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ESSENTIAL OILS AS A REPELLENT AND ANTIFEEDANT FOR SLUGS UNDER LABORATORY CONDITION

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*Plant-based biopesticides as repellents and antifeedants of animal’s pest, have been used for several years in traditional method as a useful the human, in their animals and crops protection measure against pests. Development of novel biopesticides based on plant secondary metabolic substances, are wide range of ideal properties, such as low mammalian toxicity, high target toxicity, low cost and eco-friendly. Material and methods: The current study evaluated the potency of five essential oils, Ocimum basilicum L., Curcuma longa L., Allium sativum L., Eucalyptus globulus Labill and Lavandula angustifolia Mill, for their repellency and antifeedants activities against the slugs. A 0.2%, 0.5% and 1% exposed concentrations, was prepared 10ml of each solution was dosed on a piece of fresh lettuce leaves was offered to the slugs in plastic boxes (59.2cm*39.7cm*57.7cm). The lettuce leaves were treated with 1ml of tested EOs was placed in the treated side. A further of untreated was included in control side. Each were replicated three times. Results: The choice bioassay of five EOs against the slugs revealed the high repellency A. sativum, E. globulus, L. officinalis C. longa and O. basilicum compared their untreated control. An additional antecedent analysis pointed out that, among the most feeding deterrence EOs, E. globulus, A. sativum, L. officinalis, O. basilicum and C. longa. Conclusion: It is concluded that, this study strongly suggests the use of five EOs O. basilicum, C. longa, A. sativum, E. globulus and L. officinalis as a biopesticides to control slugs under laboratory condition and environmentally safe.*

The development of biological molluscicides may help decrease dependence on synthetic chemicals. In this respect, eco-friendly (EO) molluscicides may also be effective, selective, easily biodegradable, and relatively pollutant-free for the environment. Finally, despite the repellency and antifeedants of EOs, there is still an urgent need to study the effectiveness of oils in open field conditions.

***Keywords**: essential oils, slugs, repellents, antifeedants, eco-system.*
Table 8, Fig. 4, Lit. 23.

INTRODUCTION. Slugs, recently one of the most polyphagous pests, cause high crop losses and uncontrollable threats to agricultural sustainability. Slugs are widely distributed and present on all continents. The damage caused by different slug stages is most common in countries with moist conditions, such as the European region [1]. Slugs feed on multiple hosts, both animals and plants (polyphagous). Several slug species are occurring throughout Europe, and some are pests of cash crops [10]. The amount of damage is related to agricultural practices, soil type, the spread of slug species, climate, and control methods. Slugs are a very harmful pest on vegetables, fruit trees, field corps, and wild plants [12].

Nursery damage, gardening, or vegetable fields by slugs can have a greater economic impact through slime contamination, reducing the marketing value of retailers [2]. Slugs are omnivores; if the climate is right, they cause damage to plants in gardens, nurseries, and field crops [5].

Some slugs are beneficial to humans, but not all are considered pests, such as *Helix aspersa* (Muller), the most common species used as food, which is also one of the global business strategies for food sustainability [23].

Due to the negative impacts of synthetic pesticides on environment, non-target effect, and the stringent role of environmental policies, scientists are looking for novel eco-friendly pesticides and workable techniques of integrated pest management (IPM) strategies against pests [13].

The novel biopesticides as effective alternatives to pesticides, especially those involving integrated management strategies, could have a lower phytotoxicity effect, improve production and quality of plants, and be one of the sustainable strategies for management and reduce pest inputs [3]. Developing alternative snail and slug control methods that are compatible with integrated pest management (IPM) strategies [3].

Numerous plant families that have been recommended by researchers contain bioactive compounds: Myrtaceae, Lauraceae, Rutaceae, Lamiaceae, Asteraceae, Apiaceae, Cupressaceae, Poaceae, Zingiberaceae, Piperaceae, Liliaceae, Apocynaceae, Solanaceae, Caesalpinaceae, and Sapotaceae [8].

Slugs are feeding on dead plants as well as animals on food and alive plants [9]. Damage appears on plant leaves or missing parts, girdling or scraping of the bark, or the destruction of seedlings. They hide during the day under plants, rocks, mulch, pottery, fallen leaves, or in loose soil.

O. basilicum plant and their essential oils it's used in different areas such as biopesticides, cosmetics, medicine, and pharmacology [17]. The compound curcumin from the *C. longa* roots was applied as chemotherapy for anti-carcinogenic effects [22].

A laboratory study indicated that *A. sativum had the* highest reduction damage, barrier effect, and mortality rate against slugs [18]. Several bioactive compounds extracted from *A. sativum* were found to have broad-spectrum pesticide potential [15].

Laboratory experiments revealed that baits based on extracts from E. globulus are potential alternatives for the management of slugs [6].

Money plant extracts derived from plants are used against slugs such as goldenrods (*Solidago canadensis* L., *S. gigantean* Aiton), staghorn sumac (*Rhus typhnia* L.), heaven tree (*Ailanthus altissima* Mill.), knotweeds (*Fallopia japonica* Houtt.), rinse decr, *Fallopia bohemia* (Bailey), and false indigo (*Amorpha fruticosa* L.) [14].

