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Prevalence of Nasopharyngeal Myiasis among Dromedary Camels and Influence of Cuts Dates on Chemical Composition, Antioxidant and Larvicidal Efficacy of *Mentha longifolia* L. Essential Oil against *Cephalopina titillator*

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ABSTRACT

This study aimed to determine prevalence of nasopharyngeal myiasis among camels and evaluate the variation of *Mentha longifolia* oil yield, components, antioxidant activity, and larvicidal effect against *Cephalopina titillator* during three cuts (28th of April, 4th of June, and 22nd of July). Clinical and post-mortem examinations of 300 dromedary camels slaughtered in Giza province, Egypt showed a high prevalence of nasopharyngeal myiasis (58%). Risk factors linked to high infestation were being more than 3 years old (70.8%) and cooler seasons (74%); where it reached 81.3% in Winter. The highest oil yield was obtained on the 22nd of July. The relative percentages of the main constituents (pulegone, followed by eucalyptol, then I-menthone) showed variation in all three cuts. The highest percentage of pulegone (63.79%) was on the 28th of April, followed by 62.41% on the 4th of June, and decreased to 55.25% on the 22nd of July. In contrast, the 3rd cut produced the highest Eucalyptol (18.26%) and I-menthone (9.83%). *M. longifolia* oil at 200mg/mL caused 100% mortality of 2nd and 3rd larvae within 2 and 8h, respectively. Essential oil of the 2nd cut showed superior efficacy compared to ivermectin-treated larvae, with lower LC_{50} and LC_{90} values. Light and scanning electron microscopy revealed significant damage to the oil treated larvae, including swelling, degeneration, and atrophy of the cuticle, the mouthparts and antennal lobes. These findings highlighted the danger of nasopharyngeal myiasis, effect of cut dates on *M. longifolia* oil yield and constituents, and its potent anti-*C. titillator*.

Key words: Camel nasopharyngeal myiasis, *Cephalopina titillator, Mentha longifolia,* Pulegone, Cuts dates.

INTRODUCTION

The one-humped camels (*Camelus dromedarius*) are one of the most essential multipurpose species of livestock in arid and semiarid areas (Faye 2020; Hassan et al. 2024). Through the ages, they are kept for transportation across the desert by Bedouins and for their highly nutritious milk, meat, and hide (Sazmand et al. 2019).

Among the parasitic diseases, camel nasopharyngeal myiasis is deemed a highly prevalent threat that negatively declined health and productivity, causing remarkable economic losses all over the world (Alahmed 2002; Abd El-Rahman 2010; Yao et al. 2022; Hassan et al. 2022). The obligatory camel nasal botfly deposits the first larval instars of *Cephalopina titillator* (*C. titillator*) in the camels' nostrils (Higgins and Kock 1985). Where the larvae have the ability to invade the nasopharynx and paranasal sinuses and adhere to the mucosa for around a year, which causes extreme discomfort and promote inflammatory changes. So the infested animals are suffered from serious respiratory ailments, such as excessive nasal discharge, snoring, head shaking, as well as inappetence, abortion and decreased milk production (Taylor et al. 2007; Hassan et al. 2022). According to Musa et al. (1989), pneumonia may also result from a subsequent bacterial infection. However, in certain instances, neurological conditions were noted as occurring after the larvae may have gained access to the cerebral cavity (Shakerian et al. 2011).

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There have been numerous reports of the varying prevalence of nasaopharyngeal myiasis around the world, including 91% in Saudi Arabia (Hussein et al. 1982), 58% in Nigeria (Desbordes and Ajogi 1993), 79% in Libya (Abd El-Rahman 2010), 42% in Iraq (Atiyah et al. 2011), 41.67 % in Egypt (Khater et al. 2013) and 54.2% in China (Yao et al. 2022).

Traditional treatment of nasal myiasis is mainly relied on macrocyclic lactones (Khater et al. 2013). The widespread of chemotherpeutics might have negative consequences on the food safety and increase of the concerns of establishing drug resistance (Hassan and Ghazy 2022). In light of these challenges, there has been growing interest in alternative control strategies, particularly those are environmentally friendly and sustainable. One promising approach is the use of natural essential oils extracted from plants having larvicidal effects (Abu El Ezz et al. 2018).

Mentha longifolia L. is recognized as a beneficial medicinal plant with various pharmacological and biological activities. The percentage of the main component of *M. longifolia* essential oils exhibited significant variations worldwide depending on harvest time, geographical location, environmental condition, seasonal variations (Hussain et al. 2008) which affect the regulation of the essential oil biosynthesis (Masotti et al. 2003). Hussain et al. (2010) reported a significant variation in piperitenone and piperitenone oxide contents between summer and winter samples of *M. longifolia* from Pakistan. Spring and winter samples of *M. longifolia* growing in Tunisia affected the main components of eucalyptol, pulegone, piperitenone, menthone, and isomenthone (Zouari-Bouassida et al. 2018). In addition, Tacer et al. (2018) claimed that carvone is the main compounds in *M. longifolia* var. *Schimperi*. Dihydrocarvone (cis and trans isomers) had been detected as the major compound in Croatian (Džamić et al. 2010) and Iranian samples (Nazem et al. 2019).

The essential oil of *Mentha longifolia* L. is effective in cardiovascular diseases, including improvements in lipid balance, liver and endothelial functions and reductions in blood pressure, oxidative stress and inflammation, promote vascular relaxation, and it has an inhibitory effects on diabetes emergence and angiogenesis (Sut et al. 2021). Yassin et al. (2020) assessed the anticancer efficacy of *M. longifolia*, and its ability in healing burn wound has been reported by Haikal et al. (2022). Furthermore, the essential oil of *M. longfolia* has antimicrobial activity against *S. aureus*, and *E. coli* (Jahani et al. 2021). A prominent antifungal effect of *M. longfolia* extract was observed against *Candida* strains (Yassin et al. 2020). El-Badry et al. (2010) recorded the anti-parasitic influences of menthol and menthone of *M. longifolia* extract (5-200 µg/mL) on some infectious protozoal diseases e.g. *Entamoeba histolytica* and *Giardia duodenalis*. Khani and Asghari (2012) found a remarkable anti-insect features against *Tribolium castaneum* (a pest of stored products). In the same context, it also showed insect repellent activity, in addition to an inhibitory effect on the growth of insects at the larval and pupal stage (Pascual-Villalobos and Robledo 1998).

Research indicates that *Mentha* oil can significantly reduce the activity and survival of pests like mosquitoes, ants, and cockroaches (Isman 2000; Kumar et al. 2011; Fouad et al. 2023). A study by Singh and Pandey (2018) found that Mentha oil extract effectively repelled mosquito species, making it a promising natural alternative to synthetic insecticides. Additionally, the oil's effectiveness had been attributed to its ability to disrupt the nervous systems of insects, leading to their mortality (Ben Slimane et al. 2014).

Therefore, the goal of this study is to determine the prevalence of nasapharangyeal myiasis infesting camel, and the associated risk factors along with investigating the variation of the productivity of *M. longifolia* plant, essential oil yield, constituents, antioxidant activity and larvicidal activity as a natural insecticide against *C. titillator* during three cuts dates (28th of April, 4th of June and 22nd of July).

