Efficacy of Three Herbal Essential Oils Against Rice Weevil, Sitophilus oryzae (Coleoptera: Curculionidae)

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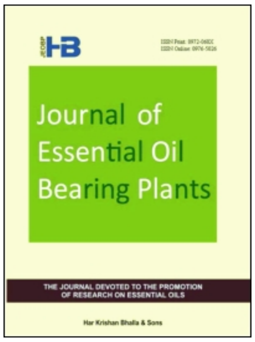

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Efficacy of Three Herbal Essential Oils Against Rice Weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae)

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Abstract: *Sitophilus oryzae* (L) were subjected to essential oils extracted from *Mentha piperita* L, *Rosmarinus officinalis* L and *Hyssopus officinalis* L. Chemical structure, repellency, fumigant toxicity and feeding reduction of the essential oils were investigated. Chemical composition of the essential oils of *M. piperita, R. officinalis* and *H. officinalis* were identified by GC-MS. Menthol (43.95 %), menthone (8.28 %) and 1,8-cineole (7.07 %) were major components of *M. piperita* oil; α-pinene (23.52 %), verbenone (11.87 %) and 1,8-cineole (8.56 %) were the main components of *R. officinalis* oil and *cis*-pinocamphone (23.39 %), *trans*-pinocamphone (17.78 %) and β-pinene (9.64 %) were major components of *H. officinalis* oil. In fumigants bioassay, *H. officinalis* (78.16 µl/L) had the highest toxicity against *S. oryzae* adults, followed by *R. officinalis* (115.63 µl/L) and *M. piperita* (299.51 µl/L), respectively. Also, the *S. oryzae* was repelled by *M. piperita* (95.0 %), *R. officinalis* (91.0 %) and *H. officinalis* (86.5 %), respectively. Based on measured nutritional indices of adults, the highest FDI and the lowest RGR, RCR, and ECI were obtained when adults were treated with *M. piperita* and *H. officinalis* at 10 µL/g food. In conclusion, *H. officinalis* essential oil was more potent for use in organic food protection.

Key words: *Sitophilus oryzae*, *Mentha piperita*, *Rosmarinus officinalis*, *Hyssopus officinalis*, toxicity, repellency, nutritional indices.

Introduction

Stored product insects reduce quality and quantity of agricultural products up to 10 % in temperate zones and up to 30 % in tropical zones [1,2]. Stored product pests include a wide range of insects such as Curculionidae, Pyralidae, Tenebrionidae and Bruchinae. One of the Coleopteran pests is rice weevil, *Sitophilus oryzae* (L) (Curculionidae), which reduces quality and quantity of stored cereals especially in the tropics [3,4]. At present, the main control method against rice weevil is application of synthetic pesticides such as organophosphates, pyrethroids or gaseous insecticides [5].

Synthetic pesticides are the most effective and accessible means to control insect pests. How-

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ever, there is a global concern about insecticides negative impact on environment and non-target organisms [6]. It is necessary to develop new pesticides, some of which are based on herbal extracts, to reduce the negative side effects of conventional pesticides. Application of herbal insecticides goes back to at least two millennia in ancient China, Egypt, Greece, and India. In Europe and North America, the application of botanicals extracts goes back to more than 150 years, that is older than discovery of synthetic chemical insecticides in 1930s to 1950s [7]. One of the main components of herbal extracts which has insecticidal effect is essential oil (EO) [8]. In recent years, application of EOs, derived from aromatic plants, has been increased, which is due to their acceptance by organic farmers and environmentally-conscious consumers. EOs are easily produced by distillation of plant material and contain many volatile, low-molecular-weight terpenes and phenolics [9].

Safety of EOs to human and environment encouraged researchers to increase application of EOs against pests and substitute chemical insecticides with EOs in IPM program [10,11]. Moreover, EOs consist of many bioactive compounds which have insecticidal, nematicidal or antifungal properties [8]. There is little concern about EOs' residue on stored grains or in water, because EOs or their constituents are highly volatile and environmentally non-persistent [12-14].

There are several reports on insecticidal effect of herbal extracts. Yildirim *et al.*[15] found mortality of adult *Sitophilus granarius* L (Col.: Curculionidae) when treated with herbal EOs of *Satureja hortensis*, *Origanum rotundifolium*, *Origanum nites*, *S. spicigera*, *Rosmarinus officinalis*, *Thymus fallax*, *Thymus sipyleus*, *Salvia hydrangea*, *Salvia multicaulis*, *Salvia sclarea* and *S. numerosa* were 100, 93, 95, 94, 93, 88, 82, 67, 55, 41 and 39 percent, respectively. The objectives of this study were 1) to extract and analysis of the EOs of three species of Lamiaceae family, 2) to study repellency and fumigant toxicity of the EOs and 3) to study changes in nutritional indices when EOs are applied against 7 to 14 days old adult rice weevil, in the laboratory.

