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CHEMICAL COMPOSITION, TOXICITY AND SIDE EFFECTS OF THREE ESSENTIAL OILS ON *BREVICORYNE BRASSICAE* (L.) (HEMIPTERA: APHIDIDAE) ADULTS UNDER LABORATORY CONDITIONS

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Abstract - The insecticidal effects of essential oils namely *Eucalyptus camaldulensis*, *Azadirachta indica* and *Thuja occidentalis* has been studied on *Brevicoryne brassicae* adults as fumigants by determining LC_{50} and LT_{50} values. LC_{50} value of Eucalyptus, Azadirachtin and Northern White Cedar fruit essential oils on cabbage aphid adults were 15.12, 38.79 and 56.02 ml / liter of air, respectively. The LT_{50} value of the three essential oils on cabbage aphid adults were 10.57, 11.90 and 13.86 hours, respectively. Regression analysis showed a significant relationship between log-concentrations and probit of mortality of *T. occidentalis*, *E. camaldulensis* and *A. indica* essential oils with R^2 (0.9995), (0.9779) and (0.9835), respectively. In essential oils of Eucalyptus, Azadirachtin and Northern White Cedar fruit 18, 35 and 22 components were identified by GC-MS analysis. According to the insecticidal properties of the essential oils on the cabbage aphid, the use of these oils as a safe pesticide is recommended.

KEY WORDS: Cabbage aphid, *Eucalyptus*, *Azadirachta*, *Thuja*, Insecticidal effect

Izvleček – KEMIJSKA SESTAVA, TOKSIČNOST IN STRANSKI UČINKI TREH ETERIČNIH OLJ NA ODRASLE MOKASTE KAPUSOVE UŠI (*BREVICORYNE BRASSICAE* (L.)) (HEMIPTERA: APHIDIDAE) V LABORATORIJSKIH RAZMERAH

Insekticidne učinke eteričnih olj vrst *Eucalyptus camaldulensis*, *Azadirachta indica* in *Thuja occidentalis* smo preizkušali na odraslih mokastih kapusovih ušeh (*Brevicoryne brassicae*) kot fumigante z določevanjem vrednosti LC_{50} in LT_{50}. Vrednosti LC_{50} eteričnih olj evkalipta, azadirahtina in plodov ameriškega kleka na mokastih kapusovih ušeh so bile 15,12, 38,79 in 56,02 ml / liter zraka. Vrednosti LT_{50} treh eteričnih olj na odraslih mokastih kapusovih ušeh so bile 10,57, 11,90 in 13,86 ur. Regresijska analiza je pokazala pomembno povezavo med koncentracijo eteričnih olj vrst *T. occidentalis* (0,9995), *E. camaldulensis* (0,9779) in *A. indica* (0,9835) in smrtnostjo. V eteričnih oljih evkalipta, azadirahtina in plodov ameriškega kleka smo z analizo GC-MS določili 18, 35 in 22 sestavin. Glede na insekticidne lastnosti eteričnih olj na mokaste kapusove uši priporočamo uporabo teh olj kot varnih pesticidov.

KLJUČNE BESEDE: mokasta kapusova uš, *Eucalyptus*, *Azadirachta*, *Thuja*, insekticidni učinek