MATERIALS AND METHODS

Animals and parasites

Three hundred Dromedary camels of age 2 to 10 years old were used in this study at Kerdasa abattoir in Giza province. Twenty-five ones were monthly examined along the period extended from February 2024 to January 2025. The camels were clinically examined for the respiratory manifestations before slaughtering. Age and sex of animals were recorded. The head region of slaughtered camels was cut longitudinally through the throat. Then, the nasopharangyl regions, the frontal sinuses and the turbinate bone were investigated for detecting *C. titillator* larvae. The larvae were gathered in sterile labelled plastic cups and transferred to the laboratory.

Cultivation of *M. longifolia* and essential oil study

This experiment was conducted at National Research Center's Agricultural Experimental Station in Nubaria, west of Egypt's Nile Delta (latitude 30º 30' 1.4" N and longitude 30º 19' 10.9" E, Egypt). The present study aimed to investigate the variation of the productivity of *Mentha longifolia* plant, essential oil yield, constituents, antioxidant activity and larvicidal activity as a natural insecticide against *C. titillator* during three cuts dates (28th of April, 4th of June and 22nd of July).

Representative soil samples were taken from one layer (0-30cm) before cultivation for physical and chemical analyses according to Jackson (1973). The soil was sandy with 1.03ds.m^{-1}EC and 7.97pH. The cations and anions were measured in meql^{-1} as follows: 3.8Ca^{++}, 0.8Mg^{++}, 6.2Na^{+}, 0.6K^{+}, 0 CO_3^{--}, 2.6HCO_3^{-}, 5.6Cl^{-} and 3.2So_4^{--}.

The seedlings were purchased from Horticulture Research Institute, Agricultural Research Centre, Egypt. Then the seedlings were transplanted in late February into the experimental field with 30cm adjusted to dripper lines, which were 75cm apart. The normal agricultural practices for *Mentha longifolia* plants were done. The plants were irrigated using the drip irrigation system (4Lhour^{-1}).

This study was performed at completely randomized design with three replications. Three cuts were taken on the 28th of April, 4th of June and 22nd of July, respectively. Fresh and dry weights of herb (g plant^{-1} and ton ha^{-1}) as well as essential oil percentage (%) and yield (mLplant^{-1} and Lha^{-1}) were recorded.

Essential oil was extracted out by hydro-distillation according to Egyptian Pharmacopoeia (1984), using

Clevenger-type apparatus. The resulting oil was dried by sodium sulphate anhydrous and stored in the refrigerator till be utilized. To identify the main constituents and to determine their relative percentages, Gas chromatography-mass spectrometry (GC-MS) device stands at Medicinal and Aromatic Plants Research Department, National Research Centre was used to analyse the essential oil following the conditions mentioned by Omer et al. (2022).

The antioxidant action of the essential oil was determined using a series of diluted essential oil in methanol with varying concentrations (5, 10, 15, 20 and 25 µL/mL). The method of Tekao et al. (1994) was used to evaluate the essential oil's ability to scavenge DPPH free radicals with some modifications of Kumarasamy et al. (2007).

Larval dipping test

The anti-larval efficacy of *M. longifolia* essential oil on the second and third instar of *C. titillator* larvae was done during three cuts on the 28th of April, 4th of June and 22nd of July. The larval instars were collected from the slaughtered camels at abattoir, and differentiated as described by Zumpt (1965). The freshly collected larval instars were thoroughly cleaned from mucous utilizing water, and then they were washed several times by PBS pH 7.2. For each treatment, a total of ten second and third larval instars; five for each, were used per replicate. Three replicates were applied. Three concentrations (50, 100, 200 mg/mL) for each cut of *M. longifolia* oils prepared using distilled water with adding of few drops of Tween 80 for emulsification, were used. Ivermectin was purchased as the reference drug from Sigma -Alderich ® developed by Merck & Co., Inc., Kenilworth, NJ, U.S. Ivermectin was used at 0.003 mg/mL. The treated larvae were transported to a mesh cloth place and exposed for sinking for 1min in a 100mL of oil. Untreated larvae were subjected to diluent, for the same period of time. Then, the treated larvae were reserved in petri-dishes having filter papers (Whatman No. 1) at room temperature 29^{o}C and 80±5% relative humidity. The larval mortality was checked by counting of dead and alive larvae. The larva which exhibited no movement or reflex after its stimulation for several times by smooth ends wooden stick, were considered dead. The mortality was recorded after varies time intervals (2, 4, 6, 8, 10, 12, 18, and 24h). Mortality percentage, LC50 and LC90 were calculated (Table 4 and 5).

Histopathological examination

The histopathological effects of *M. longifolia* essential oil on *C. titillator* larvae have been studied according to Bancroft et al. (1996). Three groups of larvae were used (G1; the untreated control exposed to saline, G2; subjected to ivermectin at dose of 0.003mg/mL and G3; exposed to the oil of the 2nd cut (4th of June) at concentration of 200 mg/mL). The larvae were incubated for 6h. The concentration of 200 mg/mL was selected according to the results obtained from the larval dipping test. Post exposures, fixation of larvae was done using 10% neutral buffered formalin. Dehydration of the specimens was performed in graded alcohols, cleaned in xylol and put in paraffin blocks then cut into about 5µm thicknesses finally; they stained with haematoxylin and eosin. The pathohistological effects were investigated using a routine light microscope and photographed through digital camera.

Scanning electron microscopy

Ultra-structure inspection was fulfilled on larval instars of groups (G1, G2 and G3) according to Hilali et al. (2015). Immersion of the larvae was immediately done in 2.5% glutaraldehyde for fixation. Then they were dehydrated utilizing upgraded ethanol degrees series. A CO_2 critical point drier (Autosamdri-815, Germany) was used for the larvae dryness and after that the larvae glued on stubs; covered with 20nm gold in a sputter coater (Spi-Module sputter Coater, UK). The electron microscope (JSM 5200, Electron probe Microanalyzer, Jeol, Japan) in the electron microscope unit at the National Research Centre, Egypt, was utilized to take images.

Statistical analysis

The analysis of the recorded data for herb yield and essential oil production was done as completely randomized design by analysis of variance (ANOVA) using the General Linear Models procedure of CoStat according to Snedecor and Cochran (1967). Least significant difference (LSD) test was recorded at 0.05 probability levels for the comparison of the means of the treatments. The analysis of the impact of risk factors was achieved using Chi square test by factual computer bundle for social science (SPSS) adaptation 15.0 (SPSS Inc., Chicago, IL), and $P<0.05$. LC50 and LC90 values were also calculated utilizing log-probit software program Ldp Line® model"Ehabsoft" (Bakr 2000). The obtained data of larval dipping test were analyzed for the mean and standard errors (SE) and statistical comparison between the means of varies treatments for the three cuts (28th of April, 4th of June and 22nd of July) of *Mentha longifolia* oil was proceeded by one way ANOVA with SPSS program version.

