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Research Article

Effects of asafoetida, geranium and walnut leaves essential oils on nutritional indices and progeny reduction on *Rhyzopertha dominica* adults (Coleoptera: Bostrychidae)

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Abstract: In this study, the efficiency of essential oils from asafoetida, *Ferula assa foetida* L., geranium, *Pelargonium hortorum* L.H. Bailey and walnut leaves, *Juglans regia* F. was investigated on nutritional indices of *Rhyzopertha dominica* (F.) adults. Wheat grains were treated with different concentrations of the oils. 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 at 28 ± 2 °C, 65 ± 5% R. H. in continuous darkness. RGR, RCR and ECI deceased as the concentration level was increased. While, FDI percent increased significantly as the concentration level increased. Asafoetida oil had the most efficiency on nutritional indices and 28.61 ppm of the oil was enough to decrease RGR, RCR and ECI% to 0.032 mg/mg/day, 0.444 mg/mg/day and 6.994%, respectively. FDI percent of adults exposed to 6.5 ppm of asafoetida oil was 13.31% which increased upto 64.62% at the concentration of 28.61 ppm. In the second experiment, the effect of tested essential oils was evaluated on F1 progeny reduction of *R. dominica.* Walnut and asafoetida leaves essential oils significantly suppressed progeny production by (59.92%) and (53.2%) respectively.

Keywords: Essential oil, lesser grain borer, progeny, Relative Growth Rate, Relative Consumption Rate.

Introduction

Many plant essential oils and their constituents have been recognized to be toxic, antifeedant, repellentand oviposition inhibitory, on stored products insect pests. They have been known as potential alternative to synthetic chemical pesticides. Their advantages include being of natural origin and having less adverse effects on human health and the environment (Isman *et al.*, 2010; Isman, 2006).

The lesser grain borer, *Rhyzopertha dominica* (Fabricius) is a worldwide primary pest of stored grains. It has been considered to be one of the most destructive and penetrative insects of stored cereals. Both adults and larvae infest whole grain cereals. The adults show a preference for the germinal region; which can be quite serious economically (Hill, 2002).

Huang *et al.* (1997) assessed the efficacy of nutmeg oil on the growth rate, food consumption and F1 progeny reduction of *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. In other researches, the antifeedant activity of *Elletaria cardamomum* (L.) Maton. essential oil (Huang *et al.*, 2000) and eugenol, isoeugenol and methyleugenol

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(Huang *et al.*, 2002) was evaluated against *T. castaneum* and *S. zeamais*. Negahban and Moharamipour (2007) investigated the efficiency of *Artemisia sieberi* Besser and *A. scoparia* Waldst et Kit on nutritional indices of *Tribolium castaneum* Herbst.

Antifeedant activity of *Mentha piperita* (L.) and *M. pulegium* (L.) essential oils were tested on nutritional indices of Indian meal moth, *Plodia interpunctella* Hübner (Saeidi and Hassanpour, 2014).

The objective of this experiment was to investigate the efficiency of asafoetida, geranium and walnut leaves essential oils on nutritional indices and progeny reduction of *R. dominica* adults.

Materials and Methods

Insects

Adults of *R. dominica* were taken from a culture that was kept on whole wheat in the Entomology laboratory-Shahid Chamran University for at least 3 years with no history of exposure to insecticides. The insects were kept at 28 ± 2 °C, 65 ± 5% relative humidity (R. H.) and continuous darkness. Adults used in the experiments were 7-14 days old.

Plant essential oils

Gum of asafoetida, *Ferula assa foetida* L., was purchased from grocery in Ahvaz-30 Metri road. Leaves and flowers of geranium, *Pelargonium hortorum* L.H. Bailey, were collected from floriculture in Islamic Azad University of Isfahan and leaves of walnut, *Juglans rejia* F., were collected from walnut orchard of Semirom city. Plant materials were dried naturally on laboratory bench at room temperature until they were crisp. The plant materials were stored at -24 °C until they were needed for experiments. Approximately 40 g of each plant material were milled and put into the Round-bottom flask over water. Essential oil was extracted by hydrodistillation using a Clevenger type apparatus at a temperature around 100 °C for 4 h. Volatile oil emitted in the reservoir was collected after 4h distillation process. Anhydrous sodium sulphate was used to remove water after extraction. Extracted oil was stored in a refrigerator at 4 °C.

