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Essential Oil Isolated from Iranian Yarrow as a Bio-rational Agent to the Management of Saw-toothed Grain Beetle, *Oryzaephilus surinamensis* (L.)

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머리대장가는납작벌레의 합리적 방제 물질로 이란 서양가새풀 정유의 살충효과 평가

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ABSTRACT: Overuse of synthetic pesticides caused negative side-effects such as environmental contamination, development of insect pests' resistance, and effects on non-target organisms. Plant origin substances without/or with low mammalian toxicity have been considered as promising alternatives to the synthetic pesticides. Fumigant toxicity of the essential oil of Iranian Yarrow, *Achillea millefolium* L., was investigated against a cosmopolitan stored-product insect pest: saw-toothed grain beetle (*Oryzaephilus surinamensis* L.). Chemical profile of this essential oil was studied by Gas Chromatography-Mass Spectrometry. Tested concentrations were significantly effective to the mortality of insect pest. A positive correlation between essential oil concentrations and pest mortality were realized. LC50 value (lethal concentration needed to 50% mortality) was achieved as 17.977 (16.195 ± 20.433) µl/l air. The main components were 1,8-Cineole (13.17%), nerolidol (12.87%), α-cubebene (12.35%), artemisia ketone (6.69%), α-terpineol (5.27%), alloaromadendrene oxide (4.71%) and borneol (3.99%). Terpenic compounds including monoterpene hydrocarbons (8.19%), monoterpenoids (44.23%), sesquiterpene hydrocarbons (21.69%) and sesquiterpenoids (22.24%) were 96.35% of the total identified compounds. Results indicated that the terpene-rich *A. millefolium* essential oil may be considered as a safe bio-agent in the *O. surinamensis* management.

Key words: *Achillea millefolium*, Essential oil, Fumigant toxicity, *Oryzaephilus surinamensis*

초 록: 유기합성 농약의 과다사용은 환경오염, 살충제 저항성 발달, 비표적 생물에 대한 영향 등 부작용의 원인이 되고 있다. 유기합성 농약의 대체약제로 포유동물에 저독성인 식물기원 물질이 각광을 받게 되었다. 이란 서양가새풀(*Achillea millefolium* L.) 정유성분의 훈증독성은 국제적 저장작물 해충인 머리대장가는납작벌레(*Oryzaephilus surinamensis* L.)의 방제제로 연구된 바 있다. 이 식물 정유의 화학적 성분을 가스크로마트 그래피(MS)를 이용하여 분석하였다. 살충실험 결과 처리농도에 따라 유의한 살충률을 나타냈다. 처리농도와 살충률 간 양의 상관관계가 있었다. 반치사농도(LC50)는 17.977 µl/L 이었다. 주요 성분은 1,8-Cineole (13.17%), nerolidol (12.87%), α-cubebene (12.35%), artemisia ketone (6.69%), α-terpineol (5.27%), alloaromadendrene oxide (4.71%) 및 borneol (3.99%) 이었다. 전체 동정된 화합물의 96.35%는 Terpenic 화합물로 monoterpene hydrocarbons (8.19%), monoterpenoids (44.23%), sesquiterpene hydrocarbons (21.69%) 및 sesquiterpenoids (22.24%)를 포함하고 있었다. 본 결과는 terpene이 풍부한 서양가새풀 정유가 머리대장가는납작벌레의 안전한 생물농약으로 고려될 수 있음을 보여주었다.

검색어: 서양가새풀, 정유, 훈증독성, 머리대장가는납작벌레

Saw-toothed grain beetle (*Oryzaephilus surinamensis* L.) as one of the cosmopolitan insect pest of processed and packaged commodities, can attack many stored-products including cereals, dried fruit and oilseeds. The larvae and adults are external feeders and are very active. It has a worldwide distribution even in cold areas (Rees, 2007). The life cycle of this pest can be completed within 20 days at 33℃ and 80%

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relative humidity (Beckel et al., 2007).

The utilization of some chemicals such as sulfuryl fluoride, methyl bromide and phosphine has been developed for management of stored-product insect pests but their abuse has resulted in negative side-effects such as threat to non-target organisms and environmental contamination (Jeyasankar and Jesudasan, 2005; Damalas and Eleftherohorinos, 2011; Carvalho, 2017). Further, there are several documents related to the resistance of stored product pests including *O. surinamensis* to the phosphine and some other chemical fumigants (Daglish, 2004; Collins et al., 2005; Pimentel et al., 2008). Therefore, search for safe and eco-friendly pesticides is necessary.

