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Efficacy of citronella and eucalyptus oils against *Musca domestica*, *Cimex lectularius* and *Pediculus humanus*

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ABSTRACT

Objective: To assess potential impacts of two indigenous plant oils: the citronella (*Cymbopogon nardus*) and eucalyptus (*Eucalyptus globulus*) for their insecticidal effect against *Musca domestica* (house fly), *Cimex lectularius* (bed bug) and *Pediculus humanus* (louse).

Methods: The oils of these two medicinal plants were applied separately at various concentrations (1, 2 and 3 mL/cup) in cups lined with filter paper containing the target insects. Mortality was evaluated after fixed intervals (6, 12 and 24 h) subsequent to the release of adult insects.

Results: Results showed that both oils exhibited concentration and time dependent mortality against the tested insects. Data pertaining to present investigation clearly showed that percentage mortality owing to these botanicals against these medical pests was significantly high (98.33%) at the rate of 3 mL for 24 h of exposure, followed by 2 and 1 mL concentrations with 12 and 6 h of exposure times.

Conclusions: The results suggest that these plant oils possess good insecticidal properties against house fly, bed bug, and louse, and are safe to humans. Furthermore, the molecular (biochemical) based study of these botanicals against diverse species of pests will be of much significance to control these pest insects.

1. Introduction

Infected arthropod species, such as mosquitoes, ticks, bugs, sandflies, and blackflies spread diseases (vector borne) through bites[1-3]. Medical pests feed on and breed in decaying matter, human waste, blood and food, and are considered as mechanical vectors for certain pathogens (bacteria, protozoa and viruses) to humans and livestock[2,4-8]. House flies [*Musca domestica* (*M. domestica*)] act as carriers of disease causing agents (*Escherichia coli*, *Shigella, Salmonella* spp.), which spread more than hundred diseases in humans and animals including amoebic dysentery, helminthic and rickettsia infections *etc*.[6,9]. The *Bartonella quintana* (a small Gram-negative bacterium) is transmitted by the body louse [*Pediculus humanus* (*P. humanus*)] which causes trench fever[10]. Nonetheless, shedding of viral DNA fragments in bed bug [*Cimex lectularius* (*C. lectularius*)] faeces and retention of hepatitis B virus through a normal moult seem to support a possibility of mechanical transmission, when bugs are crushed into abraded human skin[11].

Synthetic pesticides have been used for controlling these pests, but are highly toxic to non-target organisms and have adverse effects on the environment. Moreover, insect pests have also developed resistance to many commercially available synthetic pesticides, and henceforth their use should be minimized[6,12]. Scientists have reported the effectiveness of plant extracts/essential oils (> 2 400

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The study and associated protocols were designed based on national ethical legislative rules (according to the updated version of the declaration of Helsinki) and approved by Local Ethic Committee of Zoology Department, Buner Campus.

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plant species) against these pests as an alternative way of control[13-16]. Among such plants, citronella (*Cymbopogon nardus*) is also a potential plant having insecticidal properties. It belongs to family Cardiopteridaceae and has been reported for its strong antifungal, antimicrobial, antiacetylcholinesterase, and antioxidant properties that can be used as a successful spray deterrent against pests destroying household items[17-19]. Likewise, citronella oil is known as a natural insect repellent and its mosquito repellent qualities have been verified by researchers including effectiveness in repelling *Aedes aegypti* (*Ae. aegypti*)[20,21], body louse, head louse and stable flies[22]. Moreover, eucalyptus (*Eucalyptus citriodora*) belonging to Mytaceae family has also been reported for its significant antioxidant and insect repellent activities[23]. The present study was conducted to evaluate the toxicological effects of citronella and eucalyptus against medically important insects (*M. domestica*, *C. lectularius* and *P. humanus*) in order to find an outlet to replace synthetic products by plant based safe products.

2. Materials and methods

2.1. Test plants collection

The two plants (citronella and eucalyptus) were collected in the month of September 2013 from Bajkata Village, District Buner (Khyber Pakhtunkhwa). The plant materials were shade dried in order to avoid enzymatic degradation and fungal growth. The dried material was cleaned from dust and foreign materials and subsequently brought to the Medical Entomology Laboratory at Nuclear Institute for Food and Agriculture (NIFA), Peshawar to prepare the oils.