Introduction

Cabbage aphid, *B. brassicae* is one of the most important cabbage pests in Iran especially in the central areas and many other parts of the world that causes considerable damage to the product (Khanjani, 2005; Rivnay, 2013). This aphid has a high reproductive potential and increases its population quickly; resulting in direct damage with the formation of large colonies, feeding on plant sap and causing complexity and deformity of the leaves. On the other hand, with the transfer of plant pathogenic viruses, it can lead to indirect damage (Ellis et al., 2000; Schliephake et al., 2000). Chemical control is an effective and widely used method in pest control (Pavela, 2009). These compounds have adverse effects such as environmental pollution, toxicity to non-target organisms; causing resistance in pests and leaving left overs (Ogendo et al., 2003). Development of alternative strategies for avoiding pesticides to manage phytosanitary problems is an important need mostly for agricultural activity and tendency worldwide is a growing to organic productions (Willer et al., 2010; Marques-Francovig et al., 2014). These problems prompted the researchers to look for alternative and environment-friendly control methods to control the pests (Tapondjou et al., 2005; Laznik et al., 2010; Gombač and Trdan, 2014). The good candidates for the substitution of chemical pesticides are essential oils that many studies and patents for their use have been published in recent years (Isman, 2000; Chiasson et al., 2001). Active ingredients derived from plant extracts and essential oils have fumigation effects on pests (Maciel et al., 2010). The essential oils extracted from aromatic plants, due to the intense aroma and low toxicity for mammals, lack of a significant adverse impact on the environment and acceptance among the general public, are considered very useful compounds for pest control (Isman, 2000). Currently, more than 3000 essences have been identified, of which 300 essential oils and some of their compounds have become commercially important in the pharmaceutical, agricultural, food, health and cosmetics and perfume industries (Bakkali et al., 2008).

Plant essences have repellent (Ogendo et al., 2008), insecticide (Papachristos and Stamopoulos, 2002), fungicide (Kotan et al., 2008), antibacterial (Matasyoh et al., 2007), antivirus properties (Schuhmacher et al., 2003), deter oviposition, stop development (Papachristos and Stamopoulos, 2002) and have anti-nutritional properties (García et al., 2007). There have been many studies in this area, for example, the lethal effect of plant essential oils *Artemisia indica* (Adr. Juss) against the cabbage aphid, *B. brassicae* have been studied (Pavela, 2005). In another experiment, Ebrahimi et al. (2013) showed that essential oil of azadirachtin (*Azadirachta indica* Adr. Juss.), eucalyptus (*Eucalyptus camaldulensis* Dehnh.) and laurel (*Laurus nobilis* L.) has significant lethal effect on cotton aphid; in which azadirachtin and eucalyptus had more of a lethal effect compared with laurel. Since ancient times, the natives of America have used different parts of northern white cedar such as leaves, fruit and bark of the shrub as drug and pesticides and reports of their use have been presented as books or articles during the investigation of researchers over the years (Moussa Kéïta et al., 2001).

Considering the advantages of using compounds of natural origin to control plant pests, the insecticidal effect of three essences of eucalyptus, azadirachtin and northern white cedar fruit on cabbage aphid was investigated.

Materials and methods

Extraction and analysis of essential oil

Fruits of northern white cedar *T. occidentalis* were collected from existing trees in the Entomology area of Plant Protection Department of Agriculture Faculty of Urmia University (latitude 37.53°N, 45.08°E and 1320 m above sea level) in the spring of 2014. To prepare the essential oil, fresh fruits were crushed and 100 g of it was extracted mixed with 700 ml of distilled water at a temperature of 100°C using Clevenger apparatus in 90 minutes. Obtained essences were dehydrated with Rota Evaporator-Buchi (R-3000) to a dark brown color and refrigerated in 2 ml glass containers with aluminum covers till use. Used Eucalyptus *E. camaldulensis* and Azadirachtin *A. indica* essential oils in the tests were purchased and analyzed by Barij Esans pharmaceutical company, Kashan.

Insect rearing

The cabbage aphid rearing was started with aphids collected from gardens planted with cabbage in Urmia University. It was reared on red cabbage plant, in 27 ± 2°C and 65 ± 5% RH under a 16:8 (L:D) photoperiod. Cabbage plants and colony of the aphids were maintained in a greenhouse.

Determination of the 50% Lethal Concentration (LC_{50})

Different concentrations of the essential oils were poured on filter paper in three replications based on Kéita et al. (2001) method and placed on the inside of the 305 ml glass container lids, each containing 20 adult insects with food (red cabbage leaves). Also, in order to prevent direct contact between insects and the essential oil, a net was placed between the lid and container. Container lids were tightened using special tapes (parafilm). In the tests, counting was done after 24 hours. Insects that did not show any movement when nearing the brush were considered dead. Distilled water was used in the control treatment.

