J. Crop Prot. 2013, 2 (1): 23-31 _____

Insecticidal and repellent activities of *Artemisia khorassanica*, *Rosmarinus officinalis* and *Mentha longifolia* essential oils on *Tribolium confusum*

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Abstract: The essential oils of aerial parts of three medicinal plants *Artemisia khorassanica* Podl., *Rosmarinus officinalis* L. and *Mentha longifolia* L. were isolated by hydrodistillation and investigated for their toxicity and repellency against *Tribolium confusum* Jacquelin du Val. at 27 ± 1 ºC and 60 ± 5% RH in darkness. The mortality of the adults increased with concentration from 185 to 1111 µl/l air and with exposure time from 9 to 24 h. A concentration of 185 µl/l air and exposure time of 24 h was sufficient to obtain nearly 100% kill of the adults in all of the three essential oils tested. At the highest concentration (1111 µl/l air) *R. officinalis* oil caused 15% mortality for an exposure time of 6-h. whilst, the oil of *A. khorassanica* resulted in 1% mortality at the same exposure time. The oil of *A. khorassanica* at 1111 µl/l air caused 81% mortality for 12-h exposure time. No significant differences were observed between the lethal time (LT_{50}) values at essential oil concentrations of 741 and 1111 µl/l air. For 24-h exposure time, the LC_{50} values of *A. khorassanica, R. officinalis* and *M. longifolia* essential oils were estimated to be 22.45, 22.14 and 39.96 µl/l air respectively. Based on LC_{50} values, adults of *T. confusum* showed similar susceptibility to the *A. khorassanica* and *R. officinalis* oils, but *M. longifolia* oil proved to be less toxic. In contrast to their low fumigant properties, the essential oil of *M. longifolia* had significantly higher repellency to *T. confusum* adults than did the other two.

Keywords: Fumigant toxicity; Repellent; *Artemisia khorassanica; Rosmarinus officinalis; Mentha longifolia; Tribolium confusum*; essential oil

Introduction

Control of insect pest infestation in storage may cause special problems on stored products. In many storage systems, methyl bromide and phosphine are the most economical fumigants for management of stored-grain insect pests. EPA (2001) has proposed elimination of the production of methyl bromide by 2005 because of its ozone depletion potential. Additionally, some stored-product insects are found to have developed resistance to methyl bromide and phosphine (Subramanyam and Hagstrum, 1995; Champ and Dyte, 1977). These problems due to conventional insecticides have strongly demonstrated the need for the development of alternative products such as natural extracts derived from plants. Many types of spices and herbs are known to possess insecticidal activities (Tripathi *et al*., 1999) especially in the form of essential oils (Shaaya *et al*., 1991). They do not leave residues harmful to the environment and have lower toxicity to mammals (Duke, 1985). Among the medicinal plants, *Artemisia vulgaris*

Handling Editor: Dr. Khalil Talebi Jahromi

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Received: 19 June 2012; Accepted: 13 January 2013

[DOR: 20.1001.1.22519041.2013.2.1.7.5]

L., *Artemisia aucheri* Boiss., *Artemisia scoparia* Waldst et Kit and *Artemisia sieberi* Besser have been reported to be repellent and toxic to *Tribolium castaneum* (Herbst) (Wang *et al*., 2006; Shakarami *et al*., 2003; Negahban *et al*., 2006, 2007). The essential oil of *R. officinalis* (Rosemary) showed insecticidal activity against *Sitophilus oryzae* L. and *T. castaneum* (Shaaya *et al*., 1997; Lee *et al*., 2002). Some studies have assessed the fumigant toxicity of *Mentha piperita* L. and *Mentha arvensis* L. against T. *castaneum* (Aggarwal *et al*., 2001; Lee *et al*., 2002).

The present study was conducted to investigate the potential effects of essential oils extracted from the aerial parts of *A. khorassanica, R. officinalis* and *M. longifolia* on the confused flour beetle, *T. confusum*. Although a number of researchers have shown the effectiveness of essential oils and their constituents against adults of various stored grain insects, there are no reports on toxicity of *A. khorassanica* and *M. longifolia* on *T. confusum*

Materials and Methods

Plant materials

Aerial parts *of A. khorassanica, R. officinalis* and *M. longifolia* were collected at full flowering stage from Pole Chehel Dokhtar (Khorassan province), Karaj (Tehran province) and Youssh and Baladeh (Mazandaran province), from August to November 2007 and authenticated by Mozaffarian, Herbarium Department, Research Institute of Forests and Rangelands of Iran.