2.2. Insects collection and rearing

Adult *M. domestica* (house fly), *C. lectularius* (bed bug) and *P. humanus* (louse) were collected from the same Village Bajkata of Buner. The collected insects were kept in the sterilized plastic jars under optimum conditions in an incubator in the Department of Zoology, Abdul Wali Khan University Mardan, Buner Campus. They were reared in the screen cages of size 30 × 30 × 30 cm at 30.5 ℃ and 70.5% relative humidity according to the protocols[1,5]. Adults of house fly were fed with 10% w/v glucose + 10% w/v milk, while larvae were fed with mackerel fishes. One side of rearing cage was covered with a piece of clean net cloth tied with rubber bands and favorable conditions (temperature, food) were provided to the pests. Bed bugs were reared on chicken blood, a diet on which these insect survive for up to 3 weeks in the refrigerator and reproduce (lays eggs). The practical rearing system used a simple heating pad or hot plate to keep the containers of blood warm enough. Bed bugs accessed the blood by feeding through a membrane. The jars holding the colonies were placed upside-down onto the blood source membrane. The bed bugs crawled down (or are tapped to the bottom) to feed on this system. Colonies of the human head louse *P. humanus* were reared on an *in-vitro* feeding apparatus that was locally developed. The teneral first instars lice were placed on human hair tufts on the upper side of membrane-covered feeders (through an artificial membrane-rearing system) which were immersed bottom-side down within a vessel containing the same warmed chicken blood. They were more likely to feed through the membrane than any other stage.

2.3. Efficacy of citronella and eucalyptus oil

Efficacy of the two oils (citronella and eucalyptus) was determined by testing against these insect pests according to the protocols developed[1,5]. Oil of these plants was applied on filter papers that were already placed in a small plastic cups (5.5 cm in diameter and 1.5 cm in height). Three doses, *i.e*., 1 mL, 2 mL and 3 mL were used separately in each cup and then 50 individuals of each insect were released in each cup. Each treatment was replicated three times in screened plastic Petri dishes in a completely randomized factorial design. Mortality data were taken at 6 h, 12 h and 24 h after treatment applications. A test insect was considered dead if it did not show any movement or could not rise itself when it was prodded with forceps. Initially, it was observed that the mortality was dose and time dependent. As a result, the doses were not increased more than 3 mL because the oil at that dose showed more than 90% death rates. Data collected were analyzed statistically with the help of Statistic 8.1 software and means compared using LSD test at 0.05 level of significance.

2.4. Ethical considerations

The study and associated protocols were designed based on national ethical legislative rules (according to the updated version of the declaration of Helsinki)[24] and approved by Local Ethic Committee of Zoology Department, Buner Campus.

3. Results

Results of current study identified that both botanical oils exhibited concentration and time dependent mortality against tested insects as presented in Tables 1–3.

3.1. Efficacy of citronella oil against C. lectularius

Citronella oil used (3 mL, 2 mL and 1 mL/cup) showed significant mortality of *C. lectularius* during different exposure times. Mortality of the bed bugs was 72.66% at 3 mL of citronella oil, 63.33% at 2 mL, and 53.33% at 1 mL dose. *C. lectularius* exposed to citronella oil for 6 h caused 51.66% mortality, after 12 h it was 60.00%, while the maximum mortality was noted 77.66% after 24 h exposure. The mortality of the oils was significantly higher than the control. Highest mortality (88.00%) of *C. lectularius* was recorded at 3 mL dose and 24 h exposure time (Table 1).

3.2. Susceptibility of C. lectularius to eucalyptus oil

Eucalyptus oil applied (3 mL, 2 mL and 1 mL/cup) showed significant mortality of *C. lectularius* during different exposure times. Average mortality of *C. lectularius* was recorded as 48.33%, 61.66% and 66.66 % after applying eucalyptus oil at 1 mL, 2 mL and 3 mL/cup respectively. Exposing *C. lectularius* to eucalyptus oil for 24 h, 12 h, and 6 h caused 70.00%, 58.33% and 48.33% mortality, respectively (Table 1). The interaction between 24 h and 3

mL treatments caused highest mortality (80.00%) of *C. lectularius* (Table 1).

