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Light, Scanning Electron Microscopy and SDS-PAGE Studies on the Effect of the Essential Oil, *Citrus sinensis* Var. *balady* on the Embryonic Development of Camel Tick *Hyalomma dromedarii* (Koch, 1818) (Acari: Ixodidae)

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Abstract: GC-MSE analysis of the essential oil of fresh fruit peel of *Citrus sinensis* var. *balady* recognized two main natural toxic compounds, limonene (83.28%) as hydrocarbon compound and linalool (3.97%) as oxygenated compound. Therefore, the objective of this study was to evaluate its effect on different egg-ages of *Hyalomma dromedarii* at four concentrations of 1:40, 1:30, 1:20 and 1:15 (oil : ethanol 95%) (v/v). The LC_{50} values were 1:56, 1: 34, 1: 41, 1: 32, 1: 23, 1: 23, 1:18, 1:14 and 1:11 for egg-ages of 2, 4, 6, 9, 11, 13, 16, 18 and 20 day, respectively. Histological Examination (HE), Scanning Electron Microscopy (SEM) and Sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were done on the 9th day old-eggs treated with the essential oil 1:32 (the LC_{50} value of 9 day old-egg). HE was done on the 11, 12, 13, 14 and 15th day old eggs; SEM was done on the 11, 15 and 17th day old eggs and SDS-PAGE was done on the 10, 11, 12, 13, 14, 15 and 17th day old eggs and compared each with those of control. In control, HE showed that nuclei migrated to the periphery and became part of the cytoplasmic membrane, blastula appears as a complete ring cells. Germ layer form and the later differentiate to different organelles such as opithosoma, ambulatory segment and chelicera... etc. while incase of treated eggs, HE showed that irregular manner of ectoplasmic membrane formed, blastula gathered on one or two sides, the cells of germ layer gather on one side as small or large mass or ring shape. Cells gathered as small masses or finger shape without forming any organelles. SEM revealed that heavy small bulging wrinkles were observed on egg shells of control. These wrinkles changed into large size in treated eggs on the 11th day and disappeared at the following days to become smooth surfaced. SDS-PAGE exhibited 15, 14, 14, 12, 17, 14 and 15 bands for treated eggs on the 10, 11, 12, 13, 14, 15 and 17th day old-eggs, respectively and 14, 15, 16, 19, 17, 19 and 18 bands for control eggs at the same egg-ages. The molecular weights of these bands were different in both control and treated eggs. It was concluded that the essential oil of *C. sinensis* var. *balady* has strong toxic effect on eggs of *H. dromedarii* especially in earlier embryonic development.

Key words: *Citrus sinensis*, essential oil, embryonic development, histology, *Hyalomma dromedarii*, scanning electron microscope, SDS-PAGE

INTRODUCTION

Ixodid ticks are recognized as vectors of arboviruses and parasitic protozoa (Taege, 2000). *Hyalomma dromedarii* is one of those ticks that attack camels as a main host in Egypt (Abdel-Shafy, 2000). In the parasitic phase, larvae, nymphs and adults of *H. dromedarii* ingest a huge amount of blood. While non-parasitic phase occurs two times one at the final feeding of nymphs and the other at the final feeding period of females. Both fed nymphs and females drop on the ground of camel pens to moult to the next stage where the fed females lay large number of eggs. Blood loss resulting from tick-parasitic phase lead to anemia, consequently loss of animal strength and decrease in production of meat and milk.

One strategy to reduce disease transmission by ticks would be the identification of new molecular targets for the interruption of its life cycle (Silveira *et al*., 2006). In this respect, the non-parasitic phase that occurs after tick females complete their blood repletion, eggs are laid and embryonic development occurs. Treatment of ticks during

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this phase is tempting for two reasons; 1) it is far away from host and will not affect the camel, 2) embryonic development is strictly programmed during which an exclusive sequence of cellular and molecular process, most of which are not present in both larvae and adults (Silveira *et al*., 2006). Furthermore, egg development takes about 3 weeks and recently hatched larvae migrate to the upper part of the grasses and wait a vertebrate host (Sonenshine, 1991).

