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Oxidative effects of tarragon (*Artemisia dracunculus* L.) on biostages stages of *Drosophila melanogaster* Meigen

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ABSTRACT: Tarragon (*Artemesia dracunculus* L.) is a traditional spice often used in local food dishes. This study was undertaken to determine the effects that nutritional tarragon has on oxidative stress in various developmental stages of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). Larvae of *D. melanogaster* were reared to adulthood on artificial diets containing varying amounts of tarragon ranging from 10 to 2000 µg. The effects of the various concentrations of tarragon on major indicators of oxidative stress including lipid peroxidation products, the production of malondialdehyde (MDA) and detoxification enzyme, and glutathione-S-transferase (GST) activity were investigated in 3rd instar larvae, pupae and adult fruit flies. The results indicate that the effectiveness of tarragon as an oxidative stress agent in *D. melanogaster* is dependent on its concentration in the fly's diet.

KEYWORDS: *Drosophila*, *Artemisia dracunculus*, oxidative stress, malondialdehyde, glutathione-S-transferase.

INTRODUCTION

Foods serve for energy production by oxidative phosphorylation, and nutrition are essential for the oxidant-antioxidant network in many organisms (Sies *et al.,* 2005). Because nutritional oxidative stress shows a disturbance of the redox state resulting from excess oxidative load or from nutrient supply (proteins, fat, carbohydrates, minerals, vitamins) favoring prooxidant reactions (Sies *et al.,* 2005). Increased ingestion of natural products are associated with a diminished risk, but the organism is unable to mitigate the free radicals, damage to biological molecules may occur, formed by oxidative stress (Joanisse and Storey, 1996 a; 1996 b).

Artemesia dracunculus L. (Tarragon) is used in food and perfume industry, antiseptics, pharmaceutical (aperient, stomachic, stimulant, febrifuge), sanitary, cosmetic, antioxidant - prooxidant activity, food industries and as an appetizer in Central Anatolia. The tarragon essential oils or components have been studied in different concentration of many organisms such as bacteria, fungi, arthropods etc (Hatimi *et al.,* 2001; Lamiri *et al.,* 2001; Farzaneh *et al.,* 2006; Kordali *et al.,* 2005; Liu *et al.,* 2006; Saleh *et al.,* 2006; Van de Sande *et al.,* 2007; Bakkali *et al.,* 2008). Desiccated powder of tarragon is not genotoxic but consumed fresh it is harmful to humans (Insttitut Pasteur de lille, 2008-2010). Its essential oils have been cytotoxic capacities and damages for some tissues of various animals.

Drosophila melanogaster (Meigen) has been studied as a model organism for the research in cell and developmental biology (Adams *et al.,* 2000). Despite tarragon is used as insecticide (natural deterrent) in biological control system, there has been no report about the determination of its

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effects on developmental stages of *D. melanogaster* in oxidative stress including ROS-specific lipid damage products, the production of malondialdehyde (MDA) and detoxification enzyme, and glutathione-S-transferase (GST) activity .

Investigations were made to understand whether tarragon ingestion causes oxidative stress on insect development and what kind of effects Tarragon in peroxidation (indicate lipid damage; MDA)-detoxification (indicate antioxidant activity; GST) mechanisms created on non-target organisms.

MATERIALS AND METHODS

The experiments were maintained at 25°C, 60% humidity and 12 h light/dark photoperiodic cycle, at a density of 30-35 flies per vial. The mixed-age and mixed sex fly stocks (Wild type, W_{1118}) were cultured in glass vial (250 cc), with an artificial diet (Rogina *et al*., 2000; Lesch *et al*., 2007). Methyl 4-hydroxybenzoate (0.2%; 100 g nipagin, 700 mL 96% ethanol and 300 mL water) was added to the diet to inhibit mold growth (Dahmann, 2008). Newly eclosed flies (6 male: 18 female) were collected in separate vials, mated and fed for two days before becoming flies, after laying eggs for 18 h, flies were removed. Nutritive value of tarragon are presented in table 1, and total essential oils are presented in table 2. Tarragon seeds (Zengarden, 838H) were planted and grown in flower pots, and its fresh leaves were crushed with liquid nitrogen in sterile muller. 100 newly hatched larvae were collected and distributed (directly incorporated into freshly diets) to ~~either~~ bottles with varying concentrations of tarragon (10, 200, 600, 900, 1200, 2000 µg/mL). The control contained only water. These concentrations were used based on the results of our preliminary experiments (unpublished; Güne , 2014) within the tolerance range of. *D. melanogaster* and the results of previous studies on other insects exposed to tarragon (Azaizeh *et al.,* 2007; Bakkali *et al.,* 2008; Hifnawy *et al.,* 2001; Soliman, 2006; Tani *et al.,* 2008; Mihaljilov-Krstev *et al.,* 2014). The exposure schedule lasted until flies come to the 3rd instar larvae, puparium and adult (newly enclosed virgin female and male) stage. These samples (n=20, per concentration) were collected and frozen in the freezer (-18ºC) for 5 mins. They were transferred to a labeled micro centrifuge tubes and homogenized in 1 ml cold homogenization buffer (0.5 M potassium phosphate buffer pH 7.2) for three times using ultrasonic processor (Homogenizer, Branson) on ice. The supernatants were collected and used for biochemical analysis. All homogenates were centrifuged at 20,000g for 30 min, at 4ºC.

