Article

Synergistic effect of some essential oils on toxicity and knockdown effects, against mosquitos, cockroaches and housefly

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ABSTRACT
The toxicity and knockdown effect of *Eucalyptus globulus*, *Rosmarinus officinalis* essential oils and their mixed formulation on *Periplaneta Americana* (L.), *Blattella germanica* (L.), *Supella longipalpa*, *Culex pipiens*, *Anopheles stephensi* and *Musca domestica* were evaluated in a series of laboratory experiments. In all bioassay five different doses (0.625, 1.25, 2.5, 5 and 10%) were used by filter paper (cm^2) and aerosol (cm^3) bioassay methods, all essential oils was toxic to cockroaches, mosquitos and housefly species the lowest and the highest LC_{50} belong to mixed formulation on *B. germanica* (LC_{50} 6.1) and *E. globulus* on *P. americana* (LC_{50} 27.7) respectively. In continuous exposure experiments, Mortality (LT_{50}) values for cockroaches ranged from 1403.3 min with 0.625% *E. globulus* (for *P. americana*) to 2.2 min with 10% mixed formulation for *A. stephensi*. The KT_{50} values ranged from 0.1 to 1090.8 min for 10% and 0.625for mixed formulation and *R. officinalis* respectively. The mortality after 24 h for mixed formulation was 100% but for single essential oils ranged from 81.5 to 98.3 for *P. americana* treated with *R. officinalis* and *A. stephensi* treated with *E. globulus* respectively. Studies on persistence of essential oils on impregnated paper revealed that it has more adulticidal activity for longer period at low storage temperature. Gas chromatographic-mass spectrometric analysis of essential oil showed 14 and 16 peaks for *E. globules* and *R. officinalis* respectively. α-Pinene (39.8%), 1, 8-Cineole (13.2%), Camphene (9.1%) and Borneol (3.7%) were present in major amounts for *R. officinalis* and1,8-Cineole (31.4%), α-Pinene (15.3%), d-Limonene (9.7%) and α-Terpinolen (5.3%) were present in major amounts for *E. globulus* respectively. Our results showed that two surveyed essential oils has compatible with synergistic effect on various insect species, furthermore it is useful for applying as integrated pest management tool for studied insects management, especially in situations in which conventional insecticides would be inappropriate.

Keywords *Eucalyptus globulus*; *Rosmarinus officinalis*; toxicity; cockroaches; mosquitos; housefly.

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1 Introduction

The conventional pesticides show toxicity to the environment and harmful effects on human health. In this context, there is a growing interest in botanical insecticides due to their minimal costs and lack of ecological side effects (Khater, 2012),which makes them desirable alternatives to synthetic chemical insecticides for controlling pests. They are best suited for use in organic food production and urban area in industrialized countries but can play a much greater role in developing countries as a new class of eco-friendly products for pest control. Essential oils derived from aromatic plants are a promising new class of ecological products for insect pest's control (López and Pascual-Villalobos, 2010). They are constituted mainly by mixtures of monoterpenes, sesquiterpenes and phenylpropanoids; metabolites that confer the mixtures with organoleptic characteristics and biological activities (Stefanello et al., 2011).Apart from being a social nuisance, mosquitoes pose serious health threats to both men and animals considering that they are the principal vectors for many vector borne diseases including malaria, dengue, yellow fever, and Chikungunya (WHO, 1995; Morrison et al., 2008) in men and equine encephalitis, *Haemorrhagic septicaemia* of buffaloes, and enzootic hepatitis in animals (Christophers, 1960). In some individuals, mosquito bites also result in acute systemic allergic reactions defined by the presence of one or more of the following: urticaria, angioedema, wheezing, dyspnea, hypotension, and decrease or loss of consciousness (Peng et al., 2004). *Culex pipiens* L. (Diptera: Culicidae) is one of the most widely distributed mosquitoes in the world. The species, commonly referred to as "house mosquito", can be found in urban and suburban areas and lives near people, but feeds primarily on birds (Bernard et al., 2001). This mosquito can transmit many arbovirus encephalitides and lymphatic filariases (Cao et al., 1997; Turell et al., 2000). Several commercially available insecticides for that (e.g. temephos, chlorpyrifos-methyl, diflubenzuron) but, many of these chemical insecticides are expensive and harmful to the environment as well as to humans. *Anopheles stephensi* Liston (Diptera: Culicidae) is a major vector in the word as well as in some of the West Asian countries and has been shown to be directly responsible for about 40–50% of the annual malarial incidence (Curtis, 1994; Collins and Paskewitz 1995). Malaria, on the other hand, a life threatening disease which caused an estimated 627000 deaths in 2012 is transmitted exclusively through the bites of *Anopheles* mosquitoes (WHO, 2014). Another important insect that affects the human life in urban area is cockroaches, they 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). They have the potential to mechanically carry and transmit many pathogens, (Cochran, 1982). The Brown-banded Cockroach *Supella longipalpa* (F.) is a domiciliary pest; it is possible to find it beneath tables, behind pictures and wallpapers where it feeds on the paste. It is used to harbour in furniture, bedding, cupboards and it seldom visits kitchens, except when it is in search of food. The wide distribution of Brown-banded Cockroach in apartments makes it very difficult to control (Cornwell, 1968). American, *Periplaneta americana* (L.) (Blattodea: Blattidae), and German, *Blattella germanica* (L.) (Blattodea: Blattellidae), cockroaches remain three of the most important insect species to homeowners and in food-handling facilities (Bennett et al. 1997). Housefly (*Musca domestica* L.; Diptera : Muscidae) is an important medical and veterinary insect pests that causes irritation, spoils food and acts as a vector for more than 100 human and animal pathogenic organisms such as enteropathogenic bacteria, enterovirus and protozoa cysts (Hadan, 2013; Morey and Khandagle, 2012).

