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Original article (Orijinal araştırma)

Fumigant toxicity of essential oil of *Hypericum perforatum* L., 1753 (Malpighiales: Hypericaceae) to *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae)

Hypericum perforatum L., 1753 (Malpighiales: Hypericaceae) esansiyel yağının *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae)'a karşı fumigant toksisitesi

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Abstract

In this study, vapor of essential oil obtained by the hydrodistillation of *Hypericum perforatum* L., 1753 (Malpighiales: Hypericaceae) was tested on the different stages of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae). The larvae, pupae and adult stages of *T. molitor* were exposed to different doses of *H. perforatum* essential oil for 24 h. After exposure, mortality rate, LC_{50}, LC_{90} and LC_{99} values, antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx)], acetylcholinesterase (AChE) activity and malondialdehyde (MDA) levels were measured in the insects. *Tenebrio molitor* was cultured at Gazi University Department of Biology and all analyses were done in Yozgat Bozok University in 2017 and 2018. The results indicated that the pupae of *T. molitor* were the most tolerant and adults were the most sensitive. Mortality increased with the increasing concentration of essential oil. Also, increasing doses of essential oil caused decreasing in SOD, CAT, GST GPx and AChE activities and increasing in MDA level. These results indicate that essential oil of *H. perforatum* can be used against *T. molitor* in a pest control program.

Keywords: Antioxidant enzymes, GC-MS, *Hypericum*, insecticidal activity, meal worm, pesticide

Öz

Bu çalışmada, *Hypericum perforatum* L., 1753 (Malpighiales: Hypericaceae) distilasyonundan elde edilen uçucu yağın, *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae)'ün farklı gelişme dönemleri üzerindeki etkisi test edilmiştir. Larva, pupa ve ergin dönemdeki *T. molitor* bireyleri, 24 saat boyunca farklı dozlarda *H. perforatum* uçucu yağına maruz bırakılmıştır. Uygulama sonrası, böceklerin ölüm oranları, $LC_{50,}$ LC_{90} ve LC_{99} değerleri, antioksidan enzim aktiviteleri [süperoksit dismutaz (SOD), katalaz (CAT), glutatyon-S-transferaz (GST) ve glutatyon peroksidaz (GPx)], asetilkolinesteraz (AChE) aktivitesi ve malondialdehit (MDA) seviyeleri ölçülmüştür. *Tenebrio molitor* Gazi Üniversitesi Biyoloji Bölümü'nde kültüre alınmış ve analizler 2017 ve 2018 yıllarında Yozgat Bozok Üniversitesi'nde yapılmıştır. Sonuçlar, *T. molitor*'un pupa döneminin en toleranslı; ergin döneminin ise en hassas dönem olduğunu belirtmektedir. Ölüm oranları, maruz kalınan uçucu yağ konsantrasyonunun artmasıyla artmıştır. Ayrıca, artan uçucu yağ dozları, SOD, CAT, GST GPx ve AChE aktivitelerinde azalmaya, MDA düzeyinde ise artışa neden olmuştur. Bu sonuçlar, *H. perforatum* uçucu yağının, *T. molitor* ile mücadelede önemli bir potansiyele sahip olduğunu ortaya koymuştur.

Anahtar sözcükler: Antioksidan enzimler, GC-MS, *Hypericum*, insektisidal aktivite, un kurdu, pestisit

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Introduction

During food storage, especially corn, oat and wheat, major problems can occur because of the presence of insects like yellow mealworm *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) (Garcia et al., 2003). Insects in stored bran and grains can contaminate foods via their feces and fragments of old cuticle parts, and also indirectly by saprophytic microorganisms. All of these factors can cause quality loss (Garcia et al., 2003; Pinto, 2008). *Tenebrio molitor* adults lay elongated eggs on substances. When larvae hatch, they produce chitinous exoskeleton in a few days and their white color changes to yellow. Generally pupal stage can take up 5-6 days. After pupal stage, insects become adult and mate. Adults lay eggs in 4-17 days following mating (Siemianowska et al., 2013).

