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Research Article

Antifeedant activity of *Descurainia sophia* and *Thuja orientalis* extracts against *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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Abstract: Antifeedant activity of the aqueous and hydroalcoholic extracts of leaves of *Descurainia sophia* L. and *Thuja orientalis* L. were tested against adults of *Tribolium castaneum* (Herbst). The experiment was designed to measure the nutritional indices such as relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and feeding deterrence index (FDI). Treatments were evaluated by the method of flour disc bioassay under dark condition, at 27 ± 1 °C and 60 ± 5% RH. Ten microliter portions of each extract at concentrations (0.25-2.0%) was spread evenly on the flour discs. Results indicated that nutritional indices were significantly affected as extract concentrations increased. In this study extracts from *T. orientalis* decreased RGR, RCR and ECI significantly more than that of *D. sophia*. In addition, hydroalcoholic extracts decreased RGR, RCR and ECI significantly more than those by aqueous extracts. Both of plant extracts increased FDI as the extract concentrations were increased, showing high feeding deterrence activity against *T. castaneum*. Generally, antifeedant activity of *T. orientalis* was greater than *D. sophia* and hydroalcoholic extracts were more effective than aqueous extracts.

Keywords: *Descurainia sophia*, *Thuja orientalis*, stored products, plant extract, red flour beetle

Introduction

Damage caused by insects affects the quality, the quantity and the commercial value of the products. Many pests of stored products belong to the order Coleoptera and one of the most destructive secondary insect pests of durable stored products is the red flour beetle, *Tribolium castaneum* (Herbst) (Adarkwah *et al*., 2010). This species has a long association with human stored food and has been found in association with a wide range of commodities including grain, flour, peas, beans, cacao, nuts, dried fruits, and spices, but milled grain products such as flour appear to be their preferred food (Good, 1936; Campbell and Runnion, 2003). To control this pest, fumigants and other synthetic insecticides are widely used. Currently, malathion has been used comprehensively for controlling red flour beetle, but now, it is not so effective against this pest due to insect resistance (Horton, 1984). Therefore, this has led to the usage of other biorationl insecticides with different modes of action such as abamectin, deltamethrin, and chlorpyrifos, which might bring about a proper control of the pest. It is reported that chlorpyrifos and deltamethrin had more viability contrasted with malathion against adult red flour beetle (Mansee and Montasser, 2003). Abamectin is another expected insecticide that

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is known as antibiotic insecticide (Ware and Whitacre, 2004), and is produced by the soil bacterium *Streptomyces avermitilis* (Hayes and Laws, 1990). Considering negative effects of synthetic pesticides especially on non-target organisms caused a general perception that natural compounds are better products or generally regarded as safe (GRAS) (Scott *et al*., 2003). In order to overcome the undesirable effects of insecticide use, many researchers are looking for new biological insecticides, which provide pest control with minimal environmental hazards (Emsen *et al*., 2012a, b; Yildirim *et al*., 2012a, b; Yildirim *et al*., 2013). Plant extracts and essential oils have been reported to possess several types of bioactivities including antibacterial, antiviral, antifungal, antifeedant, insecticidal and medicinal (analgesic, sedative, anti-inflammatory, spasmolytic, locally anesthetic) (Isman, 2002; Bakkali *et al*., 2008; Adorjan and Buchbauer, 2010). The use of plant extracts, including allelochemical compounds such as essential oils, with known effects on insects, could be a useful complementary or alternative method to the heavy use of classical insecticides (Regnault-Roger, 1997). Plant allelochemicals exert a wide range of influences on insects: they can be repellent, deterrent or antifeedant. Any substance that reduces food consumption by an insect can be considered as an antifeedant or feeding deterrent (Isman, 2002).

The effect of plant extracts and essential oils on different insects and mites has been investigated using toxicity, repellency activity and antifeedant activity (Moharramipour and Nazemi Rafih, 2008; Pavela, 2009; Motazedian *et al*., 2012; Kumar and Gupta, 2013, Mobolade *et al*., 2015).

Descurainia sophia (L.) (Flixweed) belonging to the family Brassicaceae (Cruciferae) is a plant which is widely distributed throughout Europe, Asia and the Middle East (Afsharypour and Lockwood, 1985; Muhammad and Artabotryside, 2012). However, to the best of our knowledge, studies have not been yet conducted to evaluate the antifeedant activities of *D. sophia* extracts against insects.