Currently, control of these insects largely relies on chemical insecticides. Unfortunately, housefly and other above insects have developed resistance to most of chemical insecticides (Khan, 2012) and it's also adverse environment and health effect, threat of persistence and biomagnifications through the food chain (Kumar, 2012). The search for alternative pesticides and control measures that pose no risk or posing minimal risk to human health and the environment is of great interest from the preventive medicine point of view (WHO, 1999). Therefore, better alternative to synthetic chemicals, the use of botanicals to control mentioned insects is being looked upon as a main source for safer and eco-friendly insecticide. The effectiveness of essential oils in the control of various insects well established (Regnault-Roger, 1997; Isman, 2000; Kostyukovsky et al., 2002a). Naturally occurring insecticides have been used in pest control for centuries (Ebeling, 1971; Coats, 1994). Many of these compounds, including alkaloids, quinones, essential oils (including terpenoids), glycosides, and flavonoids, are secondary plant substances (Raven et al., 1992). Monoterpenoids are present in cedar, citrus, eucalyptus, mints, and a variety of spices. Many monoterpenoids are used as cosmetic, food, and pharmacological additives where they provide flavors and fragrances. Not unexpectedly, these compounds also induce a variety of responses in insects. Moreover, botanical insecticides are Biodegradable, species specific, non side effect toxic to no organisms, human, animal and environment, however, botanical insecticides from plant oils or essential oils have been used effective to control the insects (Sharma et al., 2011; Regnault-Roger et al., 2012). In this context botanical pesticides revived during recent years, because of the deleterious effects of synthetic insecticides, including lack of selectivity, impact on the environment and the emergence and spread of insect resistance. The naturally occurring pesticides appear to have a promising role in the development of future commercial insecticide for safety of the environment and public health (Bowers, 1992). Even though a wide variety of insecticidal products are available for cockroach, mosquito and housefly control, most contain synthetic organic insecticides. With homeowner's increased awareness and concern about traditional insecticides, there is a greater potential for use of less toxic materials for cockroach control.

Evaluation of essential oils against insects and isolation, identification and development of natural products from them are under the focus of numerous research programmers around the globe. So far only few insecticides of plant origin have reached the market (Cheng et al., 2003). There is a renewed interest in plant essential oils products as sources of new insect controlling agents, because they may be biodegradable to nontoxic compounds, thus minimizing the accumulation of harmful residues, leading them to be more environmentally friendly compared to synthetic compounds (Choochote et al., 2005). The purpose of this study was to determine the toxicity and knockdown effects of some essential oil against mosquitos, cockroaches and housefly alone and mixed together for evaluating the synergistic effect of them. Toxicity was determined using filter paper and LC_{50} and KD_{50} with aerosol application and continuous exposure methods.

2 Materials and Methods

2.1 Essential oils

The *Eucalyptus globulus, Rosmarinus officinalis* essential oils and their mixed formulation 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.

2.2 Insects

2.2.1 Mosquitoes

Laboratory colonies of different species of mosquitoes (*Culex pipiens* and *Anopheles stephensi*) were reared continuously for several generations in a laboratory free of exposure to pathogens and insecticides. They were maintained at 26 ± 20C and 60-80% relative humidity in the insectory University of Tehran. Larvae were fed on a mixture of commercial dog pellets and yeast powder (3:2 ratio) as nutrient. Adult mosquitoes were reared in humidified cages and fed with 10% glucose. Female mosquitoes were periodically blood-fed on rabbits for egg production.

2.2.2 Cockroaches

The population of *Periplaneta americana, Blattella germanica* and *Supella longipalpa* used in this work that was collected from dark and damp places (sewers) using food jars surrounded by dark cloth as a traps (Wang & Bennett, 2006). Traps were placed into main sewers and cockroaches were collected every two days and placed in glass containers. The collected adults and nymphs were separated in glass containers (30 × 60 × 30 cm). The containers were coated with petroleum jelly 2 cm from the top to prevent the cockroaches escaping, and supplied with water, dry dog pellets and cardboard harbor age as shelter. The cockroaches were thus kept under the laboratory condition of 25 ± 3 °C and 75 ± 5 % RH. After two weeks male and female adult cockroaches were used for the experiments. Collected nymphs were maintained until they developed to adults. The oothecae were removed to other rearing containers until hatching.

2.2.3 Houseflies

Adult houseflies were collected from the garbage site of the Tehran University, Karaj, Iran, using a sweep net method. These houseflies were reared in cylindrical boxes according to the method reported by Kumar et al. (2011a). Eggs obtained in rearing were transferred to another box containing a diet of groundnut oil cake and wheat bran mix (1:3). Hatched larvae were transferred individually to cylindrical vials (28 mm × 12 mm) containing a semi-synthetic diet (constituents: 2 g groundnut oil cake, 5 g wheat bran, 2 g milk powder, and 1 g honey mixed with 10 ml of water) which was changed daily until larvae reached the adult stage to avoid any contamination.

