Journal of Medicinal Plants Research Vol. 6(6), pp. 1065-1069, 16 February, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1244
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Efficacy of some essential oils against American cockroach *Periplaneta americana* (L.)

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Accepted 2 December, 2011

The present study was carried out to evaluate the toxicity, repellency and fumigant activity of three essential oils that is, *Cymbopogen citratus, Mentha arvensis, Eucalyptus citriodora* against *Periplaneta americana* (L.) under laboratory conditions. Of the three tested oils, *C. citratus* showed the maximum toxicity (20 to 100%) between 2 to 24 h intervals, repellency (100%) and 70 to 100% fumigation after 24 h exposure. *E. citrodora* oil was found to have least toxicity, repellency and fumigant activity. Percentage mortality (0 to 80%) between 2 to 24 h, 40 to 60% fumigant activity was observed after 24 h at different concentrations. From the present study, it was revealed that three oils were arranged according to the following order of preference that is, *C. citratus M. arvensis* and *E. citriodora.*

Key words: Toxicity, repellency, fumigation, *Cymbopogen citratus, Mentha arvensis, Eucalyptus citriodora* against *Periplaneta Americana.*

INTRODUCTION

In Pakistan, *Periplaneta americana* (L.) is a common household pest usually found in baits, sewers, basements and in moist places. Because of its presence in such places, they are associated with a number of diseases and their control is of great importance. Due to association with human wastes and certain diseases, cockroaches can become a public health problem and because of their ability to move from sewers into the homes and commercial establishments. For long, Cockroaches have been recognized as mechanical potential vectors of human intestinal parasites and animal pathogens as well as a source of human allergens. Indeed it has been found that cockroach antigen is most common in children in inner cities for asthma-inducing allergen (Arruda et al., 2001; Busse and Mitchell, 2007). Primarily, cockroaches are controlled with synthetic organic insecticides in the form of baits, foggers, aerosols and crack treatments (Frishman, 1982; Rozendaal, 1997). At present, chemical-based methods for cockroach management generally involve repeated applications of residual insecticides for example, chlorpyrifos, dichlorvos, pyrethroids and propoxur (Wei et al., 2001), stomach poisons for example, boric acid, sulfluramid and hydramethylnon (Gore et al., 1995), insect growth regulators for example, noviflumuron and lufenuron (Schal and Hamilton, 1990; Mosson et al., 1995). Essential oils are secondary plant substances (Isman, 2006) which comprises of many compounds including monoterpenoids, which gives the plant aromatic characteristics (Appel et al., 2004). They have low toxicity and are an excellent alternative to traditional insecticides (Isman, 2006). Previous literature shows that essential oils have not been used in Pakistan for the control of *P. Americana* (L.), so this study was carried out with the objective to suggest the plant oils as an alternative to chemical control of *P. americana,* examine the potential of lemongrass *(C. citratus),* eucalyptus (*E. citriodora*) and mint oil (*M. arvensis)* as controlling agent for cockroaches and to develop repellents that are economical and environment friendly.

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MATERIALS AND METHODS

Plant collection

Due the insecticidal properties Eucalyptus (*E. citriodora)*, Mint (*M. arvensis)* and lemongrass (*C. citratus)* were selected. They were collected from their natural habitats. Collected plant species were

sent to Department of Botany, Lahore College for Women University, Lahore for identification and they were given voucher number.

Collection of experimental cockroaches

Adults, nymph (male and female Cockroaches) were collected from drainage systems, sewers, basements and steam tunnels. Majority of these cockroaches were taken from sewer manholes. Some were also caught from yards, moist shady areas, outdoors, wood piles, hollow trees, and mulches. After collection they were kept in Entomology Research Laboratory, Lahore College for Women University (LCWU). These cockroaches were reared in the laboratory by feeding on sugar, bread crumbs. Water and temperature were maintained at 28 ± 2℃. Inactive and injured cockroaches were removed from the sample. While only healthy cockroaches were used for the experiments.

