Efficiency of *Mentha piperita* L. and *Mentha pulegium* L. essential oils on nutritional indices of *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae)

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Abstract

Antifeedant activity of plant extracts from *Mentha piperita* L. and *Mentha pulegium* L. were tested against the Indian meal moth, *Plodia interpunctella* (Hübner). The nutritional indices: relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and feeding deterrence index (FDI) were measured for first-instar larvae (15-d old). Treatments were evaluated using a flour disk bioassay in the dark, at 25±1°C and 60±5% R.H. Concentrations of 0, 0.1, 0.5, 0.75, 1, 1.5 and 2 μL/disk were prepared from each essential oil. After 72 h, nutritional indices were calculated. *M. piperita* oils were more effective than *M. pulegium* oils, by significantly decreasing the RGR, RCR and FDI. At the highest concentration tested (2 μL/disk), the ECI (9%) was significantly reduced.

Introduction

Stored cereals, oilseeds, pulses, spices, dried fruits, tree nuts and their processed foods are important for food and trade purposes and suffer economic and quality losses due to insect pests (Lamiri *et al*., 2001; Passino *et al*., 2004; Tapondjou *et al*., 2005; Ali & Rizvi, 2008). There are over 600 species of beetle pests and 70 species of moths capable of causing quantitative and qualitative losses (Rajendran, 2002). In developing countries, damage is between 10 to 40% (Shaaya *et al*., 1997). In some rural areas of Iran that use traditional storages, damage caused by stored product insects can be as high as 80% (Modarres-Najafabadi *et al*., 2006).

P. interpunctella (Hübner) (Indian meal moth) is considered to be the most troublesome of the moths infesting stored products in the world (Phillips *et al*., 2000; Mohandass *et al*., 2007). It attacks all cereal products, whole grains, dried fruits, pet foods, birdseed, dried milk and nuts (Arthur *et al*., 1991). The larvae are generalists, as they can feed on grain products, seeds, dried fruit, dog food, and spices (Arthur *et al*., 1990; Mohandass *et al*., 2007). Damage is caused by the larvae spinning silken threads as they feed and crawl, thus webbing the particles of food together (Simmons & Nelson, 1975). The control of this pest in storage systems mainly depends on fumigants such as methyl bromide or phosphine, and fogging with pyrethrins or dichlorvos. However, methyl bromide was banned in many countries starting in 2004, because of its ozone-depleting properties (Hansen & Jensen, 2002).

Synthetic pesticides have been considered the most effective and accessible means of controlling insect pests of stored products (Huang & Subramanyam, 2005). These chemicals are associated with undesirable effects on the environment due to their slow biodegradation and some toxic residues in products, affecting mammalian health (Benhalima *et al*., 2004; Isman, 2006; Halder *et al*., 2010). The adverse effects of synthetic pesticides have amplified the need for an effective and biodegradable pesticide.

Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts on human health and the environment. Among the various kinds of natural substances that have received particular attention as natural agents for insect management are essential oils from aromatic plants. Essential oils are renewable, non-persistent in the environment and relatively safe to natural enemies, non-target organisms and human beings (Halder *et al*., 2010).

Essential oils are defined as any volatile oil(s) that have strong aromatic components and that give a distinctive odor, flavor or scent to a plant. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites (Koul *et al*., 2008). Because of the intensity of plant-insect interactions, the plants have well-developed defense mechanisms against pests and are excellent sources of new insecticidal substances. Their components and quality vary with geographical distribution, time of harvest, growing conditions and method of extraction (Yang & Zheng, 2005). Effects of essential oils on stored-product insect pests have been reported on extensively (Ogendo *et al*., 2008; Park *et al*., 2008; Benzi *et al*., 2009; Ayvaz *et al*., 2010; Nayamador *et al*., 2010; Taghizadeh *et al*., 2010).

