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Evaluation of Toxicity of Origanum Vulgare, *Salvia rosmarinus* and *Salvia officinalis* Essential Oils on *Aculops lycopersici* in Laboratory Tests

Thomas Giordano [1], Giuliano Cerasa [1], Ilaria Marotta [1], Mauro Conte [1], Santo Orlando [1], Adele Salamone [2], Michele Massimo Mammano [2], Carlo Greco [1,*] and Haralabos Tsolakis [1,*]

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Abstract: The tomato russet mite (TRM) *Aculops lycopersici*, a destructive pest of tomato crops worldwide, is a significant challenge to growers, both in greenhouse and open-field conditions. Traditional chemical control methods are often ineffective and set up resistance and adverse environmental impact. This has prompted the exploration of alternative control methods, such as biological control and eco-friendly botanical pesticides. In this study, the acaricidal effects of essential oils (EOs) extracted by three officinal plants, *Origanum vulgare* L., *Salvia rosmarinus* Spenn., and *Salvia officinalis* L., cultivated using precision aromatic crop (PAC) techniques was evaluated against *A. lycopersici* under laboratory conditions. The chemical composition of EOs was ascertained by a solid-phase microextraction (SPME) coupled with mass spectrometry (GC-MS) analyses. Carvacrol (83.42%), ϱ-Cymene (3.06%) and γ-Terpinene (2.93%) were the major components of *O. vulgare*, while α-Pinene (28.0%), 1,8-Cineole (11.00%) and Borneol (7.72%) were present in *S. rosmarinus*. 1,8-Cineole (27.67%) was the major constituent of *S. officinalis* EO, followed by Camphor (21.91%) and Crisantenone (12.87%). Multiple concentrations (320–5000 μL L^{-1}) and exposure times (1 to 4 days) were tested to assess mortality rates. Results showed both dose and time-dependent toxic activity with significant differences among EOs. Oregano EO was found to be the most toxic of the EOs (90% of mortality at 0.5% w/v concentration after 4 days), while rosemary and sage EOs showed limited effects (46% and 42% for the latter EOs respectively). The lethal concentration (LC_{50}) values were 2,228 μL L^{-1} for oregano, 5,835 μL L^{-1} for rosemary, and 6,013 μL L^{-1} for sage, demonstrating efficacy similar to commercially available botanical pesticides. These findings support the potential of *O. vulgare* EO as a viable alternative for controlling *A. lycopersici*, contributing to integrated pest management (IPM) strategies, and highlight the need for further research for discovering botanical agents for an eco-friendly pest control.

Keywords: Medicinal and Aromatic Crops (MAPs); precision agriculture; essential oils; tomato russet mite

1. Introduction

The tomato russet mite (TRM), *Aculops lycopersici* (Tryon, 1917) (Acari: Eriophyidae), is a worldwide distributed pest, present in both tropical and temperate regions [1,2]. It infests mainly solanaceous plants, including wild species like black nightshade *Solanum nigrum* L. as well as cultivated crops such as pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), potato (*Solanum tuberosum* L.), and tomato (*Lycopersicon esculentum* Miller). *Aculops lycopersici* is especially damaging on tomato cultivation, affecting yields in greenhouses and open-field crops. The eriophyid feeds on leaf, stem, and fruit surfaces, resulting in significant cellular disruption. Mite feeding damages the adaxial and abaxial epidermal cells of leaves, causing the development of a dense layer

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of callous tissue near the parenchyma where cell death occurs [3]. The feeding behavior of *A. lycopersici* causes russeted leaves, stems and fruits and, in severe cases, can lead to plant death [4]. Recent studies suggest that *A. lycopersici*, similar to other eriophyid mites, may also transmit virus vector, increasing its overall negative impact on crops [5].

Thriving in warm, arid conditions, *A. lycopersici* populations can escalate rapidly, causing substantial damage. These mites typically cluster and feed where they initially land on the plant, only spreading upward once the population density increase. Infestations remain often undetected until visible symptoms emerge; at this point a large population has already built up, especially on drought-stressed plants that accelerate mite reproduction [6]. Recently, infestations by *A. lycopersici* have surged in European tomato crops, echoing a broader trend of increasing economic impact from eriophyoid mites globally [7].

Synthetic acaricides have long been the standard approach for controlling phytophagous mites; however, the development of resistance, along with harmful effects on native phytoseiid populations, has raised significant concerns [8]. These challenges have prompted increasing interest in biological alternatives for integrated pest management (IPM), as researchers seek more sustainable and environmentally friendly solutions.

Efforts to identify natural enemies for controlling tomato russet mites have led to a substantial list of potential predators, particularly within the Phytoseiidae family [9–13]. Several species of these predatory mites have been observed in natural association with tomato russet mites, able of feeding and reproducing on them, at least under laboratory conditions [14,15]. However, when tested under field conditions, predators often failed to suppress the eriophyid population or required several generations for adapting to tomato plants, limiting their impact towards the pest population [10,11,16–18]. Moreover, the effectiveness of these predators can be further hindered by trichome density and pollen availability [19]. Tomato trichomes hamper predator movement and provide shelter for russet mites, while toxic secondary metabolites of plants and prey can intoxicate the predators, making biological control even more complicated [7,19].

Botanical pesticides are receiving renewed attention as a potentially cost-effective alternative to synthetic chemicals in integrated pest control programs (IPM) [20]. They were commonly used before World War II, but their role diminished with the introduction of synthetic alternatives [20–23].