RESULTS

Clinical and postmortem findings

Infested camels examined before slaughtering showed various respiratory signs including heavy mucoid almost bloody nasal discharge, sneezing, snoring, shaking head and general weakness. Some expelled larvae from infested camels were found dropped in the ground of abattoir. As shown in Fig. 1a, the nasopharyngeal region of infested camels was congested where the nasal passages were plugged with dark colored mucous containing. The mucosa was edematous with hemorrhagic patches and several scattered black nodules were also noted. Some larvae were embedded firmly in the mucosa by their sharp black mouth hooks and others were loosely attached or even crawling freely within the nasal and paranasal sinuses, as well as the turbinate bones and ethmoid area. The collected larvae were of different instars (Fig. 1b).

Prevalence of nasopharayngeal myiasis

Nasopharyngeal myiasis was detected among 58% (174) camels out of three hundred ones, along the period extended from February 2024 to January 2025. Monthly detection of C. *titillator* larvae revealed significant high prevalence of infestation during February 88% (22/25); $P<0.036$ and the lowest one was during June 2024 whereas

the infestation rate reached 24% (6/25) as shown in Table 1. It was remarkable that winter season was of the highest prevalence, whereas 81.3% (61/75) followed by Autumn 66.7% (50/75), Spring, 42.7% (32/75), and Summer 34.7% (26/75) (χ value18.479; P<0.001) as shown in Fig. 2. Age of animals is considered as a risk factor for infestation. The prevalence of nasal myiasis in the adult camels of age more than three years old was prominent 70.8% (124/ 175); (P<0.004) compared with the young ones less than three years 40% (50/125). Sex of animals was not a risk factor for the infection P<0.926. The prevalence during cold season was highly significant P<0.001 where it reached 74% (111/150) Table 2.

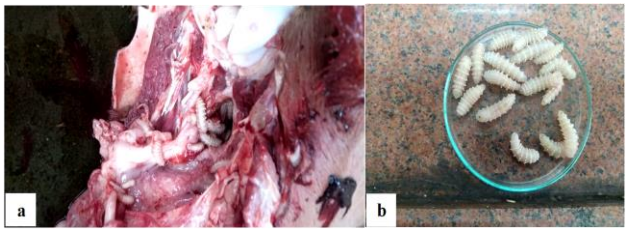

Fig. 1: a. Nasopharyngeal region of infested camels containing larvae embedded firmly to the congested, haemorrhagic and oedematus mucous membrane, and larvae loosely attached or even moving in the bloody mucous, b. *C. titillator* larvae collected from the nasopharangyl regions, the frontal sinuses and the turbinate bone of infested camels.

Table 1: Prevalence of nasopharyngeal myiasis in camels along the period extended from February 2024 to January 2025 in Giza province, Egypt

Month	Examined number	Negative	Positive	Prevalence (%)
February 2024	25	3	22	88
March 2024	25	14	11	44
April 2024	25	13	12	48
May 2024	25	16	9	36
June 2024	25	19	6	24
July 2024	25	13	12	48
August 2024	25	17	8	32
September 2024	25	10	15	60
October 2024	25	8	17	68
November 2024	25	7	18	72
December 2024	25	6	19	76
January 2025	25	5	20	80
Total	300	126	174	58
χ^2 value	20.799			
P value	0.036*			

* Significance.

Table 2: Association between nasopharyngeal myiasis and risk factors related to age, sex and seasons of studied camels

Risk factors		Total examined number	Positive	Prevalence (%)	χ^2 value	P value
Age	<3 years	125	50	40		
	>3Years	175	124	70.8	8.182	0.004**
Gender	Female	100	57	57		
	Male	200	117	58.5	0.009	0.926NS
Season	Cold	150	111	74		
	warm	150	58	38.7	11.571	0.001**

** highly significant, NS=non-significant.

Herb yield and essential oil production

The data in Table 3 revealed that the greatest fresh and dry weights of herb (g plant^{-1} and ton hectar^{-1}) of *Mentha longifolia* were obtained in the 2nd cut on the 4th of June and no further increases were recorded in the 3rd cut on the 22nd of July, while the highest values of essential oil percentage (1.43%) and yields (1.96mL plant^{-1} and 87.0L hectar^{-1}) were obtained at the 3rd cut on the 22nd of July.

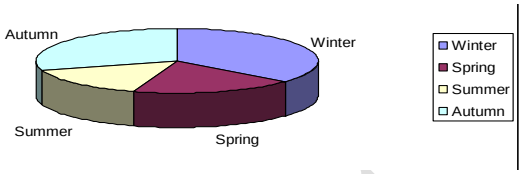

Fig. 2: Seasonal prevalence of nasopharyngeal myiasis among infested camels.

Essential oil constituents

The identified components of *M. longifolia* essential oil are shown in Table 4. The relative percentages of the main constituents showed variation in all three cuts date and oxygenated monoterpenes (86.55, 92.91 and 90.79 % on 28th of April, 4th of June and 22nd of July, respectively) were dominated. The major constituents were pulegone (63.79, 62.41, and 55.25%) followed by eucalyptol (8.88, 14.68 and 18.26%), then I-menthone (6.03, 8.32 and 9.83%) in the 1st, 2nd and 3rd cuts respectively. The highest percentage of pulegone (63.79%) was obtained from the 1st cut on the 28th of April followed by 62. 41% in the 2nd cut on the 4th of June and decreased to 55.25% in the 3rd cut on the 22nd of July. In contrast the 3rd cut on the 22nd of July produced the highest percentage of eucalyptol (18.26%) and I-menthone (9.83%).

Antioxidant activity of the essential oil

Data in Table 5 show the effect of cuts on the antioxidant activity of the essential oil of *M. longifolia* plants. It is clear that the cuts have an inversely significant effect on the antioxidant activity which recorded the highest values in the 1st cut (28th of April) and the lowest values in the 3rd cut (22nd of July). There is a significant difference between the first and third cuts in all concentrations used of the essential oil, as well as between the second and third cuts when using 15, 20, and 25µL/mL concentrations. While the first cut is significantly different from the second cut when using only 20 and 25µL/mL concentrations.

The highest antioxidant activity (79.4%) resulted by using 25µL/mL oil of the first cut, followed by 71.8 and 59.3% of the second and third cuts, respectively at the same concentration. In another word, the highest IC_{50} (20.98µL/mL) is obtained at the third cut, followed by 17.70µL/mL at the second cut and then 16.20µL/mL for the first cut, which confirms the previous results that the essential oil of the first cut led to the highest antioxidant activity.

