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# Antifeedant Activity of Nanoemulsion Formulation of Arugula *Eruca sativa* Oil on Elm Leaf Beetle *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae)

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## ABSTRACT

**The antifeedant activity of nanoemulsion formulation of arugula *Eruca sativa* Mill. oil was studied against elm leaf beetle *Xanthogaleruca luteola* (Müller)(Col.:Chrysomelidae) under laboratory conditions at 25±1ºC, 75±5% RH, and LD 16:8 hours. Ingestive $LC_{50}$ values of the oil were studied in third instar larvae. Then, physiological parameters were evaluated following 24, 48, and 72 hours post feeding at $LC_{50}$ level. $LC_{50}$ values 24, 48 and 72 hours after application were 4.940, 3.791, and 2.938 mg $mL^{-1}$, respectively. Arugula oil at $LC_{50}$ level decreased the nutritional indices including efficiency of conversion of ingested food, relative growth rate, efficiency of conversion of digested food, and relative consumption rate, but increased feeding deterrence index significantly 72 hours post feeding. Nutritional reserves such as total carbohydrate, protein, and lipid contents and also digestive enzymes containing lipase, α-amylase, and protease activity were decreased showing post-ingestive toxicity. The activity of the detoxifying enzyme glutathione S-transferase was increased, indicating that this enzyme may be involved in detoxification of arugula oil, but general esterase did not change significantly. In general, it can be concluded that arugula oil possess antifeedant activity against *X. luteola* under laboratory condition. It seems that arugula oil has a great potential to be used as effective botanical pesticides. However, further studies such as greenhouse and field experiments are necessary before recommendation and commercialization process.**

**Keywords:** Bioassay, Detoxifying enzyme, Digestive enzyme, Nutritional indices.

## INTRODUCTION

The elm leaf beetle *Xanthogaleruca luteola* (Müller) (Col.: Chrysomelidae), is one of the most destructive pests of elm trees *Ulmus* spp. The larvae and adults feed on parenchyma of leaves. Repeated infestations make the tree susceptible to other pests and environmental stresses (Arbab *et al*., 2001). Despite the existence of some predators like *Coleomegilla maculata* Lengi Timb. (Coleoptera: Coccinellidae) and some parasitoids like *Erynniopsisan tennata* (Rondani) (Diptera: Tachinidae), the damage of elm leaf beetle is still significant (Thurston, 1988; Field and Kwong, 1994). Due to the usage of elm trees in urban green space, chemical control of this pest is associated with a lot of limitations. The use of chemical pesticides against the pest is harmful to the environment and the human health (Perry *et al*., 1998). Therefore, preparation and use of safe, and efficient alternative pesticides is important (Akhtar and Isman, 2004). So far, it has been reported that some plant secondary metabolites, such as various plant essential oils and extracts, have insecticidal activity against elm leaf beetle (Valladares *et al*., 1997; Maistrello *et al*., 2005; Shekari *et al*., 2008; Huerta *et al*., 2010; Amirmohammadi and JalaliSendi, 2013; Vahabi *et al*., 2016).