Insects can be controlled with chemical treatments such as pesticides; however, using of these chemicals may have adverse effects on environment and non-target organisms (Ghini et al., 2002; Lee et al., 2004). Thus, safer control methods, like using natural extracts and secondary compounds of plants as biopesticides, are becoming important for pest control (Lima et al., 2011). Botanical pesticides are studied because they have low toxicity for humans, decreased toxic effects on environment and rapid degradation. These properties make them suitable pesticides for insects in organic agriculture. Essential oils which have botanical origin, are effective insecticides (Regnault-Roger, 1997; Cosimi et al., 2009). Essential oils can be obtained from seeds, flowers, leaves, buds, twigs, wood, bark and roots of plants, and can have anti-protozoan, antihelminthic and insecticidal activities (Upadhyay, 2010). There are many studies that report the use of essential oils against insects. Essential oils from *Conyza newii* Oliv. & Hiern, *Plectranthus montanus* Benth., *Lippia javanica* Spreng., *Lippia ukambensis* (Vatke) Verdc., *Tetradenia riparia* (Hochst.) Codd and *Tarchonanthus camphoratus* L. (Omolo et al., 2005), seven *Citrus* spp., two *Origanum* spp., three *Cymbopogon* spp., two *Pimenta* spp., two *Eucalyptus* spp., three *Mentha* spp. and two *Juniperus* spp. (Choi et al., 2003) have been reported to have insecticidal activity.

Hypericum perforatum L., 1753 (Malpighiales: Hypericaceae), St. John's wort contains essential oils that are potentially insecticidal. Recently, the consumption of compounds derived from *H. perforatum* has increased strongly, and it has become one of the most consumed medicinal plants worldwide (Wills et al., 2000). It has an extensive variety of medicinal applications, such as diseases of the alimentary tract, eczema, skin wounds and burns (Butterweck, 2003; Saddiqe et al., 2010). There are many studies that have focused on the effects of *H. perforatum* extracts against bacteria and viruses, and various diseases (Serkedjieva et al., 1990; Çakir et al., 1997). Also, *H. perforatum* can be used as antihelmintic and antiseptic (Çakir et al., 1997). In addition to medical use, *Hypericum* species have insecticidal activity. The the insecticidal effects of *Hypericum* spp. have been assessed against *Culex pipiens* (L., 1758) (Diptera: Culicidae) (Rouis et al., 2013), *Tribolium castaneum* (Herbst, 1797) (Coleptera: Tenebrionidae) (Parchin & Ebadollahi, 2016), *Manduca sexta* L., 1763 (Lepidoptera: Sphingidae) (Samuels & Knox, 1989) and *Sitophilus granarius* (L., 1758) (Coleptera: Dryophthoridae) (Kordali et al., 2012). Considering previous studies, plant materials of *Hypericum* spp. may be candidates for future works about management of pests. Therefore we aimed to evaluate the chemical composition and insecticidal effects of the essential oil extracted from *H. perforatum* against *T. molitor.*

Oxidative stress, the imbalance between free radical production and antioxidant defenses, is associated with chemical exposition. Malondialdehyde (MDA) levels and antioxidant enzyme activities [superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx)] are important indicators of oxidative stress. The antioxidant enzymes such as SOD, CAT, GPx and GST may neutralize the oxidative stress, so, increases and decreases in their levels give us important information about the cell damage caused by oxidative stress (Baş & Kalender, 2011).

Given that synthetic pesticides have many adverse effects on non-target animals and cause environmental pollution, it is important to identify alternative methods to control pests. So, we investigated the efficacy of *H. perforatum* essential oil, against *T. molitor* in this study. Data on the insecticidal activity of *H. perforatum* are limited in the literature. This study will contribute to the literature because it is the first study to investigate the effects of *H. perforatum* against *T. molitor*.

