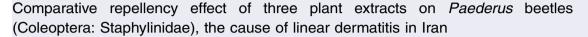
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ABSTRACT

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Keywords: Repellency effect Paederus spp. Linear dermatitis Neem Juniper Eucalyptus **Objective:** To investigate the repellent effect of neem, juniper and eucalyptus extracts as a form of protection against *Paederus* beetles, which are a cause of linear dermatitis in Iran.

Methods: After collecting and extracting plant samples, the extracts were tested on *Paederus* beetles in three concentrations (2.5%, 5.0% and 10.0%) with direct method under laboratory conditions. The data were analyzed using SPSS software (version 20). **Results:** The results indicated that there was a significant difference between neem with juniper and eucalyptus at the 2.5% and 5.0% concentrations (P < 0.05), whereas there was a significant difference between all three extracts at the 10.0% concentration (P < 0.05).

Conclusions: This is the first report on the repellent effect of these three plant extracts on *Paederus* beetles. Neem oil appeared to have the largest effect on *Paederus* spp. and juniper essential oil exhibited the second highest repellency, followed by eucalyptus.

1. Introduction

Paederus beetles belong to the family Staphylinidae (subfamily: Paederini). Adult *Paederus* beetles are predators of other insects and prefer to lay their eggs in the moist and decaying vegetation of swamps and agricultural fields, and other immature stages also occur in these areas. These beetles are small with soft bodies and are approximately 7–13 mm in length. They are mainly orange, except for the head, front wings (elytra) and the tip of abdomen, which are black. *Paederus* beetles are drawn to light sources and do not bite or sting; however, contact with human skin releases the vesicant chemical pederin, which is found in the body fluid of the beetles and causes severe linear dermatitis [1]. Acute dermatitis, inflammation and a reddish rash appear 12–36 h after exposure. The rashes then develop into blister-like lesions, which dry out to become crusted and scaly within a week. The rashes have been healed completely within 2–3 weeks. The most commonly affected body parts include the face, neck, shoulders, arms and the area around the waist.

The genus *Paederus* has been found in five provinces in Iran, including Golestan and Guilan (Northern Iran), Ardabil (Northwestern Iran), Fars (Southern Iran), and Sistan and Baluchistan (Southeastern Iran) ^[2,3]. So far, because of the importance of *Paederus* spp. in pest control as predators, several methods have been recommended to reduce the effects of *Paederus* on humans; however, most of these methods are inefficient. An alternative and safe method to avoid contact between these insects and humans is herbal or synthetic repellents. One of the best plants used as a repellent is neem [*Azadirachta indica* (*A. indica*)] and its secondary metabolites have been reported to have good repellence for mosquitoes ^[4].

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Neem has been well-known in India and its neighboring countries for more than 2 000 years for its wide spectrum of biological activity [5]. Some plants such as western juniper (*Juniperus* spp.) and eucalyptus (*Eucalyptus* spp.) have also been used as repellents [6,7].

In this study, to the best of our knowledge, for the first time, neem oil and the essential oils of juniper and eucalyptus, which are native in some parts of Iran, were tested as herbal repellents against *Paederus* beetles under laboratory conditions.

2. Materials and methods

2.1. Botanical study

2.1.1. Sample collection

All plants were collected from Jiroft County, Kerman Province, Southeastern Iran (28°40′41″ N, 57°44′26″ E). This county has a very fertile land and the weather of the city is very warm in summer and temperature is moderate in winter. It is one of the hottest places in Iran. Flowering specimens of plants were collected and sent to the Botanical Biology Group of Tehran University for identification.

2.1.2. Preparation of extracts

For the extraction of neem oil, we used an organic solvent method (*n*-hexane). Briefly, 1 kg of neem fruit was crushed and soaked in 1 L of solvent (*n*-hexane) and the neem oil was then isolated using a rotary evaporation system. Neem oil contained several chemicals but the most important component was azadirachtin. Azadirachtin caused the repellency of neem oil and its insecticidal effects had long been known [8.9]. Eucalyptus and juniper essential oils were obtained from 2 kg of wet leaves from their respective trees via steam or hydro distillation using Clevenger apparatus. Finally, three concentrations of each extract (2.5%, 5.0% and 10.0%) were prepared using ethanol as the solvent.

2.2. Entomological study

2.2.1. Study area

Live specimens were collected from Nur County $(36^{\circ}19' \text{ N}, 52^{\circ}00' \text{ E})$, which is located on the Caspian Sea coast, north of Iran.

2.2.2. Sample collection and identification

One of the most common ways to catch *Paederus* beetles was by using light traps because of their attraction to light sources at night. We used UV (black light) lamps (110 V, Ultra Violet Productions Inc., USA) as the light source. For identification and confirmation, some of the collected specimens were sent to the Museum of Medical Entomology at the School of Public Health, Tehran University of Medical Sciences, Iran.

2.2.3. Repellency experiment

To determinate the repellency of plant extracts, clean and sterile Petri dishes were covered with a piece of thick carton and a net [10]. The direct choice method was used in the repellency experiments [8,10]. Two paper semicircles were placed within each Petri dish, and essential oil was applied to one piece of paper and the other was used as the control. The concentrations used were 2.5%, 5.0% and 10.0%. Ten adult

Paederus beetles were released into each Petri dish, which was then covered with the thick carton and net. For solvent checking (ethanol), two pieces of paper were placed in one Petri dish (one was applied with solvent and the other one was not). Two papers without essential oil or solvent were placed as a control.

