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Larvicidal activity of *Eucalyptus globulus* L., *Lavandula angustifolia* L., *Cymbopogon citratus* L. and *Citrus sinensis* L. + *Illicium verum* L. essential oils against an invasive drain fly *Clogmia albipunctata* (Williston, 1893).

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ABSTRACT

Larvae of an invasive drain fly *Clogmia albipunctata* can survive in the drains of sinks in households and poses a risk to human health through the mechanical transmission of various multidrug-resistant bacteria and pathogens. We studied if the commercially available essential oils used for household cleaning could limit their survival in homes. We exposed third instar larvae from our own lab colony to 4 essential oils [*Eucalyptus globulus* L., *Lavandula angustifolia* L., *Cymbopogon citratus* L. and *Citrus sinensis* L. + *Illicium verum* L.] in 2.85, 5.7 and 8.55 µL/mL concentrations in mixture with water, 8 % acetic acid (vinegar) diluted in water (1:6) and 2 % DMSO. The lemongrass essential oil was most effective followed by eucalyptus, lavender and orange + badian essential oils. The efficacy of essential oils depended on the concentration, time of exposure and the carrier type. Drain fly larvae were very sensitive to even short time exposure to these essential oils which can help reduce their numbers in homes.

Keywords: *Citrus sinensis* L. + *Illicium verum* L., *Clogmia albipunctata*, *Cymbopogon citratus* L., drain fly larvae, essential oils, larvicidal, household, *Eucalyptus globulus L., Lavandula angustifolia* L.,

INTRODUCTION

Essential oils (EOs), consisting of mixture of various chemical components and being products of plant origin, have a variety of interesting properties, including those of insecticidal. EOs have great potential as an environmentally friendly substitute for entirely synthetic chemicals, such as insecticides and as active ingredients of botanical larvicides (1,19,27,29). EOs are used to replace the harmful toxic substances in household cleaning products to reduce exposure to chemicals in homes and because of their disinfecting capabilities and capacity to deodorize surfaces. So called, *do it yourself*, (DIY) or homemade all-purpose cleansers consisting of non-toxic, inexpensive, commonly available ingredients as vinegar, and including EOs, have become increasingly popular.

Drain fly syn. moth fly, bath fly, non-biting moth midge, sink fly, filter fly, sewer fly *Clogmia albipunctata*, Williston, 1893; Diptera: Psychodidae: Psychodinae) (Fig.1a,b,c) is non-biting, non-hematophagous species naturally occurring in the tropical and subtropical regions and prefers to stay close to shaded locations with decaying, moist organic matter (22,23).

Saprophagous larvae (Fig. 1a) inhabit aquatic habitats like swamps, small shallow pools and tree phytotelmata feeding on decomposing debris. The adults (Fig. 1c) spend most of their lifetime perched on walls and survive by drinking water or eating flower

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nectar (2,12). However, in past 40 years, the species have consistently expanded also temperate zones worldwide (21). Equally, the species has secondarily adapted to anthropogenic environments, especially wastewater from bathrooms, kitchens, sewers and toilets or even stables, which provides suitable organic pollution for larvae development and support species survival during the winter (24).

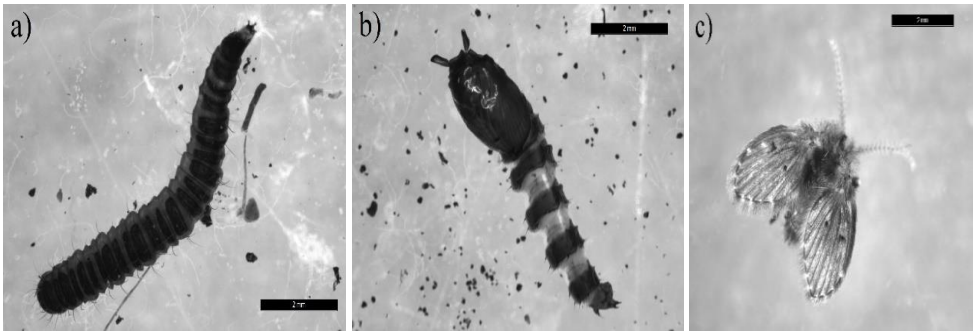

Figure 1. Larvae (a), pupae (b) and adult (c) of drain fly *Clogmia albipunctata* (Williston, 1893) Photo: Authors

The presence of adults inside household is irritating. However, larvae of *Clogmia albipunctata* also poses a risk to human health associated primarily with the mechanical transmission of various multidrug-resistant bacteria and pathogens (7,28,33) and is one of the insects responsible for accidental myiasis: urinary, intestinal, urogenital, gastrointestinal, and nasopharyngeal (6,10,17,30).