2.3 Filter paper bioassay (LC_{50})

Mosquitos, cockroaches and housefly were selected for the testing of adulticidal activities. Adulticidal bioassay was performed by WHO method (WHO, 1996). Appropriate concentrations of the essential oils were dissolved in 2.5 ml of acetone and applied on Whatman no. 1 filter papers (size 12 x15 cm2) as described earlier. Control papers were treated with acetone under similar conditions. Adulticidal activity of the oil was evaluated at five concentrations (0.625, 1.25, 2.5, 5 and 10mg/cm^2) to produce a range of mortality from 10 to 95 per cent along with control. Twenty insects were collected and gently transferred into a plastic holding tube. The insects were allowed to acclimatize in the holding tube for 1 h and then exposed to test paper for 1 h. At the end of exposure period, the insects were transferred back to the holding tube and kept 24 h for recovery period. A pad of cotton soaked with 10% glucose solution was placed on the mesh screen. Mortality of insects was determined at the end of 24 h recovery period. Percent mortality was corrected by using of Abbott's formula (Abbott, 1987). LC_{50} with their 95 percent confidence limits of the oil were determined using Log probit analysis test (Finny, 1971).

$$\%\ \text{Mortality} = \frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

2.4 Aerosol bioassay (LT_{50})

Aerosol bioassay was performed according to Umerie (1998). The insects with 3-5 days old were introduced into Peet Grundy Chamber (1 m3). Based on the preliminary results five doses viz, 0.625, 1.25, 2.5, 5 and 10%/cm3 were tested. Aerosol sample was sprayed inside the cage as aerosol repellent/adulticide. Adult mortality was recorded at 5 minutes interval up to 30 minutes. A reference control (Allethrin 0.25%) was used for comparison. A set of control was maintained in which vapor of deodorized kerosene (DOK) was used. The lethal time (LT_{50}) was recorded from the average of three replicates. LT_{50} were calculated from percentage mortality data using probit analysis (Finney, 1971). At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept 24 h for recovery period. A pad of cotton soaked with 10 per cent glucose solution was placed on the mesh screen. Mortality of insects was determined at the end of 24 h recovery period. Percent mortality was corrected by using of Abbott's formula (Abbott, 1987) (mentioned above).

2.5 Knockdown effect (KT_{50})

The experiments were conducted in a PeetGrandy Chamber of one cubic meter size. One hundred of two days old insects (female insects for mosquito) were released into the chamber for each study. The plant oil Formulation was allowed to vapourize by using the vapourizer equipment (Borah et al., 2010). The number of insects knocked down was recorded at periodic intervals of five minutes till complete knockdown. The maximum exposure period was 60 minutes. The knocked down insects were collected and placed in a recovery jar provided with 10% sugar solution to monitor mortality/recovery at 24 h period. The temperature and humidity of the chamber were maintained at 28˚C ± 2˚C and 50-70% respectively. The data obtained for knockdown were subjected to Finney's method of Probit Analysis to assess the KT_{50} values and they were drawn from four replicates.

$$\text{Knocked} - \text{down (\%)} = \frac{\text{No} \ - \ \text{of adults Knocked} - \text{down (per unit time)}}{\text{No. of adults released}} \times 100$$

2.6 Persistence

Persistence of essential oil on 10mg/ cm2 impregnated test paper stored at 4 and 26 ± 20˚Cwas studied at weekly interval for 49 days. Twenty *Culex pipiens* female were exposed to the impregnated paper (dose 10 mg/cm2) and adulticidal activity was evaluated. Percent mortality was determined at weekly intervals. After evaluation, the impregnated papers were stored at 4 and 26 ± 20C till further evaluation of adulticidal activity.

2.7 Stability

The essential oil was stored at 26 ± 20˚Cin closed vial up to six months and stability of the fraction was determined at 0, and 1, 3 and 6 months time intervals. Whatman no. 1 filter paper (size 12 x 15 cm2) was impregnated with the test fraction at the concentration of 10 mg/cm^2 during the study. Adulticidal activity was evaluated at 26 ± 20˚C and 60-80% relative humidity.

2.8 Gas chromatographic-mass spectrometric (GC-MS) analysis

The GC-MS analysis for the separation and identification of the essential oil was carried using a Shimadzu GC-2010 gas chromatograph (Simadzu Corporation, Kyoto, Japan) with capillary column BP- 20 (30 m in length, 0.25 mm internal diam. and 0.25 µm in thickness). Helium was used as a carrier gas (1.1 ml/min). GC oven program comprised of an initial temperature 700C (4 min) to 2200C at 40C /min and held at the final temperatures for 5 min. The essential oil was diluted in 1.0 ml dichloromethane and 0.25 µl of the resulting solution was injected for analysis. The identification of the compounds was performed using a mass spectral data base search (NIST, WELY and SZTERP software library of mass spectra) and spectra reported in literature.

2.9 Data analysis

Statistical analysis was performed using SPSS software package, version 15. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) Duncan (Duncan, 1957). The median Knock down time (KT_{50}) and lethal time (LT_{50}) was calculated by profit analysis (Finney, 1971). A p value of <0.05 was considered to represent significant differences. The corrected percent mortality was calculated by using Abbott's formula (Abbott, 1987).

3 Results

3.1 Toxicity

The two essential oils and their mixed formulation applied with filter paper method. The lowest LC_{50} value was 6.1 of mixed formulation for *B. germanica* and highest was 25.7 for *P. amricana*in response to *E. globules* (Table1). The lowest LC_{50} value for *R. officinalis* and *E. globulus* were 10.89 and 11.46 for *M. domestica*.

There was no control mortality for all species after 24 h of exposure. Mortality was 100% for mixed formulation in all insects but in single essential oils there was some variation for this index. The highest mortality in single application of essential oil was 98.3% for *A. stephensis* in response to *E. globules* and the lowest mortality was 81.5% for *P. amricana* in response to *R. officinalis* and there was no control mortality (Table 2).

Table 1 Adulticidal activity of the *Eucalyptus globulus*, *Rosmarinus officinalis* and Mixed formulation of essential oils on different insects.

