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TOXICOLOGICAL AND BIOCHEMICAL EFFECTS OF *Mentha pulegium L* AND *Cupressus sempervirens L* ESSENTIAL OILS ON THE PINE PROCESSIONARY CATERPILLAR: IN VITRO AND IN VIVO ASSESSMENT.

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ABSTRACT

The essential oils of *Cupressus sempervirens L* (*C. sempervirens*) and *Mentha pulegium L*(M. *pulegium*), rich in α-ocimene, δ-3-carene (27.43%) and pulegone (72.68%), respectively, were evaluated for their insecticidal activity against *Thaumetopoea pityocampa* (*T. pityocampa*) (Denis and Schiffermüller, 1775). In ingestion bioassays, *M. pulegium* EO showed the highest efficacy, reaching 100% mortality at 150 µL/mL for L4 and L5 instars, with LC_{50} values of 38.835 and 33.835 µL/mL.*C. sempervirens* essential oil exhibited the highest toxicity against third-stage *T. pityocampaSchiff* larvae, with LC_{50} values of 13.02 µL/mL (ingestion) and 47.59 µL/mL (contact), and corrected mortality up to 100%. Fumigant bioassays confirmed complete mortality of L4 larvae at 100ul/mL (LC_{50} = 22.1 µL/mL). In vivo, larval survival dropped from 100% to 0% within 18 days, and biochemical analyses revealed significant depletion of lipid, carbohydrate, and protein reserves.

Keywords: thaumetopoea pityocampa Schiff, insecticidal, *bioactive compounds,* energy biomarkers.

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1. INTRODUCTION

Recent decades have seen the use of agricultural products increase considerably worldwide to optimize food production for a growing population. Nevertheless, the uncontrolled use of these substances, particularly pesticides, has led to the formation of harmful waste in food, soil, air, and water [1]. The pine processionary, also known as *T. Pityocampa* (Denis and Schiffermüller, 1775) (Lepidoptera, Notodontidae), is a primary insect defoliator of conifers in Southern Europe and North Africa, feeding oligophagously on pines and cedars in Mediterranean nations [2]. It also limits pine forest development due to its urticating setae [3]. Currently, there is no pesticide specifically designed to control the pine processionary moth (*T. pityocampaSchiff*), and conventional methods rely on mechanical techniques such as pheromone traps or broad-spectrum larvicidal chemicals. However, these methods have increased the risk of epidemics, as well as having an impact on public health and forestry, due to climate change [4]. Indeed, the development of biological insecticides has become a sustainable strategy for pest control in recent years [5]. Plants offer a promising alternative to chemical insect control agents due to their abundant bioactive chemical content. [6, 5]. The primary botanical insecticides are essential oils, which are secondary metabolites generated by plants. These oils are of pivotal significance to the life cycles of plants, functioning as barriers against pathogens and herbivores whilst simultaneously attracting pollinators and seed dispersers [7, 8, 9]. In developing countries, the utilization of medicinal plants for first aid is prevalent, with 70-95% of first aid remedies being derived from plant sources. The primary objective of scientific research is to identify safer alternatives [10]. The study evaluates the toxicity of essential oils from *C. sempervirens L. And M. pulegium L.* on *T. pityocampa Schifflarval* stages, analysing chemical composition, compound levels, and examining insect metabolism, focusing on carbohydrate, protein, and lipid levels.

2. MATERIEL AND METHODS

2.1 Plant Materials and Extract Preparation

The plants *C. sempervirens L. and M. pulegium L.* were collected during the flowering stage from Douira forest in Algiers, 36° 40′ 10″ N, 2° 56′ 32″ E. The aerial parts were partially dried for 5 days at room temperature. (25 ± 3 °C). All EOs were obtained from the aerial parts of *C. sempervirens L* and *M. pulegium L* by hydrodistillation as described by [11].

2.2 Entomological Sample Controlled

The insecticidal effect of the EOs used focused on the processionary caterpillar in its three larval stages (L3, L4, and L5). Caterpillars were collected from Aleppo pines (*Pinus halepensis*) in the Bordj Bou Arreridj forest,

and the specimens were transported to the laboratory in their natural state and maintained in plastic containers under controlled ambient conditions (25 ± 3 °C, 75% relative humidity)."