The body of *Clogmia albipunctata* individuals (adults and larvae) are protected by the extremely fine water-repellent hair covering ensuring superhydrophobicity against large droplets and antiwetting properties. Just this unique hair covering makes *Clogmia albipunctata* difficult to drown (32). Adults and larvae are not affected by contact with most waterborne toxins such as bleach and even the eggs are highly resistant to both - chemical and thermal assault and can also withstand periods of dehydration (13). Apparently, the extermination of this pest depends mainly on the maintenance of clean household drains (32). It is questionable, how potential the standard cleansers have to limit the survival rate of its larvae.

This first study of its kind aimed to**:** (i) determine, whether four commercially available EOs (Profissimo, brand of DM Drogerie Markt GmbH & Co. KG, Germany) as components of homemade all-purpose vinegar cleanser have any larvicidal activity against drain fly larvae; (ii) laboratory breeding of *C. albipunctata* larvae for experiment and (iii) selection of testing protocol in accordance with observed drain fly larvae bionomy, behaviour and demands for the environmental condition.

MATERIALS AND METHODS

I. Essential oils

We used 4-EOs Profissimo, brand of DM Drogerie Markt GmbH & Co. KG, Germany, commercially available in the chain of DM drugstores: eucalyptus (*Eucalyptus globulus* L.), lavender (*Lavandula angustifolia* L.), lemongrass (*Cymbopogon citratus* L.)

and mix of orange + badian (*Citrus sinensis* L. + *Illicium verum* L.). These were 100 % natural from the plant parts, without alcohol or preservatives. Their major compounds are listed in Table 1.

Table 1. Major compounds of essential oils used in the experiment.

Essential Oil	**Major Compounds**
Eucalyptus globulus L.	Limonene
Lavandula angustifolia L.	Linalool, Limonene, Geraniol
Cymbopogon citratus L.	Citral, Geraniol, Linalool, Limonene, Isoeugenol, Citronellol
Citrus sinensis L. + *Illicium verum* L.	Linalyl acetate, Limonene, Linalool, Citral, Ethoxymethoxy cyclododecane

Chosen EOs are one of the most popular for use in households**,** cosmetics and pharmacy. We based EOs concentrations range on the following recipe for homemade all-purpose vinegar cleanser: 1/4 cup of 8 % vinegar and $1^{1/2}$ cups of water (150 mL) mixed with 30 drops of EO (taken as 1.5 mL). Consequently, dose of the EO per 1 mL of the overall cleanser volume 262.5 mL was determined as 5.7 µL. Except for 5.7 µL/mL, also half as low 2.85 µL/mL, and one and a half times higher 8.55 µL/mL EOs concentrations were tested for the larvicidal activity in bioassays.

II. EOs formulations for bioassays

EOs were then combined with following 3-carriers, for the bioassays:

(i). Water: To exclude the impact of vinegar (25,26) and confirm the effect of EO itself, EOs were mixed with pure distilled water for bioassay as the oil-in-water formulation (20).

(ii). Vinegar/Water (1:6): 8 % vinegar was prepared from 80% acetic acid (AA) (Centralchem ®) and then mixed with distilled water in ratio 1:6.

(iii). DMSO: To exclude the impact of vinegar on its own, evaluate effects of organic solvent and to follow standard testing protocols, EOs were diluted also in the 2 % dimethylsulfoxide. All EOs mixes were prepared using Vortex Mixer.

The pure distilled water was used as a control. Consequently, mixes of EOs in water and mixes of EOs in 2 % DMSO represented effects of pure EOs and mixes of EOs in vinegar/water (1:6) taken to represent the effect of EOs and vinegar together. We know, that the EOs are more physically dispersed then chemically diluted in vinegar/water (1:6) as well as in water. For simplicity, we used carrier instead of solvents, mix instead of solution, and mixed with instead of phrase dissolved in, are used though out the manuscript.