In contrast, botanical products usually have fewer or less severe negative effects on human health and the environment, support natural enemies of pests, and exhibit low environmental persistence [24]. Among the botanical solutions, essential oils (EOs) and their active compounds have emerged as promising biocides, particularly for their efficacy against arthropod pests, including tephritid flies, ambrosia beetles, mites, ticks, and even certain weeds [25,26]. The large-scale production of plant derived EOs for the perfume and flavouring industries also makes these substances commercially viable [26]. Current research on botanical pesticides frequently investigates plant families such as Apiaceae, Myrtaceae, Lamiaceae, Meliaceae, Annonaceae, Simaroubaceae, and, more recently, Asteraceae [23,27].

The present study aimed to assess the acaricidal effects of essential oils from *Origanum vulgare* L., *Salvia rosmarinus* Spenn., (= syn. *Rosmarinus officinalis* L.) and *Salvia officinalis* L. (Lamiaceae) cultivated using PAC techniques, on *A. lycopersici* under laboratory conditions.

2. Results

2.1. Precision Aromatic Crop (PAC) Techniques

During the spring season, oregano was in its growth phase, while rosemary remained bare and sage exhibited low vigour. On September, oregano had been harvested, leaving bare soil with visible clumps, whereas rosemary displayed medium vegetative vigour and sage reached full vigour. On October, sage maintained full vigour, and rosemary continued developing, with vigour classes increasing to 0.60–0.80 (oregano had resumed growth following the rains). More than 50% of the sage surface showed NDVI values greater than 0.40 (Figures 1 and 2).

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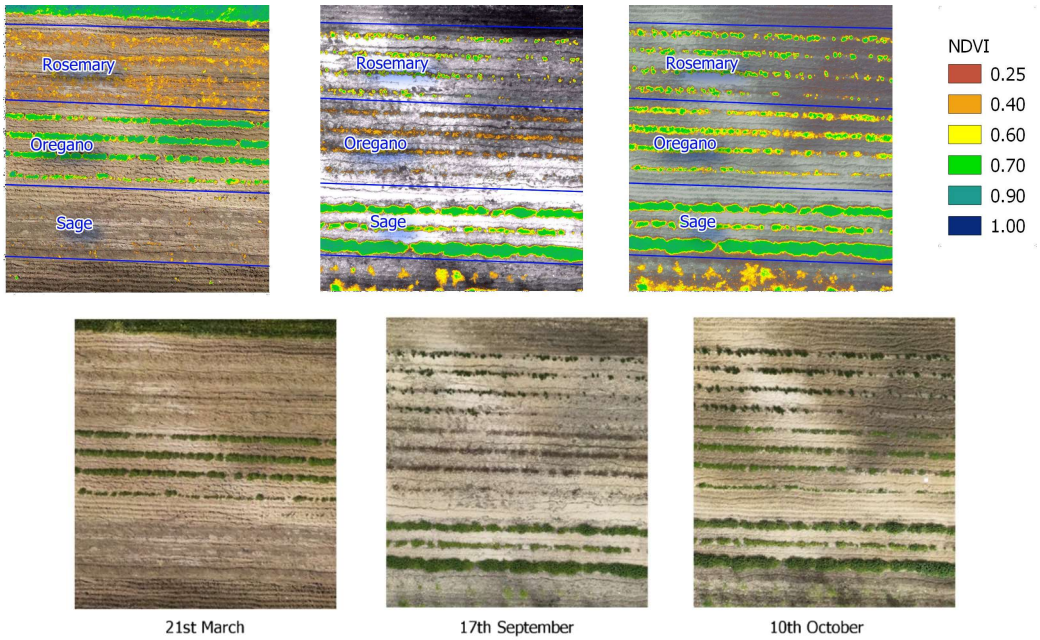

Figure 1. MAPs NDVI values during vegetative period.

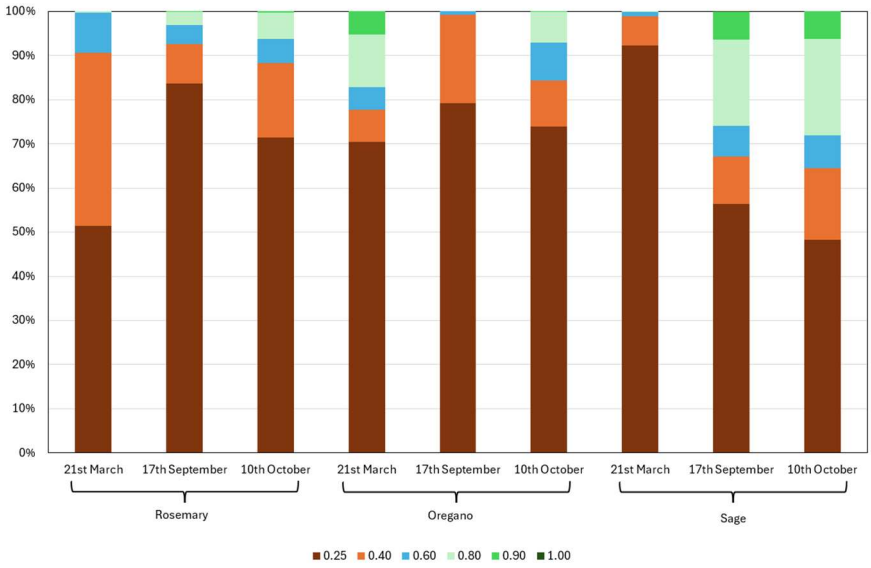

Figure 2. Percentage surface area of rosemary, oregano, and sage based on NDVI classes during the three considered periods.

Multispectral analysis enabled the identification of the optimal harvest period, corresponding to mean NDVI values of 0.85 for rosemary (in May), 0.80 for oregano (in June), and 0.75 for sage (in May and October 2024).

