

The laboratory estimation of essential oils as biological methods against black cherry aphids *M. cerasi*

M.A.S. Eisa¹, O. Matsera^{1,2*} and Ľ. Cagán¹

¹Plant protection Department, Institute of Agronomical Sciences, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Tr. A. Hlinku, 2, SK94901 Nitra, Slovakia

²Agriculture, Soil Science and Agrochemistry Department, Agronomy and Forestry Faculty, Vinnytsia National Agrarian University, Sonyacha Str., 3, UA21000 Vinnytsia, Ukraine

*Correspondence: matsera.olga.vnau@gmail.com

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Abstract. Plants' essential oils play a considerable role in the plant integrated protection systems against harmful insects. Therefore the aim of our study was to find the potential repellency and toxicity effects of essential oils (EOs) from *Allium sativum* L., *Ocimum basilicum* L., *Lavandula angustifolia* Mill, *Eucalyptus globulus*, Labill and *Curcuma longa* L., and two chemical substances DEET (N, N-diethyl-3-methylbenzamide) and 2-Undecanone on the black cherry aphids *Myzus cerasi*. Repellent peculiarities were evaluated at five different concentrations: 0.3%, 0.6%, 0.9%, 1.2% and 1.5%, with 4 replications at the interim of 15 and 30 minutes for an olfactometer test and mortality was evaluated after 2, 6 and 24 hours for insecticidal activity test. The repellent index was most influenced by the concentration of essential oil; the percentage of influence was 64%. At the same time, the highest repellency index 95% was achieved at a concentration of 1.5% when using essential oils of *A. sativum* and *E. globulus*, as well as when using 2-Undecanone within 30 minutes of waiting. A contact toxicity (mortality) test in Petri dishes showed efficacy for all tested oils and chemicals after a 6-hour treatment period. The lowest concentration that ensured 100% insect mortality was 0.9% when using *E. globulus* essential oil. The lowest lethal concentration (LC₅₀) value of 0.00240 mL/10 mL was obtained with *O. basilicum* oil and the lowest LC₉₈ value of 0.28209 mL/10 mL was obtained with *E. globulus* oil after a 6-hour treatment period.

Key words: plant protection, plants' essential oils, *Myzus cerasi*, olfactometer, repellent peculiarities, toxicity.

INTRODUCTION

Black cherry aphids *Myzus cerasi* (Fabricius, 1775) (Hemiptera: Aphididae) quickly weaken young plants by sucking out their cell sap. At the same time, it also emits poison, due to which the leaves twist, deform and die, the shoots stop growing, and the tops are twisted. Sweet secretions - waste pollutes the leafy surface, disrupts the normal life activity of plants (Thorpe et al., 2020).

Dealing with aphids is extremely difficult: it multiplies rapidly and non-stop. Therefore, we must try to get rid of it as soon as it deprives us of the harvest (Granger, 2008; Musa et al., 2020).

M. cerasi occurs throughout Europe, the Middle East, and across Asia, from India and Pakistan (Mohammad, 2001) to Siberia and the far eastern part of the Palearctic. It has been introduced more recently into Australia, New Zealand and North America (Blackman & Eastop, 1994).

Since all mankind strives for environmental protection in the technologies of plant protection against harmful organisms, which is especially important in the conditions of the modern increase in the use of pesticides (FAO, 2021) and spreading of good preconditions for development of insect resistance to pyrethroids (as main substances for insecticides) (Veromann & Toome, 2011). There is a need to find and further evaluate ecologically safe means of plant protection, in our case, it is the use of essential oils from aromatic and medicinal plants, the study their repellent and toxic properties.

The secondary metabolites produced by plants are essential oils; these oils could be used as effective sources for phytophagous pests' control especially against aphids (Smith et al., 2018).

At a time when research on the effect of essential oils on *Myzus persicae* has received a lot of attention from scientists from various countries (Digilio et al., 2008; Costa et al., 2013; Pinheiro et al., 2013; Wafa et al., 2013; Behi et al., 2017; Khaled et al., 2017; Park et al., 2017; Albouchi et al., 2018; Benelli et al., 2018; Czerniewicz et al., 2018; Pavela, 2018) there is a limited amount of information on the effect of essential oils on *Myzus cerasi*, which prompts us to conduct such studies.

Some studies have been carried out for the control of black cherry aphid. Danelski et al. (2015) has tested, with contrasting results, infusions of horsetail (*Equisetum arvense* L.), garlic (*A. sativum* L.), white mustard (*Sinapis alba* L.) and *Brassica juncea* L., as well as the extract obtained from *Quassia amara* (L.) and azadirachtin. Małgorzata et al. (2019) were studied the effectiveness of basic substances in controlling black cherry aphid (*M. cerasi* F.). For investigation were tested a water extract from dandelion plants (*Taraxacum officinale* F.H. Wigg.), and an extract from neem seeds (*Azadirachta indica* L.). (Jaastad, 2007) founded that rapeseed oil significantly reduced damage by black cherry aphid, (*M. cerasi* F.).

However, essential oils from *Allium sativum* (Mousa et al., 2013), *Curcuma longa* (Damalas, 2011), *Lavandula angustifolia* (Sampson et al., 2005; Attia et al., 2016; Minev et al., 2022), *Ocimum basilicum* (Sampson et al., 2005), *Eucalyptus globulus* (Górski & Tomczak, 2010; Oulebsir-Mohandkaci et al., 2015), according to previous studies, have largely demonstrated their repellent and toxic properties when used against individuals of the superfamily of small herbivorous insects *Aphidoidea*, so these plants' essential oils were chosen by us to investigate the effect of their properties on *M. cerasi* in the laboratory conditions.