III. Larvicide Bioassay

Lab colony establishment and maintenance

Using an entomological exhauster, wild living drain fly adults were captured from the walls and buildings in the Prešov town, Eastern Slovakia, to establish laboratory breeding of *C. albipunctata.* Flies were kept in the glass jars filled to one-fifth with an organic substrate consisting of soil (commercially available Universal substrate Agro CS, a.s., consisted a mixture of upland peat with the addition of bark compost 2:1, pH 5-6.5, combustible substances min. 70 %, electric conductivity 0.2-0.65 $mS.cm^{-1}$), a kitchen paper towel, piece of soft-wood (part of the substrate) and dog granules (Brit Premium by

Nature Adult). A paper towel and a piece of wood serve as a place for the adults to sit, while laying the eggs and thus prevent them from drowning, soaked kitchen towel served as an alternative source of organic food for larvae as well. Adding dog granules speeded up larval development (2,3).

Tap water was applied to the organic substrate and refilled regularly to create a semiaquatic environment. The glass jars were covered with a glass Petri dish to prevent emerged adults from escaping, stop water evaporation and avoid-substrate from drying out. A sufficient number of larvae (minimum = 90, maximum = 1080) can be obtained quickly (between 14 and 28 days) and cheaply (3 EUR per jar) using described breeding technique.

Bioassay selection

Firstly, contact toxicity test was performed. We assumed that this test would mimic the situation, when cleanser is applied to the various surfaces in the household, mainly in the bathroom. However, we faced following problems: as they are highly mobile in comparison to those of *Musca domestica* L., drain fly larvae were able to escape from the Petri dishes, even when dishes were reversed, loaded, or closed with parafilm; further, 1 mL of mixture was not enough to create semi-aquatic environment, even if using Petri dishes with smaller diameter and thus the majority of larvae dried up before 24 h. That is why we adopted larvicidal bioassay for mosquito larvae suggested by the World Health Organization (34) with several modifications: 10 specimens were placed in the 50 ml closable centrifuge tubes (so that the larvae did not escape) with the 1 ml of the tested EOs mix and the piece of soft paper kitchen towel. In this way, the semi-aquatic environment was achieved. However, even in repeated testing, the eucalyptus, lemongrass and lavender EOs caused the death of all larvae within 24 h of the bioassay's start. Thus, to distinguish between separate EOs larvicidal effects, the exposure time was shortened. As an advantage, the shorter duration corresponds to the real exposure of the larvae to the cleansers in the household. Finally, shortened version of bioassay was applied as described below.

Larvicidal bioassay

The third till fourth-instars drain fly larvae were used for the bioassays. The voucher specimen is kept at the Department of Ecology, FHaNS, University of Prešov in Prešov, Slovakia. Larvicidal bioassay for mosquitoes larvae suggested by the World Health Organization (34) was performed, modified according to specific bionomy of the drain fly larvae as follows: 10-larvae were placed in the 50 ml closable centrifuge tubes with 1 mL of tested EOs mix. Exposure lasted 1.0 h and larval mortality was checked after 25,45 and 60 min. Larvae that did not move were considered dead, when gently squeezed with soft entomological tweezers.

In each test, 3-experiments were done. Then, three independent tests (total n=90 larvae) were run for each EO/concentration/carrier.

All bioassays were done under laboratory conditions (temperature 22 °C, relative humidity 50 %) in the Department of Ecology FHNS Prešov University during July, August and September of 2022.

IV. Statistical Analysis

C. albipunctata larvicidal data were subjected to Finney's probit analysis for determining the LC50/LC90, LT50/LT90 and 95 % confidence intervals of upper/lower

confidence limit (UCL/LCL). Chi-square values of larval mortality data were calculated too (16). Means of larval mortality from all replicates were determined using Univariate statistics. One-way ANOVA was undertaken between treated and non-treated (control) experiments, between particular EOs, and between separated EO´s concentrations/carriers/ exposure time to find distinctions in the larval mortality. Distinctions were taken as significant by p-value ≤ 0.05. Simple linear regression was used to analyse the possible relationship between EO concentration/exposure time and larval mortality. Cluster analyse, Ward´s method was used to clustering EOs in the different carriers according to larval mortality their caused. All statistical analyses were performed in statistical program PAST Version 4.10 (11).