Chungsamarnyart and Jansawan (1996) found that the peel oils of *Citrus reticulate* and *C. maxima* (immature and mature fruits showed a high acaricidal activity against engorged females of *B. microplus* at dilution 1:10 (oil : ethyl alcohol). This was activity being 2 times higher than that of (+) – limonene. They added that *C. sinensis* and *C. maxima* peel oils (1:10) exhibited also a high larvicidal activity. However, the oil of *C. maxima* (immature fruits) showed a moderate larvicidal activity (1.5 times) stronger than that of (+) – limonene. Moreover, Abdel-Shafy and Soliman (2004) tested five essential oils against eggs, larvae and females of the cattle tick *Boophilus annulatus*. These oils were peppermint (*Mentha piperita*), spearmint (*Mentha viridis*), marjoram (*Marjorana hortensis*), lavender (*Lavandula officinalis*) and sweet basil (*Ocimum basilicum*). They found that all oils had toxic effects on all stages except *M. viridis* which was less toxic on tick females.

Most studies were concerned with bio-assay of essential oils for different stages of ticks including egg stage. As far as the effect of essential oils on embryonic development of tick eggs were not studied before. Therefore, this study tries to explain the effect of the essential oil of *Citrus sinensis* var. *balady* on the embryonic development of *H. dromedarii* eggs by the following ways; 1-Determining the toxicity of oil to eggs, 2-Investigating of various histological sections by light microscope, 3- Photograph of egg shells using SEM and 4-Detection of egg protein bands and its molecular weights using SDS-PAGE.

MATERIALS AND METHODS

Collecting of eggs: Fully engorged females of *Hyalomma dromedarii* (Koch, 1818) were collected from ground of camel pens, Burkash village, Giza governorate, Egypt and identified according to Estrada-Pena *et al*. (2004). Females were incubated at 25°C and 75% RH in plastic cups (one female/cup). Eggs were daily collected from each cup and gathered in one cup for each day to obtain eggs with different ages. Experiments were conducted in the laboratory of Parasitology and Animal Diseases Department, Veterinary Research Division, National Research Center, Egypt on February 2006.

Extraction of the essential oil: *Citrus sinensis* var. *balady* (Fam: Rutaceae) was collected at the ripening stage from trees cultivated in the experimental station of Horticultural Department, Ministry of Agriculture at El-Marg, Kalubyia, Egypt. Fresh fruits peel (flavedo and albedo) of orange samples were subjected to hydro distillation until there was no significant increase in the volume of the collected oil. The oil was dried over anhydrous sodium sulphate and kept in a dark bottle at refrigerator till performing chemical analysis and biological activity (Salido *et al*., 2004).

Chemical analysis of the essential oil: The essential oil components were determined; 20 μL of the respective essential oil were diluted with 1000 μL diethyl ether and then 2 μL of the diluted oil was injected in Perkin Elmer gas chromatograph model XL with a split ratio 1:10. The oil constituents were separated on 60 m DB-5 capillary column having 0.32 mm internal diameter according the method described by Soliman *et al*. (2003).

Toxicity test of essential oil: Toxicity test was carried out on the egg ages of 2, 4, 6, 9, 11, 13, 16, 18 and 20 day. Concentrations of the essential oil, *C. sinensis* var. *balady* were prepared by using ethanol 95% as a solvent. The test included 4 concentrations; 1:40, 1:30, 1:20, 1:15 (oil: ethanol 95%). Ethanol was used alone as a control treatment for each egg age. Each concentration or control treatment was replicated 5 times. Each replicate included 15 eggs. Treatment was applied by dipping eggs 30 sec in each concentration or alcohol in control treatment, transmitted to filter paper and incubated at 25°C and 75% RH until hatchability occurred. Calculated mortality percentages of eggs were based on eggs with brown-black color and abnormality shape, corrugated oval shape and corrected by Abbott's formula (Abbott, 1925). LC50 values for each age of egg were calculated according Finney (1971). Normal eggs were normal shape (oval) and colors (shiny brown) were left to develop until hatching occurred.

