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Research Article

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Evaluation of insecticidal efficiency of treated nets with essential oils of *Satureja khuzestanica* (karvakerol) and *Myrtus communis* (moort) using bioassay tests

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ABSTRACT

Malaria is still one of the most important infectious diseases in the world. The main problem is that many of the Anopheles mosquitoes have become resistant to various chemical pesticides and also their resistance or tolerance to pyrethroid insecticides which used for impregnating of bed nets has been reported, so the essential oils of plants with insecticidal properties are good candidates for replacements of chemical insecticides for treating of nets. Thirty-two Polyester untreated mosquito nets were impregnated with Myrtus communis (moort) (at concentrations of 1%, 5%, 10% and 30%) and Satureja khuzestanica (karvakerol) (at concentrations of 2%, 5%, 10% and 20%) essential oils. We prepared four treated nets of each concentration. Mean median knock down time (MMKDT) in continuous exposure bioassay test and mortality 24 h after 3-minute exposure test were carried out on nets. Results showed that with increasing concentrations of Moort essential oil the time for knock down (KD) is significantly increased (P = 0.006). On the contrary, by increasing the concentration of essential oil of karvakerol, the time for (KD) is significantly reduced (P <0.001). With increasing concentrations of Moort essential oil ,there was no significant increase in mortality percentage for Anopheles stephensi (P = 0.716), whereas by increasing the concentration of karvakerol essential oil , percentage of deaths and mortality was border line increased for Anopheles stephensi (P = 0.073).

Key words: Mosquito nets, *Satureja khuzestanica* (karvakerol), *Myrtus communis* (moort**),** essential oil

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INTRODUCTION

Malaria is still one of the most important infectious diseases in the world. About 40% of the world's population is at risk of this disease, and 100 countries including Iran are infected [1,2,3]. In the last 30 years, in some regions of Iran and the world this disease has been increased [2]. Presently, insecticide residual spraying (IRS) in infected areas with high cost has been carried out in Iran and other countries. About 20 years ago, the use of Insecticide Treated Nets (ITN's) for controlling of malaria in many areas of the world has been on the agenda of the World Health Organization [4,5,6,7,8,9,10]. The main problem is that many of the *Anopheles* mosquitoes have become resistant to various chemical pesticides and has also been reported their resistance to pyrethroids which used for impregnating nets, for this reason, the use of herbal essential oils with insecticidal properties of chemical pesticides is very important to replace [8, 9, 10]. Other researchers`s research have shown that the essential oil extracted from the leaves of Moort (*Myrtus communis*) with different doses can be toxic for living organisms. In a toxicity study of Moort essential oil to rats in the orally form 3/7 ml/kg (rat) and for mouse 2/2ml/ kg (mice) has been reported [1]. Insecticidal effect of different doses of extracted essential oils from Moort and the leaves of plant (*Satureja khuzestanica*) as (karvakerol) has been reported in different studies [2,3,4,5,6,7,8]

Other researchers also have been reported the antibacterial, antifungal and antiparasitic properties of essential oils of *Myrtus communis* and *Satureja khuzestanica* [9, 10, 11, 12, 13, 14, 15, 16]. According to the above description, the insecticidal effect of Moort and karvakerol essential oil has been evaluated.

EXPERIMENTAL SECTION

Extraction method

Myrtus communis (Moort, local name, Family: Myrtaceae) was collected from Khorramabad county in West of Iran (latitudes 33.4841 and longitudes 48.3525). This plant grows in West of Iran. Species and family of plant was confirmed by academic staffs of research center of Agricultural Department, Lorestan province, Iran (Shahla Ahmadi, personal communication).

The dried and powdered plant material was extracted using Hydro distillation and Clevenger-type apparatus (model BP, British Pharmacopoeia, manufacturer Ashke Shisheh Company, Iran and mantle model H610 manufacturer Fater Company, Iran). Clevenger apparatus temperature set for one hour at temperature 60^{oc} for each 0.5cc essential oil. After collecting the oil from the column, sodium sulfate was used for dewatering [17, 18, 19].

Then, after determination of the dry weight, the essential oils were kept in the dark bottle at 4 ºc temperature until use. The extract yield was 1%.

All above procedures were carried out in laboratories of Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Iran. We extracted 0.5cc pure (about 100%) essential oil of 100 gr of dried leaves of *Myrtus communis* (moort).

Essential oil of *Satureja khuzestanica* (karvakerol, Family: Lamiaceae) was bought from Khoraman Pharmaceutical Company, Khorramabad, Iran. The mentioned company culture *Satureja khuzestanica* and extracts its essential oil (karvakerol) for pharmaceutical marketing. *Satureja khuzestanica* grows in West of Iran (South of Lorestan and North of Khuzestan Provinces). The Company extracted 3cc pure (about 90% to 94%) essential oil of 100 gr of dried leaves of *Satureja khuzestanica* (Ali Salehnia, personal communication). All essential oils were kept in 4ºc.

