LARVICIDAL ACTIVITIES OF ESSENTIAL OILS AGAINST *Euphyllura olivina* Costa (Homoptera: Psyllidae)

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Abstract. The olive psylla *Euphyllura olivina* (Costa) is an economic olive pest in its native regions that attacks its various organs of olive. In this study, we were carried out in order to determine the insecticidal potential of *Thymus vulgaris*, *Satureja calamintha nepeta*, and *Eucalyptus globulus* essential oils. The contact effect of the three essential oils against the 4th and 5th larval stage of the olive psylla was investigated. While the oils of *S. calamintha nepeta*, and *E. globulus* essential oils showed the same strong insecticidal activity against *E. olivina* (LD50 = 0.13% and 0.15%, respectively), the oil of *T. vulgaris* revealed poor activity against the insect (LD50 = 0.28%). The components of the essential oils were obtained and identified. Chromatographic analysis (GC-MS) of *S. calamintha nepeta* essential oil demonstrated that Pulegone (38.75%) is the main constituent. And as for the *E. globulus* the highest components were Alpha-pinene (22.76%). And the main components of the *T. vulgaris* essential oil were determined as Carvacrol (43.30%).

Key words: *Euphyllura olivina*; larvicidal activity; biocontrol; essential oil.

INTRODUCTION

The olive tree (*Olea europaea* L.) is the main fruit species due to its social and economic importance in the countries of the Mediterranean basin [17]. Olive cultivation occupies a privileged place in Algerian agriculture [11], and it presents a remarkable flexibility and endurance, allowing him to produce in difficult conditions. In Algeria, the most important phytophagous insects that have been recorded and which are considered serious pests in the cultivation of olive trees are *Bactrocera oleae, Parlatoria oleae, Euphyllura olivina*, and *Liothrips oleae* [12]. In some studies conducted in the world, it has been reported that Olive psyllids [*Euphyllura olivina* Costa and *E. straminea* Loginova (Hemiptera: Psyllidae)] are a significant pest and cause economic damage in olive orchards [2, 5, 29, 31, 38, 50]. *Euphyllura olivina* (Costa, 1839) (Homoptera: Psyllidae) is the major pest of the olive tree, feeds by penetrating and sucking olive shoots, inflorescence and flowers, and cause damage by the formation of fumagine with the waxy and sugary substance they emit during nymphal development and led to significant losses in the olive production [29, 31, 33]. The olive psyllid is mostly abundant in spring [19, 35, 37], when olive trees are flowering and causes up to 60% yield loss in some parts of the Mediterranean Basin [25, 46]. Damage begins to appear as soon as the colony exceeds 7 to 8 late instar larvae (L4 and L5) per floral cluster [23]. Natural larvicides based on essential oils of plants present advantages over synthetic larvicides because they are prepared from renewable resources and are readily biodegradable [45]. However, the management of insect pests in many storage systems relies principally on applying synthetic insecticides. Use of these synthetic pesticides in agricultural and forest areas causes many adverse effects [41]. Recently, eco-friendly methods have been developed to control insects. Natural products are generally preferred because they are innately biodegradable and less toxic to non-target organisms. In order to solve this problem and minimize this damage, every method to be used in Integrated Pest Management (IPM) is of great significance [18]. Insecticidal activity of many plant products against various insect pests has been demonstrated by many researchers [13, 15, 20, 26, 32, 34, 49, 52]. This study was carried out with the aim of contributing to the protection of olive trees against *Euphyllura olivina* by using a bio-insecticide to better protect the environment. In the current study, three essential oils derived from the plant species *Thymus vulgaris*, *Satureja calamintha nepeta*, and *Eucalyptus globulus* were tested as contact insecticides on the 4th and 5th larval instars of *Euphyllura olivina* under laboratory conditions.

MATERIALS AND METHODS

Test insects. The insects used for bioassays were taken directly from the field, and they have not been reared in the laboratory because of the failure to provide about nutritional factors and climatic requirements of the species' biological cycle. The infested olive trees of the same age and similar in size, shape, height, vigour and infestation were chosen. From each tree, five small branches (15 cm in length) were randomly picked from each direction, from the two farms (Ain fares and oued taria) for each sampling interval. The picked samples were put in paper or polyethylene bags and transferred to the laboratory for counting stages of that insect using the binocular microscope. All the branches were examined for the 4th and 5th instars were counted and recorded; the branches were placed in cups containing a mixture of water and honey at 3%, and all branches contained 10 larvae for each stage. The larvae of *E. olivina* were placed to experiments in the laboratory at 26.7 °C, at 70% relative humidity, and at lighting conditions of 16:8 h (light, dark).

Essential oil extraction. The aerial parts of the tested plants belong to 3 different botanical species and were collected at their vegetative stage from the northwestern Algeria steppe, Mascara in 2020 (*Thymus vulgaris*, *Satureja calamintha nepeta,* and *Eucalyptus globulus*) were collected for the extraction of the essential oil. The collected plant materials were dried in a shaded place and ground in a grinder. The dried plant samples (500 g) were placed to hydrodistillation using a Clevenger-type apparatus for 3 to 4 hours. The yields were based on the dry materials of plant samples. The oil was stored at 4 °C for use in trials. The essential oils were dried over anhydrous Na_2SO_4 and stored in the refrigerator at 4 °C until used for toxicity bioassays.

The bioassay tests. In order to determine the larvicidal effect of the essential oil obtained from *T. vulgaris*, *S. calamintha nepeta* and *E.globulus* against *E. olivina* larvae, firstly all the branches were examined for the 4th and 5th instars were counted and recorded. Based on preliminary results, a range of volumic doses of 0.015 mL/µL, 0.025 mL/µL, 0.05 mL/µL, 0.1 mL/µL, 0.15 mL/µL, 0.25 mL/µL, 0.5 mL/µL, 0.7 mL/µL, 1 mL/µL, 2 mL/µL were formulated using 50% of EGO as an active ingredient, 30% solvent (ethanol) as a stabilizer and 20% Tween 80 as an emulsifier. The control formulation (T_0) consisted of 50% of distilled water, 30% of ethanol, and 20% of Tween 80 and was used as a control under the same conditions. Each sample was replicated three times at each dose. The infested branches with about 30 larvae of each stage were sprayed with different concentrations. The branches were placed in cups containing a mixture of water and honey at 3%, then covered with sterile plastic bags to avoid the moisture. The branches were stored under laboratory conditions at 25°C, 70% RH, and a 12:12 h (L: D) photoperiod. After application, the mortality of the 4th and 5th larval instars was recorded at 24, 48, and 72 hours.