2.3 GC-MS Analysis

The chromatographic analysis was accomplished using an Agilent 5975 mass spectrometer with an electron impact detector connected to a 6890 plus GC-MS system. The analysis method was performed as described by [12].

2.4 Ingestion Toxicity

The caterpillar *T. pityocampa*Schiff was subjected to an ingestion toxicity experiment using Aleppo pine leaves. The leaves were exposed to 500 µl of various essential oil dilutions, ranging from 50, 75, 100,125and 150 µl/ml. Twenty larvae were placed in boxes containing treated leaves for each larval stage. The establishment of a control group was achieved by applying 3% Tween to the leaves. The experiment was conducted in accordance with the protocol delineated by [13].

2.5 Fumigant Toxicity

This test was carried out in accordance with the protocol described by [14].

2.6 Contact Toxicity

Contact toxicity for the processionary was performed in accordance with the protocol outlined by [13], with minor adjustments. Twenty caterpillars (L3, L4, L5) were distributed evenly across the dishes, then sprayed with 500 µl of various EO dilutions of 50, 75, 100,125and 150 µl/ml. These boxes were securely sealed with lids and tape to prevent evaporation of the essential oil. The control was represented by the spray with 3% Tween.

2.7 Survival Analysis

This examination was performed to assess the toxicity of EOs on overwintering caterpillars. A survival assay was conducted under controlled laboratory conditions. A significant number of nests containing overwintering caterpillars were collected from the field and randomly allocated to designated treatment groups. Each nest was treated with 2ml of crude essential oil, and the filamentous structures were then carefully sprayed with this solution. The control nests were treated with 2ml of 3% Tween under the same conditions. The treated nests were then placed in boxes lined with perforated canvas to ensure adequate ventilation. The boxes were maintained at an ambient temperature of 25 ± 3°C and 75% relative humidity, with a 12:12 h light-dark photoperiod. Observations were conducted on a daily basis for a period of 30 consecutive days. During each inspection, nests were opened, and the number of dead larvae was recorded and removed. Survival data were analysed by the Kaplan-Meier method, and comparisons between treatment and control groups were made using the log-rank test.

2.8 Effect of Essential Oils on the Metabolic Profile

2.8.1 Homogenate preparation protocols for insect metabolic and toxicity assessments

These images provide a representation of the molecular response of the caterpillars 24 hours after treatment. A caterpillar's sample (0.1 g) was homogenized in 10 ml of ice-cold phosphate buffer (0.1 M, pH 7.4) using a mortar and pestle. The homogenate was centrifuged at 6,000 rpm for 15 minutes at low temperature, and the resulting supernatant was collected to serve as the tissue source for subsequent biochemical and centrifugation analyses. The protein concentration was measured in accordance with the method of [15]. The carbohydrate concentration was measured according to the method described by [16]. A standard calibration curve was constructed using a glucose stock solution (1 g/L), with serial dilutions yielding final concentrations of 10, 20, 40, 60, and 80 µg/mL. A 0.5 mg/mL glucose solution (5 mg in 10 mL distilled water) was used as the blank. The impact of EOs on caterpillar's lipid levels was ascertained by employing the methodology delineated by [16, 17]. The following procedure was established for the preparations. The lipid concentration for each sample was determined relative to a standard curve constructed using 25, 50, 100, 200, 400, 800, and 1200 µg of commercial vegetable oil.

2.9 Statistical Analysis

The study used statistical software GraphPad Prism and IBM SPSS Statistics for statistical analyses. Survival analyses were conducted using the Kaplan-Meier method, and differences between survival curves were assessed using the log-rank test. Median lethal dose values and 95% confidence intervals were estimated using probit regression analysis. Data were presented as mean ± standard deviation from three independent replicates. The statistical significance of the results was determined by a 5% probability level.

3. RESULTS

3.1 Essential Oils Composition

The chemical compositions of *C. sempervirens* and *M. pulegium* EO (Tables 1 and 2), with GC-MS analysis revealing major constituents. *C. sempervirens L*EO was dominated by methyl eicosadienoate and α-ocimene, accounting for 28.33% and 27.43% of the total oil, respectively.*M. pulegium L*EO contains oxygenated monoterpenes, with pulegone being the major constituent at 72.68%.

Table-1. Compounds Identified in the EO Extracted from the Plant of M. pulegium L.

