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Idin Zibaee
Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

Pooya Bahari Khorram
Graduated student in Department of Medicine, Guilan University of Medical Sciences, Rasht, Iran.

Majid Hamoni
Head of Technical Service Guilan Science and Technology Park, Rasht, Iran.

Correspondence
Idin Zibaee
Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

Evaluation of repellent activity of two essential oils and their mixed formulation against cockroaches (Dictyoptera: Blattidae, Blattellidae) in Iran

Idin Zibaee, Pooya Bahari Khorram and Majid Hamoni

Abstract
The repellency of two essential oils extracted from the plant species *Eucalyptus globulus*, *Rosmarinus officinalis* and Naphthalene as a control, were evaluated for repellent activity against the three cockroach species *Periplaneta americana* (L.), *Blattella germanica* (L.) and *Supella longipalpa* under laboratory conditions. The mixture of two essential oil showed the best repellency over single essential oils (95%, 100% and 100% for *P. americana*, *B. germanica* and *S. longipalpa* respectively). The mixed formulation of essential oils exhibited complete repellency (100%) against *longipalpa* and *B. germanica*, and also showed the highest repellency (among the essential oils tested) of about 95% against *P. americana* under laboratory conditions. In the field, two essential oils as mixed formulation with 10% active ingredient in water and some other additives, provided satisfactory repellency of up to 97% reduction in cockroaches, *P. americana*, *B. germanica* and *S. longipalpa* with a residual effect lasting for a week after treatment. The results showed that mixture of two essential oils has good potential for being used as a cockroach repellent. Further improvements in efficacy and residual activity may be obtained with more appropriate formulations.

Keywords: Essential oil, Cockroaches, *Eucalyptus globulus*, *Rosmarinus officinalis*

1. Introduction
Cockroaches may become pests in homes, schools, restaurants, hospitals, warehouses, offices and virtually in any structures that has food preparation or storage areas. They contaminate food and eating utensils, destroy fabric and paper products and impart stains and unpleasant odor to surface they contact (Rejita *et al*., 2014) [33]. They have the potential to mechanically carry and transmit many pathogens (Cochran, 1982) [6]. In one study, allergic reactions to cockroaches was second only to house dust mites in asthmatics (Kang and Morgan 1980) [23]. Twenty percent of homes without visible evidence of cockroaches had detectable levels of cockroach allergens in dust samples (Chapman *et al*. 1992) [10]. Because of their economic or medical importance all three of these groups of insects are the targets of frequent pesticide applications.
Up to now, at least 24 species of cockroaches have been reported in Iran, and the American cockroach is the most common species found in dwellings in various provinces of Iran (Fathpour *et al*., 2003) [18]. Synthetic insecticides are currently used for the control of pest cockroaches all around the world. However, their widespread use often suffers from disadvantages of residual toxicity, health hazard to humans, and development of resistance in several pest species (Collins, 1973; Dinham, 1993) [12, 16]. As reviewed by Cornwell (1976) [14], numerous reports have been published on cockroach resistance to various commercial insecticides. Recent concerns with human health and environmental safety, as well as prevalence of insect resistance to existing insecticides have prompted a revival of interest in plant-derived insecticides (Balandrin, *et al*., 1985) [5]. Among many secondary metabolites of plants, essential oils and their constituents have received considerable attention in the search for new pesticides (Singh and Klocke, 1985) [5], and have been found to possess insecticidal activities (Deb-Kirtaniya, and Ghosh, 1980; Iwuala, *et al*., 1981) [15, 22]. Many studies have been carried out in various plants for repellent effects against cockroaches, such as N, N-diethylphenylacetamide (Prakash *et al*., 1990) [31], methyl neodacanamide, propyl neodecanamide, methyl neotridecanamide, alkyl and aryl neoalkanamides (Steltenkamp *et al*.,

1992) [39, 40], citral and eugenol (Vartak *et al*., 1994) [44]. Research regarding cockroach repellents, especially those derived from plant extracts, is quite limited at this time. Essential oils are commercially used in four primary aspects: as aromas in fragrances and perfumes, as flavoring food additives, as pharmaceuticals, and as insecticides. They recently have received much attention due to their multi-functions as antimicrobial, antifungal, antitumor and insecticidal agents (Franzios *et al*. 1997) [20]. Insecticidal activity of essential oils has been shown against cockroaches (Ahmad *et al*. 1995, Appel *et al*. 2001, Ngoh *et al*. 1998) [2, 19, 3, 28, 35], mosquitoes (Watanabe *et al*., 1993), livestock ticks (Lwande *et al*. 1999) [25], house flies (Singh *et al*., 1991) [36]. Appel *et al*., (2001) [3]. Showed that good potential of Mint oil repellencyto to both American and German (Appel *et al*. 2001) [3]. A number of diverse terpenoids in essential oils, such as citronellal, citral, gernaniol and eugenol, have repellent activity against the American cockroach (Ngoh *et al*. 1998) [28, 35]. The essential oil of catnip (*Nepeta cataria* L.) was reported as having repellency against adult male German cockroaches (Peterson *et al*., 2002) [30]. To date, no studies have reported evaluation of efficacy of plant essential oils repellents against cockroaches as applied (field) research in Iran. The present study was initiated to study the repellent activity of two essential oils and their mixed formulation, against three cockroach species under laboratory conditions. The most promising essential oil was then further evaluated for repellency against cockroaches in the field.