Materials and methods

Plant material

The aerial parts of three species of Lamiaceae (pepprmint, *Mentha piperita* L; rosemary, *Rosmarinus officinalis* L and hyssop, *Hyssopus officinalis* L) were collected in full flowering stage, from the research farm of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, in July 2013. The plant material was dried in the shadow at room temperature (24°C). Voucher specimens have been deposited in the Medicinal Plants Institute Herbarium (MPIH) of Iran.

Isolation procedure

Air-dried aerial parts of the plants (100 g) were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus [16]. Anhydrous sodium sulfate was used to remove water. Extracted essential oil was stored in sealed vials at 4°C until application in the experiments.

GC and GC-MS analysis of Essential Oils

The GC and GC-MS analyses were carried out in Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, to determine the components of the volatile oils.

GC

The essential oils were analyzed by flame ionization detector gas chromatography (Younglin Acm 6000) by a 30 m × 0.25 mm (0.25 μm film thickness) BP5 capillary column. The injector temperature was 290°C. The carrier gas was helium which was adjusted at a flow rate of 0.8 ml/min. The oven temperature program was as follows: 50°C for 5 min and then heated to 240°C with a 3°C/min rate and finally heated to 300°C with a 15°C/min rate which was held at 300°C for 3 min to facilitate optimal separation.

GC-MS

The essential oils were also analyzed by an Agilent 6890 on capillary column BP-5MS (see GC). Mass Spectrometry (Agilent 5973) was done in electronic impact mode (70 eV), split injection ratio (1:50) and mass range of 40 to 500 amu. Retention indices were calculated by using retention times of n-alkanes (C_8-C_{20}) that were injected at the same temperature and conditions.

Compounds were identified by comparing retention indices (RI) with those reported in the lite-rature and their mass spectrum with Wiley library [17,18].

Insect rearing

Colonies of rice weevil were obtained from Agricultural Entomology Research Department of Iranian Research Institute of Plant Protection (Tehran-Iran). Colonies were reared on whole rice grains in plastic container in the laboratory, where all experiments were conducted (27 ± 1°C, 75 ± 5 % R.H. and 12:12 h L:D) [19,20]. The adult rice weevils which were used in the experiments were 7 to 14 days old [4].

Fumigant toxicity

To evaluate the fumigant toxicity effects of Eos extracted from *M. piperita*, *R. officinalis* and *H. officinalis* against adult rice weevil, the EOs with volumes of 5, 10, 15, 20, 25 and 30 µL were dissolved in 1 mL acetone to obtain dosages of 74.43, 142.86, 214.29, 285.71, 357.14 and 428.57 µL/L of air, respectively [16]. Treatments were applied on Whatman filter papers that were 2 cm in diameter. When the applied solvent evaporated, the treated filter papers were attached inside screw caps of 70-ml glass vials [16], into which rice was added followed by releasing 15 adults. The caps were tightly screwed on and the vials were sealed with parafilm. Each treatment had five replications. To determine LC_{50}, the mortality was recorded 72 hours after treatment.

Repellency

The repellency test was conducted based on McDonald *et al.*[21] in glass Petri dish (9 cm in diameter and 1 cm high) which contained a 9-cm filter paper. The EOs of *M. piperita*, *R. officinalis* and *H. officinalis* were diluted in acetone to prepare different concentrations (2, 4, 8 and 16 µL/30 cm^2). Pure acetone was used as the control. The filter paper was cut in half. One ml of each concentration was uniformly applied to one half of the filter paper with a micropipette. The other half (control) was treated with 1 ml of 100 % acetone. Then, both papers were air dried to completely evaporate the solvent. Then, papers were attached to each other with a paper adhesive tape. Ten adults were released at the center of each filter-paper disc and a cover was placed over the Petri dish (27 ± 1°C, 75 ± 5 % R.H. and 12:12 h L:D). Each treatment was replicated four times. The number of insects present on the control and treated regions were hourly recorded up to 5 hours after treatment. Mean number of insects present on the control (NC) and treated (NT) regions during the experiment were used to estimate the Percent Repellency (PR) which was equal to (NC-NT)/(NC+NT) × 100 [22,23]. All negative percent repellency (PR) values were considered as zero.