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), and 1,8-cineole (eucalyptol); in the case of *L. angustifolia,* they were citral, coumarin, eugenol, geraniol, limonene, and 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 [7].

Some studies have been carried out for the control of the slugs [18] reported that money plant extracts such as *Foeniculum vulgare* (Fennel), *Momordica charantia* (Bitter Gourd), *Trigonella foenum-graecum* seeds (Fenugreek), *Allium cepa* (Shallots), *Allium sativum* (Garlic), *Artocarpus heterophyllus* (Jack Fruit) leaves, *Phyllanthus emblica* (Amla), *Cymbopogon citrate* (Lemon grass), *Taraxacum officinale* (Dandelion), *Murraya koenigii* (Curry leaves), and *Curcuma longa* (Turmeric) are environmentally safe biopesticides to control slugs in laboratory and field slugs. In other reports by Kozłowski and Kozłowska [10], barriers were used as barriers for the slugs to prevent feeding. The most damaged plants were *Calamintha vulgaris, Ocimum basilicum, Brassica napus var. oleifera, Artemisia dracunculus,* and *Coriandrum sativum*.

Kumar et al. [11] found that *C. longa* leaf extract had a very distinctive smell, so it was assumed to have repellent qualities. However, *C. longa* was fruitless in deterring the slug. While limited information exists on *E. globulus* and *L. officinalis* as molluscicides, most of these oils have been shown to act on insects. Therefore, our justification could be the first to use molluscicides.

The main aim was to use *Ocimum basilicum* L., *Curcuma longa* L., *Allium sativum* L., *Eucalyptus globulus* Labill, and Lavandula *angustifolia* Mill to see if they have any potential biopesticides activities on slugs. The purpose of our laboratory study was to identify: (1) the efficacy of five EOs (*O. basilicum*, *C. longa*, *A. sativum, E. globulus,* and L. *angustifolia) that* can be used in plant protection programmes; (2) the repellency effect of the tested substances; and (3) the antifeedant effect on the slugs.

HIGHLIGHTS

1. The movement of slugs has been affected by the concentration of the essential oils on the treated side compared with those on untreated side.

2. The highest percentage of repellent (PR %) was obtained (100%) at highest concentration of *Allium sativum* L. and *Eucalyptus globulus* Labill at 2 and 24 hours. After treatment application,

3. The highest feeding deterrence percentage (FDP%) was obtained (100%) at highest concentration of *Ocimum basilicum* L., *Allium sativum* L., and *Eucalyptus globulus* Labill at 24 hours. After treatment application,

4. The exposure time had a significant effect on the movement of slugs in the plastic boxes (59.2 cm by 39.7 cm by 57.7 cm).

5. 100 percent repellency and feeding deterrence percentage were achieved at highest concentrations of 1% within 2 and 4 hours.

6. All tested oils, only *Allium sativum* L, provided lethal concentrations (LC50 = 0.02066 and LC98 = 0.38601) of the repellency activity within 24 hours.

MATERIALS AND METHODS. ***Basic experiment information.*** This study was carried out at the Laboratory of Entomology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Nitra. The experiment included choice repellency tests and feeding deterrence with *O. basilicum, C. longa, A. sativum, E. globulus,* and *L. officinalis* against different species of slugs. The study was carried out under laboratory conditions at a temperature of 25 ± 1 °C and 50 ± 70% relative humidity (RH), 10/14 light/dark regime of lightening.

Collection of Slugs. Adult specimens of the slugs were selected from and collected from lettuce leaves at a local field of the crop garden in Nitra (Slovakia) at 48°18'16.7''N and 18°05'41.3''E in June 2023. Nutrients were fresh lettuce plant leaves. The slugs were collected early in the morning and maintained in plastic containers, which were lined on the bottom with moist cotton and covered to avoid early drying of the leaves. Were incubated at the laboratory (at 9 ± 1 °C and 50% relative humidity (RH), 10/14 light/dark regime of lightening) for duration of acclimatization and experimental period.

Gas chromatography-mass spectrometry (GC/MS) analysis was used to identify chemical substances of essential oils

The chemical components of the EOs were identified by gas chromatographic-mass spectrometric analysis (GC/MS) on GC Agilent 7890B and MS Agilent 5977A, Agilent Technologies Inc., Santa Clara, CA, USA. Samples were analyzed at AgroBioTech in Nitra. EOs samples were diluted in hexane (HPLC ≥ 97%, Sigma Aldrich GmbH, Darmstad, Germany) to a concentration of 10 µL/mL. One microliter of diluted sample was injected into the inlet (250°C) operated in split mode 1:10. The separation was achieved using a HP 5 m capillary column (30m x 0.25mm x 0.25 µM film; Agilent Technologies). The temperature of oven was set to 50°C for the first 5 minutes and later increased to 240 °C at a rate of 3°C/min, where it was kept constant for 2 min. Helium was used as a carrier gas at constant flow (1.2mL/min). The mass detector had ionization energy of the filament 70eV, transfer line temperature 250°C, MS source temperature 230°C, and quadrupole temperature 150°C. Mass spectrometer was programmed under electron impact (EI) in full scan at *m/z* 40- 350 with a scanning rate of 2.4 scans/s. The identification of compounds was carried out by comparing mass spectra (over 80% match) witch a commercial database NIST 2017 and Wiley library for retention times of reference standards to compare data on occurrence in EOs with the literature [4].