Statistical analysis. Insect mortality was control-corrected [1] and Arcsine transformed when required to meet assumptions of normality and homogeneity of variances. The difference among treatments in the rate of mortality was determined by analysis of variance (ANOVA) and Tukey's method was used to compare mean significant differences among treatments ($p<0.05$) using SPSS 26 statistical software. A log probit analysis was used to determine the LCs and LTs through the R package 'ecotox' [22].

Gas Chromatography/Mass Spectrometry (GC/MS). Chromatography–mass spectrometry (GC-MS) using (GC-MS TQ 8030, USA) fitted with a detector flame ionizer (FID) using an HP-5 (phenylmethyl siloxane, 30m × 0.32mm ID × 0.25 µm df) capillary column. The carrier gas was helium and the oven temperature was held at 60ºC for 8 min, then to 230ºC (5ºC / min.), a split mode was 1:1500. The injector temperature was 230ºC and the compounds were identified by comparison to the NF ISO 4731 standard.

RESULTS

The yield of the essential oil of *Eucalyptus globulus* (0.20%) was lower than that obtained for *Thymus vulgaris* and *Satureja calamintha nepeta* with 0.94% and 1.63% respectively. Three plants EOs were tested to evaluate their larvicidal activity, and the tested oils revealed various mortality percentages at different concentrations (Table 1). The majority of the tested oils showed 100% mortality at 2 mL/µL final concentration. After 24 hours of exposure, *S. calamintha nepeta* and *E. gobulus* showed a mortality rate of more than 50% for both larval stages at 0.25 mL/µL, while *T. vulgaris* showed less than 50% mortality for the same concentration. Further, the oils started to affect the larvae life at different concentrations; the lowest concentration that caused equal or more than 10% mortality was 0.015 mL/µL for both *E. globulus* and *S. calamintha nepeta*, 0.05 mL/µL for *T. vulgaris* (Table 1). The 24h DL50 and DL90 values obtained from the larvicidal activity test of EOs extracted from the three plants are presented in Table 2. *Satureja calamintha nepeta* and *E. globulus* were the most efficient with (0.13 mL/µL and 0.15 mL/µL respectively) DL50 and (3.92 mL/µL and 3.26 mL/µL respectively) LC90, while *T.vulgaris* was the least efficient (0.28 mL/µL DL50 and 3.95 mL/µL DL90). Likewise, the influence degree of increasing one unit of EOs concentration on their larvicidal activity was different. The essential oil of *S.calamintha nepeta* is more toxic on *E. globulus* after 48 h because the DL50 and DL90 obtained are about two (2) times lower than those observed with *T. vulgaris*. The toxicity results of the extracts *T. vulgaris*, *S. calamintha nepeta* and *E. globulus* at different concentrations were significantly ($P<0.05$) effective against *E. olivina* larvae.

The Lethal Dose (LD) for the 4th and 5th larval instar of *E. olivina* treated with different concentrations of *E. globulus; S. calamintha nepeta* and *T. vulgaris* are presented in table 3 and 4. The highest efficiency was observed with the essential oils obtained from *S. calamintha nepeta* and *E. globulus* with DL50 values of (0.034 mL/µL for L4, 0.058 mL/µL for L5) and (0.047 mL/µL for L4, 0.08 mL/µL for L5); DL90 values of (0.77 mL/µL for L4, 1.78 mL/µL for L5) and (0.85 mL/µL for L4, 1.7 mL/µL for L5) respectively. The least effectiveness against olive psylla was observed for *T. vulgaris* essential oil with DL50 equal to (0.094 mL/µL for L4, 0.18 mL/µL for L5) and DL90 values equal to (1.33 mL/µL for L4 and 2.43 mL/µL for L5). In general, median lethal times to kill 25% (LT25), 50% (LT50), 75% (LT75), and 90% (LT90) decreased with increased concentrations of essential oils (Table 4 and 5). The constituents of essential oils for each test plant were analysed by gas chromatography-mass spectrometry (GC/MS) and are presented in Table 6, Figures 1, 2 and 3. Chemical analysis showed that the essential oils of *S. calamintha nepeta*, *E. globulus* and *T. vulgaris* contained mainly

Table 1. Concentrations, exposure time and cumulative mortality of 4th and 5th instar larvae *Euphyllura olivina* caused by *Eucalyptus globulus*; *Satureja calamintha nepeta* and *Thymus vulgaris* with different concentrations

Plant	H	0	0,015	0,025	0,05	0,075	0,1	0,15	0,25	0,5	0,7	1	2	F	p value
Nepeta	24	0b±0	16.7ab±0.52	23.3ab ±0.58	26.7ab ±0.63	30ab ±0.67	36.7ab ±0.72	40a±0.77	53.3a±0.84	63.3a±0.94	66.7a±0.99	73.3a±1.14	93.3a±1.44	32.875	0
	48	0b±0	30ab±0.65	33.3 ab ±0.72	40ab±0.77	43.3ab±0.8	50ab±0.99	56.7a±1.01	66.7a±1.09	70a±1.19	73.3a±1.09	90a±1.32	96.7a±1.48	21.541	0
	72	0b±0	40ab±0.8	46.7ab±0.85	53.3ab ±0.89	63.3ab±0.98	70ab±1.06	76.7ab±1.15	86.7a±1.22	90a±1.31	93.3a±1.44	100a±0.0	100a±0.0	17.825	0
Eucalyptus	24	0c±0	23.3bc±0.46	26.7bc±0.54	30bc±0.58	33.3bc±0.62	40abc±0.68	46.7ab±0.74	53.3ab±0.84	63.3ab±0.94	66.7ab±0.99	73.3ab±1.09	90a±1.32	44.647	0
	48	0b±0	33.3ab±0.6	40ab±0.67	43.3ab±0.74	46.7ab±0.77	66.7ab ±0.89	70a±0.94	73.3a±1.01	76.7a±1.07	83.3a ±1.09	90a±1.32	96.7a±1.48	28.636	0
	72	0b±0	50±0.7ab	53.3ab±0.77	56.7ab±0.85	66.7ab±0.94	70ab±1.04	76.7ab±1.1	83.3a±1.25	90a±1.31	93.3a±1.44	100a±0.0	100a±0.0	20.054	0
Thyme	24	0c±0	3.3bc±0.16	6.7bc±0.26	16.7abc±0.45	20abc±0.51	26.7abc±0.57	33.3abc±0.63	43.3abc±0.73	50ab ±0.8	60ab±0.93	66.7a±1.04	83.3a±1.09	41.417	0
	48	0c±0	10bc±0.35	16.7bc±0.45	23.3abc±0.57	30abc±0.63	33.3abc±0.68	50abc±0.8	56.7ab±0.91	66.7ab±1.02	73.3ab±1.08	80ab±1.17	93.3a±1.44	34.636	0
	72	0c±0	23.3bc±0.53	30abc±0.61	33.3abc±0.68	40abc±0.75	46.7abc±0.82	60abc±0.91	66.7ab±1.02	76.7ab±1.12	86.7ab±1.29	93.3ab±1.44	100a±0.0	37.141	0