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However, these studies have not come close to the applied stage. Arugula is a plant in Brassicaceae family, which is cultivated in Iran. It is known as a salad vegetable which is effective in reducing cancer risk (Higdon *et al*., 2007). Also, it is used as a nematode - trap crop (Melakeberhan *et al*., 2006; Riga *et al*., 2006). Arugula oil was used in the past as a fuel such as oil. There are a few reports of the insecticidal and acaricidial properties of arugula oil. Recently, acaricidal activity of arugula oil was demonstrated on *Tetranychus urticae* Koch (Masoumi, 2018). Brassicaceae plants produce glucosinolates (*β*-thioglucoside-*N*-hydroxysulfates). When plant cells are damaged, glucosinolates are converted to isothiocyanates upon hydrolysis by the enzyme myrosinase (thioglucosidase) (Shapiro *et al*., 1998). Isothiocyanates generated by the myrosinase exhibits insecticidal activity (Demirel *et al*., 2009). There are several studies on the insecticidal and antifeedant activities of isothiocyanates. Isothiocyanates toxicity has been investigated on some stored product and garden insects (Borek *et al*., 1998; Gupta *et al*., 2017; Wu *et al*., 2009; Santos*et al*., 2011). Recently, acaricidal activity of *Moringa peregrina* (Forssk.) FioriAgricolt. Containing isothiocyanate, was demonstrated on *T. urticae* (Seifi *et al*., 2018). The erucin [1-isothiocyanato-4-(methylthio) butane], is a kind of isothiocyanates that is found at high levels in arugula (Melchini and Traka, 2010). Also, it is widely cultivated in Iran. In this regard, arugula may be a good candidate for development of botanical pesticides. Feeding behavior in insects is regulated by the interaction of a number of mechanisms (Audsley and Weaver, 2009). Digestive enzymes have a major role in feeding of insects (Wigglesworth, 1984). Antifeedants are behavior-modifying substances resulting in feeding deterrence (Isman, 1994). There are many studies that show secondary metabolites produced by plants have antifeedant properties and modify digestive enzymes activities in insects (Mehrabadi *et al*., 2012; Khosravi and JalaliSendi, 2013). To investigate the effects of plant metabolites on feeding, nutritional indices such as relative consumption rates and relative growth rate can be evaluated (Scriber and Slansky, 1981). Also, activities of glutathione -S- transferases and esterases can be affected by these compounds. General esterases and glutathione –S-transferases are important detoxifying enzymes in metabolism of insecticides (Mouches *et al*., 1986; Vanhaelen *et al*., 2001). In this study, the antifeedant effects of arugula oil have been considered on elm leaf beetle. Different experiments such as survey of nutritional indices and digestive enzymes were done in this study to investigate the antifeedant activities of arugula. Also, to increase the efficiency of the oil, it has been formulated as nanoemulsion before experiment. Application of nanoemulsion formulations of pesticides enhance efficiency, quality and controlled release of these substances (Negahban *et al*., 2013). Nanoemulsion is a mixture of two immiscible liquids, one of which is dispersed in the form of nanometric scaled droplets into the other one. The generating processes for nanoemulsions are divided into low-energy and high-energy methods. High-energy methods involve the use of some devices such as high-pressure homogenizers (Tadros *et al*., 2004). Low-energy methods are related to the intrinsic physicochemical properties of the surfactants in the formulation, leading to the generation of emulsion droplets in the nanometric range. In this method, the experimental conditions are related to solvent/oil ratio. The solvent diffusion is quicker and the turbulence thereby generated causes nano-scaled droplets to form. The formulation can also include additional components such as surfactants. The system containing water, nonionic surfactant and oil, leads to the generation of nanoemulsions (Anton *et al*., 2008; Anton and Vandamme, 2009). Our nanoemulsion formulation in this study which contains ethoxylated emulsifier as nonionic surfactant, follows the rules of the low-energy nano-emulsification method.





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The purpose of this study was to investigate the antifeedant activity of nanoemulsion formulation of arugula oil on elm leaf beetle.

## MATERIALS AND METHODS

### Insect Rearing

Eggs and larvae of elm leaf beetle were collected from the elm trees in Tehran, 51° 42′ E, 35° 69′ N, in August. The adults and larvae were reared in plastic boxes (10.0 cm diameter and 2.5 cm in height) in a rearing chamber set at 25±1°C; 16:8 L:D; 75±5%RH. Fresh elm leaves were provided daily for feeding. The eggs were used to maintain the culture. Newly emerged (< 24 hours) third instar larvae of the same age were used for bioassays.

### Preparation of Arugula Oil Formulation

Arugula seeds were purchased from Gourangi Company in Shiraz. Oil seeds were extracted using cold press method. In this method the maximum temperature during extraction was less than 80°C. Seeds yielded 33% oil after extraction. To prepare nanoemulsion formulation, 60% arugula oil, 30% polyethylene glycol 400 (PEG400), and 10% nonionic ethoxylated emulsifier were used. In this method, nano-emulsification is performed by slowly pouring the oil plus surfactant mixture (homogenized beforehand with a Vortex mixer) into the slightly magnetically stirred PEG400 and nano-emulsion is rapidly formed (Anton and Vandamme, 2009). Therefore, in this study, 60% arugula oil was mixed with 10% nonionic emulsifier, then, it was added into the magnetically stirred PEG phase and the nanoemulsion was formed. The final composition was investigated by the Malvern machine (ZETA SIZER) and the particle sizes were measured in the nano range.