2.2. Analyses of Essential Oils

The essential oils extracted from the three species had a light-yellow colour and a characteristic aroma. The volatile profile of Oregano EO, includes 27 volatile compounds belonging to the following phytochemical groups: monoterpene phenol (84.31%), monoterpene hydrocarbons (9.53%), Oxygenated monoterpenes (1.91%), sesquiterpene hydrocarbons (2.87%), ethers, ketones, and alcohols each contributing less than 1%. The principal component of this EO was Carvacrol, followed by ϱ-Cymene and γ-Terpinene. Nineteen main components representing 89.3% of the Rosemary essential oil were detected: 47.53% of Monoterpene hydrocarbons, 33.37% of Oxygenated

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monoterpenes, and 8.27% of Sesquiterpene hydrocarbons (Table 1). As shown in Table 1, the sage EO yield was 0.29%, a total of 23 volatile compounds were identified by GC-MS. These compounds were categorised into monoterpene hydrocarbons (29.64%), oxygenated sesquiterpenes (2.81%), oxygenated monoterpenes (59.57%), and sesquiterpene hydrocarbons (2.72%). Additionally, other compounds not classified in the previous categories were also identified. Monoterpene hydrocarbons and oxygenated monoterpenes were the most abundant compounds in all sage leaf samples, accounting for more than 90% of the total identified compounds. Among the monoterpene hydrocarbons, camphene and crisantenone were the most prevalent (average of 9.26% and 12.87%, respectively), while eucalyptol and camphor were the dominant compounds among the oxygenated monoterpenes (average of 27.67% and 21.91%, respectively) [28].

Table 1. Percentage of components characterising the essential oils (EOs) of Oregano, Rosemary, and Sage, with their relative weights (RW %).

Component/Chemical Class	Oregano EO (RW%)	Rosemary EO (RW%)	Sage EO (RW%)
Monoterpene phenol			
Carvacrol	**83.42**	-	-
Thymol	0.89	-	-
Subtotal	*84.31*	-	-
Monoterpene hydrocarbons			
ϱ-Cymene	3.06	0.80	-
γ-Terpinene	2.93	2.10	-
β-Myrcene	1.01	1.21	2.19
α-Terpinene	0.99	0.45	-
Limonene	0.45	2.22	-
Terpinolene	0.41	0.53	-
β-Ocimene	0.42	-	-
β-Pinene	0.13	4.1	2.66
Sabinene	0.13	-	-
α-Pinene	-	**28.0**	2.66
Linalyl Acetate	-	1.12	-
Crisantenone	-	-	**12.87**
Camphene	-	**7.00**	**9.26**
Subtotal	*9.53*	*47.53*	*29.64*
Oxygenated monoterpenes			
Linalool	0.32	3.45	-
Camphor	0.27	6.20	**21.91**
Terpinen-4-ol	0.18	-	-
1,8-Cineole (Eucalyptol)	-	**11.00**	**27.67**
Borneol	-	**7.72**	2.59
β-Thujone	-	0.73	-
α-Thujone	-	-	5.32
Bornyl Acetate	-	-	1.45
4-Caranol	-	-	0.09
4-Terpineol	0.31	-	0.54
α-Terpineol	-	4.40	-
α-Thujene	0.23	-	-
α-Phellandrene	0.60	-	-
Subtotal	*1.91*	*33.37*	*59.57*
Sesquiterpene hydrocarbons			
β-Caryophyllene	1.07	6.64	1.46
α-Humulene	0.70	0.75	-
Germacrene	0.07	-	-
β-Bisabolene	0.63	-	-
Farnesene	0.40	-	-
α-Caryophyllene	-	-	0.89
Alloaromadendrene	-	-	0.24

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α-Gurjunene		-	-	0.13
Carophyllene oxide			0.88	
	Subtotal	*2.87*	*8.27*	*2.72*
Oxygenated sesquiterpenes				
Viridiflor		-	-	1.22
Palustrol		-	-	0.75
Ledol		-	-	0.53
Spathulenol		-	-	0.31
	Subtotal	-	-	*2.81*
Other compounds				
Camphol		0.70	-	-
1-Octen-3-ol		0.49	-	-
3-Octanone		0.36	-	-
Carvacrol Methyl Ether		0.03	-	-
Maool		-	-	0.62
Diethyl Phthalate		-	-	0.53
Naphthalene		-	-	0.28
	Subtotal	*1.58*	-	*1.43*

2.3. Toxicity of O. vulgare, S. rosmarinus, and S. officinalis EOs Against A. lycopersici

The three factors compared in the GLM analysis were *EO* (Oregano, Rosemary, Sage), *Concentration* (0, 320, 640, 1280, 2500, 5000) and *Time* (1, 2, 3, 4 days). The three EOs tested affected in a different way *A. lycopersici* adults ($F_{2, 648}$=5.75, p=0.003), and a significant different effect was registered both for the factor *Concentration* ($F_{5, 648}$=124.45, p<0.001) and *Time* ($F_{3, 648}$=61.67, p<0.001). The interaction between the first two factors ($F_{6, 648}$=18.42, P<0.001) and between *Concentration* and *Time* ($F_{15, 648}$=2.14, p=0.007) indicate that each EO concentration caused different toxic effects, and that the mortality had a different trend in the three adopted EOs during the test period.

The highest concentration of Oregano EO (5000 µL L^{-1}) hardly affected *A. lycopersici* after 24 hours (66% of mortality), and high mortality (90%) after 4 days was registered, while it was significantly lower with concentration of 2500 µL L^{-1} and almost null in all the remaining concentrations (Table 2). For Rosemary and Sage, the acaricidal effect remained scarce, with marginal increases during time even at the highest concentrations. No statistical differences were noted between each considered concentration for the latter extracts. As a matter of fact, only the highest concentration of oregano EO can be classified as highly toxic (class 4) according to the toxicity categories proposed by Hardman et al. [29]. At the concentration of 2500 µL L^{-1}, the effect was moderate (class 2), while at lower concentrations (1280, 640, 320 µL L^{-1}) the efficacy was classified as non-toxic (Class 1). Rosemary and Sage EOs caused similar results, falling in classes 1 and 2 (Table 2).

