphs using three bioassay methods; continuous contact toxicity, fumigant toxicity and repellent activity. The study was done in the laboratory of medical entomology, during April 2012 to September 2013.

Results: Mortality rates by the lowest concentration (2.5%) of rosemary, oregano, yarrow, eucalyptus and mint oils were 100%, 62.2%, 45 %, 36.2% and 5.2% at 24 h after exposure respectively. Rosemary oil was determined as the most toxic oil because of 100 % mortality rate at the concentration range of 2.5% to 30%. The lowest fumigation effect using 50 µl /L air was recorded from mint oil with 97.2 % mortality after 24 h, while the other oils caused 100% mortality. The most repel activity was related to oregano oil which showed 96.5–99.1% repellency at the concentration range of 2.5–30% with a residual effect lasting at least a week after treatment.

Conclusion: Oregano oil could be used as a potential repellent against *S. longipalpa*. Also, all five essential oils could be used as the safe compounds for surface treating or fumigation in cockroach control programs while rosmory and oregano oils exhibited the most toxicity.

Keywords: Essential oil, *Supella longipalpa*, Mechanical vector

Introduction

Natural contamination of cockroaches with wide range of pathogenic organisms including about 40 species of bacteria, nearly 12 species of pathogenic helminthes, the second largest group of vertebrate pathogens, and also viruses, protozoa and fungi affecting man and other vertebrate animals have been reported by numerous studies (Le Guyader et al. 1989, Rivault et al. 1994, Baumholtz et al. 1997, Cochran, 1999, Eggleston and Arruda 2001, Savoldelli and Luciano 2005). Often their movement between waste and food mate-

rials led to acquire, carry, and mechanically transfer of these pathogens. As proven or suspected carries, cockroaches play a prominent role in caring and distributing of organisms causing ddiarrhea, dysentery, cholera, leprosy, plague, typhoid fever, and viral diseases such as poliomyelitis are carries by cockroaches. They also carry the parasitic worms such as *Taenia*, *Shistosoma*, *Ascaris* and may cause allergic reactions, including dermatitis, itching, swelling of the eyelids, and more serious respiratory conditions (Stankus et al.

1990, Savoldelli and Luciano 2005). The brown-banded cockroach, *Supella longipalpa* known previously as *S. supellectilium* as a nearly cosmopolitan cockroach, has recently become a hygiene problem in the city of Ahvaz, southwestern Iran and it seems to be the dominant species of dwellings particularly in apartments (Vazirianzadeh et al. 2013, Sharififard et al. 2014). This is small cockroach measuring 10–14mm in length with pronotum of male is rather uniformly dark with lateral edges and definitely lacks of the two parallel stripes of *Blatella germanica*. The adult males appear to be very slender with its wing extending beyond the tip of the abdomen. Adult females have short wings which expose the considerable portion of their stout abdomen. The common name derives from presence of two dark-colored transverse bands on mesonotal and abdominal terga (Cochran 1999).

This cockroach species carries a variety of microorganisms (Le Guyader et al. 1989), and is a vector of pathogenic bacteria in urban environments (Rivault et al. 1994). It is also reported as an allergen source (Eggleston and Arruda 2001, Savoldelli and Luciano 2005). Twenty nine bacterial species were isolated from *S. longipalpa* caught in the hospital (Le Guyader et al. 1989). Its movement from one department to the others, inside the hospital increases potential bacterial contamination risks, for some of the species such as *Acinetobacter* and *Pseudomonas* are dangerous for some kind of patients (Le Guyader et al. 1989).

The cockroach infestations are common particularly in dwellings without proper ventilation in warm climates, hospitals, and restaurants and in business establishments with a relatively high ambient temperature and humidity (Schal et al. 1984, Le Guyader et al. 1989, Baumholtz et al. 1997, Phillips and Appel 2010). The brown-banded cockroach needs nearly much less relative humidity to complete its life cycle and spread the infesta-

tion. It may be the main reason for more distribution of this cockroach species in Ahwaz City, as a warm area, in recent years compared with same species, the German cockroach.