Repellency Assay

The experiment was conducted using the choice repellency test. 0.2%, 0.5% and 1% exposed concentrations of the essential oils *O. basilicum, C. longa, A. sativum, E. globulus* and *L. officinalis,* tested essential oils, were prepared 10 ml of each solution was dosed on a piece of fresh lettuce leaf and offered to the slugs inside the plastic boxes (59.2 cm by 39.7 cm by 57.7 cm).

The lettuce leaves were treated with 1 ml of tested essential oils and placed on the treated side (A). A further untreated was also included in the control side (B), with control suspensions with either ethanol as negative. Each were reseated three times with 9 slug nine total). The measurement started after 2 h and 24h exposure periods. Finally, a percentage repellency (PR %) (PR %) Eos was calculated according to the formula PR (%) = [(Nc−Nt) ⁄ (Nc + Nt)] ×100. Where NC is the number of slugs present in the control side (B), and Nt is the number of slugs present on the treated arm (A).

Feeding Deterrence Assay. To evaluate if the food was avoided by slugs (*Arion* spp.), exposed to three concentrations of essential oils, 10 ml of each EO (*O. basilicum, C. longa, A. sativum, E. globulus,* and *L. officinalis) and* 2 g of fresh lettuce leaves were offered to the slugs inside the plastic boxes (59.2 cm by 39.7 cm by 57.7 cm). The lettuce leaves were exposed to different concentrations of the treated oils (0.2%, 0.5%, and 1%). A further control of untreated was also included. There were three replicates per treatment with three snails each (a total of nine snails). After 24-hour exposure periods, the percentages of the consumed lettuce leaves were recorded based on the wet residual weight. The feeding deterrence percentage (FDP) was calculated with the following equation: FDP (%) = [(C-T)/C] *100.

Table 1

Dosages used in laboratory experiments

A. EOs/Dose (µl)	B. Dose (ml)	C. Solvent (ethanol) (ml)	D. Concentrations %
20	0.02	10	0.2
50	0.05	10	0.5
100	0.1	10	1

A. *The volume of EOs per (µl) before solvate,* ***B.*** *The volume of EOs per (µl) before solvate, transferred to the milliliters (ml),* ***C.*** *The volume of the solvent per milliliters (ml),* ***D.*** *The calculated concentration using the formula: V/V % = [(volume of solute) ⁄ (volume of solution)] x 100%.*

Source: based on own research

Table 2

The scheme of the experiments № 1 and 2

Factor A, concentration %	Factor B, time after treatment application		Factor C, chemicals for treatments
	Repellency Assay	Feeding Deterrence Assay	*O. basilicum*
0.2	hours	hours	*C. longa*
0.5	2	24	*A. sativum*
0.1	24	//	*E. globulus*
//		//	*L. angustifolia*

Source: based on own research

Statistical Analysis.The data were subjected to analysis using statistical software (Statistix 8.1) under a factorial design. The mean values of the data were tested with the least significant difference (LSD) test at the probability level of 5% ($P \leq 0.05$) and were considered significant.

RESULTS. *Chemical composition.* The chemical compounds identified in the five EOs consisted of three to four dominant compounds and many lesser compounds with different parts of plants (Table 3). A gas chromatography-mass spectrometry analysis (GC-MS) of the selected essential oils was from plant species *A. sativum, O. basilicum, L. angustifolia, E. globulus,* and *C. longa*. 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 [4].

Table 3

Chemical constituents (%) of essential oils *O. basilicum, A. sativum, C. longa, E. globulus* and *L. angustifolia*

Compounds and Relative amount %									
O. basilicum		*A. sativum*		*C. longa*		*E. globulus*		*L. angustifolia*	
Limonene	≤0.60	Methyl allyl sulfide	≤2.70	Limonene	≤0.26	Limonene	≤28.8	Limonene	≤0.74
Linalool	≤25.0	Dimethyl disulfide	≤11.6	beta-Ocimene	≤0.07	Linalool	≤1.66	Linalool	≤35.8
Citral	≤ 1.0	Diallyl sulfide	≤5.28	Eugenol	≤0.06	Citral	≤28.5	Citral	≤0.04
Eugenol	≤ 0.1	Methyl allyl disulfide	≤11.3	Eucalyptol	≤0.59	Eugenol	≤0.14	Eugenol	≤0.00
Carvone	≤0.03	Dimethyl trisulfide	≤0.63	Carvone	≤0.03	alloocimene	≤7.6	Caryophyllene	≤4.11
Estragole	≤76.0	Diallyl disulfide	≤37.2	Estragole	≤0.03	eucalyptol	≤85.8	Lavandulyl acetate	≤3.24
Methyl Eugenol	≤0.50	Methyl allyl trisulfide	≤8.34	Thymol	≤0.13	gamma-terpinene	≤6.93	β-ocimene	≤5.24
trans-Dihydric-arveol	≤17.8	Diallyl tetrasulfide	≤5.15	p-Cresol	≤0.03	//	//	//	//
α-bisabolene	≤2.3	//	//	Tumerone	≤26.5	//	//	//	//
//	//	//	//	quinoxaline	≤6.93	//	//	//	//