The insecticidal activity of some essential oils from Lamiaceae has been evaluated against a number of stored product insects. For example, Mollai *et al*. (2010) found strong insecticidal activity of essential oil from *Satureja hortensis* (Lamiaceae) on *P. interpunctella*. The 24-h LC_{50} values against adults were 140 μL/L air. In another experiment, Aliakbari *et al*. (2010) studied insecticidal activity of the essential oil from *Thymus daenensis* (Lamiaceae) on *Tribolium confusum* (Tenebrionidae). Mortality was evaluated after 24 and 48 h. LC_{50} and

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LC_{95} values were 50 and 169 μL after 24-h and 42 and 103 μL after 48 h, respectively. Ebadollahi & Mahboubi (2011) studied fumigant activity of the essential oil from *Lavandula stoechas* L. (Lamiaceae) on *Tribolium castaneum*. Based on the results of this research, essential oils of *L. stoechas* should be considered as a potential control agent against *T. castaneum*. Rafiei-Karahroodi *et al*. (2009) studied the effect of essential oils from *Dracocephalum moldavica*, *Lavandula officinalis*, *Melissa officinalis* and *Rosmarinus officinalis* on 1-d and 3-d-old eggs of *P. interpunctella*. Results indicated that *M. officinalis* and *R. officinalis* are suitable candidates for replacing synthetic pesticides in warehouses to control *P. interpunctella.*

The main goal of the present study was to evaluate the insecticidal activities of essential oils from *M. piperita* and *M. pulegium* grown in Iran for the control of *P. interpunctella*.

Materials and methods

Moth culture

The Indian meal moths, based on described by Silhacek & Miller (1972), were reared on artificial diet containing: cornmeal (26%), whole wheat flour (23%), glycerol (16%) honey (14%), ground dog meal (10%), brewers' yeast (5%), rolled oats (4%) and wheat germ (2%) in a chamber set to a light:dark period of (11:13) and a temperature of 28±2°C.

Collected and dried plant specimens

Two plants known to have medicinal activity, *M. piperita* L. and *M. pulegium* L., were collected from their natural habitats, from different localities in Iran. The identity of each plant species was verified by En. Shahabedin Mirinejad (botanical specialist from Agriculture and Natural Resources Researches Center of Kohgiloyeh and Boyerahmad, Yasouj), using live specimens and photographs.

Extraction of essential oils

Plant materials were air dried in the shade at room temperature (26-28°C) for 20 d and stored in darkness until distillation. The essential oils were isolated from dried plant samples by hydro-distillation using a Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample, 1:10 plant material/water volume ratio, 3 h distillation. The essential oils were collected, dried over anhydrous sodium sulfate and stored at 4°C until use.

Flour disk bioassay

According to the method of Mohandass *et al*. (2007) a suspension of 10 g wheat flour in 50 mL distilled water was prepared. A micropipette was used to transfer 200-μL aliquots from the suspension onto a plastic sheet. After 4 h at room temperature, the wheat flour suspensions in the form of spherical disks were transferred to a petri dish. Prepared disks were kept for 12 h to dry inside an oven, after which the weight of the flour disks was between 35-45 mg and their moisture content was approximately 15%. Different concentrations of essential oils from *M. piperita* and *M. pulegium* (0.1, 0.5, 0.75, 1, 1.5 and 2 μL in 1 mL of acetone) were placed on each disk separately and held for 20 min at room temperature to allow for evaporation of the acetone. In each petri dish, one flour disk was placed along with 10 first- instar Indian meal moth larvae and held at 25±1°C and 60±5% R.H. for 3 d. At the beginning of the experiment, the weight of flour disks and larvae was measured. After 3 d, flour disks and larvae were weighed again and the number of dead larvae noted. There were 5 replicates.

Nutritional indices

Nutritional indices were calculated according to Tripathi *et al*. (2002), with some modifications:

$$\text{Relative Growth Rate (RGR)}=(A-B)/(B\times \text{Day}) \quad (1)$$

where
A=weight of live insects (mg) on the third day/number of live insects on the third day;
B=original weight of insects (mg)/original number of insects.

$$\text{Relative Consumption Rate (RCR)}=D/(B\times \text{day}) \quad (2)$$

where
D=biomass ingested (mg)/number of live insects on the third day.
Percentage efficacy of conversion of ingested food (ECI)=RGR/RCR ×100. The feeding deterrent activity was calculated as a feeding deterrent index (Isman, 2006):

$$(\%\ \text{FDI})=[(C-T)/C]\times 100 \quad (3)$$

where C is the weight consumption of food in the control and T is the weight consumption food in the treatment.

Data analysis

Each of the indices was calculated using a completely randomized factorial design, and five replicates were performed. The first factor in this design included three treatments, consisting of the essential oils of *M. piperita*, *M. pulegium* and a control, and the second factor consisted of six concentrations of plant essential oils: 0.1, 0.5, 0.75, 1, 1.5 and 2 μL/disk, and a control treatment. Before statistical analysis, the ECI and FDI nutritional indices data were normalized using an Arcsin$\sqrt{X/100}$ transformation. The means were separated using Duncan's multiple range test at the 5% significance level.