RESULTS AND DISCUSSION

Essential Oils Efficacy

According to average larval mortality (Table 2) as well as LC50/LT50 and LC90/LT90 (Table 3), the lemongrass was the most effective from the EOs used, followed by eucalyptus, lavender and orange + badian consequently. When dissolved in water, vinegar/water (1:6), or 2 % DMSO, lemongrass EO had the lowest LC50 of all the EOs tested. It also differed significantly ($p < 0.05$) in comparison to LC50 of eucalyptus EO, lavender EO as well as orange + badian EO in water, in comparison to LC50 of eucalyptus EO and orange + badian EO in vinegar/water (1:6), and, in comparison to LC50 of eucalyptus EO and lemongrass EO LC50 in 2 % DMSO. Lemongrass EO in water and vinegar/water (1:6) had equally the lowest LT50. It also differed significantly ($p < 0.05$) in comparison to LT50 of orange + badian EO in water and vinegar/water (1:6). On the opposite, orange + badian EO caused the lowest larval mortality and had the highest LC50/LT50 as well as LC90/LT90 (Table 3).

Compared to zero mortality caused by pure water as control, EOs in water and EOs in 2 % DMSO taken as ~~a~~ pure EOs, and EOs in vinegar/water (1:6) taken as EOs with vinegar caused larval mortality, which was significantly higher as when no chemical substance would be applied.

To our knowledge, no prior study of this kind has been carried out. Thus we compared our results with those obtained in contact toxicity bioassays for *Musca domestica* L. larvae, as another of Diptera representatives from the, *flies group*. However, based on drain fly larvae bionomy, we also compared our results with those obtained in toxicity bioassays for mosquitos. Thus, our findings are at least consistent with Chauhan *et al.* (5), who evaluated the larvicidal activity of *Mentha piperita* L., *Cymbopogon citratus* (lemongrass), *Eucalyptus globulus* and *Citrus sinensis* (orange) essential oils against *Musca domestica* L. and *Anopheles stephensi* (Liston) (mosquitoes) through contact toxicity assay. The efficacy order of tested EOs against houseflies was lemongrass > orange > eucalyptus and against mosquitos was lemongrass > eucalyptus > orange, which means that the same as we observed. The same applied to the study of Manh *et al.* (15), when lemongrass and eucalyptus EO´s larvicidal efficacy against *Aedes aegypti* (Linnaeus 1762) was mutually compared.

Table 2. Effects of 3-applied concentrations of essential oils of eucalyptus, lavender, lemongrass and orange+badian in 3-carriers on mortality (%) of drain fly *C. albipunctata* larvae after 25, 45, and 60 min.