Conventional insecticides are used as main tool to control cockroach infestations but there are many concerns about the harmful side-effects of these chemical compounds. Also the insecticide use is restricted in places such food preparation areas, restaurants, storage buildings and apartments. These restrictions of chemical insecticide application increase demand for safer alternatives against cockroach infestations (Savoldellis and Suss 2005, Phillips and Appel 2010). Different level of resistance to many compounds of chemical insecticides including organochlorine, organophosphorus and carbamate insecticides have been documented in many field-collected strains of cockroaches from Iran. So, application of these insecticides should be stopped and replaced with other safer compounds (Nasirian et al. 2006, Nasirian 2010, Ladonni et al. 2013).

Essential oils, as secondary plant compounds responsible for the aromatic characteristics of plants, present the potential alternative to conventional insecticides (Isman 2000, 2006, Omara et al. 2013). Plant extracts and essential oils are reported to have a wide range of activity against insect and mite pests, plant pathogens, fungi and nematodes (Isman 2006). Recent reports have highlighted antimicrobial, antifungal, anti-cancer and insecticidal properties of plant essential oils (Isman 2000, 2006). They have fumigants, antifeedant and repellent effects as well as inhibiting the reproduction in cockroaches and other insects (Omara et al. 2013). They could be used in areas where chemical insecticides are prohibited. The repellent effect of essential oils has been reported against many insect pests such as cockroaches, termites, mosquitoes, ticks, ants and houseflies (Chen et al. 2002). Numerous

studies have demonstrated the toxicity and repellency of essential oils against cockroaches (Ahmad et al. 1995, Apple et al. 2001, Jang et al. 2005, Thavara et al. 2007, Ferrero et al. 2007, Ling et al. 2009, Tunza et al. 2009, Phillips et al. 2010, Phillips and Appel 2010, Zhu et al. 2012, Manzoor et al. 2012, Omara et al. 2013).

Consider to cockroaches role in transmission and distribution of many human pathogens, their resistance to many chemical insecticides, the side-effects of insecticide usage in human dwellings, high distribution of the brown-banded cockroach in Ahvaz city during recent years, and eventually in order to finding a safe alternative for chemical insecticides, the present study was done to evaluate toxicity and repellency of following five essential oils, Eucalyptus oil (*Eucalyptus* sp), Mint oil (*Mentha piprta*), Yarrow oil (*Achillea millefolium*), Oregano oil (*Origanum vulgare*) and Rosemary oil (*Rosmarinus officinalis*) against this cockroach species.

Material and Methods

Cockroach

The brown-banded cockroaches were reared in Plexiglas containers and maintained at 27 ± 2 °C, $50\pm 5\%$ RH, and the photoperiod of 12:12 (L: D) h. They were fed with dry crumbled biscuits, bread and water. Pieces of facial tissue were provided as a harborage and surface sticking of ootheca. Cockroaches were anesthetized by chilling to facilitate handling. The cockroach colony had been established at least 2–3 years before the study. The study was done in the laboratory of medical entomology, Department of Medical Entomology and Vector Control, Ahvaz Jundishapure University of Medical Sciences during April 2012 to September 2013.

Essential oils

Five essential oils including, *Eucalyptus* sp (Eucalyptus oil), *Mentha piprta* (Mint

oil), *Achillea millefolium* (Yarrow oil), *Origanum vulgare* (Oregano oil) and *Rosmarinus officinalis* (Rosemary oil) used in the tests were extracted from fresh or dried plant foliage by the hydrodistillation method using Clevenger apparatus in Herbal and Natural Product Research Center of Ahvaz Jundishapur University of Medical Science. Plant material was placed in a 2-liter round bottomed flask with distilled water (100 ml for 75g dry material and 400 ml for 200 g fresh material) and the essential oil was extracted by water distillation. The distillation period was 1 h for fresh samples and 1 h 15 min for dried samples (Charles and Simon 1990).