Essential oils from plant species *A. sativum*, *O. basilicum*, *L. angustifolia*, *E. globulus* and *C. longa* were tested in the laboratory (Cagań et al., 2022). The most important chemicals in *A. sativum* EO were diallyl disulphide, diallyl trisulphide, and diallyl sulphide, in the case of *O. basilicum* they were estragole (methyl chavicol), linalool, methyl eugenol (eugenyl methyl ether), d-limonene (p-mentha-1,8-diene), 1,8-cineole (eucalyptol), in the case of *L. angustifolia* they were citral, coumarin, eugenol, geraniol, limonene, linalool, in EO from *E. globulus* they were 1,8 cineole,

dipentene, alpha phellandrene, beta pinene and for *C. longa* they were the chemicals phellandrene and turmerone (Eisa et al., 2023).

Since the purpose of the research was to identify the repellent and toxic properties of essential oils on black cherry aphids, we had to solve the following problems:

1. **to determine** the influence of 5 essential oils (*A. sativum* L., *O. basilicum* L., *L. angustifolia* M., *E. globulus* L. and *C. longa* L.) and two chemicals (DEET (N, N-diethyl-3-methylbenzamide) and 2-Undecanone) in 5 concentrations: 0.3%, 0.6%, 0.9%, 1.2%, 1.5% on the movement of black cherry aphid *Myzus cerasi* in the olfactometer (test for repellent properties);

2. **to determine** the percentage of black cherry aphid *Myzus cerasi* mortality depending on the exposure of 5 essential oils (*A. sativum* L., *O. basilicum* L., *L. angustifolia* M., *E. globulus* L. and *C. longa* L.) and two chemicals (DEET (N, N-diethyl-3-methylbenzamide) and 2-Undecanone) in 5 concentrations: 0.3%, 0.6%, 0.9%, 1.2%, 1.5% depending on the treatment period - 2, 6 and 24 hours (contact toxicity test);

3. **to determine** the lethal and sub-lethal concentrations for black cherry aphid *Myzus cerasi* of 5 essential oils (*A. sativum* L., *O. basilicum* L., *L. angustifolia* M., *E. globulus* L. and *C. longa* L.) and two chemicals (DEET (N, N-diethyl-3-methylbenzamide) and 2-Undecanone) in 5 concentrations: 0.3%, 0.6%, 0.9%, 1.2%, 1.5% depending on the treatment period - 2, 6 and 24 hours, perform a three-factor dispersion analysis.

Highlights:

1. The movement of insects have been affected by the concentration of the essential oils in the olfactometer.

2. The highest repellent index was obtained when 2-Undecanone and *A. sativum* were applied.

3. The exposure time did not significantly effect on the movement of insects in the olfactometer.

4. 100 percent toxicity was achieved at concentrations of 0.9%, 1.2%, and 1.5% within 6 hours.

5. All tested oils, except *C. longa*, provided 100% insect mortality within 6 hours.

MATERIALS AND METHODS

This study was carried out at the Laboratory of Entomology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra. Laboratory investigations were divided into two experiments. The experiment included 'two-choice olfactometer' repellency and contact toxicity tests with five EOs, two chemicals against the *M. cerasi* (black cherry) aphid. The study was carried out under the laboratory conditions at the temperature of 20 ± 1 °C, humidity $50 \pm 1\%$, and /light/dark 16:08/ h.

Insects used

The populations of aphids *M. cerasi* (Hemiptera: Aphididae) were collected from cherry plant cultures infested by aphids at a local field of the crop garden in Nitra (Slovakia) $48^{\circ}18'16.7''N$ $18^{\circ}05'41.3''E$ in June 2022. Nutrients were fresh host plant leaves. Approximately 10000 adults were incubated. The adults of both sexes were used in the experiments.

Essential oils and chemical substances used

Essential oils from plant species *A. sativum*, *O. basilicum*, *L. angustifolia*, *E. globulus* and *C. longa* were chosen for the experiment. Essential oils were obtained from the company Mystic Moments Inc. (UK) as commercial essential oils. The extraction method of all oils selected from plant species was steam distillation, according the information published at the web-page <https://www.mysticmomentsuk.com//>. Two insect repellents: DEET and 2-Undecanone were chosen for the research, as both have an excellent safety profile and remarkable protection against mosquitoes and various other arthropods (Nguyen et al., 2023). Moreover some authors, as Costantini et al. (2004), are considering DEET as 'gold standard' according its relative efficacy and persistence profiles. Chemical substances were obtained from Sigma-Aldrich Company (DEET in 97% concentration and 2-Undecanone (methyl nonyl ketone) in 99% concentration. Detailed characteristic of concentrations and doses of essential oils used in the experiment is presented in the Table 1.

Table 1. Dosages used in laboratory experiments

The quantity of essential oils taken for solution preparation		The quantity of solvent (acetone) taken for solution preparation	The received concentration of working solution
μL	mL	(mL)	(%)
30	0.03	10	0.3
60	0.06	10	0.6
90	0.09	10	0.9
120	0.12	10	1.2
150	0.15	10	1.5

$$V/V \% = [(\text{volume of solute}) / (\text{volume of solution})] \times 100\%.$$

Application of essential oils in olfactometer test

The experiments were conducted using a Y-tube-olfactometer. The concentrations (0.3%, 0.6%, 0.9%, 1.2% and 1.5%) of essential oils solutions were placed on the piece of filter paper (No. 2, 10×20 mm), and the acetone (10 μL) was situated on the another discs. Filter paper discs with EOs were placed in one tube of olfactometer; the discs with only acetone were placed in the second tube, as control. Four replicates were used for each concentration. Twenty aphids' adults were leased individually into the olfactometer using the flexible forceps for each replicate. The measurement started after 15 and 30 minutes. The choices of the aphids were recorded. All factors used in the experiment are shown in the Table 2.