Plant parts were let dry naturally on laboratory benches at room temperature (23-27 ºC) for 7 days until they were crisp. The dried materials were stored at – 24 ºC until needed and then hydrodistilled to extract their essential oils.

Extraction of essential oils

Plant materials were milled into fine powder using a milling machine. Fifty grams of the plant samples to which 600 ml distilled water was added, were subjected to hydrodistillation for 4 h using a modified Clevenger-type apparatus. The essential oils were dried over anhydrous sodium sulfate and were stored in glass tubes at – 4 ºC in refrigerator, until they were used. The oils yielded by A. *khorassanica*, *R. officinalis* and *M. longifolia* were 0.322, 0.565 and 0.533% w/w respectively on dry weight basis.

Insect rearing

Tribolium confusum was reared on wheat flour mixed with yeast (10:1 w/w) that was covered by a fine mesh cloth for ventilation. The cultures were maintained in the dark in a growth chamber set at 27 ± 1 ºC and 65 ± 5% RH. One to seven days old adults were used for fumigation toxicity tests and repellency bioassays. All experimental procedures were carried out at the same environmental condition as those of the culture.

Fumigant toxicity tests

To determine the fumigant toxicity of essential oils, filter papers (2 cm diameter) were impregnated with oil at doses calculated to give equivalent fumigant concentrations ranging from 185 to 1111 µl /l air for all essential oils tested. Afterwards, the impregnated filter paper was attached to the under surface of the screw cap of a glass vial (volume 27 ml). The caps were screwed tightly on to the vials containing 10 (1 to 7 days old) adult insects each. Each concentration and control was replicated five times. Adults were exposed to each essential oil at concentration of 185.2, 370.4, 740.7 and 1111.1 µl /l air for 3, 6, 9, 12 and 24 h. Mortality for each concentration and exposure time was checked independently. Insects were presumed dead if they remained immobile and no leg or antennal movements were observed. Analysis of variance (ANOVA) was used to compare treatments in each experiment. Differences between the means were established by Tukey's test at 5% level (SAS Institute, 1997).

Another experiment was designed to determine median effective time to kill 50% of adults (LT_{50} values) at 740.7 and 1111.1 µl/l air

[DOR: 20.1001.1.22519041.2013.2.1.7.5]

of the respective oils. The mortality was assessed by direct observation of the insects every hour for up to end point of mortality. Time-mortality data for each experiment were analyzed by Mathematica software 6.0 (Throne *et al*., 1995) with time as the explanatory variable to derive estimated hours for 50% adult mortality.

After initial dose setting experiments another set of bioassay tests was designed to assess 50% lethal concentration (LC_{50}) as described by Negahban et al. (2006). *Tribolium confusum* were exposed to the essential oil of *A. khorassanica* at 10.71, 17.85, 25, 32.14 and 39.28 µl/l air for 24 h, without solvent. The concentrations of 7.14, 14.28, 21.42, 28.5, 35.71 µl/l air were used for *R. officinalis*, followed by 9.25, 18.51, 27.77, 37.03, 46.29, 55.55, 64.81 µl/l air for *M. longifolia*. Control insects were kept without any essential oil. Each test was replicated five times. After 24-h exposure period, the number of dead and live insects in each bottle was counted. Probit analysis (Finney, 1971) was used to estimate LC_{50} values with their confidence limits by SAS 6.12 (SAS Institute, 1997). Significant differences between LC_{50} values were determined by estimation of confidence intervals of the relative median potency using SPSS version 18.