Essential oil	Insects	LC_{50} (ppm)	95% Confidence limit	
			Lower	Upper
Eucalyptus globulus	*Periplaneta americana*	25.7	16.3	37.5
	Blattella germanica	14.3	6.5	25.3
	Supella longipalpa	15.28	9.2	39.2
	Culex pipiens	17.64	9.75	31.29
	Anopheles stephensi	18.45	12.41	43.12
	Musca domestica	11.46	6.8	35.6
Rosmarinus officinalis	*Periplaneta americana*	18.69	7.3	35.1
	Blattella germanica	17.25	8.5	27.3
	Supella longipalpa	17.8	9.4	31.1
	Culex pipiens	21.49	12.4	63.9
	Anopheles stephensi	17.21	10.7	57.5
	Musca domestica	10.89	4.6	33.2
Mixed formulation of essential oils	*Periplaneta americana*	7.94	3.5	19.7
	Blattella germanica	6.1	2.1	21.3
	Supella longipalpa	7.47	3.2	30.1
	Culex pipiens	8.59	4.5	19.2
	Anopheles stephensi	6.83	3.2	24.3
	Musca domestica	6.61	2.9	18.5

Table 2 Knockdown effect and mortality of the *E. globulus*, *R. officinalis* and mixed formulation of essential oils on different insects.

Essential oil name	Insects	Knockdown Effect at 10% in 1 h (%)	%mortality at 24 h
Eucalyptus globulus	*Periplaneta americana*	100	94.4± 1.7
	Blattella germanica	100	94.2± 4.1
	Supella longipalpa	100	95.4± 3.6
	Culex pipiens	100	91.2± 2.2
	Anopheles stephensi	100	98.3± 5.5
	Musca domestica	100	82.5± 6.3
Rosmarinus officinalis	*Periplaneta americana*	100	81.5± 1.1
	Blattella germanica	100	90.1± 4.2
	Supella longipalpa	100	96.1± 4.6
	Culex pipiens	100	89.3± 3.3
	Anopheles stephensi	100	97.7± 1.1
	Musca domestica	100	84.4± 2.2
Mixed formulation of essential oils	*Periplaneta americana*	100	100
	Blattella germanica	100	100
	Supella longipalpa	100	100
	Culex pipiens	100	100
	Anopheles stephensi	100	100
	Musca domestica	100	100
Reference Insecticide (Allethrin 0.25%)	*Periplaneta americana*	100	100
	Blattella germanica	100	100
	Supella longipalpa	100	100
	Culex pipiens	100	100
	Anopheles stephensi	100	100
	Musca domestica	100	100

Table 3 Persistence of adulticidal activity mixed formulation of essential oils against *C. pipiens* on 10 mg/cm^2 impregnated paper.

Essential oil	Days of observation	Per cent mortality of *C. pipiens**	
		Paper stored at 40C (Mean ± SD)	Paper stored at 26 ± 20C (Mean ± SD)
Eucalyptus globulus	0	100	100
	7	95.4± 4.0	87.1± 3.0
	14	92.3± 1.7	75.3± 5.4
	21	88.5± 6.1	62.7± 3.1
	28	81.7± 2.0	50.1± 2.7
	35	77.0± 5.4	47.3± 5.4
	42	63.8± 2.0	36.2± 3.0
	49	51.7± 3.0	21.1± 2.2
Rosmarinus officinalis	0	100	100
	7	97.2± 2.0	86.3± 6.0
	14	91.5± 4.1	70.1± 2.1
	21	85.3± 5.3	64.2± 6.4
	28	78.7± 3.2	53.4± 2.7
	35	69.2± 5.2	48.5± 3.3
	42	55.7± 2.7	31.6± 2.3
	49	48.8± 4.9	22.3± 3.3
Mixed formulation of essential oils	0	100	100
	7	100	100
	14	97.5± 4.1	96.2± 3.0
	21	90.3± 2.5	88.3± 6.1
	28	86.6± 2.1	80.2± 4.9
	35	79.8± 6.3	67.3± 6.3
	42	65.3± 2.0	56.1± 2.2
	49	58.7± 3.8	45.5± 3.4

*Number of each replicate: 5; Mosquitoes were exposed for 1 h and mortality was recorded at 24 h recovery period

Table 4 Stability test of mixed formulation of essential oils against *c. pipiens*

Months of extraction	Months of observation	LT_{50}	KT_{50}	Knockdown in hour 1 exposure (%)	Mortality at 24 h recovery period (%)
Eucalyptus globulus	0	3.28± 0.2	2.52± 0.4	100	100
	1	3.28± 0.1	2.82± 0.2	100	100
	3	4.21± 0.3	3.76± 0.6	100	100
	6	5.1± 0.1	4.33± 0.2	100	100
Rosmarinus officinalis	0	4.89± 0.5	3.14± 0.1	100	100
	1	4.89± 0.4	3.84± 0.5	100	100
	3	5.71± 0.1	4.61± 0.8	100	100
	6	6.53± 0.6	5.52± 0.4	100	100
Mixed formulation of essential oils	0	2.83± 0.2	0.1± 0.04	100	100
	1	2.83± 0.3	0.1± 0.03	100	100
	3	2.98± 0.1	0.2± 0.06	100	100
	6	3.2± 0.3	0.2± 0.01	100	100

Number of each replicate 3; Storage temperature 26 ± 20C

3.2 Aerosol assay (LT_{50})

All essential oils and their mixed formulation were found effective treatments in aerosol bioassay method. These essential oils were tested at five different concentrations viz., 0.625, 1.25, 2.5, 5 and 10% /cm^3. The results clearly indicated that mixed formulation killed 50% populations of *A. stephensi* within 2.2±0.5 minutes at 10% concentration. In the continuous exposure tests, LT_{50} values for American cockroaches ranged from 1403.8 min for 0.625% *E. globulus*as highest LT_{50} to 8.6 min for 10% mixed formulation (Table 5). LT_{50} values for German cockroaches ranged from 1065.4 to 5.2 min for 0.625% and 10% *E. globules* and mixed formulation, respectively (Table 5). The lowest LT_{50} was 2.2 min for *A. stephensis* in response to 10% mixed formulation. LT_{50} values for all species declined exponentially with increasing concentration of essential oils. For example LT_{50} for *S. longipalpa* was 968.5 min in 0.625% and it increased to 24.1 min in 10% in response to *R. officinalis.* All experiment indicated that essential oils and their mixed formulation has relatively low toxicity at 0.625%, but toxicity increases at a threshold value of about 5% and increases slightly with increasing concentration.