Determining the 50% lethal time (LT_{50})

An experiment was designed to determine the median effective time to kill 50% of adults (LT_{50} values) at different concentration of *T. occidentalis*, *E. camaldulensis* and *A. indica* essential oils. The mortality was assessed by direct observation of the insects in 5 times including 2, 7, 12, 18 and 24 hour to obtain the desired LT_{50}. Time-mortality data for each experiment were analyzed with time as the explanatory variable to derive estimated hours for 50% adult mortality.

Data analysis

To determine the LC_{50} from six concentrations (five concentrations and control) after 24 hours of essential oil administration and control mortality correction according to Abbott (1925) formula and to determine the LT_{50}, an oil concentration at various time points (2, 7, 12, 18 and 24 hours) was used and analyzed statistically with Probit program at SPSS (V. 20) software. All tests were conducted by fumigation method. In order to draw the graph and its regression lines, we used the SigmaPlot (V. 12.3) software. The dendrogram similarity scales that are produced by the SPSS (V. 20) software range from zero (most similarity) to 25 (least similarity). The method used was ward's (Ward, 1963). Cluster validity index called Silhouette index is applied to validate the result by MATLAB Software (Rousseeuw, 1987).

Results

According to the gas chromatography (GC/MS) analysis of essential oils, it was determined that *E. camaldulensis* essential oil is composed of 18 compounds, the most important of which are 1,8-cineole (39.91%), Para-cymene (13.98%) and gamma-terpinen (12.25%) (Table 2). *A. indica* essential oil is composed of 35 compounds, the most important ones are Azadirachtin (26.55%), Palmitic acid (18.87) and Deacetylazadirachtinol (17.22%) (Table 1). *T. occidentalis* essential oil is composed of 22 compounds and their most important ones are α-thujene (47.68%) and Fenchone (15.13%) (Table 2).

Regression analysis showed a significant relationship between log-concentrations of *E. camaldulensis* and A. *indica* and *T. occidentalis* essential oils and probit of mortality with R^2 (0.9779, 0.9835 and 0.9995), respectively (Fig. 1). The results showed that the essential oils of eucalyptus, azadirachtin and northern white cedar fruit, controlled adult cabbage aphids well for 24 hours and the used concentration of these essential oils on insects in this test are very low. LC_{50} values of the essential oils were equal to 15.12, 38.79 and 56.02 ml per liter of air, respectively (Table 3). The results obtained from the bioassay test of used LC_{50} concentration of eucalyptus, azadirachtin

Table 1. Chemical composition of *A. indica* essential oil by gas chromatography (GS/MS).

No.	Compound	Retention Index	Percentage	No.	Compound	Retention Index	Percentage
1	α-Cadinol	780	3.12	19	2,3-Butanedithiol	910	0.094
2	β-Pinene	902	2.11	20	Germacrene B	680	3.63
3	Anthracene	1311	0.093	21	α-Cadinene	788	0.18
4	Ethyl butyrate	2165	0.17	22	Azadirachtin	730	26.55
5	dl-limonene	1059	0.211	23	Sabinene	842	0.147
6	Eugenol	651	10.92	24	Isobutyl stearate	764	0.209
7	Phytol	621	10.84	25	α–Terpineol	1386	0.22
8	b-Caryophyllene	1455	0.113	26	Thiophene, 2-methoxy	878	0.078
9	Palmatic acid	1978	18.87	27	Deacetylazadirachtinol	561	17.22
10	Spathulenol	1572	0.172	28	Limonene	1521	0.188
11	Verdiflorol	1991	0.162	29	Lauric acid	1186	0.302
12	1-Octadecanol	1381	0.077	30	Valencene	1772	0.170
13	Carvone	796	0.231	31	α-Methyl-1,4-benzenedimethanol	926	0.096
14	Methyl stearate	1422	0.151	32	Aristolene	1561	0.201
15	β-Germacrene	1621	0.088	33	Cadalene	1569	0.108
16	Ethyl linoleate	2108	0.105	34	Ethyl palmitate	1822	0.076
17	1,2,4-Trithiolane, 3,5-diethyl	1359	0.11	35	Other Compounds	-	2.608
18	Pentacosane	652	0.38				