Repellency bioassay

Repellency was assessed as described by Negahban *et al*. (2006). The repellency tests were consisted of two clear plastic chambers (65 ml volume) joined to either side of a central main chamber with the same size by a small tubing (2 cm long and 3 mm in diameter). Test solutions were prepared by dissolving 50, 75 and 100 µl of *A. khorassanica*, *R. officinalis* and *M. longifolia* essential oils in 1 ml acetone. Each solution was applied on 30 seeds of wheat. In the control, the food was treated with acetone only. The treated and control seeds were air-dried under a fan for 30 min to evaporate the solvent completely, and placed in the center of treated and control chambers respectively. Four replications were used for each concentration. Fifty nonsexed adults (1-7 days old) of flour beetles were introduced into the center of each main chamber. Central chamber was covered by plastic screen but the treated and control chambers were covered by lids and the whole set up was left in darkness. After 4 h, the number of beetles at each chamber was counted and the percentage repellency (PR) values were computed using the formula of Liu *et al*. (2006):

$$\%PR = \left(\frac{C-E}{T}\right)\times 100$$

where C is the number of insects in control, E is the number of insects in oil treated chamber and T is the number of total released insects. Analysis of variance (ANOVA) was used to determine the effect of essential oil concentrations on repellency. Arcsine square-root transformation was performed on percentage repellency. Following a significant ANOVA, differences between the means were determined by Tukey's test at 5% level.

Results

Fumigant toxicity

Bioassay tests were conducted to determine if the insecticidal activity of *A. khorassanica*, *R. officinalis* and *M. longifolia* oils against *T. confusum* adults was attributable to their fumigant action. No dead insects were observed in controls. In all cases, a significant difference in mortality of the adults was observed as oil concentration and exposure time was increased. The mortality increased with increasing concentration from 185.2 to 1111.1 µl/l air and exposure time from 3 to 24 h. In all concentrations, *A. khorassanica* and *R. officinalis* oils resulted in more than 50% mortality after 12 h exposure time, which indicated that lethal time for 50% adult mortality could be a range between 9 and 12 h. At the highest concentration (1111.1 µl/l air), *R. officinalis* oil resulted in 15% adult mortality for 6-h exposure time, whilst the oil of *A. khorassanica* caused 1% mortality at the same exposure time. The oil of *A. khorassanica at* 1111 µl/l air caused 81% mortality for 12-h

[DOR: 20.1001.1.22519041.2013.2.1.7.5]

exposure time. Generally, bioassay tests indicated that *A. khorassanica* oil had higher fumigant toxicity than the other two essential oils. All tested essential oils at all concentrations revealed approximately 100% of adult mortality for 24-h exposure time (Table 1).

Increasing the essential oil concentration resulted in slight decrease in the time needed to kill 50% adult. Lethal time$_{50}$ (LT_{50}) value of *A. khorassanica* decreased from 10.03 h [95% confidence limits (CL) = 9.19-10.87] at concentration of 741 µl/l air to 9.63 h (95% CL= 8.78-10.47) at concentration of 1111 µl/lair. Increasing the concentrations of the oil, the LT_{50} value of *R. officinalis* decreased slightly from 10.77 h (95% CL = 9.70-11.84) to 9.98 h (95% CL = 8.83-11.13) but it was not significant, since 95% confidence limits overlapped. It could be concluded that LT_{50} values in doses tested do not seem to be dose-dependent (Table 2).

Table 1 Percent mortality of *Tribolium confusum* adults exposed to different concentrations of essential oil from *Artemisia khorassanica*, *Rosmarinus officinalis* and *Mentha longifolia* for various exposure periods.

Concentration (µl/l air)	Exposure time (h)	% mortality (Mean ± SE)[1]		
		A. khorassanica	***R. officinalis***	***M. longifolia***
185.2	3	0g	0f	0f
	6	0g(A)	2.00 ± 1.22f(A)	0f(A)
	9	13.00 ± 1.22f(A)	11.00 ± 2.92e(A)	2.00 ± 1.22f(B)
	12	59.00 ± 1.00d(A)	53.00 ± 4.64bc(A)	19.00 ± 2.45de(B)
	24	98.00 ± 2.00a(A)	97.00 ± 2.00a(A)	97.00 ± 2.00a
370.4	3	0g	0f	0f
	6	0g(A)	4.00 ± 2.45f(A)	0f(A)
	9	17.00 ± 1.22ef(A)	20.00 ± 1.58de(A)	15.00 ± 2.24e(A)
	12	66.00 ± 4.00cd(A)	68.00 ± 1.22bc(A)	26.00 ± 1.87bcd(B)
	24	99.00 ± 1.00a(A)	100a(A)	100a
740.7	3	0g	0f	0f
	6	0g(B)	10.00 ± 2.74e(A)	0f(B)
	9	21.00 ± 4.30ef(A)	28.00 ± 4.06d(A)	18.00 ± 2.00e(A)
	12	72.00 ± 4.64bc(A)	52.00 ± 3.39b(B)	30.00 ± 2.24bcd(C)
	24	100a	100a	100a
1111.1	3	0g	0f	0f
	6	1.00 ± 1.00g(B)	15.00 ± 2.24de(A)	0f(B)
	9	24.00 ± 1.87e(B)	44.00 ± 5.79c(A)	25.00 ± 2.74cd(B)
	12	81.00 ± 1.87b(A)	69.00 ± 2.45b(B)	36.00 ± 1.00b(C)
	24	100a	100a	100a