Histological examination of eggs: Nine day-old eggs were dipped for 30 second in the essential oil at concentration of 1 : 32 (the LC_{50} value of 9 day-old eggs in toxicity test). Control treatments were immersed at the same time in alcohol. Egg samples were taken on the following days 11th, 12th, 13th, 14th and 15th day Post Oviposition (POP). The samples were immersed in Bouin's solution for 24-48 h, dehydrated in dioxane for 10-24 h and embedded in paraplast. Serial sections of each egg sample were prepared and stained with Hematoxylin and Eosin (H and E stain) (El-Kammah *et al*. 1982).

Scanning electron microscopy of egg shells: Nine day-old eggs were dipped for 30 sec in the essential oil at concentration, 1 oil : 32 ethanol (the LC50 value of 9 day-old eggs in toxicity test). Control treatments were immersed at the same time in alcohol. Egg samples were taken on the 11th, 15 and 17th day POP. Then, egg samples of treatment and control were immersed in 2.5% glutraldehyde for 48 h, washed in buffer and post fixed in 1% osmium tetraoxide in 0.1 M cacodylate buffer before being dehydrated in an ethanol series (Cribb and Chitra, 1998). Alternatively, eggs were dehydrated in ethanol to 100%, dried at CO_2 critical point drier (Blazzer Union F1-9496 Blazer/Furstentun Liechtenstein, Germany), glued over specimens stubs and coated with 20 nm gold in a sputter coater (S150A Sputter Coater Edward, UK). Finally egg samples were examined and photographed with scanning electron microscope (JXA 840, Electron Probe Microanalyzer, Jeol, Japan).

Sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of egg proteins: Nine day-old eggs were dipped for 30 sec in the essential oil of *C. sinensis* at concentration of 1 : 32 (The LC50 value of 9 day-old eggs in toxicity test). Control eggs were immersed at the same time in alcohol. Egg samples for both treated and control were taken on the 10th, 11th, 12th, 13th, 14th, 15th and 17th POP. The samples were individually taken in 0.01M phosphate buffer saline, pH 7.2 (PBS), homogenized in an equal amount of PBS and then sonicated for 5 min under 150 watt interrupted pulse output at 50% power cycle using a sonifier cell disrupter. The sonicated eggs were subjected to a high speed centrifugation (10000 rpm) for one hour at 4°C. The resulting supernatant was collected and the protein content was determined by the Lowery method (Lowry *et al*., 1951). Ten percent SDS slabpolyacrylamide gel electrophoresis (Slab-PAGE) and running buffer consisting of 0.5M Tris, 1.92 M glycine and 10% SDS (Ph 8.3) were used as described by Hames (1987).

RESULTS

Phytochemical analysis of the essential oil: Essential oil of *C. sinensis* var. *balady* contained 7 hydrocarbon compounds and 6 oxygenated compounds. Hydrocarbon compounds; α-thujene (0.05%), α-pinene (0.64%), β-pinene (0.29%), Myrcene (2.13%), Limonene (83.28%), γ-terpinene (0.22%) and p-cymene (1.73%) were found. Oxygenated compound included; linalool (3.97%), linalyl acetate (1.56%), citral-b (1.64%), citral-a (1.97%), geraniol (0.69%) and dimethyl anthranilate (0.28%) were also detected. Total identified compounds were 98.45% of which 88.345 and 10.11% for hydrocarbon and oxygenated compounds, respectively. There were a low percentage of unidentified compounds (1.55%). Generally, the main constituent of hydrocarbon and oxygenated compounds was limonene (83.28%) and linalool (3.97%), respectively (Table 1).