and northern white cedar fruit essential oils at 3, 6, 12, 18 and 24 hours showed that eucalyptus oil at a concentration of 15.12 ml per liter of air at 10.57 hours, azadirachtin oil at a concentration of 38.79 ml per liter of air at 11.90 hours and northern white cedar fruit oil at the concentration of 56.02 ml per liter of air at 13.86 hours caused the death of 50% of adult cabbage aphids (Table 4).

Component analysis and hierarchical cluster

In order to study the likeness and relationship between essential oils (EO) composition of the previously reported samples and our studied oils, hierarchical cluster analysis (HCA) and component analysis were carried out based on similar components

Table 2. Chemical composition of *T. occidentalis* and *E. camaldulensis* essential oils by gas chromatography (GS/MS).

T. occidentalis				*E. camaldulensis*			
No.	Compound	Retention Index	Percentage	No.	Compound	Retention Index	Percentage
1	α-pinene	1004	3.14	1	α-pinene	945.35	7.45
2	4-terpineol	1548	2.03	2	α-thujene	934.88	1.3
3	α-terpinene	1149	0.30	3	Trans-Geraniol	1260.66	0.28
4	Linalool	1506	0.17	4	Valencene	1474.60	2.11
5	Camphene	1041	3.38	5	α-copaene-8-ol	1620	4.80
6	α-terpineol	1642	0.45	6	β-pinene	989.92	0.70
7	Limonene	1167	1.97	7	β-Myrcene	994.19	1.32
8	*β*-thujone	1387	8.51	8	Viridiflorol	1628.82	5.12
9	bornyl acetate	1526	2.74	9	α-Ionone	1365	0.90
10	Sabinene	1094	4.19	10	α-Terpinene	1027.68	0.25
11	*p*-cymene	1231	1.44	11	1,8-cineole	1050.83	39.91
12	Fenchone	1345	15.13	12	a-phellandrene	1015.29	1.22
13	1,8-Cineole	1450	2.06	13	terpinen-4-o1	1196.04	3.59
14	α-fenchene	1034	1.90	14	alloAromadendrene	1497.35	0.72
15	α-thujone	1373	47.68	15	α-terpineol	1207.11	2.17
16	Myrcene	1139	1.12	16	p-cymene	1039.67	13.98
17	Thymol	2110	0.27	17	γ-terpinene	1071.90	12.25
18	γ-terpinene	1210	1.11	18	Other Compounds	-	1.93
19	Borneol	1644	0.39				
20	β-pinene	1079	0.21				
21	α-terpinyl acetate	1640	1.43				
22	Terpinolene	1246	0.18				

of reported EO in the papers. Due to lack of enough similar compounds for *A. indica*, generating dendrogram was infeasible for it. The dendrogram for the HCA results using Ward´s clustering algorithm for *E. camaldulensis* is shown in Figure 2. According to the Silhouette index the best clustering for *E. camaldulensis* is five clusters (Table 5). In the đrst group (Cluster I), represented by six samples, α-pinene and terpinen-4-01 were the main components (Pappas and Sheppard-Hanger, 2000; Chalchat