### Toxicity Tests

In order to investigate the ingestive toxicity of the arugula oil, concentrations required for 50% mortality of population ($LC_{50}$) were estimated. A preliminary experiment was conducted to find concentrations to cause 20 and 80% mortality. Then, the five concentrations were tested based on logarithmic intervals. The experiment was carried out in Petri dishes with a diameter of 5 cm containing elm leaves. Fresh elm leaves were provided daily for insect feeding. The experiment was performed in four replications. The count of live and dead insects was assessed 24, 48, and 72 hours post treatment. To determine the $LC_{50}$ value, ten third instar larvae of the same age (> 1 day) were used for each replication. The experiments were carried out at 25±1°C, relative humidity of 75±5%, and LD 16:8 hours. The leaves were dipped in a solution of arugula oil and left to dry before insect introduction. After 24, 48, and 72 hours, the number of dead insects was counted in the treatments and control containers. Toxicity of water, emulsifier, and polyethylene glycol was investigated as control. $LC_{50}$ values were estimated by SPSS version 16.0 software.

### Effect of Oil on Nutritional Indices

The assay of nutritional indices was conducted at 25±1°C, 75±5% RH, and a photoperiod of L:D 16:8 hour. Samples were placed in oven at 65°C for 48 hours to obtain the dry weight. The nutritional indices were measured at $LC_{50}$ level according to the following formula (Waldbauer, 1968; Huang and Ho, 1998):

Relative Growth Rate (RGR) = P/(A×T) (1)

Where, P is dry weight gain of larvae (mg), A is dry weight of the insect over unit time (mg), and T is duration of the experimental period (day).

Relative Consumption Rate (RCR)= E/(A×T) (2)





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Where, E is dry weight of food consumed (mg).

Efficiency of Conversion of Ingested food (ECI)= (P/E)×100 (3)

Efficiency of Conversion of Digested food (ECD) = [P/(E-F)]×100 (4)

Where, F is dry weight of Feces produced (mg).

Approximate Digestibility (AD) = [(E-F)/E]×100 (5)

Feeding Deterrence Index (FDI) = [(C-W)/C]×100 (6)

Where, C is the weight consumption of food in the control (mg) and W is weight consumption of food in the treatment (mg).

### Effect of Oil on Physiological and Biochemical Parameters

The carbohydrate and lipid contents were measured according to Yuval *et al*. (1998). Total protein was measured based on the Bradford (1976) method. α-amylase activity was measured by α-amylase measuring kit (Kikkoman Corp., Chiba, Japan) (Mikani *et al*., 2012).The activity of lipase was determined by the method of Tsujita *et al*. (1989). Protease activity was measured by the method of Sakai *et al*. (2006).The activity of general esterase was determined according to Van Asperen (1962) method and glutathione *S*–transferase activity was determined using the method of Habing *et al*. (1974).

## RESULTS

### Preparation of Arugula Oil Formulation

The particles size distribution was determined in the nano range. Figure 1 shows size distribution by volume. On average, 87.6% of volume of particles in nanoemulsion formulation of arugula oil are 118.5 nanometer (Figure 1), however, only 2.8% of particle volume was dropped out of nano range. Polydispersity index (PDI), a dimensionless scale, was 0.653, and values greater than 0.7 indicate that the sample has a very broad size distribution. Therefore, it shows a polydisperse system and has greater tendency to aggregation than monodisperse system. As it can be seen in Figure 1, the particle size distribution is multimodal.

