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Excito-repellency effects of *Pelargonium roseum* wild (Geraniaceae) essential oil-treated bed nets on the malaria mosquito, *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae)

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Abstract
Pelargonium roseum essential oil was used to evaluate the efficiency of excito-repellency activity against *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae). *P. roseum* essential oil was used at different concentrations of 25, 50 and 75% (v/v) and the mosquitoes were exposed for an hour. The compound showed 50.5± 2.8%, 65.3± 3.1% and 77± 3.9% entry to exit-trap at concentrations of 25%, 50% and 75% (v/v), respectively. In addition, the mean blood feeding rates for the above concentrations were 11.3, 7.1 and 3 percent. Moreover, survival rates were 49.4± 2.9%, 34.6±3.9% and 23±4.1. 75% concentration of *P. roseum* was more effective than other concentrations in terms of irritability. Similarly, it was revealed that 25% concentration was clearly less effective. These studies clearly indicated that the populations of malaria vectors can be effectively controlled using *P. roseum* treated bednets. It seems to be a promising compound for personal protection against *An. stephensi.*

Keywords: *Anopheles stephensi*, Essential oil, Excito-Repellency, *Pelargonium roseum*

1. Introduction
Mosquitoes are the most important vectors of major human diseases such as malaria which is a crucial public health problem all over the globe, especially in the Middle East [1]. This is the most important vector-borne parasitic disease of man in most countries of the tropical world. According to the World malaria report 2012, there were about 216 million cases of malaria (with an uncertainty range of 149 million to 274 million) and an estimated 655,000 deaths in 2010 (with an uncertainty range of 537,000 to 907,000) [2]. In most urban and rural areas of Iran, mosquito populations are menacing throughout the year, except for some attenuation during summer and winter. Mosquito control by means of chemicals is an easy way, which gives immediate control. But the mosquito problem has increased ever before. The main reason is because of the indiscriminate use of chemical insecticides, resulting in mosquito resistance to many insecticides. A huge number of the pesticides owing to their toxic effects and non-biodegradable nature can be detrimental to health in man and animal [3]. Making use of repellents has been accepted as part of Integrated Vector Management (IVM) so as to control vector–borne diseases [4]. The repellency of geranium at different concentrations against species *Aedes aegypti* and *An. albimanus* has been tested and 78.4% and 61.9% repellency observed, respectively [5]. Excito-repellency is the combination of contact irritability and non-contact repellency behavioral responses. The extensive use of pyrethroid insecticides and the challenges of mosquito resistance to these chemicals are considered the main reason for undertaking this study [6]. The excito-repellency effect of some plants to mosquitoes and other pest insects were well known before the appearance of synthetic chemicals [7]. Today, the spread of vector borne diseases is also becoming a public health concern [8]. Personal protection is one commonly advocated method to the prevention of mosquito attack. This method enables an individual to choose from (or combine) avoidance techniques, exclusion of mosquitoes with physical and chemical barriers, treatment of fabric with toxicants, and the use of topical (skin) repellents [9]. The excito-repellency properties of plants to mosquitoes and other pest insects were well known before the advent of synthetic chemicals [10].

The use of repellents on exposed skin is a common personal protection practice. All the same, the effectiveness of this technique is dependent on many environmental factors and can differ greatly across mosquito species. As such, on the basis of laboratory and field tests against mosquito species of known pest/vector importance use of topical repellents was recommended [11]. It is difficult to satisfy the requirement since outdoor testing of repellents in regions with endemic mosquito-borne diseases has to be carefully performed due to the risk of human infection. In comparison, laboratory tests are safe owing to the use of pathogen-free mosquitoes. Moreover, they are more convenience, although less robust than field tests, since many of the biological and environmental factors contributing to variability in the field tests can be controlled [12]. *Pelargonium roseum* which is also called *Pelargonium graveolens* has the following characterizes: its roots grow to 15 cm in pot or up to 60 cm in soil, fresh leaves grow throughout the year if the temperature is not too low, its flowers bloom between June and October and the plant grows up to 2 meters tall in soil, but can be easily domesticated and grow indoors in a pot [13]. The main objective of this study was to investigate the efficacy of *P. roseum* -impregnated bed nets against the malaria vector, *An. stephensi*, in a baited test chamber.

2. Materials and methods

2.1 The period of study

The experiment was carried out during June and July 2014 in the national insectary at the Pasteur Institute of Iran (PII).