Bioassays

Contact Toxicity: The bioassay method of WHO (World Health Organization) (1975) was used to determine susceptibility or resistance of the cockroaches to essential oils. Essential oils were prepared in acetone as the solvent (v/v) at concentrations of 2.5%, 5%, 10%, 15% and 30%. Glass jars (600 ml) were treated uniformly with 2.5 ml of each concentration and left under room conditions to dry. The top inner surfaces of jars were smeared with a thin layer of butter to restrict cockroach movement within the jars. Groups of thirty 3rd and 4th nymph instars were anesthetized by chilling and transferred to plastic cups. After recovery, they were transferred to treated jars and left to continuous exposure to treated surface. Control group was exposed to surface treated with acetone. The mortality was calculated 24 h after recovering period.

Fumigant Toxicity: Groups including thirty 3rd and 4th nymphs instars were released in 1-lit glass jars with a 1-cm diameter cotton ball treated with 50 µl pure essential oil. To prevent direct contact of cockroaches with essential oil, it was injected to the center of each cotton ball by micro sampler. Control group was exposed to cotton ball treated

with acetone. The mortality was calculated 24 h after recovering period.

Repellency Test: The method applied that of Ferrero (2007) and Manzoor (2012) with some modifications. Circular white filter paper No 1 (15 cm diameter, Whatman) was divided in to two approximately equal pieces. Acetone was used as the solvent. One half was treated with 1 ml essential oil solution using a micro sampler and the other half was left untreated. The oils were assayed at the following 5 concentrations: 2.5, 5, 10, 15 and 30% (V/V). After solvent evaporation, filter paper was used to cover the floor of cylindrical Plexiglas jars. Thirty nymphs (3rd and 4th instars) were released into the center of each jar and distribution of the nymphs was calculated 24 h after exposure. For the control group, one half of the filter paper was treated with acetone and the other half was left untreated. All experiments were done with four replicates.

Data Analysis

Means of mortality percentages and standard errors in contact and fumigant toxicity were calculated using SPSS 16 software. Repellency values (RV) were determined using this formula:

$$\text{Repellency (\%)} = 100 - (T \times 100) / N$$

Where T stands for the number of cockroaches located in the treated area and N stands for the total number of cockroaches used (Thavara et al. 2007).

Analyses by ANOVA and comparison of mortality and repellency percentage means was done by Tukey's test ($P < 0.05$), using SPSS software (Chicago, IL, USA).

Results

Contact Toxicity

Concentrations of 30% and 15% of the five essential oils caused 100% mortality against the cockroach nymphs using contin-

uous exposure method. Mortality rates were 100%, 62.2%, 45%, 36.2% and 5.2% respectively at the concentration of 2.5% for the essential oils of rosemary, oregano, yarrow, eucalyptus and mint after 24 h (Table 1) which were significantly different ($P < 0.0001$). Furthermore, significantly difference was noted between effectiveness of the essential oils with comparison of the total means of mortality ($P < 0.0001$) against the brown-banded cockroach.

Rosemary oil was the most toxic oil against *S. longipalpa* because it caused 100% mortality of the cockroach nymphs at all concentrations (Table 1). The next most effective oils were oregano and eucalyptus oils because they killed 100% of the nymphs at the concentration range of 5–30%. At the concentration of 2.5% nymph mortality means were reduced to 62.2% and 36.2% with oregano and eucalyptus oils respectively which showed significantly difference ($P < 0.0001$). So, oregano oil was more effective than eucalyptus oil for the brown-banded cockroach (Table 1). Mortality means of the cockroach nymphs varied from 45% to 100% by yarrow oil which were significantly different ($P < 0.0001$). Mint oil caused 100% mortality at concentrations range of 10–30% but mortality reduced to 24.7% and 5.2% at the treatments of 5% and 2.5% respectively. So, mint oil was clearly determined as the least effective oil against the cockroach at lower concentrations compared to the other tested oils but its effect was similar to that of other oils at higher concentrations. All nymphs in the control group remained live 24 h after exposure, and even after a week.