Materials and Methods

Plant material, isolation and analysis of the essential oil

Hypericum perforatum was collected in June 2017 in Uşak Province of Turkey. The plant samples were cleaned and dried via herbarium techniques of Davis (1972). Two hundred g of dried aerial parts of plants were distilled for 4 h in water (3L) using a Clevenger type apparatus. The essential oil obtained was kept at 4°C until beginning of the study. Essential oil of *H. perforatum* was determined via GC-MS using Agilent Technologies (Santa Clara, CA, USA) 6890N Network GC System 5973 MSD, ionization energy: 70 eV; 19091 N-136 HP-Innowax column 60 m x 0.25 mm i.d.; helium 1 mL min^{-1}. One µl of sample was injected into the GC-MS analysis system. Injection temperature was 250°C. Column temperature was initially held at 60°C for 3 min, then raised to 280°C at a rate of 5°C/min. Then, the temperature was ramped by 5°C/min to 300°C. The amounts of essential oil components were estimated from the area under GC peaks. Identification of compounds were done by mass spectral comparison by electronic libraries (Wiley, NIST, Adams). Retention indices were calculated according to the equation given by Kovats (1958).

Tenebrio molitor culture

A culture of *T. molitor* was established at the Metin Aktaş Zoology Museum (Department of Biology, Gazi University) and all analyses were done in the General Biology and Seed Science Technology Laboratories (Yozgat Bozok University) in 2017 and 2018.

Insects were cultivated in plastic containers (30 x 25 x 15 cm). Corn flour (30%) and wheat flour (70%) were added as a food source and pasteboards were added for egg deposition. Thinly sliced potato or apple was added each week for water source and regulating for humidity of containers. Twenty individuals of different stages of the insect (larvae, pupae and adult) were selected randomly from one population.

Bioassays

Hypericum perforatum essential oil was tested for its fumigant effect against different stages of the *T. molitor*. Adult insects were placed into 1 L glass jars. Six replicates were tested for each concentration of *H. perforatum* essential oil and each replicate consisted of 20 adults. We used 2.5 × 2.5 cm filter paper strips for application of essential oil then the filter paper was attached to the bottom of the cover of glass jars. Adults of the mealworms were exposed to different concentrations of essential oil (0, 1, 2, 3, 4, 5 and 6 µl/L air) for 24 h. We determined the mortalities and values of $LC_{50,}$ LC_{90} and LC_{99}. The larval and pupal stages of *T. molitor* were handled in the same method as the adult stage of insect. Different concentrations of essential oil were applied to larvae (0, 2.5, 5, 7.5, 10, 12.5 and 15 µl/L air) for 24 h. The pupal stage of *T. molitor* was exposed to essential oil (0, 5, 10, 15, 20, 25 and 30 µl/L air) for 24 h. The control groups of larval, pupal and adult stages of *T. molitor,* involved the same conditions without essential oil. We have treated different doses to different stages of the insect because all of the adults died when we treated 6 µl/L air, but not all individuals died in the larval and pupal stages. So, we treated different doses until all died.

Toxicological assays

All of the chemicals (ethanol, thiobarbituric acid, trichloroacetic acid, pyrogallol, triton-X-100, H_2O_2, 1-chloro 2, 4-dinitrobenzene and nicotinamide adenine dinucleotide phosphate) used in this study to determine the antioxidant enzyme activities, MDA level, and AChE activity were obtained from Sigma-Aldrich (Germany).

Tissue collection and preparation

Larvae, pupae and adults were cooled on ice (5 min) then sterilized with ethanol. After this step, they were cut into small pieces and put into Eppendorf tubes which were filled with homogenization buffer at pH 7.4. Samples were kept at -80°C until examined. Before starting work, Eppendorf tubes were kept at 25°C until the samples thawed.

The extracts of larvae, pupae and adults were prepared with a homogenizer at 4°C and centrifuged (NUVE NF800) for 15 min. After centrifugation, the supernatants were taken for examination of CAT, GST, SOD, GPx, MDA and acetylcholinesterase (AChE). MDA levels and the activities of antioxidant enzymes and AChE were measured by measuring the absorbance via a UV-VIS spectrophotometer (Biotech Engineering, Spectroscan 60 DV). Protein concentrations were estimated according to the method of Lowry et al. (1951).

Measurement of malondialdehyde levels

The levels of MDA were measured by thiobarbituric acid (TBA) test as described by Ohkawa et al. (1979). First we add to 10% trichloroacetic acid to a sample then centrifuged for 10 min. The supernatant was collected and added to TBA. After incubation of tissue homogenates with TBA, MDA reacts with it to form a pink colored complex at 95°C. Next, the samples were cooled and we measured the absorbance at 532 nm. We defined the MDA level as nM/mg protein.