Number	R.T	Compound Name	Group	Area%
	9.5	α-Pinene	Monoterpene Hydrocarbon	0.04
	10.5	Camphene	Monoterpene Hydrocarbon	0.18
	10.8	α-Thujene	Monoterpene Hydrocarbon	0.58
	11.5	α-Ocimene	Monoterpene Hydrocarbon	27.43
	12.0	Camphene	Monoterpene Hydrocarbon	0.82
	13.4	4(10)-thujene	Monoterpene Hydrocarbon	0.07
	13.6	α-Thujene	Monoterpene Hydrocarbon	0.96
	13.8	β-Pinene	Monoterpene Hydrocarbon	1.11
	14.8	β-Myrcene	Monoterpene Hydrocarbon	1.74
	15.7	α-Phellandrene	Monoterpene Hydrocarbon	0.05
	16.3	δ-3-Carene	Monoterpene Hydrocarbon	11.71
	16.6	α-Terpinene	Monoterpene Hydrocarbon	0.26
	17.1	p-Cymene	Monoterpene Hydrocarbon	0.74
	17.4	D-Limonene	Monoterpene Hydrocarbon	1.97
	18.8	(E)-β-Ocimene	Monoterpene Hydrocarbon	0.04
	19.6	γ-Terpinene	Monoterpene Hydrocarbon	0.35
	21.7	(+)-δ-3-Carene	Monoterpene Hydrocarbon	1.69
	22.3	1,8-Cineole (Eucalyptol)	Monoterpene Ether	0.04
	22.6	Linalool	Monoterpene Alcohol	0.10
	22.9	trans-Verbenol	Monoterpene Alcohol	0.04
	25.0	Isoneral	Monoterpene Derivative	0.02
	25.3	α-Verbenol	Monoterpene Alcohol	0.04
	25.8	Cuminaldehyde	Aromatic Aldehyde	0.16
	26.5	Menthol derivative	Monoterpene Derivative	0.09
	26.9	Terpinen-4-ol	Monoterpene Alcohol	0.08
	28.1	Terpineol	Monoterpene Alcohol	0.44
	28.7	p-Isopropylbenzyl alcohol	Aromatic Alcohol	0.15
	29.1	α-Terpineol	Monoterpene Alcohol	0.14
	32.3	Isopiperitenone	Monoterpene Ketone	0.04
	32.9	Methyl thymol ether	Monoterpene Ether	0.14
	33.7	Linalyl acetate	Monoterpene Ester	0.03
	34.5	Terpinen-4-ol isomer	Monoterpene Alcohol	0.03
	35.5	Isoborneol	Monoterpene Alcohol	0.03
	35.7	Bornyl acetate	Monoterpene Ester	0.13
	36.6	Myrtenyl acetate	Monoterpene Ester	0.42
	37.7	(E)-4-Decenal	Aldehyde	0.06
	39.4	Isopinocarvyl acetate	Monoterpene Ester	0.33
	40.1	α-Terpineol acetate	Monoterpene Ester	2.39