Thuja orientalis (Commonly- Morpankhi, Family- Cupressaceae) is an evergreen, monoecious trees or shrubs used in various forms of traditional medicines and homeopathy in various ways. Recent researches in different parts of the world have shown that *T. orientalis* and its active component thujone have the great potential against a various health problems. It can be used as antioxidant, anticancer and anti-inflammatory agent. Instead of these effects, it can be also used as insecticidal, molluscicidal and nematicidal activity against different pests (Srivastava *et al*., 2012).

The aim of the present study was to investigate antifeedant activities of *D. sophia* and *T. orientalis* extracts against adults of *T. castaneum*.

Materials and Methods

Insect culture

T. castaneum was reared on wheat flour mixed with yeast (10: 1 w/w). The cultures were maintained in the dark at 27 ± 1 °C and 60 ± 5% RH. All adults used in the experiments were 1-7 days old.

Preparation of plant extracts

Leaves of *D. sophia* and *T. orientalis* were collected from campus of Miandoab (Urmia University) in North West Iran. The collected plant materials were dried at room temperature (23-24 °C). The dried materials were stored at 4 °C. The air-dried ground (80 mesh) plant material (20g for each sample) was extracted with each of the solvents-aqueous and hydroalcoholic (70 ethanol: 30 water) (200ml)-for 6 hours at room temperature in an orbital shaker. The extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The residues were extracted twice with the same fresh solvent and extracts combined. Plant extracts were stored in a refrigerator at 4 °C.

Antifeedant assay

Flour discs were prepared according to the method of Xie *et al*. (1996), in brief, 10g of flour was mixed with 50ml of distilled water. Using a micropipette, 200µl of the prepared suspension was poured on a nylon sheet to convert the suspension to tablet-like discs. The discs were placed in normal room conditions for 4 h. and then transferred to sterile petri dishes with the help of fine forceps. The flour discs were stored for 12h. inside the hood to dry completely. Preliminary experiments were done, then the concentrations to be tested were determined. Flour discs were treated with 10 µl of the extracts prepared at 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0% (v/v). Discs to which only the solvent had been applied were used as the control. The solvent was allowed to evaporate for 10 min at room temperature. *T. castaneum* adults were starved for 48h prior to the experiment. In each container 2 flour discs carrying the same extract and dose, and 10 starved adults were placed. Each experiment was replicated four times and set at the above stated conditions. The weight of the flour discs, insects and plastic containers (130ml) were separately measured and recorded at the beginning and at 3 days after the start of the experiment. The nutritional indices were calculated according to Huang *et al*. (2000) with some modifications as follows:

Relative Growth Rate (RGR) = (A-B)/(B × day).

Where: A: weight of live insects on the third day (mg)/ number of live insects on the third day, B: original weight of insects (mg)/ original number of insects

Relative Consumption Rate (RCR) = D/(B × day).

Where: D: biomass ingested (mg)/number of live insects on the third day

Efficacy of Conversion of Ingested Food (ECI%) = RGR/RCR × 100

Feeding Deterrence Index (FDI%) = [(C-T)/C] × 100.

Where: C: the food consumption in control discs (mg), T: food consumption in treated discs (mg), for antifeedant activity, the formula described by Isman *et al*. (1990) was modified in calculating the feeding deterrence index.

Statistical Analysis

Statistical analysis were done using SPSS 19.0 software. Normality of the data was tested by Kolmogorov-Smirnov method. One-way analysis of variance and General Linear Model (GLM) with Duncan's multiple range tests ($P < 0.05$) were used to determine differences between means. In addition, correlations between measured factors were calculated.

Results

Effect of *D. sophia* and *T. orientalis* extracts on nutritional indices

The feeding efficiency of *T. castaneum* adults that were fed on discs treated with *D. sophia* and *T. orientalis* extract was greatly affected. The antifeedant activity varied significantly based on the solvents used for extraction. The difference between aqueous and hydroalcoholic extracts was significant ($P < 0.05$) and hydroalcoholic extracts were more effective than aqueous extracts. For both plant extracts the values of RGR, RCR and ECI were reduced significantly ($P < 0.05$) with increasing *D. sophia* and *T. orientalis* extracts concentration. Results on the effect of *D. sophia* extracts on nutritional indices of *T. castaneum* adults are presented in Table 1. It was shown that *T. orientalis* extract had a strong effect on feeding behavior and growth of this pest. Results of the effect of *T. orientalis* extract on reduced feeding efficiency are presented in Table 2.