Fumigant Toxicity

No mortality was observed in the control group during 24 h test period. Fumigation of all the five essential oils caused 97.2% to 100% mortality in the brown-banded cockroach nymphs at 24 h after exposure or even earlier. Mint oil exhibited lower fumigant ef-

fect compared to the other essential oils and it caused a mean mortality rate of 97.2% in nymph population that was not significantly different with the other oils ($P > 0.05$).

Repellency Effect

All tested essential oils showed high repellency against the brown-banded cockroach at the prepared concentrations (Table 2). Evaluations for repellent activity were different according to the essential oil and the concentration. The highest and the lowest repellent effects were recorded in oregano oil and eucalyptus oil respectively compared with the control group. Repellent effect of oregano oil at different concentrations ranged from 96.5 to 99.1% which were not significantly different together ($P > 0.05$), while the highest repellency (99.1%) was observed at the lower concentration of 2.5% (Table 2). In the repellency test with oregano oil, nearly all the released cockroaches were concentrated at the same place in untreated area and they were not close to the treated area even after a week.

Eucalyptus oil was determined as the least repellent compound, it caused 27.7–49.8% re-

pellency at different concentrations against the brown-banded cockroach compared to the other oils. Its repell effects were a little significantly different ($P < 0.05$) at different concentrations.

Mint oil caused 59.1–68.8% repellency against the brown-banded cockroach and that repellency of this oil at lower concentration (2.5%) was not significantly different with higher concentrations ($P > 0.05$).

Repellent effect of yarrow oil was varied from 79.3 to 92.8% that was not significantly different ($P > 0.6$). The most repellent activity was related to the concentration of 5% that was 92.8% but decreased to 79.3% at the concentration of 2.5%. While the difference between repellency at the concentration of 2.5% and 5% was not significantly ($P = 0.149$).

The repellent effect of rosemary oil was tested with only two concentrations. While the highest effect was recorded at the lower concentration of 2.5% (94.3%) but it was not differ significantly with the concentration of 5% (86.2%).

The difference was significant between the repellency of the essential oils with control group ($P < 0.0001$).

Table 1. Contact toxicity of essential oils against *Supella longipalpa* at different concentrations at 24 h after recovering period. (Department of Medical Entomology, Ahvaz Jundishapure University of Medical Sciences during April 2012 to September 2013)

Essential oil	Concentrations (%)	Mortality
	(acetone was used as solvent)	Means (%)±SE
Rosemary oil	30	100
	15	100
	10	100
	5	100
	2.5	100
Oregano oil	30	100
	15	100
	10	100
	5	100
	2.5	62.2±1.4
Eucalyptus oil	30	100
	15	100
	10	100
	5	100
	2.5	36.2±1.5

Table 1. Continued...

	30	100
	15	100
Yarrow oil	10	93±3
	5	79.9±3.3
	2.5	45±2.9
	30	100
	15	100
Mint oil	10	100
	5	24.7±4.1
	2.5	5.2±1.9
Control (treated with acetone)	100	0

Table 2. Repellency effects of plant essential oils against the brown-banded cockroach, *S. longipalpa* at 24 h after recovery period (Department of Medical Entomology, Ahvaz Jundishapure University of Medical Sciences during April 2012 to September 2013.

Essential oil	Concentrations (%) (acetone was used as solvent)	Repellency Means (%)±SE
	30	96.5± 3.5
	15	96.8 ± 3.8
<i>Origanum vulgare</i>	10	96.03± 2.4
	5	98.8± 1.2
	2.5	99.1± 0.9
	30	27.7± 3.9
	15	33.3 ± 3.1
<i>Eucalyptus sp</i>	10	43.2± 2.8
	5	51.7. ± 0.9
	2.5	49.8± 4.9
	30	63.3± 4.7
	15	60.1 ± 3.8
<i>Mentha piprita</i>	10	59.01± 2.8
	5	68.8± 3.2
	2.5	63.3± 3.7
	30	86.7 ± 4.9
	15	84.2± 3.5
<i>Achillea millefolium</i>	10	83.3± 4.5
	5	92.8± 1.7
	2.5	79.3± 3.9
	30	-
	15	-
<i>Rosmarinus officinalis</i>	10	-
	5	86.2± 4.3
	2.5	94.5± 0.87
Control (treated with acetone)	100	6.6± 2.3