Plant essential oils, with low persistence in the environment, have several volatile compounds such as terpenic and aromatic constituents (Bakkali et al., 2008) and their toxicity to the mammals is low (Cloyd et al., 2009; El Asbahani et al., 2015). Recent studies indicated that they are effective against different orders of pests and can be considered as safe and available bio-pesticides (Isman and Grieneisen, 2014; Ebadollahi and Jalali-Sendi, 2015).

Achillea species from Asteraceae family with aromatic leaves and flowers become globally known medicinal herbs and subjected to numerous pharmacological and biological studies (Nemeth, 2005; Nemeth and Bernath, 2008). Yarrow, *A. millefolium* L., has been showed significance pharmacological activities and become a most important among *Achillea* species (Csupor-Loffler et al., 2009; Saeidnia et al., 2011; Demirci et al., 2017).

Susceptibility of *O. surinamensis* to the essential oils was considered in the recent researches, in which the essential oils of *Agastache foeniculum* (Pursh) Kuntze (Ebadollahi et al., 2010), *Artemisia argyi* Levl et Vant (Lü et al., 2011), *Eucalyptus dundasii* Maiden (Parsia-Aref et al., 2015), and *Ocimum gratissimum* L. (Ogendo et al., 2008) presented significant toxicity. Based on the ongoing world-wide researches to the screening of plant-derived agents, the main objective was to assess the insecticidal effect of essential oil isolated from Iranian *A. millefolium* against *O. surinamensis*. Further, evaluation of the chemical composition of this oil was the other aim.

Materials and Methods

Essential oil extraction and analysis

Aerial parts of *A. millefolium* were collected from Sardabeh county, Ardabil province, Iran. Flowers were separated after air-drying at room temperature (27 ± 2℃) in 10 days. Samples were crushed into powder using an electric grinder and were hydro-distilled using a Clevenger apparatus. Essential oil extraction conditions were: 100 g plant material, 2000 ml distilled water and 180 min distillation period.

The chemical components of essential oil isolated from *A. millefolium* was investigated by a Hewlett-Packard Gas Chromatography (HP, Palo Alto, CA) equipped with a mass sensitive detector (5975C) according to the our recent study: Ebadollahi et al (2017). Identification was made through comparison of their patterns and coincidence fragmentation with the standard spectra present in the library of the instrument (Adams, 2004): Wiley 7n.1 mass computer library and NIST (National Institute of Standards and Technology).

Rearing of insect pest

Parent adults of *Oryzaephilus surinamensis* were collected from contaminated rice grains of Ardabil city, Iran. It was reared in 1-liter glass containers comprising the wheat flour. Containers were covered with a mesh cloth for ventilation. Adult insects with 1-3 days old were used for bioassays. Insects were kept in the dark in an incubator set at 27 ± 2˚C and 65 ± 5% Relative Humidity.

Insecticidal activity

Based on a preliminary experiment, concentrations of 13.33, 15.23, 17.37, 19.85 and 22.67 µl/l air were selected for evaluation of the toxicity of *A. millefolium* essential oil on the adults of *O. surinamensis*. Required concentrations were poured on 2 × 3 cm filter papers (Whatman No. 1) which were positioned at the bottom of 750 ml glass containers. Twenty adult insects (1-3 days old) were located in the 3.5 × 5 cm tubes covered with cloth mesh. In general, 120 adults' insects were used for each replication. Tubes were then hung at the midpoint of containers and air-tightly closed. The same procedures were considered for control groups without oil concentrations. Four replications were made and the insect mortality was determined after 24 h exposure time.

Statistical analysis

Data were analyzed by ANOVA (analysis of variance) with SPSS software (Version 16) and differences were confirmed by the results of Tukey's test at $P < 0.05$. Lethal concentrations were calculated through Probit analysis and linear regression analysis was accomplished to designate insect mortality in contradiction of oil concentrations.

Results

Chemical composition of essential oil

Chemical components of *A. millefolium* essential oil are shown in Fig. 1 and Table 1. Sixty-two components representing 99.81% of the total oil were recognized, in which 1,8-Cineole (13.17%), nerolidol (12.87%), α-cubebene (12.35%), artemisia ketone (6.69%), α-terpineol (5.27%), alloaromadendrene oxide (4.71%) and borneol (3.99%) were the main components. Terpenic compounds including monoterpene hydrocarbons (8.19%), monoterpenoids (44.23%), sesquiterpene hydrocarbons (21.69%) and sesquiterpenoids (22.24%) were 96.35% of the total identified compounds (Fig. 1 and Table 1).