Table 1

Mortality of *C. lectularius* after exposing to 1, 2 and 3 mL/cup doses of citronella and eucalyptus oils for 6, 12 and 24 h.

Group		Average mortality (%)	
		Citronella oil	Eucalyptus oil
Dose	1 mL	53.33^{c}	48.33^{c}
	2 mL	63.33^{b}	61.66^{b}
	3 mL	72.66^{a}	66.66^{a}
	Std. Error	1.165	1.018
	LSD value	2.448	2.139
Time	6 h	51.66^{c}	48.33^{c}
	12 h	60.00^{b}	58.33^{b}
	24 h	77.66^{a}	70.00^{a}
	Std. Error	1.165	1.018
	LSD value	2.448	2.139
Dose * Time	1 mL * 6 h	40.00^{g}	40.00^{g}
	1 mL * 12 h	50.00^{f}	50.00^{f}
	1 mL * 24 h	70.00^{c}	55.00^{e}
	2 mL * 6 h	55.00^{e}	50.00^{f}
	2 mL * 12 h	60.00^{d}	60.00^{d}
	2 mL * 24 h	75.00^{b}	75.00^{b}
	3 mL * 6 h	60.00^{d}	55.00^{e}
	3 mL * 12 h	70.00^{c}	65.00^{c}
	3 mL * 24 h	88.00^{a}	80.00^{a}
	Std. Error	2.018	1.763
	LSD value	4.240	3.705

Different superscript letters show significant differences.

3.3. Efficacy of citronella oil against P. humanus

Citronella oil was applied at 3 mL, 2 mL and 1 mL/cup against *P. humanus*. Thee mL dose of citronella oil reported 82.66% mortality of *P. humanus* while 2 mL and 1 mL caused 71.66% and 55.00% mortality, respectively (Table 2). After 6 h, mortality was 56.66%, after 12 h 68.33% and after 24 h 84.33%. The oil application was dose and time dependent and highest mortality of 98.00% was achieved at 3 mL dose and 24 h exposure time (Table 2).

Table 2

Mortality of *P. humanus* at 1, 2 and 3 mL/cup doses of citronella and eucalyptus oils after exposing for 6, 12 and 24 h.

Group		Average mortality (%)	
		Citronella oil	Eucalyptus oil
Dose	1 mL	55.00^{c}	51.66^{c}
	2 mL	71.66^{b}	66.66^{b}
	3 mL	82.66^{a}	77.77^{a}
	Std. Error	1.227	1.200
	LSD value	2.578	2.521
Time	6 h	56.66^{c}	48.33^{c}
	12 h	68.33^{b}	65.00^{b}
	24 h	84.33^{a}	82.77^{a}
	Std. Error	1.227	1.200
	LSD value	2.578	2.521
Dose * Time	1 mL * 6 h	40.00^{g}	35.00^{g}
	1 mL * 12 h	55.00^{f}	50.00^{f}
	1 mL * 24 h	70.00^{d}	70.00^{d}
	2 mL * 6 h	60.00^{e}	50.00^{f}
	2 mL * 12 h	70.00^{d}	70.00^{d}
	2 mL * 24 h	85.00^{b}	80.00^{b}
	3 mL * 6 h	70.00^{d}	60.00^{e}
	3 mL * 12 h	80.00^{c}	75.00^{c}
	3 mL * 24 h	98.00^{a}	98.33^{a}
	Std. Error	2.125	2.078
	LSD value	4.465	4.367

Different superscript letters show significant differences.

3.4. Efficacy of eucalyptus oil against P. humanus

Eucalyptus oil was applied at 3 mL, 2 mL and 1 mL/cup against *P. humanus*. Eucalyptus oil applied at 3mL/cup caused 77.77% mortality of *P. humanus*, while 2 mL and 1 mL caused 66.66% and 51.66 mortality, respectively (Table 2). After 6 h exposure, the mortality was 48.33%, after 12 h 65.00% and after 24 h, the mortality was as high as 82.77%. The oil application was dose and time dependent and highest mortality of 98.33% was achieved at 3 mL dose and 24 h exposure time (Table 2).

