© by PSP Volume 25 – No. 7/2016, pages 2620-2627 Fresenius Environmental Bulletin

INSECTICIDAL EFFECTS OF ESSENTIAL OILS OBTAINED FROM SIX PLANTS AGAINST *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE), A PEST OF COWPEA (*VIGNA UNGUICULATA*) (L.)

Ayse Usanmaz Bozhüyük[1], Saban Kordali[2], Memis Kesdek[3], Mahmut Alper Altinok[4], Murat Varcin[5] and Mehmet Ramazan Bozhüyük[6]

[1]Department of Plant Protection, Agriculture Faculty, Iğdir University, 76100, Iğdir-Turkey
[2]Department of Plant Protection, Agriculture Faculty, Atatürk University, 25240, Erzurum-Turkey
[3]Department of Environment Protection Technologies, Fethiye Ali Sıtkı Mefharet Koçman Vocational High School, Muğla Sıtkı Koçman University, 48300, Fethiye, Muğla-Turkey
[4]Department of Plant Protection, Seyrani Agriculture Faculty, Erciyes University, Develi, Kayseri-Turkey
[5]Graduate School of Natural and Applied Sciences, Ecological Science, Muğla Sıtkı Koçman University, 48000, Muğla-Turkey
[6]Department of Horticulture, Agriculture Faculty, Atatürk University, 25240, Erzurum-Turkey

ABSTRACT

Callosobruchus maculatus (F.), cowpea seed beetle (Coleoptera: Bruchidae) is one of the most serious pests of cowpea (the black-eyed pea) grains, worldwide. In this study, essential oils of *Artemisia dracunculus* L., *Artemisia santonicum* L., *Artemisia spicigera* C. Koch, *Origanum onites* L., *Satureja thymbra* L. and *Thymus sipyleus* Boiss. were tested for their insecticidal activities against three day-old adults of *C. maculatus* at 25±2 °C, 65±5 % r.h. in dark conditions**,** at different exposure times (12, 24, 48 and 72 h), and doses (5, 7.5 and 10 µl). It was found that there were the mortalities in all exposure doses and durations. The percentage mortality of adults of *C. maculatus* increased with increasing the concentration of different oils and exposure times. Minimum mortality rate in *C. maculatus* adults (16.6%) was recorded at 12 h with 5 µl essential oil of *O. onites*, while the peak mortality was registered as 100%, at 72 h with 10 µl essential oil of *A. dracunculus*. Additionally, LD_{25}, LD_{50} and LD_{90} values of each essential oil were estimated for *C. maculatus*. Results suggested that the essential oils from the tested plants could be used as potential control agents against *C. maculatus* adults in stored cowpea (the black-eyed pea) protection.

KEYWORDS:
Callosobruchus maculatus, cowpea seed beetle, essential oils, insecticidal effect, mortality percentage.

INTRODUCTION

In recent years, scientists have focused on increasing food production for the need of the rapidly expanding population of the world. Unfortunately, crop loss is still keeping on due to plant diseases, caused by insects, plant pathogen fungi, bacteria and viruses. *Callosobruchus maculatus* (F.), cowpea seed beetle (Coleoptera: Bruchidae), is one of the most important pests of cowpea (the black-eyed pea) grains in the cowpea storages. This pest requires great attention because it is very fairly distributed throughout the tropical and sub-tropical regions of the world. It feeds on cowpea (*Vigna unguiculata* (L.)), also chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), soybean *(Glycine max* Mer.) and haricot beans (*Phaseolus vulgaris* L.). The leguminous plants are a very important source of vegetable protein for millions of people around the world, especially in tropical and subtropical regions. Cowpea seeds have seriously affected by *C. maculatus* and cause maximum damage of 2 to 5 kg seeds with in 45 to 90 days in optimum temperature (30^0±1 ^{0} C) and moisture conditions (75 ± 3 %) [29]. In the past, many residual insecticides such as malathion and pirimiphosmethyl were largely used to control this pest in stored cowpea. However, these chemicals caused hazardous effects such as environmental pollution, toxicity to non-target organisms, pest resistance, pesticide residues, direct toxicity to users and also ozone depletion [1, 16, 41]. However, the natural products are relatively less damaging to environment and mammalian health as compared with synthetic chemicals. Although many studies reported to insecticidal activity of plant essential oils [3, 41, 24, 8, 19, 6, 45, 32], there are few reports to insecticidal properties of essential oils against *C. maculatus* [40, 33, 20].