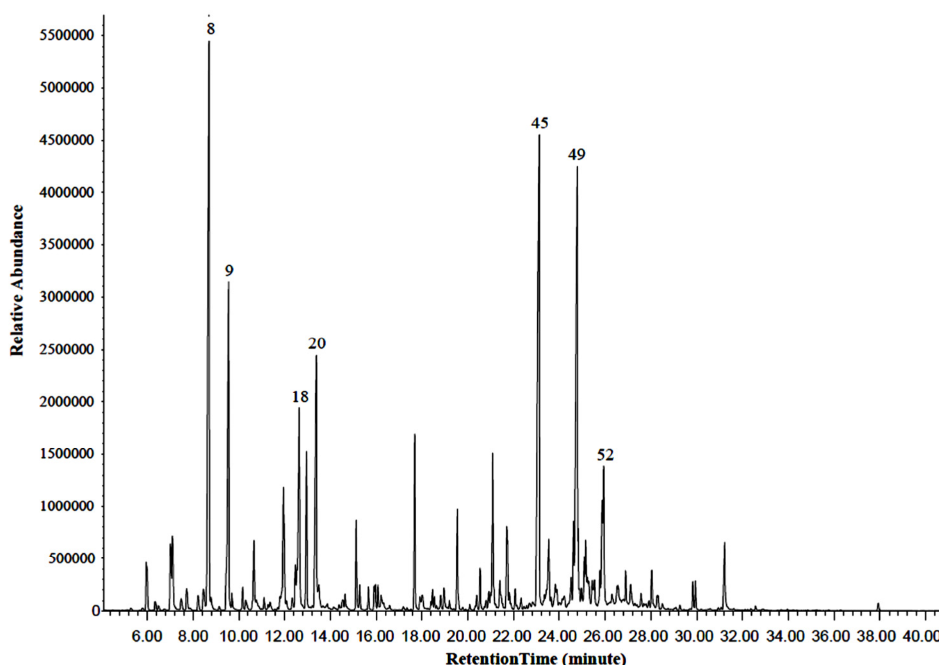

Fig. 1. GC-MS chromatogram of the essential oil isolated from *Achillea millefolium*.

Table 1. Chemical composition of essential of Iranian *Achillea millefolium*

Compound	Retention time (minute)	Percentage	Formula
α-Pinene	5.976	1.06	$C_{10}H_{16}$
Camphene	6.357	0.18	$C_{10}H_{16}$
Sabinene	7.062	2.78	$C_{10}H_{16}$
2,3-Dehydro-1,8-cineole	7.489	0.27	$C_{10}H_{16}O$
Yomogi alcohol	7.729	0.44	$C_{10}H_{18}O$
α-Terpinene	8.227	0.31	$C_{10}H_{16}$

Table 1. Continued

Compound	Retention time (minute)	Percentage	Formula
Cymene	8.460	0.40	$C_{10}H_{14}$
1,8-cineole	**8.673**	**13.17**	$\mathbf{C_{10}H_{18}O}$
Artemisia ketone	**9.527**	**6.69**	$\mathbf{C_{10}H_{16}O}$
Sabinene hydrate	9.708	0.18	$C_{10}H_{18}O$
Artemisia alcohol	10.167	0.35	$C_{10}H_{18}O$
Linalool	10.653	1.05	$C_{10}H_{18}O$
Sabinol	11.105	0.18	$C_{10}H_{18}O$
Chrysanthenone	11.345	0.27	$C_{10}H_{14}O$
Camphor	11.953	2.48	$C_{10}H_{16}O$
Dill ether	12.341	0.20	$C_{10}H_{16}O$
Chrysanthemol	12.464	0.34	$C_{10}H_{18}O$
Borneol	**12.619**	**3.99**	$\mathbf{C_{10}H_{18}O}$
4-Terpineol	12.949	2.44	$C_{10}H_{18}O$
α-Terpineol	**13.363**	**5.27**	$\mathbf{C_{10}H_{18}O}$
Myrtenol	13.499	0.17	$C_{10}H_{16}O$
7-Propylidenebicyclo[4.1.0]heptane	14.527	0.14	$C_{10}H_{16}$
1-Imidazol-1-yl-3-methylbut-2-en-1-one	14.644	0.16	$C_8H_{10}N_2O$
Linalyl acetate	15.122	1.21	$C_{12}H_{20}O_2$
Chrysanthenyl acetate	15.284	0.30	$C_{10}H_{18}O_2$
4-Thujen-2α-yl acetate	15.659	0.31	$C_{12}H_{18}O_2$
Bornyl acetate	15.937	0.58	$C_{12}H_{20}O_2$
Lavandulyl acetate	16.073	0.27	$C_{12}H_{20}O_2$
Thymol	16.254	0.34	$C_{10}H_{14}O$
Carene	17.677	2.59	$C_{10}H_{16}$
β-Phellandrene	17.988	0.52	$C_{10}H_{16}$
Neryl Acetate	18.486	0.59	$C_{12}H_{20}O_2$
β-Elemene	18.810	0.22	$C_{15}H_{24}$
Jasmone	18.978	0.37	$C_{11}H_{16}O$
Caryophyllene	19.540	1.55	$C_{15}H_{24}$
α-Humulene	20.375	0.29	$C_{15}H_{24}$
α-Gurjunene	20.537	0.59	$C_{15}H_{24}$
4-Methyl-2-(3-methyl-2-butenyl)-furan	20.776	0.17	$C_9H_{15}O$
1,3-Diisopropenyl-6-methylcyclohexene	20.905	0.14	$C_{13}H_{20}$
Germacrene	**21.086**	**3.13**	$\mathbf{C_{15}H_{24}}$
Geranyl acetate	21.733	2.27	$C_{12}H_{20}O_2$
β-cadinene	22.083	0.36	$C_{15}H_{24}$
Humulen-(v1)	22.316	0.20	$C_{15}H_{24}$
Nerolidol	**23.079**	**12.87**	$\mathbf{C_{15}H_{26}O}$
(1α, 3β, 4β, 5α, 7β)-1,4-Dimethyladamantane	23.506	2.06	$C_{12}H_{20}$
(E,Z)-α-Farnesene	23.842	0.80	$C_{15}H_{24}$
Longifolene	24.185	0.35	$C_{15}H_{24}$

