



SCIENCE OF YOUTH



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Faculty of Horticulture
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FLOODS
AND CLIMATE CHANGE



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THE LABORATORY INVESTIGATION OF *CURCUMA LONGA* L. ESSENTIAL OIL AS REPELLENT AGAINST KINDS OF ORCHARD APHIDS

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Abstract

The aim of the study was to find the potential repellency of *Curcuma longa* L. essential oil (EO) and two chemical substances DEET, (N, N-diethyl-3-methylbenzamide) and 2-Undecanone on *Aphis pomi* DeGeer., *Dysaphis plantaginea* Passerini and *Myzus cerasi* F. 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. Results showed that repellency was increased with increasing the concentration and expecting time. The results showed that the volatiles from *C. longa* at 30 minutes, significantly repellent to aphids in an olfactometer. The strong repellent activity was recorded on *Aphis pomi* (2.25 pcs.). We concluded that the EOs may have potential as an alternative to chemical control, and therefore, they could be included into integrated pest management of aphids, in the Europe and all over the world.

Keywords: repellent, olfactometer, aphids, essential oils.

1. Introduction

Aphids are one of the foremost dangerous bugs of almost all developed crops. Given their brief life

cycle, aphids can have as numerous as 40 generations or more annually beneath optimal conditions, which suggest that theoretically, one female can deliver billions of relatives yearly, in the event that there's no mortality (Dedryver et al. 2010). In addition to debilitating the plant by sucking sap, frequently coming about in leaf distortions, aphids act as vectors for various plant viruses (Jarošová et al. 2016).

In the modern world, there is a rapid increase in the number of aphid populations, which is the result of a successful combination of intensification of technologies in crop production, monoculture and climate change, which all together creates a favourable environment for the growth of the number of these insects (Blackman and Eastop 2017).

Pyrethroids, neonicotinoids, carbamates and insect growth regulators are the classes of active substances that are the basis of chemical plants protection products against aphids. However, in the control against aphids, insecticides do not always provide a reliable result, since insects are already resistant to some classes of insecticides, and the fight is complicated by the fact, that aphids often feed on the underside of leaves (Bass et al. 2014). In addition, the use of some synthetic insecticides or their residues can be dangerous to non-target organisms, including humans (Costa 2018).

This is the reason for the search for new alternative ways to resist damage to plants by aphids. Currently, botanical insecticides (BIs) based on the insecticidal efficacy of secondary plant metabolites are considered a suitable alternative for plant protection against pests (Ofuya et al. 2023) including aphids. The BIs are usually produced from extracts of medicinal plants or plants used in the food industry, and they are viewed as products associated with minimum health and environmental risks. Highly promising plant metabolites with useful insecticidal activities also include the group of plant essential oils (Ikbāl, C. and Pavela, R. 2019).

Turmeric essential oil is one of the promising oil in the system of plant protection (Divekar 2023; Islam et al. 2020; Lee et al. 2001; Salama et al. 2023), and at the same time, not well studied enough. *C. longa* (turmeric) is a small rhizomatous perennial herb of Zingiberaceae (Ginger family) originating from south-eastern Asia, most probably from India. The plant produces fleshy rhizomes

of bright yellow to orange colour in its root system, which are the source of the commercially available spice turmeric (Damalas 2011; Singh 2017).

The EOs, extracts, and curcuminoids extracted from *Curcuma* species have been found effective against a wide range of microbial pathogens and pest insects damaging food crops, either in storage or in the field (Abdelmaksoud et al. 2023; Golafshan & Dorosti 2015; Hasan et al. 2021; Vázquez-Aguilar et al. 2023;). The fresh juice, water extracts and essential oils of *C. longa* show insecticidal activity against insect pests and act as mosquito repellents (Said et al. 2015; Uzair et al. 2018).

Research efforts so far and data from the international literature have shown a satisfactory potential of turmeric as a natural repellent/pesticide for possible use in crop protection and thus a highly promising future towards this direction, that is, the possibility of effective control of certain pests of agricultural importance with the use of turmeric products as a cheap and more environmentally friendly alternative to chemical pesticides already used for the same purpose.