	40.4	α-Terpinyl acetate	Monoterpene Ester	0.13
	42.3	Geraniol	Monoterpene Alcohol	0.02
	43.5	Longifolene	Sesquiterpene Hydrocarbon	0.07
	44.0	α-Cedrene	Sesquiterpene Hydrocarbon	0.19
	44.5	β-Caryophyllene	Sesquiterpene Hydrocarbon	0.26
	45.1	α-Caryophyllene (Humulene)	Sesquiterpene Hydrocarbon	0.05
	46.6	α-Humulene	Sesquiterpene Hydrocarbon	0.14
	47.2	cis-Muurola-4(15),5-diene	Sesquiterpene Hydrocarbon	0.11
	48.1	γ-Cadinene	Sesquiterpene Hydrocarbon	0.16
	48.3	(-)-Germacrene D	Sesquiterpene Hydrocarbon	0.47
	48.9	2-Phenylethyl isovalerate	Ester (aromatic)	0.08
	49.2	α-copaene	Sesquiterpene Hydrocarbon	0.12
	49.5	α-Muurolene	Sesquiterpene Hydrocarbon	0.12
	50.3	γ-Cadinene	Sesquiterpene Hydrocarbon	0.12
	50.9	δ- Cadinene	Sesquiterpene Hydrocarbon	0.39
	54.4	Caryophyllene oxide	Sesquiterpene Oxide	0.06
	55.5	Cedrol	Sesquiterpene Alcohol	1.46
	56.0	Spathulenol	Sesquiterpene Alcohol	0.17
	56.4	Junenol	Sesquiterpene Alcohol	0.05
	57.0	α-Acorenol	Sesquiterpene Alcohol	0.08
	57.7	τ-Muurolol	Sesquiterpene Alcohol	0.12
	58.0	Ledol	Sesquiterpene Alcohol	0.04
	58.4	α-Cadinol	Sesquiterpene Alcohol	0.15
	59.0	Isolongifolenone	Sesquiterpene Ketone	0.03
	71.0	Methyl palmitate	Fatty Acid Methyl Ester	3.33
	72.1	Methyl 4,6-dimethyloctanoate	Fatty Acid Methyl Ester	0.77
	72.3	Methyl 2-propylheptanoate	Fatty Acid Methyl Ester	0.65
	75.6	Abietatriene	Diterpene Hydrocarbon	0.17
	78.6	Abietane	Diterpene	0.03
	80.4	Methyl eicosadienoate	Fatty Acid Methyl Ester	28.33
	81.7	Methyl stearate	Fatty Acid Methyl Ester	1.23
	82.5	Linoleic acid	Fatty Acid	3.31
	89.0	Glycidyl palmitate	Fatty Acid Ester	0.25
	94.2	Glyceryl linoleate	Fatty Acid Ester	0.13
	96.1	Phytol derivative	Diterpene Alcohol Derivative	2.33
	97.3	Glycidyl myristate	Fatty Acid Ester	0.14
Total				97.85%

Table-2. Compounds Identified in the EO Extracted from the plant of C.sempervirens L.

N	R.T	Compound Name	Group	Area%
	11,1	α-Pinene	Monoterpene	0,43
	12,0	3-Methylcyclohexanone	Miscellaneous (Ketone)	0,12
	13,6	Sabinene	Monoterpene	0,09
	13,8	β-Pinene	Monoterpene	0,3
	14,4	3-Octanone	Miscellaneous (Aliphatic ketone)	0,07
	14,8	β-Myrcene	Monoterpene	0,05
	15,1	3-Octanol	Oxygenated Monoterpene (Alcohol)	1,39
	17,1	p-Cymene	Monoterpene (Aromatic)	0,09
	17,4	D-Limonene	Monoterpene	0,7
	17,5	1,8-Cineole	Oxygenated Monoterpene (Ether)	0,12
	20,3	γ-Terpinene	Monoterpene	0,09
	21,6	Camphor	Oxygenated Monoterpene (Ketone)	0,36
	24,4	Octyl acetate	Miscellaneous (Ester)	0,15
	25,6	(+)-Camphor	Oxygenated Monoterpene (Ketone)	0,13
	26,1	Terpineol (α-Terpineol)	Oxygenated Monoterpene (Alcohol)	0,23
	26,4	cis-p-Menthone	Oxygenated Monoterpene (Ketone)	2,52
	26,6	Terpinen-4-ol	Oxygenated Monoterpene (Alcohol)	0,22
	27,4	dl-Menthol	Oxygenated Monoterpene (Alcohol)	9,77
	28,1	trans-p-Menthone	Oxygenated Monoterpene (Ketone)	2,16
	33,3	Pulegone	Oxygenated Monoterpene (Ketone)	72,68
	34,3	3-Methyl-3-decen-2-one	Miscellaneous (Aliphatic ketone)	0,07
	35,1	Neomenthyl acetate	Oxygenated Monoterpene (Ester)	0,12
	36,0	Isopiperitenone derivative	Oxygenated Monoterpene (Ketone derivative)	0,33
	39,4	Isopulegone	Oxygenated Monoterpene (Ketone)	0,14
	39,7	γ-Decalactone	Lactone	0,08
	40,0	Terpineol acetate	Oxygenated Monoterpene (Ester)	0,22
	42,3	Longifolene	Sesquiterpene	0,1
	42,8	Germacrene D	Sesquiterpene	0,05
	44,5	β-Caryophyllene	Sesquiterpene	0,32
	45,0	Possibly a Linalyl acetate derivative	Oxygenated Monoterpene (Ester derivative)	0,08
	45,5	p-Mentha-1(7),8-dien-2-one (Menthone isomer)	Oxygenated Monoterpene (Ketone)	0,1
	46,0	α-Humulene	Sesquiterpene	0,06
	46,6	1-Chlorooctadecane	Miscellaneous (Chlorinated Alkane)	0,4
	47,2	(-)-Germacrene D	Sesquiterpene	0,07
	48,3	δ-Cadinene	Sesquiterpene	0,16
	50,9	Caryophyllene oxide	Oxygenated Sesquiterpene (Epoxide)	0,18
	54,4	Cedrol	Oxygenated Sesquiterpene (Alcohol)	0,12
	55,4	No clear common name	Miscellaneous	1,01

