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Antifeedant and growth inhibitory activities of essential oils from *Eucalyptus globulus* and *Eucalyptus camaldulensis* on *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

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Abstract
The cowpea seed beetle, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae: Bruchinae) is one of the major pests infesting stored pulses and is distributed worldwide. In this research, efficiency of plant extracts from *Eucalyptus globulus* Labill. and *Eucalyptus camaldulensis* Dehnh. were tested against the cowpea seed beetle for its antifeedant activity**.** Several experiments were 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 using a flour disk bioassay in the dark, at 27±1°C and 65±5% relative humidity concentrations of 0, 100, 500, 750, 1000, and 1500 mg/l were prepared from each essential oil and 10 adult insects were introduced into each treatment. After 72 h, nutritional indices were calculated. *E. globulus* oils were more effective than *E. camaldulensis* oils, by significantly decreasing the RGR, RCR and ECI. Both of plant essential oils, with the same activity, increased FDI as the oil concentration was increased, showing high feeding deterrence activity against *C. maculatus*. Generally, antifeedant activity of *E. globulus* was more effective than *E. camaldulensis*.

Keywords: *Eucalyptus* oils, nutritional indices, cowpea seed beetle

Introduction
Insect pests are one of the main causes of extensive damage in stored grains and their products. Universal pest damage to the stored products is 10 to 40% (Aliakbari *et al*., 2010; Park *et al*., 2008). Among these pests, coleopteran stored pests are the fiercest pests that are continually reproductive and extremely high generation in optimal conditions. The stored pest can damage to the agricultural products in a short time (Arthur *et al*., 1990; Ayvaz *et al*., 2010).

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The cowpea seed beetle, *Callosobruchus maculatus* (Fab.) (Coleoptera: Chrysomelidae: Bruchinae) is a major pest of economically important leguminous grains, such as cowpeas, lentils, green gram, and black gram (Benhalima *et al*., 2004; Benzi *et al*., 2009; Halder *et al*., 2010). They are important pests of pulse crops in Asia and Africa under storage conditions (Ali & Rizvi, 2008; Arthur *et al*., 1991; Ebadollahi & Mahbobi, 2011; Huang *et al*., 2000).

In order to keep these stored grains free from pest attack, various synthetic pesticides have been used (Huang & Subramanyam, 2005). Chemicals insecticides are currently used for stored product pest (Hansen and Jensen, 2002; Isman, 2006). In this regard, synthetic pesticides came into existence, but uncontrolled use of these chemicals causes great environmental hazards due to their persistent nature, increased risk of neurotoxic, carcinogenic, teratogenic and mutagenic effects in non-target animals (Lamiri *et al*., 2001; Koul *et al.*, 2008).

Over the past few decades, many investigations have been conducted on different plant products in order to obtain safer and more effective alternatives rather than chemical insecticides for controlling store-product insects. Natural products are an excellent alternative to synthetic pesticides as a means to reduce negative impacts to human health and the environment. Use of plant products as insecticide is one of the important approaches of insect pest management and it has many advantages over synthetic insecticides (Lee *et al*., 2001; Modarres –Najafabadi *et al*., 2006). Plant essential oils could be an alternative source for insect pest control because they constitute a rich source of bioactive chemicals and are commonly used as flavoring agents in foods.

These materials may be applied to food crops shortly before harvest without leaving excessive residues. Moreover, medically safe of these plant derivatives has emphasized also. For these reasons, much effort has been focused on plant essential oils or their constituents as potential sources of insect control agents. Many researches are conducted for managing this pest by various essential oils. Huang *et al*. (2000) reported that *Cassia sophera* L. extract is effective in reducing *C. maculatus* infestation. Nayamador *et al*. (2010) indicated that essential oils of *Cymbopogon schoenanthus* and piperitone had toxic effects on adults of *C. maculatus*. Ogendo *et al*., (2008) reported that citrus peel essential oil as potential fumigant and may be used as a safe pesticide for the management of cowpea seed beetle. The fruit peels of some citrus species have been reported to have insecticidal properties against *C. maculatus* (Mollai *et al*., 2010; Passino *et al*., 2004; Rafiei-Karahroodi, 2009). Shaaya *et al*. (1997) indicated that essential oil of *Cupressus arizonica* is effective in reducing of cowpea seed beetle infestation. Taghizadeh *et al*. (2010) reported that *Chenopodium ambrosioides* Linn. extract is effective in reducing population of cowpea seed beetle.