All the above-mentioned served as a driving mechanism for our research. We have decided to find the appropriate concentration of curcuma essential oil, which will provide good/excellent repellent effectiveness against three types of aphids – rosy apple (*D. plantaginea*), black cherry (*M. cerasi*) and green apple (*A. pomi*).

2. Material and methods

2.1 Basic experiment information

This study was carried out at the Laboratory of Entomology, Faculty of Agrobiolgy and Food Resources, Slovak University of Agriculture in Nitra. The experiment included "two-choice olfactometer" repellency tests with *C. longa* and two chemicals against three types of aphids. The study was carried out under the laboratory conditions at the temperature of 20 ± 1 °C, humidity 50 ± 1 %, and /light/dark 16:08/ h.

2.2 Insects used

Parthenogenetic populations of aphids were collected from apple and cherry cultures infested by aphids at a local field of the crop garden in Nitra (Slovakia) 48°18'16.7"N 18°05'41.3"E in June 2022. Nutrients were fresh host plant leaves. Approximately 10000 adults were incubated at 9 ± 1 °C and 50 % relative humidity (RH) under a long day (16:08 hours) in plastic boxes (22 cm diameter x 13 cm height). Insects were maintained in plastic containers that were lined on the bottom with moist tissue and covered with a tight lid to avoid early drying of the leaves. The adults of both sexes of different age were used in the experiments.

2.3 Essential oils and chemical substances used

Essential oils were obtained from a Mystic Moments Inc. (UK) as commercial essential oils. In the *C. longa* essential oil were founded (by chemical analysis) the chemicals phellandrene and turmerone, which were the most effective against insects. Chemical substances were obtained from Sigma-Aldrich Company (DEET (N, N - diethyl - 3 methylbenzamide) in 97 % concentration; 2-Undecanone (methyl nonyl ketone) in 99 % concentration.

2.4 Laboratory Bioassay

Frist "two-choice olfactometer" test of *C. longa* on *D. plantaginea*, *M. cerasi* and *A. pomi*

"Two-choice olfactometer" test was used in the experiment. An EO of *C. longa* and two chemical substances were used in the experiment with the three species of aphids (*D. plantaginea*, *M. cerasi* and *A. pomi*). Four replicates were used for each tested dose (0.3%, 0.6%, 0.9%, 1.2% and 1.5%). Liquids for the experiment were prepared by diluting each EOs and chemicals with 10 ml acetone (v/v). The experiments were conducted using a Y-tube-olfactometer. A 10 µl of each solution was dosed on a piece of filter paper (ADVANTEC, No. 2, 10×20mm), odour sources were placed in the treatment arm, and a filter paper treated with 10 µl of acetone was placed in the control side. In each arm used one leaf of the apple tree as aphids' food. The three species of aphids (*D. plantaginea*, *M. cerasi* and *A. pomi*) were released individually into the olfactometer using flexible

forceps. The air was taken with the help of standard compressor and it was filtrated with an air delivery system. The total airflow for each chamber was 300-400 ml per minute. The measurements were done after 15 and 30 minutes. The aphids were moved to treated and untreated arms from the release point at 10 cm distance to the Y-junction. The choices of the aphids were recorded. Each replicate consisted of 20 responding aphids (= total 240 of all three aphids) per three treatments. Odour sources (chemicals and EOs odours) were replaced after each replicate. Experiments were conducted at temperature 20°C and relative humidity 50 %. Absence of aphids in treated arm leaves was considered a repellency effect (repellence=oriented movement away from a stimulus).

3 Results and Discussion

We have started our experiment (the scheme of experiment is in the Table 1) form verification of curcuma essential oil (*C. longa*) on three types of aphids – rosy apple (*D. plantaginea*), black cherry (*M. cerasi*) and green apple (*A. pomi*), using 5 levels of oil concentrations in the olfactometer (Table 2).