	55,9	Patchoulol	Oxygenated Sesquiterpene (Alcohol)	0,09
	56,0	τ-Muurolol	Oxygenated Sesquiterpene (Alcohol)	0,05
	57,7	α-Cadinol	Oxygenated Sesquiterpene (Alcohol)	0,09
	58,4	Methyl palmitate	Fatty Acid Ester	0,12
	72,5	Ledol or similar	Oxygenated Sesquiterpene (Alcohol)	0,55
	75,6	Longifolene isomer	Sesquiterpene	0,2
	78,6	Methyl linoleate	Fatty Acid Ester	0,04
	80,4	Methyl oleate	Fatty Acid Ester	1,77
	80,7	Methyl stearate	Fatty Acid Ester	1,55
	81,9		Monoterpene	0,17
				99%

3.2 Ingestion Toxicity

The results of the ingestion test using *M.pulegium L* and *C. sempervirens* (EOs) against*T. Pityocampa* Schiff showed extremely high and rapid efficacy. The CM% (corrected mortality) rate varied significantly according to the type of EO used, with F-values of 6.541 (P = 0.000), 6.295 (P = 0.000), and 6.321 (P = 0.000) for the L3, L4, and L5 larval stages, respectively. It was also found that there was a significant effect of the applied concentration, with F-values of 2.291 (P = 0.000), 1.065 (P = 0.000), and 1.545 (P = 0.000) for L3, L4, and L5, respectively. The study found that the EO of *M. pulegium* (EO) was more toxic than the essential oil of *C. sempervirens L* (EO) at all stages of caterpillar development. CM% rate values increased with concentration. *M. pulegium achieved* CM% rates of 51.67% to 78.33%, 90% to 100% and 75% to 100% at concentrations of 50 to 150 µL/mL for L3, L4, and L5 stages, respectively. LC_{50} values for *C.sempervirens* and *M. pulegium L* EO against the three larval stages of the tested caterpillar species showed significant variations in toxicity, according to both oil type and larval stage. *M. pulegium L* demonstrated lower LC50 values across stages 4 and 5 at 38,835 and 33,343 (ul/ml), respectively, suggesting a more pronounced insecticidal effect in comparison to *C.sempervirens L*. (Table-3). LC_{50} values of contact, Ingestion, and fumigant toxicity of *C. sempervirens L* and *M. pulegium L* EOs against *T. pityocampa* Schiff. Values represent the $\chi 2 \pm$ Chi-Square. LC_{50} median lethal concentration.

Table-3.

Plant	Test	Laval stage	R^2	$\chi 2$	LC_{50} (ul/ml)
C.sempervvirens L	Ingestion	L3	0,347	2,3	13,1
		L4	1	3.7	39,1
		L5	0,292	19,1	60,1
	Fumigant	L3	1	0,6	18,9
		L4	1	0,7	22,1
		L5	0,959	1,8	97,1
	Contact	L3	0,900	1,9	47,6
		L4	0,840	1,9	50.9
		L5	0,539	9,1	56.3
M. pulegium L	Ingestion	L3	0,941	0,3	55,8
		L4	1	0,9	38,9
		L5	0,829	3,5	33,4
	Fumigant	L3	0,829	1,1	17,4
		L4	0,796	5,4	53,6
		L5	0,745	2,6	33,6
	Contact	L3	0,943	2,8	71,6
		L4	1	0,6	18,8
		L5	0,721	0,8	8,2