Flour disk bioassay

Aliquots of 100 ml of a water suspension of wheat flour (10 g in 50 ml) were poured onto a Petri dish to form flour disks [24,25]. The disks were dried in a fume hood, after which they were equilibrated at 27 ± 1°C and 70 ± 5 % R.H. The flour disks weighed 95 ± 5 mg, and their moisture content was 13.5 ± 0.1 % [26]. Flour disks were treated with acetone solutions (25 µl) containing various EO concentrations (2, 4, 6 and 10 µl) of *M. piperita*, *R. officinalis* and *H. officinalis*. The control was acetone. After evaporation of the solvent, the disks were placed in Petri dishes (9 cm in diameter and 1.5 cm high). Ten adult rice weevils which were 7 to 14 days old were weighed and added onto the flour disks in Petri dishes. Each treatment had four replications. After 72 h, the remaining flour disk and live insects were weighed again and mortality of insects was recorded. Nutritional indices (Huang *et al*) [27] were estimated as follows:

Relative growth rate (RGR) = (A-B)/(B×day),

where A = weight of live insects on the third day (mg)/No. of live insects on the third day, B = original weight of insects (mg)/original No. of insects;

Relative consumption rate (RCR) = D/(B×day)

where D = biomass ingested (mg)/No. of live insects on the third day.

Efficiency of conversion of ingested food (ECI) (%) = (RGR/RCR) × 100.

Feeding deterrence index (FDI) (%) = (C–T)/C × 100,

where C is the consumption of control disks and T the consumption of treated disks.

Data analysis

The Polo-Plus software was used to estimate the mortality rate and lethal concentration [28]. Percentage insect mortality was calculated by probit analysis [29]. Data of repellency test and nutritional indices were analyzed using procedures of SAS® (SAS Institute Inc. 2002) based on a completely randomized design. The normality of the untransformed and transformed data and also normality of residuals after analysis of variance were checked using stem-leaf and normal probability plots. Homoscedasticity was checked by observing graphical distribution plots of variance by mean (PROC PLOT). Data were square-root transformed. A general linear model for analysis of variance (PROC GLM) was used to compare treatments. Comparisons among treatments were made using the Tukey test where analysis of variance showed significant differences among means. In all experiments, differences between treatments were considered significant at $P< 0.05$ and mean values are given as the mean ± SE.

Results

Essential oils

The yield of EOs in dried aerial parts of *M. piperita* and *R. officinalis* and *H. officinalis* were 1.5, 1.5 and 0.5 %, respectively. The results also revealed that major essential oil components of *M. piperita* were menthol (43.95 %), menthone (8.28 %) and 1,8-cineole (7.07 %). Major essential oil components of *R. officinalis* were α-pinene (23.52 %), verbenone (11.87 %) and 1,8-cineole (8.56 %). The main essential oil components of *H. officinalis* were *cis*-pinocamphone (23.39 %), *trans*-pinocamphone (17.78 %) and β-pinene (9.64 %) (Table 1, 2 and 3).

Fumigant toxicity

LC_{50} values of *M. piperita*, *R. officinalis* and *H. officinalis* essential oils against adult rice weevil were 299.51, 115.63 and 78.16 µL/L air, respectively (Table 4).

Table 1. Chemical composition of the essential oil extracted from *Mentha piperita* L.

No.	Component	RT	% Composition	KI[1] Sample	KI Adams [17]	Type
1	2E-Hexenal	8.30	0.11	846	855	Others
2	α-Pinene	11.72	0.69	934	939	MH[2]
3	Sabinene	13.80	0.46	975	975	MH
4	β-Pinene	14.06	0.93	980	979	MH
5	Myrcene	14.63	0.21	992	991	MH
6	3-Octanol	15.18	0.24	1003	991	Others
7	α-Terpinene	16.12	0.33	1021	1017	MH
8	o-Cymene	16.62	0.15	1030	1026	MH
9	Limonene	16.78	2.46	1033	1029	MH
10	1,8-Cineole	16.97	7.07	1037	1031	MO[3]
11	Z-β-Ocimene	17.11	0.12	1040	1037	MH
12	γ-Terpinene	18.32	0.59	1063	1060	MH
13	*cis*-Sabinene hydrate	19.03	0.33	1077	1070	MO
14	Terpinolene	19.71	0.15	1090	1089	MH
15	Linalool	20.53	0.39	1106	1097	MO
16	Camphor	23.11	0.16	1158	1146	MO
17	Menthone	23.55	8.28	1166	1153	MO
18	Menthofuran	23.85	4.07	1172	1164	MO

table 1. (continued).