2. Materials and Methods

2.1 Essential oils

Two essential oils and their mixed formulation were evaluated for repellent activity against three cockroach species (the American cockroach, the German cockroach and Brown-banded cockroach) under laboratory conditions. These essential oils were derived from *Eucalyptus globulus* and *Rosmarinus officinalis*. These oils were selected for this study because the plants are commonly available in Iran and the oils are available commercially. These oils were purchased from Giah Essence Industry Co. Ltd. Golestan Province, Iran. Naphthalene was used as a control since it is commonly used as cockroach repellent. The essential oil of mixture of two essential oils was selected for further evaluation against cockroaches in the field because of its high efficacy under laboratory conditions. This oil was prepared at various concentrations in ethanol [5, 10, 20, 50 and 100% (undiluted)] and tested against three cockroach species (the American cockroach, the German cockroach and Brown-banded cockroach) under laboratory conditions. For field testing, the essential oil was then formulated as 10% (w/w) (totally) liquid repellent in water with some additives. As test cockroaches, three cockroach species *Periplaneta Americana* (L.) (The American cockroach), *Blattella germanica* (L.) (The German cockroach) and *Supella longipalpa* (Brown-banded cockroach) were laboratory-reared and used in the present study. These cockroaches have been reared according to the standard protocols of the Biology and Ecology Section, National Institute of Health, Thailand, and maintained in the insectary of the institute (Thavara *et al*., 2007) [42]. The colonies were maintained in the insectary under ambient temperature (24-30 ºC) and humidity (70-90% RH). Adult *P. americana* (aged 3-5 months), *B. germanica* (aged 6-8 weeks) and *S. longipalpa* (4-5 weeks) were employed for repellent testing under laboratory conditions. Both males and females of each cockroach species were used in the laboratory tests.

2.2 Laboratory tests

A stainless steel square-box (50×50×15 cm, with the top open) was employed in the repellent tests. All four walls of the box were smeared with Vaseline to prevent escape of cockroaches. A piece of filter paper (Whatman No.1, 50×50 cm) was marked by a pen to divide it into 2 equal parts (treated and control areas) and then placed at the bottom of the box. The test repellent (1.25 ml) was applied (equal to dosage of 10 ml/m2) by placing drops from a pipette on the treated area to cover the treated portion of the paper, whereas the control area was untreated. Naphthalene, the control standard, is solid. Two pieces of naphthalene (1 g each) were placed together as a treatment on the treated side. Each set of containers of food and drink for the cockroaches was placed at both sides of the box (treated and control areas). Twenty adult cockroaches (10 males and 10 females) were anesthetized with CO2 and released into the box at the central point. The box was then placed in a Peet Grady chamber (180×180×180 cm) surrounded by cloth curtains to keep a dark environment and to prevent disturbances from surroundings. The cockroaches located in the treated and control areas were carefully observed and counted at 48 hours after treatment. Repellency against the cockroaches was calculated with the following equation:

Repellency (%) = 100 - [T × 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. The average repellency was calculated from the values obtained in six replicates.

2.3 Field estimation - City-scale trials

The mixture of two essential oils (*Eucalyptus globulus* and *Rosmarinus officinalis*) was selected for further evaluation in the field because it showed the highest repellent activity against the three cockroach species tested under laboratory conditions. Field evaluation of the repellent formulated from these essential oils was carried out in Guilan Province, Iran. Three cities in Guilan province (Rasht, Langrod and Astara) were selected for the study. Rasht city in center of Province (51 houses) and Langrod city in east of the Province (52 houses) were designated as the treatment sites, whereas Astara city in west of the Province (50 houses) was designated as the control site. All sites were surveyed for cockroach species and densities using sticky traps (HOY HOY, produced by Earth Chemicals, Japan) before and after treatment. The sticky trap is a simple device that can be folded into a trapezoid paper-house (10×15×3 cm), having four entrances for cockroaches and has been shown to be an effective tool for cockroach surveys in the field (Tawatsin *et al*., 2001) [41]. The sticky area for catching cockroaches inside the trap is about 9.5×15 cm. Cockroaches are lured into the trap by built-in attractants located in the middle of the sticky area. At least 40 houses in each experimental site were randomly sampled for cockroaches by placing two sticky traps in the kitchen of each house and left there for one night. The cockroaches caught in each trap were identified by species following the relevant references (Cornwell, 1968; Bell, 1981; Cochran, 1982, 1999) [13, 6, 11] and then counted. Three days after the preliminary survey, the test repellent was applied at a dosage of 10 ml/m2 by a hand-trigger window sprayer on the floor in the kitchen area in each house of the two treated sites, whereas the houses

in the control site were untreated. The treatment was carried out only once in each house of the two treated sites. To assess the degree of cockroach infestation, the sticky traps were again placed in the kitchens of each house at the three experimental sites and left there for one night. Then, all the traps were collected and the cockroaches caught in each trap were identified by species and counted. Assessment of the cockroach densities and species in each house at the three experimental sites was carried out on three additional occasions at 6-, 9- and 12-days post-treatment. Another field evaluation of the repellent formulation from the two mixed essential oils was conducted in Yousef abad zone, Tehran Province, Tehran, Iran. Two urban communities (one treated and one control site) were selected for this evaluation. Yousef abad-1 (the treated site) included 52 houses, whereas Yousef abad-2 (the control site) consisted of 50 houses. The evaluation carried out in the field in Tehran was similar to that in Rasht as described above. However, the assessments were carried once a week for four weeks post-treatment during the course of this study. After each weekly assessment, the repellent was reapplied at the same dosage in the previously treated areas in each house of the treated sites. The average number of collected cockroaches per house (mean) and standard error of the mean (SE) were calculated for each study site in each assessment in the field. The percentage reduction in cockroach number following treatment at each treated site was calculated by Mulla's formula (Mulla *et al.*, 1971) [27]:

Reduction (%) = 100 - [(C1/T1) × (T2/C2)] 100

Where: C1 = average number of cockroaches per house at the control site (pre-treatment),

T1 = average number of cockroaches per house at the treated site (pre-treatment), C2 = average number of cockroaches per house at the control site (post-treatment), T2 = average number of cockroaches per house at the treated site (post-treatment). These values, mean SE and percentage reduction (%), are presented in the figures.