Table 5 Knock down time (KT50) of effective volatile oils at five concentrations against *P. americana*, *B. germanica*, *S. longipalpa*, *C. pipiens*, *A. stephensi* and *M. domestica* as determined by aerosol assay.

Essential oil	Insects	Lethal time* (Mean ± SD) min LT_{50}					Knockdown time* (Mean ± SD) min KT_{50}				
		0.625%	1.25%	2.5%	5%	10%	0.625%	1.25%	2.5%	5%	10%
Eucalyptus globulus	*Periplaneta americana*	1403.8± 113.7	872± 17.2	367.2± 10.5	121.6± 21.4	38.3± 5.1	1090± 122.8	701.6± 27.2	190.8± 19.5	83.3± 17.9	25.3± 2.1
	Blattella germanica	1065.4± 124.5	401.6± 13.7	170.3± 43.8	93.5± 5.2	28.6± 2.5	802± 22.4	298.4± 15.7	98.5± 28.1	39.4± 5.2	6.8± 3.5
	Supella longipalpa	1063.3± 243.5	389.7± 34.1	203.4± 15.9	88.1± 7.2	28.3± 2.4	820.2± 12.4	278± 26.3	170.9± 29.4	32± 7.3	7.1± 0.1
	Culex pipiens	29.3± 0.2	17.2± 0.1	13.7± 0.2	5.79± 0.3	3.28± 0.1	20.3± 4.2	14.8± 5.4	8.2± 2.5	3.3± 0.2	2.5± 0.4
	Anopheles stephensi	27.6± 2.1	18.5± 6.4	12.7± 1.2	6.3± 3.1	3.6± 2.4	23.9± 6.2	15.6± 3.2	9.7± 1.2	4.8± 0.3	2.2± 0.9
	Musca domestica	729± 12.9	235± 26.7	168± 10.4	119± 32.7	41.9± 5.1	520.4± 18.2	189.2± 3.1	137.8± 2.2	93.5± 3.1	29.4± 4.3
Rosmarinus officinalis	*Periplaneta americana*	1117.5± 123.2	679.4± 23.2	201.6± 24.2	99.7± 3.6	42.7± 3.9	970.2± 31.6	589.7± 27.2	165.6± 16.4	87.6± 5.5	30.9
	Blattella germanica	1074.1± 135.1	452± 19.2	191.4± 11.6	77.3± 4.9	26.8± 2.1	948.7± 14.8	234± 14.1	89.8± 3.2	43.5± 16.7	5.9± 0.6
	Supella longipalpa	968.5± 12.6	289.6± 24.2	191.4± 13.3	83.4± 6.2	24.1± 3.3	779.6± 12.2	143.7± 19.4	71.8± 5.5	35.8± 7.2	6.5± 0.3
	Culex pipiens	35.2± 2.3	19.5± 2.4	8.5± 0.2	6.0± 0.3	3.1± 0.5	24.7± 5.1	15.3± 2.6	6.8± 0.7	5.1± 0.4	4.8± 0.6
	Anopheles stephensi	29.5± 2.4	19.9± 0.1	13.5± 0.2	7.6± 0.4	3.8± 0.1	24.5± 3.9	17.3± 4.6	9.7± 0.3	5.9± 0.8	4.7± 0.4
	Musca domestica	341± 26.1	220± 34.3	173± 16.7	149± 19.6	40.3± 8.1	232.8± 14.1	160.7± 23.7	119.5± 16.3	81.1± 3.1	28.5± 3.9
Mixed formulation of essential oils	*Periplaneta americana*	567.4± 16.3	240.2± 12.4	100.3± 3.5	19.8± 6.2	8.6± 0.5	48.7± 0.4	22.0± 5.1	12.7± 0.6	5.4± 0.3	2.3± 0.3
	Blattella germanica	453.9± 12.7	198.6± 11.3	87.8± 2.5	16.3± 4.3	5.2± 0.7	22.4± 5.1	20± 4.2	14.5± 3.3	2.3± 0.9	0.7± 0.3
	Supella longipalpa	351.7± 13.4	138.4± 16.3	69.1± 4.3	14.2± 2.3	3.7± 0.1	18.9± 3.7	17.4± 6.1	10.3± 5.3	2.1± 0.4	0.6± 0.7
	Culex pipiens	16.9± 0.3	10.6± 0.4	3.82± 0.6	3.83± 0.3	2.8± 0.5	25.3± 4.6	19± 3.2	12.0± 5.6	1± 0.1	0.1± 0.6
	Anopheles stephensi	18.3± 0.6	11.2± 0.1	9.8± 0.6	4.2± 0.3	2.2± 0.5	23.7± 7.3	21± 3.1	13.8± 2.6	1± 0.3	0.1± 0.5
	Musca domestica	46.3± 5.1	27.6± 6.4	19.7± 2.3	7.9± 0.7	4.2± 0.4	32.9± 4.4	19.5± 7.1	8.4± 0.6	1.7± 0.4	0.3± 0.6
Reference Insecticide (Allethrin 0.25%)	*Periplaneta americana*			2.9± 0.4					0.5± 0.04		
	Blattella germanica			2.3± 0.6					0.3± 0.006		
	Supella longipalpa			1.9± 0.2					0.1± 0.008		
	Culex pipiens			2.1± 0.4					0.1± 0.003		
	Anopheles stephensi			2.1± 0.3					0.1± 0.006		
	Musca domestica			3.1± 0.6					0.2± 0.007		