Effectiveness of *M. longifolia oils* on *C. titillator* larvae

M. longifolia essential oil significantly affected the 2nd and 3rd larval stages across three different cut dates (28th of April, 4th of June and 22nd of July), compared to the control group. Mortality percentages, detailed in Table 6 and 7, varied depending on the concentration of the oil and the cut date. For the second larval stage, complete mortality

Table 3: Fresh and dry weights of herb yield as well as essential oil percentage and yield of *Mentha longifolia* during three cuts dates

Cuts	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Fresh yield (ton ha^{-1})	Dry yield (ton ha^{-1})	Oil percentage (%)	Oil yield (mL plant^{-1})	Oil yield (L ha^{-1})
1st Cut	90.0	17.2	4.0	0.76	0.77	0.70	30.9
2nd Cut	143.0	31.8	6.4	1.41	1.12	1.60	71.3
3rd Cut	136.8	30.7	6.1	1.37	1.43	1.96	87.0
LSD at 5%	23.02	3.68	1.00	0.169	0.202	0.431	19.00

Table 4: Essential oil constituents of *Mentha longifolia* during three cuts dates

RT	KI	Compounds	MF	Area %		
				1st Cut	2nd Cut	3rd Cut
4.93	930	α-Pinene	C10H16	0.96	0.93	1.17
5.87	965	Sabinene	C10H16	0.85	0.94	1.30
5.96	968	β-Pinene	C10H16	1.52	1.48	2.09
6.40	982	β-Myrcene	C10H16	0.63	0.41	0.92
7.34	1012	β-Cymene	C10H14	0.2	--	0.19
7.49	1018	Eucalyptol	C10H18O	8.88	14.68	18.26
7.59	1022	D-Limonene	C10H16	3.09	0.77	--
10.18	1102	β-Linalool	C10H18O	0.5	0.50	0.44
11.77	1143	l-Menthone	C10H18O	6.03	8.32	9.83
12.52	1160	Isopulegone	C10H16O	1.27	1.30	1.48
12.72	1165	L-à-Terpineol	C10H18O	0.3	0.36	0.75
13.11	1173	Terpinen-4-ol	C10H18O	0.31	--	0.26
13.84	1188	α-Terpineol	C10H18O	0.95	1.15	1.73
15.35	1222	Pulegone	C10H16O	63.79	62.41	55.25
15.75	1232	Piperitone	C10H16O	0.15	--	--
17.20	1264	Bicyclo[3.2.0]heptan-2-one, 5-formylmethyl-6-hydroxy-3,3-dimethyl-6-vinyl	C13H18O3	0.39	1.78	0.83
19.34	1309	cis-Verbenone	C10H14O	3.98	2.41	1.96
23.38	1399	Caryophyllene	C15H24	1.78	--	0.47
24.78	1433	Humulene	C15H24	0.14	--	--
25.87	1458	Germacrene D	C15H24	0.41	--	--
27.24	1489	γ-Cadinene	C15H24	0.34	--	--
29.88	1553	Caryophyllene oxide	C15H24O	--	0.60	0.42
32.40	1613	tau.-Cadinol	C15H26O	1.97	0.50	0.66
Total Identified				98.44	98.54	98.01
Monoterpenes hydrocarbons				7.25	4.53	5.67
Oxygenated Monoterpenes				86.55	92.91	90.79
Sesquiterpenes hydrocarbons				2.67	0.00	0.47
Oxygenated Sesquiterpenes				1.97	1.10	1.08

RT: Retention time, KI: Kovat index

Table 5: Antioxidant activity (%) of the essential oil of *Mentha longifolia* during three cuts dates

Cuts	Concentration					IC_{50} (µg/mL)*
	5 µL/mL	10 µL/mL	15 µL/mL	20 µL/mL	25 µL/mL	
1st Cut	17.6	29.8	45.0	59.8	79.4	16.20
2nd Cut	16.2	28.0	42.6	54.3	71.8	17.70
3rd Cut	14.8	25.5	36.5	47.5	59.3	20.98
L.S.D.	1.92	1.74	2.91	2.68	3.02	

***** IC_{50} signifies concentration (µg/mL) for 50% Inhibition.

(100%) occurred within 2h at concentrations of 200mg/mL from the 1st and 2nd cuts (on 28th of April and 4th of June), while the 3rd cut resulted in 100% mortality after 10h of exposure. At 100mg/mL concentration, 100% mortality among the 2nd larval stage was observed 4h after exposure to oil from the 2nd cut (Table 6). The third larval stage showed 100% mortality after 8h at 200 mg/mL from the 2nd cut (Table 7). Mortality reached 93.3% after 24 hours of exposure to 100 and 200mg/mL oil concentrations from the 1st and 3rd cuts, respectively. In contrast, the reference drug, ivermectin, achieved 100% mortality in the second and third larval stages after 10 and 24h, respectively.

Lethal concentration values (LC50 and LC90) were calculated for both larval stages at 2h and are presented in Table 6 and 7 for each cut. The second larval stage exhibited the highest LC50 and LC90 values at the 3rd cut, with values of 79.698mg/mL and 254.372mg/mL, respectively. The lowest LC50 and LC90 values were recorded at the 2nd cut, at 1.18mg/mL and 74.987mg/mL. For the third larval stage, the highest LC50 and LC90 values were 294.253mg/mL and 2391.451mg/mL, respectively, at the 1st cut, while the lowest values were 119.174mg/mL and 256.31mg/mL at the 2nd cut.

Light microscopic observations

The cuticle of the third instar of *C. tittilator* of non-treated control (group 1) is composed of an epicuticle, and a procuticle. The procuticle is consist of exocuticle, endocuticle, and inner epidermal cells (Fig. 3a and Fig. 3b). The cuticle of the third instar of *C. tittilator* epicuticle layer treated with ivermectin at dose of 0.003mg/mL (group 2) showed wrinkling and corrugation (Fig. 3c and Fig. 3d).

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Table 6: Mortality Percentages (Mean±SE) of *C. titillator* 2nd larval stage treated with different concentrations of *M. longifolia* three cuts essential oils at various time intervals

Time interval/hr	The first cut			The second cut			The third cut			Reference drug	Negative control	F1	P1
	Concentration (mg/mL)												
	50	100	200	50	100	200	50	100	200				
2	26.7±6.7^{bC}	46.6±13.3^{bB}	100±0.0^{aA}	86.6±6.7^{aB}	93.3±6.7^{aA}	100±0.0$^{a\ A}$	40±11.5^{bC}	40±0.00^{bC}	93.3±6.7^{aA}	26.6±6. 7^{bC}	0.0±0.0^{c}	26.7	0.0
4	86.6±6.7^{aB}	93.3±6.7^{aA}		93.3±6.7^{aAB}	100±0.0^{aA}		66.6±6.7^{bB}	66.6±6.7^{bB}	93.3±6.7^{aA}	33.3±6. 7^{cC}	0.0±0.0^{d}	37.1	0.0
6	100±0.0^{a}	100±0.0^{aA}		100±0.0^{aA}			86.6±6.7^{abAB}	80±11.5a^{bAB}	93.3±6.7^{aA}	66.6±17.6^{bB}	0.0±0.0^{c}	18.5	0.0
8							86.6±6.7^{abAB}	80±11.5^{bAB}	93.3±6.7^{abA}	93.3±6. 7^{abA}	0.0±0.0^{c}	35.9	0.0
10							93.3±6.7^{abA}	86±6.7^{bAB}	100±0.0^{aA}	100±0.0^{aA}	0.0±0.0^{c}	110.3	0.0
12							93.3±6.7^{abA}	86±6.7^{bAB}			0.0±0.0^{c}	110.3	0.0
18							93.3±6.7^{aA}	100±0.0^{aA}			0.0±0.0^{b}	223.0	0.0
24							93.3±6.7^{aA}				0.0±0.0^{b}	223.0	0.0
P1	0.000	0.000	0.089	0.089	0.466	-	0.001	0.000	7.619	0.000	-	-	-
F1	59.357	12.543	2.214	2.214	1.000	-	6.571	7.619	0.571	17.814	-	-	-
LC50	82.829			1.18			79.698			-	-	-	-
LC90	174.927			74.987			254.372			-	-	-	-

SE: Standard error of mean values. Values with different letters (a, b, c…) are significantly different (P<0.05) within hours for each concentration; Values with different letters (A, B,..) are significantly different (P<0.05) within concentrations of each cut (based on the nonoverlapping confidence limits). F1 and P1 :variants and significance between groups of each concentrations; F2, P2:variants and significance between groups for each cut. LC50, LC90: Lethal concentration that kill 50% and 90% of larvae after 2 hr, respectively.