The analysis of mean survival times further corroborated these trends, indicating that at the highest concentration of 5000 µL L^{-1} of oregano EO, the mean survival time of *A. lycopersici* adults was about one day after treatment. A higher survival time was recorded using 2500 µL L^{-1} of the latter EO, although it was statistically lower compared to the control (Table 2). In contrast, survival times at lower concentrations did not differ to those observed in the control tests. This highlights that the acaricidal efficacy of oregano EO is evident only surpassing the critical concentration threshold of 2500 µL L^{-1}.

The lethal concentrations of the three essential oils were calculated by probit analysis. The Pearson goodness of fit test indicated that LC values obtained by oregano EO, does not fit the linear model (χ^2 = 21.62; p< 0.001), because of the high discrepancies of toxic effects registered at the highest dose (90% of mortality) and the lower doses (less than 36% of mortality). On the other hand, LC values calculated for the other two EOs well fitted to the linear model (Table 3).

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Table 2. Susceptibility of adult stages of *Aculops lycopersici* to different concentrations of *Origanum vulgare, Salvia rosmarinus* and *Salvia officinalis.*

Concentrations		Cumulative mortality (%) (mean ± SE)				Survival time (days)	Abbott's corrected mortality	Toxicity class
Plant extracts	**(µL L^{-1})**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**(mean** ± SE)	**(%)**	*****
O. vulgare	5000	66.00 ± 7.92	74.00 ± 8.46	80.00 ± 7.30	90.00 ± 4.47 a	0.90 ± 0.203 a	89.13	4
	2500	14.00 ± 4.27	20.00 ± 2.98	34.00 ± 6.00	36.00 ± 7.77 bc	2.96 ± 0.216 bcd	30.43	2
	1280	0.00 ± 0.00	4.00 ± 2.67	12.00 ± 4.42	22.00 ± 5.54 efg	3.62 ± 0.114 ed	15.22	1
	640	2.00 ± 2.00	4.00 ± 2.67	12.00 ± 3.27	20.00 ± 5.16 ef	3.62 ± 0.124 ed	13.04	1
	320	2.00 ± 2.00	6.00 ± 3.06	10.00 ± 3.33	16.00 ± 4.00 efg	3.66 ± 0.127 ed	8.70	1
	Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	8.00 ± 3.27 fg	3.92 ± 0.038 e	0.00	-
S. rosmarinus	5000	16.00 ± 6.53	36.00 ± 9.33	42.00 ± 9.17	46.00±10.3 b	2.60 ± 0.234 b	46.00	2
	2500	12.00 ± 4.42	24.00 ± 4.99	34.00 ± 4.27	40.00±5.16 bc	2.90 ± 0.214 bcd	40.00	2
	1280	2.00 ± 2.00	12.00 ± 4.42	20.00 ± 5.16	26.00±3.06 de	3.40 ± 0.159 cde	26.00	2
	640	2.00 ± 2.00	16.00 ± 4.99	16.00 ± 4.99	22.00±6.96 e	3.44 ± 0.165 cde	22.00	1
	320	4.00 ± 2.67	6.00 ± 3.06	6.00 ± 3.06	12.00±4.42 efg	3.72 ± 0.128 cd	12.00	1
	Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00±0.00 g	4.00 ± 0.00 e	0.00	-
S. officinalis	5000	12.00 ± 5.33	32.00 ± 9.52	42.00 ± 9.17	42.00 ± 9.17 bc	2.72 ± 0.225 bc	39.58	2
	2500	12.00 ± 6.80	32.00 ± 6.80	40.00 ± 7.30	54.00 ± 8.46 b	2.62 ± 0.216 bc	52.08	3
	1280	10.00 ± 4.47	28.00 ± 6.11	32.00 ± 8.00	34.00 ± 6.70 bcd	2.96 ± 0.218 bcd	31.25	2
	640	0.00 ± 0.00	14.00 ± 4.27	22.00 ± 5.54	22.00 ± 5.54 cde	3.42 ± 0.159 cde	18.75	1
	320	4.00 ± 2.67	16.00 ± 4.00	18.00 ± 4.67	28.00±3.27 bcde	3.34 ± 0.173 bcde	25.00	1
	Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.00±2.67 e	3.96 ± 0.028 e	0.00	-

Different letters indicate significant differences among extracts and concentrations for survival time and corrected mortality. Tukey's multiple comparison tests ($p<0.05$) were applied after GLM analysis. * Toxicity classes were defined on corrected mortality after Hardman et al. [29].

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Table 3. Lethal concentrations of the essential oils of *Origanum vulgare, Salvia rosmarinus* and *Salvia officinalis* against adult stages of *Aculops lycopersici.*