***Eucalyptus globulus* EO**									
Concentration	2.85 µL/mL			5.7 µL/mL			8.55 µL/mL		
Exposure time (min)	*25*	*45*	*60*	*25*	*45*	*60*	*25*	*45*	*60*
EO in Water	7.78*	8.89*	21.1*	27.78	40.0*	56.7*	44.4*	88.9*	95.6*
EO in Vinegar+Water	6.67	25.6*	41.1*	23.33	44.4*	65.6*	52.2*	77.8*	97.8*
EO in 2 % DMSO	6.67	13.3*	24.4*	37.78	70.0*	82.2*	44.4*	92.2*	98.9*
***Lavandula angustifolia* Essential Oil**									
Concentration	2.85 µL/mL			5.7 µL/mL			8.55 µL/mL		
Exposure time (min)	*25*	*45*	*60*	*25*	*45*	*60*	*25*	*45*	*60*
EO in Water	5.56*	15.6*	28.9*	4.44	13.3*	30.0*	12.2*	33.3*	55.6*
EO in Vinegar+Water	3.33	17.8*	31.1*	30.0*	61.1*	86.7*	16.7*	37.8*	62.2*
EO in 2 % DMSO	4.44	18.9*	27.8*	15.6*	43.3*	61.1*	14.4*	33.3*	68.9*
***Cymbopogon citratus* Essential Oil**									
Concentration	2.85 µL/mL			5.7 µL/mL			8.55 µL/mL		
Exposure time (min)	*25*	*45*	*60*	*25*	*45*	*60*	*25*	*45*	*60*
EO in Water	12.5*	42.5*	76.5*	15.6*	30.0*	56.7*	26.7*	52.2*	72.2*
EO in Vinegar+Water	11.1*	32.2*	64.4*	32.2*	64.4*	80.0*	21.1*	54.4*	91.1*
EO in 2 % DMSO	12.2*	47.8*	64.4*	23.3*	46.7*	83.3*	34.4*	68.9*	98.7*
***Citrus sinensis* +*Illicium verum* Essential Oil**									
Concentration	2.85 µL/mL			5.7 µL/mL			8.55 µL/mL		
Exposure time (min)	*25*	*45*	*60*	*25*	*45*	*60*	*25*	*45*	*60*
EO in Water	1.1	1.1	4.4	1.1	7.8**	20.0*	2.2	7.7	26.7*
EO in Vinegar+Water	12.1*	25.6*	43.3*	6.7*	21.1*	47.8*	16.7*	50.0*	84.4*
EO in 2 % DMSO	2.2	4.4	4.4	3.3	5.6*	14.4*	5.5*	16.7*	27.8*

Significant differences between experiments and control i.e. pure water which caused zero larvae mortality assessing with One-way ANOVA test at three levels of significance (*p ≤0.05; **p ≤0.01; ***p ≤ 0.001) are indicated.

We haven't personally evaluated the essential oils' chemical composition. However, we suppose-that Citronellol was responsible for the highest lemongrass EO efficacy. Since the other major constituents were almost dentical in all EOs, Citronellol was only presented in lemongrass EO. Nine EO constituents were evaluated by Lima *et al.* (14) against *Anopheles gambae* (Giles) larvae and just the Citronelool was the most effective and the more effective compared to Citral - presented in orange + badian EO, the less effective EO in our study.

Table 3. Larvicidal effects of eucalyptus, lavender, lemongrass and orange + badian essential oils (EO) on Probit analysis: LC50-concentration of a EO that was lethal to 50 % of drain flies larvae in a toxicity test, LC90-concentration of a EO that was lethal to 90 % of drain flies larvae in a toxicity test, LT50-exposure time in minutes after which 50 % of exposed drain flies larvae became dead in a toxicity test, LT90-exposure time in minutes after which 90 % of exposed drain flies larvae became dead in a toxicity test and 95 % confidence intervals of upper confidence limit (UCL) and lower confidence limit (LCL), results of an Chi test ($\chi 2$) results, df -degrees of freedom.