Table 1 The scheme of the experiment. EO from *C. longa* was used to repel the aphids: rosy apple (*D. plantaginea*), black cherry (*M. cerasi*) and green apple (*A. pomi*).

Factor A – concentration, %	Factor B – time after treatment, min	Factor C – Aphid's species
1. 0.3	1. 15	<i>D. plantaginea</i> (rosy apple)
2. 0.6	2. 30	<i>M. cerasi</i> (black cherry)
3. 0.9		<i>A. pomi</i> (green apple)
4. 1.2		
5. 1.5		

Source: made by authors based on the own research

The average amounts (after 4 replications) of aphids are shown in the table 2. This means that for

experiment we took 5 insects, and after the placement of odour source and insects in olfactometer, checked the amount of insects on the treated by EO side (tube) of olfactometer (second tube of olfactometer contained cotton pad without odour source). The concentration amplification influenced on the movement and thus on the appearance of repellent peculiarities of curcuma EO.

Table 2 The effect of *C. longa* essential oil concentrations and waiting time on the movement of aphids in the two-choice olfactometer test

Concentration, %	Time after, min	Variation indexes		Data variation, %	RI, %
		Average amount, pcs. $\bar{X} \pm S_x$	Standard deviation, S		
<i>D. plantaginea (rosy apple)</i>					
0.3	15	4,500	0,577	1,28	10
	30	3,750	0,500	1,33	25
0.6	15	3,750	0,957	2,55	25
	30	4,000	0,816	2,04	20
0.9	15	2,750	0,500	1,82	45
	30	2,000	0,816	4,08	60
1.2	15	1,500	0,577	3,85	70
	30	1,250	0,500	4,00	75
1.5	15	0,250	0,500	20,00	95
	30	0,750	0,500	6,67	85
<i>M. cerasi (black cherry)</i>					
0.3	15	4,250	0,500	1,17	15
	30	4,000	1,155	2,88	20
0.6	15	3,250	0,957	2,94	35
	30	3,000	0,816	2,72	40
0.9	15	2,000	0,816	4,08	60

	30	1,500	1,000	6,66	70
1.2	15	1,250	0,500	4,00	75
	30	1,250	0,957	7,65	75
1.5	15	0,750	0,500	6,66	85
	30	0,750	0,500	6,66	85
<i>A. pomi</i> (green apple)					
0.3	15	4,250	0,957	2,25	15
	30	4,500	0,577	1,28	10
0.6	15	3,500	0,577	0,00	30
	30	2,750	0,957	3,48	45
0.9	15	2,500	0,577	2,31	50
	30	2,000	0,816	4,08	60
1.2	15	1,500	0,577	3,85	70
	30	1,000	0,816	8,16	80
1.5	15	0,500	0,577	11,55	90
	30	0,500	0,577	11,55	90

Source: made by authors based on the own research

Thereby these numbers allowed us to calculate the repellency index of curcuma essential oil. Repellency index (%) is the dimension which shows how does some substance repel / not attract insects. According Saxena & Sayyed (2018) *C. longa* EO showed 73% RI. The highest RI – 95%, according our observation, was received at concentration 1.5% after checking the olfactometer in 15 min when it was applied for *D. plantaginea* (rosy apple aphids). Also good RI value – 90% were achieved at the same concentration (1.5%) after 15 and 30 min when curcuma EO was applied for *A. pomi* (green apple aphids). When EO was applied for *M. cerasi* (black cherry aphids) the highest RI – 85% were observed at the same concentration – 1.5%. The lowest values of RI, which is expectable, were noticed at the lowest concentrations. Similar results were observed by Cheng et al. (2020) and Azeem et al. (2020) were investigated the antifeedant activity of curcuma EO. In

every case this oil showed good results.

The application of correlation-regression analysis made it possible to construct correlation fields with polynomial, linear and logarithmic trend lines (Figures 1-3) and make mathematical models of dependences between the concentrations of *C. longa* EO and investigated types of aphids (Table 3).