Biochemical analysis:

The MDA content and GST activity (EC 2.5.1.18) of each supernatants were assayed via measuring the absorbance of the samples in spectrophotometer (Biochrom Libra S22) as described previously (Jain and Levine, 1995; Habig *et al.,* 1974; Fig. 1). At the same time protein concentrations were determined according to the method of Lowry *et al.* (1951) by using bovine serum albumin (BSA) as a standard. Data graphics were calculated using the computer program (Microsoft Excel). All chemicals used in this experiment were analytically pure and obtained from Sigma-Aldrich.

Statistical analysis:

The experiments were performed four times. Experimental data were expressed as means ± S.E. The data (MDA and GST activity) were subjected to statistical analysis by one-way analysis of variance (ANOVA) was followed by lest significant difference (LSD) test to determine significant differences between means. A values of $p<0.05$ was considered significant (SPSS, 1997).

RESULTS

The MDA contents and GST activities obtained from larvae, pupae and adults stages were shown in Figure 2 and 3. The effective Tarragon concentration was determined to be 10 µg in larva. It was determined that the MDA content was found lower, and GST activity was found higher in 100 µg/L plant application, but these two parameters were increased and stabilized in higher concentrations. It is thought that while the lower

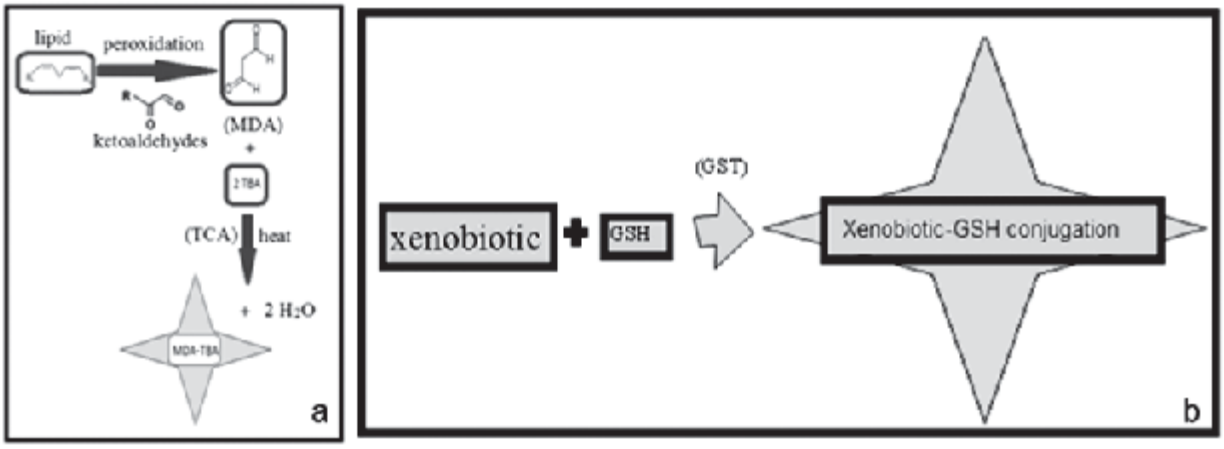

Figure 1. The principles of biochemical analysis (a: MDA content, b: GST activity)