Table 2. Toxicity of *Eucalyptus globulus, Satureja calamintha nepeta* and *Thymus vulgaris* according to time for fourth and fifth instar olive psyllid

Plant	Time/ Hours	DL10	DL30	DL50	DL90	DL99	Slope.SE	Chi²	P
Nepeta	24	0.0046	0.0336	0.134	3.9277	61.6724	0.8736 ± 0.084	11.0493	0.9449
	48	0.0019	0.012	0.0423	0.9261	11.4712	0.9559 ± 0.0917	19.4361	0.4937
	72	0.0014	0.0064	0.019	0.2664	2.295	1.1172 ± 0.1129	16.5901	0.6794
Eucalyptus	24	0.0078	0.0465	0.1599	3.2632	38.1489	0.9784 ± 0.0862	11.5951	0.9293
	48	0.003	0.0176	0.0604	1.2361	14.4847	0.9775 ± 0.0897	19.4978	0.4897
	72	0.0027	0.0107	0.0275	0.2778	1.832	1.2754 ± 0.1161	11.5879	0.9295
Thyme	24	0.021	0.0985	0.2877	3.9503	33.4272	1.1265 ± 0.0911	12.6795	0.8907
	48	0.0106	0.0467	0.131	1.6246	12.657	1.1719 ± 0.0918	13.8281	0.8391
	72	0.0059	0.0244	0.0651	0.7147	5.0382	1.2318 ± 0.0985	21.0971	0.3914

Table 3. Lethal Dose (LD) for the 4th and 5th larval instar of *E. olivina* treated with different concentrations of *E. globulus; S. calamintha nepeta* and *T. vulgaris*

Plant	Larva	DL10	DL 30	DL 50	DL 90	DL 99	Slope.SE	Chi²	p
Nepeta	L4	0.0015	0.0096	0.0343	0.778	9.9187	0.9451 ± 0.0762	92.5531	0
	L5	0.0019	0.0144	0.0584	1.7819	28.9243	0.8632 ± 0.0706	73.0791	0
Eucalyptus	L4	0.0025	0.0142	0.0468	0.8597	9.2315	1.0135 ± 0.076	82.8555	0
	L5	0.0037	0.0228	0.0797	1.7035	20.6754	0.9637 ± 0.0716	74.435	0
Thyme	L4	0.0067	0.0319	0.0943	1.3336	11.5578	1.114 ± 0.0746	59.4433	0.0016
	L5	0.0144	0.0655	0.1871	2.439	19.7808	1.1493 ± 0.0741	53.1143	0.008

Table 4. Lethal Time (LT) for 4th and 5th instar *E. olivina* treated with different concentrations of *E. globulus*; *S. calamintha nepeta* and *T. vulgaris*.

Plant	Larva	LT25	LT50	LT75	LT90	Slope.SE	Chi²	P
Nepeta	**L4**	7.8371	22.893	66.8731	175.493	1.4488 ± 0.2016	363.8908	0
	L5	9.2422	29.8966	96.7086	278.1894	1.3229 ± 0.1977	304.0891	0
Eucalyptus	**L4**	8.0273	25.0976	78.4682	218.9096	1.3624 ± 0.1995	363.7047	0
	L5	10.2204	33.9148	112.5411	331.2508	1.2948 ± 0.197	325.3221	0
Thyme	**L4**	9.2047	35.399	136.1362	457.5891	1.153 ± 0.1962	359.6965	0
	L5	14.2171	58.6425	241.8873	865.9501	1.096 ± 0.1975	349.4	0

Table 5. Lethal Time (LT) in day (mL/µL) values of *Eucalyptus globulus, Thymus vulgaris* and *Satureja calamintha nepeta* essential oils against *Euphyllura olivina*.

	Dose	LT25	LT50	LT75	LT90	Slope.SE	Chi²	P
	0.015	25.4578	69.2305	188.2675	463.2536	1.5524 ± 0.5013	1.1322	0.8891
	0.025	19.9182	55.8223	156.4462	395.5312	1.507 ± 0.491	1.4822	0.8298
	0.05	15.4567	46.9016	142.3175	386.4887	1.3992 ± 0.485	2.0781	0.7214
	0.075	15.0416	38.6113	99.1137	231.5389	1.6474 ± 0.4872	2.3373	0.674
	0.1	12.7313	28.3521	63.1388	129.7919	1.9398 ± 0.4983	2.5023	0.6442
Eucalyptus	0.15	12.0596	25.2466	52.8537	102.7707	2.1021 ± 0.5098	2.0554	0.7256
	0.25	10.5106	21.1302	42.4796	79.6413	2.224 ± 0.5324	2.4024	0.6622
	0.5	8.3422	16.4275	32.3489	59.529	2.2919 ± 0.5785	4.5311	0.3389
	0.7	7.0542	14.8197	31.1338	60.729	2.0921 ± 0.576	6.9385	0.1392
	1	6.9761	12.004	20.6557	33.6658	2.8615 ± 0.8234	5.9964	0.1994
	2	4.9554	8.344	14.05	22.4572	2.9805 ± 1.2961	1.9336	0.748
	0.015	32.5158	98.5893	298.9273	811.2278	1.4001 ± 0.5158	0.8564	0.9307
	0.025	22.6015	75.5081	252.2614	747.0312	1.2875 ± 0.4958	1.3449	0.8537
	0.05	19.6183	54.5026	151.4165	379.8108	1.52 ± 0.4905	1.6398	0.8016
	0.075	18.3299	44.5475	108.2643	240.7648	1.7489 ± 0.4902	1.7786	0.7764
	0.1	14.7582	33.3074	75.171	156.3938	1.908 ± 0.4929	3.7453	0.4416
Nepeta	0.15	12.9739	28.4971	62.5935	127.0856	1.9738 ± 0.499	3.1181	0.5383
	0.25	10.9867	22.233	44.9915	84.8514	2.2033 ± 0.5254	3.0291	0.553
	0.5	7.6668	16.5803	35.8567	71.7897	2.0135 ± 0.5495	4.8568	0.3023
	0.7	7.0542	14.8197	31.1338	60.729	2.0921 ± 0.576	6.9385	0.1392
	1	8.5533	13.952	22.7581	35.3503	3.1741 ± 0.823	3.8996	0.4198
	2	2.3716	4.6852	9.2558	17.0822	2.2811 ± 1.3104	2.3513	0.6714
	0.015	67.517	167.618	416.1297	943.3182	1.708 ± 0.6217	4.4306	0.3509
	0.025	51.9996	118.7737	271.2941	570.5577	1.8803 ± 0.5843	2.3743	0.6673
	0.05	30.5415	112.7946	416.5682	1350.0869	1.1887 ± 0.5106	4.5774	0.3335
	0.075	22.6978	83.2266	305.1695	982.6745	1.1953 ± 0.4963	4.3546	0.3601
	0.1	17.5092	62.0251	219.7195	685.8929	1.2279 ± 0.4876	4.3808	0.3569
Thyme	0.15	14.0317	41.6942	123.8915	330.1607	1.4261 ± 0.4842	0.8428	0.9326
	0.25	9.5915	27.5654	79.222	204.8767	1.4711 ± 0.4884	2.7876	0.594
	0.5	8.6927	21.3404	52.3903	117.5726	1.7292 ± 0.5069	2.6411	0.6196
	0.7	6.9991	15.8193	35.7547	74.4863	1.9046 ± 0.5465	4.1917	0.3807
	1	5.4803	11.8203	25.4948	50.9219	2.0205 ± 0.6133	6.6258	0.157
	2	5.1737	9.3592	16.9306	28.8651	2.62 ± 0.9009	2.4255	0.658