The given images allow us to make possible conclusions that there is strong negative connection between concentrations of *C. longa* EO and amount of aphids.

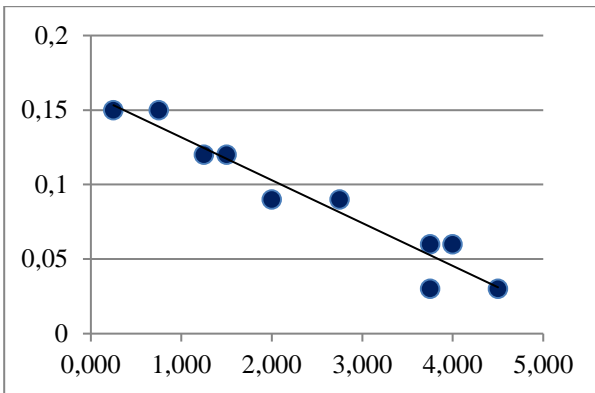


Figure 1 The correlation field between average amount of rosy apple aphids (X) and concentration levels of *C. longa* EO (Y).

Source: made by authors based on the own research

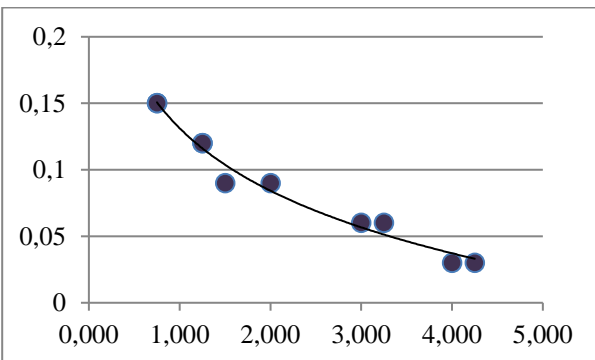


Figure 2 The correlation field between average amount of black cherry aphids (X) and concentration levels of *C. longa* EO (Y).

Source: made by authors based on the own research

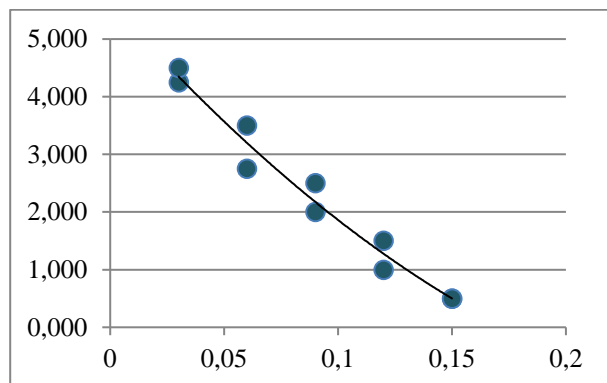


Figure 3 The correlation field between average amount of green apple aphids (X) and concentration levels of *C. longa* EO (Y).

Source: made by authors based on the own research

Table 3 Mathematical models of the influence of curcuma oil concentrations and waiting time on the number of aphids (in the olfactometer)

Index	Regression equation	Correlation coefficient, r	Determination coefficient, R ²
<i>D. plantaginea</i> (rosy apple)			
concentration	$y = -0,0287x + 0,1604$	-0,966	0,92
<i>M. cerasi</i> (black cherry)			
concentration	$y = -0,068\ln(x) + 0,1312$	-0,972	0,94
<i>A. pomi</i> (green apple)			
concentration	$y = 69,444x^2 - 44,583x + 5,625$	-0,981	0,96

Source: made by authors based on the own research

For statistical assessment of experiment we were used the correlation and determination coefficients, which shown that the correlation between concentrations of curcuma EO and number of all three types of aphids had strong negative connection; for simple conception it is mean that the amount of aphids depended from concentration, in the increasing of its was decreasing of individuals amount. One of the indicators describing the quality of the built model in statistics is the coefficient of determination, which is also called the approximation reliability value. R² numerically shows how much of the variation in the dependent variable is explained by the model.