Essential oils	LC_{10} µL L^{-1} (95% CI)	LC_{30} µL L^{-1} (95% CI)	LC_{50} µL L^{-1} (95% CI)	LC_{90} µL L^{-1} (95% CI)	LC_{95} µL L^{-1} (95% CI)	Intercept ± SE	Slope ± SE	Goodness of fit χ^2 *(d.f.)*
Origanum vulgare	369.33 (208.57-530.18)	1,068.17 (799.56-1362.01)	2,228.90 (1740.28-3050.91)	13,451.79 (8015.49-31804.60)	22,391.85 (12084.54-63217.89)	-5.49 ± 0.71	1.64 ± 0.22	21.62 (3) p = 0.000
Salvia rosmarinus	207.16 (33.57-428.25)	1,488.81 (907.55-2451.9)	5,835.39 (3281-22288.8)	164,369.0 (35154.4-10573529.9)	423,447.9 (67507.2-61877106.9)	-3.32 ± 0.67	0.88 ± 0.21	0.53 (3) p = 0.911
Salvia officinalis	26.62 (0.002-146.38)	654.53 (80.06-1292.05)	6,013.81 (2630.4-261607.4)	1,358,595.0 (74342.1-20,379,803,862,870.6)	6,315,387.0 (184,714.1-3,651,741,272,548,380.0)	-2.05 ± 0.61	0.54 ± 0.19	5.73 (3) p = 0.125

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The LC_{50} values were 2228.90 μL L^{-1} for oregano EO, 5835.39 μL L^{-1} for rosemary EO, and 6013.81 μL L^{-1} for sage EO. The probability plot built on probit data indicates a more sensitive response for mortality in oregano EO doses, in comparison to the other two EOs (IQR=0.864). The probability of 50% of mortality was almost the same for rosemary and sage EOs but the different values of IQR (1.526 and 2.478 for the latter EOs respectively) indicate a more immediate effect of rosemary EO than of the sage one (Figure 3).

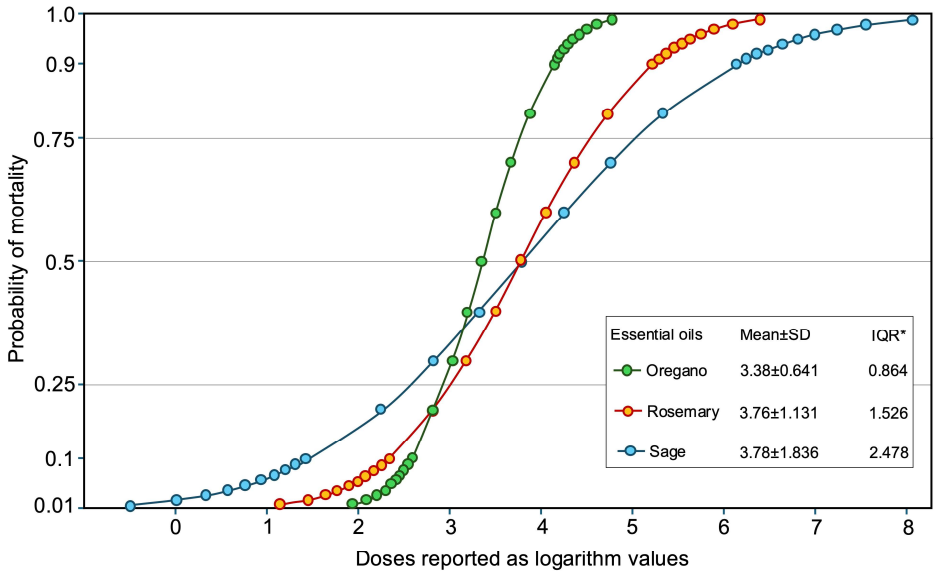

Figure 3. Parametric cumulative failure plot for probability of mortality based on Probit data and maximum likelihood method. * Interquartile range.

3. Discussion

The three Lamiaceae species used in the present study are wide-spread in the Mediterranean basin and used from millennia for their therapeutic and culinary properties [30,31]. Moreover, the three species are included in the BELFRIT list [32], making easier a potential future biopesticide registration.

Oregano EO is mainly composed by carvacrol, thymol and monoterpenes [33]. However, the predominant compound identified in this study, was carvacrol, accounting for about 83% of the total composition. This confirms monoterpenoids as the dominant class of volatile organic compounds (VOCs) in oregano EO. This composition places this EO within the carvacrol oregano chemotype, primarily due to its high carvacrol content (~80%). Similar volatile profiles have been reported for oregano from other Mediterranean regions, suggesting that the essential oil composition is more strongly influenced by the oregano variety than by the geographical origin of cultivation [34].

Recent research has shown that carvacrol, along with other monoterpenoids, is highly toxic to a range of invertebrate pests, including insects, acari, and nematodes [35–37]. Furthermore, these compounds are environmentally friendly, as they biodegrade or dissipate quickly, and exhibit low toxicity to mammals, fish, and other non-target organisms [38]. These properties make monoterpenoids like carvacrol a viable alternative to synthetic pesticides for pest management.

The percentage composition of key components in rosemary EO indicates that α-pinene, 1,8-cineole and borneol are the major constituents, as previously reported [39]. Furthermore, other researchers have noted that each rosemary EO typically contains nine major compounds, collectively

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accounting for over 90% of its composition, with 1,8-cineole being the predominant component (>52%) [40].

From a chemical perspective, rosemary EO can be classified into distinct chemotypes based on the relative abundance of its primary constituents. The main chemotypes identified include *cineoliferum*, characterised by a high 1,8-cineole content, *camphoriferum*, with camphor levels exceeding 20%, and *verbenoniferum*, where verbenone exceeds 15%. Additionally, chemotypes with high levels of α-pinene have been identified in specific regions, including Italy and Morocco [41].

The biological activity of α-pinene has been extensively investigated. In addition to its well-documented antifungal, antibacterial, and antiviral properties, α-pinene exhibits insecticidal and nematocidal effects [42], underscoring its potential for various control activities. According to a review by Jankowska et al. [43], α-pinene was found to be one of the most effective volatile organic compounds (VOCs) in inhibiting acetylcholinesterase (AChE), which may explain the higher entomotoxic activity of rosemary EO, in which α-pinene is generally the second most abundant compound. Moreover, several other constituents of rosemary EO, such as camphor, eucalyptol, and α-pinene, have been reported to exert a cytotoxic mode of action, leading to cell membrane damage [44,45].