No.	Component	RT	% Composition	KI[1] Sample	KI Adams [17]	Type
19	*iso*-Menthone	23.98	1.95	1175	1163	MO
20	*neo*-Menthol	24.18	4.27	1179	1166	MO
21	Menthol	24.67	43.95	1189	1172	MO
22	*iso*-Menthol	25.14	0.94	1198	1183	MO
23	*neoiso*-Menthol	25.27	0.32	1201	1187	MO
24	α-Terpineol	25.50	0.44	1206	1189	MO
25	Pulegone	27.60	2.63	1250	1237	MO
26	Piperitone	28.39	0.37	1267	1253	MO
27	*neo*-Menthyl acetate	28.90	0.69	1278	1274	MO
28	Menthyl acetate	29.74	8.35	1296	1295	MO
29	Thymol	30.07	0.13	1303	1290	MO
30	*iso*-Menthyl acetate	30.45	0.43	1312	1305	MO
31	β-Bourbonene	33.89	0.52	1389	1388	SH[4]
32	β-Elemene	34.11	0.19	1394	1391	SH
33	E-Caryophyllene	35.48	2.69	1426	1419	SH
34	β-Copaene	35.93	0.11	1437	1432	SH
35	Z-β-Farnesene	36.70	0.32	1456	1443	SH
36	α-Humulene	37.04	0.12	1464	1455	SH
37	Germacrene D	38.11	2.05	1489	1485	SH
38	Bicyclogermacrene	38.71	0.28	1504	1500	SH
39	Caryophyllene oxide	42.31	0.20	1595	1583	SO[5]
40	Veridiflorol	42.81	0.64	1608	1593	SO
	Total Identified		98.33			

[1]KI = Kovats index;
[2]MH = Monoterpene hydrocarbons;
[3]MO = Oxygenated monoterpenes
[4]SH = Sesquiterpene hydrocarbons;
[5]SO = Oxygenated sesquiterpenes

Table 2. Chemical composition of the essential oil from *Rosmarinus officinalis* L.

No.	Component	RT	% Composition	KI[1] Sample	KI Adams [17]	Type
1	α-Thujene	11.16	0.27	923	930	MH[2]
2	α-Pinene	11.72	23.52	934	939	MH
3	Camphene	12.60	4.93	952	954	MH
4	Thuja-2,4(10)-diene	12.83	0.50	956	960	MH
5	β-Pinene	14.05	0.74	980	979	MH
6	1-Octen-3-ol	14.32	0.28	986	979	Others
7	Myrcene	14.61	5.88	991	991	MH
8	3-Octanol	15.18	0.22	1003	991	Others
9	α-Phellanderen	15.56	0.21	1010	1003	MH

table 2. (continued).

No.	Component	RT	% Composition	KI[1] Sample	KI Adams [17]	Type
10	α-Terpinene	16.11	0.44	1020	1017	MH
11	p-Cymene	16.61	0.75	1030	1025	MH
12	Limonene	16.77	3.29	1033	1029	MH
13	1,8-Cineole	16.96	8.56	1037	1031	MO[3]
14	γ-Terpinene	18.30	0.63	1063	1060	MH
15	Terpinolene	19.69	0.69	1089	1089	MH
16	Linalool	20.50	2.01	1105	1097	MO
17	Chrysanthenone	21.80	0.17	1131	1128	MO
18	*trans*-Verbenol	22.98	0.31	1155	1145	MO
19	Camphor	23.16	7.98	1159	1146	MO
20	Menthone	23.52	0.35	1166	1153	MO
21	*trans*-Pinocamphone	23.79	0.53	1171	1163	MO
22	Borneol	24.37	6.12	1183	1169	MO
23	Menthol	24.59	2.50	1187	1175	MO
24	Terpinen-4-ol	24.71	0.98	1190	1177	MO
25	p-Cymen-8-ol	25.16	0.15	1199	1183	MO
26	α-Terpineol	25.49	1.77	1206	1189	MO
27	Verbenone	26.19	11.87	1220	1205	MO
28	Pulegone	27.59	0.14	1250	1237	MO
29	Carvone	27.94	0.10	1258	1243	MO
30	Isobornyl acetate	29.52	2.86	1291	1286	MO
31	E-Caryophyllene	35.48	2.16	1426	1419	SH[4]
32	α-Humulene	37.04	0.27	1464	1455	SH
33	Germacrene D	38.10	0.32	1489	1485	SH
	Total Identified		91.50			

[1]KI = Kovats Index
[2]MH = Monoterpene Hydrocarbons
[3]MO = Oxygenated Monoterpenes
[4]SH = Sesquiterpene Hydrocarbons

Table 3. Chemical composition of the essential oil from *Hyssopus officinalis* L.

