

Antifeedant Activities of Essential Oils of *Satureja hortensis* and *Fumaria parviflora* against Indian Meal Moth *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae)

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Abstract

Environmental friendly pesticides with a natural origin can be used as an alternative to chemicals in pests control programs. Efficiency of plant extracts from *Satureja hortensis* and *Fumaria parviflora* was tested against the Indian meal moth, *Plodia interpunctella* Hübner for its antifeedant activity. The nutritional indices: relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and feeding deterrence index (FDI) were analysed for first-instar larvae (15 d-old). Treatments were evaluated by the method of flour disk bioassay in the dark, at $27 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ R.H. Concentrations at 0, 0.1, 0.5, 0.75, 1, 1.5 and 2 $\mu\text{L}/\text{disk}$ were prepared from each essential oil and 10 first instar larvae (15 day-old) were introduced into each treatment. After 72 h, nutritional indices were calculated. Results indicated that *S. hortensis* oil was highly effective compared to *F. parviflora* and significantly decreased the relative growth rate and relative consumption rate. Also, in higher concentration (2 $\mu\text{L}/\text{disk}$), the efficiency of conversion of ingested food (9.843%) was significantly low. The *S. hortensis* oil was more effective on feeding deterrence index than *F. parviflora*.

Keywords: Nutritional indices; *Satureja hortensis*; *Fumaria parviflora*; *Plodia interpunctella*

Introduction

Stored cereals, oilseeds, pulses, spices, dried fruits, tree nuts and their processed foods which are important for food and trade purposes, suffer economic and quality losses due to insect pests attack [1-4]. There are over 600 species of beetle pests and 70 species of moths capable of causing quantitative and qualitative losses [5]. Damage caused by pests of stored products in countries that have not advanced storage technology is accounted as 10 to 40 percent [6]. In some rural areas of Iran that use traditional storages, damage caused by stored product insects are 80% and more [7].

The Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is a very common household pest, feeding principally on stored food products. This pest is a major pest during the processing and storage of products including cereal, walnut, almond, pistachio and dates in Iran. The larvae are general feeders, as they are found in grain products, seeds, dried fruit, dog food, and spices [8]. Damage caused by the larvae is spinning silken threads as they feed and crawl, thus webbing the particles of food together [9]. 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 [10].

Synthetic pesticides have been considered the most effective and accessible means of controlling insect pests of stored products [11]. These chemicals are associated with undesirable effects on the environment due to their slow biodegradation, some toxic residues in products and affect mammalian health [12-14]. 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 to 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 [14].

Essential oils are defined as any volatile oil(s) that have strong aromatic components with 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 [15]. 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 [16]. Effects of essential oils on stored-product insect pests have been reported extensively [17-22].

The insecticidal activity of some essential oils from Lamiaceae has been evaluated against number of stored product insects [23] found strong insecticidal activity of essential oil from *Satureja hortensis* (Lamiaceae) on *P. interpunctella*. Aliakbari et al. [24] studied insecticidal activity of the essential oil from *Thymus daenensis* (Lamiaceae) on *Tribolium confusum* (Tenebrionidae). Ebadollahi and Mahboubi [25] studied fumigant activity of the essential oil from *Lavandula stoechas* L. (Lamiaceae) on *Tribolium castaneum*. Rafiei-Karahroodi et al. [26] 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*. The main goal of the present

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study was to evaluate the insecticidal activities of essential oils from *S. hortensis* and *F. parviflora* for the control of *P. interpunctella*.

Materials and Methods

Insect culture

The Indian meal moths 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 $28 \pm 2^\circ\text{C}$.

Collection of plant materials: Two plants known to have medicinal activity, *S. hortensis* and *F. parviflora* were collected from different localities in Iran. The identity of plant species was verified by Shahabedin Mirinejad (botanical specialist from Agriculture and Natural Resources Researches Center of Kohgiluyeh va Boyerahmad, Yasouj).

Extraction of essential oils

Plant materials were shade dried at room temperature ($26-28^\circ\text{C}$) 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 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 and 45 mg and their moisture content was approximately 15%. Different concentrations of essential oils from *S. hortensis* and *F. parviflora* (0.1, 0.5, 0.75, 1, 1.5 and 2 mL 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 $25 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ 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 was noted. For each experiment 5 replicates were maintained.

Nutritional indices

Nutritional indices were calculated according to Tripathi et al. [27] 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 ($\text{ECI} = \text{RGR} / \text{RCR} \times 100$). The feeding deterrent activity was calculated as a feeding deterrent index [16]:

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

where C is the weight consumed in control and T is the weight consumed in treatment.

Data analysis

Each of the indices was calculated using completely randomized factorial design, and five replicates were performed. The first factor in this design included three treatments, consisting of the essential oils of *S. hortensis*, *F. parviflora* 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 mL/disk, and a control. 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 5% significance level.

Results

Effect of essential oil on Relative Growth Rate (RGR) of larvae

Analysis of variance showed that effect of essential oil *S. hortensis* and *F. parviflora* on relative growth rate larvae of *P. interpunctella* at different concentrations there are significant differences with each other. Also, interaction between essential oil and concentration was significant at 1% level (Table 1). In Table 2 showed that *S. hortensis* significantly more effective than *F. parviflora* and relative growth rate (RGR) has reduced. Also, the essential oils at all concentrations show significant differences with control (Table 3). Results of the effect of increasing concentrations on RGR showed that concentration of 2 μL /disk could be more effective than other concentration. Also, between concentrations of 0.1 and 0.5 μL /disk, 0.5 and 0.75 μL /disk and 1 and 1.5 μL /disk no significant difference was observed.