3.5. Efficacy of citronella oil against M. domestica

The *M. domestica* was exposed to citronella oil at 1 mL, 2 mL, and 3 mL/cup for a period of 6 h, 12 h and 24 h. Citronella oil applied at 3 mL/cup caused 89.22% mortality of *P. humanus*, while 2 mL and 1 mL doses caused 73.33% and 61.66% mortalities, respectively (Table 3). After 6 h exposure, mortality was 61.66%, after 12 h 75.00% and after 24 h, the mortality was as high as 87.55%. The oil application was dose and time dependent and highest mortality of 97.66% was achieved at 3 mL dose and 24 h exposure time (Table 3).

3.6. Efficacy of eucalyptus oil against M. domestica

The *M. domestica* was exposed to eucalyptus oil at 1 mL, 2 mL, and 3 mL/cup for a period of 6 h, 12 h, and 24 h. Eucalyptus oil applied at 3mL/cup caused 82.66% mortality of *P. humanus*, while 2 mL and 1 mL doses caused 76.66% and 56.66% mortalities,

Table 3

Mortality of *M. domestica* after exposing to 1, 2 and 3 mL/cup doses of citronella and eucalyptus oils for 6, 12 and 24 h.

Group		Average mortality (%)	
		Citronella oil	Eucalyptus oil
Dose	1 mL	61.66^{c}	56.66^{c}
	2 mL	73.33^{b}	76.66^{b}
	3 mL	89.22^{a}	82.66^{a}
	Std. Error	1.069	1.042
	LSD value	2.247	2.189
Time	6 h	61.66^{c}	58.33^{c}
	12 h	75.00^{b}	71.66^{b}
	24 h	87.55^{a}	86.00^{a}
	Std. Error	1.069	1.042
	LSD value	2.247	2.189
Dose * Time	1 mL * 6 h	45.00^{g}	40.00^{h}
	1 mL * 12 h	60.00^{f}	60.00^{g}
	1 mL * 24 h	80.00^{d}	70.00^{e}
	2 mL * 6 h	60.00^{f}	65.00^{f}
	2 mL * 12 h	75.00^{e}	75.00^{d}
	2 mL * 24 h	85.00^{c}	90.00^{b}
	3 mL * 6 h	80.00^{d}	70.00^{e}
	3 mL * 12 h	90.00^{b}	80.00^{c}
	3 mL * 24 h	97.66^{a}	98.00^{a}
	Std. Error	1.852	1.805
	LSD Value	3.892	3.792

Different superscript letters show significant differences.

respectively (Table 3). After 6 h exposure, the mortality was 58.33%, after 12 h, 71.66% and after 24 h, 86.00%. Again, the oil application was dose and time dependent and highest mortality of 98.00% was achieved at 3 mL dose and 24 h exposure time (Table 3).

4. Discussion

Due to the long-term side effects and overmedication of synthetic drugs and sprays, the researchers again are turning to treatments of insect pests with natural products that have good efficacy against the pests but are safe to humans and other non-targeted species. In the present study, the oil of citronella and eucalyptus caused noticeable mortality of more than 98.33% of medical pests at very low dose (3 mL). Palacios *et al*.[25] evaluated the insecticidal activity of nine essential oils from medicinal plants belonging to Lamiaceae family against *M. domestica* and recorded LC_{50} of 46.9 mg/dm^3 in 30 min. Our result is in accordance to their work as we observed significant insecticidal activity of the plants (citronella and eucalyptus) (Tables 1–3) against the medical pests at very small doses.

It is commonly assumed that plant-based products are safer and easily available than synthetic chemicals because they are natural and cheaper. Kwon *et al*.[26] demonstrated the effects of citronella against different insects. They reported that the potential molecules in this plant have strong activity in the form of activating cation channels as its mode of action. They observed that this effect is similar to the mode of action of pyrethrin (a synthetic compound). The development of plant based product is extremely fertile due to the presence of insecticidal compounds, which are defensive against insects[27].