Essential oils are genuine plant extracts that contain natural flavours and fragrances grouped as monoterpenes (hydrocarbons and oxygenated derivatives), sesquiterpenes (hydrocarbons and oxygenated derivatives) and aliphatic compounds (alkanes, alkenes, ketones, aldehydes, acids and alcohols) that provide characteristic odours. Many essential oils isolated from various plant species belonging to different genera contain a relatively high amount of monoterpenes. Insecticidal properties of numerous essential oils and some monoterpenes have been extensively studied against various insect species [12, 27, 28, 37, 17, 22, 36, 2, 35, 23].

The genus *Artemisia* is an important genus of the family Asteraceae, which comprises about 1000 genera and over 20,000 species widespread throughout the world. Among them, about 500 species of the genus *Artemisia* are found in Asia, Europe and North America. There are *Artemisia dracunculus* L. and *Artemisia santonicum* L. species of *Artemisia* genus in Turkish flora. The species of *Artemisia* genus are known in Anatolia as ‘‘Acı pelin”, “Ak pelin”, “Yavşan” , “Deniz yavşanı” and “Acı yavşan”. *Artemisia* species is industrially important due to its insecticidal, antifungal, antibacterial, allelopathic and other properties. This genus is very rich in essential oils and bitter substances, including flavon and pinen [46,40].

The genus *Origanum* (oregano) is an important genus within the Lamiaceae family and comprises about 900 species, widespread throughout the world. There are about 20 species of *Origanum* genus in Turkish flora and this genus is very rich in essential oils and bitter substances [11, 5, 14]. The species of *Origanum g*enus are known in Anatolia as ‘‘Yalancı kekik”, ‘‘Kekik”, ‘‘İstanbul kekiği” and ‘‘Keklik otu”. *Origanum* species are traditionally used as a spicy additive for food flauvoring instead of thyme, as sedative, diuretic, degasifier, sweater and antiseptic, and also in the treatment of gastrointestinal diseases and constipation [5]. There are numerous reports on the chemical composition and their various biological activities of *Origanum* species [13, 44, 7, 46, 21, 14].

The genus *Thymus* and *Satureja*, belonging to Lamiacaeae family, are well known as aromatic and medicinal plants and are distributed in northern Anatolia [11, 5]. In Anatolia, *Thymus* L.(thyme) and *Satureja* L.(savory) species are frequently used as herbal tea or additives in commercial spice mixtures of many foods to offer aroma and flavour [5]. The genus *Thymus* L. consists of over 300 evergreen species of herbaceous perennials and subshrubs, native to Southern Europe and Asia [26]. Members of the genus *Thymus* L. are called “kekik” and “sipil kekik” in Turkish and used as herbal tea and condiments. *Satureja* species have been widely used as folk medicines and locally known as “sivri kekik” in Turkey. *Satureja* species are widely grown in Mediterranean region of the world. In Turkey, there are 15 *Satureja* species and five of them are endemic. Among them, the *Satureja thymbra* are consumed as species or herbal teas by the local people [43, 5].

Turkey flora is characterized by the abundance of aromatic plants among its components. The feature differentiating these plants from all others, although they belong to many different families, is the production of chemically related secondary compounds, the low molecular weight and volatile isoprenoids. This remarkable presence of aromatic species is important in determining the candidates with insectidal potential within this ecosystem.

Thus, the aim of the present study was to assess insecticidal effects of the essential oils isolated from six plant species, *Artemisia dracunculus, Artemisia santonicum, Artemisia spicigera, Origanum onites, Satureja thymbra* and *Thymus sipyleus* in different localities of Turkey against three day-old adults of *Callosobruchus maculatus*, the cowpea seed beetle.