The *Salvia* genus comprises about 900 species mainly distributed throughout the world, and some of which are used also in perfumery and cosmetics. The essential oil of many species of sage is characterized by the presence of 1,8 cineole (eucalyptol), -thujone, camphor, borneol, and -cymene which is attributed with the antimicrobial activity against many microorganisms [46,47].

The insecticidal efficacy of sage EO is attributed to its richness in monoterpenoids, which are widely recognised for their potent insecticidal effects against a broad spectrum of insects. For example, 1,8-cineole and α-pinene, two key monoterpenoid constituents of this EO, have been shown to inhibit erythrocyte acetylcholinesterase activity [48]. Moreover, it has been demonstrated that sage EO exhibits significant insecticidal activity on *Spodoptera littoralis* Boisduval, with mortality increasing proportionally to the concentration after 24 hours of exposure [49]. It has also been demonstrated that essential oils of sage EO possess both contact toxicity and repellent effects on *Tetranychus urticae*. Generally, the terpenoids in essential oils exert various effects on insects, including toxicity, reduced maturity, and diminished reproductive capacity. These oils are also a complex mixture of neurotoxic compounds with acute effects on insects, acting by interfering with the octopaminergic transmitters in arthropods. The action of these essential oils is likely due to the synergy or antagonism between the major compounds [50].

Interest in the use of plant extracts and essential oils as tools for controlling phytophagous populations has recently increased considerably, due to their ability to reduce environmental impact and preserve non-target organisms [24,26,51]. However, research has mainly focused on a limited number of phytophagous species, neglecting others that, although less studied, may be just as harmful or even more damaging. Regarding Acari, studies on vegetal products have mainly focused on *T. urticae* [23,52–54], while the knowledge available on the effectiveness of biopesticides against other damaging pests such as the tomato rust mite is still very limited. To the best of our knowledge, this is the first study on the effects of essential oils against *A. lycopersici*.

Among the tested oils, oregano EO exhibited the highest acaricidal activity. However, significant efficacy was observed only at the highest concentration (5000 µl/L), suggesting that its toxic effect manifests only beyond a specific concentration threshold. Probably, the main toxic effect of oregano EO is attributed to carvacrol, as it exhibits various bioactive properties, including antioxidant effects, inhibition of antibiotic-resistant bacteria, suppression of microbial and fungal toxins, and potential anticancer activity [34]. However, the primary mode of action of carvacrol remains unclear, although it has shown limited acetylcholinesterase inhibition in certain insect species, such as house flies, ticks, and cockroaches [48].

Regarding rosemary and sage EOs, Laborda et al. [53] assessed high toxic effects after 24 h exposure (79 to 100% of mortality), against *T. urticae* at concentrations ranging from 1500 to 2500 µl/L. Our results on *A. lycopersici* revealed very low toxicity after 24 h (2 to 12%) and also after 4-days

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exposure (26 to 54% of mortality), at comparable concentrations (1280 and 2500 µl/L). However, the sage EO used by the abovementioned authors had a different chemical profile: the main component was α-Thujone (42.3%), followed by Camphor (11.0%) and 1,8-Cineole (10.3%); the latter two were the principal components of our sage EO (21.91 and 27.67% for the two components respectively), while Camphor was not detected in our sage EO. The rosemary EO used by Laborda et al. [53] showed also a different compound composition to that used in our experiments. The four main components of their rosemary EO were 1,8-cineole, α-pinene, camphor and camphene (26.7, 18.6, 17.5 and 11.8 for the four compounds respectively). However, concentration of the above compounds in our rosemary EO was quite different (11.0, 28.0, 6.2 and 7.0 for the above components respectively). The lower toxic effects on *A. lycopersici* could be attributed to the different concentrations of the components of the essential oils adopted but also to a different susceptibility of the two mite species.

4. Materials and Methods

4.1. Cultivation of Officinal Plants with Precision Aromatic Crops (PAC) Techniques, and Essential Oil Extraction Methods

4.1.1. Plants Cultivation Method

Cultivation of *O. vulgare* (Oregano), *S. rosmarinus* (Rosemary), and *S. officinalis* (Sage) carried out at Morreale's Farm in Grotte (Agrigento Province, Italy) (37°22'52.284"N 13°40'24.067"E) (World Geodetic Coordinate System 1984). The soil moisture regime is xeric, bordering on aridic, and the temperature regime is thermic. The experimental field includes 1,520 plants of *O. vulgare*, 1,980 plants of *S. rosmarinus* and 2,485 plants of *S. officinalis*, arranged with 35 cm spacing along the rows and 180 cm between the rows. Periodically, inter-row surface tillage was performed to control weed growth and disrupt soil capillarity. No organic or mineral fertilization was carried out during the growth period.

4.1.2. Precision Aromatic Crop (PAC) Techniques

Precision Aromatic Crop (PAC) techniques were applied to optimize the cultivation and monitoring of Medicinal and Aromatic Plants (MAPs). Specifically, unmanned aerial vehicles (UAVs) equipped with multispectral cameras, combined with post-processing software, have become a widely adopted technique for assessing vegetation indices (VIs) in the management of MAPs. This method seeks to modernize agricultural practices by minimizing resource use and boosting productivity [55–57].

Advanced technologies for spatially variable crop condition monitoring, focusing on the use of UAVs equipped with multispectral cameras and a spectroradiometer to assess MAPs were adopted.