Table 1: Main chemical compositions of essential oil of orange peel, *C. sinensis* var. *balady*

Chemical group	Compound	Relative (%)
	α-thujene	0.05
	α-pinene	0.64
	β-pinene	0.29
	Myrcene	2.13
	Limonene	83.28
	λ-terpinene	0.22
Hydrocarbon	p-cymene	1.73
Total	-	88.34
	Linalool	3.97
	Linalyl acetate	1.56
	Citral-b	1.64
	Citral-a	1.97
	Geraniol	0.69
Oxygenated	Dimethyl anthranilate	0.28
Total	-	10.11
Total identified	-	98.45
Total unidentified	-	1.55

Toxicity of the essential oil: Mortality increased with increasing oil concentrations. Youngest aged-eggs were more sensitive to oil than the old eggs. There was a significant difference between mortalities of two high concentrations (1:20 and 1:15) and control through all egg-ages. While, the low concentration 1:40 achieved significant mortalities with respect to control at 2, 6 and 9 days. The concentration 1:30 recorded significant mortalities with respect to control at all egg-ages except at 18 day. The LC_{50} values were 1:56, 1:34, 1.41, 1.32, 1:23, 1:23, 1:18, 1:14 and 1:11 for 2, 4, 6, 9, 11, 13, 16, 18 and 20 days, respectively (Table 2). It was also observed that embryonic development occurred inside all healthy treated egg-ages but hatchability occurred only on the 20 day old-eggs.

Effect of the essential oil on embryonic development: Cross section of 11 day old-egg POP or 2 days post treatment (PTM) showed cleavage nuclei migration to the periphery and formation of a cytoplasmic membrane (cm) in control section. These processes continue until the blastula (morula) is fully formed 11 days POP (Fig. 1A). While, cleavage nuclei migration occurred in an irregular manner and a cytoplasmic membrane formed in sections of treated eggs (Fig. 1B and C).

Cross section of 12 day old-egg POP or 3 days PTM showed that the fully formed blastula appears as a complete ring of cells, blastoderma (b) ranged in a single layer dorsally (d) or two layers in few spots around blastula in control section (Fig. 2A). In case of sections of treated eggs, cells of the blastula gathered on one side or formed two masses on two sides (Fig. 2B and C).

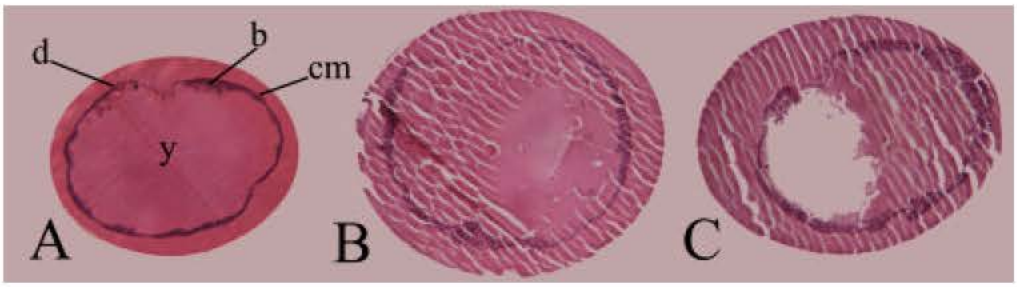

Fig. 1: Cross sections of eleven day old -eggs of *H. dromedarii*, A: Control shows cleavage and blastula formation, B and C: Exposed to essential oil of *C. sinensis*, b = blastoderm, cm = cytoplamic membrane, d = dorsal surface, y = yolk