Bioassays

Antifeedant bioassay

Wheat grains variety Ahvazi with moisture content of 11.5% were used in the experiments. Wheat grains (10 g) were treated with different concentrations of the essential oils (obtained by a preliminary test) in glass vials. The concentrations were 6.5, 7.8, 10.4, 18.7 and 28.61 mg/kg for asafoetida oil, 175.5, 220.5, 310.7, 581 and 742.27 mg/kg for geranium oil and 16.42, 25, 32.84, 65.69 and 81.35 mg/kg in the case of walnut leaves essential oil. A total of 20 adults, that were kept hungry for 48 h, were added to each vial. Adults' weight was measured before releasing into the vials and almost equal weight was considered for the adults. The experiment was conducted at 28 ± 2 °C, 65 ± 5% R. H. in continuous darkness with six replications. After 48 h, the wheat and adults were weighed and nutritional indices were calculated as follows (Huang *et al.*, 2000):

1. Relative Growth Rate (RGR)

$$= \frac{(A-B)}{(B \times Day)}$$

Where A = weight of live insects (mg)/number of live insects;
B = original weight of insects (mg) / original number of insects.

2. Relative Consumption Rate (RCR)

$$= \frac{D}{(B \times Day)}$$

Where D = biomass ingested (mg)/number of live insects.

3. Efficacy of Conversion of ingested Food (ECI%)

$$= \frac{RGR}{RCR} \times 100$$

4. Feeding Deterrence Index (FDI%)

$$= \frac{(C-T)}{C} \times 100$$

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

Progeny reduction bioassay

Another experiment was conducted to assess the efficiency of tested essential oils on progeny reduction of *R. dominica*. Wheat grains (50 g) were poured in the glass vials (200 ml). Fifty adults of mixed sex were introduced to each vial and the vials were kept in incubator set at experimental conditions. After 12 days, all of insects were discarded from the vials. Then, wheat grains were treated with LC_{95} concentration of each essential oil obtained from previous study on fumigant toxicity of the oils in wheat commodity (Personal communication). According to Hill (2002) the life cycle of *R. dominica* is about 3-4 weeks at about 34 °C and 70% R. H. Therefore, the vials were kept in incubator for additional 30 days. The experiments were replicated six times and untreated wheat was served as a control. Then, the numbers of emerged adults were counted and the percent of reduction in progeny production was calculated as follow:

$$= \frac{(NP_c - NP_t)}{NP_c} \times 100$$

Where; NP_c = number of progeny in control and NP_t = number of progeny in treatment.

Data analysis

The data were analyzed using Analysis of Variance (ANOVA). Means were separated by Tukey multiple range test at P = 0.01 using SPSS software (SPSS, 2007).

Results

Results indicated that asafoetida essential oil significantly affected nutritional indices of *R. dominica*. The oil reduced growth rate (RGR), consumption rate (RCR) and conversion of ingested food (ECI). Also, asafoetida oil had a strong effect on the adults feeding deterrence (FDI%) and FDI% increased with increasing concentrations of asafoetida oil (Table 1).

In the case of geranium essential oil the RGR, RCR and ECI of *R. dominica* adults decreased significantly in a concentration-dependent manner. In addition, geranium oil showed a great effect on FDI% and FDI% increased from 20.94% at the concentration of 175.5 ppm to 44.54% when adults were exposed to wheat treated with 742.3 ppm of the oil (Table 2).

A significant reduction in RGR, RCR and ECI was observed in *R. dominica* adults at 81.35 ppm of walnut leave essential oil. Feeding deterrence percent (FDI%) obtained was 22.99% when adults were exposed to 16.42 ppm of the oil which increased to 54.10% at the concentration of 81.35 ppm (Table 3).

Percent of reduction in progeny production of *R. dominica* exposed to three tested essential oils is presented in Figure 1. Significant reduction in progeny production was observed in walnut leaves (59.92%) and asafoetida (53.2%) essential oil.

Table 1 Effects of asafoetida essential oil on relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and feeding deterrence index (FDI) of *Rhyzopertha dominica*.

Nutritional indices	Concentrations (ppm)					
	6.5	7.8	10.4	18.7	28.61	$F_{4, 25}$, P
RGR (mg/mg/day)	0.077 ± 0.001a	0.063 ± 0.002b	0.046 ± 0.001c	0.039 ± 0.001d	0.032 ± 0.001e	158.5, < 0.001
RCR (mg/mg/day)	0.936 ± 0.005a	0.835 ± 0.018b	0.715 ± 0.010c	0.585 ± 0.013d	0.444 ± 0.018e	212.8, < 0.001
ECI (%)	8.195 ± 0.113a	7.517 ± 0.186ab	6.422 ± 0.262bc	6.717 ± 0.156bc	6.994 ± 0.258c	11.83, < 0.001
FDI (%)	13.31 ± 1.311a	29.71 ± 2.388b	48.72 ± 1.36c	56.07 ± 1.24d	64.62 ± 0.708e	190.2, < 0.001

Means followed by the same letter(s) in each row are not significantly different using Turkey's Test at $P < 0.05$.