The DJI Mavic 3 Multispectral (M3M) drone was used to capture high-resolution images across visible and near-infrared bands, including Green (G), Red (R), Red Edge (RE), and Near-Infrared (NIR) (Figure 4). The Hand Held 2 ASD spectroradiometer used in the tests have a sensitivity in the region 325-1075 nm. A solar irradiance sensor and a GNSS system with RTK correction (< 2 cm accuracy) ensure precise data collection. Flight parameters were carefully planned to use DJI GS Pro software to avoid issues like shadows, with flights scheduled at noon for optimal lighting conditions in the period from March to October 2024.

Ground Control Points (GCPs) were placed and georeferenced using an RTK-enabled GNSS receiver. After image collection, data were processed in Agisoft Metashape Professional to create a multiband ortho-mosaic, which was then calibrated and orthorectified. Spectral canopy data were extracted and analysed using QGIS software, calculating NDVI values to monitor vegetation health and optimize harvest timing for MAPs [55–57]. To assess vegetation dynamics and health, NDVI values were calculated for three key periods: March, September, and October, to identify vegetation areas. Zonal statistics in QGIS were used to determine the surface area corresponding to different NDVI classes, providing valuable data for effective land management. Spectral data also facilitated

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the generation of false-colour images, enhancing the visualisation of vegetation and offering critical insights into the health and vigour of rosemary, oregano, and sage. The collected biomass was intended for the extraction of EOs process, so it was necessary to identify the time of highest vegetative vigor for MAPs. The NDVI index is sensitive toward crop biophysical properties, like nitrogen, chlorophyll, vigor, biomass etc. In order to choose the optimal harvest time, the MAPs NDVI values were calculated in the period from 1st March and 30th October 2024 (Figure 5)

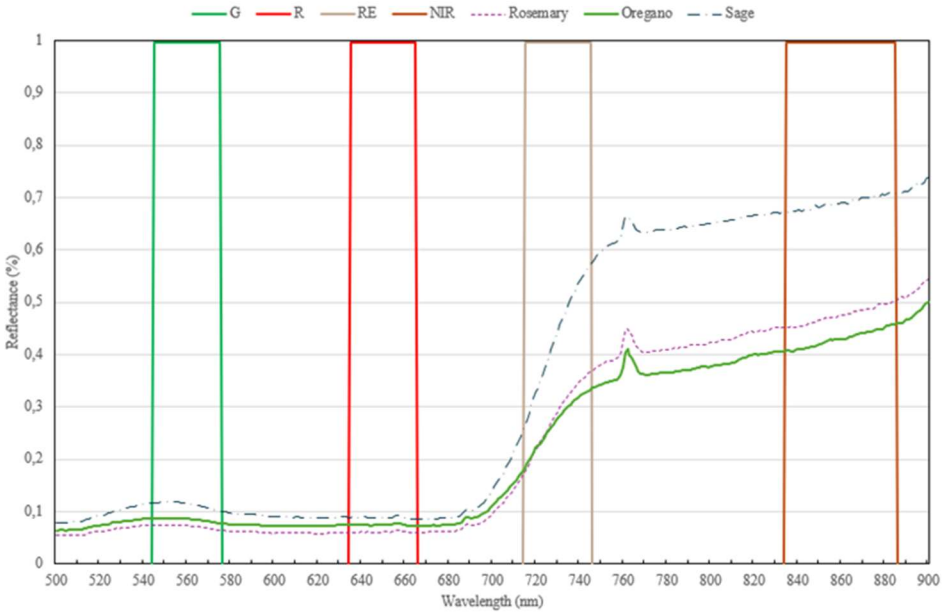

Figure 4. Reflectance of Rosemary, Oregano and Sage in the 500-900 nm spectrum region measured with the spectroradiometer and spectral sensitivity of the M3M camera (G–Green, R–Red, RE–Red Edge and NIR–Near-Infrared).

Figure 5. MAPs monitoring through Precision Agriculture.

4.1.3. Extraction and Analyses of Essential Oils

The oregano EO was extracted from 500 g of dried leaves and flowers [55,56,58], using a 12 L conventional steam distiller for aromatic plants (Spring Extractor, Albrigi Luigi, Verona, Italy). After the distillation process, the EO was separated from its vegetation water and stored at 4°C. Yield of EO was expressed in percentage (volume/weight).

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Volatile organic compounds (VOCs) in the oregano EO were analyzed using solid-phase microextraction (SPME) coupled with GC-MS, with a dilution of the oil in hexane at a ratio 1:100. The SPME fiber (DVB/CAR/PDMS, 50 mm, Supelco) was exposed to the diluted oils while stirring at 60°C. After 5 minutes of extraction, the fiber was placed in a GC splitless injector, and the VOCs were desorbed for 1 minute at 250°C. Chromatographic separation was achieved using a DB-624 capillary column (Agilent Technologies, 60 m length, 0.25 mm diameter, and 1.40 µm film thickness). The oven temperature was initially set at 40°C for 5 minutes, then increased linearly by 5°C per minute up to 200°C, where it was held for 2 minutes. Helium was used as the carrier gas at a flow rate of 1 mL/min, and the interface temperature was set to 230°C. Mass spectra were recorded in the m/z range of 40-400 amu using full-scan acquisition mode. Individual VOCs were identified by comparing the mass spectra with the NIST05 commercial library. The analysis was performed in triplicate, and the results are expressed as percentages relative to the most significant peak [34].