MATERIALS AND METHODS

Insect material. *Callosobruchus maculatus* adults were collected from private store houses in Bozkurt/Denizli, Turkey and kept on cowpea (the black-eyed pea), (*Vigna unguiculata*) seeds. The cultures were maintained in Deparment of Environment Protection and Technologies, Fethiye Ali Sıtkı Mefharet Koçman Vocational School, Sıtkı Koçman University, Fethiye, Muğla, Turkey. In addition, the cowpea seeds were purchased from a local market and maintained in a freezer at −15˚C in order to control any arthropod pests prior to use for bioassay during two days. After, *C. maculatus* adults were reared in 1 L jars containing cowpea seeds. The cultures were maintained in the dark conditions in a growth chamber set at 25±2 ˚C and 65±5% r.h. without exposure to any insecticide for several generations. Adult insects, three day-old, were used for the fumigant toxicity test. All experimental procedures were carried out under the same environmental conditions as the cultures.

Bioassays. In order to test the toxicity of the oils from six different plants, 20 individuals of *Callosobruchus maculatus* adults (all three-day old) were used. Cowpea seeds were placed in each of Petri dishes (9 cm x 1.5cm). Adults of *C. maculatus* in the Petri dishes were exposed separately to essential oils of *A. dracunculus, A. santonicum, A. spicigera, O. onites, S. thymbra* and *T. sipyleus.*

The amounts of essential oils were applied at rates of 5, 7.5 and 10 µl corresponding to 38.46, 50.6 and 76.92 µl/L air oils impregnated into Whatman no. 1 filter paper, which was stuck onto the inner top of the Petri dishes. A filter paper was placed at the bottom of each Petri dish (9cm×1.5cm deep) and 20 adults of *C. maculatus* were placed onto filter paper containing 20 g cowpea (*Vigna unguiculata*) seeds. This prevented direct contact between the oils and *C. maculatus* individuals. The Petri dishes were covered with the lid and transferred to an incubator, and then kept under standard conditions of 25±2 °C, 65±5 r.h. and in the darkness for two days. Mortalities of the adults were then counted at 12, 24, 48 and 72 h. A Petri dish treated with only sterile water was used as control. Each assay was repeated three times for each dose and exposure time combination, and insecticidal activity of the oils was expressed as percent mean mortality of the adults.

Plants And Essential Oils. *Artemisia dracunculus* L., *Artemisia santonicum* L., *Artemisia spicigera* C. Koch, *Origanum onites* L., *Satureja thymbra* L. and *Thymus sipyleus* Boiss. (Lamiaceae) were collected at the flowering stage from different localities of Turkey between June and August of 2013 and 2014. Voucher specimens have been deposited in the herbarium of Ataturk University, Faculty of Agriculture, the Department of Plant Protection, Erzurum, Turkey. Aerial parts of the plants were dried in shade and ground in a grinder. The dried plant samples (500 g) were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. The oil yields of *A. dracunculus*, *A. santonicum*, *A. spicigera*, *O. onites*, *S. thymbra* and *T. sipyleus* were 1, 0.21, 0.25, 4.5, 1.17 and 0.98 %(w/w, dry weight basis), respectively. The yield was based on dry materials of plant samples. The essential oils were stored in a freezer at 4 °C for further tests.

Statistical analysis. The differences among the insecticidal activity of tested essential oils were determined according to analysis of variance (ANOVA) test contained in SPSS 17.0 software package. Differences between means were tested through Duncan tests and values with $p<0.01$ were considered significantly different.

TABLE 1
Values followed by different letters in the same column differ significantly at p<0.01 according to Duncan multiple test.
Mean±SE of three replicates, each set up with 20 adults.