(73.97%, 72.37% and 93.32% respectively). The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. The GC–MS analyses of *S. calamintha nepeta* EO identified 38 constituents. The toxic effects of *S. calamintha nepeta* could be attributed to major constituents such as Pulegone (38.75%), piperitone (7.10%), aromadendrene (3.69%) and p-menthone (2.96%) which were the most abundant chemical compounds. The chromatographic analysis of the essential oil *E. globulus* by GC/MS allowed the recording of 27 compounds. The majority of the compounds in this oil are Para-cymene (22.76%), Cryptone (11.13%), Spathulenol (10.13%), Eucalyptol (9.45%) and Carvacrol (3.36%). This composition is different from previously published data for the same species with a rate of (64.80%). Chromatographic analysis conducted on *T. vulgaris* essential oil with 18 compounds, consists mainly of a high rate of carvacrol (43.30%), thymol (20%), para-cymene (11.55%) and y-Terpinene (9.17%).

DISCUSSION

The leaves of *Satureja calamintha nepeta* and *Thymus vulgaris* produce more essential oil than that of *Eucalyptus globulus*. Indeed, *S. calamintha nepeta* showed an extraction yield of 1.63%; *T. vulgaris* showed a remarkable yield of 0.94%; on the other hand, *E. globulus* oil has the lowest yield of 0.2%. This result is fairly close to that obtained by Hassani et al. (2017) [21] in Algeria, who obtained by hydrodistilation method a fairly high yield in *T. vulgaris* with 1,8%. Comparable yields are also recorded in samples of *S. calamintha nepeta* from different regions of Algeria and Morocco [7, 30]. On the other hand, the essential oil yield of *E.globulus* which is 0.2% is very low compared to that obtained by the essential oil yields in the values found in the literature, but according to Russo et al. (2015) [51], there are no significant differences in the essential oil yield of young and mature leaves of *E. globulus*. These differences are likely due to several factors including:

geographical origin, ecological factors includeing climatic factors (temperature and humidity), the plant species itself, the plant organ, the stage of growth, the picking period, conservation of plant material and extraction methods, even genetic factors [4, 10, 16]. Due to their high volatility, essential oils and their constituents exert insecticidal effects and can reduce or disrupt the growth of the insect. In the current study, the chromatographic analysis of the three plants tested showed the presence of important quantities of chemicals compounds. The toxic effects of *T. vulgaris* could be attributed to major constituent such as carvacrol (43.30%), and thymol (20%). This monoterpenoid phenol has very interesting biological effects [18, 54]. Other authors conclude that the powder of *Santoline chamayciparisus* and *Thymus vulgaris* at doses of 0.5 and 1 g play a very important role as a bio insecticide to test their biocidal effect on different biological parameters studied on *C. chinensis*; they had life expectancy, low fertility and a longer life cycle compared to the control [47, 48]. The essential oil doses of LC 10, LC 30 and LC 50 were estimated to

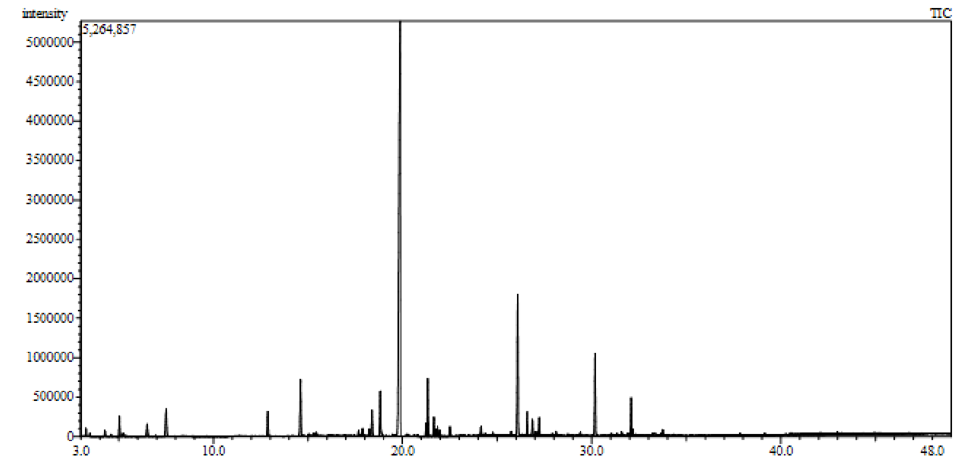

Figure 1. GC-MS chromatogram of *Satureja calamintha nepeta* essential oil.