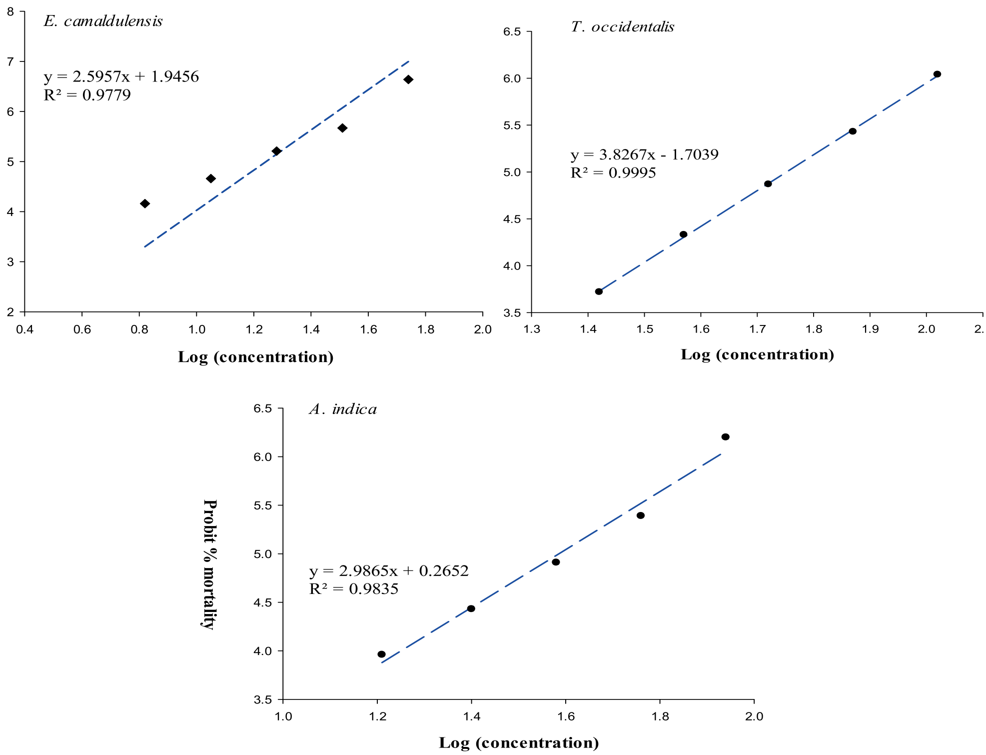

Fig 1. The relationship between log concentration of three essential oils and probit of percentage mortality after 24 hours.

et al., 2001; Verdeguer et al., 2009; Grbović et al., 2010; Khubeiz et al., 2016; Knezevic et al., 2016). Cluster II with one sample has α-pinene and *p*-Cymene as the major compounds (Cheng et al., 2009). Cluster III included two samples with high β-Pinene (Oyedeji et al., 2000; Coffi et al., 2012). Our studied EO formed an individual group from the previous reports, characterized by 1,8-cineole (Cluster IV). In Cluster V with one sample, 1,8-cineole was the main component (Faria et al., 2011).

Table 3. Estimated values of LC_{50} and LC_{95} of *E. camaldulensis*, *A. indica* and *T. occidentalis* essential oils on adult cabbage aphid after 24 hours.

LC_{95}(µL/L air)	LC_{50}(µL/L air)	χ^2(df=3)	Intercept(a)+5	Slope±SE	Total insect	Plant species
68.91 (51.88-105.24)	15.12 (12.89-17.52)	1.64	2.06	0.28±2.50	300	*E. camaldulensis*
138.97 (106.86-207.49)	38.79 (34.29-44.01)	1.24	0.28	0.34±2.97	300	*A. indica*
150.62 (122.98-203.98)	56.02 (50.88-61.94)	0.05	-1.69	0.42±3.83	300	*T. occidentalis*

Table 4. Estimated values of LT_{50} and LT_{95} of *E. camaldulensis*, *A. indica* and *T. occidentalis* essential oils on adult cabbage aphid.

LT_{95}(h)	LT_{50}(h)	χ2(df=3)	Intercept(a)	Slope±SE	Total insect	Plant species
38.16 (20.84-471.56)	10.57 (5.89-18.24)	12.41	1.98	0.30±2.95	300	*E. camaldulensis*
40.60 (31.10-57.26)	11.90 (10.49-13.53)	7.35	1.68	0.32±3.09	300	*A. indica*
47.69 (36.76-70.29)	13.86 (12.22-15.86)	4.19	1.5	0.34±3.07	300	*T. occidentalis*

Table 5. The result of average Silhouette index for *E. camaldulensis*.