3.3 Knockdown activity (KT_{50})

The essential oils were evaluated for their knockdown effect (expressed in minutes) against *P. americana, B. germanica, S. longipalpa, C. pipiens, A. stephensi* and *M. domestica*. Mixed formulation of essential oilswas the most promising one showing KT_{50} values of 2.3 min and 0.1 min, respectively against *P. amricana* and *A. stephensi* as highest and lowest KT_{50} values. The remaining oils showed good knockdown activity in 10% concentration with KT_{50} values ranging from 30.9 to 2.2 min for *P. amricana* and *A. stephensi* respectively (Table 5).

Table 6 Comparative the percentage of the main chemical composition of Eucalyptus oils.

No.	Compound	Percent %
1	1,8-Cineole	31.4
2	α-Pinene	15.3
3	d-Limonene	9.7
4	α-Terpinolen	5.3
5	β-Terpineol	4.72
6	1,4-Cineol	4.07
7	β-Pinene	3.70
8	o-Cymene	3.68
9	-Terpinen	2.92
10	Terpinenol	2.42
11	Linalool acetate	2.35
12	Linalool	2.34
13	Terpinenol	2.42
14	β-Myrcene	1.88
	Total	95.2

Table 7 Comparative the percentage of the main chemical composition of Rosemary oils.

No.	Compound	Percent %
1	α-Pinene	39.8
2	1, 8-Cineole	13.2
3	Camphene	9.1
4	Borneol	3.7
5	β,-Myrcene	3.5
6	Verbenene	2.9
7	Bornyl acetate	2.8
8	Camphor	2.4
9	Verbenone	2.3
10	Verbenol	2.2
11	β,-Pinene	1.9
12	Linalool	1.7
13	β,-Caryophyllene	1.6
14	3-Octanone	1.4
15	β,-Phellanderene	1.3
16	Limonene	1.2
	Total	91

3.4 Chemical composition analysis

GC–MS analysis of oil components In the present study, *E. globulus* essential oil showed the presence of 14 components, which formed 95.2% of the total oil composition with 1,8-Cineole (31.4%), α-Pinene (15.3%), d-Limonene (9.7%) and α-Terpinolen (5.3%) as major components (Table 6). The 1,8-cineole content of *E. globulus* oil reported in the literature varied between 18 and 65% while –pineneaccounted for 2–20% of the composition (Jimenez-Carmona and Luque de Castro, 1999; Cimanga et al., 2002; Baranska et al., 2005;

Sacchetti et al., 2005). This wide variation in chemical composition of oil could be attributed to environmental and agronomic factors (Ahmadouch et al., 1985; Hernandez et al., 1988) as well as on extraction procedure (Jimenez-Carmona and Luque de Castro, 1999). And chemical composition of essential oils of rosemary (*R. officinalis*L.) to be rich in α-Pinene (39.8%), 1, 8-Cineole (13.2%), Camphene (9.1%), Borneol (3.7%), β,-Myrcene (3.5%) and Verbenene (2.9%) which formed 91% of the total oil composition(Table 7), the Since the insecticidal efficacy of essential oil is reportedly dependent upon its monoterpene content, its characterization may help to derive a general idea about the oil constituents responsible for its insecticidal activity. As compared to the literature, the content of major monoterpene was found to be moderate in the present study.

4 Discussion

Essential oils are usually safe to humans and the environment (Yang et al., 2005). Insecticides of plant origin are expected to be target selective and biodegradable leading to fewer harmful effects on human and other animals and are environmentally safe as compared to synthetic compounds (Jeyabalan et al., 2003; Prabhakar and Jebanesan, 2004).Essential oils have been shown to be relatively nontoxic to fish, birds and mammals and easily biodegrade in the environment (Stroh et al., 1998; Kumar et al., 2012b). Some essential oils or their volatile constituents have been used to prevent and treat illness due to perceived antibacterial, antiviral, antioxidant and antidiabetic properties (Edris, 2007). Essential oils have been used in sensitive areas, such as homes, schools, restaurants, and hospitals (Batish et al., 2008; Palacios et al., 2009b). We determined to study the effect of essential oils from herbal plants to control of the various insects. Essential oils are generally known to have fumigant insecticidal properties, and the mode of action may involve elements of acetylcholinesterese inhibition and octopaminergic effects (Isman, 2000). Additional effects can be seen in behavior modification (attraction/repellency) and contact toxicity for different life stages (Koul et al., 2008). In this study data showed that combination of two essential oils has synergistic effect on mortality, LC_{50}, LT_{50} and KT_{50}values, in mixed formulation the lowest and highest LC_{50} were recorded for *B. germanica* and *P. amricana* respectively (LC_{50} 6.1 and 8.59 respectively).