The average number of *Paederus* beetles on the control and treated papers was counted during 1 h and interval every 10 min. All of the experiments were repeated five times and the repellency index (RI) was calculated using the following formula:

RT (%) = 100
$$\left(\frac{PC - PT}{PC + PT}\right)$$

where PC is the number of *Paederus* beetles on the control paper and PT is the number of *Paederus* beetles on the treated paper.

2.3. Statistical analysis

The data were subjected to statistical analyses using SPSS software (version 20). For statistical comparison among several means, One-way ANOVA was used, followed by Tukey's test.

3. Results

Neem and juniper samples were identified as *A. indica* and *Juniperus polycarpus*, respectively. However, for the eucalyptus, we could not classify the species of the tree. All adult beetles sent to the Museum of Medical Entomology were identified as *Paederus* spp.

The RI was calculated for each concentration for each replicate (Table 1) and the means were calculated and compared (Table 2). For the neem oil, there was a significant difference between the RIs for all the concentrations (P < 0.05). In juniper, no significant difference was found between the RIs for the 0.0% (solvent) and 2.5% concentrations. However, with increasing concentration from 2.5% to 5.0% and 10.0%, the repellency rate increased and these differences were statistically significant (P < 0.05). The strength of repellency was not significantly different between the 2.5% and 0.0% (solvent) solutions of eucalyptus essential oil (P > 0.05). When the concentration was increased from 2.5% to 5.0%, the RI also increased from (7.83 ± 1.17)% to (24.17 ± 1.14)%, representing a significant difference when compared with pure solvent (P < 0.05).

Table 1

RI of three plant extracts against *Paederus* spp. at different concentrations.

Plants	Concentration (%)	RI (%)				
Neem	0.0^{*}	14.28	7.69	14.28	7.69	6.66
	2.5	33.33	8.33	33.33	27.27	33.33
	5.0	60.00	40.00	45.45	55.55	60.00
	10.0	66.66	55.55	77.77	75.00	77.77
Juniper	0.0^{*}	6.66	6.66	0.00	0.00	0.00
	2.5	14.28	14.28	7.69	7.69	6.66
	5.0	23.07	23.07	27.27	16.66	23.07
	10.0	60.00	45.45	40.00	40.00	45.45
Eucalyptus	0.0^{*}	6.66	0.00	5.88	6.66	6.66
	2.5	6.66	6.66	12.50	6.66	6.66
	5.0	23.07	23.07	28.57	23.07	23.07
	10.0	23.07	23.07	38.46	33.33	33.33

*: Solvent.

Table 2

Means of RI against *Paederus* spp. for three extracts at various concentrations. Mean \pm SE.

Concentration (%)	Repellency (%)				
	Neem	Juniper	Eucalyptus		
0.0^{*}	10.12 ± 1.71	2.66 ± 1.63	5.17 ± 1.30		
2.5	27.12 ± 4.84	10.12 ± 1.71	7.83 ± 1.17		
5.0	52.20 ± 4.04	22.63 ± 1.70	24.17 ± 1.14		
10.0	70.55 ± 4.27	46.18 ± 3.66	30.25 ± 3.08		

*: Solvent.

A significant difference was found between neem with juniper and eucalyptus for the 2.5% and 5.0% concentrations (P < 0.05), as well as between all three extracts for the 10.0% concentration (P < 0.05).

4. Discussion

One of the most phytochemical pesticides discovered in recent years is derived from the neem tree (*A. indica*) of the Meliaceae family, which is native to many tropical and sub-tropical regions of the world. This species was originally imported into Iran about 50 years ago [11,12]. According to the results, it seems that neem oil has the greatest repellent effect on *Paederus* beetles and the RI increases with increasing concentration.

Azadirachtin, the active component derived from the neem tree, is one of the most potent natural repellents against many medical and agricultural pests [13]. The repellency of neem oil has also been documented against several anopheline and culicine mosquitoes, though results have been variable [4,13–15]. Although neem (*A. indica*) is a well-known botanical repellent against mosquitoes, sand flies, and biting midges, this is the first report of these three plants being used as repellents for *Paederus* beetles [16].

Juniper essential oil exhibited the second highest repellency, followed by eucalyptus, whereas, eucalyptus is one of the herbs whose repellency property is promising [17].

Over 130 species of eucalyptus trees have been imported to Iran, though only a few of them have been able to survive in the Iranian climate. Since several species of this tree were moved in the past years into Iran, as yet, no adequate classification system has been devised for this species. Therefore, we cannot know their diversity and species [11].

In 2014, Frances *et al.* reported that the eucalyptus formulation provided 32% protection against mosquitoes ^[18], which was similar to our finding. In our experiments, the maximum repellency of eucalyptus essential oil was $(30.25 \pm 3.08)\%$.

Juniperus polycarpus is becoming more common in many of the northern highlands and mountains of Southeastern Iran, including the Kerman Province. The Persian name for this plant is "Ardoj" [11]. Repellent activities of juniper essential oil were tested on tick which was not very effective [6].

Nowadays, with the growing use of chemicals to control insects and the side effects of these compounds on the environment, human health and the health of other creatures, efforts have been made toward the use of nontoxic and low-risk compounds. Natural repellents are considered as relatively safe alternatives to synthetic repellents against many insects. In particular, the complex chemistry of neem, which interferes with insect growth regulator and has ovicidal properties, makes it an ideal candidate as a repellent against *Paederus* beetles [19].

Conflict of interest statement

We declare that we have no conflict of interest.

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