Cluster	2	3	5
Index	0.7029	0.7999	0.9105

The Fig. 3 dendrogram presents the results from *T. occidentalis*. The dendrogram was divided into three groups, according to the Silhouette index (Table 6). Our study with one sample was the first cluster in the dendrogram with α-thujone as the main component (Hosseinzadeh et al., 2014). Cluster II is divided into two groups (Cluster II 1-2). Cluster II-1 included the ones with high α-thujone and β-thujone (Akkol et al., 2015; Jasuja et al., 2015); while Cluster II-2 with one sample with α-thujone and sabinene as the major constituents (Lis et al., 2016). Cluster III with one sample had α-thujone and β-thujone as the main compounds (Szołyga et al., 2014).

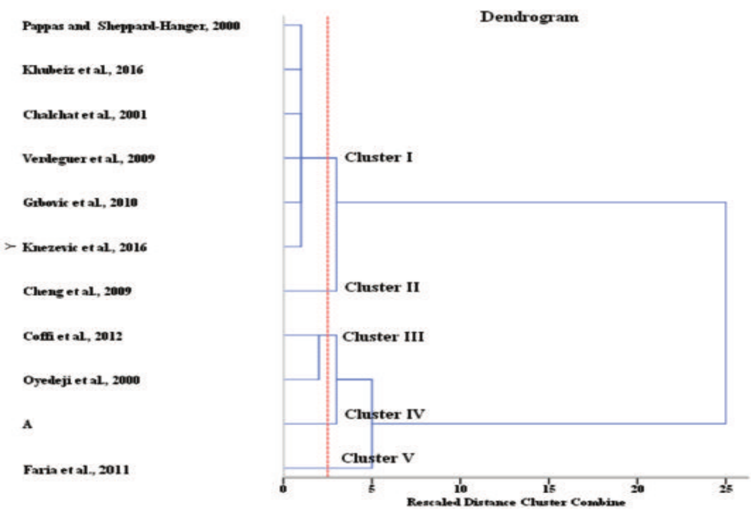

Fig 2. Dendrogram generated of cluster analysis from *E. camaldulensis* EOs based on the chemical compounds of the investigated sample (A) and those from the articles.

Table 6. The result of average Silhouette index for *T. occidentalis*.

Cluster	2	3	4
Index	0.7029	0.7999	0.9105

Discussion

Aphid control is typically done using three main categories of pesticides containing organophosphates, carbamates and pyrethroids. Long-term use of these pesticides has caused resistance in aphids and made their control difficult. Usage of essential oils to control aphids is essential due to increased reports of pest resistance to chemical pesticides and remainder of these toxins in products and environmental pollution (Sadeghi et al., 2009).

The major components of *T. occidentalis*, *E. camaldulensis* and *A. indica* essential oils in our research were the same as in previous studies and differences between this analysis and other works can be related to the time and place of the plant harvested that might influence the chemical composition of the plant essential oil (Kurose and Yatagai, 2005; Tsiri et al., 2009; Ashraf et al., 2010; Alzogaray et al., 2011; Szołyga et al., 2014).

In this study, the insecticidal properties of three essential oils of eucalyptus (*E. camaldulensis*), azadirachtin (*A. indica*) and northern white cedar fruits (*T. occidentalis*) have been studied on cabbage aphid. The results of this study show that these essential oils have a lethal effect on the tested pest and mortality rate increased with increasing concentration of oil. In recent years, extensive surveys have been carried out in order to verify the insecticidal properties of essential oils and their compounds on various pests and a number of them have had favorable effects. For example, Mareggiani et al.