The RGR index is significantly reduced compared to the control even in the lowest concentration used. The treatment at higher concentration (2%) of *D. sophia* caused 97% (for aqueous) and 140% (for hydroalcoholic extracts) reduction of relative growth rate in adults respectively. Hydroalcoholic extract of *T. orientalis* decreased RGR more than 79% at concentrations higher than 1.0% compared to control (Table 2). Aqueous extract of *T. orientalis* decreased RGR more than 69% at concentrations higher than 1.0% compared to control. The reduction rate gradually increased with increasing extract concentration. The results also indicated that the reduction rate of RCR was higher in hydroalcoholic extracts than aqueous extracts of the two medicinal plants. Similar to the RGR trend, the reduction rate of the ECI increased with increasing extract concentration. In contrast, the FDI values increased significantly (P < 0.05) with increasing plant extracts concentration. Hydroalcoholic extracts of *D. sophia* and *T. orientalis* increased FDI from 0 to 76% and 90%, but aqueous extracts increased FDI to 63% and 77%, respectively. GLM analysis showed that the difference of nutritional indices between plants was significant (P < 0.05), also the effects of extracts, treatments and extract × treatment were significant.

Table 1 The effect of *Descurainia sophia* extracts on nutritional indices (mean ± SE) of *Tribolium castaneum* adults.

Concentration % (v/v)	RGR (mg/mg/day)		RCR (mg/mg/day)		ECI (%)		FDI (%)	
	Aqueous	Hydroalcoholic	Aqueous	Hydroalcoholic	Aqueous	Hydroalcoholic	Aqueous	Hydroalcoholic
Control	0.251 ± 0.01^{a}	0.245 ± 0.01^{a}	0.752 ± 0.02^{a}	0.748 ± 0.02^{a}	37.56 ± 1.45^{a}	34.19 ± 1.09^{a}	0.000^{g}	0.000^{f}
0.25	0.203 ± 0.01^{ab}	0.194 ± 0.01^{b}	0.636 ± 0.01^{b}	0.617 ± 0.01^{b}	32.76 ± 0.95^{a}	31.43 ± 1.56^{ab}	18.22 ± 1.60^{a}	21.81 ± 1.03^{e}
0.5	0.148 ± 0.01^{bc}	0.134 ± 0.01^{c}	0.509 ± 0.02^{c}	0.459 ± 0.02^{c}	30.54 ± 0.72^{a}	29.21 ± 0.99^{bc}	25.38 ± 1.13^{a}	27.53 ± 1.48^{d}
0.75	0.107 ± 0.01^{c}	0.078 ± 0.01^{d}	0.280 ± 0.03^{d}	0.194 ± 0.01^{d}	29.86 ± 0.71^{a}	28.80 ± 0.78^{bc}	39.47 ± 3.98^{a}	49.74 ± 1.36^{c}
1.0	0.100 ± 0.01^{c}	0.065 ± 0.01^{d}	0.224 ± 0.01^{de}	0.212 ± 0.01^{d}	27.45 ± 1.16^{a}	25.67 ± 1.68^{c}	50.18 ± 6.28^{a}	66.64 ± 1.54^{b}
1.5	0.033 ± 0.006^{d}	-0.057 ± 0.01^{e}	0.230 ± 0.01^{de}	0.216 ± 0.01^{d}	-0.37 ± 10.31^{b}	-27.08 ± 2.91^{d}	61.88 ± 6.13^{a}	77.89 ± 1.71^{a}
2.0	0.007 ± 0.006^{d}	-0.102 ± 0.01^{f}	0.181 ± 0.02^{e}	0.144 ± 0.01^{e}	-9.60 ± 13.12^{b}	-44.27 ± 1.18^{e}	63.13 ± 4.94^{a}	76.02 ± 1.13^{a}
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F (df = 6, 21)	15.506	185.179	144.521	239.486	8.223	409.394	32.171	536.667

Means with different letters in the same column indicate significant differences (Duncan's multiple range test, P < 0.05).

Table 2 The effect of *Thuja orientalis* extracts on nutritional indices (mean ± SE) of *Tribolium castaneum* adults.