### Toxicity Tests

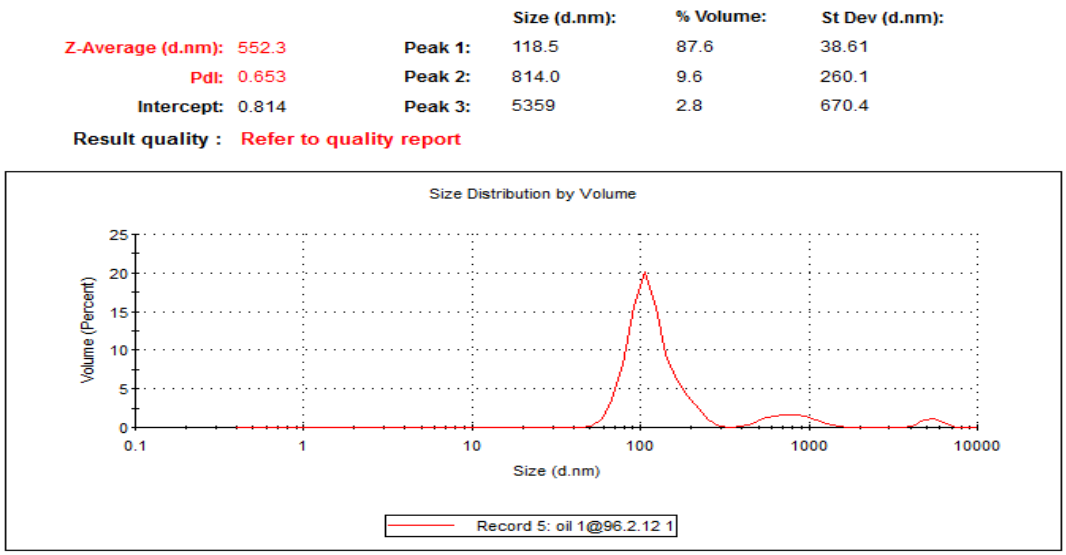


**Figure 1**. Particle size distribution of nanoemulsion formulation of arugula oil.





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**Table 1.** The effect of emulsifier, polyethylene glycol and arugula oil on mortality percentage of 3rd instar larvae of elm leaf beetle.

| Treatments | Mortality (%) |
|---|---|
| Water | 0 |
| Water+Emulsifier (10%) | 0 |
| Water+Polyethylene glycol (30%) | 0 |
| Arugula oil+Emulsifier (1 mg $mL^{-1}$) | 0 |

The results showed that emulsifier and polyethylene glycol did not affect elm leaf beetle mortality (Table 1). The results showed that increasing the duration of exposure, resulted in $LC_{50}$ decrease:. $LC_{50}$ values 24, 48, and 72 hours after application were 4.940, 3.791 and 2.938 mg $mL^{-1}$, respectively. Based on the results, the arugula oil has ingestive effects on elm leaf beetle (Table 2). Also, Table 2 shows the amounts of $\chi^2$ and P-values. These values show that $\chi^2$ decreased with increasing P-value. When the values are bigger than 0.15, heterogeneity is not considered to estimate the 95% confidence limits. Therefore, the confidence limits are sufficiently accurate.

### Effect of Oil on Nutritional Indices

The results showed that arugula oil changed nutritional indices significantly at $LC_{50}$ level as compared with the control. The RCR and RGR fell significantly from 3.958 and 0.853 mg $mg^{-1}$ $d^{-1}$ in the control to 1.582 ($F= 2.70$; $df_{t,e}= 3,16$; $P< 0.0001$) and 0.205 mg $mg^{-1}$ $d^{-1}$ ($F= 1.76$; $df_{t,e}= 3,16$; $P< 0.0001$) in treated larvae after 72 hours, which showed 60.03 and 75.97% reduction, respectively. Also, both ECI and ECD fell from 21.564 and 23.577 mg $mg^{-1}$ $d^{-1}$ in the control to 12.987 ($F= 615.79$; $df_{t,e}= 3,16$; $P< 0.0001$) and 14.990 mg $mg^{-1}$ $d^{-1}$ ($F= 441.81$; $df_{t,e}= 3,16$; $P< 0.0001$) in treated larvae, 72 hours post feeding, which showed 39.77 and 36.42% reduction, respectively. Although the AD showed significant reduction in treated larvae, this change was not high compared to other indices ($F= 285.53$; $df_{t,e}= 3,16$; $P< 0.0001$). Also, FDI showed significant increment in treated larvae (F=