No.	Component	RT	% Composition	KI[1] Sample	KI Adams [17]	Type
1	α-Thujene	11.33	0.17	927	930	MH[2]
2	α-Pinene	11.72	0.55	934	939	MH
3	Sabinene	13.80	1.16	975	975	MH
4	β-Pinene	14.07	9.69	981	979	MH
5	Myrcene	14.63	1.07	992	991	MH
6	α-Terpinene	16.12	0.79	1021	1017	MH
7	o-Cymene	16.61	0.41	1030	1026	MH
8	Limonene	16.78	0.67	1033	1029	MH

table 3. (continued).

No.	Component	RT	% Composition	KI[1] Sample	KI Adams [17]	Type
9	β-Phellandrene	16.90	2.39	1036	1030	MH
10	E-β-Ocimene	17.65	0.17	1050	1050	MH
11	γ-Terpinene	18.31	1.50	1063	1060	MH
12	*cis*-Sabinene hydrate	19.03	0.13	1077	1070	MO[3]
13	Terpinolene	19.70	0.34	1090	1089	MH
14	Linalool	20.51	0.70	1105	1097	MO
15	*trans*-Sabinene hydrate	20.66	0.44	1108	1098	MO
16	*cis*-Thujone	21.57	0.10	1127	1102	MO
17	*cis*-p-Menth-2-en-1-ol	21.86	0.52	1132	1122	MO
18	*trans*-Pinocamphone	23.82	17.88	1172	1163	MO
19	Pinocarvone	23.92	1.59	1174	1165	MO
20	Borneol	24.39	0.30	1183	1169	MO
21	*cis*-Pinocamphone	24.63	23.53	1188	1175	MO
22	Terpinen-4-ol	24.72	9.08	1190	1177	MO
23	α-Terpineol	25.49	3.21	1206	1189	MO
24	*cis*-Piperitol	26.12	0.21	1219	1196	MO
25	Geraniol	27.94	0.20	1258	1253	MO
26	*neo*-Menthyl acetate	29.73	0.12	1296	1274	MO
27	δ-Elemene	31.49	0.44	1335	1338	SH[4]
28	Neryl acetate	33.66	0.11	1384	1362	SH
29	β-Bourbonene	33.88	0.21	1389	1388	SH
30	β-Elemene	34.10	0.16	1394	1391	SH
31	Methyl eugenol	34.90	0.38	1413	1404	Others
32	(E)-Caryophyllene	35.48	3.56	1426	1419	SH
33	α-Humulene	37.03	0.49	1463	1455	SH
34	*allo*-Aromadendrene	37.21	0.91	1468	1460	SH
35	Germacrene D	38.10	4.31	1489	1485	SH
36	Viridiflorene	38.46	0.15	1498	1497	SH
37	Bicyclogermacrene	38.70	2.92	1504	1500	SH
38	γ-Cadinene	39.43	0.20	1522	1514	SH
39	δ-Cadinene	39.56	0.15	1525	1523	SH
40	*trans*-Calamenene	39.77	0.10	1531	1529	SH
41	Elemol	40.89	1.47	1559	1550	SO[5]
42	Spathulenol	42.12	0.64	1590	1578	SO
43	Caryophyllene oxide	42.31	0.76	1595	1583	SO
44	Globulol	42.44	0.10	1598	1585	SO
45	10-*epi*-γ-Eudesmol	44.23	0.28	1645	1624	SO
46	*epi*-α-Cadinol	44.60	0.23	1655	1640	SO
47	β-Eudesmol	45.17	0.34	1670	1651	SO
	Total Identified		94.83			

[1]KI = Kovats Index; [2]MH = Monoterpene Hydrocarbons
[3]MO = Oxygenated Monoterpenes; [4]SH = Sesquiterpene Hydrocarbons
[5]SO = Oxygenated Sesquiterpenes

Repellency

There was a significant difference among treatments regarding their repellency effect (GLM ANOVA: $F_{11,228}$ = 28.74, P<0.0001). The EOs of *M. piperita*, *R. officinalis* and *H. officinalis* strongly repelled *S. oryzae*. EOs of *H. officinalis* at 2 μL had the least repellency. The EOs of *M. piperita* and *R. officinalis* at 16 μL were the most repellent compounds. The repellency of EOs increased with concentrations of EOs (Table 5).