Table 7: Mortality Percentages (Mean±SE) of *C. titillator* 3rd larval stage treated with different concentrations of *M. longifolia* three cuts essential oils at various time intervals

Time interval/hr	The first cut			The second cut			The third cut			Reference drug	Negative control	F2	P2
	Concentration (mg/mL)												
	50	100	200	50	100	200	50	100	200				
2	13.3±6.7^{deA}	26.6±6.7^{cdE}	40±0.0^{bcD}	6.7±6.7^{deE}	40±11.5bcB	80±0.0^{aC}	20±11.5^{cdeB}	26.6±6.7^{cdC}	53.3±6.7^{bC}	6.7±6.7^{deC}	0.0±0.0e	11.567	0.000
4	33.3±17.6^{bcdA}	40±0.0^{bcDE}	53.3±6.7^{abCD}	13.3±6.7^{cdDE}	66.6±13.3^{abAB}	86.6±6.7^{aBC}	53.3±6.7abA	46.6±13.3^{bcBC}	53.3±6.7abAB	40±20^{bcBC}	0.0±0.0d	4.800	0.001
6	33.3±17.6^{bcdA}	46.6±6.7^{bcCD}	60±11.5abcBCD	20±11.5^{cdCDE}	73.3±17.6^{abAB}	93.3±6.7^{aAB}	60±11.5abcA	53.3±6.7^{abcABC}	66.7±13.3abAB	60±23a^{bcAB}	0.0±0.0d	3.933	0.004
8	46.6±13.3^{aB}	46.6±6.7^{bCD}	66.6±6.7abBC	40±11.5bBCD	73.3±17.6abAB	100±0.0^{aA}	60±11.5^{bA}	60±0.0b^{ABC}	66.6±13.3abAB	66.7±17.6^{abAB}	0.0±0.0c	5.087	0.001
10	46.6±13.3^{bA}	46.6±6.7^{bCD}	73.3±6.7abAB	46.6±6.7bBC	73.3±17.6abAB		66.6±6.7^{abA}	80±11.5^{abAB}	86.6±13.3^{aAB}	73.3±17.6^{abAB}	0.0±0.0^{c}	6.248	0.000
12	53.3±17.6^{bA}	60±0.0^{bBC}	80±11.5^{abAB}	60±11.5^{bAB}	80±11.5^{abAB}		80±11.5^{abA}	80±11.5^{abAB}	86.6±13.3^{abAB}	73.3±17.6^{abAB}	0.0±0.0^{c}	5.206	0.001
18	60±11.5^{cA}	66.6±6.7^{bcB}	93.3±6.7^{abA}	80±11.5^{abcA}	93.3±6.7^{abA}		80±11.5^{abcA}	86.6±13.3^{abcA}	93.3±6.7^{abA}	86.7±13.3^{abcAB}	0.0±0.0^{d}	9.257	0.000
24	60±11.5^{bA}	80±0.0^{abA}	93.3±6.7^{aA}	86.6±6.7^{aA}	100±0.0^{aA}		80±11.5^{abA}	86.6±13.3^{aA}	93.3±6.7^{aA}	100±0.0^{aA}	0.0±0.0^{c}	16.077	0.000
P1	0.325	0.000	0.002	0.000	0.148	0.002	0.015	0.007	0.054	0.025			
F1	1.270	9.943	5.818	10.134	1.839	5.429	3.650	4.314	2.600	3.210			
LC50	294.253			119.174			196.491			-	-	-	-
LC90	2391.451			256.31			1282.357			-	-	-	-

SE: Standard error of mean values. Values with different letters (a, b, c…) are significantly different (P<0.05) within hours for each concentration; Values with different letters (A, B,..) are significantly different (P<0.05) within concentrations of each cut (based on the nonoverlapping confidence limits). F1 and P1:variants and significance between groups of each concentrations; F2, P2:variants and significance between groups for each cut. LC50, LC90:Lethal concentration that kill 50% and 90% of larvae after 2 hr, respectively

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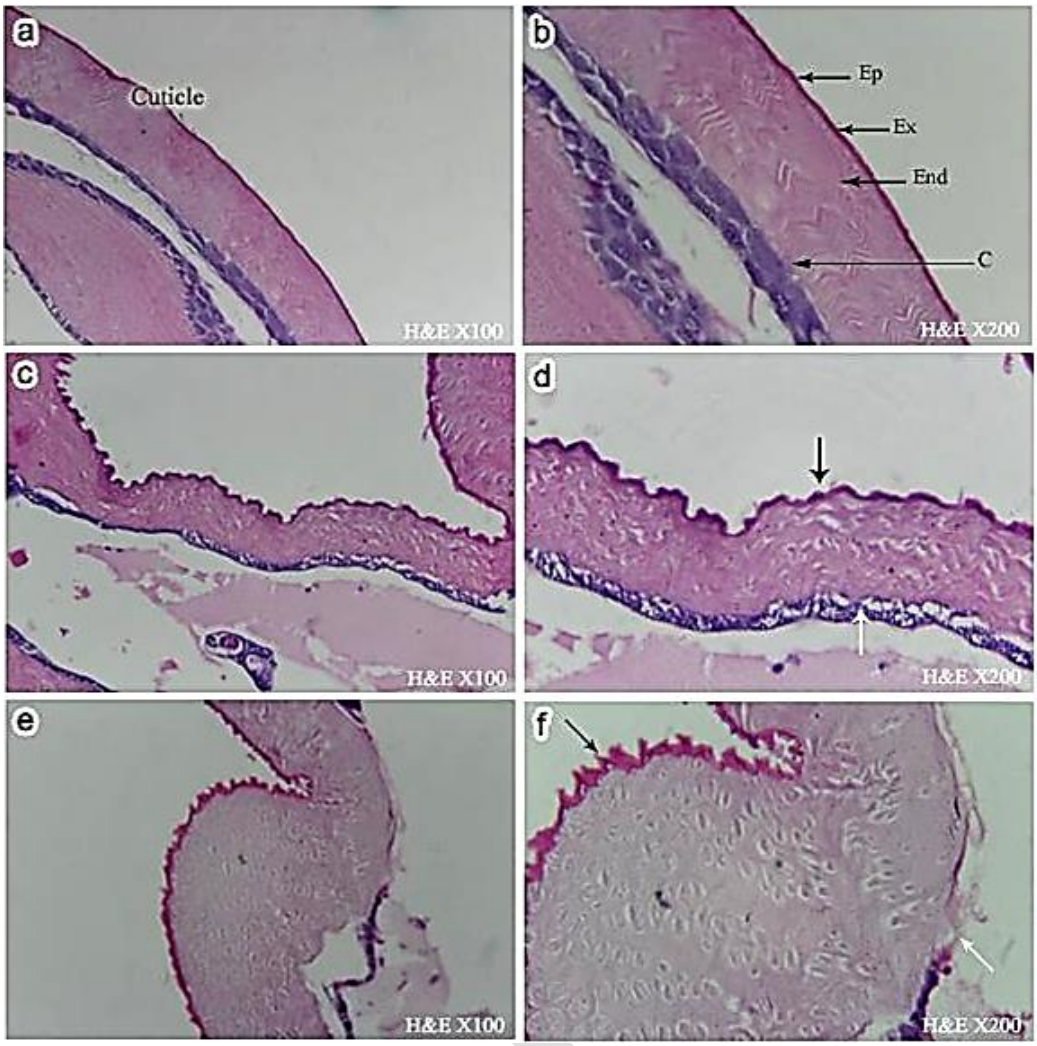