Treatment Essential oils	Dose(µl/l)	Mortality(%) Exposure time(h) 12	24	48	72
Artemisia dracunculus	5	31.6 ± 1.66 fg	76.6 ± 1.66 fgh	88.3 ± 3.33 efg	95.0 ± 2.88 def
	7.5	35.0 ± 2.88 gh	78.3 ± 1.66 gh	90.0 ± 2.88 fg	96.6 ± 1.66 ef
	10	38.3 ± 1.66 hı	81.6 ± 1.66 h	95.0 ± 0.0 gh	100.0 ± 0.0 f
Artemisia santonicum	5	20.0 ± 2.88 bc	58.3 ± 1.66 d	68.3 ± 1.66 b	90.0 ± 2.88 cd
	7.5	21.6 ± 1.66 bcd	63.3 ± 1.66 d	76.6 ± 3.33 c	95.0 ± 2.88 def
	10	30.0 ± 2.88 efg	73.3 ± 1.66 efg	90.0 ± 2.88 fg	98.3 ± 1.66 ef
Artemisia spicigera	5	26.6 ± 1.66 def	70.0 ± 2.88 e	81.6 ± 1.66 cde	93.3 ± 1.66 cde
	7.5	35.0 ± 2.88 gh	78.3 ± 1.66 gh	86.6 ± 1.66 def	95.5 ± 2.88 def
	10	41.6 ± 1.66 ı	81.6 ± 1.66 h	93.3 ± 1.66 fgh	98.3 ± 1.66 ef
Origanum onites	5	16.6 ± 1.66 b	41.6 ± 1.66 b	68.3 ± 1.66 b	88.3 ± 1.66 bc
	7.5	25.0 ± 2.88 cde	46.6 ± 1.66 bc	65.0 ± 2.88 b	85.0 ± 2.88 b
	10	26.6 ± 1.66 def	51.6 ± 1.66 c	70.0 ± 2.88 b	93.3 ± 1.66 cde
Satureja thymbra	5	25.0 ± 2.88 cde	70.0 ± 2.88 e	81.6 ± 3.33 cde	93.3 ± 1.66 cde
	7.5	26.6 ± 3.33 def	76.6 ± 1.66 fgh	86.6 ± 1.66 def	95.0 ± 2.88 def
	10	30.0 ± 2.88 efg	78.3 ± 1.66 gh	91.6 ± 1.66 fgh	98.3 ± 1.66 ef
Thymus sipyleus	5	21.6 ± 1.66 bcd	71.6 ± 1.66 ef	80.0 ± 2.88 cd	93.3 ± 1.66 cde
	7.5	30.0 ± 2.88 efg	78.3 ± 1.66 gh	93.3 ± 1.66 fgh	98.3 ± 1.66 ef
	10	31.6 ± 3.33 fg	73.3 ± 4.4 efg	88.3 ± 4.40 efg	98.3 ± 1.66 ef
Positive Control (Izoldesis)	10	86.6 ± 1.66 j	91.6 ± 1.66 ı	98.3 ± 1.66 h	100.0 ± 0.0 f
Control (Sterile water)	-	0.0 ± 0.0 a	1.67 ± 1.39 a	3.10 ± 1.43 a	4.44 ± 0.0 a

RESULTS AND DISCUSSION

In the present study, the toxicity effects of essential oils isolated from *A. dracunculus*, *A. santonicum*, *A. spicigera*, *O. onites*, *S. thymbra* and *T. sipyleus* were tested against adults of *C. maculatus* at the 5, 7.5 and 10 µl doses and at 12, 24, 48 and 72 h exposure times (Table 1; Figure 1, 2, and 3). All essential oils obtained from *A. dracunculus*, *A. santonicum*, *A. spicigera*, *O. onites*, *S. thymbra* and *T. sipyleus* were displayed toxicity against adults of *C. maculatus* in comparison to control, but the effects of these essential oils varied among each plant species. However, the mortality rates increased with increasing doses and exposure times for essential oils of tested plant species (Table 1; Figure 1, 2 and 3). The highest mortality rate (100%) was observed in the 10 µl dose at an exposure time of 72 h with the essential oil of *A. dracunculus*, while the lowest mortality rate (16.6%) was obtained in the 5 µl at an exposure time of 12 h with the essential oil of *O. onites*. In general, all essential oils caused over 90% mortality rate (except 5 and 7.5 µl doses of *O. onites* oil) after 72 h exposure (Table 1; Figure 1, 2 and 3). The results show that essential oils of *A. dracunculus*, *A. santonicum*, *A. spicigera*, *O. onites*, *S. thymbra* and *T. sipyleus* have a remarkable insecticidal activity on adults of *C. maculatus*. Statistical analysis results indicated that the effects of the essential oil, doses and exposure times on insect mortality were all statistically very significant ($p < 0.01$) (Table 1).