Source: based on own research

Repellency activity. The most effective concentration was 1% after 2 hours. Four essential oils provided 100% repellency at this level: *C. longa, A. sativum, E. globulus,* and *L. angustifolia*. After 24 hours, two essential oils, *A. sativum* and *E. globulus,* obtained 100% (PR %) at the same concentration of 1%. Within increasing concentrations, the percent of repellency was increasing. This allows us to make the assumption that preferment of concentration values can cause the emergence of positive effects.

The lowest concentration value was reached at the lowest percentage repellency of slugs (Table 4).

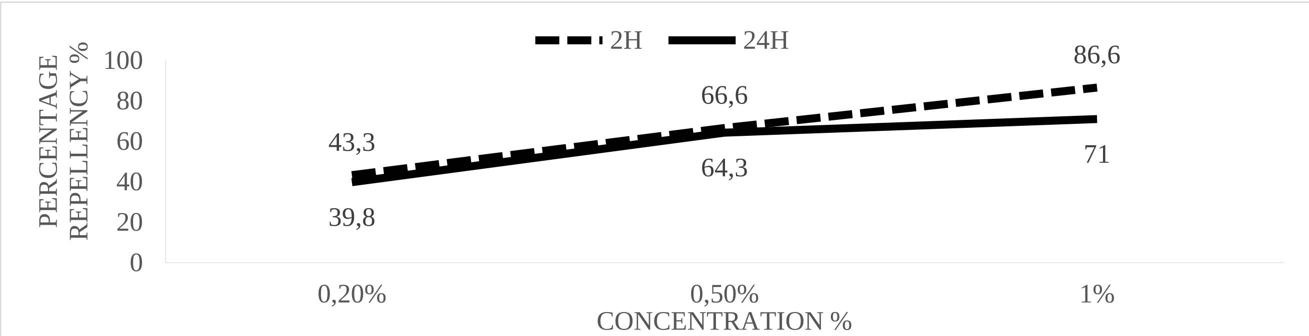
Table 4

Percentage repellency (PR) of *O. basilicum, C. longa, A. sativum, E. globulus,* and *L. officinalis* against slugs in binary-choice bioassays after 2 and 24 hours at doses of 0.2%, 0.5%, and 1%. PR (%) = [(Nc−Nt)∕(Nc + Nt)] ×100. Where Nc is the number of slugs present on the control side and Nt is the number of slugs present on the treated side. Positive values indicate repellent effects

Oils	Time after 2h			Time after 24h		
	Concentration %					
	0.2	0.5	1	0.2	0.5	1
O. basilicum	(58±33.3)AB	(58±66.7)AB	(58±67.7)AB	(38±22)B	(38±77.7)AB	(19±77.7)AB
C. longa	(29±66.7)AB	(0±100) A	(0±100) A	(58±11)B	(51±44.3)AB	(19±66.7)AB
A. sativum	(0±0.0) B	(58±33.3)AB	(0±100) A	(51±44.3)AB	(38±77.7)AB	(0±100) A
E. globulus	(58±33.3)AB	(58±66.7)AB	(0±100) A	(51±44.3)AB	(51±44.3)AB	(0±100) A
L. officinalis	(50±50) AB	(58±66.7)AB	(0±100) A	(38±22)B	(58±66.7)AB	(19±77.7)AB
F	1.24			1.45		
P	0.31			0.22		

Values followed by different letters in the same column differ significantly at P≤0.05 according to the least significant differences (LSD) multiple test. ***F.*** *F-value,* ***P.*** *P-value.*

Source: based on own research



Source: made by authors based on the own research.

Figure 1. **The correlation field between percentage repellency of Arion spp. (X) and concentration levels of all EOs (Y) after treatment duration 2h and 24h.**