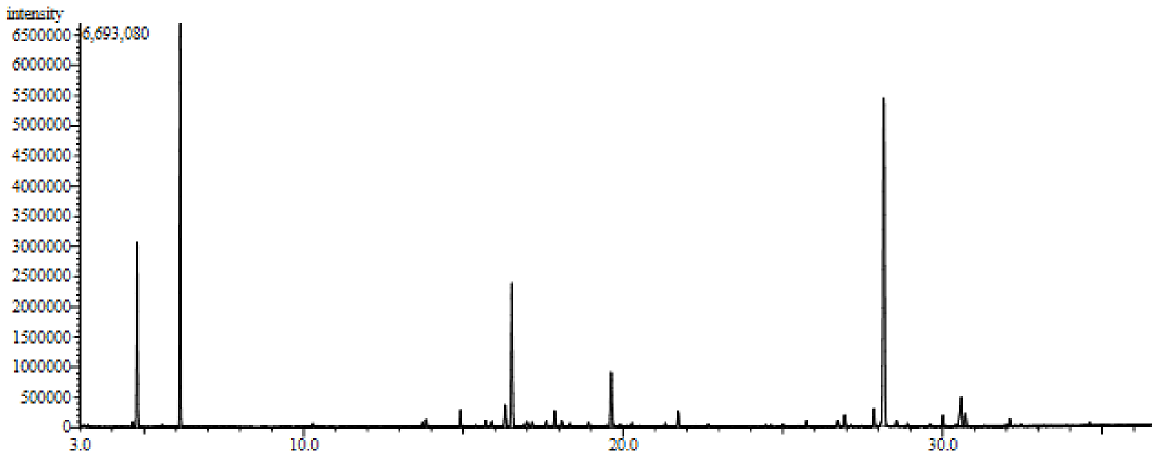

Figure 2. GC-MS chromatogram of *Eucalyptus globulus* essential oil.

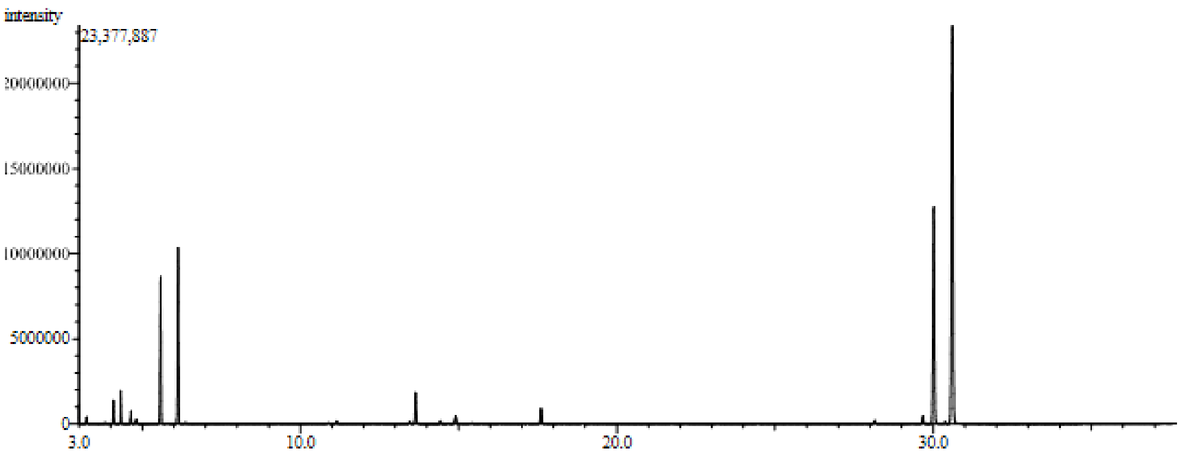

Figure 3. GC-MS chromatogram of *Thymus vulgaris* essential oil.

be 0.107%, 0.188% and 0.279% for *T. vulgaris*, on the feeding efficiency of 4th instar larva of *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae); was more toxic [55] In a previous study, the LC_{50} values of *T. vulgaris* was recorded as 57.48 mg/L against *P. shantungensis* nymphs using the leaf dipping bioassay, and 75.80 mg/L against *P. shantungensis* adults using the spray bioassay method. Regarding volatile components identified in *T. vulgaris* oil, the LC_{50} values of carvacrol and thymol using the leaf dipping bioassay against *P. shantungensis* nymphs were 56.74 and 28.52 mg/L, respectively [44]. This EO was previously assessed by Nabti and Bounechada (2019) [42] against the *Culiseta longiareolata* Macquart. Larvae (Diptera: Culicidae) larvae; however, its toxicity against *C. longiareolata* (LC50=25.64 ppm, LC90=50.53 ppm) was more than that shown by our *T. vulgaris* EO. Conversely, *T. vulgaris* essential oil showed a lower potency in the larval toxicity test compared to the other two plants tested. This can be explained by its richness in highly volatile monoterpenes such as pulegone, cryptone, Menthone and 1,8-cineole as reported in many aromatic plants [24]. The main compounds of the thyme oil are oxygenated monoterpens thymol, carvacrol, and linalool, and non-oxygenated monoterpene p-cymene [27]. The essential oil of *S. calamintha nepeta* was the most effective against the 4th and 5th larval instars of *E. olivina*. indeed, the higher insecticidal potential of *S. calamintha nepeta* could be attributed to the high levels of pulegone, menthone and menthol, three oxygenated monoterpenes well known for their high insecticidal activity [6] .Concerning the essential oil of Satureja the lethal effect on olive psyllid is due to the important toxic effect of the major compounds pulegone. Our results are similaire to [8, 9], who noted that the chemical composition of the essential oil of the species of Serbian origin contains predominantly pulegone (75.5%) by comparison with that of Morocco, Ech–chahad et al. (2013) [16], noted the dominance of borneol (34.52%). Toxicity and repellency of three Algerian medicinal plants against pests of stored product: *Ryzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) showed that the essential oil of *S. calamintha nepeta* has a highly significant biocidal effect with a 100 % mortality at a concentration of 5 µL (F Cal = 3.49 et F Théo = 0.61) even the first hour of treatment [49]. The results obtained in our work showed a high efficiency of *E. globulus* essential oil. This later had one of the major repellent activities on *E. olivina*. The essential oil of *E. globulus* is known to have bio insecticides, repellents and antiappetizing [20,

Table 6. Chemical composition (%) of *Satureja calamintha nepeta*, *Eucalyptus globulus* and *Thymus vulgaris* essential oils