Table 2 Effects of geranium essential oil on relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and feeding deterrence index (FDI) of *Rhyzopertha dominica*.

Nutritional indices	Concentrations (ppm)					
	175.5	220.5	310.7	581.0	742.3	$F_{4,25}$, P
RGR (mg/mg/day)	0.089 ± 0.000a	0.084 ± 0.000b	0.077 ± 0.000c	0.068 ± 0.001d	0.068 ± 0.001e	159.0, < 0.001
RCR (mg/mg/day)	0.435 ± 0.000a	0.426 ± 0.000ab	0.420 ± 0.001b	0.407 ± 0.004c	0.388 ± 0.004d	41.80, < 0.001
ECI (%)	20.56 ± 0.143a	19.77 ± 0.105b	18.29 ± 0.105c	16.70 ± 0.199d	16.17 ± 0.223d	129.8, < 0.001
FDI (%)	20.94 ± 0.577a	25.50 ± 0.637b	31.88 ± 0.539c	39.71 ± 1.134d	44.54 ± 0.817e	159.0, < 0.001

Means followed by the same letter in each row are not significantly different using Turkey's Test at $P < 0.05$.

Table 3 Effects of walnut leaves essential oil on relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and feeding deterrence index (FDI) of *Rhyzopertha dominica*.

Nutritional indices	Concentrations (ppm)					
	16.42	25.00	32.84	65.69	81.35	$F_{4,25}$, P
RGR (mg/mg/day)	0.087 ± 0.000a	0.081 ± 0.000b	0.073 ± 0.000c	0.063 ± 0.001d	0.052 ± 0.001e	141.1, 0.000
RCR (mg/mg/day)	0.541 ± 0.000a	0.540 ± 0.000a	0.540 ± 0.000a	0.540 ± 0.000a	0.500 ± 0.012b	10.10, 0.000
ECI (%)	16.10 ± 0.151a	15.04 ± 0.192b	13.42 ± 0.165c	11.61 ± 0.319d	10.38 ± 0.179e	125.63, 0.000
FDI (%)	22.99 ± 0.665a	28.38 ± 0.879b	35.71 ± 0.847c	44.45 ± 1.525d	54.10 ± 1.107e	141.1, 0.000

Means followed by the same letter in each row are not significantly different using Turkey's Test at $P < 0.05$.

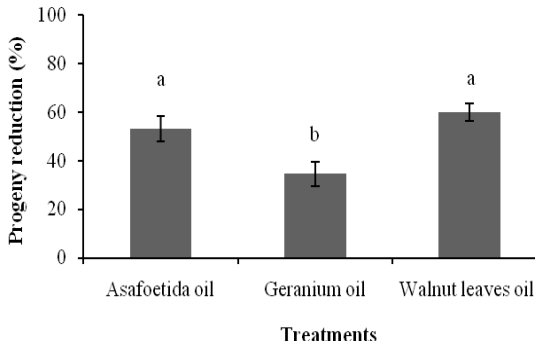

Figure 1 Percent reduction in progeny production of *Rhyzopertha dominica* exposed to asafoetida, geranium and walnut leaves essential oils.
Means bearing the same letter are not significantly different using Turkey's test at $P < 0.05$.

Discussion

Effects of different essential oils on nutritional indices of stored product insects have been studied by different researchers (Huang *et al.*, 2002; Huang and Ho, 1998; Huang *et al.*, 2002; Negahban and Moharamipour, 2007; Saeidi and Hassanpour, 2014). According to our findings nutritional indices (RGR, RCR and ECI) of *R. dominica* adults decreased with increasing concentration level of EOs. These findings are similar to those of EOs from *A. sieberi* and *A. scoparia* (Negahban and Moharamipour, 2007). They reported that the RGR, RCR and ECI percent of *T. castaneum* adults increased as the concentration increased; while FDI percent increased with increasing concentration level.

In our observation, asafoetida essential oil has more antifeedant activity on *R. dominica* adults. Growth rate (RGR), consumption rate (RCR) and conversion of ingested food (ECI) decreased as the concentration level increased. Our results are in agreement with those of Nazemi Rafie *et al.* (2004). They stated that asafoetida extract had the greater effect on nutritional indices of second instar larva of *Ephestia kuehniella* Zeller than the other two tested plant extracts (*Nerium oleander* L. and *Lavandula officinalis* Chaix). Therefore, asafoetida essential oil is effective even at lower concentrations in reducing *R. dominica* adults feeding indices.

Negahban and Moharamipour (2007) found that *A. sieberi* was highly effective compared to *A. scoparia* oil and significantly decreased the RGR and RCR of *T. castaneum*. They stated that the ECI (9.81%) was significantly very low even in the *A. sieberi* oil.