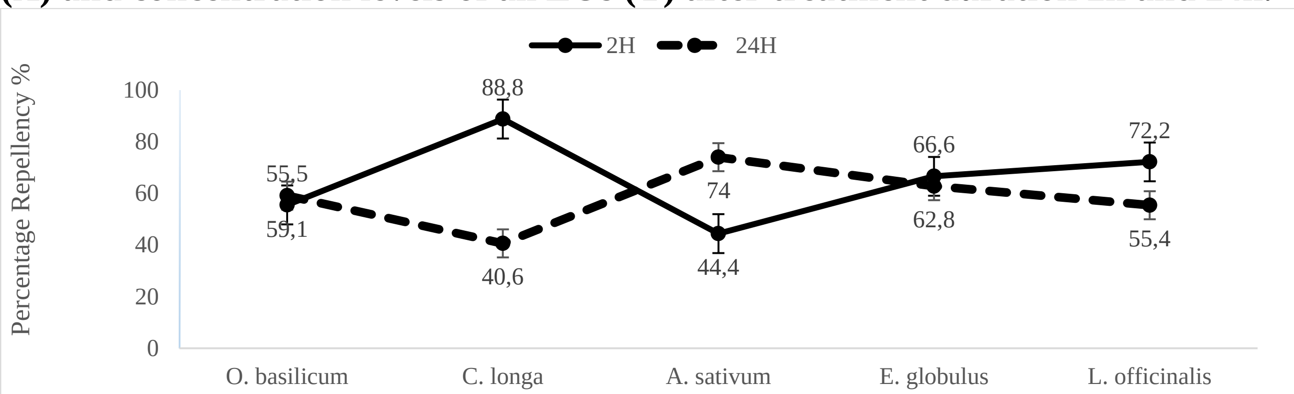


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Figure 2. **The correlation field between percentage repellency of Arion spp. (X) and overall treated EOs (Y) after treatment duration 2h and 24h.**

Feeding deterrent (FD) activity. The result of lettuce leaf consumption showed a weight reduction in the slugs feeding activity after 24 hours of exposure to the three different concentrations of *O. basilicum, C. longa, A. sativum, E. globulus,* and *L. officinalis* essential oils compared with that of the untreated control. The feeding deterrent percentage (FDP) observed that the essential oils extended a potent feeding deterrent effect (Table 5). The feeding deterrent percentage ranged from 42.2% to 100% for *O. basilicum;* the highest concentration 1% was obtained at the highest feeding deterrent percentage 100%, from 54% to 91% for *C. longa;* the highest concentration 1% showed the highest feeding deterrent percentage 91%, from 78.3% to 100% for A. sativum; and at the highest concentration 1%, the highest feeding deterrent percentage 100%, from 81% to 100% for E. globulus. The concentrations of 0.5% and 1% indicated that the highest feeding deterrent percentage was 100%, and from 73.3% to 94.3% for L. officinalis, the highest FDP% was 94.3% obtained at the highest concentration of 1%.

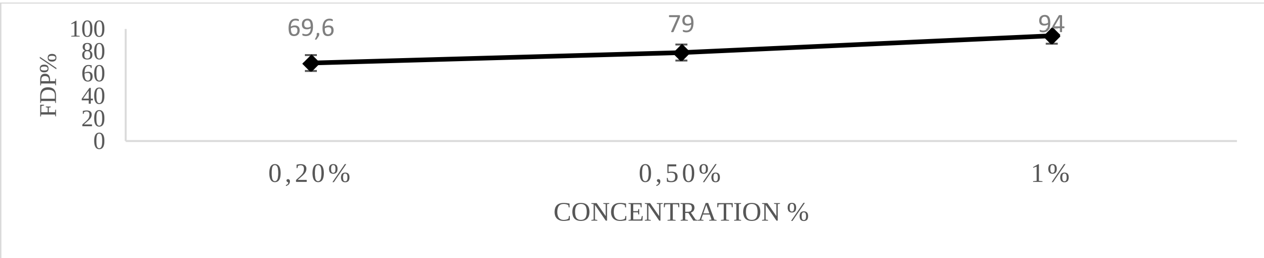
Table 5

Feeding deterrent activity of *O. basilicum, C. longa, A. sativum, E. globulus,* and *L. officinalis* against slugs in binary-choice bioassays after 24 hours at doses of 0.2%, 0.5%, and 1 FDP (%) = [(C-T)/C] *100. Where C is the consumed lettuce leaves on the control side and T is the consumed lettuce leaves on the treated side. Positive values indicate repellent effects

Oils	Time after 24h		
	Concentration %		
	SD ± Feeding Deterrence Percentage (FDP) %		
	0.2	0.5	1
O. basilicum	(12.0±42.3) C	(44.0±68.3) ABC	(0.0±100) A
C. longa	(30.0±54)BC	(16.0±65 ABC	(15.0±91)AB
A. sativum	(38.0±78.3)ABC	(23.0±86.6)AB	(0.0±100)A
E. globulus	(33±81)ABC	(0.0±100)A	(0.0±100)A
L. officinalis	(46.0±73.3)ABC	(26.0±79.3)ABC	(10.0±94.3)AB
F	0.89		
P	0.53		

Values followed by different letters in the same column differ significantly at P≤0.05 according to the least significant differences (LSD) multiple test. ***F.*** *F-value,* ***P.*** *P-value.*

Source: based on own research



Source: made by authors based on the own research.