The lowest mortality rate was fixed as 16.6% in the 5 µl dose after 12 h treatment with essential oil of *O. onites* on the adults of *C. maculatus*, while the highest mortality rate was determined as 41.6% in the 10 µl dose for the essential oil of *A. spicigera* (Table 1; Figure 1, 2 and 3). However, the minimum mortality rate after 24 h treatment with essential oil of *O. onites* was acquired as 41.6% (in the 5 µl), but the maximum mortality rate was observed as 81.6% in the 10 µl doses for the essential oils of *A. dracunculus* and *A. spicigera* (Table 1; Figure 1, 2 and 3). Similarly, the least mortality rate 48 h after the treatments at was counted as 65.0% for *O. onites* essential oil (in the 7.5 µl), whereas the highest mortality rate was observed as 95.0% for essential oil of *A. dracunculus* (in the 10 µl) (Table 1; Figure 1, 2 and 3). In addition to these, the highest mortality rate at 72nd h after the treatment was determined as high as 100%, with the 10 µl dosage of *A. dracunculus* oil. But, the least mortality rate was found as 85.0% for *O. onites* oil (in the 7.5 µl). Besides, the mortality rates after a 72 h exposure were observed between 90% and 100% for all essential oils of all plants (with the exception of *O. Onites*, 85% for the 7.5 µl and 88.3% for the 5 µl) (Table 1; Figure 1, 2 and 3). In comparison with all essential oils of the tested plants, the highest mortality rates were observed with essential oil of *A. dracunculus* in all doses (5, 7.5 and 10 µl) and at all exposure times (12, 24, 48 and 72 h) on the adults of *C. maculatus*, while the essential oil of *O. onites* yielded the lowest mortality rates, among others (Table 1; Figure 1, 2 and 3). According to the test results, it was determined that *A. dracunculus* essential oil had an important insecticidal effect against adults of *C. maculatus*. However, the mortality rates were found to be 100% at the longest exposure period and with the maximum dose in the positive control (Table 1).

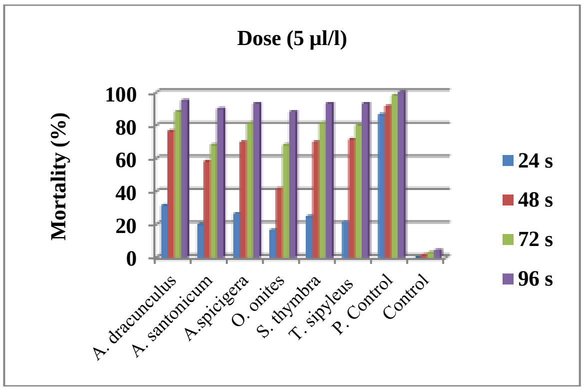

FIGURE 1
Percent mortality of adults of *Callosobruchus maculatus* after treatment with essential oils and treatment times in the 5 µl dose. *Statistically significant (p<0.01).

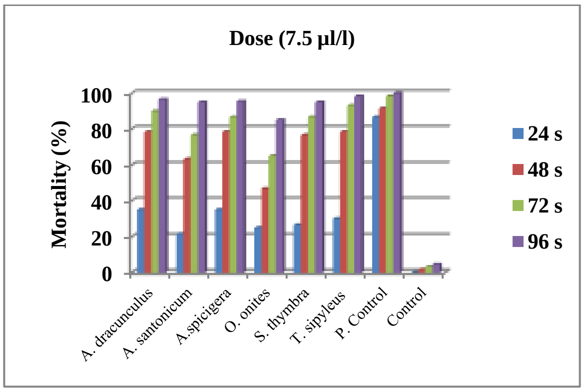

FIGURE 2
Percent mortality of adults of *Callosobruchus maculatus* after treatment with essential oils and treatment times in the 7.5 µl dose. *Statistically significant (p<0.01).