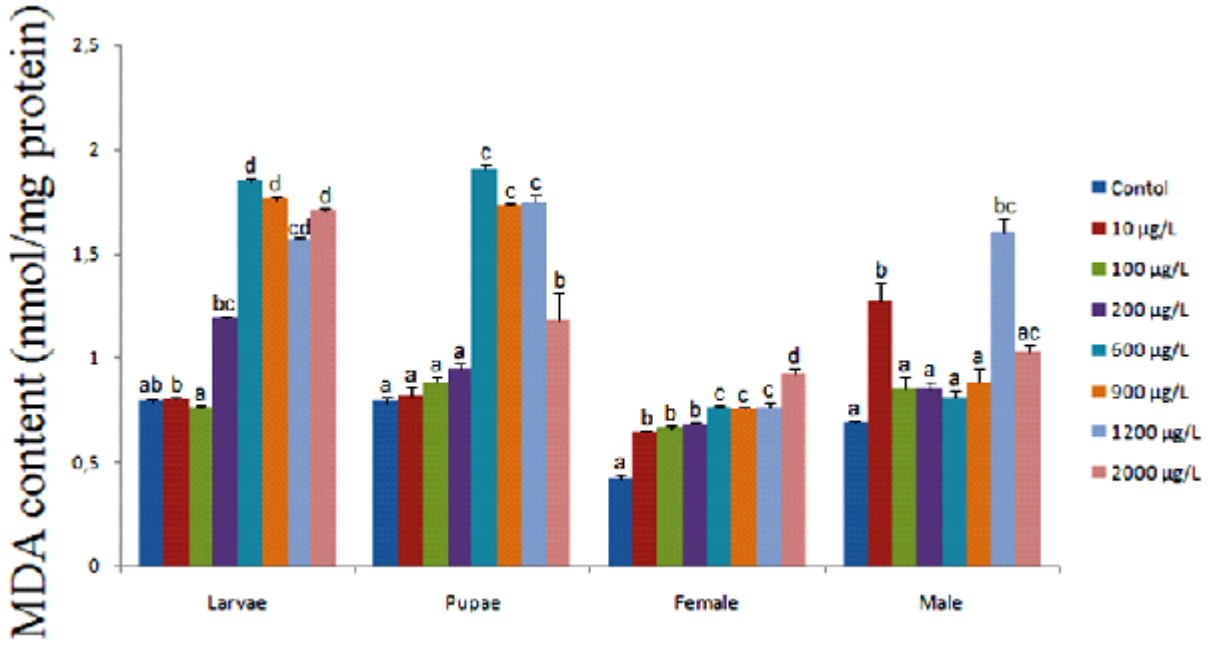

Figure 2. The Tarragon effects of MDA content were indicated on larvae, pupae and adults (female and male) of *D. melanogaster*. Samples with increasing concentration of Tarragon: control (0.00 mg/L); 10 µg/mL; 200 µg/mL; 600 µg/mL, 900 µg/mL, 1200 µg/mL and 2000 µg/mL. Each histogram bar represented the mean of four replicates (± S.E., n=20) in each of treatment groups.

concentrations of the plant can be tolerated, the toxic impact in higher concentration cannot be tolerated by the insect.

MDA contents were not significantly different from 0.0 to 200 µg/L tarragon concentration in pupae. GST activity increased sharply in pupae after 200 µg/L plant, but it decreased slightly from 600 to 2000 µg/L (Fig. 2). It was determined that the MDA contents were observed as gradual increases dependent on Tarragon concentration in female, and as well as the GST activities were increased with the activation of the detoxification mechanisms when we compared to control group.

In addition, the males' MDA contents were not significantly ($p>0.05$) different from 0.0 to 900 µg/L, but MDA content was significantly increased oxidative stress by feeding with 1200 µg/L tarragon, and there were parallel increase in these GST