**Table 2.** The ingestive $LC_{50}$ values of nanoemulsion formulation of arugula oil**,** on 3rd instar larvae of elm leaf beetle, 24, 48, and 72 hours post treatment.[a]

| Time (h) | n | $\chi^2$ (df) | P-value | Slope±SE | $LC_{50}$ (95% CL) (mg $mL^{-1}$) |
|---|---|---|---|---|---|
| 24 | 200 | 3.040 (3) | 0.386 | 6.770±1.143 | 4.94 (4.574-5.262) |
| 48 | 200 | 0.492 (3) | 0.921 | 6.653±1.232 | 3.791 (3.518-4.042) |
| 72 | 200 | 3.198 (3) | 0.362 | 8.248±1.407 | 2.938 (2.760-3.095) |

[a] n: Number of tested insects; 95% CL: 95% Confidence Limits.

**Table 3.** The effect of nanoemulsion formulation of *Eruca sativa* oil at $LC_{50}$ level on nutritional indices of 3rd instar larvae of *Xanthogaleruca luteola*.[a]

| Time of feeding (h) | RCR (mg $mg^{-1}$ $d^{-1}$) | RGR (mg $mg^{-1}$ $d^{-1}$) | ECI (%) | ECD (%) | AD (%) | FDI (%) |
|---|---|---|---|---|---|---|
| 0 | 3.958 ± 0.037a[b] | 0.853 ± 0.004a | 21.564 ± 0.292a | 23.577± 0.340a | 91.467 ± 0.086a | |
| 24 | 3.456 ± 0.002b | 0.646 ± 0.000b | 18.688 ± 0.008b | 20.870± 0.035b | 89.545 ± 0.148b | 17.063 ± 0.001a |
| 48 | 2.448 ± 0.001c | 0.343 ± 0.000c | 14.031 ± 0.005c | 15.818± 0.007c | 88.703 ± 0.003c | 38.173 ± 0.041b |
| 72 | 1.582 ± 0.017d | 0.205 ± 0.000d | 12.987 ± 0.140d | 14.990± 0.188d | 86.643 ± 0.163d | 60.048 ± 0.970c |

[a] Relative Consumption Rate (RCR), Relative Growth Rate (RGR), Efficacy of Conversion of Ingested food (ECI), Efficacy of Conversion of Digested food (ECD), Approximate Digestibility (AD), and Feeding Deterrence Index (FDI). [b] Means followed by the same letters in each column are not significantly different (Tukey's test, $P < 0.05$).





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7.35; $df_{t,e}$= 2,12; P< 0.0001) (Table 3).

### Effect of Oil on Physiological and Biochemical Parameters

The results indicated that arugula oil interfered in physiological and biochemical parameters of treated elm leaf beetle. Arugula oil decreased essential energy sources in treated larvae. The total carbohydrate (F= 97.40; $df_{t,e}$= 3,12; P< 0.0001), total lipid (F= 423.99; $df_{t,e}$= 3,12; P< 0.0001) and total protein content (F= 320.50; $df_{t,e}$= 3,12; P< 0.0001) decreased significantly at $LC_{50}$ level as compared with the control. Carbohydrate content fell from 0.501 ug/larvae in the control to 0.221 ug $larvae^{-1}$ in treated larvae, 72 hours post feeding. Protein and lipid content fell from 0.074 and 0.653 ug $larvae^{-1}$ in the control to 0.041 and 0.304 ug $larvae^{-1}$ in treated larvae, respectively. Effects of arugula oil were evaluated on digestive and detoxification enzymes in treated elm leaf beetle. The results indicated that the activity of α - amylase (F= 94.81,$df_{t,e}$= 3,8; P< 0.0001), protease (F= 34.30, $df_{t,e}$= 3,8; P< 0.0001), and lipase enzymes (F= 32.81,$df_{t,e}$= 3,8; P< 0.0001) was decreased significantly compared to the control, 48 h post feeding. α-Amylase content fell from 128.265 mU in control to 95.687 mU in treated larvae, 48 hours post feeding. Protease and lipase content fell from 121.533 and 160.213 mU in the control to 96.666 and 115.655 mU in treated larvae, respectively. The activity of glutathione *S*–transferase increased compared to the control and the highest activity was observed 72 hours post feeding (F= 11.66, $df_{t,e}$= 3,20; P< 0.0001); but the activity of esterase did not change significantly even after 72 hours (F= 1.93, $df_{t,e}$= 3,20; P= 0.15) (Table 4).