Measurement of antioxidant enzyme activities

We measured the enzyme activity of CAT based on procedure of Aebi (1984). This procedure is based on determining the hydrolysis of H_2O_2. The absorbance was determined at 240 nm using spectrophotometer. The activity was given as mM/mg protein. The enzymatic activity of GPx was measured by the procedure which was identified by Paglia & Valentine (1987). The reaction was searched at 340 nm and the activity was indicated as nM/mg protein. The method of Marklund & Marklund (1974) was used for assessment of SOD activity at 440 nm and the enzymatic activity was indicated as U/mg protein. GST activity was measured at 340 nm (Habig et al., 1974). This procedure is based on assaying the generation of 1-chloro-2,4-dinitrobenzene and glutathione conjugate. The GST activity was estimated as μM/mg protein.

AChE enzyme activity

The effects of the *H. perforatum* essential oil on activity of AChE was measured by the procedure of Ellman et al. (1961). The assay solution contained 0.015 M acetylthiocholine iodide, 0.01 M 5,5'-dithiobis(2-nitrobenzoic acid), 0.1 M Na-K phosphate buffer at pH 8.0 and ethopropazine. The reaction was monitored at 412 nm wavelength using a spectrophotometer. The activity was estimated as U/mg protein.

Statistical analysis

The data were analyzed by one-way analysis of the variance in SPSS program 20.0 for Windows and Tukey test for multiple comparisons at a significance level of 0.05. LC_{50}, LC_{90} and LC_{99} values were estimated by probit analysis with SPSS (Abbott, 1925).

Results

Chemical compounds of *Hypericum perforatum*

The chemical composition of *H. perforatum* essential oil (25 compounds were determined by GC-MS) is shown in Table 1. α-Pinene (51.2%), 3-carene (7.3%), α-caryophyllene (5.2%) are the main compounds of essential oil.

Table 1. Percentage composition of the essential oil of *Hypericum perforatum*

Compounds	RI (Retention Index)	Percantage (%)
)α-pinene	939	51.2
Sabinene	976	2.4
β-pinene	979	3.2
β-Myrcene	990	3.6
2-carene	1001	1.0
Eugenol	1356	0.6
α-phellandrene	1004	0.4
3-carene	1010	7.3
p-cymene	1024	0.7
Limonene	1029	2.0
ɣ-terpinene	1058	2.2
Myrtenol	1194	0.6
α-longipinene	1351	0.3
α-copaene	1376	0.5
β-caryophyllene	1419	0.8
Aromadendrene	1439	0.3
α-caryophyllene	1454	5.2
Allo-	1461	0.5
ɣ-muurolene	1477	0.3
Germacrene-D	1482	0.6
ɣ-cadinene	1513	0.3
Calamenene	1518	0.5
Longifolene	1556	0.4
Cedrol	1601	0.3
Cadalene	1674	3.2
Terpenes	87.8	
Monoterpenes	74.6	
Sesquiterpenes	13.2	
Non-terpenes	0.6	
Unidentified	11.6	
Total	88.4	

Fumigant activity results

The mortality percentages of life stages increased with increment of the concentrations of essential oil (adults $F = 30.5$, $df = 5,114$, $P < 0.05$; pupae $F= 87.2$, $df = 5,114$, $P < 0.05$; larvae $F = 51.5$, $df = 5,114$, $P < 0.05$).

Considering the results of probit analysis, $LC_{50,}$ LC_{90} and LC_{99} values of the essential oil were 3.06, 4.99 and 6.56; 13.3, 23.6 and 32.0; and 8.24, 13.9 and 18.5 µl/L air for the adult, pupal and larval stages of *T. molitor* respectively (Table 2).

The fumigant effects of essential oil were more significant on the adults than the larvae and pupae of *T. molitor*. The most resistant developmental stage of the insect was the pupae. The data of this research determined that the fumigant toxicity of *H. perforatum* essential oil differed between the life stages of the insect.