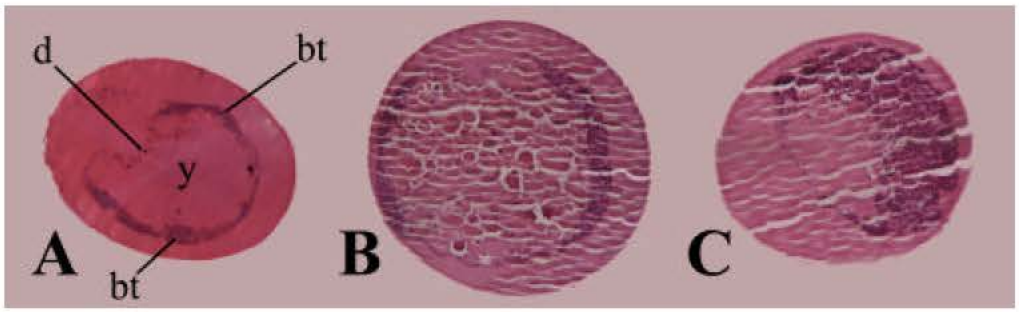

Fig. 2: Cross sections of twelve day old-eggs of *H. dromedarii*, A: Control shows blastula formation, B and C: Exposed to essential oil of *C. sinensis*, bt = blastoderm thickning, d = dorsal surface, y = yolk

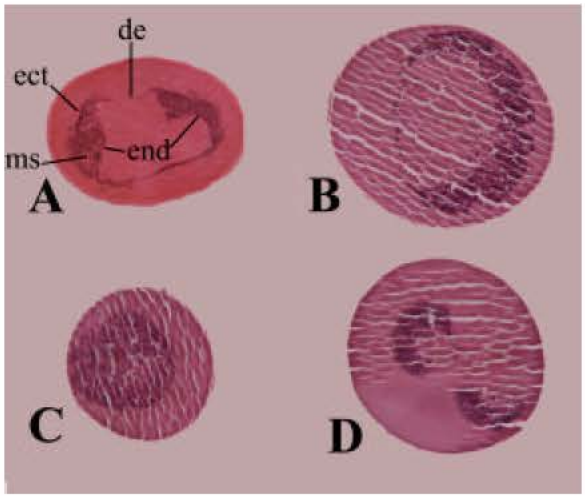

Fig. 3: Cross sections of thirteen day old-eggs of *H. dromedarii*, A: Control shows formation germ layer, B-D: Exposed to essential oil of *C. sinensis*, de = dorsal ectoderm, ect = ectoderm, end = endoderm, ms = mesoderm

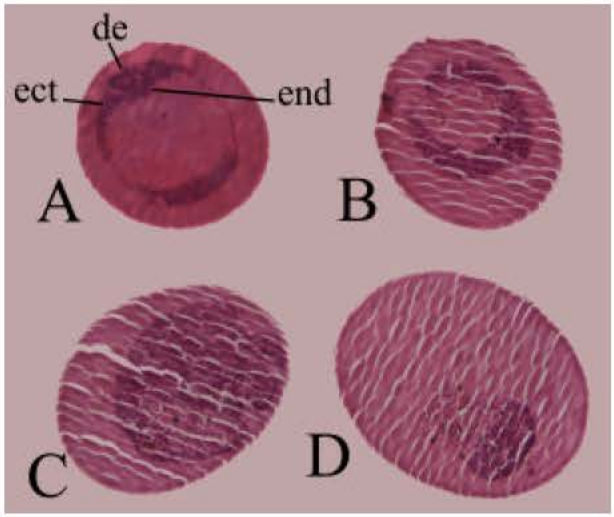

Fig. 4: Cross sections of fourteen day old-eggs of *H. dromedarii*, A: Control shows formation of germ layer, B-D: Exposed to essential oil of *C. sinensis*, de = dorsal ectoderm, ect = ectoderm, end = endoderm