Table 1. Continued

Compound	Retention time (minute)	Percentage	Formula
α-Cubebene	**24.741**	**12.35**	**$C_{15}H_{24}$**
α-Eudesmol	25.168	2.34	$C_{15}H_{26}O$
α-Selinene	25.479	0.89	$C_{15}H_{24}$
Alloaromadendrene oxide	**25.886**	**4.71**	**$C_{15}H_{24}O$**
Santolina triene	26.300	0.21	$C_{10}H_{16}$
Aromadendrene	26.552	0.60	$C_{15}H_{24}$
Nerolidyl acetate	26.895	0.39	$C_{17}H_{28}O_2$
Hernandulcin	27.122	0.44	$C_{15}H_{24}O_2$
β-Costol	27.562	0.24	$C_{15}H_{24}O$
2,3,4,5-Tetramethyltricyclo[3.2.1.0^{2,7}]oct-3-en	28.040	0.62	$C_{12}H_{18}$
Calarene	28.286	0.36	$C_{15}H_{24}$
1-[1-Methyl-1-(4-methyl-cyclohex-3-enyl)-ethyl]-1H-pyrrole	29.826	0.33	$C_{14}H_{21}N$
4-Thujen-2α-yl acetate	29.942	0.33	$C_{12}H_{18}O_2$
6-(3-Isopropenyl-3-methyl-1-cyclopropen-1-yl)-6-methyl-2-heptanon	31.210	1.25	$C_{15}H_{24}O$
Tetracosane	37.931	0.15	$C_{24}H_{50}$
Monoterpene hydrocarbons		8.19	
Oxygenated monoterpenes		44.23	
Sesquiterpene hydrocarbons		21.69	
Oxygenated sesquiterpenes		22.24	
Other components		3.46	
Total		99.81	

Insecticidal activity

Essential oil isolated from an aerial part of *A. millefolium* had strong fumigant toxicity against *O. surinamensis*. Analysis of variance revealed tested concentrations were meaningfully toxic to the pest ($F = 156.689$, $df = 4, 15$ and $P < 0.0001$). There wasn't any mortality in the control groups. Created mortalities through all essential oil concentrations were taken different letters by Turkey's test at $P = 0.05$ (Fig. 2).

According to R^2 value in Table 2, a positive correlation between essential oil concentrations and mortality of the pest was recognized. LC_{50} was calculated as 17.977 (16.195 ± 20.433) µl/l air (Table 2).

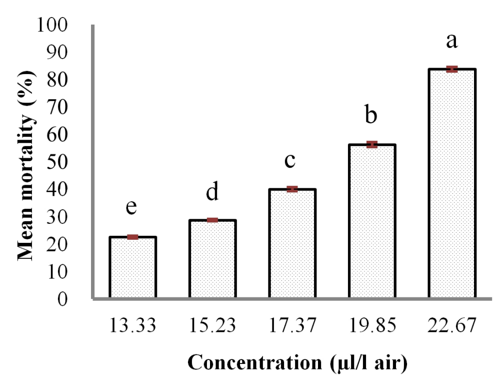

Fig. 2. Mean mortality of *O. surinamensis* affected by the different concentrations of *A. millefolium* essential oil. Dissimilar letters display significant differences based on Tukey test at $p < 0.05$. Vertical bars show standard error (±).