## DISCUSSION

Arugula plant (Brassicaceae family) contains a large quantity of glucoerucin (4-methylthiobutylglucosinolate) (Zhang *et al*., 1992; Fahey *et al*., 2001) that is converted to erucin (1-isothiocyanato-4-(methylthio) butane), a kind of isothiocyanates (Barillari *et al*., 2005; Azarenko *et al*., 2014). Although isothiocyanates exhibit insecticidal activity (Demirel *et al*., 2009), however, there is no information on the activity of *E. sativa*as insecticide. The present study was performed to survey the effect of arugula oil against the elm leaf beetle *X. luteola*. Our findings confirmed that arugula oil possess antifeedant activity on *X. luteola*. Until now, different studies have been conducted on the activity of plant secondary metabolites on the elm leaf beetle (Huerta *et al*., 2010; Amirmohammadi and JalaliSendi, 2013; Khosravi and JalaliSendi, 2013; Vahabi*et al*., 2016). However, the toxicity of isothiocyanateshas been reported on a

**Table 4.** Effect of nanoemulsion formulation of *Eruca sativa* oilat $LC_{50}$ level for each time on physiological and biochemical parameters in 3rd instar larvae of *Xanthogaleruca luteola.*

| Entries | Time of feeding (h) | | | |
|---|---|---|---|---|
| | Control | 24 | 48 | 72 |
| α-Amylase (mU) | 128.265 ± 2.202a [a] | 121.922 ± 2.442a | 95.687 ± 2.726b | 71.259 ± 3.253c |
| Protease (mU) | 121.533 ± 3.655a | 118.333 ± 3.756a | 96.666 ± 4.630b | 76.433 ± 1.596c |
| Lipase (mU) | 160.213 ± 0.009a | 148.123 ± 0.316a | 115.655 ± 0.491b | 85.533 ± 0.346c |
| Carbohydrate (ug $larvae^{-1}$) | 0.501 ± 0.000a | 0.451 ± 0.003a | 0.271 ± 0.027b | 0.221 ± 0.003b |
| Lipid (ug $larvae^{-1}$) | 0.653 ± 0.007a | 0.566 ± 0.011b | 0.391 ± 0.007c | 0.304 ± 0.002d |
| Protein (ug $larvae^{-1}$) | 0.074 ± 0.001a | 0.071 ± 0.001a | 0.048 ± 0.001b | 0.041 ± 0.001c |
| GST (umol gc $min^{-1}$ $mg^{-1}$ protein) [b] | 0.673 ± 0.073a | 0.757 ± 0.128ab | 1.282 ± 0.210bc | 1.777 ± 0.155c |
| Esterase (umol n $min^{-1}$ $mg^{-1}$ protein | 0.472 ± 0.034a | 0.503 ± 0.028a | 0.551 ± 0.069a | 0.608 ± 0.022a |

[a] Means followed by the same letters in each row are not significantly different (Tukey's test, P< 0.05). [b] GST: Glutathion S-Transferase, gc: Glutathion conjugated, n: Naphthol.