Extraction of essential oils

Oils were extracted by Steam Distillation (Reserve Dean-Stark Method).

Laboratory bioassays: Contact toxicity

For testing toxicity of essential oils, the method was as adopted by Ferrero et al. (2007) and Appel (2001) with some modifications. Serial dilution of each of the essential oil was prepared as 30, 25, 20, 15, 10, 8, 7, 6, 5 and 4%. Each concentration was prepared in Ethanol (v/v). Desired volume of the essential oil was added in ethanol. Filter paper (Whatman No. 1.9 cm) was impregnated with 1.5 ml of the appropriate essential oil concentration. Group of 10 cockroaches were used for experimentation. Control group was treated with 1.5 ml of the ethanol On the other hand experimental group was treated with 1.5 ml of the oil concentration. Each oil was assayed at four different concentrations which were 4, 5, 6, 7% (v/v). After 15 min of solvent evaporation, insects were placed between the inverted glass plates. This allowed the insects to come into contact with filter papers. Four concentrations were tested per oil and this was replicated at least three times. This set-up was kept at 28±2ºC. Mortality was recorded after 2, 4, 6 and 24 h. Each batch of the insects was then transferred to clean glass jars with mouse pellet and water. Mortality of cockroaches was made sure by following steps:

By exposure of light and observing their movements.
By touching their legs with a forceps and noticing their movements.

After exposure to light and touching with fore cap, if there were no movement then the cockroaches were considered dead. The mean percentage mortality was calculated by the abbott's formula

Fumigant toxicity

For testing fumigant toxicity, method was as adopted by Appel et al. (2001) and Yoon et al. (2008) with modifications. Fumigant activity of each oil was assessed by sealing either 6 or 10 adult cockroaches in 0.95-liter square glass jars with a 1 cm diameter cotton ball treated with 100 µl of the 4, 5, 6 and 7% of each oil concentration. Each oil concentration was injected into center of each cotton ball to allow volatilization while preventing the cockroaches from contacting the residue. After 24 h, numbers of cockroaches knocked down were determined. Control jars had cockroaches and untreated cotton ball. At least four concentrations were tested per chemical and this was replicated at least three times. The criterion for knockdown for this experiment was the same as that used in the contact toxicity study.

Repellency test

The method of Ferrero (2007) was adopted with some modifications. Circular white filter papers No1 (9 cm diameter, Whatman International Limited), divided in two halves were used. One of the halves was treated with 1 ml of ethanol; the other half was treated with 1 ml ethanol solutions plant oil. Each oil was assayed at four concentrations: 4, 5, 6 and 7% (v/v). After solvent evaporation (15 min), the filter paper were fitted together to make a single layer and used to cover the floor of a square plastic glass jars. For control, circular white filter papers divided in two halves, one treated with solvents and the other untreated, were used. Ten insects were released in the centre of each plastic glass jars, and their distribution was recorded 24 h later. Each experiment was repeated three times. Finally, a repellency value (RV) was determined as a measure of the repellent effect of the plant oil. It was calculated as:

$$RV = (T / NT)$$

Where, T, the number of insects on the treated zone and; NT, the number of insects on the untreated zone.

Data analysis

The data were analyzed by using Prism Version 4.00 for window 2003, Graph pad software, San Deigo California USA, (www.graphpad.com). Tukeys' standardized range test was used to compare the difference between time intervals. One-way ANOVA test was applied to calculate the significant difference in the relative distribution of cockroaches in contact toxicity and fumigant test. Mean±SE and Mean ±SD was also calculated for the analysis of variance. Results with $P<0.05$ were considered statistically significant, (*) represents the significant difference.