3.3 Contact Toxicity

The contact toxicity bioassay revealed that both *C. sempervirens L*and *M.pulegium L* (EOs) exhibited strong insecticidal activity against *T. pityocampa*Schiff caterpillars. Corrected mortality varied according to the type of EO (F = 15,745; P = 0.000*) and the applied concentration (F = 9,576; P = 0.000*). C. sempervirens EOs on third-instar caterpillars demonstrated a significant dose-related mortality trend. Following a 24-hour exposure period, the recorded CM% rate was 96.67% at 75 µl/ml after 72 hours. The LC50 values indicated that *C. sempervirens* EO was the most toxic for caterpillars at the L3 stage, with a value of 47,589 µL/mL (χ^2 = 1,923). In comparison, the *M pulegium L*. EO exhibited a higher toxicity level of 71,617 µL/mL (χ^2 = 2,883). The results of the study demonstrated that *T. pityocampa*Schiff caterpillars on L3 insect pests were sensitive to *M. pulegium L*, with a CM% rate ranging from 65% at 150 µl/ml. Following 48 hours, there was a significant increase in. CM% rate for all doses, with 100% mortality recorded at 100, 125, and 150 µl/ml, corrected mortality varied according to the type of EO (F = 6,507; P = 0.000*),(F = 28,466; P = 0,000*), the applied concentration (F = 7,250; P = 0.000*), (F = 8,307; P = 0.000*)for L4and L5 respectively, *C. sempervirens L*EO exhibited insecticidal toxicity against fourth- stage larvae. After 24 hours. CM% rate ranged from 50% to 80% at concentrations between 50 and 125 µL/mL after 72 hours, complete. CM% rate (100%) was maintained at 100 µL/mL, demonstrating a more toxic effect than C. sempervirens LEO. The LC_{50} values indicated that *M. pulegium L* EO was more toxic against *T. pityocampa*Schiff on L4 andL5withLC_{50} = 18,824µL/mL (χ^2 = 0,646), and LC_{50} = 8,197µL/mL (χ^2 = 0,878) (Table 3). CM% rate increased from 100% mortality being achieved at 125 µl/ml. Following 72 hours of experimentation, the essential oils of *M.pulegium L* and *C. sempervirens L were* observed to achieve mortality rates ranging from 53.75% to 97.5% at concentrations of 100 µl/ml and 125 µl/ml, respectively. These concentrations were found to be effective against *T. pityocampa* Schiff on the fifth instar.

3.4 Fumigant Toxicity

During Fumigant toxicity bioassays, both *M. pulegium and C. sempervirens L* (EOs) exhibited marked insecticidal activity against *T. pityocampa*Schiff caterpillars at different larval stages (L3, L4, and L5). The (CM %) varied significantly with the type of EO, with F-values of 4.197 (P < 0.006) for L3, 5.937 (P < 0.001) for L4, and 8.837 (P < 0.001) for L5.

Concentration significantly influenced mortality at all larval stages (F = 4.660, P< 0.004 for L3; F = 15.745, P< 0.000 for L4; F = 9.576, P< 0.000 for L5). *M. pulegium L* EO showed higher toxicity compared to *C.sempervirens* L EO, with the CM% values increased progressively with concentration for both EOs. *M.*

pulegium L (EO) at concentrations ranging from 50 to 150 µL/mL induced CM% values of 100%, 65-75%, 75–150 µL/mL, and 20% to 150 µL/mL for L3, L4, and L5 larvae, respectively. The LC50 values indicated that *M.Pulegium L*EO was found to be more toxic to *T. pityocampa*Schiff caterpillars on L3 and L5, with an LC50 of 17,388 µL/mL (χ^2 = 1,149) and an LC50 of 33,651 µL/mL (χ^2 = 2,688) (see Table 3 for details). In contrast, *C. sempervirens L* EO at the same concentrations resulted in lower CM% ranges: 20–88, 33-100% to 50-100, 125, and 150µL/mL (L3), 90-100% to 50-100, 125, and 150µL/mL (L4), and 20-48% to 50-100µL/mL (L5).