Concentration % (v/v)	RGR (mg/mg/day)		RCR (mg/mg/day)		ECI (%)		FDI (%)	
	Aqueous	Hydroalcoholic	Aqueous	Hydroalcoholic	Aqueous	Hydroalcoholic	Aqueous	Hydroalcoholic
Control	0.579 ± 0.01^{a}	0.600 ± 0.01^{a}	0.714 ± 0.02^{a}	0.891 ± 0.01^{a}	81.29 ± 1.34^{a}	67.39 ± 1.42^{a}	0.000^{f}	0.000^{e}
0.25	0.452 ± 0.01^{b}	0.445 ± 0.01^{b}	0.567 ± 0.03^{b}	0.656 ± 0.02^{b}	73.25 ± 1.13^{b}	64.66 ± 2.10^{a}	11.91 ± 1.18^{e}	12.23 ± 1.15^{d}
0.5	0.338 ± 0.02^{c}	0.318 ± 0.01^{c}	0.389 ± 0.02^{c}	0.565 ± 0.03^{c}	69.50 ± 0.88^{b}	50.81 ± 1.34^{b}	33.69 ± 1.99^{d}	58.39 ± 2.42^{c}
0.75	0.229 ± 0.01^{d}	0.224 ± 0.01^{d}	0.375 ± 0.01^{c}	0.350 ± 0.01^{d}	57.40 ± 1.85^{c}	50.67 ± 1.14^{b}	54.97 ± 1.20^{c}	70.02 ± 1.23^{b}
1.0	0.174 ± 0.01^{e}	0.125 ± 0.01^{e}	0.186 ± 0.01^{d}	0.241 ± 0.01^{e}	52.39 ± 2.53^{d}	35.06 ± 1.59^{c}	65.75 ± 1.55^{b}	82.25 ± 1.33^{b}
1.5	0.134 ± 0.01^{f}	-0.009 ± 0.01^{f}	0.173 ± 0.01^{d}	0.150 ± 0.01^{f}	38.73 ± 1.57^{e}	3.25 ± 1.40^{d}	75.09 ± 1.03^{a}	86.45 ± 1.24^{a}
2.0	0.058 ± 0.01^{g}	-0.091 ± 0.01^{g}	0.137 ± 0.01^{d}	0.146 ± 0.01^{f}	32.46 ± 1.94^{f}	-52.86 ± 1.65^{e}	77.13 ± 1.75^{a}	90.64 ± 2.31^{a}
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F (df = 6, 21)	205.009	433.225	172.099	344.265	114.758	769.114	498.629	541.247

Means with different letters in the same column indicate significant differences (Duncan's multiple range test, P < 0.05).

Discussion

From an ecological point of view, antifeedants are very important since they rarely kill the target insects directly and let them be available to their natural enemies and help in the maintenance of natural balance (Arivoli and Tennyson, 2013). These plant materials are used mainly on stored product pests. In fact, management of stored product pests, using substances of natural origin, is nowadays the subject of many studies (Isman, 2006). Aromatic plants are known for their healthful and medicinal properties. Methanolic extract of *T. occidentalis* has insecticidal (on *T. castaneum*) and antioxidant activity (Ahmad *et al*., 2013). Leaf oil of *T. orientalis* has shown natural larvicidal effect against *Aedes aegypti* and *Culex pipiens pallens* (Ju-Hyun *et al*., 2005). Ethanolic extract of *T. orientalis* leaf at 20, 40, 60, and 80%, used 3 times at intervals, caused mortality of eggs and juveniles of *Meloidogyne incognita* (Cannayane and Rajendran, 2002).

The results obtained from the antifeedant assays clearly demonstrated that the aqueous and hydroalcoholic extracts obtained from *D. sophia* and *T. orientalis* leaves have antifeedant effects on *T. castaneum* adults. The antifeedant and repellent effects of various plant extracts and essential oils have been reported on the cosmopolitan red flour beetle, *T. castaneum*, is one of the world's most important pests of cereal products (Kumar and Gupta, 2013; Guruprasad and Akmal, 2014). In our study, hydroalcoholic extracts of *D. sophia* and *T. orientalis* leaves had higher antifeedant activity than the aqueous extracts for *T. castaneum*, causing 76.02 and 90.64 percent reduction in feeding respectively at 2% (Table 1-2). Compounds with feeding deterrents are generally toxic to insects or cause physiological disturbances in their development or oviposition (Nawrot and Harmantha, 1994). For example azadirachtin is well known antifeedant that interferes with insect growth (Siddig, 1980). There are several reports on antifeedant activity of essential oils. Abbasipour *et al*. (2011) stated that *Datura stramonium* L. (Solanaceae) extract had significant effects on the nutritional indices and mortality of *T. castaneum* adults at different concentrations. They showed that, at higher concentrations, this extract significantly reduced the growth rate, food consumption and utilization in *T. castaneum* adults which is similar to our findings. Ahmadi *et al*. (2015) reported the antifeedant action of the *Perovskia atriplicifolia* essential oil on the red flour beetle adults and larvae. Sahaf and Moharramipour (2009) also reported that antifeedant activity of *Carum copticum* was stronger than *Vitex pseudo-negund* essential oil. These studies support the validity of our experiments however, *T. orientalis* extracts was more effective than *Datura stramonium*, *Perovskia atriplicifolia* and *Carum copticum* on *T. castaneum*.