Tapondjou *et al*. (2005) studied fumigant activity of the essential oil from *Salvia bracteata* L., *Artemisia aucheri* Boiss and *Nepeta cataria* L. on cowpea seed beetle. Based on the results of this research, essential oil of *S. bracteata* is merit to be considered as a potential control agent against *C. maculatus*. Rafiei-Karahroodi *et al*., (2009) studied effect of essential oils from *Lavandula angustifolia*, and *Zataria multiflora* on one-day and three-day old adults of *C. maculatus*. Results indicated that, *L. angustifolia* and *Z. multiflora* are suitable candidates for replacing them with synthetic pesticides in warehouses to control *C.maculatus*.

Therefore, the main goal of the present study was to evaluate the insecticidal activities of essential oils from *E. globulus* and *E. camaldulensis* grown in Iran in the control of cowpea seed beetle, *C. maculatus*.

Materials and methods

Insect rearing

A culture of the *C. maculatus* was established on the seeds of unshelled brown cowpea, *Vigna ungiculata* L. in one litter wide-mouthed glass jars under laboratory conditions. Parent adults were obtained from laboratory stock culture maintained at the Entomology Department, University of Urmia, Iran. The culture was maintained in the dark (similar to storage conditions) in a growth chamber set at 27 ± 1°C and 65 ± 5 % relative humidity. All experiments were carried out under the same environmental conditions. The 1-2 days old adults of *C. maculatus* were used in bioassay tests.

Collected and dried plant specimens

Two plants known to have medicinal activity, *Eucalyptus globulus* and *Eucalyptus camaldulensis* were collected from Gachsaran region, Kohgiluyeh Va Boyer Ahmad province. The identity of each plant species mentioned was verified and confirmed by botanical specialist using photographs.

Extraction of essential oils

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

Bioassay by the method of flour disk

According to the method of Silhacek *et al*. (2007) a suspension of 10 g wheat flour in 50 mL distilled water was prepared. A micropipette was used to transfer 200-mL 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 *E. globulus* and *E. camaldulensis* (0.0, 100, 500, 750, 1000 and 1500 ppm 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 adults of cowpea seed beetle and held at 25±1°C and 60±5% Relative humidity. For 3 d. At the beginning of the experiment, the weight of flour disks and larvae was measured. After 3 d, flour disks and adults were weighed again and the number of dead adults noted. There were 5 replicates.

Nutritional Indices

Nutritional indices were calculated (Tripathi *et al*. 2002) with some modifications:
Relative Growth Rate (RGR) = (A-B) / (B × Day),

Where A = weight of live insects (mg) on the third day / number of live insects on the third day and
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.
Percentage efficacy of conversion of ingested Food (ECI) = RGR / RCR × 100.
The feeding deterrent action was calculated as Feeding Deterrent Index (Isman 2006):
(% FDI) = [(C – T) / C] × 100,
Where C is the weight consumption food of control and T is the weight consumption food of treatment.

Data Analysis

Each of indices in factorial design based on completely randomized design and in five replicates was performed. The first factor in this design included three treatments consisting of essential oils *E. globulus, E. camaldulensis* and control and the second factor consisted of six concentrations of plant essential oils to the extent of 100, 500, 750, 1000 and 1500 mg/l and with a control treatment. Before statistical analysis, nutritional indices of ECI and FDI with using the equation Arcsin√X/100 were normal. The means were separated using the Duncan's test at the 5 % level.

Results and Discussion

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

Analysis of variance showed that effect of essential oil *E. globulus* and *E. camaldulensis* on relative growth rate larvae of *C. maculatus* 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 *E. globulus* significantly more effective than *E. camaldulensis* 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.

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

According to table 1, effects of essential oil of *E. globulus* and *E. camaldulensis* and concentrations on relative consumption rate larvae of *C. maculatus* showed that essential oils, concentrations and interactions between them are all significant at 1% level. Essential oil of *E. globulus* compared with essential oil *E. camaldulensis* 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 *E. globulus* and *E. camaldulensis* on efficiency of conversion of ingested food larvae of *C. maculatus* 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. According to table 1 although, property of anti-feeding essential oil of *E. globulus* from *E. camaldulensis* 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 *E. globulus* is higher than essential oil *E. camaldulensis*. 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 *E. globulus* and *E. camaldulensis* on feeding deterrence index larvae of *C. maculatus* at different concentrations there are significant differences with each other (Table 1). Generally, essential oil of *E. globulus* had large effect on feeding deterrence index and FDI value significantly compared with essential oil of *E. camaldulensis* 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).