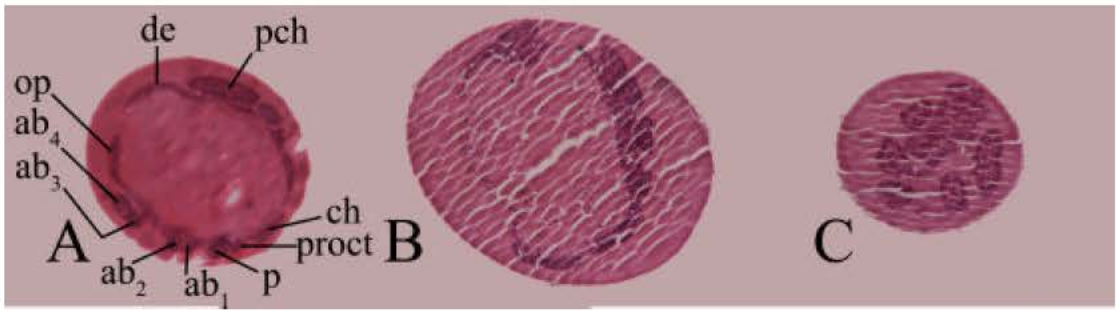

Fig. 5: Cross sections of fifteen day old-eggs of *H. dromedarii*, A: Control shows segmentation of germ band, B and C: Exposed to essential oil of *C. sinensis*, pch = precheliceral lobe, ch = chelicera, p = pedipalps, proct = proctodaeum, ab_1-ab_4 = ambulatory segments, op = opithosoma, de = dorsal ectoderm

Table 2: Mortality rates of different ages of *H. dromedarii* eggs exposed to various concentrations of the essential oil, *C. sinensis* var. *balady*

	Old-egg (day) ± SE								
Conc.	2	4	6	9	11	13	16	18	20
Control	74.67±5.33^{a}	68.00±3.27^{a}	37.33±2.67^{a}	33.33±2.11^{a}	33.33±3.65^{a}	32.00±3.27^{a}	26.67±2.98^{a}	24.00±4.00^{a}	13.33±2.98^{a}
1:40	94.67±5.33^{b}	77.33±2.67^{a}	60.00±5.96^{b}	53.33±8.94^{b}	42.67±9.09^{a}	41.33±4.42^{a}	36.00±3.40^{ab}	29.33±4.00^{a}	21.33±4.42^{ab}
1:30	100±0.00^{b}	93.33±4.22^{b}	86.67±4.71^{c}	74.67±6.80^{c}	64.00±7.48^{b}	64.00±6.53^{b}	48.00±5.73^{bc}	30.67±2.67^{a}	29.33±2.40^{bc}
1:20	100±0.00^{b}	96.00±4.00^{b}	94.67±3.89^{c}	93.33±4.22^{d}	73.33±4.71^{b}	72.00±2.49^{b}	54.67±3.89^{c}	48.00±2.49^{b}	38.67±2.49^{cd}
1:15	100±0.00^{b}	98.67±1.33^{b}	96.00±2.67^{c}	93.33±2.98^{d}	78.67±2.49^{b}	77.33±6.18^{b}	73.33±4.21^{d}	57.33±4.00^{b}	45.33±3.27^{d}
F-value	3.59*	6.29**	37.42**	12.75**	10.68**	16.59**	18.63**	16.28**	14.56**
LC_{50}	1:56	1:34	1:41	1:32	1:23	1:23	1:18	1:14	1:11

Small letters represent significant differences between treatments and control. * Significant at ($p<0.05$), ** Significant at ($p<0.01$). Conc. = Concentration = Oil: Ethyl alcohol

Cross section of 13 and 14 days old-egg POP or 4 and 5 days PTM showed that formation of the germ layers begins bay rapid division of the peripheral nuclei and migration of cells ventrally and dorsally. When complete arrangement of this mass has been accomplished, two layers could be identified in section, the outer ectoderm (ect) and inner endoderm (end) layer. The remainder of the blastoderm attenuates to form a dorsal extra embryonic ectoderm (de). The segmentation cavity is gradually enlarged in control sections (Fig. 3A and 4A). In sections of treated eggs, the embryo retains the spherical shape during gastrulation. Cells of the germ layer formation were