Figure 3. **The correlation field between feeding deterrent percentage of Arion spp. (X) and concentration levels of all EOs (Y) after treatment duration 24h.**

Source: based on own research

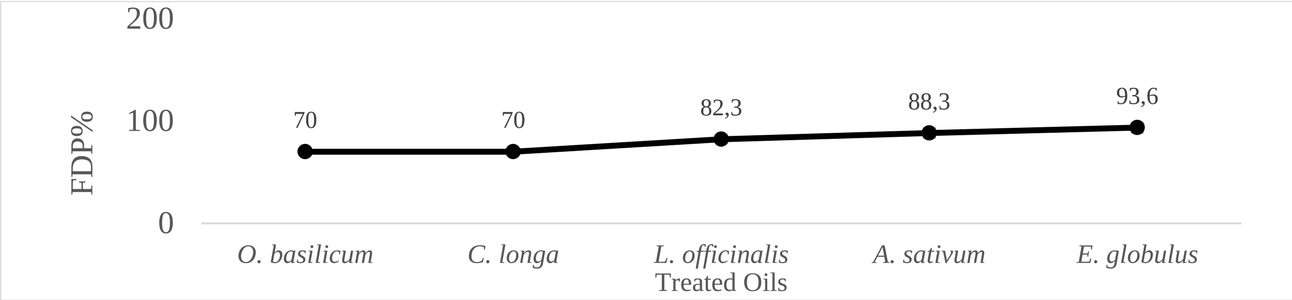


Figure 4. **The correlation field between feeding deterrent percentage of Arion spp. (X) and overall treated EOs (Y) after treatment duration 24h.**

Source: made by authors based on the own research

Source: based on own research

The repellency activity of the five evaluated EOs is presented in Table 6. The EO of C. longa displayed the highest repellency activity against slugs. With lethal concentration, LC50 values of 0.01109 ppm and A. sativum had LC98 values of 0.17469 ppm after 2 h exposure periods, respectively. The repellency activity of A. sativum was highest with a lethal concentration, with LC50 and LC98 values of 0.02066 ppm and 0.38601 ppm after 24 h exposure periods, respectively (Table 7). The EO of O. basilicum, E. globulus, and L. officinalis exhibited the lowest repellency activity up to mg/L.

Table 8 shows the feeding deterrent activity of the five EOs that were evaluated. The ecological activity of L. officinalis showed the highest feeding deterrent activity against slugs, with lethal concentrations (LC50 values of 0.00396 ppm) and E. globulus with lethal concentrations (LC98 values of 0.32088 ppm) after 24 hours of exposure periods, respectively.

Table 6

Lethal concentrations of the repellency activity of investigated essential oils after 2 hours of treatment

Treatment	$LC_{50,}$ %			$LC_{98,}$ %		
	ppm	lower	upper	ppm	lower	upper
O. basilicum	0.01491	0.00089	0.25023	5540.03038	330.01860	93000.62652
C. longa	0.01109	0.00488	0.02519	0.27164	0.11961	0.61691
A. sativum	0.04550	0.03210	0.06447	0.17469	0.12328	0.24755
E. globulus	0.02352	0.01232	0.04492	0.37007	0.19379	0.70670
L. officinalis	0.02047	0.00994	0.04216	0.45140	0.21918	0.92964

Indexes	Treatment				
	O. basilicum	*C. longa*	*A. sativum*	*E. globulus*	*L. officinalis*
Slope	0.369	1.507	3.522	1.743	1.560
Intercept	5.674	7.950	9.727	7.844	7.641
SD (σ)	2.709	0.663	0.284	0.574	0.641
SE	0.625	0.182	0.077	0.143	0.160
R^2	0.816	0.816	0.992	0.836	0.768
Chi-test (χ2) Sig	0.931	0.895	0.869	0.780	0.768
df	2	2	2	2	2
Chi-Test	NS	NS	NS	NS	NS

Source: made by authors based on the own research

The EO of O. basilicum, C. longa, and A. sativum exhibited the lowest repellency activity up to mg/L.

Table 7

Lethal concentrations of the repellency activity of investigated essential oils after 24 hours of treatment

Treatment	LC $_{50}$, %			LC $_{98}$, %		
	ppm	lower	upper	ppm	lower	upper
O. basilicum	0.02163	0.00431	0.10857	32.04437	6.38354	160.85763
C. longa	0.03881	0.00834	0.18063	42.19051	9.06467	196.37115
A. sativum	0.02066	0.01033	0.04134	0.38601	0.19293	0.77236
E. globulus	0.02321	0.01156	0.04659	0.48984	0.24401	0.98335
L. officinalis	0.02390	0.00463	0.12337	40.31625	7.80981	208.12292

Indexes	Treatment				
	O. basilicum	*C. longa*	*A. sativum*	*E. globulus*	*L. officinalis*
Slope	0.649	0.676	1.636	1.601	0.637
Intercept	6.082	5.955	7.760	7.626	6.033
SD (σ)	1.540	1.478	0.611	0.625	1.570
SE	0.357	0.341	0.154	0.154	0.364
R^2	0.816	0.993	0.856	0.678	0.947
Chi-test (χ2) Sig	0.884	0.975	0.822	0.680	0.941
df	2	2	2	2	2
Chi-Test	NS	NS	NS	NS	NS