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number of stored product pests (Worfel *et al*., 1997; Demirel *et al*., 2009; Wu *et al*., 2009). Also, acaricidal activity of isothiocyanate containing plants such as *M. peregrina* (Seifi *et al*., 2018) and arugula (Masoumi, 2018) has been reported against two-spotted spider mite *T. urticae*. The results of this study show that arugula oil has antifeedant activity on elm leaf beetle.According to the studies of Isman (1994), most botanicals pesticides are described as "stomach poisons" and antifeedant that is a peripherally-mediated behavior-modifying substance resulting in feeding deterrence. Plant antifeedants have been the subject of several reviews (Frazier, 1986; Isman, 1994). Until now, antifeedant activity of some plant secondary metabolites such as extracts of *Daphne gnidium* L. (Maistrello *et al*., 2005), *Artemisia annua* L. (Shekari *et al*., 2008) and *Melia azedarach* L. (Valladares *et al*., 1997) has been demonstrated on elm leaf beetle. Researches indicated that vapors of isothiocyanates have mostly respiratory action (Wu *et al*., 2009; Santos *et al*., 2011), however, this study is one of the few studies demonstrating post feeding toxicity of isothiocyanates on phytophagous insects. Our findings demonstrated that nutritional indices such as RCR, RGR, ECI, and ECD were significantly affected in treated larvae. Moreover, significant increment of FDI after 72 hours indicates feeding deterrence of the oil. Reduction of food consumption (pre-ingestive) and ECD (post- ingestive) show that the toxic responses were formed by both feeding deterrence and post-ingestive responses mechanism. Feeding suppression could be a consequence of an action either on the insect's nervous system or on the alimentary canal (Isman, 1994). Probably, insect uses the energy of the food to detoxify the toxic compound and thus only small amount is used for growth, causing changed nutritional indices. Digestive enzymes have a major role in insects because of converting complex food materials into smaller absorbable molecules that are necessary to provide energy and synthesis of other metabolites (Wigglesworth, 1984). Based on the results, arugula oil decreased α–amylase, protease and lipase activity post feeding. Also, it decreased essential energy sources such as carbohydrate, lipid, and protein contents. It shows that the toxic effect of isothiocyanate is mediated by a post-ingestive mechanism. There are examples of toxic compounds changing feeding behavior and physiology through a post-ingestive mechanism (Glendinning and Slansky, 1995; Glendinning, 1996). Plant secondary compounds can change an insect's gustatory response to nutrients (Glendinning, 1996) and cause lower feeding by insect.We know that higher level of energy consumption occurs during detoxification of toxic compounds by insects. Detoxification and deconstruction of plant secondary metabolites requires energy and protein to build detoxification enzymes (Manson and Thomson, 2009). Also, there are hypotheses that isothiocyanate reacts with some proteins and inactivate the thiol group of some proteases (Kawakishi and Kaneko, 1987; Hassall, 1990). It can explain the reason for protein and protease reduction, respectively. The mode of action of isothiocyanate is not well understood. It is believed that the reaction of the electrophilic isothiocyanate group with amino acid residues of proteins causes cleavage of disulfide bonds (Kawakishi and Kaneko, 1987). Sequestration and metabolizing are ways to detoxification of isothiocyanates in insects. Glutathione-S- transferases are a kind of enzymes that are involved in the detoxification of various xenobiotic compounds such as insecticides (Manneryik and Danielson, 1988). Francis *et al*. (2005) showed that glutathione S–transferase are involved in detoxifying plant secondary metabolites such as isothiocyanates of the family Brassicaceaein aphid *Myzus persicae* (Sulzer) (Hem.: Aphididae). Also, the studies of Wadleigh and Simon (1988) showed that glutathione S– transferase plays an important role in detoxification of isothiocyanates in phytophagous insects such as lepidopteran. In the present study,