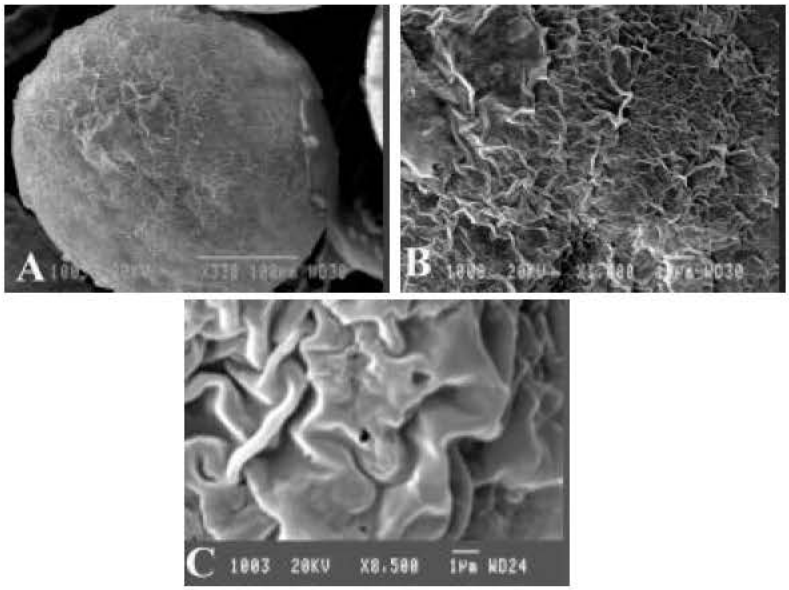

Fig. 6: Scanning electron micrographs of untreated egg shell of *H. dromedarii* (control), A: 330X, B: 1000X, C: 8500X

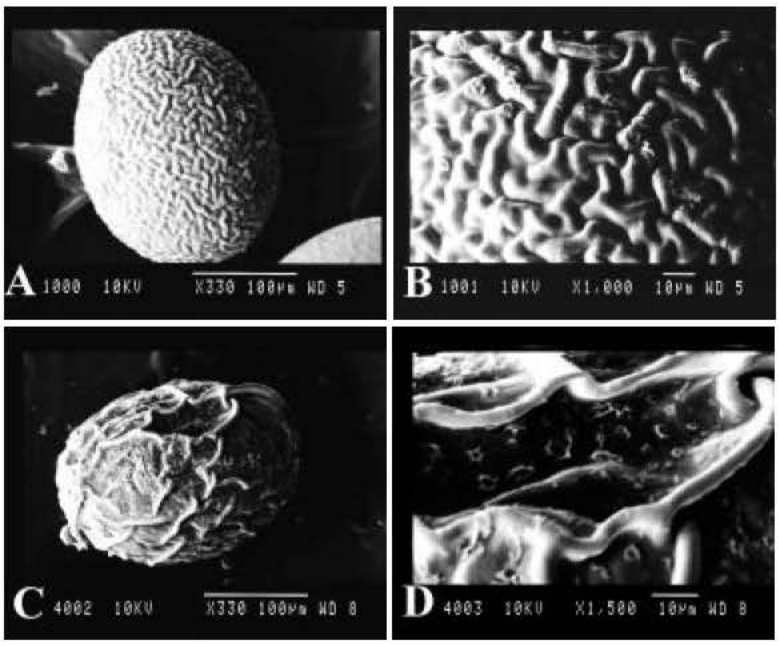

Fig. 7: Scanning electron micrographs of treated egg shells of *H. dromedarii* with the essential oil, *C. sinensis* at 11 day POP: A: 330X, B: 1000X, C: 330X, D: 1500X

gathered on one side of the egg as a small or large mass and a ring shape occurred (Fig. 3 B-D and 4 B-D).

Cross section of 15 days old-egg POP or 6 days PTM showed that the germ band is began to differentiate. The ectoderm cells proliferate in the anteroventral area to the appendage segments of the embryo. It forms a bilateral V-shape asymmetrical band with apices lying on the dorsal surface of the yolk mass. The opithosoma (OP) and ambulatory segments (ab) are formed on one side of the germ band and the precheliceral (pch), cheliceral (ch) and