Results

Plant source and dose significantly affected all nutritional indices (Table 1). For RCR, ECI and FDI there was a significant interaction

Table 1. Analysis of variance of essential oils of *Mentha piperita* and *Mentha pulegium* on nutritional indices of larvae of *Plodia interpunctella.*

Source of variation	Degrees of freedom	Mean squares			
		RGR	RCR	ECI	FDI
Plant	1	$7.01\times10^{-4*}$	0.066^{**}	99.836^{**}	5167.934^{**}
Concentration	6	0.002^{**}	0.032^{**}	26.125^{**}	1998.654^{**}
Plant×Concentration	6	3.086×10^{-5ns}	0.003^{**}	16.822^{**}	176.234^{**}
Error	42	2.620×10^{-5}	3.736	4.354	0.023

RGR, relative growth rate; RCR, relative consumption rate; ECI, efficiency of conversion of ingested food; FDI, feeding deterrence index; ns, non-significant; *, ** respectively significant differences at 5 and 1% level.

Table 2. Effect of essential oils of *Mentha piperita* and *Mentha pulegium* on nutritional indices of larvae of *Plodia interpunctella*.

Essential oil	RGR (mg/mg/day)	RCR (mg/mg/day)	ECI%	FDI%
Mentha piperita	0.026±0.002[b]	0.219±0.160[b]	11.94±0.76[a]	34.9±3.8[a]
Mentha pulegium	0.028±0.001[a]	0.294±0.006[a]	9.64±0.44[b]	14.2±2.2[b]

RGR, relative growth rate; RCR, relative consumption rate; ECI, efficiency of conversion of ingested food; FDI, feeding deterrence index. [a,b]Values in the same column followed by different letters are significantly different (P<0.01, Duncan's multiple range test).

Table 3. Total average effect of essential oils of *Mentha piperita* and *Mentha pulegium* at various concentrations on nutritional indices of larvae of *Plodia interpunctella*.

Concentration (μL/disk)	Standard error±average nutritional indices			
	RGR (mg/mg/day)	RCR (mg/mg/day)	ECI%	FDI%
0.00 (Control)	0.051±0.001[a]	0.3131±0.015[a]	16.288±0.453[a]	-
0.1	0.035±0.001[b]	0.253±0.014[b]	13.833±0.765[a]	13.281±2.050[f]
0.5	0.031±0.001[bc]	0.230±0.017[c]	13.478±0.749[ab]	20.098±2.710[e]
0.75	0.027±0.000[c]	0.210±0.015[d]	12.857±0.512[abc]	25.889±3.112[d]
1	0.019±0.002[d]	0.169±0.016[e]	11.242±0.765[abc]	32.705±3.256[c]
1.5	0.016±0.001[d]	0.116±0.023[f]	13.793±1.682[cb]	48.949±6.014[b]
2	0.010±0.003[e]	0.102±0.039[g]	9.384±1.812[c]	52.829±5.235[a]

RGR, relative growth rate; RCR, relative consumption rate; ECI, efficiency of conversion of ingested food; FDI, feeding deterrence index. [a,b,c,d,e,f,g]Dissimilar letters in each column with using Duncan's test at level of 1% together have significant differences.