Nutritional indices

There were significant differences among treatments regarding the FDI (GLM ANOVA: $F_{11,36}$ = 13.83, P<0.0001), RGR (GLM ANOVA: $F_{14,45}$ = 6.61, P<0.0001), RCR (GLM ANOVA: $F_{14,45}$ = 23.08, P<0.0001) and ECI (GLM ANOVA: $F_{14,45}$ = 4.57, P<0.0001) (Table 6).

When adults were treated with EOs of *M. piperita*, RGR and RCR were ca. three times less; and ECI was ca. two times less at 10 μL/g food compared to the lower concentrations. However, the FDI was not different among concentrations of *M. piperita*. The FDI range was 31 % to 58 % (Table 6).

When adults were treated with EOs of *R. officinalis*, none of the nutritional indices had a significant difference among concentrations. The FDI range was 6 % to 19 % (Table 6).

When adults were treated with EOs of *H. officinalis*, RGR and RCR were ca. 4 and 1.5 times less at 10 μL/g food compared to the lower concentrations. At 10 and 6 μL/g food, FDI was 3 and 2 times more, respectively, compared to the lower concentrations.

In general, at 10 μL/g food, EOs of *M. piperita*, compared with *R. officinalis*, significantly reduced the RGR and RCR. However, it was not different from *H. officinalis*. Similar result was observed for RCR. Regarding the ECI, the low-

Table 4. Fumigant toxicity of the essential oils extracted from three species of Lamiaceae against adult rice weevil, *Sitophilus oryzae*, in the laboratory

Essential oils	LC_{50} (μL/ L air)	LC_{95}) (μL/ L air)	Chi-square	Heterogeneity
Mentha piperita L.	299.51 (264.19-339.71)*	576.43 (465.85-922.43)	8.15	2.039
Rosmarinus officinalis L.	115.63 (63.89-156.59)	357.28 (243.83-1067.19)	13.09	3.273
Hyssopus officinalis L.	78.16 (73.40-83.45)	125.60 (109.54-161.79)	0.23	0.056

* 95 % lower and upper fiducial limits are shown in parenthesis

Table 5. Repellent effect of the essential oils extracted from three species of Lamiaceae against adult rice weevil, *Sitophilus oryzae*,in the laboratory

	Repellency % (Mean* ± SE) at different concentration of essential oils (μL/30 cm²)			
Essential oils	2	4	8	16
Mentha piperita L.	85.0 ± 2.67 abc	87.0 ± 2.72 abc	88.5 ± 3.27 ab	95.0 ± 1.36 a
Rosmarinus officinalis L.	68.5 ± 6.93 c	84.0 ± 3.51 abc	85.5 ± 2.46 abc	91.0 ± 3.15 ab
Hyssopus officinalis L.	17.0 ± 4.59 e	45.0 ± 5.83 d	74.0 ± 5.45 bc	86.6 ± 5.35 abc

* Means followed by same letters do not differ significantly based on Tukey test (á=5%). (SE=Standard Error)

Table 6. The effects of the essential oils extracted from three species of Lamiaceae on feeding and nutritional indices of adult rice weevil, *Sitophilus oryzae*,in the laboratory