2.4 Data analysis

Comparison of repellency among tested repellents was carried out by using the one way analysis of variance (ANOVA) with Duncan's multiple range test. All differences were considered significant at $p \leq 0.05$.

3. Results

3.1 Laboratory repellency

The essential oil of *E. globulus*, *R. officinalis* provided good repellency (100%) against *P. americana*, and a high degree of repellency was also obtained from the essential oils of mixed formulation of two medicinal plants (90%, 89.3%, respectively) (Table 1). It is interesting to note that all the essential oils in this experiment provided better repellencies than did the standard repellent naphthalene (82%, 84%, 86% for *P. Americana*, *B. germanica* and *S. longipalpa* respectively). Regarding the repellent tests against *P. Americana*, the mixed formulation of two medicinal plants exhibited the highest repellency (95%) of the tested repellents, whereas the essential oil of *R. officinalis* provided the lowest repellency of about 89.3% (Table 1). Essential oils of *R. officinalis* had low repellency levels among the essential oils with 93% and 94% repellency for *B. germanica* and *S. longipalpa*. The repellency of *E. globulus* for *B. germanica* and *S. longipalpa* were 96% and 97% respectively. Highest repellency belonged to mixed formulation with 95%, 100% and 100% for *P. Americana*, *B. germanica* and *S. longipalpa* respectively. The repellency of essential oil of the *E. globulus* and *R. officinalis* at various concentrations against *P. Americana*, *B. germanica* and *S. longipalpa* is shown in Fig 1. The 50% concentration and undiluted essential oil provided excellent repellency from 95 to 100% against *P. Americana* cockroaches. The essential oil at 20% concentration exhibited a moderate level of repellency at an average of about 84.5%. The essential oil diluted to 10% and 5% showed lower repellency against

P. Americana, about 60% and 71%, respectively. Regarding repellency against *B. germanica*, the 50% concentration and the undiluted essential oil of mixed formulation also provided excellent repellency (95-100%), whereas the essential oil at 20% concentration showed an average repellency of about 85%. The results against *B. germanica* in 10% and 5% exhibited repellency of 75% and 58% and the same essential concentration against the *S. longipalpa* Cockroach's were 72% and 49%, respectively.

3.2 Field repellent test against cockroaches in Gilan Province

3.2.1 Rasht city (the 1st treated site).

A total of 50 houses in the first treated site (Rasht) were surveyed and a total of 247 cockroaches were found in 38 houses (76% positive) during the preliminary inspection before treatment. The average number of cockroaches collected at Rasht prior to treatment was 4.94 cockroaches/house (Fig 2). Three days after treatment with the test repellent (10% mixed formulation), 194 cockroaches were caught from 30 houses (60.23% positive). The average number of captured cockroaches dropped to 3.88 cockroaches/house with a reduction of about 11% using the formula of Mulla *et al.* (1971) [27], taking both the treated and control populations (Fig 2). Subsequently, 26 houses (52.3%) were positive for 175 cockroaches during the inspection at 6 days after treatment. An average of 3.5 cockroaches/house was obtained in this assessment with 22.5% reduction (Fig 2). The number of houses infested with cockroaches declined to 22 houses (44.1% positive) at 9 days post-treatment with only 71 cockroaches found in this survey. As shown in Fig 2, the mean number of captured cockroaches dropped to 1.42 cockroaches/house with the reduction reaching a peak of 62%. Finally, 13 houses (26.7%) were positive for a total of 69 cockroaches in the last period of survey (12 days post-treatment). The reduction remained at 71.1% with an average of 1.38 cockroaches/house (Fig 2). Frequency of investigated species in Rasht city includes *P. Americana*, *B. germanica* and *S. longipalpa* with 35%, 25% and 22% respectively and 18% other species.

3.2.2 Langrod city (the second treated site)

The preliminary survey conducted at Langrod (the second treated site) revealed that 34 (65.38%) of 52 houses were infested with a recovery of 207 cockroaches. The average number of cockroaches captured at Langrod before treatment was 3.98 cockroaches/ house (Fig 2). A total of 22 houses (40.2%) were positive for cockroaches 3 days after treatment with the test repellent (10% mixed formulation) and 143 cockroaches were captured. An average of 2.75 cockroaches/house was obtained in this survey with a reduction rate of 21% according to Mulla's formula compared to pretreatment at the control site (Fig 2). The numbers of houses infested with cockroaches decreased to 16 (30% positive) during the survey carried out 6 days post-treatment with a total of 117 cockroaches. The average number of cockroaches captured in this assessment was 2.25 cockroaches/house, a reduction of 37.4% (Fig 2). In the survey

conducted 9 days after treatment, 12 houses (23.4%) were positive for cockroaches and only 57 cockroaches were collected. An average of 1.09 cockroaches/ house were found in this survey. The reduction reached a peak of about 63.5% (Fig 2). Twelve days after treatment, the number of houses infested with cockroaches decreased to 14 houses (28% positive) with 62 cockroaches captured. As seen in Fig 2, the average number of collected cockroaches was about the same as the previous survey (1.19 cockroaches/house), however, the reduction declined to 68.5%. Cockroaches were
Collected from Langrod during five surveys in this study. Including *P. Americana* (30%), *B. germanica* (33%), *S. longipalpa* (35%) followed by other species less than 2%.