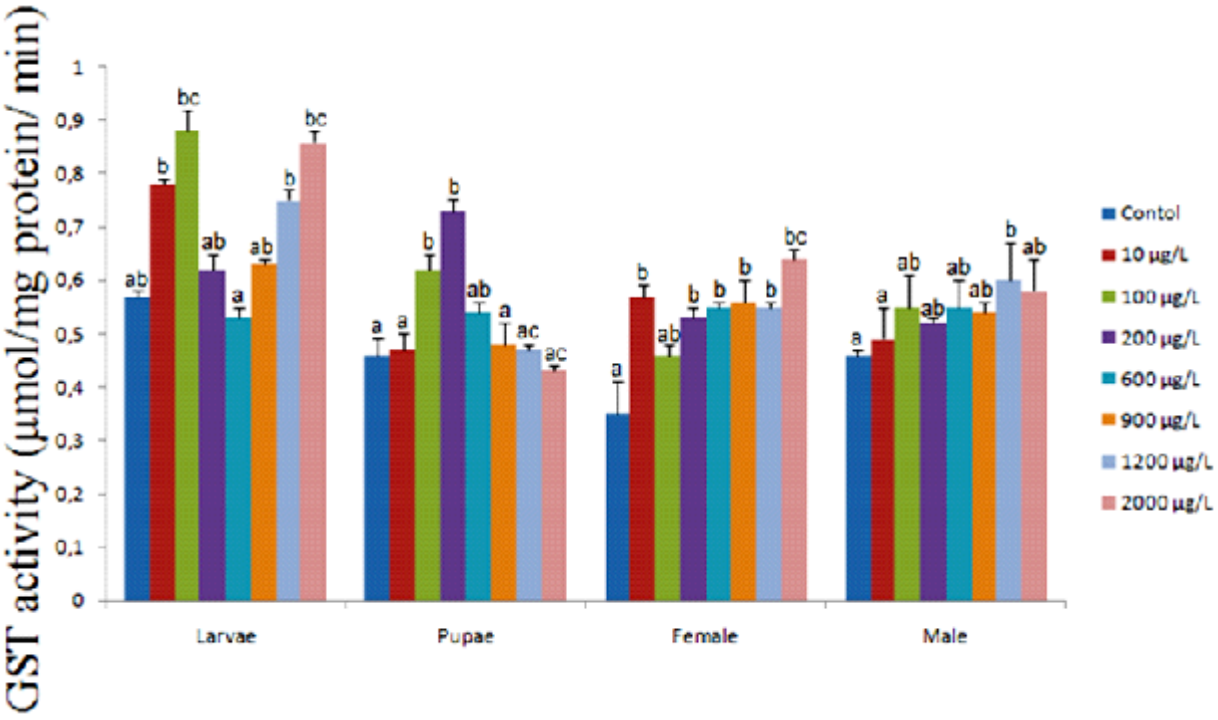

Figure 3. The Tarragon effects of GST activity were indicated on larvae, pupae and adults (female and male) of *D. melanogaster*. Samples with increasing concentration of Tarragon: control (0.00 mg/L); 10 µg/mL; 200 µg/mL; 600 µg/mL, 900 µg/mL, 1200 µg/mL and 2000 µg/mL. Each histogram bar represented the mean of four replicates (± S.E., n=20) in each of treatment groups.

Table 1. Approximate composition of Tarragon /100g of edible portion (Farrell, 1990)

Energy (kcal)	295	
Protein (g)	22.8	
Fat (g)	7.2	
Total carbohydrates (g)	50.2	
Minerals (mg)	Calcium	1139
	Fe	32
	Mg	347
	P	313
	K	3020
	Na	62
	Zn	4
Vitamins (mg)	Riboflavin	1
	Niacin	9
	Vitamin A (IU)	4200
Fibre (g)	7.2	

Table 2. The chemical constituents of the *A. dracunculus essential oil* (Sayyah *et al.,* 2004)

Component	Kovats' index	Content (%)
á-Pinene	922.7	5.1
â-Pinene	959.5	0.8
Limonene	1015.5	12.4
á-*trans*-Ocimene	1026.7	20.6
á-Terpinolene	1069.2	0.5
Allo ocimene	1113.4	4.8
trans-Anethole	1195.3	21.2
Bornyl acetate	1259.4	0.5
Methyl eugenol	1364.8	2.2
Bicyclogermacrene	1470.2	0.5

activities. As can be seen in Figure 2 and 3., minimum LPO levels and detoxificatin activities were observed in females, and these parameters maximum levels observed in the third instar larvae of *D. melanogaster*.

DISCUSSION

The experimental organisms are influenced by nutrition, genotype, age, and various aspects of the environment. Nutrition has influences on development, fertility, longevity, immune defense in variety of animals (Piper *et al.,* 2005; Unckless *et al.,* 2015). *D. melanogaster* is suitable for experimental design for detailed nutritional studies, and it provides an overview (Piper *et al.,* 2005). A great deal of literature has been published concerning the effects of nutritions, quantitative nutritional requirements, food or dietary restrictions, diet interaction drive phenotype and etc. on *Drosophila* (Sang, 1956; Piper *et al.,* 2005; Reed *et al.,* 2010; Sisodia and Singh, 2012; Wong *et al.,* 2014; Unckless *et al.,* 2015).