Satureja calamintha nepeta		***Eucalyptus globulus***		***Thymus vulgaris***	
Composition	**(%)**	**Composition**	**(%)**	**Composition**	**(%)**
Pulegone	38.75	Para-cymene	22.76	Carvacrol	43.3
Piperitone	7.1	Eucalyptol	9.45	Thymol	20
Borneol	2.64	Cryptone	11.13	Para-cymene	11.55
P-Menthone	2.96	Aromadendrene	10.13	y-Terpinene	9.17
Para-cymene	1.52	Carvacrol	3.36	Linalool	2.46
Isomenthol	2.63	Pulegone	0.59	α-Terpinene	1.73
Aromadendrene	3.69	Pinocarveol « trans »	1.66	Borneol	1.31
Carvacrol	1.84	Phellandral	1.37	β-Pinene	1.28
Limonene	0.92	Benzaldehyde	4.91	Limonene	0.7
y-Terpinene	0.68	4-Isopropylphenol	1.07	Terpinen	0.71
Eucalyptol	0.24	Thujone	0.4	Caryophyllene	0.28
β-Pinene	0.27	Globulol	1.14	Aromadendrene	0.31
3-Octanol	1.2	Trans-piperitol	0.46	4-Thujanol	0.24
D-Germacrene	0.68	Spathulenol	0.45	D-Carvone	0.06
Piperitenone-oxide	0.8	Pinocarvone	0.37	Isospathulenol	0.03
Delta- Cadimene	0.71	Limonene	0.31	Verbenol	0.1
Caryophyllene	1.36	Terpinen-4-ol	1.28	Pelargol	0.01
D-Carvone	0.32	β-Pinene	0.12	Deta-Cadinene	0.08
Naphthalenol	0.36	Bicyclopropyl	0.23		
Isospatholenol	0.16	y-Terpinene	0.17		
α-Elemol	0.22	α-Terpineol,dihydro	0.06		
Dehydroxy-isocalamindiol	0.27	1,2-Expoxylinalool	0.12		
Sabinene	0.12	Isomenthone	0.08		
α-Terpinene	0.1	Lanceol « Cis »	0.27		
4-Thujanol	0.13	Neodihydro-carveol	0.29		
P-Mentha-3.8-diene	0.15	Campholaldehyde	0.07		
Camphor	0.1	α-Fenchol	0.12		
Linalool	0.24				
α-Gurjunene	0.09				
Trans-Carveol	0.09				
Humulene	0.12				
Verbenol	0.09				
D-Germacrene	0.68				
Myrtenol	0.11				
Caryophyllene-oxide	1.18				
1.2-Campholide	0.24				
Bicyclogermacrene	0.47				
Piperitenone-oxide	0.83				

51, 52]. It also has a strong repellent action against two stored grain insect pests *Tribolium castaneum* and *Sitophilus oryzae* [39], and low repellent activity against *Aedes aegypti* and larvae of *Cydia pomonella* [53]. The contact toxicity observed in the essential oil of *E. globulus* can be attributed to the richness of para-cymene, Spathulenol, cryptone and eucalyptol. The insecticidal properties of *E. globulus* essential oil on psylla is poorly documented. Under laboratory conditions, *E. globulus* is reported to have insecticidal activity on aphids: *Aphis nerii, Aphis gossypii* and *Aphis fabae* [20, 36, 52], on stored insect pests *T. castaneum* and *Tribolium confusum* [14, 50] and even an acaricide action against *Tetranychus urticae* [3]. Recently, Russo et al. (2018) [52] also showed the insecticidal properties of essential oil against *A. nerii*, after 30 min of exposure, treatment with 0.20 $\mu L/cm^2$ had 35% dead adults, while all insects died at the highest concentrations (0.15 and 0.20 $\mu L/cm^2$) at 24 and 48 hours, respectively. The same authors found a LD50 value of 0.099 $\mu L/cm^2$ on filter discs at 12 h exposure under laboratory conditions. In open field testing, Mousa et al. (2013) [40] also showed that *E. globulus*, the 3% diluted oil, is effective in reducing by 80.66% the populations of two aphids species *Macrosiphum sp*. and *Aphis craccivora* in Faba bean fields. The essential oil of *Eucalyptus globulus* grown in Dehradun, India has shown that the dominant compounds being Eucalyptol (1,8- cineole) 54.79%, α-pinene 18.54% α-pinene 11.46 %, α-eudesmol 4.68%, α-phellandrene 2.06% Para cymene 1.60% and Gamma-eudesmol 1.20% [28]. In this study, the results justify the repellent and biocidal potential of the three plants studied against the 4th and 5th larval instar of the olive psylla. The EOs extracted from the aromatic medicinal plants and their principal components may serve as safe products to control *Euphyllura olivina* larvae in Algeria; nevertheless, their practical application remains a fundamental step to evaluate their field efficacy and to note their possible secondary effects on non-targeted organisms.

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REFERENCES

[1] Abbott, W.S., (1925): A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

[2] Abou-Kaf, N., Hamoudi, O., (1999): Evaluation of damage caused by Olive psylla *Euphyllura straminea* Loginova (Homoptera: Aphalaridae) in Syria. Arab Journal of Plant Protection, 17(2): 71-76.

[3] Afify, A.M., Ali Fatma, S., Turky, A.F., (2012): Control of *Tetranychus urticae* Koch by extracts of three essential oils of chamomile, marjoram and Eucalyptus. Asian Pacific Jouranl of Tropical Biomedicine, 2: 24-30.

[4] Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., (2008): Biological effects of essential oils–a review. Food and Chemical Toxicology, 46(2): 446-475.

[5] Bene, G., Gargani, E., Landi, S., Del Bene, G., (1997): Observations on the life cycle and diapause of *Euphyllura olivina* (Costa) and *Euphyllura phillyrea* Foerster (Homoptera Aphalaridae). Advanced in Horticultural Sciences, 11(1): 10-16.

[6] Boudjema, K., Bouanane, A., Gamgani, S., Djeziri, M., Mustapha, M.A., Fazouane, F., (2018): Phytochemical profile and antimicrobial properties of volatile compounds of *Satureja calamintha* (L) Scheel from northern Algeria. Tropical Journal of Pharmaceutical Research, 17(5): 857-864.

[7] Bouzidi, N., Mederbal, K., Bouhadi, D., (2018): Chemical composition of the essential oil of *Satureja calamintha* sub sp. *Nepeta* of west Algerian. Moroccan Journal of Chemistry, 6(2): 213-217.

[8] Butnariu, M., Sarac, I., Pentea, M., Samfira, I., Negrea, A., Motoc, M., Buzatu, A.R., Ciopec, M., (2016): Approach for analyse stability of Lutein from *Tropaeolum majus*. Revista de Chimie, 67(3): 503-506.

[9] Butu, A., Rodino, S., Golea, D., Butu, M., Butnariu, M., Negoescu, C., Dinu-Pirvu, C.E., (2015): Liposomal nanodelivery system for proteasome inhibitor anticancer drug bortezomib. Farmacia, 63(2): 224-229.