Table 2. The scheme of the experiment

Factor A – concentration (%)	Factor B – time after treatment (min)		Factor C – sources for treatments
1. 0.3	olfactometer, toxicity,		1. <i>C. longa</i>
2. 0.6	minutes hours		2. <i>E. globulus</i>
3. 0.9	1. 15	1. 2	3. <i>L. angustifolia</i>
4. 1.2	2. 30	2. 6	4. <i>O. basilicum</i>
5. 1.5		3. 24	5. 2-Undecanone
			6. <i>A. sativum</i>
			7. DEET (<i>N,N</i> -Diethyl- <i>meta</i> -toluamide)

Contact toxicity bioassay on *Myzus cerasi*

The filter paper disk method was used for the bioassay. Discs were sprayed by five different concentrations (%) of investigated EOs and two chemicals (0.3%, 0.6%, 0.9%, 1.2% and 1.5%), were prepared by diluting each EOs and chemicals with 10 mL of acetone (v/v). The filter papers were dried for 5 min. Then, discs were placed upside down in petri dish (10 cm diameter); 10 healthy aphids' adults were placed on the treated discs surface. Four replicate batches of aphids' adults (i.e., 40 insects) were used per each concentration of each essential oil. Petri dishes containing aphids were carefully closed by cover and kept at 25 ± 1 °C to count died individuals and record mortality percentages after 2, 6, and 24 h of application (Karim & Hossein, 2018). To avoid aphids' mortality caused by starvation the pieces of cherry leaves were placed in the middle of the down part of Petri dish. Mortality was confirmed by touching the aphid with a fine brush. Aphids that appeared no realistic movement were considered as dead.

Statistical Analysis

The data were subjected to analysis of variance followed by the Tukey HSD (Honestly Significant Difference) test. The probability of 0.05 and 0.01 were considered as significant. For LC calculating was used Finney's probit analysis (Mekapogu, 2021).

RESULTS AND DISCUSSION

Three-factor variance analysis was done to find out the percent effect of each investigated factors while test for repellency was carrying out. Among the investigated factors, the concentration of the essential oil had the greatest influence on the results 64%, the influence of the waiting time from treatment was 2%, and the result depended on the type of essential oil by 7%. The influence of the interaction of the concentration and type of essential oil on the result was 3%, and the influence of the interaction of all factors was 2% (Table 3).

Repellency index was calculated after all EOs were used in olfactometer (Table 4). The calculation showed 95% repellency index was received after *A. sativum* and *E. globulus* EOs treatment and when 2-Undecanone was used at the concentration level 1.5%. The lowest repellency index - 10% was obtained at the concentration 0.3% after *E. globulus* and *L. angustifolia* EOs were used.

Table 3. The dispersions of the three-factor variance analysis of data for experiment

Dispersion	The sum of squares	Degrees of freedom	Root mean square	F
General	525.57	279.00	\	\
Replications	8.92	3.00	\	\
A-concentration (%)	338.62	4.00	84.66	151.29
B-time after treatment (min)	8.57	1.00	8.57	15.33
C (chemicals for treatments)	34.44	6.00	5.74	10.26
AB	0.34	4.00	0.08	0.15
AC	13.63	24.00	0.57	1.01
BC	0.90	6.00	0.15	0.27
ABC	4.31	24.00	0.18	0.32
Remainder	115.83	207.00	0.56	\

Table 3 (continued)

The table of effects and <i>LSD</i>		
Factor	The power of influence	<i>LSD</i> at 0.05
A (concentration, %)	0.64	0.17
B (time after treatment, min)	0.02	0.11
C (chemicals for treatments)	0.07	0.21
AB	0.00	0.25
AC	0.03	0.46
BC	0.00	0.29
ABC	0.02	0.65
Remainder	0.24	\\

Table 4. Repellency index (%) calculated after application the essential oils when olfactometer was used

Concentration (%)	Duration of treatment (min)	EOs and chemicals						
		<i>C. longa</i>	<i>A. sativum</i>	<i>E. globulus</i>	<i>L. angustifolia</i>	<i>O. basilicum</i>	2-Undecanon	DEET
0.3	15	15	30	10	10	30	35	30
	30	20	35	15	20	35	45	30
0.6	15	35	40	35	30	40	55	35
	30	40	45	50	35	60	60	35
0.9	15	60	55	55	40	60	70	45
	30	70	70	60	50	55	85	50
1.2	15	75	75	70	50	70	85	65
	30	75	85	75	70	75	90	80
1.5	15	85	90	85	75	85	90	85
	30	85	95	95	85	85	95	90

Summarizing the obtained results, it can be concluded that all investigated factors influence on movement of insects, but each factor has its own power of effect (Table 5).

Table 5. The factors' averages of olfactometer experiment (repellency test)

Factor A		Factor B		Factor C	
Concentration (%)	Number of insects (individuals)	Duration of treatment (min)	Number of insects (individuals)	Chemicals for treatments	Number of insects (individuals)
0.3	3.71			<i>C. longa</i>	2.20
0.6	2.88	15	2.29	<i>E. globulus</i>	2.25
0.9	2.05	30	1.94	<i>L. angustifolia</i>	2.68
1.2	1.29			<i>O. basilicum</i>	2.03
1.5	0.63			2-Undecanone	1.45
				<i>A. sativum</i>	1.90
				DEET	2.28

The lowest amount of black cherry aphids (*M. cerasi*) - 1.45 pcs was obtained when 2-Undecanone was used and 1.90 pcs - when *A. sativum* EO was used. On the highest concentration 1.5% also was the lowest amount of insects 0.63 pcs, and time factor effected on results 2.29 pcs insects after 15 min and 1.94 pcs after 30 min.