***Eucalyptus globulus* Essential Oil**				
Treatments	LC50 LCL-UCL (95 % confidence limit) µL/mL	LC90 LCL-UCL (95 % confidence limit) µL/mL	$\chi 2$	df
EO in Water	4.43 (3.43 - 5.70)	8.23 (6.38 - 10.63)	0.629	1
EO in Vinegar+Water (1:6)	3.73 (2.24 - 6.27)	15.9 (9.22 - 27.71)	0.804	1
EO in 2 % DMSO	3.83 (2.96 - 4.97)	6.91 (5.33 - 9.22)	0.833	1
	LT50 LCL-UCL (95 % confidence limit) min.	LT90 LCL-UCL (95 % confidence limit) min.	$\chi 2$	df
EO in Water	52 (31 - 90)	249 (138 - 456)	0.749	1
EO in Vinegar+Water (1:6)	46 (31 - 67)	131 (89 - 193)	0.799	1
EO in 2 % DMSO	31 (22 - 44)	76 (54 - 106)	0.986	1
***Lavandula angustifolia* Essential Oil**				
	LC50 LCL-UCL (95 % confidence limit) µL/mL	LC90 LCL-UCL (95 % confidence limit) µL/mL	$\chi 2$	df
EO in Water	8.88 (3.97 - 21.3)	124 (44.3 - 376)	0.427	1
EO in Vinegar+Water (1:6)	3.76 (2.26 - 6.48)	21.4 (10.1 - 39.8)	0.294	1
EO in 2 % DMSO	4.91 (3.16 - 7.64)	16.4 (10.5 - 25.9)	0.725	1
	LT50 LCL-UCL (95 % confidence limit) min.	LT90 LCL-UCL (95 % confidence limit) min.	$\chi 2$	df
EO in Water	113 (66 - 200)	369 (206 - 678)	0.540	1
EO in Vinegar+Water (1:6)	34 (25 - 46)	76 (56 - 104)	0.725	1
EO in 2 % DMSO	51 (36 - 71)	126 (89 - 179)	0.859	1
***Cymbopogon citratus* Essential Oil**				
	LC50 LCL-UCL (95 % confidence limit) µL/mL	LC90 LCL-UCL (95 % confidence limit) µL/mL	$\chi 2$	df
EO in Water	2.02 (0.93 - 4.53)	18.2 (7.87 - 43.1)	0.911	1
EO in Vinegar+Water (1:6)	1.88 (1.06 - 3.42)	8.36 (4.48 - 15.9)	0.903	1
EO in 2 % DMSO	1.78 (1.18 - 2.81)	15.2 (3.61 - 206)	0.929	1
	LT50 LCL-UCL (95 % confidence limit) min.	LT90 LCL-UCL (95 % confidence limit) min.	$\chi 2$	df
EO in Water	44 (34 - 57)	85 (66 - 108)	0.700	1
EO in Vinegar+Water (1:6)	34 (24 - 47)	82 (58 - 114)	0.888	1
EO in 2 % DMSO	39 (30 - 52)	82 (62 - 108)	0.494	1
***Citrus sinensis +Illicium verum* Essential Oil**				
	LC50 LCL-UCL (95 % confidence limit) µL/mL	LC90 LCL-UCL (95 % confidence limit) µL/mL	$\chi 2$	df
EO in Water	21.4 (9.66 - 48.3)	150 (63.4 - 361)	0.752	0
EO in Vinegar+Water (1:6)	3.94 (2.41 - 6.65)	21.0 (11.3 - 40.6)	0.476	1
EO in 2 % DMSO	24.4 (9.85 - 63.2)	178 (68.8 - 480)	0.178	0
	LT50 LCL-UCL (95 % confidence limit) min.	LT90 LCL-UCL (95 % confidence limit) min.	$\chi 2$	df
EO in Water	95 (64 - 143)	220 (145 - 338)	-	1
EO in Vinegar+Water (1:6)	62 (48 - 81)	118 (89 - 156)	-	1
EO in 2 % DMSO	916 (160 - 5 941)	54 191 (8 122 - 362 887)	-	1

EOs carriers comparison

There were no significant differences in the larvicidal activity of eucalyptus or lemongrass EOs when mixed with the different carriers. Lavender EO in 8 vinegar/water (1:6) showed significantly stronger larvicidal activity in comparison to when the lavender EO was mixed with the water. But, eucalyptus EO acted significantly faster when mixed with 2 % DMSO in comparison to be mixed with water or vinegar/water (1:6); lemongrass EO when mixed with vinegar/water (1:6) and 2 % DMSO in comparison to be mixed with water; and lavender EO when mixed with vinegar/water (1:6) in comparison to be mixed with 2 % DMSO. Nevertheless, the highest larval mortality as well as lowest LC50/LT50, LC90/LT90 were generally achieved, when EOs were mixed with vinegar/water (1:6) or 2 % DMSO in comparison to be mixed with water. The pattern was confirmed also by cluster analyze. We suppose, that in the end, after 60 minutes, the efficacy of EOs mixed with various carriers becomes relatively equal, although different carriers can reduce the exposure time required to achieve 50 % larval mortality.

When orange + badian EO was mixed just with the vinegar/water (1:6), it was found to have the lowest LC50/LT50, LC90/LT90 and achieved the highest larval mortality at all concentrations and exposure times in comparison to those mixed with water or 2 % DMSO.