RESULTS

Table 1 reveals the physical properties of the three plant oils that is, Eucalyptus (*E. citriodora*), Mint (*M. arvensis*) and Lemongrass oil (*C. citratus*). In contact toxicity test, *P. americana* (L.) were exposed to four different concentrations (4, 5, 6 and 7%) of Eucalyptus (*E. citriodora*), Mint (*M. arvensis*) oil. LC_{50} values of eucalyptus (*E. citriodora*), mint (*M. arvensis*) and lemongrass oil (*C. citratus*) against *P. americana* (L.) were recorded at 2, 4, 6 and 24 h intervals. For eucalyptus (*E. citriodora)* after 2, 4, 6 and 24 h LC_{50} values were 8.268, 10.392, 7.050 and 4.814, respectively. For mint (*M. arvensis*) oil after 2, 4, 6 and 24 h, LC_{50} value were 8.122, 8.013, 6.004 and 4.640, respectively. In case of lemongrass oil, LC_{50} values were 8.013, 7.050, 4.897 and 3.399, respectivley. It was revealed from the results that least LC_{50} value was recorded for lemongrass (*C. citratus*) that has the highest toxicity rate. To evaluate the fumigant activity of the selected essential oils against *P. Americana* (L.) four

Table 1. Physico-chemical properties of essential oils used in study.

S/N	Physical properties	Values		
		Eucalytpus citriodora	***Cymbopogen citratus***	***Mentha arvensis***
1	Color	Greenish (light)	Pale sherry	Pale yellow
2	Odor	Lemon scent	Pungent citrus like	Sweet and pleasant
3	Yield	0.72%	0.24%	0.14%
4	Solubility in an alcohol (70%)	1.3 to 1.5 vol	Soluble in 70% alcohol	Soluble in 70% alcohol
5	Specific gravity	0.8670 (at 20℃)	0.846 (at 20℃)	0.89500 to 0.8990 (at 20℃)
6	Refractive index	1.455	1.474	1.44900 to 1.42600
7	Acid value	3.00	3.116	0.3 to 5
8	Ester value	12 to 45%	2.244 to 12.1	210 to 255

Table 2. Fumigant activity of Eucalyptus (*E. citriodora),* Mint *(M. arvensis) and* lemonmgrass *(C. citratus)* against *P. americana* (L.) recorded after 24 h interval.

Time	Concentrations used (%)	Mean ±SE	KD_{50} (95%Cl)	χ^2	P
Eucalyptus citriodora	4	4.33±0.33	4.897 (0.320-0.999)	0.020	0.020
	5	4.67±0.33			
	6	5.67±0.33			
	7	6.67±0.33			
Mentha arvensis	4	5.67±0.33	3.776 (0.256-0.999)	0.978	0.023
	5	6.0±0.0			
	6	7.33±0.33			
	7	8.05±0.0			
Cymbopogen citratus	4	6.67±0.33	3.399 (0.870-0.999)	0.631	0.0027
	5	7.67±0.33			
	6	8.67±0.33			
	7	10.0±0.0			

different concentrations (4, 5, 6 and 7%) for each oil were used and knockdown value at four different concentration were recorded after 24 h for each oil separately. No knockdown value was seen in control group.

Analysis of variance revealed that there were significant differences in the fumigant activity of three oils at these concentrations as P = 0.0234, df = 2, 9 and F = 5.866. Table 2 shows that eucalyptus (*E. citriodora*) has minimum fumigant potential while lemongrass oil has highest fumigant potential. Repellent values of three essential oils are shown in Table 3. It was evident from the table that minimum repellency was observed by eucalyptus (*E. citriodora*) and the highest repellency was noted in case of lemongrass (*C. citratus*) against *P. americana* (L.) after 24 h interval. Analysis of variance showed that there were significant differences in the potential of eucalyptus (*E. citriodora*), mint (*M. arvensis*) and lemongrass oil (*C. citratus*) at 4, 5, 6 and 7% concentration as P = 0.0001, df = 6, 2 and F = 5.800.