Fig. 3: Light microscopy of the cuticle cross section of the third instar larvae. a, b Untreated control larvae. c, d Following 6h post treatment with 0.003mg/mL ivermectin. e, f Following 6h post treatment with 200mg/mL *M. longifolia* oil. Note distortion of inner cellular layer of epidermal cells. Ep: epicuticle, Ex: exocuticle, End: endocuticle, Epid: epidermal cell.

The effects of a 200mg/mL concentration of *M. longifolia* oil from the 2nd cut on cuticle morphology (group 3) were examined (Fig. 3e and Fig. 3f) where severe distortion and wrinkling of epicuticle layer were observed. Swelling of the cuticle led to moderate degeneration and atrophy of the fibrils in both the exocuticle and endocuticle was recorded. Furthermore, the inner epidermal cells showed substantial damage (Fig. 3e and Fig. 3f).

The SEM findings

Untreated control *C. titillator* 3rd instars (group1) appeared to have tiny antenna lobes and an unarmed pseudocephalon with an antenno-maxillary sensory complex consisting of the antenna and maxillary palp, as well as a set of center small coeloconic sensilla and a few outlying sensilla (Fig. 4a). The buccal funnel is already well-structured with powerful mouth hooks (maxillae). The maxillae are highly pointed and ventrally curved, with wrinkle areas and dorsolateral grooves. There are no mandibles (Fig. 4a). A band of tiny spines with irregular rows can be seen ventrally. There are numerous curricular semi-grooves in the abdominal segments, many of which include pits and deep pores (Fig. 4d). The abdominal respiratory spiracles were located at the posterior end of the larval body, contained inside a cuticle ring and connecting to the dorsal and ventral lips (Fig. 4g). The spiracles plate showed signs of significant sclerotization and was packed with many respiratory units that were dispersed throughout the spiracles plates. Every breathing unit featured a slit encircled by rima.

After 6h, third instar larvae of both the reference drug (group 2) (Fig. 4c, Fig. 4f and Fig. 4i) and 200mg/mL of *M. longifolia* oil second cut (group 3) (Fig. 4b, Fig. 4e and Fig. 4h) exhibited unusual features. The majority of investigated specimens showed significant integument edema. The front cuticle surface of group 3 appeared to be more sloughed and corroded than group 2 ivermectin treated, while inter-segmental spins swelled for both groups. Also, *M. longifolia* oil caused significant internal structural damage and shrinking at posterior spiracles more obviously than ivermectin treated one.

DISCUSSION

Prevalence of nasopharayngeal myiasis

Infestation by *C. titillator* is a widely spread among camels and it is associated with severe financial losses (Abu El Ezz et al. 2018). Periodic surveillance of nasopharyngeal myiasis is a need for applying efficient management and control measures. Several studies have reported varying prevalence of nasopharyngeal myiasis, with 67.6% and 52% in Saudi Arabia (Hussein et al. 1983; Fatani and Hilali 1994), 25% in Egypt (Morsy et al. 1998), 41% in Saudi Arabia (Alahmed 2002), 41.67% in Egypt (Khater et al. 2013), 46% in Jordan (Al-Ani and Amr 2016), 40.07% in Iraq (Al-Jindeel et al. 2018) and 54.2% in China (Yao et al. 2022).