Take into account that:

(i) The orange+badian EO had the lowest larvicidal effect from all EOs used; (ii) its highest efficacy was achieved when it was mixed with vinegar/water (1:6) and the efficacy was even higher than when mixed with 2 % DMSO as organic solvent and (iii) the results of Pangnakorn *et al.* (25,26) - who tested and confirmed the effect of wood vinegar and extracts from some medicinal plants to control housefly (*Musca domestica* L.). We concluded that vinegar can act against drain fly larvae by itself. Consequently, it was confirmed in a separate larvicidal bioassay using single vinegar diluted 1:6 in water.

Since even pure vinegar itself is used as cleanser in homes, it appears that in combination with EOs, a real effective remedy against drain fly larvae can be obtained in this way. According to our findings, we recommend adding widely available ethanol to EO based homemade prepared cleansers, because organic solvents (like DMSO in our case) boost EOs solubility. Thus, higher EOs larvicidal effect against drain flies can be achieved when used in households.

Concentrations and Time of Exposure

When successive EO´s concentrations were mutually compared, the corresponding larval mortality was significantly increased (Table 2). It paid for eucalyptus, lavender, and lemongrass EOs when dissolved in water, vinegar/water (1:6) or 2 % DMSO, but not for orange + badian EO. However, only eucalyptus EO in water and lemongrass and orange + badian EO in 2 % DMSO provided a significant linear dependence between EO concentration and larval mortality. Along with the longer exposure, larval mortality increased as well. Larval mortality after a 60-minute exposure was significantly higher than that after a 25-minute exposure, regardless of EO carrier or concentration. The lowest 2.85 µL/mL concentration of orange + badian EO in water and 2 % DMSO was an exception. However, only eucalyptus EO in vinegar/water (1:6), lavender EO in vinegar/water (1:6) and 2 % DMSO and lemongrass in water provided a significant linear dependence between exposure time and larval mortality. There was no dependence observed by orange + badian EO.