The extraction of EO from Rosemary is the same as that used for oregano, using 300 g of fresh plant material consisting mainly of flowering tops and leaves. After 3 hours of distillation, a light yellow EO with camphor odor was obtained. Chemical composition was determined by a GC/MS method using an Agilent 6890 Gas-Chromatography coupled with an Agilent 5973 Mass Spectrometer equipped with silica capillary column HP5-MS (30 m x 0.25 mm, film thickness 0.25 mm). The oven temperature was held at 60°C for 8 min, increased to 180°C with a gradient of 4°C/min, and then held for 2 min at 180°C. The components were identified by helium as carrier gas (1 ml/min) and injector temperature and ion-source temperature were 250°C and 280°C, respectively. The identification of Rosemary EO compounds was made by comparison with their relative retention time (RT) with those of original samples or by comparison with their relative retention index (RI) to the series of n-hydrocarbons and computer matching against commercial library and homemade library mass spectra made up from pure substances and components of known oils and MS literature data (NIST90). The Kovats index was calculated agreed with that reported by Adams [59].

For sage EO extraction and analysis, 200 g of leaves were manually chopped into small pieces and subjected to water distillation for three hours using a Clevenger apparatus. The extracted EO was dried with anhydrous sodium sulfate and stored in the dark at 4°C until analysis. The EO was analyzed using a gas chromatograph-mass spectrometer (Shimadzu GC-MS QP2010 Ultra), following the method outlined by Zito et al. [60]. The GC-MS system was equipped with an AOC-20i self-injector (Shimadzu, Kyoto, Japan) and a ZB-5 column (5% phenyl-polysiloxane; 30 m length, 0.32 mm internal diameter, and 0.25 µm film thickness, Phnomenex). For each analysis, 1.3 µL of the sample was injected at 280°C in a 1:1 ratio, with a helium carrier gas flow rate set to 3 mL/min. The oven temperature was held at 60°C for 1 minute, then increased at a rate of 10°C/min until reaching 300°C, where it was maintained for 5 minutes. The MS interface was set to 300°C, and the ion source operated at 200°C. Mass spectra were recorded at 70 eV (EI mode) from 30 to 450 m/z. The GC-MS data were processed using the GC-MS Solution software, version 4.11 [28]. The yields of EOs extracted from the species under study were expressed as a percentage (volume/weight)

4.2. Aculops lycopersici Experimental Set-Up

4.2.1. *Solanum nigrum* Seedlings for *A. lycopersici* Breeding

Seeds of *S. nigrum* (black nightshade) were isolated from fruits gathered from field (38° 6'25.03"N, 13° 21' 0.19"E), in autumn 2023, dried on filter paper for three days at room temperature and stored in a glass container in a refrigerator at 9°C, 35% of RH and photoperiod of 0:24 (light:dark) for 6 months. In April 2024, seeds were sown in plastic pots (22x22x26 cm) using a substrate mixture of blonde peat and perlite and placed in a greenhouse.

Aculops lycopersici was collected from *S. nigrum* plants in the garden of the Department SAAF, in May 2024 and used for infesting *S. nigrum* potted plants placed inside entomological cages (150 x 150 mesh, 160 µm aperture), in a controlled conditions (25±1°C, 70±5% RH, 16:8 light:dark photoperiod).

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4.2.2. Adult Cohort for the Experiments

To obtain coetaneous for the experiments, 200 adults of *A. lycopersici* were transferred onto the adaxial surface of four *S. nigrum* leaves placed in Petri dishes (Ø150 mm, h 10 mm) on cotton wool saturated with distilled water. Adults were allowed to oviposit, and after a 24-hour period, they were removed. The presence of juvenile stages was recorded daily, and the postembryonic development was monitored until attaining adulthood. Since it was not possible to distinguish females from males under the stereomicroscope, a mixed population of both sexes was used in the trials.

4.2.3. Experimental Units

The experimental unit (EU) consists in a leaf disc (Ø 1.6 cm) placed on cotton wool moistened with distilled water in a Petri dish (Ø100 mm, h 10 mm). The adaxial surface of the leaf was used as experimental surface.

4.2.4. Effects of Essential Oils on *A. lycopersici*

Five adults of *A. lycopersici* were transferred on each EU, using a specialized pen with micro clamping mandrel, into which a human eyelash was inserted at the tip. The flexibility of the eyelash and the presence of micro-sculptures on its surface enabled the delicate collection of the specimens without injure them.

Essential oils were tested at five different concentrations: 320, 640, 1280, 2500 and 5000 μL L^{-1}. Each EO was initially dissolved in pure acetone to ensure homogeneous mixing and afterwards distilled water was added in a 3:2 ratio (for water and acetone, respectively). For each concentration, 10 replications were carried out for a total of 50 *A. lycopersici* adults per test. The negative control replications were treated with only water and acetone at ratio 3:2.

Each replicate was treated with 8 ml of the solution using a Potter Precision Spray Tower (Burkard Manufacturing Co. Limited, Woodcock Hill Industrial Estate, Rickmansworth, Hertfordshire WD3 1PJ, England), set to a pressure of 62.05 kPa. The EUs were checked at 24h intervals for 4 days after the treatment; the cotton wool was replenished daily with distilled water. Adult mortality was registered daily until the conclusion of the tests.

The categories proposed by Hardman et al. [29] for toxicity classes was applied on corrected mortality data [61]: 1) not toxic (mortality <25%), 2) slightly toxic (mortality between 26% and 50%), 3) moderately toxic (mortality between 51% and 75%), 4) very toxic (mortality >76%).

4.2.5. Statistical Analysis

The Johnson and Kotz [62] transformation was applied on the mortality data before the General linear model analysis (GLM). In the presence of significant differences between treatments, the averages were separated by the Tukey's HSD test ($P<0.05$). Mortality was corrected by Abbott's [61] formula before the Probit analysis. The lethal concentrations corresponding to the mortality of 10% (LC_{10}), 30% (LC_{30}), 50% (LC_{50}) and 90% (LC_{90}) were determined by means of a probit model implemented in the Minitab software, considering a 95% of confidence level. All analyses were performed using Minitab 19.0 software (Minitab Inc., State College, PA, USA).

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