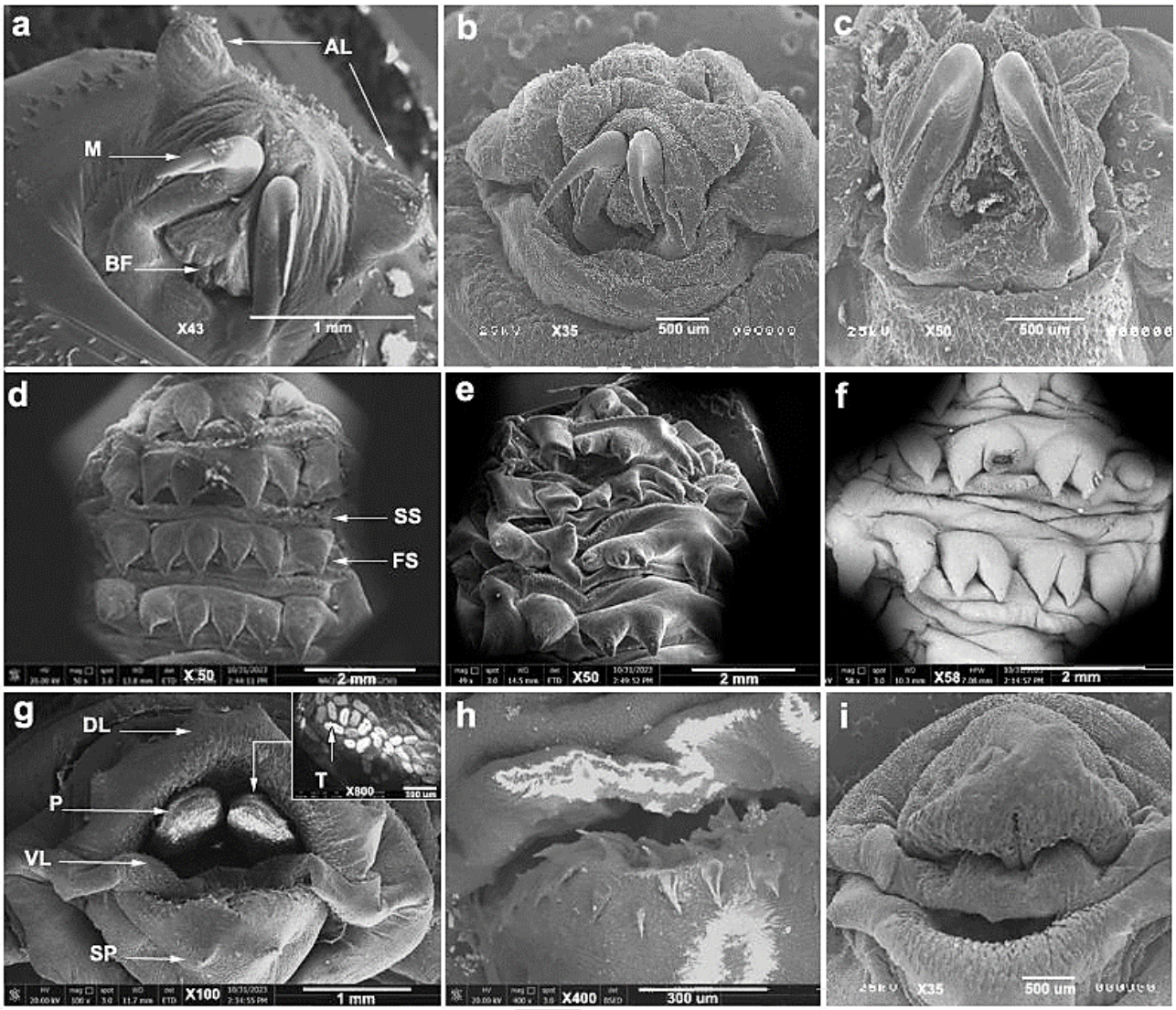

Fig. 4 (a-i): Scanning electron micro-graphs of the third instar *C. tittilator* (L.). a) Normal anterior end and buccal funnel. b) After being exposed for 6 h to 200mg/mL of *M. longifolia* 2nd cut, the anterior end showed severe corrosion. c) After 6h of ivermectin treatment, the anterior end showed sever damage, d) Normal appearance of integument and spines. e, f) The mid region of the larvae treated with *M. longifolia* 2nd cut and ivermectin, respectively, displayed swelling of the intersegmental spine and integument, g) Typical kidney-shaped posterior spiracles with spiracle units. h,i) The treated larvae with *M. longifolia* 2nd cut and ivermectin, respectively, exhibited severe corrosion swelling of the spines in the posterior region, distortion of posterior spiracles, sunken respiratory units, and lost linear shape of respiratory slits. M: maxillae; BF: buccal funnel; AL: antennary lobe; SS: intersegmental spine; FS: fleshy spine; DL: dorsal lip; P: spiracle plate; VL: ventral lip; SP: sensory papillae; T: respiratory slit.

These variations in prevalence across various nations could be due to multiple factors such as environmental conditions, camel populations, and rearing facilities in each region. This study revealed the highest prevalence of infestation in February (88%), with the lowest prevalence observed in June (24%). Notably, the winter season showed the highest overall prevalence at 81.3% ($P<0.001$). In contrast, the lowest prevalence was observed during the summer season, with a rate of 34.7%. Additionally, the prevalence during the cold season (74%) was significantly higher than in the warm season (38%).

These findings aligned with those obtained by Oryan et al. (2008) in Iran, who observed a higher prevalence of nasal myiasis in colder months, reaching 69.8%, compared to 36.2% in warmer months. Similarly, Jalali et al. (2016) observed a higher prevalence in cold seasons (62.5%) compared to hot seasons (32%) in Iraq. Furthermore, Yao et al. (2022) in China found that the infestation rate was higher in the cold season (64.2%) than in the warm season (48.4%). Morsy et al. (1998) also reported that autumn had the highest prevalence of nasal myiasis among camels in Egypt. The higher prevalence of nasopharyngeal myiasis in camels during the cold season might be attributed to the increased survival and development of larvae in lower temperatures and/or the management and feeding systems of the animals.

The results of the current study revealed a higher infestation rate in camels older than three years (70.8%) compared to those younger than three years (40%). This finding was consistent with previous studies by Fatani and Hilali (1994), El Bassiony et al. (2005), Sanchez (2010), and Yao et al. (2022). According to Bekele and Molla (2001) and Shakerian et al. (2011), adult camels with mature, heavier bodies were more tolerant to infestation, allowing flies to deposit eggs around their nostrils, while

younger camels tended to actively avoid egg deposition near their nostrils. The present study also found that the sex of the animals was not a significant risk factor for infestation (P>0.05). This result aligned with findings by Shakerian et al. (2011) and Yao et al. (2022), who found that there was no significant difference in nasal myiasis infestation between male and female camels.

Up till now the traditional approach of nasopharyngeal myiasis treatment is using chemotherapeutics, despite their inefficacy. Production of eco-friendly alternatives is a need to overcome the medical, economical and environmental concerns of emerging drug resistance.

Herb yield and essential oil production

Harvest cut may have an impact on herb yield due to temperature, sunshine accumulation, and available day length (Figueiredo et al. 2008). These results are agreed with Omer et al. (2022) who showed that when harvesting dates were postponed until July 12 for pennyroyal and July 26 for apple mint, the fresh and dry weight of the herb increased. Aziz et al. (2019) stated that the highest herb yield of *Achillea millefolium*, and the best essential oil content and composition of it can be obtained according to the harvest time and climatic conditions.

Essential oil constituents

The cut dates had a significant impact on the main components of *M.* l*ongifolia* essential oil, and displayed significant variances in the percentage and the identified constituents. These results agreed with Shahbazi et al. (2021) who revealed that pulegone (47.20%), 1,8 cineole (22.72%) and menthone (13.44%) are the main components of the essential oil of *M. longifolia*. Also, pulegone, E-Menthone, Z-menthone, eucalyptol were the major constituents of *M. longifolia* essential oil (Abdel-Gwad et al. 2022). Fouad et al. (2023) stated that the 2nd cut on the 12th of June was found to be suitable for producing the greatest percentage of pulegone in *M. pulegium* and *M. Longifolia*, linalyl acetate in *M. suaveolens*, as well as increasing the value carvone in *M. spicata.* However, the biosynthesis of Linalool and eucalyptol in *M. suaveolens* as well as D-limonene in *M. spicata* and *M. viridis* increased when the third cut was delayed until August 1. Omer et al. (2022) showed that pulegone was found to be the major constituent of pennyroyal oil and the highest relative concentration (94.89 %) was obtained at the 2nd harvest date on 17th of May. The later harvest date in July 26th produced the highest relative concentration of ρ-menthan-3-one,cis (48.14 %) and the lowest concentration of pulegone (46.91%). Increasing plant age increased the biosynthesis of cis-ρ-menthan-3-one and decreased pulegone amount. Also, the transformation rate of cis-ρ-menthan-3-one to pulegone is influenced by the environmental factors and conditions.

Antioxidant activity of the essential oil

The highest antioxidant activity of the essential oil of the first cut could be attributed to its highest content (63.79%) of the main component (Pulegone) compared to the other two cuts. Pulegone has very strong antioxidant activity as mentioned by Ruberto and Baratta (2000), Demirci et al. (2011) and Silva et al. (2012). Moreover, the first cut has the highest content of monoterpenes hydrocarbons (7.25%) which may be considered as active antioxidants (Ruberto and Baratta 2000).

Effectiveness of *M. longifolia* oil on *C. titillator* larvae

The current *in vitro* treatment of *C. titillator* showed that the mortality rates of the larvae varied depending on the concentrations of *M. longifolia* oils and the dates of the cuts. The second larval stage exhibited complete mortality (100%) within 2h when exposed to 200mg/mL concentrations from the 1st and 2nd cuts (28th of April and 4th of June), and within 10h for the 3rd cut (22nd of July). At 100mg/mL, the second larval stage showed 100% mortality 4h after exposure to oil from the 2nd cut. This high efficacy indicates that *M. longifolia* oil is highly effective at various concentrations and exposure times, particularly for the second larval stage. Similarly, the third larval stage exhibited 100% mortality 8h post-exposure to 200mg/mL oil from the 2nd cut. Although the mortality rate reached 93.3% after 24h of exposure to 100 and 200mg/mL concentrations from the 1st and 3rd cuts, respectively, the third larval stage was slightly less susceptible than the second stage under the same conditions. The differential effectiveness observed in *M. longifolia* oil based on cut dates. Garcia et al. (2018) suggested that the chemical composition of essential oils plays a critical role in their effectiveness. Similar findings have been reported for other essential oils, such as *Eucalyptus globulus*, where both composition and efficacy varied depending on the harvest season and extraction method (Santos et al. 2019).