In this study, to compare the anti-nutritional effects essential oils of *E. globulus* and *E. camaldulensis* parameters as indicators of nutrition were used. Also, from non-free method of choice that the forced feeding of insect food which had been was impregnated with various concentrations of essential oils. Thus, during these experiments, two important factors could be measured. The first weight loss insects compared to control in the time specified in this experiment, called the characteristic index RGR was measured and expressed. Second, compared to control insect had been given to avoid eating food or taking less than the R.C.R. index measured and was expressed. Effective factor in weight loss could be related to the impact of essential oil on insect food (Koul *et al*., 2008), and the clarify avoidance of insect feeding from the feeding deterrence index (FDI) was used. In this experiment, was observed with increasing concentration and changing the essential oil type, amount of RGR and RCR is reduced. So that, in essential oil with high concentrations, the effect is greater and in terms of essential oil type *E. globulus* is more effective. Mechanism of action to respond to this decrease, the differences in ECI and FDI be created, it is clear that low concentrations of essential oil *E. globulus* and *E. camaldulensis* did not show significant differences in terms of ECI, but with increasing of very high concentration of essential oil value of ECI is reduced. While the essential oil of *E. globulus* and *E. camaldulensis* even at lower concentrations the inhibitions of insect feeding were significantly effective. Therefore, the effective factors on the RGR and RCR can be attributed to the effects of feeding deterrence or FDI.

Therefore, the essential oils of *E. globulus* and *E. camaldulensis* even at lower concentrations can effectively be effective in avoiding insect feeding. It is also used by other researchers have been studying. There are some studies that demonstrate *C. maculatus* is sensitive to essential oil of plants (Ebadollahi, 2011; Isman, 2000; Javaid & Poswal, 1995). In this study, to prove the anti-nutritional properties of essential oils of eucalyptus from cowpea seed beetle as a model was used and proved that if the concentration essential oils at warehouse to reach sub-lethal concentration can prevent the insects from feeding the stored product. Therefore, we can conclude that if there is a possibility of mixing the essential oils with some storage products, these essential oils can effectively be effective in controlling pests.

Table 1. Comparison of different concentrations essential oils *E. globulus* and *E. camaldulensis* on Relative Growth Rate (RGR), of adults of *C. maculatus*

Concentration (mg/l)	RGR (mg/mg/day) (Mean ± S.E)		t-student	P-value
	E. globulus	*E. camaldulensis*		
0.00	0.0379 ± 0.0000 [a]	0.0455 ± 0.0000 [a]	-	-
100	0.0303 ± 0.0000 [b]	0.0371 ± 0.0004 [b]	-15.59	0.000
500	0.0227 ± 0.0000 [c]	0.0307 ± 0.0000 [c]	-21.00	0.000
750	0.0193 ± 0.0007 [d]	0.0239 ± 0.0004 [d]	-5.56	0.001
1000	0.0152 ± 0.0000 [e]	0.0208 ± 0.0004 [e]	-15.00	0.000
1500	0.0068 ± 0.0004 [f]	0.0136 ± 0.0000 [f]	-15.59	0.000
Mean	0.0220 ± 0.0021	0.0286 ± 0.0022	-2.16	0.036

a,b,c,d,e,f, Dissimilar letters in each column with using Duncan's test at level of 5% together have significant differences.

Table 2. Comparison of different concentrations essential oils *E. globulus* and *E. camaldulensis* on Relative Concenteration Rate (RCR), of adults of *C. maculatus*

Concentration (mg/l)	RGR (mg/mg/day) (Mean ± S.E)		t-student	P-value
	E. globulus	*E. camaldulensis*		
0.00	0.0398 ± 0.0004 [a]	0.0470 ± 0.0000 [a]	-19.00	0.000
100	0.0341 ± 0.0004 [b]	0.0386 ± 0.0004 [b]	-7.35	0.000
500	0.0292 ± 0.0004 [c]	0.0348 ± 0.0000 [c]	-15.00	0.000
750	0.0254 ± 0.0007 [d]	0.0303 ± 0.0000 [d]	-6.79	0.000
1000	0.0208 ± 0.0004 [e]	0.0269 ± 0.0004 [e]	-11.31	0.000
1500	0.0152 ± 0.0000 [f]	0.0167 ± 0.0006 [f]	-2.45	0.050
0.00	0.0274 ± 0.0017	0.0324 ± 0.0020	-1.91	0.063

a,b,c,d,e,f, Dissimilar letters in each column with using Duncan's test at level of 5% together have significant differences.