3.2.3 Astara (control site).
Cockroach surveys were also carried in Astara (control site) during the same period as the trial in Rasht and Langrod. On a preliminary survey, 35 out of 51 houses (68.62%) were positive for cockroaches. A total of 199 cockroaches were caught in this survey with an average of 3.9 cockroaches/house (Fig 2). Three days after the preliminary survey, the number of houses infested with cockroaches declined to 27 houses (52%) and 174 cockroaches were caught (average 3.41 cockroaches/house). Later, 30 houses (58%) were positive for cockroaches in the survey conducted 6 days after the preliminary survey and 180 cockroaches were captured during this inspection. An average number of 3.52 cockroaches/house was obtained in this inspection. A total of 149 cockroaches were found in 26 houses (50% positive) in the control site in the fourth survey carried out nine days after the preliminary survey, with an average of 2.92 cockroaches/house. Finally, 30 houses (58%) were still positive with a total of 189 cockroaches at the last inspection (12 days after the preliminary survey). The average number of captured cockroaches remained at 3.7 cockroaches/house (Fig 2). Identified cockroaches caught from Astara (control) from five surveys in this study. *P. Americana* (53%) was the predominant species found in all surveys, *B. germanica* and *S. longipalpa* were 28% and 10% respectively. The other species was 9% in that survey.

3.3 Field repellent test against cockroaches in Tehran Province Tehran
3.3.1 Yousef abad-1 (the treated site).
A total of 37 (71.15%) out of 52 houses were positive for cockroaches in the pre-treatment survey carried out in the treated site (Yousef abad-1 Community) and 260 cockroaches were collected in this survey with an average number of 5 cockroaches/house (Fig 3). One week after treatment with the test repellent (10% mixed formulation), the number of infested houses became 29 (55%) and the number of collected cockroaches declined to 106. As a result, the average number dropped to 2.03 cockroaches/house with a 53% reduction. The number of infested houses decreased slightly to 22 (42%) in the survey conducted two weeks post-treatment and 71 cockroaches were captured. In this inspection, an average number of 1.36 cockroaches/house was obtained, whereas the reduction rate increased to 70.8% (Fig 3). In the survey carried out three weeks after treatment, the number of houses positive for cockroaches declined to 16 (30%) and 43 cockroaches were collected. As shown in Fig 3, the average number of 0.82 cockroaches/house was achieved with a reduction of about 81.5%. Finally, the number of houses infested with cockroaches remained at 7 (40%) in the final inspection conducted four weeks post-treatment and 21 cockroaches were captured in the survey. An average number of 0.13 cockroaches/house was found in the assessment with a high reduction rate of 97.3% (Fig 3). The cockroaches collected from the five surveys carried out at Yousef abad-1 Community includes *P. americana* (42%), *B. germanica* (39%) and *S. longipalpa* (16%) the other species (3%).

3.3.2 Yousef abad -2 (control site)
The cockroach surveys were also carried out in Yousef abad-2 Community (the control site) during the same period as the study in Yousef abad -1 Community for comparison. The results of pre-treatment survey showed that 35 (70%) out of 50 houses were infested with cockroaches and 213 cockroaches were caught. An average of 4.26 cockroaches/house was obtained from this assessment (Fig 3). One week after the preliminary survey, 30 houses (60.1%) were positive with a total of 183 cockroaches (average 3.66 cockroaches/house). Subsequently, it was found that 38 houses (76%) in the control site were infested with 198 cockroaches during the inspection at two weeks after the preliminary survey with an average of 3.96 cockroaches/house. In the third week post-treatment, the inspection revealed that 27 houses (54%) were positive for a total of 190 cockroaches and the average captured cockroaches declined to 3.8 cockroaches/house. Finally, 31 houses (62.3%) in the control site were found positive for cockroaches with 202 cockroaches collected in the last inspection. The average number of cockroaches at this assessment increased to 4.04 cockroaches/house. Frequency of species captured in Yousef abad-2 Community during the course of this study were *P. americana* (47%), *B. germanica* (29%), *S. longipalpa* (19%), and the other species (5%).

4. Discussion
The laboratory repellency results indicated differences in susceptibility to volatile chemicals derived from essential oils among the three species of tested cockroaches. *S. longipalpa* was the most sensitive species in this study, followed by *B. germanica*, and *P. Americana*. All the essential oils in this study provided better repellencies against *P. americana*, *B. germanica* and *S. longipalpa* than naphthalene (Table 1).This method of bioassay was selected because of its reliability among several attempts that had been made previously (Thavara *et al*., 2007) [42].
Many experiments have been performed in other plants also in addition to the selected plant species and have shown commendable effects on insects especially cockroaches. Seven commercial essential oils extracted from the plant species *Boesenbergia rotunda (L.) Citrus hystrix., Curcuma longa* L., *Litsea cubeba* (Lour.), *Piper nigrum L., Psidium guajava* L. and *Zingiber officinale* and naphthalene as a control, were evaluated for repellent activity against the three cockroach species *P. americana* (L.), *B. germanica* (L.) and *Neostylopyga rhombifolia* under laboratory conditions (Faujan *et al*., 1995) [19]. The toxic and repellent properties of nine major constituents of essential oils, comprising benzene derivatives and terpenes, against *P. Americana* (L.) were evaluated, verified and analyzed in many earlier experiments. Contact and fumigant toxicities to adult females and repellency to nymphs were determined. The decreasing order of knockdown
Activity via contact was methyl eugenol>isosafrole=eugenol>safrole. The benzene derivatives were generally more toxic and repellent to *P. americana* than the terpenes (Shay *et al*., 1998).