In a former study, it was reported that *A. dracunculus* oil had a toxic effect on adults of *Aphis gossypii* [34]. In another research, it was determined that the essential oil from *A.*

dracunculus had the mortality effects both on first instar larvae and eggs of *Plodia interpunctella* [38]. In the current study, it was determined that *A. dracunculus* oil had an insecticidal effect between 31.6% (in the 5 µl dose**,** after 12 h of exposure) and 100% (in the 10 µl dose, after 72 h of exposure) mortality rates on the adults of *C. maculatus* (Table 1; Figure 1, 2 and 3).

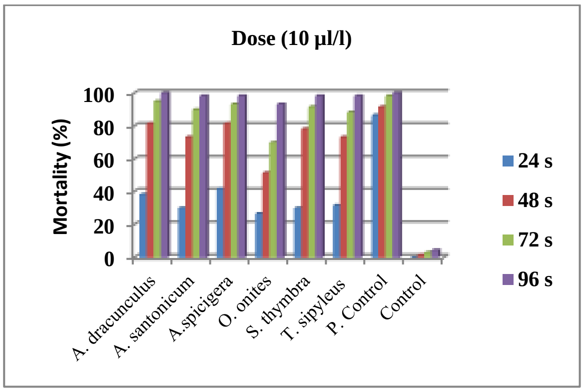

FIGURE 3
Percent mortality of adults of *Callosobruchus maculatus* after treatment with essential oils and treatment times in the 10 µl dose. *Statistically significant (p<0.01).

It had been previously stated that the essential oil recovered from *A. santonicum* had a toxic effect on *Sitophilus granarius* and showed about 80–90% mortality at a dose of 9 µl/l air after 48 h of exposure [23]. Similarly, it was indicated that essential oil of *A. santonicum* had an insecticidal effect on adults of *Sitophilus zeamais* and after 96 h of exposure, in the 20 µl/l air occured up to 100% mortality against this pest [25]. The present study proved that the essential oil of *A. santonium* had a toxic effect in all exposure times and at treatment doses, mortality rates of the adults of *C. maculatus* varied between 20.0% (in the 5 µl dose after 12 h exposure) and 98.3% (in the 10 µl dose after 72 h exposure) (Table 1; Figure 1, 2 and 3).

It was reported that essential oil of *A. spicigera* had a toxic effect against the larvae of *Dendroctonus micans*, the mortality of the larvae of *D. micans* increased significantly after treatment with the essential oil [15]. In another study, it was found that the essential oil of *A. spicigera* had an insecticidal effect on *Sitophilus granarius* [23]. In the current study, it was determined that *A. spicigera* essential oil lead to a minimum of 26.6% (in the 5 µl dose after 12 h of treatment) and a maximum 98.3% mortality (in the 10 µl dose after 72 h of treatment) of adults of *C. maculatus* (Table 1; Figure 1, 2 and 3).

In a previous study, it was stated that the essential oil of *O. onites* had a larvicidal effect on 4th and 5th larval instars of *Thaumetopoea wilkinsoni* (Çetin *et al*., 2006). Another study with *O. onites* showed that essential oil of *O. onites* was highly toxic against the house mosquito, *Culex pipiens* L. (Diptera: Culicidae) [9]. Also, it was determined that *O. onites* essential oil had 80% larval mortality on the larvae of *Culex pipiens* with concentrations between 50-200 ppm [9]. Likewise, *O. onites* oil resulted up to 100% mortality on the larval instars of *Ephestia kuehniella* and *Plodia interpunctella* [4]. In addition, it was found that the essential oils of *O. onites* had a toxic effect on adults of *Sitophilus granarius* [48]. In the present study, the essential oil of *O. onites* lead to the mortality rates between 16.6% (in the 5 µl dose after 12 h) and 93.3% (in the 10 µl dose after 72 h), on the adults of *C. maculatus* (Table 1; Figure: 1, 2 and 3).