[10] Caunii, A., Butu, M., Rodino, S., Motoc, M., Negrea, A., Samfira, I., Butnariu, M., (2015): Isolation and separation of Inulin from *phalaris arundinacea* Roots. Revista de Chimie, 66(4): 472-476.

[11] Chafaa, S., (2013): Contribution à l'étude de l'entomofaune de l'olivier, *Olea europaea* et de la dynamique des populations de la cochenille violette *Parlatoria oleae* Colvée, 1880 (Homoptera: Diaspididae) dans la région de Batna. Doctoral thesis. National Superior School of Agronomy, El-Harrach, Algeria.

[12] Chafaa, S., Mimeche, F., Chenchouni, H., (2019): Diversity of insects associated with olive (Oleaceae) groves across a dryland climate gradient in Algeria. Canadian Entomologist, 151: 629-647.

[13] Chun-xue, Y., Zhang, W.J., Shan-shan, G., Chengfang, W., Kai, Y., Jun-yu, L., Ying, W., Zhu-feng, G., Shu-shan, D., Zhi-wei, D., (2015): Chemical composition of essential oils extracted from six Murraya species and their repellent activity against *Tribolium castaneum*. Industrial Crops and Products, 76: 681-687.

[14] Ebadollahi, A., (2011): Antifeedant activity of essential oils from *Eucalyptus globulus* Labill. and *Lavandula stoechas* L. on *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Biharean Biologist, 5: 8-10.

[15] Ebadollahi, A., (2013): Essential oils isolated from Myrtaceae family as natural insecticides. Annual Research and Review in Biology, 3: 148-175.

[16] Ech–chahad, A., Farah, H., Bouyazza, L., (2013): Composition chimique de l'huile essentielle de *Satureja calamintha* (L.). Afrique Science, 3: 77-81.

[17] Fabbri, A., Lambaradi, M., Tokatli, Y.O., (2009): Olive breeding in breeding plantation tree crops: tropical species. Springer, New York, United States of America, 423-465.

[18] Gokturk, T., Saban Kordali, S., Ak, K., Kesdek, M., Bozhuyuk, A.U., (2020): Insecticidal effects of some essential oils against *Tribolium confusum* (du Val.) and *Acanthoscelides obtectus* (Say), (Coleoptera:

Tenebrionidae and Bruchidae) adults. International Journal of Tropical Insect Science, 7 p.

[19] Guessab, A., Elouissi, M., Lazreg, F., Elouissi, A., (2021): Population dynamics, seasonal fluctuations and spatial distribution of the olive psyllid *Euphyllura olivina* Costa (Homoptera, Psyllidae) in Algeria. Arxius de Miscellània Zoològica, 19: 183–196.

[20] Harizia, A., Benguerai, A., Boukhari, Y., (2020): Toxicity and repellency of *Eucalyptus globulus* L. essential oil against *Aphis fabae* Scopoli, 1763 (Homoptera : Aphididae). Journal of Entomological Research, 44(1): 147-152.

[21] Hassani, A., Sehari, N., Sehari, M., Bouchenafa, N., Labdelli, F., Kouadria, M., (2017): Etude des propriétés insecticides et bactéricides de l'huile essentielle de *Thymus vulgaris* L. dans la lutte contre les ravageurs des semences et denrées stockées. Revue Ecologie-Environnement, 13: 5-11.

[22] Hlina, B.L., Birceanu, O., Robinson, C.S., Dhiyebi, H., Wilkie, M.P., (2019): The relationship between thermal physiology and lampricide sensitivity in larval sea lamprey (*Petromyzon marinus*). Journal of Great Lakes Research, 47(1): 272-284.

[23] Hmimina, M., (2009): Les principaux ravageurs de l'olivier, la mouche, la teigne, le psylle et la cochenille noire. Bulletin Mensuel D'Information Et De Liaison du PNTTA, 4 p.

[24] Hosseinzadeh, S., Kukhdan, A.J., Hosseini, A., Armand, R., (2015): The application of *Thymus vulgaris* in traditional and modern medicine: a review. Global Journal of Pharmacology, 9: 260-266.

[25] Hougardy, E., Wang, X., Hogg, B.N., Johnson, M.W., Daane, K.M., Pickett, C.H., (2020): Current distribution of the olive psyllid, *Euphyllura olivina*, in California and initial evaluation of the Mediterranean parasitoid, *Psyllaephagus euphyllurae* as a biological control candidate. Insects, 11(3): 146-157.

[26] Isman, M.B., (2006): Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, 51: 45-66.

[27] Jevremović, S., Lazarević, J., Kostić, M., Krnjajić, S., Ugrenović, V., Radonjić, A., Kostić, I., (2019): Contact application of Lamiaceae botanicals reduces bean weevil infestation in stored beans. Archives of Biological Sciences, 71: 665-676.

[28] Joshi, A., Sharma, A., Bachheti, R.K., Pandley, D.P., (2016): A comparative study of the chemical composition of the essential oil from *Eucalyptus globulus* growing in Dehradun (India) and around the world. Oriental Journal of Chemistry, 32(1): 331-340.

[29] Kaplan, M., Alaserhat, İ., (2020): Population development, bio-ecology and damage of *Euphyllura straminea* Log. (Hemiptera: Psyllidae) causing damage in olive orchards. Erwerbs-Obstbau, 62(1): 97-106.

[30] Kerbouche, L., Hazzit, M., Baaliouamer, A., (2013): Essential oil of *Satureja calamintha subsp. nepeta* (L.) Briq. from Algeria: analysis, antimicrobial and antioxidant activities, TBAP. Journal of Biologically Active Products from Nature, 3(4): 266-272.

[31] Keçecioglu, E., (1984): Identification of the olive cotton-bit *Euphyllura olivina* (Costa) (Homoptera: Aphalaridae) which is damaged on olives in Antalya province and around, its short biology and natural enemies. Publications of General Directorate of Agricultural Struggle and Agricultural Quarantine, Ministry of Agriculture of Turkey, Volume 1, Ankara. pp.

[32] Kesdek, M., Kordali, Ş., Usanmaz Bozhüyük, A., Güdek, M., (2020): Larvicidal effect of *Achillea biebersteinii* Afan. (Asteraceae) essential oil against larvae of pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae). Turkish Journal Agriculture and Forestry, 44: 451-460.