Many earlier studies focused on cockroach repellents which caused the movement of pests away from treated surfaces. (Steltenkamp *et al*., 1992; McGovern and Burden 1985; Pandey *et al*., 1994) [39, 40, 26, 29] These repellents are useful in difficult-to-reach hidden places such as electrical and plumbing systems, which may serve as runways for cockroaches and facilitate their dispersal between apartments. Furthermore, non-toxic and relatively volatile repellents may be applied to surfaces

Through cleaning solutions which protect merchandise in transport and storage, and sensitive equipment from being disrupted by pest insects. For such applications, repellents must have low mammalian toxicity and relatively low residual activity.

Naphthalene is the most common chemical used as a cockroach repellent. However, naphthalene is hazardous to human. Long time exposure to naphthalene by inhalation, ingestion or dermal contact, may result in hemolytic anemia, liver toxicity, or neurological damage in infants (ATSDR, 1995) [1]. Perhaps the most attractive aspect of using essential oils and/or their constituents as crop protectants (and in other contexts for pest management) is their low to zero mammalian toxicity. The two essential oils and mixed to their repellency nature as illustrated in many earlier findings. The results found in the present study, with respect to the formulation of essential oils, tested and therefore qualified against *P. Americana* and *B. germanica* and *S. longipalpa*. The reason for selecting these plants was their common availability and to compare their efficiencies (attractancy or repellency) so that it can be recommended to wide commercial applications as they are environmental friendly techniques as compared to other hazardous pesticides. However, mixed formulation was the most effective repellent tested providing complete repellency (100%) against *P. americana*, *B. germanica* and *S. longipalpa* and the highest repellency (among all the essential oils tested) of about 100% against *B. germanica* and *S. longipalpa*. It was selected for further evaluation in the field at a dosage of 10% mixed formulation in total.

The application of mixed formulation from essential oil (10%), in field, against cockroaches showed satisfactory repellency in the treated areas in both Guilan and Tehran Province. In Guilan, the repellent activity reached its peak at nine days post-treatment at both treated sites (62 and 81.5% reduction). Therefore, we assessed repellent activity with weekly applications in the field evaluation carried out in Tehran. We found that weekly surveys and re-applications of repellents were practical and effective. Although the repellent could not eliminate cockroaches completely, it could basically reduce the numbers of cockroaches as well as the numbers of houses infested with cockroaches in the treated sites compared to the control sites. The infestation rate and number of cockroaches captured in the treated site in Tehran declined substantially, especially two weeks post-treatment. This could be due to the accumulated residual activity of the repellent that was applied weekly. Faujan *et al*., (1995) [19]. studied the essential oil extracts of six Malaysian plants, i.e. *Curcuma longa, Zingiber officinale, Pandanus odorus, Cinnamomum zeylanicum, Syzygium aromaticum* and *Cymbopogon citratus*, were evaluated for repellent activity against *Periplaneta americana* using a modification of the 'two-cylinders' method. Dose-dependent repellency ranging from 57.1 to 100% was exhibited by all six extracts at the lowest concentration tested (12 ppm) (Faujan *et al*., 1995) [19] and in other study by Ling. *et al.* (2009) where repellency against the *B. germanica* nymphs increased with increasing concentrations of 2AP (PC = 65-93%), whereas repellency increased with decreasing concentrations of pandan essence (PC = 67-85%) and hexane pandan extract (PC = 68-83%). 2AP is a highly effective repellent as its repellency is projected to increase until it tapers off at an optimum efficiency level with higher concentrations, making it possible for its efficiency level to be controlled. The reduction of cockroaches and infestation rates in the treated sites may have been partially affected by trap catching; however, this factor is minor as seen in the results of the control site.

In this study, Mulla's formula was used for assessing the degree of reduction in cockroach number for each treated site following the treatment with the assumption that the treated and control sites were uniform in regard to factors contributing to changes in cockroach populations (Mulla *et al*., 1971) [27]. In practice, this formula was powerful for assessing the level of reduction of cockroaches in this study as it compares the number at the treated site and the control site both pre- and post- treatment. According to this formula, no reduction occurs in the cockroach numbers because of treatment if the factor [(C1/T1) x (T2/C2)] is greater than 1. This phenomenon appeared once in the treated site at Wang Itok Village (6 days post-treatment). In fact, it occurred because the average number of cockroaches post-treatment at the treated site (T2) was greater than that of post-treatment in the control site (C2). The interesting point in application of mixed formulation was acceptance of this formulation between people at the treated sites, some of residents acknowledged that mosquito- biting activity was decreased at night in the treated areas along with the cockroach reduction, this finding were compatible with Thavara *et al.* (2007) [42]. Trigg (1996) [43] showed that Quwenling, as a popular *Eucalyptus*-based repellent product, contains a mixture of *p*-menthane-3,8-diol (PMD), isopulegone and citronellol. Quwenling has largely replaced dimethyl phthalate as the insect repellent of choice in China (Trigg, 1996) [43]. *Eucalyptus* oil itself, the principal ingredient of which is PMD, provided protection comparable to DEET in repelling *Anopheles* mosquitoes in field studies (Trigg, 1996) [43]. Although repellent to *Culicoides impunctatus*, *Eucalyptus* oil was attractive to *C. imicola* (Braverman *et al*., 1999) [7]. These results indicate the potential for the use of the mixed formulation of essential oil as a cockroach and mosquito repellent in the future. This is the first study of repellents derived from plant extracts against cockroaches in Iran. More research is needed to develope more effective formulations. There is limited research about repellents against cockroaches, especially in agents derived from plant extracts. Peterson *et al.* (2002) [30] investigated the repellent activity of catnip essential oil (*Nepeta cataria*), two purified isomers of nepetalactone and deet (N, N-diethyl-methylbenzamide) against male German cockroaches (*B. germanica*) in a choice- test arena and found that E,Z-nepetalactone was the most active of the compounds tested, being significantly more active than equivalent doses of the essential oil, Z,E-nepetalactone, or deet. Other studies have evaluated chemicals for repellent effects against cockroaches. N, Ndiethylphenylacetamide (DEPA), at a dosage of 0.5 mg/cm2 showed residual repellency against