Discussion

There are many studies on evaluation of essential oils against other species of cockroaches, but this is the first time to evaluate the efficacy of these components against the

brown-banded cockroach, *S. longipalpa*. The tested essential oils showed contact toxicity, fumigant toxicity and repellent activity against the brown-banded cockroach compared to

control group during this study. Results of continuous contact toxicity showed considerable effect at different concentrations, but yarrow oil was determined as the most toxic oil. It caused 100% mortality even at the lowest concentration of 2.5%. Comparison contact toxicity of rosemary, oregano, yarrow, eucalyptus and mint oils at the lowest concentration (2.5%) showed mortality rates of 100%, 62.2%, 45%, 36.2% and 5.2% respectively at 24 h after treatment. These mortality rates were significantly different together and with control group and confirmed that rosemary oil was the most toxic oil against the brown-banded cockroach nymphs.

The highest repellency was related to oregano oil and the most repellent activity was observed at lower concentrations of 2.5% that was 99.1% with a residual effect lasting at least a week after treatment. Oregano and rosemary oils were more efficient at the concentration of 2.5% with 99.1% and 94.5% repellency. Yarrow, mint and eucalyptus oils showed the most repellent effects at the concentration of 5% but the differences were not significant with the repell activity of 2.5% (*P* values were 0.15 and 0.97 and 0.99 for yarrow, mint and eucalyptus oils respectively). Repellency of these oils was 92.8%, 68.8% and 51.7% at the concentration of 5% and it was 79.3%, 63.3% and 49.8% at the concentration of 2.5%. So it could be concluded that the essential oil concentration of 2.5% is the favorite concentration to recommend for repellency against the brown-banded cockroach for further evaluation.

Many previous studies have evaluated the efficacy of different essential oils against cockroaches.

Evaluation of repellency and fumigant toxicity of clove (*Syzygium aromaticum*) and sesame (*Sesamum indicum*) oils against the American cockroach (*Periplaneta americana*) showed complete repellency (100%) against first nymph at concentration of 2% for clove oil and 6% for sesame oil. Same result was

obtained against fourth nymph at concentration of 10% of sesame oil after 48 h. While clove oil completely repelled all fourth nymphs after 24 h at concentration of 8%. For adult stage, the greatest repellency percentages were recorded by clove oil ($90.00 \pm 5.77\%$) and sesame oil ($83.33 \pm 3.33\%$) after 48 h at a concentration of 10% (Omara et al. 2013). The repellency value of clove oil at concentration of 8% against the fourth instar nymphs of the American cockroach is nearly close to our repellency value of oregano oil at concentration ranges of 2.5–10% which repel 96.03–99.1% of the brown-banded cockroach nymphs after 24 h and event lasted for a week. Both essential oils have considerable repellency against cockroaches but the cockroach species, essential oil type led to a little difference in the obtained results.

Toxicity and repellent evaluation of *Eucalyptus citriodora*, *Mentha arvensis* and *Cymbopogon citratus* against *P. americana*. *C. citratus* exhibited the maximum toxicity and repellency with 20% to 100% toxicity between 2 to 24 h intervals, 100% repellency and 70–100% fumigation after 24 h exposure. Minimum repellency ranged from 11–67% was observed from eucalyptus oil against *P. americana* (Manzoor et al. 2012). The minimum repellency against *S. longipalpa* was also recorded for eucalyptus oil in our study that ranged from 27.7–51.7%. Differences in the obtained result could be probably related to difference in cockroach species.

In another study, the essential oil of *Citrus hystrix* exhibited 100% repellency effect against *P. americana* and *Blattella germanica*, and also about 87.5% against *Neostylopyga rhombifolia* under laboratory conditions. The essential oil caused an 86% reduction in cockroaches with a residual effect lasting a week after treatment in the field (Thavara et al. 2007). These results are also similar to repellency values of oregano oil that continued for at least a week or even 10 days after treatment.