Source: made by authors based on the own research

Table 8

Lethal concentrations of the feeding deterrent activity of investigated essential oils after 24 hours of treatment

Treatment	LC $_{50}$, %			LC $_{98}$, %		
	ppm	lower	upper	ppm	lower	upper
O. basilicum	0.02181	0.01099	0.04330	0.40686	0.20495	0.80768
C. longa	0.01107	0.00141	0.08720	116.07013	14.73852	914.08580
A. sativum	0.01288	0.00516	0.03215	0.58549	0.23456	1.46142
E. globulus	0.00831	0.00321	0.02149	0.32088	0.12407	0.82989
L. officinalis	0.00396	0.00028	0.05626	495.43231	34.87868	7037.34149

Indexes	Treatment				
	O. basilicum	*C. longa*	*A. sativum*	*E. globulus*	*L. officinalis*
Slope	1.645	0.512	1.258	1.315	0.403
Intercept	7.738	6.001	7.380	7.737	5.969
SD (σ)	0.608	1.955	0.795	0.761	2.478
SE	0.152	0.457	0.203	0.211	0.588
R^2	0.807	0.856	0.775	0.816	0.841
Chi-test (χ2) Sig	0.778	0.928	0.851	0.917	0.947
df	2	2	2	2	2
Chi-Test	NS	NS	NS	NS	NS

Source: made by authors based on the own research

In the past, slugs were controlled by synthetic pesticides based on metaldehyde, carbamates, and phosphate (iron) [5]. However, molluscicides have become toxic to vertebrates and entomophagous invertebrates [5]. There are also reports of the Sid-effect of molluscicides on non-target organisms [13]. This synthetic chemical is already being withdrawn from use following a resentment ban by the European Commission [13].

Slugs are a serious pest of cultural plants and private sector gardens and are an important model of a decomposing organism in the terrestrial ecosystem [1]. Many biopesticides based on plant secondary metabolic substances have beneficial properties, such as low mammalian toxicity, high target toxicity, low cost, and eco-friendliness (fast biodegradability) [13].

An essential oil could be useful in IPM strategies where the goal is not to kill animal pests but rather to prevent product losses. The product losses may be reduced by the inactivating effect of essential oils on slugs [16]. These essential oils are extracted from different parts of the plant, such as leaves, stems, bark, roots, flowers, and fruits, rather than seeds, and then separated from the plant parts by distillation. Although there are various methods for essential oil extraction from natural plant material, four major methods are commonly used, such as (i) hydrodistillation, (ii) steam distillation, (iii) dry distillation, and (iv) mechanical processes [20].

In our study, we showed that C. *longa, A. sativum, E. globulus,* and *L. officinalis* can cause 100% repellency at a concentration of 1% (v/v) at 2 hours after treatment application. Application of biological tests, which indicate the potential benefit of these plant essential oils as novel molluscicides. Of these, *A. sativum and E. globulus* had the highest repellency to slugs, even after 24 hours, when we received PR100% at the highest concentration of 1%.

According to many authors' suggestions, their activity might be successfully implemented as an alternative method of plant protection against slug repellency and feeding deterrents [10]. They have been reported as being used as barriers for the slugs to prevent feeding. The most damaged plants were *Calamintha vulgaris* (92.1%), *Ocimum basilicum* (89.2%), *Brassica napus var. oleifera* (81.7%), *Artemisia dracunculus* (80.8%), and *Coriandrum sativum* (76.7%), respectively [10]. In a direct comparison to our study, we did not receive 100% repellency of *O. basilicum* at a higher concentration of 1% (v/v) after 24 hours under laboratory bioassay, indicating that the potential of *O. basilicum* showed a semi-attractive activity.

Kumar et al. [11] found that *C. longa* leaf extract had a very distinctive smell, so it was assumed to have repellent qualities. However, *C. longa* was fruitless in deterring the slug. On the other hand, *A. sativum* oils, which have a strong potential smell, proved to be effective in repelling slugs [11].

In our bioassay, we found C. *longa* can cause 100% repellency at a concentration of 1% (v/v) at 2 hours after treatment application under laboratory conditions.

Schröder et al. [18] reported that several plant extracts, such as *Foeniculum vulgare* (Fennel), *Momordica charantia* (Bitter Gourd), *Trigonella foenum-graecum* seeds (Fenugreek), *Allium cepa* (shallots), *Allium sativum* (garlic), *Artocarpus heterophyllus* (Jack Fruit) leaves, *Phyllanthus emblica* (Amla), *Cymbopogon citrate* (Lemon grass), *Taraxacum officinale* (Dandelion), *Murraya koenigii* (Curry leaves), and *Curcuma longa* (Turmeric) are environmentally safe biopesticides to control slugs in laboratories and field slugs. Furthermore, the result indicated that *A. sativum* had a strong repellency effect against slugs, with 65% and 72.5% barrier efficacy (Schröder et al. 2008). Our result shows that *A. sativum* can cause 100% repellency at 1% (v/v) concentration in laboratory bioassay, indicating the potential of *A. sativum* as a novel molluscicides. We observed an LC50 and LC98 of 0.02066 ppm and 0.38601 ppm for *A. sativum* at 24 h and LC50 and LC98 of 0.01109 ppm and 0.17469 ppm for *C. longa* and *A. sativum* at 2 h after treatment application.