Net treatment with essential oils

Thirty-two Polyester untreated mosquito nets were impregnated with moort (at concentrations of 1%, 5%, 10% and 30%) and karvakerol (at concentrations of 2%, 5%, 10% and 20%) essential oils. We prepared four treated nets of each concentration.

Pure ethanol (99.6%) was used as solvent of essential oils and each untreated net absorbed 500cc essential oil with its solvent ethanol. Four untreated nets were treated with 99.6% ethanol as positive controls. We used 500cc ethanol for each untreated net. Four untreated nets were used as negative controls as well. For treating of nets, each net with 500cc of essential oil dissolve in solvent (99.6% ethanol) was put in a plastic container and was massaged as much as to make sure that all liquid was absorbed by net fibers. After impregnation, the nets were dried in the shade horizontally [20, 21, 22].

Bioassay tests

All nets (treated nets with essential oils, positive controls and negative controls) were transferred to Kazeroun field station (Fars province, South Iran, related to Tehran University of Medical Sciences) for bioassay tests. Mean median knock down time (MMKDT) in continuous exposure bioassay test and mortality 24 h after 3-minute exposure test were carried out on nets with collaboration of staff of station. 3 to 5 days old female blood fed *Anopheles stephensi* as main vector of malaria in the area were reared in insectary and used for bioassay tests. For each mosquito net four continuous exposure test and four 3-minute exposure test were carried out on three sides and the top of nets. We used a cubic metal frame (10cm x 10cm x 10cm) to wrap of net around it and then a group of 11 *An. Stephensi* were put inside the cubic frame using mouth aspirator. In continuous exposure tests, the times of each mosquito that was knocked down were recorded in special forms and the time of the sixth mosquito that was knocked down was considered for statistical analysis (MMKDT). After each knockdown, mosquito was removed immediately from floor of the cage by mouth aspirator. Similarly in 3-min exposure tests again 11 mosquitoes were put inside the frame and after 3 minutes they were removed from the cage and were put in a paper cup and transferred to insectary (27 ^{oc} and 70% to 90% humidity) and held there for 24 hours. Cups were supplied with glucose solution on cotton to prevent dying of mosquitoes due to starvation. Percent mortality of mosquitoes were recorded after 24 hours holding in insectary. Percent mortality were recorded after 24 hours holding of mosquitoes in insectary following 15 minutes expose of mosquitoes to the negative control nets. We did one test on negative control between nine tests on treated nets. The overall mortality was recorded during one day tests of nets. The

mortality rates of negative control nets were usually zero, but if it exceeded from 5%, we repeated tests on treated nets and positive controls in the next day [20, 21, 22, 23].

Statistical analysis

The SPSS version 19 was used for analysis of data. Out puts of two dependent variables, mean median knock down times (MMKDT) and 24 h mortality after 3-min exposure (% mortality) were considered for analysis.

RESULTS

-Bioassay test results (continuous exposure bioassay test)

Bioassay test results (continuous exposure bioassay test) for impregnated mosquito nets with different concentrations of Moort and karvakerol essential oil are reported in Figure 1.As shown in Figure 1, with increasing concentrations of Moort essential oil the time for knock down (KD) is significantly increased (P = 0.006). On the contrary, by increasing the concentration of essential oil of karvakerol, the time for (KD) is significantly reduced (P <0.001).

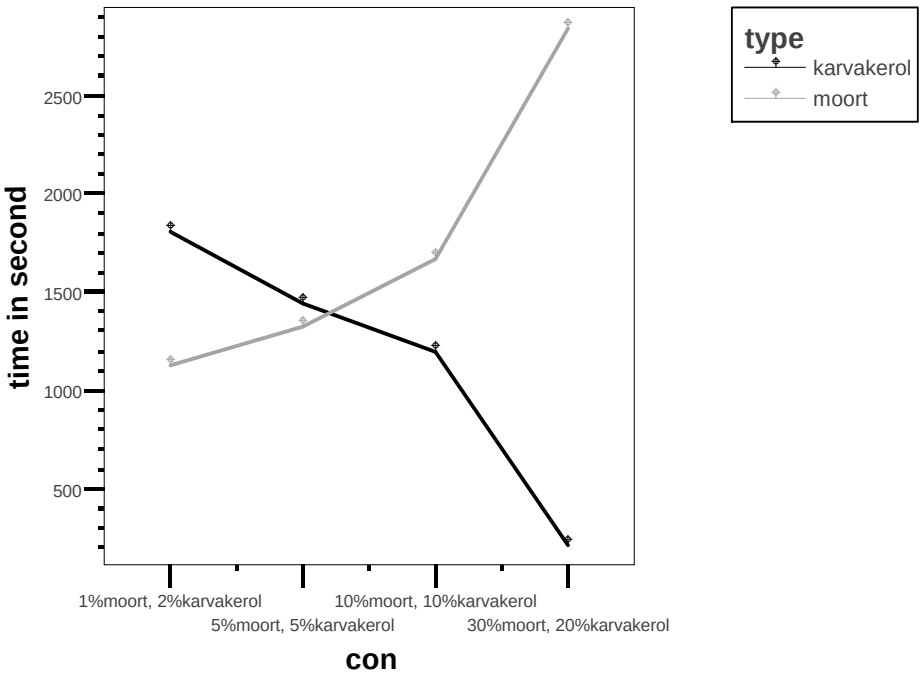

Figure 1: Knock down time of *Anopheles stephensi* after continuous exposure to treated nets with different concentrations of moort and karvakerol essential oils