P. Americana, *B. germanica* and *S. longipalpa* for 4, 3 and 2 weeks, respectively (Prakash *et al*., 1990) [31]. Steltenkamp *et al.* (1992) [39, 40]. Demonstrated that alkyl and aryl neoalkanamides with a total carbon number between 11 and 14 exhibited highly repellent effects against male *B. germanica*. In addition, methyl neodecanamide, propyl neodecanamide and methyl neotridecanamide were also found highly repellent against females and nymphs of *B. germanica*, and male *P.*

Americana (Steltenkamp *et al.*, 1992) [39, 40]. It is interesting to note that these chemicals showed relatively specific repellency against certain species, sexes and developmental stages of cockroaches. Vartak *et al.* (1994) [44] showed that citral and eugenol were effective as repellents against *P. americana* under laboratory conditions when used at the dosages of 25-100 mg per 4 × 4 cm filter paper. However, none of these chemicals is currently marketed as a commercial repellent product against cockroaches.

In the present study, the mixed formulation of two essential oil derived from *E. globulus, R. officinalis* exhibited complete repellency (100%) against *B. germanica*, *S. longipalpa* and 95% against *P. americana*, and also showed the highest repellency (among all essential oils tested) of about 100%, against *B. germanica* and *S. longipalpa* under laboratory conditions. In addition, the repellent containing 10% mixed essential oil formulated in water and some additives also showed satisfactory repellency yielding up to 97% reduction in cockroaches, mostly *B. germanica* and *S. longipalpa*, in field tests with residual effects for a week after treatment. The present study reveals the potential for mixed formulation essential oil to be used as a cockroach repellent. Further improvements in efficacy and longevity are expected with more appropriate formulations.

5. Acknowledgements

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Table 1: Repellency of essential oils and naphthalene against *P. Americana*, *B. germanica* and *S. longipalpa* cockroaches in the laboratory.

Plant essential oils/chemical	Mean repellency* (%) ± SE		
	P. americana	***B. germanica***	***S. longipalpa***
Eucalyptus globulus	90 ± 2.6 a	96 ± 1.8 a	97 ± 2.1a
Rosmarinus officinalis	89.3 ± 3.3 b	93 ± 2.9 b	94 ± 1.5b
Mixture of two medicinal plants	95 ± 1.8 c	100 ± 0.0 c	100 ± 2.3c
Naphthalene	82 ± 1.3d	84 ± 2.7d	86 ± 1.7d

*Repellency against the same species (in the same column) followed by the same letter is not significantly different from each other ($p \geq 0.05$, by one-way ANOVA and Duncan's multiple range test).

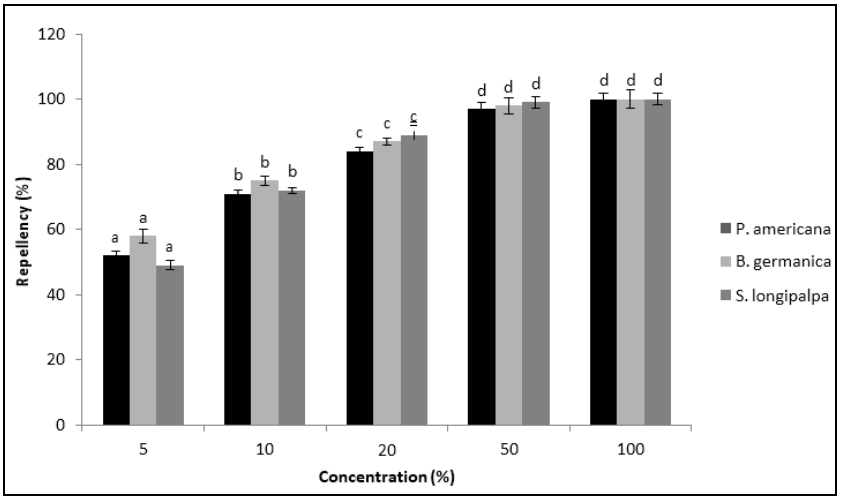

Fig 1: Comparison of repellency of mixed essential oil (*E. globulus, R. officinalis*) at various concentrations in water against *P. mericana*, *B. germanica* and *S. longipalpa* cockroaches under laboratory conditions.

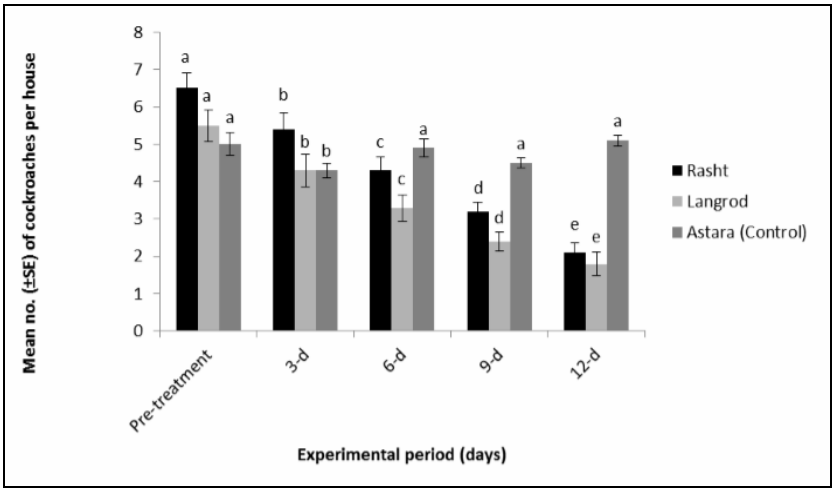

Fig 2: Field evaluation of formulated repellent containing 10% mixed essential oil of (*E. globulus, R. officinalis*) against cockroaches conducted in three city of Rasht, Guilan Province, Iran.