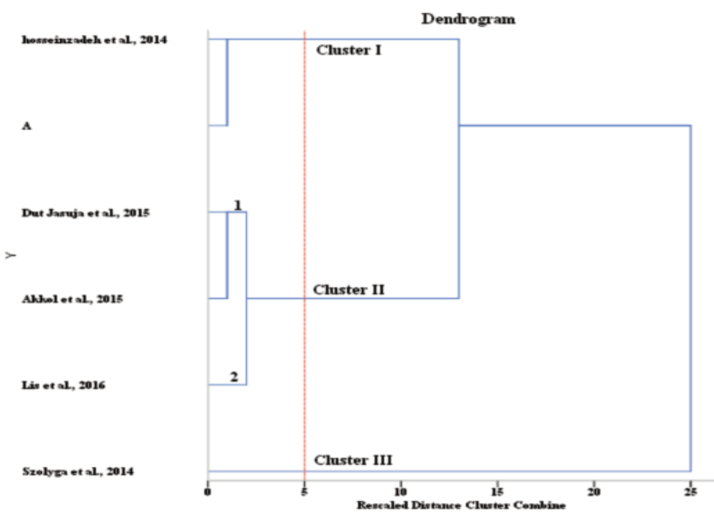

Fig. 3. Dendrogram generated of cluster analysis from *T. occidentalis* EOs based on the chemical compounds of the investigated sample (A) and those from the articles.

(2008) proved the high insecticidal activity of *Eucalyptus globules* essential oil against cotton aphid. In this regard, Ebrahimi et al. (2013) tested the plant essence of azadirachtin (*A. indica*), eucalyptus (*E. camaldulensis*) and laurel (*L. nobilis*) to control cotton aphid and concluded that azadirachtin and eucalyptus had more of a lethal effect than laurel. Also, Kraiss and Cullen (2008) studied the insecticide effect different formulations of essential oil of azadirachtin on bean aphid nymphs *Aphis glycines* Matsumura and came to the conclusion that the essence has had a high controlling effect on the pest. The findings of this study correspond with the results of the experiments done by Mareggiani et al. (2008), Ebrahimi et al. (2013) as well as Kraiss and Cullen (2008), stating that essential oils of eucalyptus and Azadirachtin show a significant lethal effect on aphids from the family Aphididae. In another experiment, controlling effect of azadirachtin and eucalyptus leaf powder on bean beetle was studied and the results showed that they have significant insecticidal and egg-killing effect (Javaid and Mpotokwane, 1997). Also Moussa Kéïta et al. (2001) proved the insecticidal effect of northern white cedar fruit essential oil with kaolin powder on the eggs and adults of bean beetle, *Callosobruchus maculatus* F. The results of these two studies are consistent with the results of this research on the toxicity of eucalyptus, azadirachtin and northern white cedar fruit on pests. In an experiment, Işık and Görür (2009) proved the effect of plant essential oils against the cabbage aphid (*B. brassicae*). Also, Pavela (2005) reported the lethal effect of *Artemisia indica* plant essential oil against the cabbage aphid (*B. brassicae*) and insecticidal activity of both laurel (*L. nobilis*) and eucalyptus (*E. camaldulensis*) essential oil on this pest (*B. brassicae*), respectively. Therefore, the obtained results by this research correspond with the findings of these researchers on insecticidal activity of essential oils such as eucalyptus on cabbage aphid. According to recent findings, various studies have been done on the insecticidal activity of essential oils on various species of aphids from the family Aphididae including the insecticidal effect of 23 plant essential oils and their main compounds against adult turnip aphid (*Lipaphis pseudobrassicae* Davis) (Sampson et al., 2005), respiratory toxicity of 12 Mediterranean species essential oils against pea aphids (*Acyrthosiphon pisum* Harris) and green peach aphid (*Myzus persicae* Sulzer) (Digilio et al., 2008), intense insecticidal activity of a number of plant essences against foxglove aphid (*Aulacorthum solani* Kalt.) (Gorski and Tomczak, 2010).

The results of this study indicate the high insecticidal activity of these essential oils on adult cabbage aphid. Therefore, a place can be reserved for these essences in pest control programs for the effective control of pests as well as reducing the use of chemical insecticides.

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