Plant species	Concentration (µL/g food)	RGR (mg/mg/d) (Mean ± SE)	RCR (mg/mg/d) (Mean ± SE)	ECI (%)	FDI (%)
Mentha piperita	0	0.0409 ± 0.0012 a	0.2673 ± 0.0062 bc	15.34 ± 0.74 a	-
	2	0.0350 ± 0.0019 ab	0.3446 ± 0.013 4a	10.23 ± 0.85 a	31.49 ± 7.19 ab
	4	0.0324 ± 0.0024 abc	0.3254 ± 0.0071 ab	9.99 ± 0.90 a	41.13 ± 6.70 ab
	6	0.0255 ± 0.0044 abcd	0.3472 ± 0.0173 a	7.26 ± 0.97 a	43.63 ± 4.87 ab
	10	-0.0142 ± 0.0092 f	0.1380 ± 0.0201 e	-13.15 ± 8.01 b	58.13 ± 7.52 a
Rosmarinus officinalis	0	0.0192 ± 0.0008 bcd	0.3054 ± 0.0080 abc	6.31 ± 0.28 a	-
	2	0.0181 ± 0.0022 bcd	0.3117 ± 0.0148 abc	5.78 ± 0.51 a	5.93 ± 2.74 d
	4	0.0172 ± 0.0067 bcd	0.3053 ± 0.0080 abc	5.54 ± 2.08 a	7.56 ± 2.90 cd
	6	0.0138 ± 0.0029 cd	0.3063 ± 0.0089 abc	4.46 ± 0.88 a	10.81 ± 4.70 cd
	10	0.0101 ± 0.0037 de	0.2505 ± 0.0116 cd	3.89 ± 1.22 a	19.42 ± 5.55 bcd
Hyssopus officinalis	0	0.0309 ± 0.0063 abc	0.3724 ± 0.0126 a	8.24 ± 1.62 a	-
	2	0.0245 ± 0.0083 abcd	0.3351 ± 0.0106 a	7.52 ± 2.74 a	20.62 ± 3.23 bcd
	4	0.0161 ± 0.0108 cd	0.3048 ± 0.0071 abc	5.14 ± 3.53 a	23.50 ± 1.24 bc
	6	0.0163 ± 0.0036 cd	0.2474 ± 0.0180 cd	6.84 ± 1.70 a	42.17 ± 3.73 ab
	10	-0.0038 ± 0.0063 ef	0.1893 ± 0.0195 de	-2.70 ± 3.06 ab	59.03 ± 4.35 a

Means in a column followed by at least one same letter are not significantly different based on Tukey test (α=0.05)
(RGR=Relative Growth Rate
RCR=Relative Consumption Rate
ECI=Efficiency of Conversion of Ingested food
FDI=Feeding Deterrence Index
SE=Standard Error
mg=Mili Gram, d= Day)

est value was estimated for EOs of *M. piperita* at 10 µL/g food, while the all other treatments were not different (Table 6). The highest FDI were obtained when adults were treated with *M. piperita* and *H. officinalis* at 10 µL/g food. The lowest FDI was obtained when adults were treated with *R. officinalis* at 2 µL/g food (Table 6).

Discussion

Essential oils belong mainly to two phytochemical groups of terpenoids, i.e. monoterpenes and sesquiterpenes of low molecular weight. The toxicity of many plant EOs are due to monoterpenoids [9], which are lipophilic volatile compounds that can rapidly penetrate into insects and interfere with their physiological functions [30]. Due to high volatility, the toxic effects of *M. piperita*, *R. officinalis* and *H. officinalis* could be due to some well-known toxic compounds from *M. piperita* such as menthol (43.95 %), menthone (8.28 %) and 1,8-cineole (7.07 %). Based on Golparvar and Hadipanah [31], extract of *M. piperta* includes 12.37 % and 13.89 % menthol and menthone, respectively. The major components of *M. piperita* essential oil, analyzed in Serbia, were menthol (37.4 %), menthyl acetate (17.4 %) and menthone (12.7 %) [32]. The leaves of *M. piperita* grown in Korea had linalyl acetate (28.2 %), menthol (33.4 %), 1,8-cineole (46.1 %), limonene (64.5 to 94.2 %), and *p*-menth-2-en-ol (34.5 %) [33]. The components of peppermint oil vary slightly from year to year. This may be mostly due to changes in climate conditions and the effect of climate on chemotypes of mints. Yazdani *et al* [34] reported that the highest menthol content in essential oil of *Mentha piperita* was (56.4 %) from Sari province in Iran.

In this study, main components of the essential oil of *R. officinalis* were α-pinene (23.52 %), verbenone (11.87 %), 1,8-cineole (8.56 %) and camphene (4.93 %). In other reports, main components of the essential oil of *R. officinalis* collected from Lalehzar region (Kerman Province of Iran) were α-pinene (43.9 %), 1,8-cineole (11.1 %), camphene (8.6 %) and verbenone (2.6 %); while in Kerman suburb, the main components were α-pinene (46.1 %), 1,8-cineole (11.1 %), camphene (9.6 %) and verbenone (2.3 %) [35]. Roomiani *et al*.[36] reported that 1,8-cineole (78.6 %), α-pinene (15.9 %) and camphene (4.2 %) were main components of the essential oil of *R. officinalis* collected from Karaj region in Iran.