Table 2. LC_{50}, LC_{90} and LC_{99} values (µl/L air) of *Hypericum perforatum* essential oil against different life stages of *Tenebrio molitor*

Time (24 h)	N	LC_{50}	LC_{90}	LC_{99}	df	Chi-Square	Slope	Sig.	
Pupae	20	13.3	23.6	32.0	5	2,61	0,12±0,02	0,760	a
95% confidence limits		11.0-15.4	20.7-28.0	27.6-39.3					
Larvae	20	8.24	13.9	18.5	5	4,37	0,23±0,03	0,497	a
95% confidence limits		7.10-9.44	12.3-16.6	16.0-22.9					
Adult	20	3.06	4.99	6.56	5	2,08	0,67±0,09	0,838	a
95% confidence limits		2.64-3.48	4.44-5.84	5.73-7.94					

N, number of the tested stages; a, since the significance level is greater than 0.15, no heterogeneity factor is used in the calculation of confidence limits.

AChE enzyme activity, MDA levels and antioxidant enzyme activity results

The MDA levels of insects increased with the increasing exposure doses of essential oil of *H. perforatum* against different stages of *T. molitor,* significantly (Figure 1).

The antioxidant enzyme activities (CAT, GST, SOD and GPx) decreased by increasing application doses of essential oil of *H. perforatum* against different stages of *T. molitor,* significantly (Tables 3 to 5).

The AChE enzyme activity decreased by increasing exposure doses of essential oil of *H. perforatum* against different stages of *T. molitor,* significantly (Tables 3 to 5).

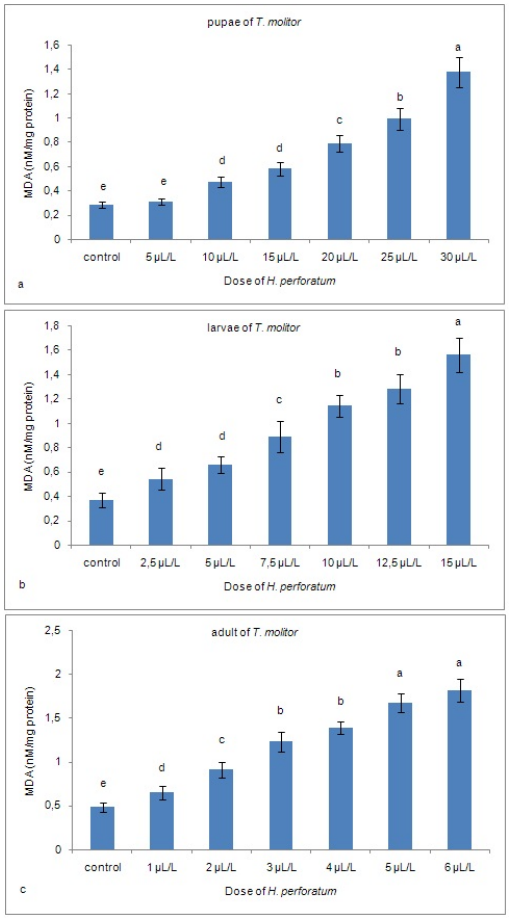

Figure 1. Effect of *Hypericum perforatum* essential oil on MDA levels of different life stages of *Tenebrio molitor*: a) pupae, b) larvae and c) adults. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different. Error bars indicate standard deviation (SD) of means.

Table 3. Effect of *Hypericum perforatum* essential oil doses on enzyme activities of pupae of *Tenebrio molitor*

Enzyme	Control		5 µl/L		10 µl/L		15 µl/L		20 µl/L		25 µl/L		30 µl/L	
CAT (mM/mg protein)	47.0±3.21	a	41.4±2.42	b	36.3±2.58	c	31.8±1.96	d	26.5±3.15	e	20.8±2.51	f	18.5±2.87	f
GPx (nM/mg protein)	0.221±0.011	a	0.218±0.02	a	0.183±0.013	b	0.156±0.011	c	0.13±0.021	c	0.094±0.012	d	0.068±0.012	e
SOD (U/mg protein)	0.03±0.0018	a	0.0268±0.0011	b	0.0241±0.0014	c	0.0212±0.0011	d	0.0179±0.002	e	0.014±0.0016	f	0.0127±0.0023	f
GST (µM/mg protein)	3.12±0.171	a	2.72±0.21	b	2.32±0.165	c	1.95±0.187	d	1.6±0.132	e	1.29±0.141	f	0.27±0.223	f
AChE (U/mg protein)	0.025±0.0022	a	0.0237±0.0019	a	0.0194±0.0012	b	0.0182±0.0024	b	0.0143±0.0011	c	0.0108±0.0017	d	0.0072±0.0015	e

Values are mean±standard deviation. Significance at P < 0.05. Within each row, means followed by the same letter are not significantly different.