Besides, all five essential oils showed nearly complete fumigant toxicity (97.2–100%) against the brown-banded cockroach at the concentration of 50 µl per 1 lit air of pure oil. Apple et al. (2001) investigated that fumigant toxicity of mint oil at the concentration of 50 µl per 1 lit air of pure oil killed 100% of both *P. americana* and *B. germanica* after 24 h and caused 92.3–100% repellent effect on both cockroach species during each day of the 14-day experiment (Appel et al. 2001). Repel activity of mint oil ranged from 59.1–68.8% against *S. longipalpa* with the concentration of 2.5–30% and the fumigant toxicity was 97.2% at 24 h after exposure in our study. It seems that the fumigant toxicity of mint oil in the mentioned concentration is not significantly difference against the three cockroach species whereas the repellent activity shows considerable difference. This difference could be related to cockroach species or essential oil component.

Clove oil provided highly fumigant toxicity against nymphs and adults of *P. americana* after 24 and 48 h, respectively. Complete mortality (100%) was recorded at a concentration of 7.5 µl/L of air for first nymph, 10 µl for fourth one and 17.5 µl for adults after 48 h of fumigation (Omara et al. 2013).

In the another study, the fumigant toxicity of 12 essential oil components including: carvacrol, 1,8-cineole, trans-cinnamaldehyde, citronellol, eugenol, geraniol, S-(-)-limonene, (-)-linalool, (-)-menthone, (+)-alpha-pinene, (-)-beta-pinene, and thymol was determined against adult male, adult female, gravid female, and large, medium, and small nymphs of the German cockroach, *B. germanica*. 1,8-Cineole was the most toxic essential oil component to adult males and females, gravid females, and large nymphs, with LC₅₀ values of 6.8, 8.4, 5.3, and 11.4 mg/liter air at 24 h, respectively (Phillips and Appel 2010).

Evaluation of fumigant toxicity of some plant essential oils by Tunza et al. determined

that Allyl isothiocyanate (monoterpen oil component) was the most toxic compound, followed by essential oil of *Allium sativum* against *B. germanica* (Tunza et al. 2009).

While all five essential oils exhibited complete fumigant toxicity against *S. longipalpa* which is comparable with the findings of other studies but it is necessary to test lower concentrations.

The findings of current study are confirmed by all the mentioned studies in that essential oils can be favorite alternative to conventional chemical insecticides to control cockroach infestations especially in situations where the use of chemical insecticides has many harmful side effects. Differences in the obtained results could be probably related to difference in cockroach species, type of essential oil, bioassay method and exposure time.

Plant essential oils offer the potential repellent agents to cockroach infestations in hidden and hard-to-reach areas and to eliminate such infestations (Steltenkamp et al. 1992, Ngoh et al. 1998). This strategy would increase the efficacy of non-repellent, insecticide treated areas because cockroaches in the hidden areas will be get out and they will be exposed to such treated areas and finally killed (Steltenkamp et al. 1992). Besides, essential oils could be applied as a safe treatment and used to treat surfaces for food preparation in order to deter cockroach infestations (Steltenkamp et al. 1992). Essential oil applications may also be effective around cockroach infested places that cannot be treated with chemical insecticides, such as food stores, sensitive equipment, clothes lockers and in beds (Koehler et al. 1995). The repellent effects of essential oils could be applied as a flushing agent during inspections of cockroach infestation in order to determine the degree of an infestation.

Conclusion

Given that *S. longipalpa* can be found in

all parts of the buildings such as clothes lockers, beds, furniture and cabinets and that ootheca stick in hard-to-reach or obscure places, application of chemical insecticide spraying in such places is hard and unacceptable by the people. However, the use of essential oils presents a safe alternative for indoor application against cockroaches because of less toxicity to humans and no toxic residues. Oregano oil showed considerable repellent effect against *S. longipalpa*, so, it could be recommended as a potential repellent compound for further evaluation on a larger scale and under field conditions. Contact and fumigant toxicity of the five selected essential oils were considerable but additional study is required for the practical application and developing favorable formulation of them against the brown-banded cockroach and also other cockroach's species.

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