With increasing concentrations of hydroalcoholic extracts, the feeding deterrence index increased at the same rate, also the efficiency of conversion of ingested food decreased. These results show that the hydroalcoholic extracts, in addition to the feeding deterrence, have considerable role in post-ingestive toxicity. As shown in tables 1 and 2, with increasing the concentration of aqueous and hydroalcoholic extracts of *D. sophia*, the FDI also increased, while the ECI decreased. Increasing the concentration of aqueous extracts of *T. orientalis*, the FDI increased, but the ECI did not decrease, considerably. Although at 2%, it caused 77% feeding deterrence yet the ECI was not decreased as compared with the control accounting for 32%. The results indicated that the feeding deterrency effect of aqueous extracts of *T. orientalis* was more than post-ingestive toxicity. However, comparing the hydroalcoholic extracts of studied plants indicated that *D. sophia* extracts have feeding deterrency effect (63%) and post-ingestive toxicity, while *T. orientalis* extracts mainly have feeding deterrency effect (90%). Consequently, antifeedant activity of hydroalcoholic extract of *T. orientalis* was greater than *D. sophia* (Tables 1-2). In the present study there were positive significant correlations ($P < 0.01$, $r^2 > 0.806$) between RGR and RCR or ECI in treated adults with aqueous extracts of *D. sophia*, whereas $r^2 > 0.873$ was estimated for hydroalcoholic extracts. In treated adults with extracts of *T. orientalis* $r^2 > 0.9$ for both extracts, indicates that aqueous and hydroalcoholic extracts showed no significant difference in correlation values.

Based on the results it could be concluded that the hydroalcoholic extracts of *D. sophia* and *T. orientalis* play a role in pest control due to its antifeedant and post-ingestive toxicity effects. In addition, the feeding deterrency effect of *T. orientalis* extracts was more than *D. sophia* extracts. The results of the investigation would indicate a significant potential for this plant as a possible source of natural insecticide. Therefore, hydroalcoholic extracts of *T. orientalis* may be applied as an alternative to synthetic insecticides for stored-products insect pest management.

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فعالیت ضدتغذیه‌ای عصاره‌های گیاهی خاکشیر *Descurainia sophia* و سرو *Thuja orientalis* علیه شپشه آرد، *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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چکیده: فعالیت ضدتغذیه‌ای عصاره‌های آبی و هیدروالکلی برگ خاکشیر، *Descurainia sophia* L. و سرو، *Thuja orientalis* L. علیه حشرات کامل شپشه آرد، *Tribolium castaneum* مورد بررسی قرار گرفت. جهت ارزیابی شاخص‌های تغذیه‌ای مانند: نرخ رشد نسبی (RGR)، نرخ مصرف نسبی (RCR)، کارایی غذای خورده شده (ECI) و شاخص بازدارندگی تغذیه‌ای (FDI)، آزمایش‌هایی طراحی شد. تیمارها به روش دیسک آردی در دمای ۱ ± ۲۷ درجه سلسیوس و رطوبت نسبی ۵ ± ۶۰ درصد و تاریکی ارزیابی شدند. در این آزمایش ۱۰ میکرولیتر از غلظت‌های (٪۲-۰/۲۵) عصاره هر دو گیاه به‌همراه شاهد به‌طور یکنواخت روی دیسک‌های آردی پخش شدند. نتایج نشان داد که افزایش غلظت عصاره دو گیاه روی شاخص‌های تغذیه شپشه آرد به‌طور معنی‌داری مؤثر بوده است. در این تحقیق، عصاره سرو نرخ رشد نسبی، نرخ مصرف نسبی و کارایی تبدیل غذای خورده شده توسط شپشه آرد را به‌طور معنی‌دار بیش از عصاره خاکشیر کاهش داد. به‌علاوه، عصاره‌های هیدروالکلی نرخ رشد نسبی، نرخ مصرف نسبی و کارایی تبدیل غذای خورده شده را به‌طور معنی‌دار بیش از عصاره‌های آبی کاهش داد. با افزایش غلظت، شاخص بازدارندگی تغذیه هر دو گیاه افزایش یافت، که نشان‌دهنده اثر بازدارندگی تغذیه‌ای بالا روی شپشه آرد است. به‌طور کلی خاصیت ضدتغذیه‌ای عصاره سرو بسیار مؤثرتر از خاکشیر و عصاره هیدروالکلی بسیار مؤثرتر از عصاره آبی است.

واژگان کلیدی: *Descurainia sophia*، *Thuja orientalis*، محصولات انباری، عصاره گیاهی، شپشه قرمز آرد