Our research shows that emulsions of *E. globulus* and *L. officinalis* can cause 100% repellency at a concentration of 1% (v/v) at 2 hours after treatment application. On the other hand, for an antifeedant activity, we found an LC50 of 0.00396 ppm for *L. officinalis* and an LC98 of 0.32088 ppm for *E. globulus* at 24 hours after treatment application. While limited information exists on *E. globulus* and *L. officinalis* as molluscicides, most of these oils have been shown to act on insects. Therefore, our justification could be the first to use molluscicides.

The laboratory experiments clearly showed that applying the essential oils *O. basilicum, C. longa, A. sativum, E. globulus,* and *L. officinalis* into molluscicides reduced slugs attracting and feeding on lettuce leaves and thus should be preferred to the side of lettuce leaves without EOs or untreated control. The highest concentrations successfully reduced slug’s attraction and feeding in the laboratory. The essential oils evaporated quickly; we suggest that repeat application of essential oils could counteract the evaporation of EOs, thus maintaining a high slug’s repellency and the antifeedants of the EOs. However, this could be low-cost, eco-friendly, and thus applicable in the garden and open field. The use of a higher EO concentration could be a more promising approach that is still to be tested in the laboratory and field.

CONCLUSIONS. This study addressed the potential effects of a selection of plant EOs on slugs, which have been strongly investigated up to now. An extensive choice bioassay of five EOs against the slugs revealed the high repellency of *A. sativum, E. globulus, L. officinalis,* C. *longa*, and *O. basilicum* compared to their untreated control. An additional antecedent analysis pointed out that, among the most feeding-deterrence EOs, *E. globulus, A. sativum, L. officinalis, O. basilicum, and* C. *longa* to address these hypotheses, future work should explore the effect of EOs compounds as well as additional EOs doses and exposure times in more scientific and development stages of the slugs with interaction with the plant.

The development of biological molluscicides may help decrease dependence on synthetic chemicals. In this respect, eco-friendly (EO) molluscicides may also be effective, selective, easily biodegradable, and relatively pollutant-free for the environment. Finally, despite the repellency and antifeedants of EOs, there is still an urgent need to study the effectiveness of oils in open field conditions.

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АНОТАЦІЯ

ОЦІНКА РЕПЕЛЕНТНИХ ТА АНТИФІДЕНТНИХ ВЛАСТИВОСТЕЙ БІОЛОГІЧНИХ ПРЕПАРАТІВ У ЛАБОРАТОРНИХ УМОВАХ

Рослинні біопестициди, зокрема репеленти та засоби боротьби зі шкідниками тваринного походження, традиційно використовуються для захисту людей, тварин і сільськогосподарських культур. Розроблення нових біопестицидів на основі вторинних метаболітів рослин є перспективним напрямом завдяки їхнім потенційним перевагам, зокрема низькій токсичності для ссавців, високій біологічній активності щодо цільових організмів, відносно низькій вартості та екологічній безпечності.

Матеріали та методи. У дослідженні оцінювали репелентну та антикормову активність п'яти ефірних олій – Ocimum basilicum L., Curcuma longa L., Allium sativum L., Eucalyptus globulus Labill. та Lavandula angustifolia Mill. проти слимаків. Ефірні олії використовували у концентраціях 0,2, 0,5 та 1,0%. Для проведення біологічного тесту по 10 мл кожного розчину наносили на шматок свіжого листка салату, який розміщували у пластиковій ємності зі слимаками. Оброблену та необроблену частини листка використовували відповідно як дослідний і контрольний варіанти. Кожен варіант повторювали тричі.

Результати. Біологічний тест показав виражену репелентну дію ефірних олій A. sativum, E. globulus, C. longa, O. basilicum та L. angustifolia порівняно з необробленим контролем. Найвищу репелентну активність продемонстрували ефірні олії E. globulus, A. sativum, L. angustifolia, O. basilicum та C. longa.

Висновки. Отримані результати свідчать про перспективність використання ефірних олій O. basilicum, C. longa, A. sativum, E. globulus та L. angustifolia як рослинних біопестицидів для контролю чисельності слимаків у лабораторних умовах. Застосування біологічних молюскоцидів може сприяти зменшенню залежності від синтетичних хімічних засобів захисту рослин. Ефірні олії можуть бути перспективною альтернативою завдяки їхній біологічній активності, здатності до біодеградації та потенційно низькому впливу на навколишнє середовище. Водночас для підтвердження їхньої практичної ефективності необхідні подальші дослідження в умовах відкритого ґрунту.

Ключові слова: *біологічні продукти, сільськогосподарські шкідники, репеленти, засоби від шкідників, екосистема.*

Табл. 8, Рис. 4, Літ. 23.

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