In a previous study, the essential oil obtained from *S. thymbra* caused an insecticidal activity on adults of *Ephestia kuehniella*, *Plodia interpunctella* and *Acanthoscelides obtectus* [4]. Similarly, it was stated that *S. thymbra* oil had a toxic effect on the larvae of *Culex pipiens* [31]. In this study, the essential oil of *S. thymbra* lead to minimum 25.0% mortality (in the 5 µl dose after 12 h of treatment) and maximum 98.3% mortality (in the 10 µl dose after 72 h of treatment) (Table 1; Figure 1, 2 and 3). In some earlier studies, the toxicity tests of *T. sipyleus* oil showed that 10 µl of *T. sipyleus* oil resulted with 73% and 72% mortality against *S. granarius* adults and *Ephestia kuehniella* larvae, respectively [47, 48]. In another study, the essential oil of *T. sipyleus* caused between 90% and 100% mortality in the 10 µl dose after 24, 48, 72 and 96 h on the 2nd, 3rd and 4th larval instars of *T. pityocampa* [18]. Similarly, in the present study, it was determined that essential oil of *T. sipyleus* had an important insecticidal effect in the 10 µl dose and at all exposure times (12, 24, 48 and 72 h) between 31.6% and 98.3% with mortality rates on *C. maculatus* adults (Table 1; Figure 1, 2 and 3).

Furthermore, according to LD values (LD_{25}, LD_{50} and LD_{90}), the lowest LD values of *C. maculatus* was recorded for the essential oils of *O. onites* as 0.064 (LD_{25}) and *S. thymbra* 0.218 (LD_{25}), whereas the essential oils of *A. dracunculus* (LD_{25}) and *A. santonicum* (LD_{25}) had the lowest toxicity as 1.016 and 0.979, respectively (Table 2). However, the lowest LD_{50} value was found for the essential oil of *A. santonicum* as 1.722 against *C. maculatus* adults. Similarly, the least toxicity by means of LD_{90} value was obtained with the essential oil of *O. onites*, as 8.029 (Table 2).

TABLE 2
The LD values of essential oils obtained from six plants against adults of *Callosobruchus maculatus.*

Essential oils	LD_{25}	LD_{50}	LD_{90}	X^2	Slope ± SE
Artemisia dracunculus	1.016	1.639	4.064	4.428	3.250 ± 2.031
Artemisia santonicum	0.979	1.722	5.044	5.238	2.748 ± 1.415
Artemisia spicigera	0.383	0.861	4.024	4.902	1.915 ± 1.444
Origanum onites	0.064	0.340	8.029	3.197	0.933 ± 1.033
Satureja thymbra	0.218	0.569	3.512	5.691	1.621 ± 1.459
Thymus sipyleus	0.577	1.108	3.834	5.205	2.378 ± 1.632

In conclusion, the development and wide use of natural or biological insecticides may help to decrease negative effects of synthetic chemicals such as residues in the stored products, insect resistance and environmental pollution. In this respect, natural insecticides appear as easily bio-degradable solutions with relatively low environmental pollution. In the present study, the essential oils isolated particularly from *A. dracunculus*, *A. santonicum*, *A. spicigera*, *S. thymbra* and *T. sipyleus* were found to be more toxic against *C. maculatus* adults, but *O. onites* oil had a lesser effect. In many cases, their toxicities were also found identical in comparison to the toxicity of current commercial insecticides, widely used as insect reagents to protect the cowpea against adults of *C. maculatus*. Therefore, in the light of the present findings, it can be suggested that these plant essential oils can be used as new and effective insecticidal reagents against adults of *C. maculatus*. However, further studies need to be conducted to evaluate the cost and safety of these reagents, along with the efficiencies under various environmental conditions.

ACKNOWLEDGEMENTS

The authors thank to Mrs. Yeşim Kulapa (9 Eylül University, İzmir, Turkey) for her valuable grammatical improvements on drafts of this manuscript, also to the editors for their critical reviews.

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Received: **16.11.2015**
Accepted: **12.03.2016**

CORRESPONDING AUTHOR

Ayse Usanmaz Bozhüyük
Igdir University Faculty of Agriculture Department of Plant Protection 76000, Igdir, TURKEY

e-mail: ayseusanmaz@hotmail.com