3.5 Biochemical Impact of Essential Oils on the Energy Metabolism of *T. Pityocampa*Schiff

The protein, lipid, and carbohydrate levels in adult *T. pityocampa*Schiff caterpillars on L5 exposed to the EOs of *M. pulegium*, *C. sempervirens at LC50 are* shown in Figure-1. ANOVA analysis revealed a noteworthy and significant difference in the impacts of these EOs on the assessed metabolic parameters. For LC_{50} concentrations, the decrease in protein lipid, and carbohydrate levels was statistically significant [F (2, 12) = 184,9; P<0,0001] and lipid levels [F (5, 22) = 29.573; P<0,0001], whereas protein levels [F (2, 12) = 12,25, P=0,0013]. The protein levels in *T. pityocampa*Schiff caterpillars on L5 significantly declined when exposed to *M. pulegium*, *C. sempervirens L* at LC_{50}, yielding values of 1.03, 0.81 µg/mg, respectively, relative to the control measurement of 1.22 µg/mg (Figure-1). Carbohydrate levels showed a notable decrease when treated with LC_{50} exposure to these EOs over a period of eight days. Formulations of *M. pulegium L* and C. *sempervirens L* demonstrated the most significant reduction in carbohydrate levels, recording 0,63 and 0,9 µg/mg in comparison to 3.4 µg/mg of control(Fig 1). The lipid content of caterpillars treated with the bioinsecticide was markedly reduced compared to the untreated control. The measured values in treated groups were 6,405 and 20,825µg/mg for treatments of *M. pulegium L* and C. *sempervirens* relative to the control measurement of 30,01µg/mg. (Figure-1).

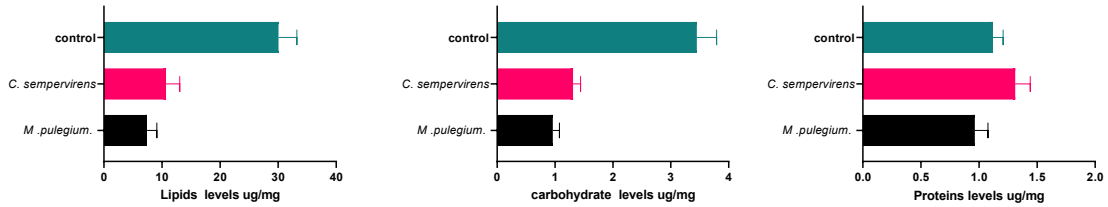

Figure-2. The quantities of total protein, carbohydrate, and lipids in *T. pityocampa* Schiff-treated with M. pulegium L and C. semperviren L EOs.

3.6 Survival Response of Caterpillar to *M. pulegium L* and *C. Sempemvirens L* Treatments

In the survival bioassay, the lifespan analysis was conducted to investigate the effect of *M. pulegium L* and *C. sempervirens* L on the survival of *T. pityocampa*Schiff caterpillars on L5. The Kaplan–Meier survival analysis revealed clear differences in mortality dynamics between the treated group, which was exposed to the bioinsecticide for 30 days, and the control group. Our results showed that *M. Pulegium L*and *C. semperviren resulted* in a 24.69% reduction in the lifespan of caterpillars (log-rank test, χ^2 = 37.78, df = 1). The mean duration of survival was found to be 7.1 days (±15 days) ($p < 0.0001$) and 9.23% (log-rank test, χ^2 = 29.80, df = 2, $p < 0.0001$), respectively, in comparison with the control group. After 15 days, 100% of the control group had survived. The rate of decline was more pronounced in the treatment of *M. pulegium L* and *C.semperviren L,* which achieved complete mortality by day 18, whereas the control group showed a slower but still substantial reduction in survival, reaching 0% by the end of the observation period. The estimated median lethal times (LT_{50}) were ascertained to be 15 days for treatment *M. pulegium L*and and 12 days for treatment *C. semperviren L*. This finding serves to confirm the greater potency of treatment *C. semperviren under* the tested conditions. (Figure-2).