These variations highlighted the importance of optimizing harvest times to enhance the larvicidal potential of essential oils. Notably, the study compared these results with the effectiveness of the reference drug, ivermectin, which caused 100% mortality in both the 2nd and 3rd larval stages at 10 and 24h post-treatment, respectively. This comparison emphasized the potential of *M. longifolia* oil as a promising alternative to traditional chemical treatments.

Likewise, Khater et al. (2013) mentioned the anti - parasitic action of lavender (50%) on *C. titillator* larvae. Moreover Abu El Ezz et al. (2018) recorded the priority of the effect of camphor oil than ginger and cinnamon oils on the third larvae of *C. tittilator*.

Moreover, the LC50 and LC90 values, further illustrated the effectiveness of the essential oils. The 2nd larval stage exhibited the lowest values of LC50 and LC90 with 1.18 and 74.987mg/mL, while, for the 3rd larval stage were 119.174 and 256.31mg/mL at the 2nd cut. These findings suggest that the effectiveness of *M. longifolia* oil can vary significantly depending on the cut date and concentration, with the 2nd cut on being particularly potent. The differential effectiveness observed between the different larval stages and cut dates can be attributed to the variations in the chemical composition of the essential oils extracted at different times (Garcia et al. 2018). The potent larvicidal activity, especially at the 2nd cut on 4th of June, highlights the importance of optimizing harvest times to maximize the efficacy of essential oils as bio-insecticides.

Light and electron scanning microscopic observations

The cuticular structure of the third instar of *C. tittilator* (L.) is essential for its protection and interaction with the environment. Significant damage to the inner epidermal cells was also observed. These cells are crucial for the

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synthesis and maintenance of the cuticle (Gillott 2005). Damage to the epidermal cells can impair the production of new cuticular material and the repair of existing structures, leading to long-term detrimental effects on the insect's health and development. These findings align with other research indicating the potential of botanical extracts to interfere with insect cuticle formation and function. For instance, essential oils from plants like *Azadirachta indica* (neem) have been shown to disrupt cuticle synthesis and cause morphological abnormalities on mosquito vector *Culex pipiens* (L.) (Ahmed et al. 2023). The observations from the SEM analysis of *C. titillator* (L.) third instars after 6h of treatment with both the reference drug and 200mg/mL of *M. longifolia* oil of the second cut reveal several noteworthy changes in larval morphology. Recent studies have similarly observed integumentary disruptions in insect larvae subjected to various treatments. Jiménez-Durán et al. (2021) reported that exposure to certain phytochemicals led to pronounced edema and structural degradation of the cuticle in *Spodoptera litura*. These findings are consistent with our observations of integument edema and surface corrosion. The swelling of inter-segmental spines observed in our study aligns with the findings of Stadler and Buteler (2009) and Abu El Ezz et al. (2018) who detected severe cell membrane disruption and darkening following exposure to essential oils. Najar-Rodríguez et al. (2007) showed the capability of essential oils to sneak the cell membranes and accumulate within the cytoplasm prompting dehydration of the cell and condensation of DNA inside the nucleus. The insecticidal activity of *M. longifolia* oil was primarily attributed to oxygenated monoterpenes, Pulegone, a monoterpene, is an active compound in *M. longifolia* essential oil that can penetrate bacterial cell walls, leading to protein denaturation and disintegration of the cell membrane. This causes cytoplasmic leakage, cell lysis, and ultimately cell death (Oussalah et al. 2007). Pulegone has been shown to effectively combat *S. aureus*, *S. typhimurium*, and *E. coli* (Farhanghi et al. 2022). The lipophilic nature of essential oils helps increase membrane permeability or cause membrane destruction due to the activity of enzymes like protein kinase in the cell membrane (Vogt 2010). This suggested that *M. longifolia* essential oil could serve as a viable alternative to traditional chemical control strategies. Additionally, Khani and Asghari (2012) found that the essential oil demonstrated anti-insect properties against *Tribolium castaneum*, a pest of stored products, and showed inhibitory effects on insect growth at the larval and pupal stages, along with insect-repellent activity (Pascual-Villalobos and Robledo 1998).

Thus, these findings suggested that mint species' essential oils can serve as natural larvicidal agents, offering a safer and more efficient alternative to chemical insecticides for humans, livestock and the environment.

Conclusion

The study drew attention to the high prevalence of nasopharyngeal myiasis in camels in Giza Governorate, Egypt. The risk factors associated with high infestation included the cooler seasons particularly winter and autumn, and being older camels. The cuts dates affect the rate of accumulation of the major constituents of *M. longifolia* essential oil and revealed great variations in the percentage and the identified components. The highest relative concentration of pulegone (63.79%) was obtained from the 1st cut on the 28th of April followed by 62. 41% in the 2nd cut on the 4th of June. This study also highlighted the potent larvicidal effects of *M. longifolia* oil, based on harvest timing for both 2nd and 3th larval instars of *C. titillator* particularly at concentration 200mg/mL oil from the 2nd cut compared to control untreated and ivermectin treated larvae. It could be concluded that *M. longifolia* essential oil could serve as a promising natural insecticides, offering an environmentally friendly alternative to traditional treatments or complementary with the chemotherapeutics.

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Availability of data and materials: All data obtained is included in this manuscript and is available on request from the corresponding author.

Ethical Approval and Consent to participate: Ethical permission for the use of animals was approved by institutional guidelines of the National Research Centre's Animal Research Committee (0444 0125). Informed consent has been obtained from all sheep owners for this study.

Author's Contribution: NMFH: Planned the study of the prevalence, and the larvicidal assessment experiments, conducted the clinical and postmortem examinations, determined the prevalence and the risk factors, also conducted larval dipping test, histopathological examination, scanning electron microscopy, discussed results, wrote, and reviewed the final manuscript. AE: conducted histopathological examination and scanning electron microscopy, statistical analysis, discussed results, wrote and reviewed the final manuscript. HS: carried out histopathological examination, scanning electron microscopy, and reviewed the final manuscript. NMTA: inspired the idea of study, conducted the larval dipping test, Scanning electron microscopy, and reviewed the final manuscript. HA: conducted the larval dipping test, histopathological examination, and reviewed the final manuscript. RF: was responsible for cultivating plants, fertilization, antioxidant activity, analyzing the oil using GC-MS and calculating the Kovat index. HF: was responsible for cultivating plants, recording vegetative parameters, calculating the yield, extracting the essential oil and conducting statistical analysis of growth parameters. EEA: proposed the research plan of the agricultural experiment, provided the fertilizers and wrote the sections of the field experiment, essential oil

constituents and antioxidant activity in the manuscript. EAO: proposed the research plan of the agricultural experiment, designed the agricultural experiment, provided the used tools and chemicals and reviewed the manuscript.

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