To solve our second task, the experiment in Petri dishes was done. It was used the acetone as control in the experiment, the results is shown in the Table 6.

According to the results of Tunç et al. (2004) the time required for 99% mortality in the adults of *T. cinnabarinus*, *A. gossypii* and adults and larvae of *F. occidentalis* was 95.5, 67.6, 34.7 and 34.3 h, respectively, at 80 acetone/l air. Our results show not high percent of insects' mortality (%), because the exposure time was not long. This finding would agree with the data collected

by Dessenbe et al. (2022) who have demonstrated the hexane fraction was more effective than that of acetone and methanol because it induced more mortality and it appears that *S. zeamais* is more vulnerable than *C. maculatus* to the different extracts. While time factor included 3 terms for mortality checking 2, 6 and 24 hours, 100% mortality was founded only after 6 hours expectation. According the results of Oulebsir-Mohandkaci et al. (2015) which show that the mortality rate increases with the increase of dose by advancing in time, our results were confirmed this assertion - we received 100% mortality after 6 hours of expectation at the highest concentration levels 1.2 and 1.5%, and after 24 hours expectation the mortality percent was going down not reaching 100% for no any oil or concentration.

All chemicals and essential oils showed 100% effectiveness excepting *C. longa*. This allows us to make the assumption that the effectiveness of using some chemicals and essential oils as insecticides is affected by time and concentration (Table 7).

The most effective concentration value was 1.2% after six hours expectation - three essential oils provided 100 % mortality at this level - *L. angustifolia*, *O. basilicum* and *A. sativum*, within increasing of concentration value the percent of mortality was decreasing, this allows us to make assumption that preferment of concentration values can caused to emersion of negative effects. The lowest concentration value, where was reached 100% mortality of insects, was 0.9%, also after six hours expectation, under the application of the *E. globulus* essential oil. For two chemicals DEET (*N, N-Diethyl-meta-toluamide*) and 2-Undecanone the highest effectiveness was reached at 1.5% concentration.

Results of Sajjad et al. (2014) painted that percent mortality was directly proportional to increasing concentration of extracts. Both *A. sativum* and *C. longa* significantly reduced the larval, pupal and adult emergence as well as percent weight loss (at α 5%) but *A. sativum* performed better as compared to the *C. longa*.

According our research, the treatment by *O. basilicum* EO provided 100% mortality of aphids on concentration 1.2% after 6 hours after treatment (contact method), and good results in the olfactometer test 2.03 pcs., 1.9 pcs were got after olfactometer test of *A. sativum* also and this number of insects was one of the lowest, excepting the results provided by using 2-Undecanone 1.45 pcs. The results obtained after *A. sativum* using against aphids are similar to results of Mousa et al. (2013), where was achieved 100% of population reduction of aphids in the open field condition and this botanical essential oil was more effective than used in experiment chemical insecticides, but in our investigation the lowest amount of insects were founded after using the 2-Undecanone, and obtained results after using the *A. sativum* EO where on 0.55 pcs higher than after using chemical origin insecticides.

Table 6. The mortality effect of acetone on black cherry aphids (*M. cerasi*) depending on the time after treatment

Time after treatment, hours	Mortality, %
2	17.5
6	20.0
24	7.5
Total	45.0

Table 7. Toxicity (mortality) effect of essential oils and chemicals on black cherry aphids (*M. cerasi*)

Concentration, (%)	Treatment ± SE _x							<i>F</i> statistic	<i>P</i> - value	Tukey
	2- Undecanon	<i>L.</i> <i>angustifolia</i>	<i>E.</i> <i>globulus</i>	<i>O.</i> <i>basilicum</i>	<i>A.</i> <i>sativum</i>	<i>C.</i> <i>longa</i>	DEET			HSD
0.3	70.0 ± 9.46	70.0 ± 9.46	70.0 ± 9.46 ^A	80.0 ± 11.81	77.5 ± 9.46	47.5 ± 6.29 ^A	72.5 ± 11.81	1.2677	0.3139	-
0.6	77.5 ± 9.46	77.5 ± 9.46	85.0 ± 11.90	82.5 ± 11.81	80.0 ± 11.81	55.0 ± 8.66	77.5 ± 9.46	0.9292	0.4946	-
0.9	82.5 ± 11.81	82.5 ± 11.81	100.0 ± 0.00 ^C	85.0 ± 11.90	92.5 ± 4.78	62.5 ± 8.53	85.0 ± 11.90	1.4114	0.2567	-
1.2	90.0 ± 7.07	100.0 ± 0.00 ^b	92.5 ± 4.78	100.0 ± 0.00 ^d	100.0 ± 0.00 ^e	75.0 ± 8.66 ^f	92.5 ± 4.78	3.2805	0.0195	0.023 b ^x f 0.023 d ^x f 0.023 e ^x f
1.5	100.0 ± 0.00	92.5 ± 4.78	95.0 ± 2.88	92.5 ± 4.78	95.0 ± 2.88	87.5 ± 6.29 ^E	100.0 ± 0.00	1.3469	0.2811	-
Average	84.0	84.5	88.5	88.0	89.0	65.5	85.5			
<i>F</i> statistic	2.0593	2.3598	3.0595	0.6549	1.6440	4.2103	1.5635			
<i>P</i> - value	0.1373	0.1002	0.0498	0.6324	0.2151	0.0176	0.2349			
Tukey HSD	-	-	0.0427 A ^x C	-	-	0.0175 A ^x E	-			
<i>p</i> -value										

Within each column, means followed by the same uppercase letter are not significantly different, post-hoc *Tukey HSD Test* at $p = 0.05$. Within each row, means followed by the same lowercase letter are not significantly different, post-hoc *Tukey HSD Test* at $p = 0.05$. Where no letters exist, no significant differences were recorded. SE_x – standard deviation of mean.