Indicates how well the obtained observations confirm the model. In the conditions of classical linear multiple regression, the coefficient takes a value from 0 to 1. It is believed that the closer the coefficient is to 1, the better the model is. Depending on the level of the coefficient of determination, it is customary to divide the models into three groups: 0.8-1 – a good quality model; 0.5- 0.8 – a model of acceptable quality; 0-0.5 - poor quality model. With regard to this we can assess our models as models with good quality and in this case the quality of the model indicates the possibility of using it for forecasting.

The three-factor variance analysis of data was done (Figure 4). According the received calculation was founded that factors B and C, exactly time after treatment and type of aphid don't effected on the moving and amount of aphids – the power of influence was 0. The combination of concentration and time after treatment (AB) showed the power of effect – 1 %. The power of influence merely concentration (A), % of essential oil was 57 %. Obtained results depend from combination of concentration of EO, % and aphid's species (AC) on 2 %, from combination of time after treatment, min and aphid's species (BC) – on 1 %. The power of influence on obtained results caused by interaction of all three factors was not significant and constituted 4 %.

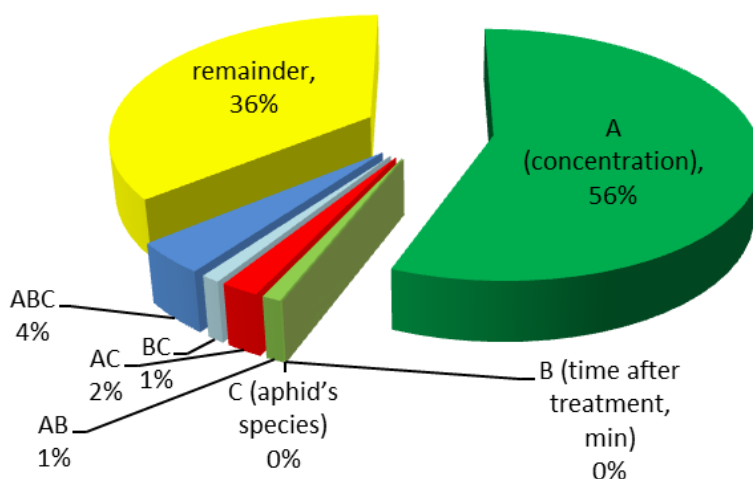


Figure 4 The power of influence caused on the movement of aphids – *A. pomi*, *D. plantaginea* and *M. cerasi* done by investigated factors (%) Source: made by authors based on the own research



Figure 5 The average amount of *A. pomi*, *D. plantaginea* and *M. cerasi* through experiment depending on the effect of concentrations and time after treatment. Source: made by authors based on the own research

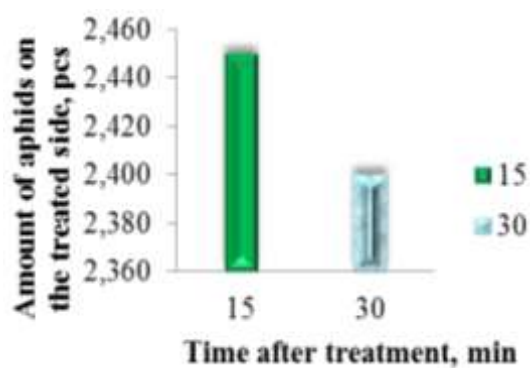


Figure 6 The effect of time after treatment on the amount of *A. pomi*, *D. plantaginea* and *M. cerasi* through experiment. Source: made by authors based on the own research