DISCUSSION

As described earlier chemical control agents is a common method of cockroach control but it has been limited by several factors: the development of natural resistance by cockroaches and the negative impact on human health. Hence, there is a great focus on finding alternative repellents, that is, essential oils which are environment friendly and ecologically safe (Jang et al., 2005; Thavara et al., 2007). Present study highlights the importance of use of essential oils in cockroach control. The oils are generally composed of complex mixtures of biogenetically related phenols, monoterpenes and sesquiterpenes. It has been reported that 1, 8-cineole is the major constituent of oils from rosemary and eucalyptus; menthol, citral and limonene from lemongrass oil (Isman, 2000). The oil of lemongrass is well known as powerful and strong insect repellent, used in aromatherapy and perfumery. The eucalyptus oil is a complex mixture of a variety of sesquiterpenes,

Table 3. Repellence value of the Eucalyptus (*E. citriodora),* Mint *(M. arvensis)* and Lemongrass (*C. citratus)* oils against *P. americana* recorded after 24 h using four concentrations.

Treatment	Concentration used	Repellence value
Eucalyptus citriodora	4%	0.67+/-0.11
	5%	0.43+/-0.22
	6%	0.25+/-0.0
	7%	0.11+/-0.0
Mentha arvensis	4%	0.43+/-0.33
	5%	0.25+/-0.0
	6%	0.11+/-0.11
	7%	0+/-0.0
Cymbopogen citratus	4%	0.25=+/-0.0
	5%	0.11+/-0.11
	6%	0+/-0.0
	7%	0+/-0.0
Control	Ethanol	-

monoterpenes and oxides, ethers, esters, alcohols, aromatic phenols, ketones and aldehydes (Brooker and Kleinig, 2006). Botanical insecticides including essential oils can act as a natural repellent, fumigant or toxic.

During the present work there was significant repellency observed in treated groups but no repellency was observed in control group. Oils were considered toxic if some or all cockroaches behaved sluggishly were moribund or dead. It was evident from Table 3 that lemongrass oil was considered highly repellent at 4, 5, 6 and 7% concentration after 24 h treatment, mint oil was the second most effective in repellent activity at the same dose while eucalyptus oil was least repellent. The LC_{50} value was estimated for three essential oils against *P. americana* (L.) using EPA Probit analysis program. The LC_{50} for eucalyptus, mint and lemongrass was 8.268, 8.122 and 8.013 after 2 h; 10.392, 8.013 and 7.050 after 4 h; 7.050, 6.004 and 4.897 after 6 h and 4.814, 4.640 and 3.399 after 24 h. These values proved that lemongrass was highly toxic, mint was moderate and eucalyptus was least effective one. Fumigant toxicity of essential oils and their constituents has been reported for various insects including cockroaches, house flies, etc (Rice and Coats, 1994).

Fumigant activity as shown in Table 1 suggested that the highest knockdown value was recorded for lemongrass oil; mild knockdown was given by mint while least values were given by eucalyptus oil. So according to order of toxicity oils can be arranged in the following ascending order of preference: *C. citrates M. arvensis* and *Eucalyptus citriodora.*

It was further revealed that the reason for the highest efficacy rate of lemongrass might be due to the presence of citral (3, 7-dimethyl 2, 6-octadienal), the most important member of acyclic monoterpenoids was found in lemongrass oil. The toxicity and repellency of mint oil extract was due to the presence of pulegone (0.86%) that made an ideal compound for use in a comprehensive integrated pest management program against cockroaches due to its low mammalian toxicity and status as a natural insecticide. Therefore mint oil has been formulated as an aerosol for direct spray onto cockroaches and other pest arthropods. Eucalyptus proved to be less toxic due to high content of oxides. The differences of activity may be due to of the target insect species. This reason explains the absence of activity of eucalyptus which contain predominately 1, 8-cineole. From the present work it was concluded that plant oils of *E. citriodora, M. arvensis* and *C. citratus* in ethanol solvent had insecticidal properties. Results showed that the investigated plant oils can be used as a source of naturally produced chemicals that could be valued as cockroach repellent. Such oils with multiple insecticidal properties will be given priority in future tests and their mode of action should be determined because of their high potential and environment friendly relationship.

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