A study[28] evaluated the biocidal activity of acetone, chloroform, ethyl acetate, hexane, and methanol leaf and flower extracts of *Ocimum sanctum* against fourth instar larvae of *Ae. aegypti* and *Culex quinquefasciatus*. They observed the highest larval mortality in leaf extract of *Ocimum sanctum* against *Ae. aegypti* and *Culex quinquefasciatus* larvae. This report is similar to the present results in terms of the lethal effects (90% mortality after 24 h of exposure in case of lice) of the alcoholic extract of citronella and eucalyptus. The difference in both the studies might be due to the different species of insect and plant. This study further suggests about the molecular based evaluation of these plants.

Verbal interviews of the people on subjects used for the study showed that there was no adverse impact in the form of rashes, irritation, pains or other skin problems during the study of these medicinal plants oils. Historically, many plant oils and extracts, such as tea tree, myrrh and clove, have been used as topical antiseptics and reported to have antimicrobial properties[29].

The efficacy of eucalyptus oil has also been evaluated for the control of some piercing-sucking insect pests associated with faba bean (*Vicia faba*) in open field[30]. They observed 43.27% toxicity of this oil after 10 days. Their report regarding the toxicity is quite similar to the present results of the same plant because we observed 98% mortality at 3 mL after 6 h. The difference between the two studies might be due the concentration, time of treatment and the tests species.

Another study[31] reported the repellent activity of citronella and eucalyptus plants oil against *Ae. aegypti* and *Anopheles dirus* (*An. dirus*). They observed that the oil of these plants exhibited protection against biting from two mosquitoes *Ae. aegypti* and *An. dirus*. Essential oil from lemon grass exhibited protection against biting from two mosquito species, for *Ae. aegypti* [(98.66 ± 11.56) min protection time and 0.97% biting rate] and for *An. dirus* [(98.00 ± 15.28) min protection time and 0.80% biting rate]. The sweet of this plant was effective as repellent and feeding detergent against *Ae. aegypti* [(98.87 ± 10.28) min protection time and 0.90% biting rate] and *An. dirus* [(210.00 ± 10.70) min protection time and 0.93% biting rate]. In the present study, it was observed that these two plant oils showed repellency against the medical pests at 3 mL for up to 24 h in case of lice, up to 6 h in case of bed bugs and showed 98% repellency in case of house fly in 3 h of treatment. The similar repellency response by the species (*Ae. aegypti*, *An. dirus* and *M. domestica*) to the oil may be that they belong to the same order (Diptera). However, in case of bed bugs these oils showed biocidal activity at the dose of 1 mL up to 6 h.

A recent study by Tennyson *et al*.[32] has documented that citronella (*Cymbopogon nardus*) and eucalyptus (*Eucalyptus globulus*) have insecticidal activity against the third instar larvae of *Ae. aegypti*. They observed larval mortality after 24 h at 125, 250, 500 and 1 000 ppm concentrations, and they also reported that the best insecticidal activities were observed for citronella and eucalyptus against the *Aedes* mosquitos (vectors for dengue virus). Similarly, the current study found much effectiveness of the above discussed two plants against the dipterous species (*M. domestica*). This efficiency got increased with the rise in exposure time (3 to 24 h of treatment) of the test insects to citronella and eucalyptus oils even above 90% in case of bed bugs. The differences may be due to difference in test models (*Aedes* mosquito) used for the evaluation of repellent potential of these oils. It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds[33]. As a result, an integrated vector management (IVM) is a sustainable approach to manage insects that combines biological, cultural, physical and chemical tools in a way that minimizes economic, health and environmental risks[34]. The results of the current study also supports the traditional application of the plant products and suggests that the plant extracts possess compounds with insecticidal properties that can be used as lethal or repellent agents against medical pests in preparation of lotion, spray and creams. In order to further exploit insecticidal activities of these indigenous medicinal plants and to come up with a potent, safe and economically affordable formulation, further investigations are required. Conclusively, the tested products are the excellent botanical insecticides for house fly, bed bug and louse controls, safe for human health and environmental friendly. However, integrated vector management is a justifiable tactic to manage insects and diseases that they spread in a way which reduces economic, health and environmental dangers.

Conflict of interest statement

We declare that we have no conflict of interest.

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