1. Means followed by the same lower-case letters in a column and upper-case letters in a row are not significantly different using Tukey's test at 5% level.

Table 2 Lethal time$_{50}$ (LT_{50}) values of the two highest concentrations of oils against *Tribolium confusum* adults.

Plant species	Concentration (µl/l air)	LT_{50}[1] (h)	Slope ± SE	Degree of freedom	Chi-square (χ^2)
A. khorassanica	741	10.03 (9.19 - 10.87)	0.03 ± 0.31	9	12.59
	1111	9.63 (8.78 - 10.47)	0.03 ± 0.31	10	11.27
R. officinalis	741	10.77 (9.70 - 11.84)	0.02 ± 0.24	10	14.67
	1111	9.98 (8.83 - 11.13)	0.02 ± 0.23	11	16.45
M. longifolia	741	12.01 (11.15 - 12.87)	0.03 ± 0.30	9	11.59
	1111	11.23 (10.38 - 12.08)	0.03 ± 0.30	10	11.93

1. 95% lower and upper confidence limits are shown in parenthesis.

[DOR: 20.1001.1.22519041.2013.2.1.7.5]

The results of probit analysis showed that *T. confusum* adults were comparatively more susceptible to *A. khorassanica* (LC_{50} = 22.45 µl/l air) and *R. officinalis* oils (LC_{50} = 22.14 µl/l air) than to *M. longifolia* oil (LC_{50} = 39.96 µl/l air) (Table 3). Moreover, as shown in table 4, relative median potency of *A. khorassanica* and *R. officinalis* oils versus *M. longifolia* oil was significant, confirming the more tolerance of the *T. confusum* to *M. longifolia* than the other two oils.

Repellency

In the present study, the repellency of *A. khorassanica*, *M. longifolia* and *R. officinalis* were evaluated against *T. confusum* adults.

The essential oil of *M. longifolia* strongly repelled the flour beetle in all concentration except 50 µl/l air acetone. Therefore, repellency of *M. longifolia* was significantly higher than *A. khorassanica* and *R. officinalis* oils to the *T. confusum* adults with overall repellency (*A. khorassanica*: F = 22.56, df = 2 and P < 0.001; *R. officinalis*: F = 22.10, df = 2 and P < 0.001; *M. longifolia*: F = 54.59, df = 2 and P < 0.001). In general, the repellency increased with increasing concentration of essential oils in all cases (Table 5).

Table 3 The LC_{50} values of *Artemisia khorassanica*, *Rosmarinus officinalis* and *Mentha longifolia* oils against *Tribolium confusum* adults resulting from 24-h laboratory fumigations.

Plant species	LC_{50}[1] (µl/l air)	Slope ± SE	Degree of freedom	Chi-square (χ^2)
A. khorassanica	22.45 (19.10 - 26.02)	0.47 ± 2.77	3	0.446
R. officinalis	22.14 (18.61 - 26.71)	0.43 ± 2.43	3	0.561
M. longifolia	39.69 (32.97 - 50.19)	0.31 ± 1.77	5	0.404

1. 95% lower and upper confidence limits are shown in parenthesis

Table 4 Relative potency of the LC_{50} values of *Artemisia khorassanica*, *Rosmarinus officinalis* and *Mentha longifolia* oils tested on *Tribolium confusum* adults

Plant A	Plant B	Relative potency (LC_{50} A/ LC_{50}B)	95% confidence limits
A. khorassanica	*R. officinalis*	1.014ns	0.767 - 1.328
A. khorassanica	*M. longifolia*	0.566*	0.412 - 0.767
R. officinalis	*M. longifolia*	0.558*	0.400 - 0.776

*: significant
ns: non-significant

Table 5 Percent repellency (mean ± SE) of the essential oils from *Artemisia khorassanica*, *Rosmarinus officinalis* and *Mentha longfolia* on *Tribolium confusum* adults using treated filter paper test.