Table 4. Effect of *Hypericum perforatum* essential oil doses on enzyme activities of larvae of *Tenebrio molitor*

Enzyme	Control		2.5 µl/L		5 µl/L		7.5 µl/L		10 µl/L		12.5 µl/L		15 µl/L	
CAT (mM/mg protein)	58.2±4.1	a	55.8±3.72	a	48.3±3.53	b	46.0±2.78	b	39.4±2.84	c	32.1±3.07	d	24.0±4.21	e
GPx (nM/mg protein)	0.27±0.012	a	0.243±0.013	b	0.235±0.011	b	0.203±0.013	c	0.18±0.016	c	0.145±0.014	d	0.101±0.021	e
SOD (U/mg protein)	0.036±0.0021	a	0.034±0.0015	a	0.0311±0.0012	b	0.0297±0.0023	b	0.0257±0.0014	c	0.0214±0.0023	d	0.019±0.0026	d
GST (µM/mg protein)	4.76±0.224	a	4.68±0.18	a	4.29±0.174	b	4.07±0.235	b	3.62±0.164	c	3.23±0.188	d	2.81±0.197	e
AChE (U/mg protein)	0.037±0.0026	a	0.035±0.0014	a	0.029±0.0017	b	0.024±0.0015	c	0.02±0.0018	d	0.017±0.0026	d	0.011±0.0021	e

Values are mean±standard deviation. Significance at P < 0.05. Within each row, means followed by the same letter are not significantly different.

Table 5. Effect of *Hypericum perforatum* essential oil doses on enzyme activities of adults of *Tenebrio molitor*

Enzymes	Control		1 µl/L		2 µl/L		3 µl/L		4 µl/L		5 µl/L		6 µl/L	
CAT(mM/mg protein)	53.8±4.01	a	46.1±2.91	b	39.0±3.52	c	32.2±3.27	d	29.8±2.98	d	23.5±3.04	e	21.7±2.74	e
GPx (nM/mg protein)	0.24±0.013	a	0.231±0.011	a	0.196±0.022	b	0.157±0.014	c	0.14±0.023	c	0.105±0.011	d	0.097±0.013	d
SOD (U/mg protein)	0.032±0.0011	a	0.0292±0.0013	b	0.0288±0.0022	b	0.0251±0.0011	c	0.022±0.0015	d	0.0185±0.0018	e	0.00178±0.0021	e
GST (µM/mg protein)	3.95±0.227	a	3.51±0.192	b	3.01±0.23	c	2.58±0.166	d	2.56±0.236	d	2.1±0.191	e	1.97±0.248	e
AChE (U/mg protein)	0.031±0.0015	a	0.028±0.0019	a	0.023±0.0016	b	0.0022±0.0021	b	0.018±0.0014	c	0.016±0.0023	c	0.011±0.0013	d

Values are mean±standard deviation. Significance at P < 0.05. Within each row, means followed by the same letter are not significantly different.

Discussion

Chemical control is the mostly used method against insect pests (Jembere et al.,1995). However, these harmful insects have developed resistance against many chemicals (Upadhyay, 2010). Also, other problems have been observed such as adverse effects on non-target organisms, especially natural enemies and toxicity to users and mammals, residue problems and environmental pollution (Cosimi, 2009). So, we evaluated an alternative method in this study. Potential insecticidal compounds have been extracted from plants which have shown growth inhibition of and toxicity to many harmful insects which damage field crops (Koul et al., 2000; Upadhyay, 2010). Previous studies have shown that essential oils of these plants have insecticidal activity against insects in stored cereals (Tripathi et al., 2000; Verma et al., 2000), field crops (Isman et al., 2001) and households (Singh et al., 2000). These studies on essential oils motivated us to focus on essential oil experiments for finding alternative methods.