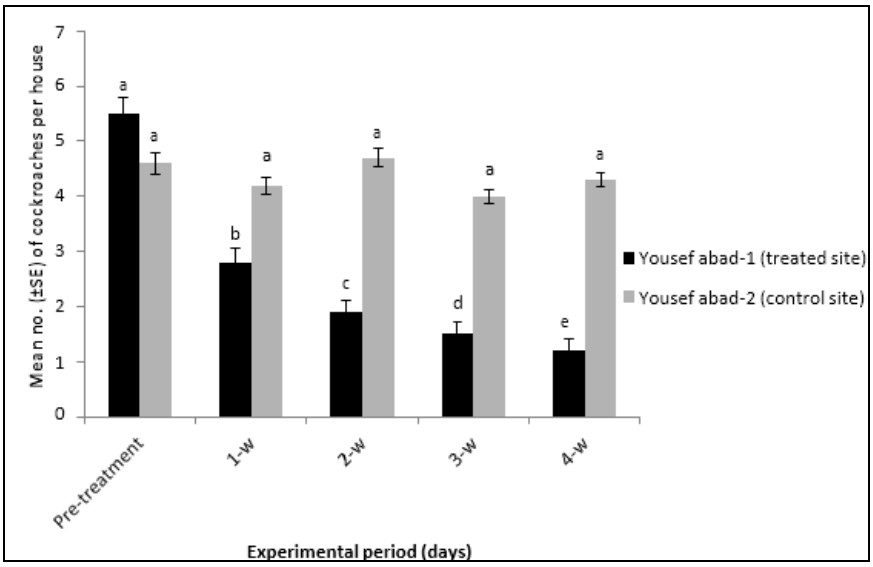

Fig 3: Field evaluation of formulated repellent containing 10% mixed essential oil of (*E. globulus, R. officinalis*) applied weekly against cockroches in two urban communities of Yousef abad, Tehran, Iran.

6. References

1. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for naphthalene (update). Atlanta: Department of Health and Human Services, 1995.
2. Ahmad FBH, Mackeen MM, Ali AM, Mashirun SR, Yaacob MM. Repellency of essential oils against the domiciliary cockroach, *Periplaneta americana*. Insect Science and its Application 1995; 16:391-393.
3. Appel AG, Gehret MJ, Tanley MJ. Repellency and toxicity of mint oil to American and German cockroaches (Dictyoptera: Blattidae and Blattellidae). Journal of Agricultural and Urban Entomology. 2001; 18:149-156.
4. Asahina S. Domiciliary cockroach species in Thailand. Tokyo: National Institute of Health, 1983, 12.
5. Balandrin M F, Klocke J A, Wurtele E S, Bollinger W H. Natural plant chemicals: sources of industrial and medicinal materials. Science (Washington), 1985; 228:1154-60.
6. Bell WJ. The laboratory cockroach. London: Chapman & Hall, 1981: 161 pp. Cochran DG. Cockroach: biology and control. WHO/VBC/1982; 52(82):856.
7. Braverman Y, Chizov-Ginzburg A, Mullens BA. Mosquito repellent attracts *Culicoides imicola* (Diptera: Ceratopogonidae). Journal of Medical Entomology 1999; 36(1):113-115.
8. Carpenter DO, Arcaro KF, Bush B, Niemi WD, Pang SK, Vakharia DD. Human health and chemical mixtures: an overview. Environ Health Perspect 1998; 106(6):1263-1270.
9. Carpy SA, Kobel W, Doe J. Health risk of low-dose pesticides mixtures: a review of the 1985-1998 literature on combination toxicology and health risk assessment. Journal of Toxicology and Environmental Health B Crit Rev. 2000; 3:1-25.
10. Chapman MD, Vailes LD, DE Mullins, Squillace SM, Gelber LE, Platts-Mills TAE. Cockroach allergens in urban environments are a major risk factor for acute asthma attacks. In: Robinson, W.H (ed.): Proceedings of the National Conference on Urban Entomology 1992, 161.
11. Cochran DG. Cockroaches: their biology, distribution and control. WHO/CDS/WHOPES/1999; 83(99):3.
12. Collins WJ. German cockroach resistance. I. Resistance to diazinon includes cross-resistance to DDT, pyrethrins and propoxur in laboratory colony. J Econ. Entomol. 1973; 66:44-7.
13. Cornwell PB. The cockroach, London: Hutchinson, 1968; 1:391.
14. Cornwell PB. The cockroach Associated Business Programmes Ltd, London 1976; 2:557.
15. Deb-Kirtaniya S, Ghosh KV, Adityachaudhury N, Chatterjee A. Extracts of garlic as possible source of insecticides. Indian Journal of Agricultural Sciences 1980; 50:507-9.
16. Dinham B. WHO/UNEP, Public Health Impact of Pesticides used in Agriculture, WHO, Geneva 1990. In The Pesticide Hazard: A Global Health and Environmental Audit. Zeb Books, London, 1993, 33-9.
17. El-Masri HA, Readon KF, Yang RSH. Integrated approaches for the analysis of toxicological interactions of chemical mixtures. Critical Reviews in Toxicology 1997; 27:175-197.
18. Fathpour H, Emtiazi G, Ghasemi E. Cockroaches as reservoirs and vectors of drug resistant *Salmonella* spp. Iranian Biomedical Journal 2003; 7(1):35-38.
19. Faujan BHA, Ahmad Muhammad M, Mackeen Abdul MA, Siti RM, Yaacob M. Repellency Of Essential Oils Against The Domiciliary Cockroach *Periplaneta americana*. International Journal of Tropical Insect Science. 1995; 16:391-393.
20. Franzios G, Mirotson M, Hatziapostolou E, Kral J, Scouras ZG, TsipidouP M. Insecticidal and genotoxic activities of mint essential oils. Journal of Agricultural and Food Chemistry. 1997; 45:2690-2694.
21. Isman MB. Plant essential oils for pest and disease management. Crop Protection 2000; 19:603- 608.
22. Iwuala MOE, Osisiogu IUW, Agbakwuru EOP. Dennettia oil, a potential new insecticide: Tests with adults and nymphs of *Periplaneta americana* and *Zonocerus variegatus*. J Econ. Entomol. 1981; 74:49-52.
23. Kang B, Morgan C. Incidence of allergic skin reactivities of asthmatics to inhalant allergens. Clinical Research 1980; 28:426.
24. Kongpanichkul A, Vichyanond P, Tuchinda M. Allergen skin test reactivities among asthmatic Thai children. J Med Assoc Thai 1997; 80:69-75.