-Bioassay test results (mortality 24 h after 3-minute exposure test)

Bioassay test results (mortality 24 h after 3-minute exposure test) are shown in Figure 2. As indicated in Figure 2, with increasing concentrations of Moort essential oil, there was no significant increase in mortality percentage for *Anopheles stephens*i (P = 0.716), whereas by increasing the concentration of karvakerol essential oil, percentage of deaths and mortality was border line increased for *Anopheles stephens*i (P = 0.073).

-Comparison the percentage of mortality between Moort and karvakerol essential oils

To compare mortality rate between the two essential oils regression model was used, and thus two essential oils were significantly (P <0.001) different in mortality rate 24 h after 3-minute exposure test. In other words, the percentage of deaths for *Anopheles stephens*i which were in contact with karvakerol for 3 minutes were significantly more than the Moort essential oil (P <0.001) .

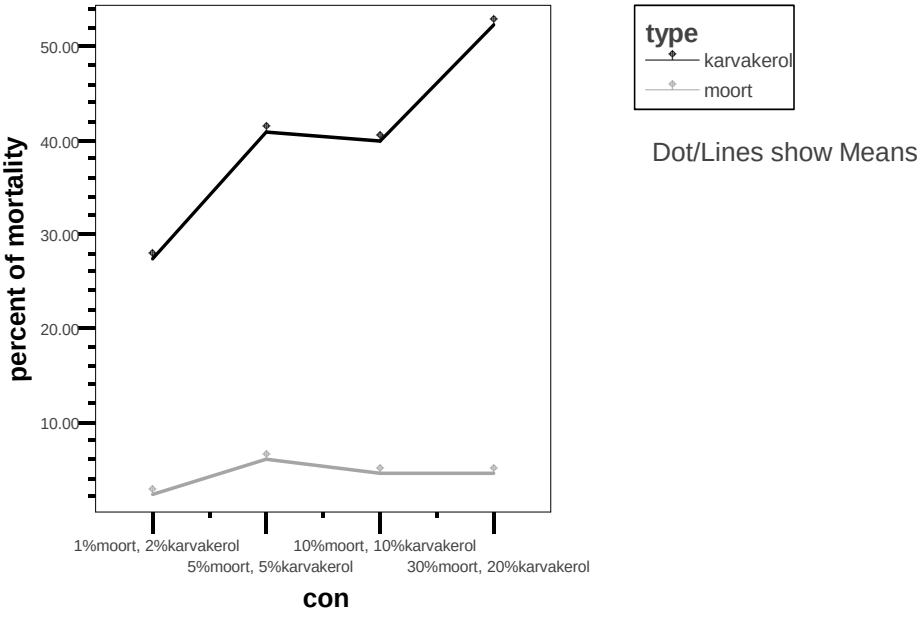

Figure 2: Percentage mortality of *Anopheles stephensi* 24 hours after 3 min contact via mosquito nets impregnated with different concentrations of moort and karvakerol essential oils

DISCUSSION AND CONCLUSION

The findings of the present study show that there was 50% mortality of *Anopheles stephens*i 24 h after 3-minute exposure to 20% concentration of karvakerol in comparison to 5% mortality of *Anopheles stephens*i 24 h after 3-minute exposure to 30% concentration of moort. So it has been concluded that even with higher concentrations of moort essential oil (above30%) in mosquito nets, there was no insecticide efficacy in nets.

Karvakerol treated nets with concentration more than 10% after impregnating have strong odor and repellent activity and cause breathing difficulty , and have respiratory symptoms such as coughing and throat irritation and itching that actually sleeping under mosquito nets is impossible. At concentrations as low as 5% or even less, sleeping under the mosquito nets are not recommended. Mortality rate under 30% for *Anopheles stephens*i in bioassay tests (MOR) is not acceptable for apply on mosquito nets that had been impregnated with a concentration of karvakerol essential oil in 2% concentration. In addition, sleeping under these nets are also not recommended. Due to contact with the body during sleeping under nets, the karvakerol oil concentration more than 10% can cause skin problems such as redness, burning and itching. The higher the concentration the more severe complications occur, but after hanging up to the mosquito net
after 2 to 3 hours , all symptoms disappear completely.

Knock down time of *Anopheles stephens*i increases proportionally with increasing concentrations of moort essential oil. This increasing may be is due to repellency properties of the essential oil. Repellency effect of moort and karvakerol essential oils have been investigated [17].

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