Many species of *Artemisia* plants (Compositae) have been identified and they are known to have pharmaceutical (treatment, drug, antioxidant, antitumor, antifungal) and industrial properties (Zani *et al.,* 1991; Meepagala et al., 2002; Ribnickya *et al.,* 2004; Sayyah *et al.,* 2004; Kordali *et al.,* 2005; Emami *et al.,* 2009; Shahriyary and Yazdanparast, 2009; Hatami *et al.,* 2014). Tarragon, also known as *A. dranculus*, has been safely and widely used as a food in Central Anatolia (seasoning, salads, vinegar etc.). Some studies have shown that it has a safe use as a dietary supplement or in functional foods (Ribnickya *et al.,* 2004; Kordali *et al.,* 2005). *Drosophila* needs of the salts such as K, O, Mg, Na (Sang, 1956), and these materials are available in sufficient amounts for tarragon-feeding. It has been shown that the LD_{50} for Tarragon is greater than 2000 µg/L on different developmental stages of *D. melanogaster*. Previous studies have indicated that toxic effect of Tarragon is started especially in higher concentrations (Bakkali *et al.,* 2008; Emami *et al.,* 2009; Güneş, 2014). Similar studies have been concluded that some *Artemisia* species (for example *A. absinthium*) are toxic for developing insect larvae such as *M. domestica* and *D. melanogaster* (Bezzi and Caden,1991; Mihaljilov-Krstev *et al.,* 2014). Because of this feature, it may be effective on insecticidal and radical scavenging activity (Saadali *et al.,* 2001; Parejo *et al.,* 2002; Sayyah *et al.,* 2004).

The amount of nutrients and supplements consumed by organisms a strong impact on stress and resistance (Sisodia and Singh, 2012). The crude plants were evaluated for pesticidal activity and used in pest management to adults, *Artemisia* essential oil was tested in larvacidal, insecticidal activities against house flies (Hifnawy *et al.,* 2001;

Soliman, 2006; Ebadollahi, 2008; Tani *et al.,* 2008). Several studies have demostrated that the tarragon toxic effects are dose (concentration) dependent (not linear) and diminishes rapidly at low exposures that levels can be detoxified by organisms. This is concentration depent effect in range of 10 through 2000 µg/mL. A similar effect observed for some other studies (Azaizeh *et al.,* 2007; Emami *et al.,* 2009). In previous studies, some monoterpens (The most abundant essential oil in Tarragon) have protective effects, cytotoxic (at 1.6 mg/mL) and genotoxic/antigenotoxic (Sayyah *et al.,* 2004; Fernandes *et al.,* 2013). Concentration-dependent of tarragon increase in GST activities may not be able to protect the organism beyond a particular limit. Therefore, it seems that the detoxified effects of *A. dracunculus* may be related dose–response relationship in developmental phases. In addition, *trans-anethole* is the main component of tarragon oil. Its esential oils have low toxicity (Sayyah *et al.,* 2004), and this finding may support the low toxicity of our feeding experiments.