Table 3. Comparison of different concentrations essential oils *E. globulus* and *E. camaldulensis* on Efficiency of Conversion of Integrated Food (ECI), of adults of *C. maculatus*

Concentration (mg/l)	RGR (mg/mg/day) (Mean ± S.E)		t-student	P-value
	E. globulus	*E. camaldulensis*		
0.00	95.26 ± 0.089 [a]	96.77 ± 0.00 [a]	-1.86	0.112
100	88.93 ± 1.14 [b]	96.08 ± 0.04 [a]	-7.57	0.000
500	77.96 ± 0.99 [c]	88.4 ± 1.09 [b]	-6.47	0.001
750	76.06 ± 0.67 [c]	78.75 ± 1.25 [bc]	-1.92	0.103
1000	72.80 ± 1.37 [c]	77.45 ± 0.33 [c]	-3.30	0.016
1500	45.00 ± 2.89 [d]	82.16 ± 3.07 [c]	-7.94	0.000
Mean	76.00 ± 3.36	86.54 ± 1.70	-2.74	0.009

a,b,c,d,e,f, Dissimilar letters in each column with using Duncan's test at level of 5% together have significant differences.

Table 4. Comparison of different concentrations essential oils *E. globulus* and *E. camaldulensis* on Feeding Deterrence Index (FDI), of adults of *C. maculatus*

Concentration (mg/l)	RGR (mg/mg/day) (Mean ± S.E)		t-student	P-value
	E. globulus	*E. camaldulensis*		
100	14.29 ± 1.10 [e]	17.74 ± 0.93 [e]	-2.39	0.054
500	26.67 ± 0.95 [d]	25.81 ± 0.00 [d]	-0.88	0.413
750	36.19 ± 1.82 [c]	35.48 ± 0.00 [c]	-0.37	0.725
1000	47.62 ± 0.95 [b]	42.74 ± 0.81 [b]	-3.91	0.008
1500	61.90 ± 0.00 [d]	64.52 ± 1.32 [a]	-1.98	0.095
Mean	37.33 ± 3.80	37.26 ± 3.70	-0.03	0.974
100	14.29 ± 1.10 [e]	17.74 ± 0.93 [e]	-2.39	0.054

a,b,c,d,e,f, Dissimilar letters in each column with using Duncan's test at level of 5% together have significant differences.

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فصلنامه گیاه‌پزشکی
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اثرات بازدارندگی و ضد تغذیه ایی اسانس *Eucalyptus globulus* و *Eucalyptus camaldulensis* روی سوسک چهار نقطه ایی حبوبات *Callosobruchus maculatus* (Coleoptera: Chrysomelidae)

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چکیده

سوسک چهار نقطه‌ایی حبوبات *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) یکی از آفات کلیدی حبوبات بوده و در اغلب نقاط دنیا دیده می شود. در این تحقیق کارایی بازدارندگی و ضد تغذیه ایی عصاره های گیاهی *Eucalyptus globulus* و *Eucalyptus camaldulensis* روی سوسک چهار نقطه ایی حبوبات بررسی شد. نرخ رشد نسبی، نرخ مصرف نسبی، کارایی تبدیل غذای خورد شده و شاخص بازدارندگی تغذیه برای ارزیابی اثر ضد تغذیه ای اسانس ها اندازه گیری شد. تیمارها به روش دیسک آردی در شرایط کنترل شده در دمای ۱ ± ۲۷ درجه سلسیوس و رطوبت نسبی ۵ ± ۶۵ درصد و تاریکی ارزیابی شدند. در این آزمایش ها ۱۰ میکرولیتر از غلظت های ۱۵۰۰ -۱۰۰ پی پی ام از اسانس هر دو گیاه به همراه شاهد (فقط حاوی ۱۰ میکرولیتر استون) به طور یکنواخت روی دیسک های آردی پخش شدند. پس از تبخیر حلال در هر تکرار ۱۰ حشره کامل سوسک چهار نقطه ای حبوبات روی دو عد دیسک آردی قرار داده شد. پس از گذشت ۷۲ ساعت از شروع آزمایش، شاخص های تغذیه محاسبه شدند. نتایج نشان داد که اسانس *E. globulus* نرخ رشد نسبی، نرخ مصرف نسبی و کارایی تبدیل غذای خورده شده توسط سوسک چهار نقطه ای حبوبات را به طور معنی داری بیش از اسانس *E. camaldulensis* کاهش داده است. با افزایش غلظت، شاخص بازدارندگی تغذیه هر دو گیاه به طور معنی داری افزایش یافت و مانع از تغذیه حشرات کامل از غذایی که در اختیار آنها بود، گردید. به طور کلی نتایج نشان داد که خاصیت ضد تغذیه ای اسانس *E. globulus* بسیار موثرتر از اسانس *E. camaldulensis* است.

واژه‌های کلیدی: *E. globulus*، *E.camaldulensis*، شاخص بازدارندگی تغذیه، سوسک چهار نقطه‌ای حبوبات

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