In case of cockroachour data showed that mixed formulation of *E. globulus* and *R. officinalis* has highest toxicity, the lowest toxicity was 6.1 for *B. germanica* (Table1) Appel et al. (2001) stated that mint oil was toxic to the American cockroach in topical application experiments. Similar results were recorded by Ling et al. (2009) using the essential oil of *Piper aduncum.* Manzoor et al. (2012) recorded that the three essential oils of *Cymbopogon citratus* Stapf, *Mentha arvensis* L. and *Corymbia citriodora* (Hook.) Hill & Johnson showed contact toxicity to American cockroaches between 2 and 24 h of continuous exposure to the residues. The highest mortality in this research were recorded for mixed formulation in all insect but in single application *E. globulus* showed 98.3% mortality on *A. stephensis* as highest mortality and lowest mortality recorded for *R. officinalis* with 81.5% on *P. americana*. Aroiee et al. (2005) showed that essential oil of rosemary at 5 ppm resulted in 72.7 % mortality to the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood by topical application. Ngoh et al. (1998) and Enan (2005) on the American cockroach adults, Samarasekera et al. (2005) on *Musca domestica* (L.) and Phillips et al. (2010) on the German cockroach adults, who mentioned that eugenol and some terpenoids derived from plant essential oils such as clove and ginger oils had contact toxicity to the tested insects by topical application. The highest LT_{50} were recorded for *P. americana* with 1403.8 values and lowest for 3.7 for *S. longipalpa* respectively. Whereas Appel et al. (2001) stated that LT_{50} values for adult *P. americana* ranged from 469.9 min for 3 % mint oil to 10.4 min for 30 % in continuous exposure test. Tunaz et al. (2009) recorded that the estimated LT_{50} was 6.51 h for *Blattela germanica* (L.) adults at the concentration of 2.5 µl/l of air of allyl isothiocyanate (component of horseradish). Also, Phuakbuakao and Soonwera (2010) found that herbal essential oil from clove had the highest effect to *P.*

Americana adults (LT_{50} value was 0.50 min) in topical application at dose of 0.01 µl of essential oil and they also showed the Lemon grass mixed with clove oil had the highest effect to nymphs (LT_{50} value was 0.22 min).

The mosquitos surveyed as another insect the data showed that lowest and highest LC_{50} was 6.83 and 21.49 for *A. stephensis* and *C. pipiens* in response to mixed formulation and *R. officinalis* respectively. Insecticidal properties of essential oils against adult mosquitoes have been reported by many workers (Yang et al., 2005; Mansour et al., 2000; Panella et al., 2005).In the present study the all essential oils and their mixed formulation showed adulticidal activity against different mosquitoes.Eucalyptus oil showed LC_{90} value of 247.18 and 294.0 ppm against *A. stephensi* and *C. quinquefasciatus*, respectively at 24 h exposure (Manimaran et al., 2012). Zhu et al. (2006) reported that LC_{50} value of 84 and 66 ppm against *A. albopictus* and *C. pipiens*, respectively. At 24 h exposure, eucalyptus oil showed LC_{90} value of 274.00 and 264 ppm against *A. albopictus* and *C. pipiens*, respectively. Chaiyasit et al. (2006) reported the adulticidal activity of essential oils derived from five plant species, celery (*Apium graveolens*), caraway (*Carumcarvi*), zedoary (*Curcuma zedoaria*), long pepper (*Piper longum*), and Chinese star anise (*Illicium verum*), against *A. aegypti*. Traboulsi et al. (2005) reported that LC_{50} values for oils from *Citrus sinensis* and *Eucalyptus* spp were 60.0 and 120.0 ppm, respectively against *C. pipiens*larvae. Jeyabalan et al. (2003) have reported the adulticidal effect of *Pelargonium citrosa* on *A. stephensi*, with LC_{50} and LC_{90} values as 1.56% and 5.22% respectively. Prajapati et al. (2005) have studied 10 essential oils viz., *Cinnamomum zeylanicum*, *Cuminum cyminum*, *Cyperus scariosus*, *Curcuma longa*, *Juniperus macropoda*, *Ocimum basilicum*, *Rosmarinus officinalis*, *Nigella sativa*, *Pimpinella anisum*, and *Zingiber officinale* for adulticidalt activity against three mosquito species; *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus.* Omoloa et al. (2005) have reported the fumigant toxicity of essential oils from 15 species of African plants against *Anopheles gambiae*in the laboratory. Duaet al. (2010) reported the adulticidal activity of the essential oil of *Lantana camara* against different mosquitoes species on 0.208 mg/cm2 impregnated papers. In this study the knockdown times (KT_{50} and KT_{90}) values of the mixed essential oil against *Periplaneta americana, Blattella germanica, Supella longipalpa, Culex pipiens, Anopheles stephensi* and *Musca domestica*were 20, 18, 15, 12, and 14 min and 35, 28, 25, 18, and 23 min respectively and percent mortality of 93.3%, 95.2%,100%, 100%, and 100% respectively.