2.2 Mosquito rearing

Adults of *An. stephensi* were used for excito-repellency tests. The laboratory bred *An. stephensi* (type strain, at Bandar-abbas province) was reared and maintained at 25-30 °C and 70% relative humidity in the national insectary at the Pasteur Institute of Iran (PII). This colony has been maintained in laboratory at Pasteur Institute of Iran (PII) since 2008. This strain is used as a susceptible strain in the insectary. In the present experiment, non-blood fed 5-7 day old females *An. stephensi* were used.

2.3 Cage design

Excito-repellency test cage of Evans [14] and Das [15] were slightly modified for insecticide sensitivity studies. The improved version of the excito-repellency (E-R) test system is shown in Figure 1. The exposure chamber is constructed with six aluminum sides, each side wall measuring 30×30 cm^2, forming a cube. In order to lead mosquitoes into the chamber, a small entry opening (radius = 5 cm) equipped with a short (15 cm length) netting sleeve on the outside is sealed on the front face of the exposure (or mosquito release) chamber (Figure 1).

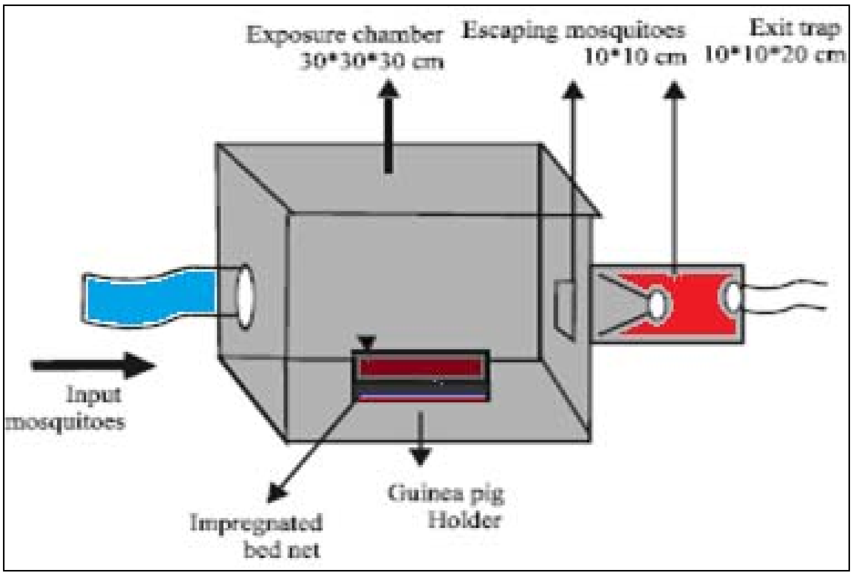

Fig 1: Excito-repellency test boxes

On the opposite face, a rear exit portal is composed of a horizontal opening, 10 cm long and 10 cm wide, at the end of an outward projecting funnel. A rectangular cube, 20 cm long and 10 cm wide, serving as an exit trap for collection of the 'escaped' mosquitoes with an aspirator at the end of each test, is attached over the exit funnel. On the distal side of this rectangle, like the entry opening, an orifice is cut which is equipped with a netting sleeve on the outside. A cylindrical, 25-mesh wire screen, serving as an animal (guinea-pig) bait holder, 10 cm long and 7 cm diameter, is inserted onto the floor of the exposure chamber. Insecticide treated (test) or untreated (control) nets cover the bait holder accordingly. The whole E-R test apparatus is run in complete darkness (covered by a black hood).

2.4 Net impregnation

The bed net was 100% polyester, white in color and with a mesh size of 2 mm and 1 m^2 area, which absorbed about 27 ml of water per 1m^2 based on the following formula:

$$\text{water absorption} / 1m^2 \text{ bed net} = \text{Wet weight} - \text{Dry weight}$$

The nets were impregnated with *P. roseum* using standard dipping procedures [16]. The compound was first diluted in water and the nets were then immersed for 10 min in solutions and well shaken. The nets were then removed and hanged on a plastic rope under shadow till dried out. After 24 h, they were collected and stored for later use in black plastic bags in the fridge. Nets were thus impregnated with 25, 50 and 100 mg a.i. m^2 of three concentration of *P. roseum*. The control nets were left untreated.

2.5 *Pelargonium roseum*
The essential oil (90% v/v) of *P. roseum* (figure 2) was used in this study and purchased from Barijessence Company, Iran.

Fig 2: Illustration of *Pelargonium roseum* plant showing the leaves and purple color flowers.