In this study, main components of the essential oil of *H. officinalis* were *cis*-pinocamphone (23.39 %), *trans*-pinocamphone (17.78 %) and β-pinene (9.64 %). However, in the other countries, components of the essential oil of *H. officinalis* were different. For example, in Turkey, the main components were pinocarvone (29.2 %), *trans*-pinocamphone (27.2 %) and β-pinene (17.6 %) [37]; while in Egypt, they were *trans*-pinocamphone (15.9 %) and β-pinene (20.4 %) [38].

In general, the main components of the EOs of plants, grown in different climates and locations, are different quantitatively and qualitatively. Therefore, the difference in efficacy of herbal EOs obtained from the same species of plants which are grown in different habitats might be due to difference in their EO components. Soil texture, climate and altitude, plant part, methods of extraction, ecological and geographical conditions can affect EO components [35,39,40].

The insecticidal properties of EOs varied considering the plant species, type of compound and the exposure time. The most active EO was *H. officinalis*, followed by *R. officinalis* and *M. piperita*. 1,8-Cineole is highly effective against *S. oryzae* when applied for 24 h at 0.1 ml/720 ml volume [41] and the monoterpene β-pinene has insecticidal effects against *S. oryzae* [42]. Also toxicity effect of limonene against *Tribolium castaneum* was reported by Lee *et al*.[43].

The essential oils of *M. piperita*, *R. officinalis* and *H. officinalis* had fumigant toxicity, as well as repellent activity against *S. oryzae*. The insecticidal and repellent activities varied with concentrations of the oil and exposure time. The results showed higher mortality rate due to *H. officinalis* followed by *R. officinalis* and *M. piperita*. However, the repellent activity was more marked by the *M. piperita* EO followed by *R. officinalis* and *H. officinalis*.

This was the first report on fumigant effect of *H. officinalis* against *S. oryzae*. The average mortality rate of *Sitophilus granarius* due to *H. officinalis* (at 10 µl) was 66 % [44]. Based on Laznik *et al* [45], the essential oil of *R. officinalis* was the most effective fumigant, causing more than 60 % mortality in adult *S. granarius*. Also, Yildirim *et al.*[15] reported that essential oils of *Origanum onites*, *Origanum rotundifolium*, *Rosmarinus officinalis*, *Salvia hydrangea*, *Satureja hortensis*, *Satureja spicigera*, *Thymus fallax* and *Thymus sipyleus* had insecticidal effects on adult *S. granarius*. However, variation in toxicity of EOs against one species, reported in different researches, may be due to difference in their texture, decrease in penetration, or biochemical and physiological condition of insect [9,46,47].

Essential oils from *M. piperita*, *R. officinalis* and *H. officinalis* modified the nutritional indices of *S. oryzae*. At maximum tested concentration (10 µL/g food), the EOs from *M. piperita* and *H. officinalis* had significantly reduced the nutritional indices. However, *R. officinalis*, at maximum tested dose (10 µL/g food) did not show any difference with control. Also, due to low PCR and high feeding deterrence indices, *H. officinalis* inhibited the feeding behavior.

The results obtained in this study indicated that the essential oils of *M. piperita, R. officinalis* and *H. officinalis* had antifeeding deterrence effect against *S. oryzae*. This study also demonstrated that the essential oil of *H. officinalis* had fumigant toxicity and feeding inhibitory effect against *S. oryzae* adults. Similar results in stored-product pests have been reported by other authors [27,48,49].

Some studies showed that EOs have neurotoxic, cytotoxic, phototoxic and mutagenic activities against different organisms [50,51]. The findings of Kiran & Prakash [52] revealed that the toxicity of EO might be associated with inhibition of AChE activity and oxidative imbalance.

In conclusion, EOs of *H. officinalis* might be useful for managing populations of *S. oryzae* in storages. More research is required to clarify the field efficacy of these compounds and develop the formulations to improve potency and stability, as well as to reduce the production expenses.

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Efficacy of Three Herbal Essential Oils Against Rice Weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae)

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Abstract

Sitophilus oryzae (L) were subjected to essential oils extracted from *Mentha piperita* L, *Rosmarinus officinalis* L and *Hyssopus officinalis* L. Chemical structure, repellency, fumigant toxicity and feeding reduction of the essential oils were investigated. Chemical composition of the essential oils of *M. piperita, R. officinalis* and *H. officinalis*

were identified by GC-MS. Menthol (43.95%), menthone (8.28%) and 1,8-cineole (7.07%) were major components of *M. piperita* oil; α-pinene (23.52%), verbenone (11.87%) and

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