Eucalyptus oil in the present study showed the KT_{50} values of 25.3 6.8, 7.1, 2.5, 2.2 29.4 min in 10% against *Periplaneta americana, Blattella germanica, Supella longipalpa, Culex pipiens, Anopheles stephensi* and *Musca domestica.*, respectively. This is in agreement with Tolazaetal. (2010) who recorded KT_{50} value of 31.39 min against head lice for Eaculyptus oil. Rosmary oil in the present study showed KT_{50} values of 4.7 and 4.8 min against *A. stephensi* and *C. pipiens*. This value was much lower than 45.02 min which was observed in the same oil against *A. aegypti* by Zaridahetal (2006). Xue et al.(2003)evaluated the toxicity of 16 commercial insect repellents (6 botanicals and 10 synthetic organic products) in spray formulations were evaluated in the laboratory for adult knock down and mortality of laboratory reared female *A. aegypti*, *A. albopictus,* and *A. quadrimaculatus.* All tested products produced significant post treatment knockdown effect and 24 h mortality in all three mosquito species. In the present study the essential oils showed variation in adulticidal activity of essential oils when tested single or mixed together. Yang et al. (2005) have evaluated adulticidal activity of five essential oils against *C. quinquefasciatus*. Ethanol extract of *Apium graveolence* exhibited adulticidal activity against *A. aegypti* with LD_{50} and LD_{90} values of 6.6 mg/cm^2 and 66.4 mg/cm^2. In this study mixed essential oil formulation showed more adulticidal activity against insects compared to earlier reports (Yang, 2005; Singh et al., 1984; Choochote et al., 2004)and almost all insects showed signs of paralysis at exposure to 10 mg/ with aerosol method within 10 to 15 min, and at the end of 1 h exposure all insects become inactive. At 24 h holding period percent mortality ranged from 81.5 to 100 percent against all test insects. The symptoms observed in adult mosquitoes were similar to those caused by nerve poisons *i.e*., excitation, convulsion, paralysis and death (Choochote et al., 2004). Studies on persistence of essential oil on impregnated paper revealed that it possessed more adulticidal activity for longer period stored at low temperature. This may be due to low volatile nature of oil. Panella et al. (2005) evaluated adulticidal activity of 15 natural products isolated from essential oils of yellow cedar against *A. aegypti*, five of which demonstrated residual activity for up to six weeks. Stability test of essential oil against *A. Stephensi* during six months observation revealed that the oil was stable at room temperature.

The housefly response to essential oils showed 6.1, 10.89 and 11.46 LC_{50} value for *E. globulus*, *R. officinalis*, Mixed formulation of essential oils respectively. Palacios et al. (2009a,b) some plants were reported that have insecticidal effects against adult houseflies include *Minthostachys verticillata*, *Hedeoma multiflora*, *Citrus sinensis*, *Citrus aurantium*, *Eucalyptus cinerea* and *Artemisia annua* with LC_{50} values of 0.5, 1.3, 3.9, 4.8, 5.5 and 6.5 mg/fly at 30 minutes, respectively. The essential oil of *Pogostemon cablin* had an LD_{50} value of 3 µg/ cm^2 after topical application and *Mentha pulegium* oil had an LD_{50} value of 4.7 µg/cm^2 (Pavela, 2008). Palacios et al. (2009a,b) examined the efficacy of essential oils of 21 medicinal and edible plants against housefly of the edible plants, essential oils from orange peel and eucalyptus leaves were the most toxic to flies; the principal components of these oils were limonene (92.5%) and 1,8-cineole (56.9%), respectively. In this study highest and lowest for *M. domestica* LT_{50} were 29.2 and 0.3 for *E. globulus* and mixed formulation respectively. Tarelli et al. (2009) reported that the knockdown time (KT_{50}) values obtained for orange oil was 10.1 minutes against the housefly. The medicinal plants were most toxic to house flies were those whose essential oils were high in pulegone, menthone, limonene, and 1,8-cineole. In a survey of 34 plants conducted by Pavela (2008), essential oils of rosemary (*Rosemarinus officinalis*) and pennyroyal mint (*Mentha pulegium*) had high activity against adult flies in both fumigant and contact toxicity assays. Essential oils of peppermint (*Mentha piperita*) and blue gum (*Eucalyptus globulus*) were the most effective of 6 plant extracts examined by Kumar et al. (2011) and had both insecticidal and repellent properties. Application of an emulsifiable concentrate formulation of peppermint oil in field tests resulted in over 95% control of house flies (Kumar et al., 2011).

Natural oils are complexes of many biologically active constituents including terpenes, acyclic monoterpene alcohols, monocyclic alcohols, aliphatic aldehydes, aromatic phenols, monocyclic ketones, bicyclic monoterpenic ketones, acids, and esters (Koul et al., 2008). The composition of oils from a particular plant species can be affected by the plant tissues extracted, cultivar variation, climatic and growth conditions, and the methods used for extraction and analysis. For this reason, there have been considerable efforts to examine the effects of individual components that are common to those essential oils known to have insecticidal properties (Isman, 2000; Koulet al., 2008). The GC results in this research showed that 1,8-Cineole and α-Pinene were highest compound in *E. globulus* and *R. officinalis* essential oils that confer the important role of them in insecticidal activity that data similar to Jamshidi et al. (2009) and Kumar et al. (2012) that revealed Oil of rosemary and Eucalyptus is high in α -pinene and 1,8-cineole respectively. Similarly Koul et al. (2008) reported that 1,8-Cineole and α-Pinene in essential oils conferring in insecticidal activity, in contrast Palacios et al. (2009b) showed that the principal components of peppermint oil are menthone (20.9%) and menthol (41.5%). Urzua et al. (2010) recently found that essential oils from *Haplopappus foliosus* (Asteraceae) had high activity against adult house flies; limonene was the most abundant component in the extract. Taken together, these results do not point to any single component of essential oils that stand out as the critical element that accounts for activity against house flies. The results in our laboratory indicate that this combination of essential oils is effective as a space spray and a residual surface treatment for all tested insects in adult stage. Complex interactions may occur among major and minor constituents in an unforeseen manner that affects insecticidal activity. Similarly, mixtures of essential oils from different plants may have higher activity than individual extracts in ways judicious use of synergists could improve efficacy further. Addition of piperonylbutoxide can reduce the LC_{50} of essential oils and their individual constituents by several orders of magnitude (Waliwitiya et al., 2008). Further research on blends of essential oils and improved formulations and delivery systems could lead to substantial improvements in the performance of botanicals for vector and sanitary insect control. In the present investigation, essential oils of *E. globulus, R. officinalis* and their mixed formulation showed adulticidal activity against important vector insects. The present finding may be utilized for the development of plant-based pesticides as supplementary and replacement to synthetic insecticides.

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