2.6 Behavioural tests
Following the assembly of exposure chamber, a guinea pig was placed in the animal bait holder which was then inserted into the exposure chamber. The test method consisted of enclosing 25 female mosquitoes in a chamber containing animal bait covered with *P. roseum* -treated or untreated (control) test nets. The exposure chamber had an exit portal for mosquitoes to escape to a receiving cage. A full test consisted of a pair of treatment chambers and a pair of control chambers such that the tests were performed in the same time and place.
The results of mosquitoes' behavior were recorded after 1 hour as dead, survived, recovered, blood-fed and retrieved in the exit trap. At the end of each test, the animal bait was removed. Live and dead (or knocked-down) mosquitoes were collected with aspirator and forceps from exit and exposure chambers. They were transferred to paper cups covered with nets and containing cotton pads soaked with dilute sucrose as food. The escaped mosquitoes and the remaining test specimens collected from the exposure chamber were held separately for observation and scoring of mortalities after 24 h holding periods.

2.7 Tests performed
Only *An. stephensi* females were used in excito-repellency tests. Each test was replicated at least 4 times. To fulfill the goals of this research, tests were performed to compare the three concentrations of *P. roseum* and survival versus recovery rates among mosquitoes.

2.8 Data analysis
The one-way ANOVA was performed using the Statistical software, SPSS, so as to determinate if there were significance differences in the outcome of various treatments. Significant differences between the means were measured on the LSD test basis at $P<0.05$. Additionally, arcsine-transformed data ($Y = \text{Arc Sin} \sqrt{P}$) were used for analyzing proportions.

3. Results
3.1 Blood feeding and mortality of mosquitoes
The results obtained from E-R tests on bed nets impregnated with three concentrations of *P. roseum* on *An. stephensi*, and their comparison with control mosquitoes are presented in Table 1. As indicated, lower blood feeding rates were observed in *P. roseum*-treated trials compared with untreated controls. The mean blood feeding rates of female mosquitoes were statistically significant ($p<0.01$) differences at different concentrations (Figure 3). In addition, as regards mortality rate the results indicated that there was no significant difference between *P. roseum* treated- nets and control ($p> 0.05$). There was almost no knockdown or mortality of control mosquitoes exposed to untreated net.

Table 1: The E-R test results of three concentrations against *An. stephensi* under laboratory conditions

Concentrations	Repeat	Mosquito Number	Location		Total	Status	
			Exposure chamber (Survival) Rate (%)±SE	Exit Trap Rate(%)±SE		Mortality Rate (%)±SE	Blood feeding Rate (%)
75%	4	100	23(23)±4.1	77(77)±3.9	100	5(5)±.05	3(3)±2.3
50%	4	98	34(34.6)±3.9	64(65.3)±3.1	98	3(3.06)±1	7(7.1)±1.4
25%	4	97	48(49.4)±2.9	49(50.5)±2.8	97	2(2.06)±0.01	11(11.3)±2.1
Control	4	99	97(97.9)±1.8	1(1.01)±0.5	99	0(0)±0.2	78(78.7)±0.01

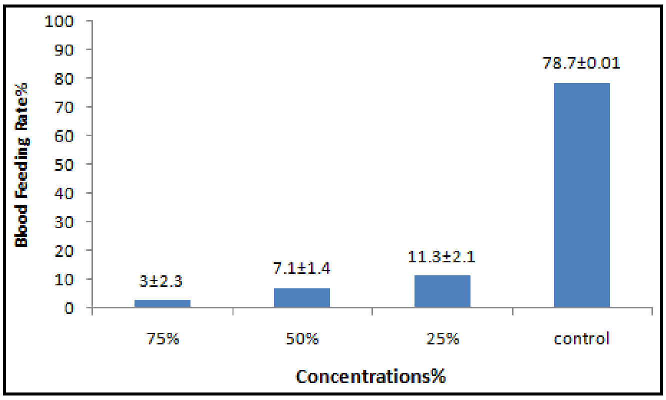

Fig 3: Blood feeding rate of *An. stephensi* on guinea pigs inside the holder covered with nets impregnated with three concentrations of *P. roseum.* The assays were repeated four times and the data indicated as Mean± SE.

3.2. Exit trap rate

The entry indices of mosquitoes in the exit trap also indicated that only 1.01% of control mosquitoes attempted to escape, while in treated trials on average 64.4% entered the exit trap and there was statistically significant differences ($p<0.01$). The highest mean entry index (i.e., deterrence) was recorded for 100% concentration (77%). However, there was significant evidence of a difference in entry indices between the three concentrations ($p<0.01$) (Figure 4).