It was decided to find the lethal concentrations of essential oils and 2 chemical sources after 2 (Table 8), 6 (Table 9) and 24 hours of treatment (Table 10).

Table 8. Lethal concentrations of investigated essential oils and chemicals after two hours of treatment

Treatment	LC ₅₀			LC ₉₈		
	mL/10 mL	lower	upper	mL/10 mL	lower	upper
<i>C. longa</i>	0.08515	0.04903	0.14789	2.08434	1.20012	3.62005
<i>E. globulus</i>	0.02572	0.01106	0.05983	3.01776	1.29758	7.01833
<i>L. angustifolia</i>	0.04383	0.02359	0.08141	1.54653	0.83257	2.87273
<i>O. basilicum</i>	0.01496	0.00496	0.04510	7.34700	2.43655	22.1537
2-Undecanone	0.03826	0.01587	0.09225	6.28143	2.60508	15.1459
<i>A. sativum</i>	0.03907	0.01850	0.08251	2.91701	1.38130	6.16006
DEET (N,N-Diethyl-meta-toluamide)	0.04262	0.02249	0.08075	1.70694	0.90085	3.23431

Indexes	Treatment						
	<i>C. longa</i>	<i>L. angustifolia</i>	<i>E. globulus</i>	<i>O. basilicum</i>	<i>A. sativum</i>	2-Undecanone	DEET
Slope	1.485	1.345	0.999	0.770	1.106	0.931	1.302
Intercept	6.588	6.828	6.588	6.403	6.559	6.320	6.784
SD (σ)	0.674	0.744	1.001	1.298	0.904	1.074	0.768
SE	0.122	0.137	0.187	0.245	0.166	0.195	0.142
R ²	0.923	0.855	0.829	0.83	0.821	0.848	0.814
Chi-test (χ^2) Sig	0.992	0.998	0.999	1.000	0.997	0.997	0.993
df	3	3	3	3	3	3	3
Chi-Test	ns	ns	ns	ns	ns	ns	ns

The analysis of the table 8 demonstrate that lowest dose which could cause to 50% mortality of aphid population is 0.01496 mL/10 mL of *O. basilicum* oil and reaching of 98% of mortality could be caused by *L. angustifolia* essential oil in dose 1.54653 mL/10 mL after two hours obtained results.

The results of Attia et al. (2016) showed that *L. angustifolia* oil can provide valuable pesticide activity with significantly lower LC₅₀ values by fumigation. According the results obtained after our experiments is clear that in olfactometer test using *L. angustifolia* EO against black cherry aphids was provide the highest amount 2.68 pcs of insects on the treated side, this allows us to assume that this essential oil is not effective enough against this species of aphids in a concentration from 0.3 to 1.2%, also in this case the time factor can play a significant role, i.e. if time increases or decreases, it is possible there is a probability of achieving better results. In the same time, the results of contact test in Petri dish, lavender essential oil provided 100 percent mortality of aphids at a concentration value of 1.2% after 6 hours of waiting, and the calculation of LC of the studied essential oils showed that the use of lavender essential oil would provide LC₉₈ at the lowest concentration value - 1.54653 mL/10 mL among all investigated oils, under the conditions of the time factor 2 hours. The results obtained after conducting the experiment in Petri dishes are reinforced by the results obtained by Pavela, (2006) after lavender oil testing where this oil was demonstrate good results among investigated oils.

Table 9. Lethal concentrations of investigated essential oils and chemicals after six hours of treatment

Treatment	LC ₅₀			LC ₉₈		
	mL/10 mL	lower	upper	mL/10 mL	lower	upper
<i>C. longa</i>	0.04110	0.02402	0.07033	0.86870	0.50770	1.48641
<i>E. globulus</i>	0.01358	0.00694	0.02658	0.28209	0.14417	0.55195
<i>L. angustifolia</i>	0.01322	0.00617	0.02830	0.57538	0.26872	1.23204
<i>O. basilicum</i>	0.00240	0.00064	0.00903	1.22382	0.32487	4.61026
2-Undecanone	0.01174	0.00518	0.02663	0.69959	0.30849	1.58654
<i>A. sativum</i>	0.01028	0.00474	0.02226	0.30882	0.14258	0.66888
DEET (N,N-Diethyl-meta-toluamide)	0.01223	0.00574	0.02608	0.47425	0.22244	1.01114

Indexes	Treatment						
	<i>C. longa</i>	<i>L. angustifolia</i>	<i>E. globulus</i>	<i>O. basilicum</i>	<i>A. sativum</i>	2-Undecanone	DEET
Slope	1.583	1.263	1.56	0.777	1.382	1.167	1.307
Intercept	7.194	7.371	7.912	7.021	7.764	7.248	7.497
SD (σ)	0.632	0.792	0.641	1.286	0.724	0.857	0.765
SE	0.119	0.169	0.149	0.294	0.171	0.181	0.168
R ²	0.825	0.920	0.996	0.658	0.864	0.91	0.855
Chi-test (χ^2) Sig	0.991	0.989	0.994	0.993	0.994	0.993	0.993
df	3	3	3	3	3	3	3
Chi-Test	ns	ns	ns	ns	ns	ns	ns

After six hours expectation from treatment the mortality percent was rise up and the doses which could cause the 50% population mortality of aphids decreased. The *O. basilicum* oil using in dose 0.00240 mL/10 mL was the lowest dose, which caused the half of population mortality, among investigated oils and chemicals. The lowest concentration which caused 98% mortality of individuals was 0.28209 mL/10 mL of *E. globulus* essential oil.