Plant species	Concentration of essential oil (µl /food)		
	50	75	100
A. khorassanica	2.00 ± 0.82b (B)	9.00 ± 1.00b (A)	13.00 ± 0.58b (A)
R. officinalis	9.00 ± 0.58a (B)	11.50 ± 0.50b (B)	16.50 ± 1.26b (A)
M. longifolia	7.50 ± 0.50a (B)	29.00 ± 3.00a (A)	24.00 ± 0.82a (A)

1. Means followed by the same letter in a column (small letters) and within a row (capital letters) are not significantly different using Tukey's test at p < 0.01.

[DOR: 20.1001.1.22519041.2013.2.1.7.5]

Discussion

In this study, three essential oils were tested for their fumigant toxicity against adults of *T. confusum*. The insecticidal activity varied with plant-derived material, concentration and exposure time. The chemical constituents of many plant essential oils are mainly composed of monoterpenoids (Coats *et al*., 1991; Regnault-Roger and Humraoui, 1995; Ahn *et al.*, 1998). Monoterpenoid compounds have been considered as potential pest control agents because they are acutely toxic to insects and possess repellent (Watanabe *et al.*, 1993) and antifeedant properties (Hough-Goldstein, 1990). It has been reported that α-thujone (43.4%), β-thujone (16.2%) and camphor (12.6%) are the major constituents of A. *khorassanica* essential oil (Barazandeh, 2003). Therefore the toxic effects of *A. khorassanica* in part, could be attributed to such monoterpenoid compounds. The monoterpene camphor has been reported to possess insecticidal activity against a number of stored product beetles (Obeng-Ofori *et al.*, 1998). GC and GC/MS analyses of rosemary essential oil have shown that 1,8 cineole (34.5%), α-pinene (15.5%), β-pinene (10.1%), camphor (8.4%) and camphene are the major constituents of the oil. 1,8 cineole has been reported as the most toxic fumigant against *Sitophilus oryzae* L. in rosemary essential oil (Lee *et al*., 2004). α-pinene has been reported to be toxic to *T. confusum* (Ojimelukwe and Alder, 1999). The chemical constituents of *M. longifolia* oil, collected from Tehran province extracted by the same conditions as described in this study were comprised of carvone (61.8%) and limonene (19.4%) as the major constituents (Monfared, 2002). As insecticidal activity of limonene has been demonstrated by Coats *et al*. (1991), the fumigant toxicity of the *M. longifolia* oil could in part be attributed to this compound

Based on the LC_{50} values of *A. khorassanica* and *R. officinalis* oils obtained in this study, it could be estimated that the aforementioned essential oils are more toxic that the essential oil of *Carum copticum* C. B. Clarke (Sahaf *et al*., 2007).

Our results did not show that fumigant toxicity is necessarily correlated with the high repellency. As Talukder and Howse (1993) reported that in spite of high toxicity of Pithraj *Aphanamixis polystachya* Wall and Parker seeds against *Callosobruchus chinensis* (L.), it exhibited a weak repellency. Findings of this study suggest that there may be different modes of action of the oil on insecticidal activity and repellency.

Our observations showed that fumigant activity of *A. khorassanica*, *R. officinalis* and *M. longifolia* oil was characterized by hyperactivity, convulsion, paralysis and quick knock down followed by death. Needless to say, that there is an urgent need for environmentally safe alternatives to conventional fumigants phosphine and methyl bromide, for the control of stored product insects, . As fumigants play major role in insect pest control in storage, there is a global interest in alternative strategies including development of plant products such as essential oils and their constituents. Therefore, large quantities of plant materials have to be processed in order to obtain essential oil in quantities sufficient for commercial scale tests (Tunc *et al.*, 2000). This should encourage the breeding or selection of plant varieties that produce such compounds in geater amounts. Synthetic production of these compounds may also be an option in order to gain enough material for practical use as plant protection products. It is also necessary to generate toxicity data to examine if oil of these essential oils has nontarget toxicity and whether consumer of the products stored in its presence can detect any residual volatile oils. In conclusion essential oil of *A. khorassanica*, *R. officinalis* and *M. longifolia* provided promising results to be used as a stored product protectant against insect attack.