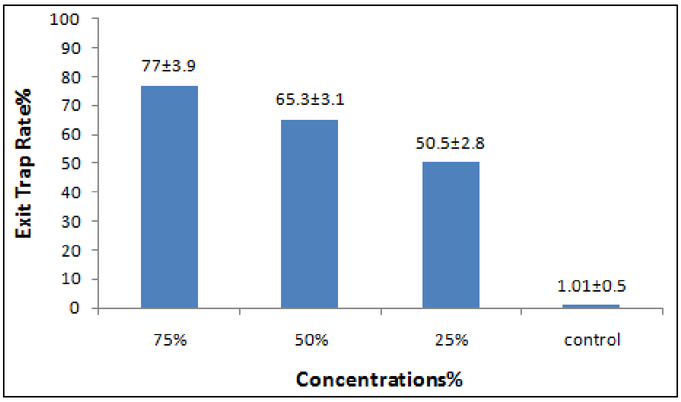

Fig 4: Exit trap rate of *An. stephensi* on guinea pigs inside the holder covered with nets impregnated with three concentrations of *P. roseum*. The assays were repeated four times and the data indicated as Mean± SE.

3.3 Survival rate in exposure chamber

The survival rate, or the rate at which hungry female mosquitoes remained alive despite being exposed to animal bait covered with *P. roseum*-treated or untreated bed nets, was near 100% in the control specimens.

The mean survival rate at various concentrations of *P. roseum* was about 35 %. The mean survival rate of female mosquitoes exposed to 25% concentration was twice that of 100%. This difference was statistically very significant ($p<0.001$) (Figure 5).

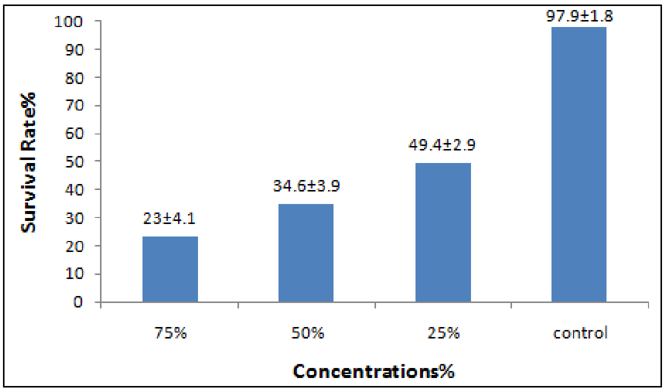

Fig 5: Survival rate of *An. stephensi* on guinea pigs inside the holder covered with nets impregnated with three on centrations of *P. roseum*. The assays were repeated four times and the data indicated as Mean± SE.

4. Discussion

According to the World malaria report 2012, the treated nets are considered as one of the major preventive tools in the global control of malaria [17]. One of the accessible aims of the public use of treated nets is a reduction in mean age of the local mosquito population, so that fewer vector mosquitoes could harbor the malaria parasites. On treated bed nets, the activity of *P. roseum* evaluated in four ways: blood feeding rate due to the presence of some volatiles, irritancy (or excito-repellence) due to a brief contact or minor exposure, survival rate for rein from *P. roseum* and mortality rate due to an effective exposure to *P. roseum*. The results revealed that there was a significant difference among concentrations of *P. roseum* ($p<0.05$) in the sequential effects of blood feeding, entry to exit trap. Comparing the three concentrations of *P. roseum*-treated bed nets indicated that 75% concentration was more effective than other concentrations. As a result, *P. roseum* compound appears to be a repellent for *An. stephensi* that is in agreement with another study [5]. In the recent years, the use of botanical products has attracted a great deal of attention as repellent, and therefore, several botanicals have been tested for the repellent activity against mosquitoes [18]. In ancient medicine Lemon *Citrus limon* Burm and *Melissa officinalis* L. have long been used as natural insect repellents in the world [7, 19]. Some recent studies have addressed the phytochemical composition of *P. roseum* leaves having repellency effect against a few vector mosquitoes [5]. The percentages of landing and biting mosquitoes on the area treated by Geranium (*P. graveolens*) essential oil were 2.8 and 0.4 for *An. stephensi*, respectively [5]. In our study, the percentage of landing for blood feeding was 3% on *P. roseum* treated nets with 75% concentration. Another study has also demonstrated that the use of Geranium extract resulted in a significant reduction in the blood feeding of sand fly [20]. Recently, the study has indicated that Pelargonium essential oils had no statistically effect on the mortality rate of *Aedes aegypti* [21] which is consistent with our study (Table 1). In addition, our study revealed that entry to exit-trap is different significantly in three concentrations ($p<0.05$). The same effects have been reported using ITNs conducted by Maxwell *et al.* and Soremekun *et al.* [22-23].

5. Conclusion

All in all, the *P. roseum* may give the same protection against other mosquito vector species. As such, the further studies should be carried out against as much different malaria vector as possible under both laboratory and field conditions. Moreover, several methods enhancing the efficacy of repellent, such as the purification of the active fraction, increase in persistence and the duration of repellency need to be studied.

6. Acknowledgments

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