The conducted comparison of investigated essential oils and chemical effectiveness allows us to conclude that *O. basilicum* essential oil is the most effective even at low concentrations/doses. Among the investigated oils, basil caused the 50% death of individuals at the lowest doses, both after 2 and after 6 hours of waiting after treatment. Essential oils from *L. angustifolia* and *E. globulus* were more effective after six hours, causing 98% mortality at the lowest doses.

Using eucalyptus EO in the investigation showed similar results with results obtained by Costa et al. (2015). These scientist founded that mortality of *M. persicae* was 85.5% using essential oil at 1% (w v⁻¹). Estimated values of LC₅₀ (0.40% w v⁻¹) and LC₉₀ (1.15% w v⁻¹) for *M. persicae* evidences the potential use of *E. citriodora* essential oil in controlling this pest. We received the 100% mortality of *M. cerasi* adults at the concentration of 0.9% after 6 hours in Petri dish experiment; estimated value of LC₉₈ for 6 hours expectation time was 0.28209 mL/10 mL and was the lowest value among all investigated substances for this expectation time, the LC₅₀ was 0.0135 mL/10 mL. However Górski & Tomczak (2010) after determine the efficacy of natural essential oils, such as basil oil, citronella oil, eucalyptus oil, juniper oil and patchouli oil did not reach 100% mortality of insects after using eucalyptus oil, herewith after application of

citronella oil and patchouli oil at a concentration of 0.05 and 0.10% and juniper oil at 0.10%, 100% mortality of insects was observed.

Table 10. Lethal concentrations of investigated essential oils and chemicals after 24 hours of treatment

Treatment	LC ₅₀			LC ₉₈		
	mL/10 mL	lower	upper	mL/10 mL	lower	upper
<i>C. longa</i>	0.06128	0.03453	0.10875	1.72805	0.97375	3.06665
<i>E. globulus</i>	0.03336	0.01454	0.07650	3.81236	1.66228	8.74346
<i>L. angustifolia</i>	0.03346	0.01472	0.07604	3.75961	1.65420	8.54473
<i>O. basilicum</i>	0.02297	0.00804	0.06563	9.15729	3.20475	26.1661
2-Undecanone	0.03460	0.01480	0.08089	4.63846	1.98417	10.8435
<i>A. sativum</i>	0.01154	0.00384	0.03466	4.61892	1.53837	13.8682
DEET (N,N-Diethyl-meta-toluamide)	0.03092	0.01332	0.07176	3.73853	1.61091	8.67622

Indexes	Treatment						
	<i>C. longa</i>	<i>L. angustifolia</i>	<i>E. globulus</i>	<i>O. basilicum</i>	<i>A. sativum</i>	2-Undecanone	DEET
Slope	1.432	1.01	0.998	0.792	0.792	0.972	0.988
Intercept	6.737	6.49	6.475	6.298	6.535	6.42	6.492
SD (σ)	0.698	0.99	1.002	1.263	1.263	1.029	1.012
SE	0.127	0.182	0.184	0.233	0.244	0.188	0.187
R ²	0.862	0.834	0.966	0.902	0.844	0.888	0.918
Chi-test (χ^2) Sig	0.990	0.998	1.000	1.000	1.000	0.999	0.999
df	3	3	3	3	3	3	3
Chi-Test	ns	ns	ns	ns	ns	ns	ns

After twenty four hours expectation from treatment the mortality percent was rise down and the doses which could cause the 50% population mortality of aphids increased, and the *A. sativum* essential oil solution among all investigated chemicals showed the lowest concentration value - 0.01154 mL/10 mL, and using 1.72805 mL/10 mL of *C. longa* essential oil solution can cause the 98% of pest individuals mortality.

CONCLUSIONS

The purpose of the study was to evaluate essential oils, which included testing their repellent and toxic properties against aphids *M. cerasi*. According to the results of our study, *A. sativum* and *E. globulus* essential oils were the most effective. When using them in an olfactometer at a concentration of 1.5% and 30 minutes after establishing the source of the smell, it was possible to achieve a repellent index of 95%, the same effectiveness was shown by 2-Undecanone, which is a chemical substance. The same oils ensured 100% mortality of aphids in Petri dishes, that is, when applied by contact after 6 hours of waiting at concentrations of 1.2% when using eucalyptus and 1.5% when using garlic oil. General average mortality index after using these oils was the highest between investigated oils: 88.5% for the *E. globulus* and 89.0% for *A. sativum*. Although all essential oils provided 100% contact toxicity, except for *C. longa* essential oil, our assessment is based on a comprehensive approach, considering all parameters studied.

The research we conducted and the results obtained allow us to recommend the use of solutions of essential oils of garlic and eucalyptus in the conditions of organic farming to struggle the black cherry aphid. However, there is still an urgent need to study the effectiveness of oils in open ground conditions.