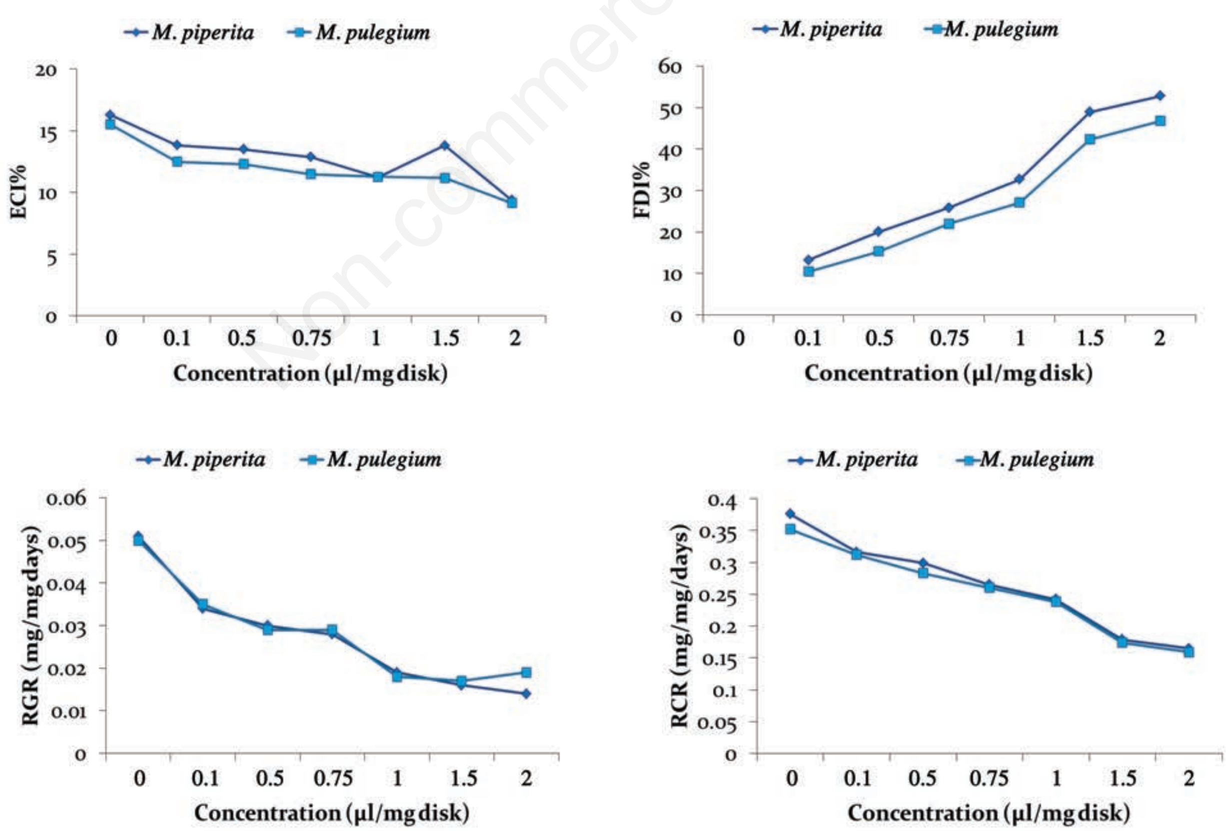

Figure 1. Effect of essential oil *Mentha piperita* and *Mentha pulegium* at various concentrations on nutritional indices larvae of *Plodia interpunctella*.

between plant source and dose, indicating that the effect of plant source varied significantly with dose (Table 1). Essential oils of *M. piperita* had a significantly greater negative impact on nutritional indices than did the essential oils of *M. pulegium* (Tables 2 and 3). However, these differences were small (Figure 1).

Discussion and conclusions

In this study, to compare the anti-nutritional effects of essential oils of *M. piperita* and *M. pulegium,* parameters as indicators of nutrition were used by employing no-choice tests of the insects' food, which had been impregnated with various concentrations of the essential oils. In these experiments, two primary outcomes were measured. The first was weight loss of the insects compared with the control during the duration of this experiment, expressed as the RGR index. Second, the RCR index was measured and compared with control insects to measure whether test insects had taken less or avoided eating treated food. The effective weight loss could be related to the impact of essential oils on insect food (Koul *et al*., 2008), and to clarify avoidance of insect feeding, FDI was used. In this experiment, it was observed that increasing the concentration and changing the type of essential oil reduced the RGR and RCR values, so that there was a greater effect with essential oil at high concentrations, and regarding essential oil type, *M. piperita* was found to be more effective. In terms of the mechanism of action in response to this decrease, it is clear that low concentrations of essential oils of *M. piperita* and *M. pulegium* did not show significant differences in terms of ECI, but with very high essential oil concentrations, the value of the ECI was reduced. Even at lower concentrations of the essential oils of *M. piperita* and *M. pulegium,* there was significant inhibition of insect feeding. Therefore, the impact on the RGR and RCR can be attributed to the effects of feeding deterrence or FDI. Even at lower concentrations, these essential oils can effectively reduce insect feeding, as noted in the studies of other researchers.

In this study, to examine the anti-nutritional properties of mint essential oils, Indian meal moth was used as a model, and showed that sub-lethal concentrations of essential oils in warehouse use could prevent the insects from feeding on the stored product. Therefore, we would conclude that a method to combine these essential oils with some storage products could be effective in controlling pests.

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