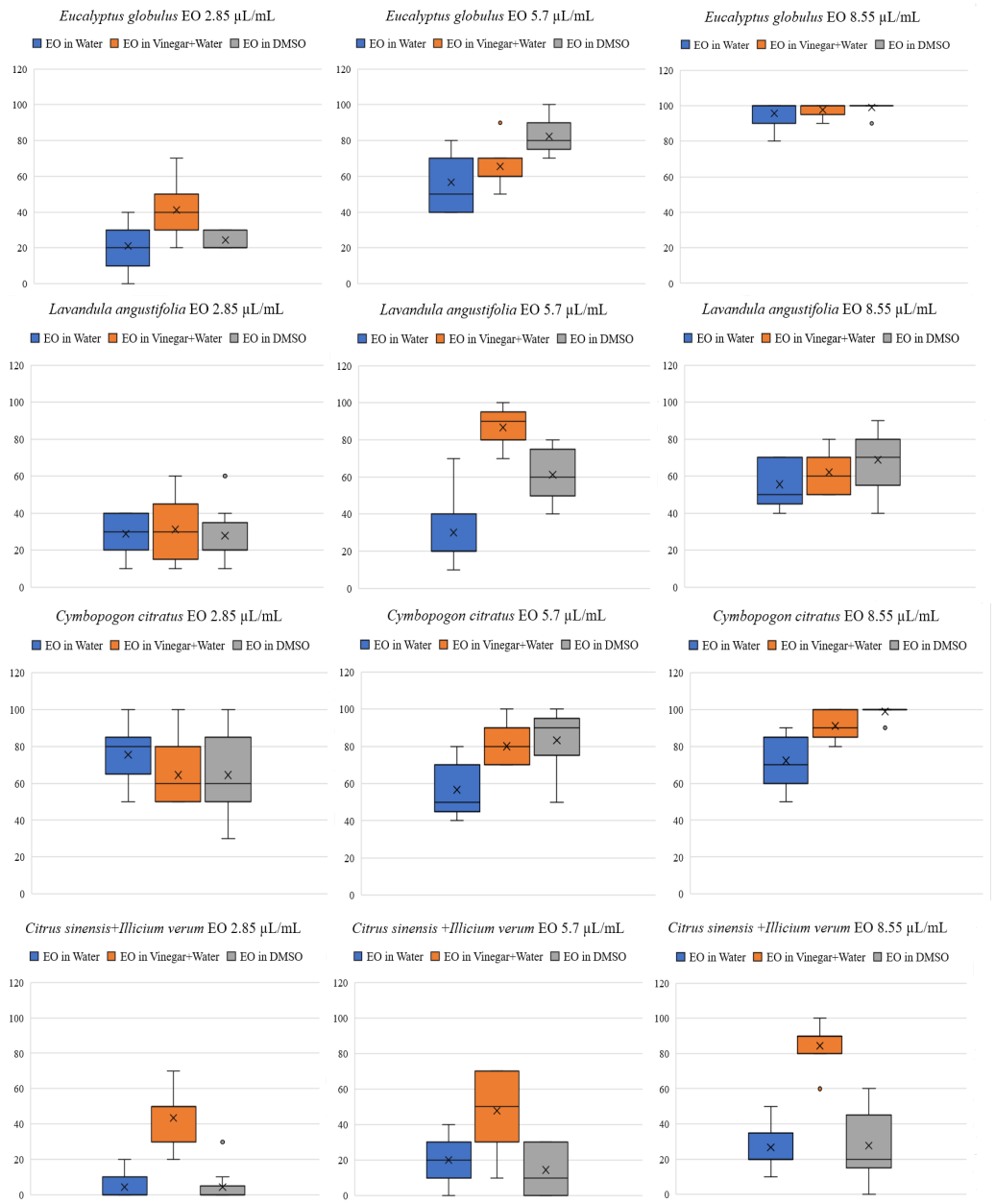

Figure 2. Effects of different concentrations of essential oils in 3-types of carriers (EO in Water, EO in Vinegar+Water (1:6), EO in 2 % DMSO) on mortality of drain fly *C. albipunctata* larvae after 60 min exposure. Control i.e. pure water caused zero larvae mortality Mean: Transverse line in Boxes, X: Median (Second quartile) values. Upper side of Box: First quartile; Bottom Box: Third quartile.

As mentioned previously, all larvae were killed by eucalyptus, lemongrass, and lavender EOs within 24 h of the bioassay's start. Since shorter exposure time than the standard 24 h was required, EOs can obviously act very quickly against drain fly larvae. However, the distinctions between laboratory and home exposure must hardly be considered. Nevertheless, we may at least advise prolonged exposure or letting the homemade, EOs based cleanser work for a longer period of time after being pureed in the sink. Secondary effects of EOs might also be considered simultaneously: due to EOs' antibacterial properties, their use in sinks can sterilize organic waste, slow down its decomposition and lessen both, larval and adult attraction to it (9,18,31,35).

Nevertheless, basic 5.7 µL/mL EO concentration (corresponding 30 drops of EO in 262.5 mL) from the original homemade cleanser recipe was confirmed to be effective enough with eucalyptus or lemongrass EOs. Therefore, we do not recommend unnecessarily increasing EOs dose in order to achieve a better effect because, despite being entirely natural, EOs may have some ecotoxic effects that should be considered (8).

CONCLUSIONS

The drain fly larvae appeared extremely sensitive to essential oils. EOs worked pretty fast, and the concentration, carrier type, and time of exposure affected their efficacy. However, we hardly take into account the differences between drain fly larvae exposure to EOs in a lab and exposure at home. Nevertheless, regular use of a EO based homemade all-purpose vinegar cleanser + ethanol reduced the number of drain fly larvae that survive in homes. Lemongrass or eucalyptus essential oils proved best of those we tested. Equally, prolonged exposure, or letting the cleanser work for a longer period after being pureed in the sink should contribute to better efficacy. Nevertheless, further research is required, and we intend to use several approaches like the dipping method and feeding trial.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. The research was conducted according to Regulation of the Government of the

Slovak Republic no. 377/2012 Coll. dated 14 November 2012 and Decree of the Ministry of Agriculture and Rural Development of the Slovak Republic SR no. 436/2012 Coll. from 14 December 2012 establishing requirements for the protection of animals used for scientific or educational purposes. Ethical protection is granted by the Slovakian legislative to animals listed in the attachment no. 1 of the regulation, but *Clogmia albipunctata* do not fall in this category, and this research is thus in concordance with the current state of ethical legislation in the Slovak Republic.

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