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the activity of glutathione S-transferase increased, which is in agreement with the above contents and shows that this enzyme may be involved in detoxification of isothiocyanate in elm leaf beetle. In this regard, continuous exposure to arugula oil may cause adaptation of the elm leaf beetle toisothiocyanate. Further investigations are needed to find definitive result. In conclusion, our findings show that arugula oil has antifeedant activity on *X. luteola*. Although impacts of a lot of plant natural products have been demonstrated on insects in laboratory, there are only a few botanical insecticides in widespread use or in the stages of commercialization. The reason is that the industry focuses on acute and rapid insecticidal action (Isman, 1994).This has led to neglect of the role of botanical insecticides in pest control industry. Screening and evaluation of antifeedants can be an effective step in pest control. Some plant metabolites like plant essential oils cannot be used in the stages of commercialization because of limitations such as low persistence, high cost, phytotoxicity, and the need to frequent reapplication in the field (Koul *et al*., 2008). Glucosinolates and some of their degradation products like isothiocyanates are almost stable and persistent formulations that can be produced (Van Eylen *et al*., 2006; Dekker *et al*., 2009). Also, abundance and dependability are the most important subjects to supply plant material for commercialization. *E. Sativa* is a plant that is widely cultivated as a vegetable, thus plant material is available from a regionally abundant and renewable resource and can be used in biopesticide productions. Due to the low cost of arugula oil in Iran and the reasonable lethal dose as a botanical pesticide, this oil may have a potential to be produced commercially. In conclusion, our findings indicated that nanoemulsion formulation of arugula oil possess antifeedant activity against *X. luteola* under laboratory condition, but further studies on the insecticidal and antifeedant effects of arugula oil on elm leaf beetle such as greenhouse and field experiments are suggested. Also, there are reports that continuous exposure to isothiocyanates causes adaptation of the pest (Agut *et al*., 2018), so, further studies are necessary. Moreover, due to the existence of some useful predators and parasitoids in urban spaces, further experiments are needed to test the effects of isothiocyanates on natural enemies. Lastly, it is necessary to note that evaluation of isothiocyanate based on its antifeedant effects alone may overlook physiological effects that could prove to be even more useful on elm leaf beetle. Therefore, further investigations should be carried out to complete the results of this study.

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# بررسی اثر ضدتغذیه ای فرمولاسیون نانوامولسیون روغن منداب *Eruca sativa* روی سوسک برگخوار نارون(*Xanthogaleruca luteola* (Coleoptera: Chrysomelidae)

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## چکیده

فعالیت ضدتغذیه ای فرمولاسیون نانوامولسیون روغن منداب *Eruca sativa* Mill. روی سوسک برگ نارون *Xanthogaleruca luteola* (Müller) (Col.: Chrysomelidae) در شرایط آزمایشگاهی در دمای ۱ ± ۲۵ درجه سلسیوس، رطوبت نسبی ۵ ± ۷۵ درصد و دوره نوری ۱۶ ساعت روشنایی و ۸ ساعت تاریکی مورد مطالعه قرار گرفت. مقادیر $LC_{50}$ گوارشی روغن منداب روی لارو سن سوم ارزیابی شد. سپس پارامترهای فیزیولوژیکی حشره، ۲۴ ، ۴۸ و ۷۲ ساعت پس از تغذیه، در سطح $LC_{50}$مورد بررسی قرار گرفت. مقادیر $LC_{50}$ ۲۴، ۴۸ و ۷۲ ساعت پس از تغذیه ، به ترتیب ۴.۹۴۰ و ۳.۷۹۱ و ۲.۹۳۸ میلی گرم در میلی لیتر بود. روغن منداب۷۲ ساعت بعد از تغذیه در سطح $LC_{50}$ باعث کاهش شاخص های تغذیه ای شامل نرخ رشد نسبی، نرخ مصرف نسبی، کارایی تبدیل غذای خورده شده، کارایی تبدیل غذای هضم شده و شاخص تقریبی هضم‌شوندگی و همچنین افزایش معنی دار شاخص بازدارندگی تغذیه، شد. میزان ذخایر غذایی مانند کربوهیدرات کل ، پروتئین و لیپید و نیز فعالیت آنزیم های گوارشی شامل لیپاز ، α- آمیلاز و پروتئاز کاهش یافت که نشان دهنده سمیت بعد از گوارش روغن منداب است. همچنین فعالیت آنزیم سم زدای گلوتاتیون S - ترانسفراز افزایش یافت که نشان می دهد این آنزیم ممکن است در سم زدایی روغن منداب دخیل باشد اما استراز تغییر معنی داری نکرد. به طور کلی می توان نتیجه گرفت که روغن منداب در شرایط آزمایشگاهی، دارای فعالیت ضدتغذیه ای روی سوسک برگخوار نارون است. به نظر می رسد روغن منداب پتانسیل زیادی جهت استفاده به عنوان آفت کش گیاهی موثر دارد. با وجود این، انجام تحقیقات بیشتری مانند آزمایش های گلخانه ای و مزرعه ای قبل از مرحله توصیه و تجاری سازی، ضروری است.





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