There are a wide variety of chemical compounds such as terpenes, sesquiterpenes, and aromatic compounds in essential oils (Ogendo et al., 2008). These natural compounds may have volatile properties so, they may have fumigant activity and this character can be used against pests. These volatile compounds have harmful effects against insects and effect reproduction and longevity of different life stages of them. Table 1 shows the percentage composition of the volatile oil of *H. perforatum*.

Essential oils from pennyroyal, eucalyptus, rosemary and marjoram have shown insecticidal activity against *Pediculus humanus* subsp. *capitis* De Geer, 1778 (Psocodea: Pediculidae). In this study, LT_{50} values were 14.7, 12.6, 22.4 and 19.6, respectively (Yang et al., 2004). Essential oils from some plants grown in Africa have shown fumigant effects against *Anopheles gambiae* Giles, 1902 (Diptera: Culicidae). LD_{50} values were 3.8×10^{-3} for *T. camphoratus*; 4.3×10^{-3} for *L. javanica*; 2.8×10^{-3} for *P. montanus;* 4.4×10^{-3} for *T. riparia* and 4.7×10^{-3} for *L. ukambensis* (Omolo et al., 2005). Essential oil from *Ipomoea cairica* (L.) Sweet have shown insecticidal effect against larvae of *Aedes aegypti* (L., 1762)*, Culex tritaeniorhynchus*

Giles, 1901*, Culex quinquefasciatus* Say, 1823 and *Anopheles stephensi* Liston, 1901 (Diptera: Culicidae). The LC_{50} and LC_{90} values estimated for *C. tritaeniorhynchus, A. aegypti, A. stephensi* and *C. quinquefasciatus* were 14.8 and 78.3; 22.3 and 92.7; 14.9 and 110; and 5.9 and 162 ppm, respectively (Thomas et al., 2004). Essential oils, carvacryl, mentha, eucalyptus and citronella, have shown high repellency to *Aedes albopictus* Skuse, 1894 (Diptera: Culicidae) (Yang & Ma, 2005). In another study, *Cinnamomum camphora* (L.) J.Presl. and *Artemisia princeps* Pamp. have shown toxicity and repellency to *Bruchus rufimanus* Boheman, 1833 (Coleptera: Chrysomelidae) and *Sitophilus oryzae* Schoenherr, 1838 (Coleptera: Dryophthoridae) (Liu et al., 2006). Also, the toxicity of essential oils from the bark and leaves of *Drimys winteri* Forst. and *Laurelia sempervirens* Tul. against *T. castaneum* were studied (Zapata & Smagghe, 2010). In another study, 43 essential oils were studied against *Lycoriella ingenua* (Dufour, 1839) adults (Choi et al., 2006). Fumigant toxicity of essential oil of *Lippia origanoides Kunth* against *T. molitor* was investigated in a work (Lima et al., 2011). Also, the fumigant effect of essential oils of nine plant species from Clusiaceae and Asteraceae against *S. granarius* were assessed in another study (Kordali et al., 2012). In our study, it was revealed that *H. perforatum* essential oil has toxicity to *T. molitor*, like these studies. $LC_{50,}$ LC_{90} and LC_{99} values of the essential oil of *H. perforatum* were 3.06, 4.99 and 6.56; 13.3, 23.6 and 32.0; and 8.24, 13.9 and 18.5 µl/L air against adults, pupae and larvae of *T. molitor,* respectively. These values indicate that *H. perforatum* may be an alternative biopesticide against *T. molitor.* Similar results to our study were obtained in previous studies on different target insects. The insecticidal effects of *Hypericum* spp. have been assessed against *T. castaneum* (Parchin & Ebadollahi, 2016), *C. pipiens* (Rouis et al., 2013), *S. granarius* (Kordali et al., 2012) and *M. sexta* (Samuels & Knox, 1989). Despite these results Dastagir et al. (2016) reported that *H. perforatum* did not show cytotoxic, insecticidal and antibacterial activity in vitro at different doses. However, it is possible that the doses applied in that study were too low.