[33] Keyhanian, A.A., Taghadossi, M.V., Farzaneh, A., (2000): Biology and seasonal population fluctuations of the olive psylla (*Euphyllura olivina* Costa.) (Hom., Aphalaridae) in Tarom Oulia of the Zandjan Province. Applied Entomology and Phytopathology, 67(1&2): 78.

[34] Kherroubi, M., Zerrouk, I.Z., Rahmoune, B., Zaidat, S.A.E., Messadi, O., Mouhouche, F., (2021): Evaluation of the potential insecticide activity of three plants essential oil against the chickpea seeds beetles, *Callosobruchus maculatus*. Analele Universităţii din Oradea, Fascicula Biologie, 28(1): 97-102.

[35] Laoudi, T., (2014): Bioécologie du psylle de l'olivier *Euphyllura olivina* (Hemiptera : Aphalaridae) Costa 1839 et l'entomofaune de l'olivier dans quatre oliveraies de la région de Tizi-Ouzou. Magister Sciences Biologiques, 89 p.

[36] Mareggiani, G., Russo, S., Rocca, M., (2008): *Eucalyptus globulus* (Mirtaceae) essential oil: efficacy against *Aphis gossypii* (Hemiptera: Aphididae), an agricultural pest. Revista Latinoamericana de Química, 36: 16-21.

[37] Meftah, H., Boughdad, A., Bouchelta, A., (2014): Infestation et cycle biologique d'*Euphyllura olivina* Costa (Homoptera, Psyllidae) au centre du Maroc. Science Lib Éditions Mersenne, 6: 1-25.

[38] Mestar, N.G., Malika N. Boudiaf, M.N., Lahcene, S., Abbaci, H., Aiche, G.I., Metna, B., Saadoun, N.S., Taibi, F., Houali, K., (2018): Bio-insecticidal effects of oleaster leaves aqueous extracts against psylla larvae (*Euphyllura olivina* (Costa)), a primary pest of *Olea europaea* L. Cellular and Molecular Biology, 64(15): 35-40.

[39] Mishra, B.B., Tripathi, S.P., Tripathi, C.P.M., (2012): Repellent effect of leaves essential oils from *Eucalyptus globulus* (Mirtaceae) and *Ocimum basilicum* (Lamiaceae) against two major stored grain insect pests of Coleopterans. Nature and Science, 10: 50-54.

[40] Mousa, K.M., Khodeir, I.A., El-Dakhakhni, T.N., Youssef, A.E., (2013): Effect of garlic and eucalyptus oils in comparison to organophosphat insecticides against some piercing-sucking faba bean insect pests and natural enemies populations. Egyptian Academic Journal of Biological Sciences, 5: 21-27.

[41] Mozaffari, F., Abbasipour, H., Sheikhi Garjan, A., Saboori, A., Mahmoudvand, M., (2013): Toxicity and oviposition deterrence and repellency of *Mentha pulegium* (Lamiacaeae) essential oils against *Tetranychus urticae* Koch (Teteranychidae). Taylor and Françis, 16(5): 575-581.

[42] Nabti, I., Bounechada, M., (2019): Larvicidal activities of essential oils extracted from five algerian medicinal plants against *Culiseta longiareolata* Macquart. Larvae (Diptera: Culicidae). European Journal of Biology, 78(2): 1-6.

[43] Ouvrard, D., (2022): Psyl'list – The World Psylloidea Database. http://www.hemipteradatabases.com/psyllist - searched on 17 Janvier 2022.

[44] Park, J.H., Jeon, Y.J., Lee, C.H., Chung, N., Lee, H.S., (2017): Insecticidal toxicities of carvacrol and thymol

derived from *Thymus vulgaris* Lin. against *Pochazia shantungensis* Chou & Lu., newly recorded pest. Scientific Reports, 7: 40902.

[45] Pavela, R., (2015): Essential oils for the development of eco-friendly mosquito larvicides: A review. Industrial Crops and Products, 76: 174-187.

[46] Percy, D.M., Rung, A., Hoddle, M.S., (2012): An annotated checklist of the psyllids of California (Hemiptera: Psylloidea). Zootaxa, 3193: 1-27.

[47] Righi, A.F., Kkelil, M.A., Medjdoub-Bensaad, F., Righi, K., (2010): Efficacy of oil and powder of some medical plants in biological control of the pea weevil (*Callosobruchus chinensis* L.). African Journal of Agricultural Research, 12: 1474-1481.

[48] Righi, A.F., Righi, K., Khelil, A.M., Pujade-Villar, J., (2014): Biological control against the cowpea weevil (*Callosobruchus Chinensis* L., Coleoptera: Bruchidae) using essential oils of some medicinal plants. Journal of Plant Protection Research, 3: 211-217.

[49] Righi, K., Righi, A.F., Boubkeur, A., Boungab, K., Elouissi, A., Djendara, A.C., (2018): Toxicity and repellency of three Algerian medicinal plants against pests of stored product: *Ryzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae). Banat's Journal of Biotechnology, 17: 50-59.

[50] Rolli, K., (1974): Plant protection in Tunisian olive groves. Z Pflanzenkrankh Pflanzenschutz, 81(12): 705-710.

[51] Russo, S., Cabrera, N., Chludil, H.D., Yaber Grass, M.A., Leicach, S.R., (2015): Insecticidal activity of young and mature leaves essential oil from *Eucalyptus globulus* Labill. against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). Chilean Journal of Agricultural Research, 75(3): 375-379.

[52] Russo, S., Yaber Grass, M.A., Fontana, H.C., Leonelli, E., (2018): Insecticidal activity of essential oil from *Eucalyptus globulus* against *Aphis nerii* (Boyer) and *Gynaikothrips ficorum* (Marchal). Agriscientia, 35: 63-67.

[53] Trongtokit, Y., Rongsriyam, Y., Komalamisra, N., Apiwathnasorn, C., (2005): Comparative repellency of 38 essential oils against mosquito bites. Phytotherapy Research, 19: 303-309.

[54] Vardanian, A., Kurzbaum, E., Farber, Y., Butnariu, M., Armon, R., (2018): Facilitated enumeration of the silicate bacterium *Paenibacillus mucilaginosus* comb. nov. (formerly *Bacillus mucilaginosus*) via tetrazolium chloride incorporation into a double agar-based solid growth medium. Folia Microbiologica, 63(3): 401-404.

[55] Yazdani, E., Sendi, J.J., Hajizadeh, J., (2014): Effect of *Thymus vulgaris* L. and *Origanum vulgare* L. essential oils on toxicity, food consumption, and biochemical properties of lesser mulberry pyralid *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). Journal of Plant Protection Research, 54(1): 53-61.

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