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REFERENCES

- Albouchi, F., Ghazouani, N., Souissi, R., Abderrabba, M. & Boukhris-Bouhachem, S. 2018. Aphidicidal activities of *Melaleuca styphelioides* Sm. Essential oils on three citrus aphids: *Aphis gossypii* Glover; *Aphis spiraeicola* Patch and *Myzus persicae* (Sulzer). *South African Association of Botanists* **117**, 149–154.
- Attia, S., Lognag, G., Heuskin, S. & Hance, T. 2016. Insecticidal activity of *Lavandula angustifolia* Mill. Against the pea aphid *Acyrtosiphum pisum*. *J. Entomol Zool Stud* **4**, 118–122.
- Behi, F., Bachrouch, O., Fekih, I.B. & Boukhris-Bouhachem, S. 2017. Insecticidal and synergistic activities of two essential oils from *Pistacia lentiscus* and *Mentha pulegium* against the green peach aphid *Myzus persicae*. *Tunisian Journal of Plant Protection* **12**, 53–65.
- Benelli, G., Pavela, R., Petrelli, R., Cappellacci, L., Santini, G., Fiorini, D., Sut, S., Dall'Acqua, S., Canale, A. & Maggi, F. 2018. The essential oil from industrial hemp (*Cannabis sativa* L.) by-products as an effective tool for insect pest management in organic crops. *Industrial Crops and Products* **122**, 308–315.
- Blackman, R.L. & Eastop, V.F. 1994. Aphids on the world's trees: an identification and information guide. Wallingford, UK: CAB International.
- Cagaň, L., Fusková, M., Hlávková, D. & Skoková Habuštová, O. 2022. Essential Oils: Useful Tools in Storage-Pest Management. *Plants* **11**(22), 1–15.
- Costa, A.V., Pinheiro, P.F., de Queiroz, V.T., Rondelli, V.M., Marins, A.K., Valbon, W.R & Pratisoli, D. 2015. Chemical composition of essential oil from *Eucalyptus citriodora* leaves and insecticidal activity against *Myzus persicae* and *Frankliniella schultzei*. *Journal of Essential Oil Bearing Plants* **18**, 374–381.
- Costa, A.V., Pinheiro, P.F., Rondelli, V.M., de Queiroz, V.T., Tuler, A.C., Brito, K.B., Stinguel, P. & Pratisoli, D. 2013. *Cymbopogon citratus* (Poaceae) essential oil on *Frankliniella schultzei* (Thysanoptera: Thripidae) and *Myzus persicae* (Hemiptera: Aphididae) [Oleoessencial de *Cymbopogon citratus* (Poaceae) sobre *Frankliniella schultzei* (Thysanoptera: Thripidae) e *Myzus persicae* (Hemiptera: Aphididae)]. *Journal of Biosciences* **29**, 1840–1847.
- Costantini, C., Badolo, A. & Ilboudo-Sanogo, E. 2004. Field evaluation of the efficacy and persistence of insect repellents DEET, IR3535, and KBR 3023 against *Anopheles gambiae* complex and other Afrotropical vector mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **98**(11), 644–652. <https://doi.org/10.1016/j.trstmh.2003.12.015>
- Czerniewicz, P., Chrzanowski, G., Sprawka, I. & Sytykiewicz, H. 2018. Aphidicidal activity of selected Asteraceae essential oils and their effect on enzyme activities of the green peach aphid, *Myzus persicae* (Sulzer). *Pesticide Biochemistry and Physiology* **145**, 84–92.
- Damalas, C. 2011. Potential Uses of Turmeric (*Curcuma longa*) Products as Alternative Means of Pest Management in Crop Production. *Plant Omics* **4**(3), 136–141.
- Danelski, W., Badowska-Czubik, T. & Rozpara, E. 2015. Assessment of the effectiveness of plant-derived pesticides in controlling the black cherry aphid *Myzus cerasi* F. in organic growing of sweet cherry. *Journal of Research and Applications in Agricultural Engineering* **60**(3), 21Wafa24.

- Dessenbe, T., Nukenine, E. & Mbailao, M. 2022. Effect of hexane, acetone and methanol extracts of *Plectranthus glandulosus* on the mortality of the adults of *Callosobruchus maculatus* and *Sitophilus zeamais*. *Journal of Entomology and Zoology Studies* **10**(2), 20Wafa27.
- Digilio, M.C., Mancini, E., Voto, E. & De Feo, V. 2008. Insecticide activity of Mediterranean essential oils. *Journal of Plant Interactions* **3**, 17–23.
- Eisa, M.A.S., Matsera, O. & Ludovít Cagaň, L. 2023. Insects Pest Repellent, Essential Oils, Is Can Be an Efficacious Alternative to Synthetic Pesticides. *International Journal of Agriculture and Food Science* **5**(1), 117–25.
- FAO. 2021. World Food and Agriculture – Statistical Yearbook 2021. Rome. <https://doi.org/10.4060/cb4477en>
- Górski, R. & Tomczak, M. 2010. Usefulness of natural essential oils in the control of foxglove aphid (*Aulacorthum solani* Kalt.) occurring on eggplant (*Solanum melongena* L.). *Ecological Chemistry and Engineering* **17**, 345Wafa349.
- Granger, A. 2008. Resistance to *Myzus cerasi* in an open-pollinated population of sweet cherry. *Acta Horticulture* **795**, 87Wafa88. doi: 10.17660/ActaHortic.2008.795.7
- Jaastad, G. 2007. Late dormant rapeseed oil treatment against black cherry aphid and cherry fruit moth in sweet cherries. *Journal of Applied Entomology* **131**, 284Wafa8. doi: 10.1111/j.1439-0418.2007.01155.x.
- Karim, S. & Hossein, P. 2018. Insecticidal Activity of Four Plant Essential Oils against Two Stored Product Beetles. *Entomology, Ornithology & Herpetology: Current Research* **7**(3), 213.
- Khaled, W., Fekih, B.I., Chaieb, I., Souissi, R., Harbaoui, I. & Boukhris-Bouhachem, S. 2017. Insecticidal activity assessment of *Thymus capitatus* essential oils in combination with natural abrasives against *Myzus persicae*. *Tunisian Journal of Plant Protection* **12**, 49–59.
- Małgorzata, T., Witold, D., Elżbieta, R. & Eligio, M. 2019. Possibility of limiting the number of Black Cherry Aphid (*Myzus cerasi* F.) with basic substances for organic sweet cherry. *Journal of Research and Applications in Agricultural Engineering* **64**(4). <https://www.researchgate.net/publication/340606798>
- Mekapogu, A.R., 2021. Finney's probit analysis spreadsheet calculator (Version 2021). Available at: <https://probitanalysis.wordpress.com/>.
- Minev, N., Matev, A., Yordanova, N., Milanov, I., Sabeva, M. & Almaliev, M. 2022. Effect of foliar products on the inflorescence yield of lavender and essential oil. *Agronomy Research* **20**(3), 660–671.
- Mohammad, I. 2001. Aphids and Their Biological Control in Pakistan. *Pakistan Journal of Biological Sciences* **4**. doi: 10.3923/pjbs.2001.537.541
- Mousa, K.M., Khodeir, I.A., El-Dakhakhni, T.N & Youssef, A.E. 2013. Effect of garlic and eucalyptus oils in comparison to organophosphate insecticides against some piercing-sucking faba bean insect pests and natural enemies populations. *Academic Journal of Biological Sciences* **5**, 21Wafa27.
- Musa, F., Krasniqi, D. & Musa, S. 2020. Aphid complex associated with potato in agro-climatic conditions of Kosovo. *Agronomy Research* **18**(1), 206–215.
- Nguyen, Q.D., Vu, M.N. & Hebert, A.A. 2023. Insect repellents: An updated review for the clinician. *Journal of the American Academy of Dermatology*, Jan; **88**(1), 123Wafa130. doi: 10.1016/j.jaad.2018.10.053
- Oulebsir-Mohandkaci, H., Kaki, S.A & Doumandji-Mitiche, B. 2015. Essential oils of two Algerian aromatic plants *Thymus vulgaris* and *Eucalyptus globulus* as bio-insecticides against aphid *Myzus persicae* (Homoptera: Aphididae). *Wulfenia Journal* **22**, 185–197.
- Park, B., Lee, M.J., Lee, S.K., Lee, S.B., Jeong, I.H., Park, S.K., Jeon, Y.J. & Lee, H.S. 2017. Insecticidal activity of coriander and cinnamon oils prepared by various methods against three species of agricultural pests (*Myzus persicae*, *Tetranychus urticae* and *Plutella xylostella*). *Journal of Applied Biological Chemistry* **60**, 137–140.