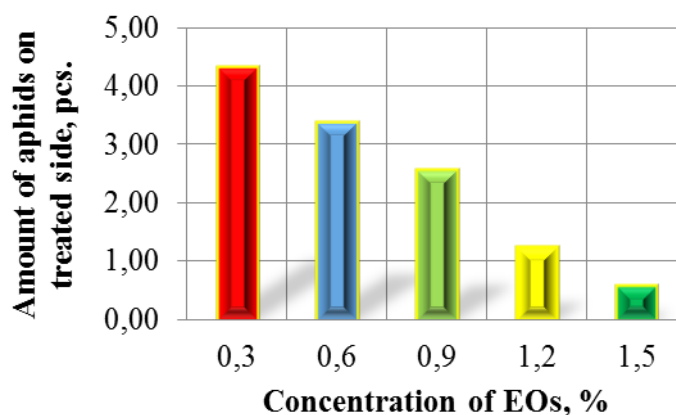


Figure 7 The effect of *C. longa* EO concentrations on the amount of *A. pomi*, *D. plantaginea* and *M. cerasi*

through experiment. Source: made by authors based on the own research

According this calculation is possible to conclude that the lowest amount of insects – 0.58 pcs was founded at the highest concentration – 1.5 % (Figure 7), this arrangement of the obtained data corresponds to the data obtained from the previously conducted correlation analysis, and that is, the concentration of essential oil has an effect on the number and movement of insects. If analyse the time factor, it is visible that the lowest amount of insects – 0.240 pcs was founded after 30 minutes of treatment (Figure 6), and the smallest amount of insects was demonstrated by green aphids – *Aphis pomi* – 2.25 pcs (Figure 5).

The Tukey HSD test was used to find statistical significances between investigated factors; the results are shown in the Table 4.

Table 4 Tukey HSD p-value at 0.05 and 0.01 inferences for *C. longa* olfactometr test against three types of aphids – *A. pomi*, *D. plantaginea* and *M. cerasi*

Concentration, %	Time, min	Concentration, %						
		0,6	0,9	0,9	1,2	1,2	1,5	1,5
		Time, min						
		30	15	30	15	30	15	30
0,3	15		0.0181 p* ; 0.0136 b* ; 0.0464 g*	0.0010 p**; 0.0013 b**; 0.0039 g**	0.0010 p**; 0.0010 b**; 0.0010 g**	0.0010 p**; 0.0010 b**; 0.0010 g**	0.0010 p**; 0.0010 b**; 0.0010 g**	0.0010 p**; 0.0010 b**; 0.0010 g**
0,3	30	0.0464 g*	0.0399 b* ; 0.0141 g*	0.0181 p* ; 0.0010 g**; 0.0044 b**	0.0010 p**; 0.0010 g**; 0.0013 b**	0.0010 p**; 0.0010 g**; 0.0013 b**	0.0010 p**; 0.0010 g**; 0.0010 b**	0.0010 p**; 0.0010 g**; 0.0010 b**
0,6	15		0.0010 p**;	0.0181 p*	0.0399 b* ; 0.0141 g*	0.0399 b*; 0.0010 p**; 0.0010 g**	0.0010 g**; 0.0010 p**; 0.0044 b**	0.0010 p**; 0.0010 g**; 0.0044 b**

0,6	30			0.0044 p**;	0.0010 p**	0.0464 g* ; 0.0010 p**	0.0136 b* ; 0.0010 p**; 0.0039 g**	0.0136 b* ; 0.0010 p**; 0.0039 g**
0,9	15						0.0141 g* ; 0.0010 p**	0.0141 g* ; 0.0044 p**
0,9	30						0.0181 p*	

* Tukey HSD inferences at $p < 0.05$; ** Tukey HSD inferences at $p < 0.01$;

g – green apple aphids *A. pomi*; **p** – pink apple aphids *D. plantaginea*; **b** – black cherry aphids *M. cerasi*.

4 Conclusion

A search of the scientific literature showed that the essential oil of turmeric, as well as the powder from the roots of the plant, have long been known for their insecticidal effect, while we did not find any studies on the effect of the essential oil solution on *D. plantaginea* Passerini, *M. cerasi* and *A. pomi*, in this context, this research has scientific novelty and relevance. Thus, according to the results of our research on the effect of curcuma essential oil on aphids, it was established that there is a persistent negative correlation between the increase in the concentration of the essential oil in the solution and the decrease in the number of insects in the treated part of the olfactometer. Therefore, in this way, turmeric essential oil shows good repellent properties against garden pest species.

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