The major component of *H. perforatum* was found to be as α-pinene (51.2%). Also, Çakir et al. (1997) was found that α-pinene is main compound (61.7%) which found in *H. perforatum* essential oil. α-pinene is an organic compound of the terpene class and monoterpenoids were known to have insecticidal activity. In a previous study, monoterpenes were described to have an insecticidal effect on some substantial pests (Lee et al., 1997). So, α-pinene may have caused the mortalities seen in this study. The results show that essential oils of both plants were toxic to all stages of *T. molitor*. Data of this study showed that the adults of *T. molitor* was more sensitive to essential oil than other stages (LC_{99} 6.56 µl/L air). Pupae of *T. molitor* was found to be more tolerant than other stages (LC_{99} 32.0 µl/L air). We obtained complete mortality by vapor of *H. perforatum* essential oil in 6, 15 and 30 µl/L air for 24 h against adults, larvae and pupae of *T. molitor,* respectively. Gözek (2007) demonstrated that adult and larval stages of *Tribolium confusum* Jaquelin Du Val, 1868 (Coleptera: Tenebrionidae) were the most tolerant stages to treatments of garlic essential oil. On the other hand, Sümer Ercan et al. (2013) found that adult of *Ephestia kuehniella* Zeller, 1879 (Lepidoptera: Pyralidae) was the most sensitive stage to exposure of *Prangos ferulaceae* Lindl. essential oil. The larval stage of *E. kuehniella* was more tolerant than the egg stage after exposure to essential oils of *Thymus argaeus* Boiss. & Balansa and *Thymus sipyleus* Boiss. (Ercan et al., 2018). So, the effectiveness of different essential oils may differ between different stored product pests.

MDA is the major product of polyunsaturated fatty acid peroxidation process. It indicates the level of lipid peroxidation (LPO) and is used as a marker of oxidative stress (Büyükgüzel et al., 2010; Baş & Kalender, 2016). In this research, oxidative stress evidenced by increased MDA level might be related to antioxidant enzyme activity reduction. Antioxidant enzymes such as CAT, GPx, GST and SOD are substantial cell protectors against oxidative stress caused damage (Baş & Kalender, 2011; Messarah et al., 2012). SOD enzyme is responsible for superoxide radical dismutation into H_2O_2 and O_2. CAT is responsible for catalyzing H_2O_2 conversion into H_2O and O_2 (Büyükgüzel & Kalender, 2009). The role of GPx enzyme is preventing oxidative damage of cell membranes by catalyzing H_2O_2 conversion to H_2O (Baş & Kalender, 2011; Messarah et al., 2012). GST is one of the major antioxidant enzymes, it has important

roles in oxidative damage inhibition via detoxifying LPO products (Büyükgüzel & Kalender, 2009). There are many studies that investigated antioxidant enzyme activities and MDA levels for examining degree of oxidative stress in insect tissues (Büyükgüzel & Kalender, 2007; Büyükgüzel et al., 2010; Aslanturk et al., 2011). Our research demonstrated a significant decrease in SOD, CAT, GST and GPx activities in all of the examined developmental stages of *T. molitor*. Changes reported in this study on antioxidant enzyme activities may be due to the reactive oxygen species generation. Moreover, some components of essential oils can show neurotoxic effects on pests and monoterpenes can be act as competitive inhibitors of AChE in insect tissues (Kostyukovsky et al., 2002). In this research *H. perforatum* essential oil caused decreasing of AChE activity. A previous study suggested that AChE enzyme activity may be decreased by essential oil exposing (Polatoğlu et al., 2016). Mortalities of *T. molitor* may be observed by these changes on activities of antioxidant enzymes and AChE and MDA values.

Conclusions

This is the first report on the insecticidal fumigant activity of *H. perforatum* essential oil against different developmental stages of *T. molitor.* This study showed that mortality rates and MDA levels were increased, and CAT, GST, SOD and GPx activities were decreased by the essential oil. The tested doses of essential oil gave complete mortality of pupae, larvae and adults of *T. molitor*. The results support the hypothesis that *H. perforatum* essential oil causes oxidative stress and induces LPO process. So, *H. perforatum* essential oil has been demonstrated to have toxicity to *T. molitor* and could be used as an alternative to synthetic chemical control of this insect. The use of *H. perforatum* essential oil may be a more healthy and reliable method for controlling insects.

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