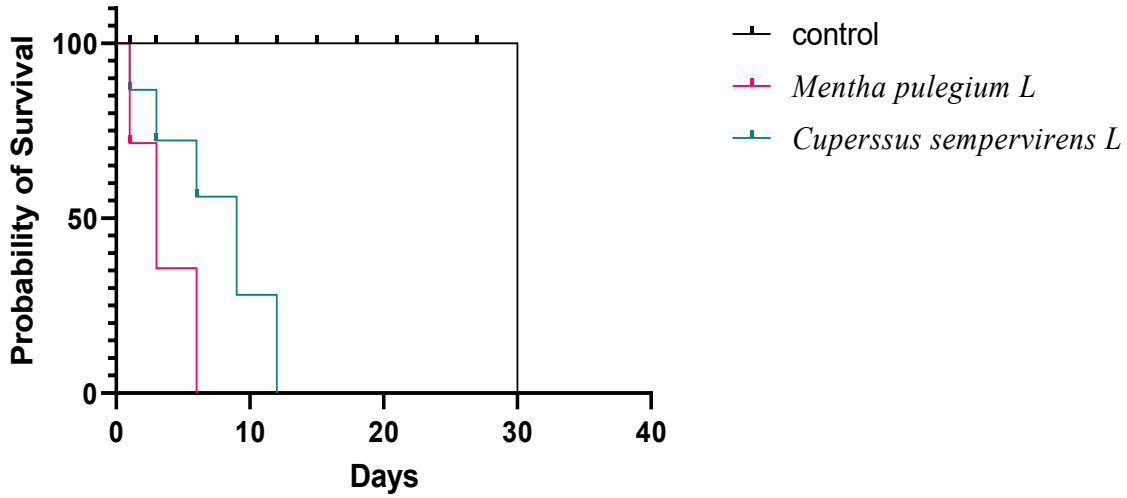

Figure-2. Survivorship curves of T. pityocampa exposed to C. sempervirens, L and M.pulegium L EOs. Graphs were plotted using the Kaplan-Meier survival method, and the results are presented as the mean±SEM.

4. DISCUSSIONS

This study confirms the strong insecticidal activity of *C. sempervirens* and *M. pulegium L*EOs against T. *pityocampa*. The GC-MS analysis of the complex chemical composition of these oils revealed pulegone (72.68%) in *M. pulegium L* and δ-3-carene (27.43%) in *C. sempervirens* as major active compounds. Many research studies have been reported, with α-pinene and δ-3-carene identified as dominant constituents in *C. sempervirens L* [18]. The study reports LC50 values obtained through ingestion and contact tests at various larval stages. In the contact test, LC50 values were 18.6 µl/ml and 7.8 µl/ml at the fourth and fifth stages, respectively. The ingestion test produced LC50 values of 38.5 µl/ml and 33.1 µl/ml at the LA and L5 stages. The results are consistent with previous findings, demonstrating a high degree of similarity in LC50 values across different studies for both testing methods and larval stages [19]. Say that *C. sempervirens L*EO had a lot of bioactivity. Fumigant at 100 µL/L exhibited the greatest insecticidal activity, and the oil exhibited significant repellency (66.25%). The study's ANOVA analyses revealed that the insecticidal effectiveness of essential oils from *C. sempervirens L*. and *M. pulegium L* against *T. pityocampa* larvae depended on both the larval stage and the application method (ingestion, fumigant, contact). The (CM %) showed significant variation based on the type of EO used. Specifically, fumigation treatments resulted in notable differences in mortality rates across larval stages L3, L4, and L5, with F-values of 4.197, 5.937, and 8.837, respectively, all statistically significant. Regarding the insecticidal effect of the two oils tested, *C. sempervirens L* and *M. pulegium L,* the mortality rate of *T. pityocampa Deest is* generally higher after 72 hours of exposure, often reaching 100% mortality at the highest doses. For C. sempervirens, La 100% (CM%) rate on L4 larvae of *T. pityocampa D* after 48 h of exposure by ingestion, fumigant, or contact at a concentration of 100 µl/ml and more. This result is consistent with the findings of [20]. Who reported the high insecticidal activity of cypress oil against flour moth larvae? This study investigated the impact of various (EOs) on different biological parameters. The results indicated that, at LC50 concentrations, these oils significantly reduced the levels of protein, lipids, and carbohydrates in the organisms tested. Specifically, Schiff caterpillars exposed to *M. pulegium and C. sempervirens* exhibited notable reductions in protein levels, measuring 1.03 and 0.81 µg/mg, respectively, compared to the control value of 1.22 µg/mg. Numerous studies have demonstrated that essential oils derived from plants can interfere with vital biochemical and physiological processes, thereby disrupting insect metabolism and development [14, 21]. The decreased protein content observed in both pests may be due to a number of factors, such as a reduction in protein synthesis or an increase in the breakdown of proteins to detoxify bioactive essential oil molecules [22 and 23].

5. CONCLUSIONS

The study shows that essential oils from *C. sempervirens* and *Mentha pulegium L*. have significant insecticidal properties. This indicates their potential for use in creating bioinsecticides and suggests that they could be a promising, sustainable approach to pest management.

ETHICAL APPROVAL

All experiments involving the pine processionary caterpillar (*Thaumetopea pityocampa*) were conducted in accordance with ethical standards for the handling of insects in laboratory and field studies. No endangered or protected species were involved in this research.

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