Huang *et al.* (1997) stated that the feeding deterrence index of *T. castaneum* in a flour disc treated with nutmeg oil was 7%, whereas that of *S. zeamais* was 33%. Eugenol, the major constituent of cloves oil also significantly reduced food consumption (RCR) in the adults of *S. zeamais* at a concentration of 13.2 mg/g food. While, for *T. castaneum*, concentrations of 35 and 99 mg/g food is required to reduce the growth rate (RGR) and RCR, respectively.

Saeidi and Hassanpour (2014) reported that *M. piperita* essential oil was more effective than *M. pulegium* by significantly reducing *P. interpunctella* nutritional indices. Therefore, most of the essential oils have antifeedant activity and their potential on nutritional indices should be studied. Our study indicated that asafoetida oil was generally a more effective essential oil in reducing the growth rate, food consumption and food utilization of *R. dominica*.

Huang *et al.* (1997) noted that the F1 progeny production was suppressed for both *T. castaneum* and *S. zeamais*. The most progeny reduction was recorded at the concentration of 1.05 g/100 g rice for *T. castaneum* and 0.35 g/100 g rice in the case of *S. zeamais*. Our results indicated that walnut leaves essential oil has the most efficacy on F1 progeny reduction (59.9%) followed by asafoetida oil (53.2%). The reduction in progeny production may be in accordance with the efficacy of the oils on oviposition, egg hatch or survival of the larvae (Huang *et al.*, 1997). The suppression of progeny production in both *T. castaneum* and *S. zeamais* exposed to *E. cardamomum* essential oil was also reported (Huang *et al.*, 2000). Cardamom oil suppressed egg hatching and larval survival of *T. castaneum*; while, the oil didn't prevent oviposition of the adults. They concluded that cardamom oil was ovicidal to the eggs of *T. castaneum*. However, more studies are required to determine the main reason for the progeny reduction of the test results.

Our study suggests that asafoetida essential oil may be a potential grain protectant according to its antifeedant activity. Further research should be conducted to formulate asafoetida oil to increase the oil antifeedant efficiency before commercial application can be considered.

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اثر اسانس‌های آنغوزه، شمعدانی، و برگ گردو روی شاخص‌های تغذیه و کاهش تولید نتاج حشرات بالغ *Rhyzopertha dominica*

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چکیده: در این مطالعه، کارایی اسانس آنغوزه، *Ferula assa foetida* L.، شمعدانی، *Pelargonium hortorum* L.H. Bailey و برگ گردو، *Juglans rejia* F. روی شاخص‌های تغذیه‌ای حشرات بالغ *Rhyzopertha dominica* (F.) مورد بررسی قرار گرفت. دانه‌های گندم با غلظت‌های مختلف از اسانس گیاهان تیمار شدند. شاخص‌های تغذیه‌ای شامل نرخ نسبی رشد (RGR)، نرخ نسبی مصرف (RCR)، کارایی تبدیل غذای خورده شده (ECI) و شاخص بازدارندگی تغذیه (FDI) در دمای ۲ ± ۲۸ درجه سلسیوس و رطوبت نسبی ۵ ± ۶۵ درصد در شرایط تاریکی اندازه‌گیری شد. مقدار RGR، RCR و درصد ECI با افزایش غلظت کاهش یافت. درصورتی‌که، درصد FDI به‌طور معنی‌داری با افزایش سطح غلظت افزایش نشان داد. اسانس آنغوزه دارای بیش‌ترین کارایی روی شاخص‌های تغذیه بوده و ۲۸/۶۱ پی‌پی‌ام از آن برای کاهش مقدار RGR، RCR و درصد ECI به‌ترتیب تا ۰/۰۳۲ میلی‌گرم/میلی‌گرم/ روز، ۰/۴۴۴ میلی‌گرم/میلی‌گرم/روز و ۶/۹۹۴ درصد کافی بود. درصد FDI حشرات بالغ تیمار شده با غلظت ۶/۵ پی‌پی‌ام از اسانس آنغوزه ۱۳/۳۱ درصد بوده و در غلظت ۲۸/۶۱ پی‌پی‌ام تا ۶۴/۶۲ درصد محاسبه شد. در آزمایش دوم، اثر اسانس‌های تست شده روی کاهش تعداد نتاج نسل اول *R. dominica* بررسی شد. اسانس برگ گردو (۵۹/۹۲ درصد) و آنغوزه (۵۳/۲ درصد) به‌طور معنی‌داری باعث کاهش تولید نتاج شدند.

واژگان کلیدی: اسانس گیاهان، سوسک کشیش، نتاج، نرخ نسبی رشد، نرخ نسبی مصرف