Table 2. Results of Probit analysis of fumigant toxicity of essential oil isolated from *A. millefolium* against *O. surinamensis*

LC_{50} value with 95% confidence limits (µl/l air)	LC_{90} value with 95% confidence limits (µl/l air)	Slope	χ^2 (df = 3)	P-value	R^2
17.977 (16.195 ± 20.433)	27.111 (22.807 ± 46.051)	7.182 ± 0.857	6.004	0.111	0.906

Discussion

The chemical composition of *A. millefolium* essential oil was investigated in recent studies. For example, Orav et al. (2006) indicated that some terpenic components including sabinene, β-pinene, 1,8-cineole, artemisia ketone, and linalool were the main components in *A. millefolium* essential oils from some European countries. Some of these compounds such as sabinene, 1,8-cineole, and artemisia ketone were also recognized in this study. Orav et al. (2006) were also showed that samples from Estonia, Hungary, and Greek contained high amounts of Monoterpene hydrocarbons, and essential oils from France, Belgium and Russia were rich in oxygenated monoterpenes. They also realized the essential oils from Greece, Estonia and Moldavia were rich in sesquiterpenes. According to results of our study, Iranian *A. millefolium* essential oil was rich in terpenic compounds (96.35%) in which monoterpenoids (44.23%) has high amount. Nadim et al. (2011) showed that borneol, bornyl acetate, 1,8-cineole, α-pinene, β-pinene, sabinene, and terpinine-4-ol had high amount in the *A. mellifolium* essential oil from India. Some of these compounds were also recognized in the present study but with some quantitative differences. For example, sabinene, 1,8-cineole and bornyl acetate were 17.58%, 13.04% and 7.98% in the study of Nadim et al. (2011) but these compounds respectively were 2.78%, 13.17% and 0.58% in the present work. Therefore, there are differences in the kind and quantity of the components between previous reports and the results of present study. The differences may be due to a number of endogenous (genetic makeup and plant stages) and exogenous factors (method of essential oil extraction and geographical position) which affect composition of essential oils (Ozguven et al., 2008; Ben Jemâa et al., 2012; Khanavi et al., 2013; Zandi-Sohani and Ramezani, 2015).

Although pesticidal activity of *Achillea* essential oils was evaluated against some economical pests (Calmasur et al., 2006; Rafiei Karahroodi et al., 2009; Dehghani and Ahmadi, 2013), the toxic effect of *A. millefolium* essential oil was tested against *O. surinamensis* for first time in this work. Moreover, the toxicity of some terpenic components was investigated against some insect pests by previous researchers. For example, fumigant toxicity of 20 naturally occurring monoterpenoids was assessed against *O. surinamensis* and it was found that 1,8-cineole and terpineol produced 100% mortality at 50 mg/ml air (Lee et al., 2003). In the other studies, fumigant toxicity of some terpenic compounds including 1,8-cineole, camphor, eugenol, linalool, carvacrol, thymol, borneol, and bornyl-acetate was evaluated against major stored-product insect pests: *Sitophilus oryzae* L., *Rhyzopertha dominica* F. and *Tribolium castaneum* Herbst (Rozman et al., 2007). Nerolidol was also showed considerable toxicity (LD_{50} = 29.30 μg/adult) against *Sitophilus zeamais* Motsch (Yang et al., 2011). Consequently, the toxicity of *A. millefolium* essential oil may be related to the bioactive constituents such as 1,8-cineole, nerolidol, terpineol and borneol.

Plant essential oils have been known as secondary metabolites in the chemical defense mechanisms towards aggressive organisms such as arthropod pests (Prakash and Rao, 1997). Essential oils contain evolutionary origin complex of chemical constituents which are responsible for their bio-activities. For this reason, pests' resistance to these materials will be low (Isman, 2006). They are also considered as safe and available bio-rational agents in insect pests' management (Regnault-Roger et al., 2012). The essential oil of *A. millefolium* indicated promising toxicity against *O. surinamensis*, in the present study. Based on the results of present and previous works, the essential oil of *A. millefolium* is useful in the management of *O. surinamensis*. However, additional researches are needed to achievement of new practical formulations and cost reduction.

Acknowledgements

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