Increased public concern over the residual toxicity of insecticides applied to stored grain, the occurrence of resistant insect strains and the necessary precautions to work with traditional insecticides calls for new approaches to control stored-product insect pests (Yildirim *et al*., 2001). Essential oils of medicinal plants such as

[DOR: 20.1001.1.22519041.2013.2.1.7.5]

Artemisia, Rosemary and mints have extensive use as food supplements, flavors, perfumes, decongestants and antiseptics to chemical. These plants may have potential as alternative fumigants, because they pose fewer risks to human health and less harmful effects on environment.

Acknowledgements

We gratefully acknowledge Dr Valiollah Mozafarian who confirmed the identification of the plant samples. This research was partly supported by a grant from the center of Excellence for the Integrated Pests and Diseases Management of Oil Crops of Iran, which is gratefully appreciated.

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[DOR: 20.1001.1.22519041.2013.2.1.7.5]

خواص حشره‌کشی و دورکنندگی اسانس‌های *Artemisia khorassanica*، *Rosmarinus officinalis* و *Mentha longifolia* روی *Tribolium confusum*

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دریافت: ۳۰ خرداد ۱۳۹۱؛ پذیرش: ۲۴ دی ۱۳۹۱

چکیده: اسانس اندام‌های هوایی سه گیاه دارویی درمنه خراسانی *Artemisia khorassanica* Podl.، رزماری *Rosmarinus officinalis* L. و پونه کوهی *Mentha longifolia* L. به روش تقطیر با آب استخراج شد و سمیت تنفسی و دورکنندگی آن روی شپشه آرد *Tribolium confusum* Jacquelin du Val. در دمای ۱ ± ۲۷ درجه سلسیوس و رطوبت نسبی ۵ ± ۶۰ درصد در تاریکی مورد بررسی قرار گرفت. مرگ و میر حشرات کامل با افزایش غلظت از ۱۸۵ تا ۱۱۱۱ میکرولیتر بر لیتر هوا و پس از ۹ تا ۲۴ ساعت، قرار گرفتن در معرض اسانس افزایش یافت. غلظت ۱۸۵ میکرولیتر بر لیتر هوا و ۲۴ ساعت قرارگیری در معرض اسانس تقریبا، موجب مرگ و میر ۱۰۰ درصد از حشرات کامل شد. در بالاترین غلظت (۱۱۱۱ میکرولیتر بر لیتر هوا) اسانس رزماری پس از ۶ ساعت، موجب مرگ و میر ۱۵ درصد از حشرات شد، درحالی‌که اسانس درمنه خراسانی در زمان مشابه موجب مرگ و میر ۱ درصد از حشرات کامل شد. اسانس درمنه خراسانی در غلظت ۱۱۱۱ میکرولیتر بر لیتر هوا طی ۱۲ ساعت، مرگ و میر ۸۱ درصد از حشرات را رقم زد. هیچ اختلاف معنی‌داری بین LT_{50} به‌دست آمده از اسانس‌ها در غلظت‌های ۷۴۱ و ۱۱۱۱ میکرولیتر بر لیتر هوا مشاهده نشد. مقادیر LC_{50} درمنه خراسانی، رزماری و پونه کوهی در ۲۴ ساعت قرارگیری در معرض اسانس، به‌ترتیب ۲۲/۴۵، ۲۲/۱۴ و ۳۹/۹۶ میکرولیتر بر لیتر هوا به‌دست آمد. براساس مقادیر LC_{50}، حشرات کامل شپشه آرد حساسیت مشابهی را به اسانس درمنه خراسانی و رزماری از خود نشان دادند، اما اسانس پونه کوهی سمیت کمتری از خود نشان داد. علی‌رغم پایین بودن خواص سمیت تنفسی اسانس پونه کوهی، خاصیت دورکنندگی آن نسبت به سایر اسانس‌های مورد مطالعه بالاتر بود.

واژگان کلیدی: اسانس، سمیت تنفسی، خواص دورکنندگی، *Artemisia khorassanica*، *Rosmarinus officinalis*، *Mentha longifolia* و *Tribolium confusum*

[DOR: 20.1001.1.22519041.2013.2.1.7.5]