25. Lwande W, Ndakala AL, Hassanali L, Moreka E, Ndungu M, Amiani H, *et al*. Gynandropsis gynadra essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents. Phytochemistry 1999; 50:401-405.
26. McGovern TP, Burden GS. Carboxamides of 1, 2, 3, 6-tetrahydropyridine as repellents for the German cockroach, *Blattella germanica* (Orthoptera (Dictyoptera): Blattellidae). Journal of Medical Entomology 1985; 22:381-4.
27. Mulla MS, Norland L, Fanara DM, Darwazeh HA, McKean DW. Control of chironomid midges in recreational lakes. Journal of Economic Entomology 1971; 64:300-7.
28. Ngoh SP, Choo LE, Pang FY, Huang Y, Kini MR, Ho SH. Insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach, *Periplaneta americana* (L.). Pesticide Science 1998; 54:261-268.
29. Pandey KS, Prakash S, Rao KM. Vaidyanathaswamy R. Tetrahydropyran esters as new attractants for cockroaches. Bioscience, Biotechnology, and Biochemistry 1994; 58:647-51.
30. Peterson CJ, Nemetz LT, Jones LM, Coat JR. Behavioral activity of catnip (Lamiaceae) essential oil compounds to the German cockroach (Blattodea: Blattellidae). Journal of Economic Entomology 2002; 95:377-80.
31. Prakash S, Srivastava CP, Kumar S, Pandey KS, Kaushik MP, Rao KM. N,N-diethylphenyl acetamide-a new repellent for *Periplaneta americana* (Dictyoptera: Blattidae), *Blattella germanica* and *Supella longipalpa* (Dictyoptera: Blattellidae). Journal of Medical Entomology. 1990; 27:962-7.
32. Pumhirun P, Towiwat P, Mahakit P. Aeroallergen sensitivity of Thai patients with allergic rhinitis. Asian Pac J Allergy Immunol. 1997; 15:183-5.
33. Rejitha TP, Reshma JK, Mathew A. Study of Repellent Activity of Different Plant Powders against Cockroach (*Periplanata americana*). International Journal of Pure & Applied Bioscience. 2014; 2(6):185-194.
34. Seed J, Brown RP, Olin SS, Foran JA. Chemical mixtures: current risk assessment methodologies and future directions. Regulatory Toxicology and Pharmacology 1995; 22:76-94.
35. Ngoh SP, Choo LEW, Fung YP, Huang Y, Kini MR, HO SH. Insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach. Journal of Pest Management Science. 1998; 54(3):261-268.
36. Singh D, Singh A-K. Repellent and insecticidal properties of essential oils against housefly, *Musca domestica* L. Insect Science and its Application. 1991; 12:487-491.
37. Singh G, Upadhyay RK. Essential oils: a potent source of natural pesticides. Journal of Scientific & Industrial Research. 1993; 52:676-83.
38. Sriwichai P, Nacapunchai D, Pasuralertsakul S, Rongsriyam Y, Thavara U. Survey of indoor cockroaches in some dwellings in Bangkok. Southeast Asian Journal Trop Med Public Health 2002; 33(3):36-40.
39. Steltenkamp RJ, Hamilton RL, Cooper RA, Schal C. Alkyl and aryl neoalkanamides: highly effective insect repellents. Journal of Medical Entomology. 1992; 29:141-9.
40. Steltenkamp RJ, Hamilton RL, Cooper RA, Schal C. Alkyl and aryl neoalkanamides: highly effective insect repellents. Journal of Medical Entomology 1992; 29:141-9.
41. Tawatsin A, Thavara U, Chompoosri J, Kongngamsuk W, Chansang C, Paosriwong S. Cockroach surveys in 14 provinces of Thailand. Journal of Vector Ecology. 2001; 26:232-8.
42. Thavara U, Tawatsin A, Bhakdeenuan P, Wongsin kongman P, Boonruad T, Bansiddhi J *et al*. Repellent activity of essential oils against cockroaches (Dictyoptera: Blattidae, Blattellidae, and Blaberidae) in Thailand Southeast The Southeast Asian Journal of Tropical Medicine and Public Health. 2007; 38:4.
43. Trigg JK. Evaluation of a eucalyptus-based repellent against Anopheles spp. in Tanzania. Journal of the American Mosquito Control Association. 1996; 12(2):243-246.
44. Vartak PH, Tungikar VB, Sharma RN. Comparative repellent properties of certain chemicals against mosquitoes, houseflies and cockroaches using modified techniques. Journal of Common Disease 1994; 26:156-60.