Source of variation	Degrees of freedom	Mean squares			
		RGR	RCR	ECI	FDI
Plant	1	$7.05 \times 10^{-4**}$	0.075**	96.639**	5187.825**
Concentration	6	0.002**	0.044**	24.342**	1994.564**
Plant×Concentration	6	3.096×10^{-5ns}	0.005**	14.560**	181.651**
Error	42	2.720×10^{-5}	3.845	4.654	0.033

*and ** respectively significant differences at 5 and 1 % level, ns: Non-significant Abbreviations: RGR: Relative Growth Rate; RCR: Relative Consumption Rate; ECI: Efficiency of Conversion of Ingested Food and FDI: Feeding Deterrence Index

Table 1: Analysis variance of essential oils of *Satureja hortensis* and *Fumaria parviflora* on nutritional indices larvae of *Plodia interpunctella*.

Essential oil	RGR (mg/mg/day)	RCR(mg/mg/day)	ECI %	FDI %
<i>Satureja hortensis</i>	0.028 ± 0.002^b	0.219 ± 0.160^b	15.564 ± 0.349^a	38.219 ± 5.78^a
<i>Fumaria parviflora</i>	0.030 ± 0.001^a	0.294 ± 0.006^a	13.118 ± 529^b	17.348 ± 4.457^b

Dissimilar letters in each column with using Duncan test at level of 1% together have significant differences.

Abbreviations: RGR: Relative Growth Rate; RCR: Relative Consumption Rate; ECI: Efficiency of Conversion of Ingested Food and FDI: Feeding Deterrence Index

Table 2: Effect of essential oil *Satureja hortensis* and *Fumaria parviflora* on nutritional indices 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)	0.049 ± 0.00 ^a	0.3129 ± 0.014 ^a	15.265 ± 0.453 ^a	
0.1	0.033 ± 0.001 ^b	0.250 ± 0.012 ^b	12.786 ± 0.765 ^a	12.653 ± 2.065 ^f
0.5	0.029 ± 0.001 ^{bc}	0.228 ± 0.015 ^c	12.542 ± 0.749 ^{ab}	18.077 ± 2.780 ^e
0.75	0.025 ± 0.000 ^c	0.208 ± 0.012 ^d	12.756 ± 0.410 ^{abc}	23.795 ± 3.118 ^d
1	0.017 ± 0.002 ^d	0.166 ± 0.014 ^e	11.354 ± 0.567 ^{abc}	30.569 ± 3.653 ^c
1.5	0.014 ± 0.001 ^d	0.112 ± 0.020 ^f	13.799 ± 1.862 ^{cb}	45.781 ± 6.011 ^b
2	0.010 ± 0.003 ^e	0.101 ± 0.026 ^g	9.843 ± 1.718 ^c	50.238 ± 5.543 ^a

Dissimilar letters in each column with using Duncan's test at level of 1% together have significant differences.

Abbreviations: RGR: Relative Growth Rate; RCR: Relative Consumption Rate; ECI: Efficiency of Conversion of Ingested Food and FDI: Feeding Deterrence Index.

Table 3: Total average effect of essential oil *Satureja hortensis* and *Fumaria parviflora* at various concentrations on nutritional indices larvae of *Plodia interpunctella*.

Effect of essential oil on Relative Consumption Rate (RCR) of larvae

According to Table 1, effects of essential oil of *S. hortensis* and *F. parviflora* and concentrations on relative consumption rate larvae of *P. interpunctella* showed that essential oils, concentrations and interactions between them are all significant at 1% level. Essential oil of *S. hortensis* compared with essential oil *F. parviflora* significantly had a large impact so that relative consumption rate much more reduced (Table 2). Results in Table 3 also showed that essential oils at all concentrations have significant difference with control. Increasing concentrations of essential oils caused of a significant decrease in the amount of relative consumption rate and greatest effect on the concentration 2 µL/disk so that significantly value of relative consumption rate compared with control is reduced.

Effect of essential oil on Efficiency of Conversion of Ingested Food (ECI) of larvae

Analysis of variance showed that effect of essential oil *S. hortensis* and *F. parviflora* on efficiency of conversion of ingested food larvae of *P. interpunctella* at different concentrations there are significant differences with each other. Also, interaction between plant and concentration was significant at 1% level (Table 1). The results showed that effect type of essential oil two thyme plants is concentration-dependent. Although, property of anti-feeding essential oil of *S. hortensis* from *F. parviflora* is higher but significant differences occur only in certain concentrations. Therefore, generally not possible to judge that in all cases of anti-nutritional properties of essential oil *S. hortensis* is higher than essential oil *F. parviflora*. According to Table 3, between concentrations of 1.5 and 2 µL/disk significant differences was observed with the control, but there was no significant difference between control and other concentrations. Therefore, effect of essential oils at high concentrations increased and caused were significant decrease of efficiency of conversion of ingested food.

Effect of essential oil on Feeding Deterrence Index (FDI) of larvae

Analysis of variance showed that effect of essential oil *S. hortensis* and *F. parviflora* on feeding deterrence index larvae of *P. interpunctella* at different concentrations there are significant differences with each other (Table 1). Generally, essential oil of *S. hortensis* had large effect on feeding deterrence index and F.D.I. value significantly compared with essential oil of *F. parviflora* increased (Table 2). There was no significant difference between all concentrations (Table 3), and the feeding deterrence index was higher with increasing concentration. Also, interactions between plant and the concentration were significant (Table 1).

Discussion and Conclusions

In this study, to compare the anti-nutritional effects of essential oils of *S. hortensis* and *F. parviflora*, 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 [18], 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, *S. hortensis* 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 *S. hortensis* and *F. parviflora* 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 *S. hortensis* and *F. parviflora*, 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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