- Pavela, R. 2006. Insecticidal Activity of Essential Oils Against Cabbage Aphid *Brevicoryne brassicae*. *Journal of Essential Oil Bearing Plants* **9**(2), 99Wafa106, <https://doi.org/10.1080/0972060X.2006.10643479>
- Pavela, R. 2018. Essential oils from *Foeniculum vulgare* Miller as a safe environmental insecticide against the aphid *Myzus persicae* Sulzer. *Environmental Science and Pollution Research* **25**, 10904–10910.
- Pinheiro, P.F., de Queiroz, V.T., Rondelli, V.M., Costa, A.V., Marcelino, T.D. & Pratisoli, D. 2013. Insecticidal activity of citronella grass essential oil on *Frankliniella schultzei* and *Myzus persicae*. *Ciência e Agrotecnologia* **37**, 138–144.
- Sajjad, A., Muhammad, S., Mansoor, H., Muneer, A., Faisal, H., Muhammad, F., Dilbar, H., Muhammad, S. & Abdul, G. 2014. Insecticidal activity of turmeric (*Curcuma longa*) and garlic (*Allium sativum*) extracts against red flour beetle, *Tribolium castaneum*: A safe alternative to insecticides in stored commodities. *Journal of Entomology and zoology studies* **2**(3), 201Wafa205.
- Sampson, B.J., Tabanca, N., Kirimer, N., Demirci, B., Baser, K.H.C., Khan, I.A., Spiers, J.M. & Wedge, D.E. 2005. Insecticidal activity of 23 essential oils and their major compounds against adult *Lipaphis pseudobrassicae* (Davis) (Aphididae: Homoptera). *Pest Management Science* **61**, 1122–1128.
- Smith, G.H., Roberts, J.M. & Pope, T.W. 2018. Terpene based biopesticides as potential alternatives to synthetic insecticides for control of aphid pests on protected ornamentals. *Crop Protection* **110**, 125–130.
- Thorpe, P., Escudero-Martinez, C.M., Akker, S. & Bos, J.I.B. 2020. Transcriptional changes in the aphid species *Myzus cerasi* under different host and environmental conditions. *Insect Molecular Biology* **29**, 271–282. [10.1111/imb.12631](https://doi.org/10.1111/imb.12631)
- Tunç, İ., Ünlü, M. & Dağlı, F. 2004. Bioactivity of acetone vapours against greenhouse pests, *Tetranychus cinnabarinus*, *Aphis gossypii* and *Frankliniella occidentalis* /Bioaktivität von Aceton-Dämpfen auf Gewächshauschädlinge, *Tetranychus cinnabarinus*, *Aphis gossypii* und *Frankliniella occidentalis*. *Zeitschrift Für Pflanzenkrankheiten und Pflanzenschutz/ Journal of Plant Diseases and Protection* **111**(3), 225–230. <http://www.jstor.org/stable/43226878>
- Veromann, E. & Toome, M. 2011. Pollen beetle (*Meligethes aeneus* Fab) susceptibility to synthetic pyrethroids – pilot study in Estonia. *Agronomy Research* **9**(1–2), 365–369.
- Wafa, K., Khaoula, Z., Salaheddine, S., Chedi, G., Wafa, T., Asma, L. & Ikbal, C. 2013. Chemical composition of essential oils of fruits of three ripening stages of bitter orange and their insecticidal potential against the aphid (*Myzus persicae*). *Microbiologie Hygiène Alimentaire* **25**, 107–111.