A potential source of cellular damage associated with nutrient is through the production of reactive oxygen species (ROS) and respiration mechanism (under aerobic or anaerobic conditions) (Sies *et al.,* 2005). So, the physiological or biological condition of an organism under this stress factors (metabolic and environmental oxidative stress, photooxidative stress, drug-dependent oxidative stress, or nitrosative stress etc.) can be assessed using different biochemical (like antioxidant enzyme activities) and molecular markers (Sies, 2000; Siddique *et al.,* 2007). Some pathways (JNK) and enzyme systems (GST, SOD etc.) can protect fruit flies against oxidative damage. *Drosophila* possess both enzymatic and non enzymatic defenses to cope with reactive oxygen species (ROS) such as Catalase (CAT), Superoxide dismutase (SOD), Reduced glutathione (GSH), Glutathione reductase (GR), GST, Disulfide reductase, Methionine sulfoxide reductase (MSR) and Thioredoxin peroxidase (TRXP) (Moskovitz *et al.,* 1997; Missirlis *et al.,*2003; Valko *et al.,* 2006; Siddique *et al.,* 2007). Insects exploit a series of antioxidant and detoxification enzymes such as GST that may form a combined response to chemicals or food supplements (Felton and Summers, 1995; Krishnan *et al.,* 2007) and MDA is an indicator of cellular oxidation (Shahriyary and Yazdanparast, 2009). The determination of MDA content was often accompanied with a measurement of GST or SOD activity (Lei *et al.,* 2014). For example, Fennel was contributed to the daily antioxidant diet (Shahat *et al.,* 2011; Amkiss *et al.,* 2013). Inorganic insecticides, plant esential oils or food supplements lead to oxidative stress and altered GST activities and MDA content in virtual tissues (Hyrsl *et al.,* 2007; Ebadollahi, 2008). Some researchers have shown that 0.8 and 4 mg/mL of hawthorn extracts (increased CuZn-SOD, CAT enzyme activity but decreased MDA levels; Rosemary extract (1-5 mg/mL) can improve the antioxidant enzyme activity (SOD, CAT), inhibit the lipid peroxidation (MDA) in *Drosophila* (Zhang *et al.,* 2012; Zhang *et al.,* 2014). *Kunlun Chrysanthemum* flowers (China herb) have shown antioxidative effect (improved SOD activity and decreased MDA content) feeding with 0-0.6 % doses on *Drosophila* (Jing *et al.,* 2015). We infer from these findings that Tarragon influences life history parameters of *D. melanogaster*. The results indicate that the diet containing the highest tarragon concentration led to increased MDA content and GST activity but not of the pupal stages in whole body and the effect was dose dependent. MDA contents increased in pupal stages, probably caused by the use of the lipid storage as in Lepidoptera (Warbrick-Smith et al., 2006). Tarragon exhibited low toxicity to the adult stages and higher toxicity to the larval and pupal stages. Previous studies shown that oxidative effects of a dietary supplements on development depends on its interaction with feeding for instance *Artemisia ssp.* (49 mg/mL) is toxic for developing insect larvae after 15 days (Mihaljilov-Krstev *et al.,* 2014), because the flies are fed in adult and larval stages. Feeding can affect developmental stages such as growth and reproduction, and larval nutrition may affect a range of different stages as well as response to cellular stress in adult (Sisodia and Singh, 2012). Normal growth and development are suspended during stress (Tettweiler *et al.,* 2005), the dietary supplements such as essential oil also affected by the development of insect larvae and delayed achievement of the pupal stage

(Mihaljilov-Krstev *et al.,* 2014). In addition, the high Tarragon exposure demonstrated to induce an increase in oxidative stress, including an increase in MDA and decreases in GST activities. Because the level of MDA content and GST activity reflects the level of cells attacked by free radicals and oxygen free radical scavenging ability (Lei *et al.,* 2014).

Tarragon was known with numerous polyphenols compounds such as phenyl carboxylic acids, flavonoids and coumarins (Obolskiy *et al.,* 2011; Pirvu *et al.,* 2014). It was also noted that the females MDA content and GST activity was concomitant increased compared to the control, as in similar studies (Navarro *et al.,* 2010). Polyphenols shows antioxidant features such as eugenol that is induced phase 2 antioxidant enzymes; *A. dracunculus* polyphenolic compounds used for preventing the diseases (Alma *et al.,* 2003; Miguel *et al.,* 2003; Scalbert *et al.,* 2005; Govorko *et al.,* 2007; Kim *et al.,* 2014). Some studies showed that plant compounds have antioxidant potential (El-Massry *et al.,*2002; Kim *et al.,* 2014). For example, females and males of *Drosophila* were fed either containing curcumin and supplemented at 0.5-1.0 mg/g of diet, MDA levels decreased and SOD activity increased in both diets (Shen *et al.,* 2013). It was highlighted in another study, black garlic extracts were possessed strong antioxidant capacity in vitro in a dose-dependent manner and the content of MDA was decreased by improving SOD and CAT activities (Lei *et al.,* 2014). Thus, it might have prevented the toxicity related disorders by feeding high concentration of tarragon. Furthermore, if the food contains a high concentration of plant, MDA contents will increase, and this is probably caused by starvation or malnourishment, because insects are changing their feeding behaviour in response to prevent oxidative damage (Povey et al., 2009). Also positive correlation has a ratio between lipid content and starvation resistance among individuals of *Drosophila* (Sisodia and Singh, 2010).

The results indicate that the effectiveness of tarragon as an oxidative stress agent in *D. melanogaster* is dependent on its concentration in the fly's diet. This data suggests that adverse effects at lower levels (antioxidant activities) of daily exposure would not be expected and it would be taken through food chains on directly non-target organisms. It is belived that these increases in lipid peroxides are probably due to an tarragon accumulation. This work will serve as a point for studies seeking to understand the usage of tarragon as a nutrition in *Drosophila* whose nutrient-related signalling pathways are known to be similar with mammalian.

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