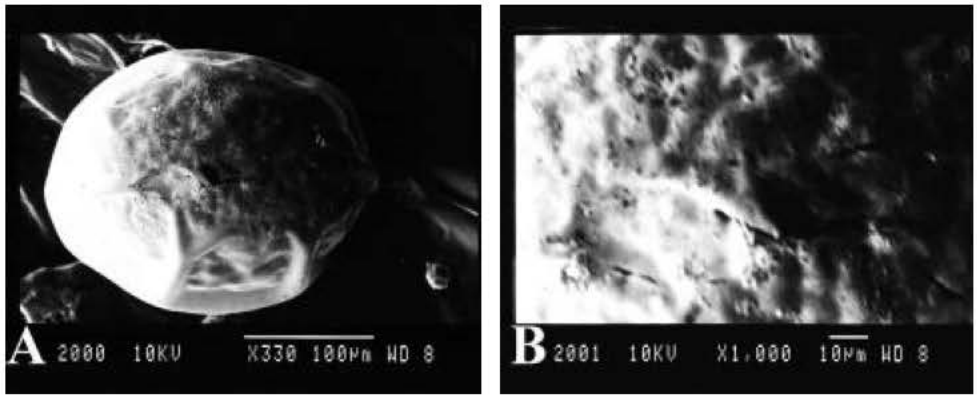

Fig. 8: Scanning electron micrographs of treated egg shells of *H. dromedarii* with the essential oil, *C. sinensis* at 15 day OP: A: 330X, B: 1000X

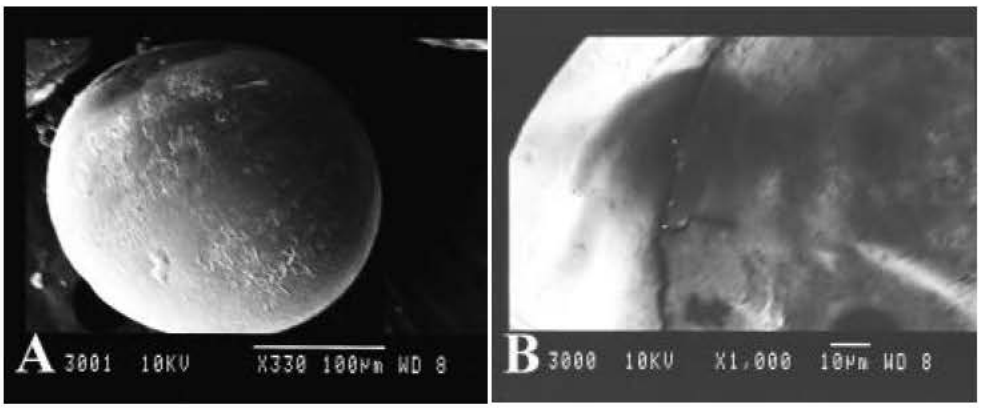

Fig. 9: Scanning electron micrographs of treated egg shells of *H. dromedarii* with the essential oil, *C. sinensis* at 17 day POP: A: 330X, B: 100s0X

Table 3: Molecular weights of egg extracts of *H. dromedarii* treated with essential oil, *C. sinensis* across different days post oviposition period (POP)

Day post oviposition period (day post treatment period)													
10 (1)		11 (2)		12 (3)		13 (4)		14 (5)		15 (6)		17 (8)	
Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.
273.08	195.54	276.77	280.46	278.62	289.69	280.46	286.00	315.54	295.23	311.85	306.31	322.92	304.46
252.77	145.69	254.62	260.15	254.62	267.54	263.85	271.23	289.69	273.08	287.85	295.23	297.08	276.77
236.69	106.97	230.62	247.23	230.62	256.46	249.08	260.15	263.85	260.15	263.85	282.31	263.85	263.85
193.69	88.25	171.54	234.31	162.31	247.23	199.23	247.23	236.15	250.92	239.85	260.15	238.00	256.46
154.92	59.22	134.62	166.00	125.38	193.69	149.38	184.46	177.08	193.69	184.46	217.69	182.62	190.00
121.69	50.27	107.76	125.38	88.25	156.77	98.30	143.85	143.85	140.15	91.06	178.92	91.53	134.62
90.60	46.41	87.79	119.85	83.57	93.576	80.76	90.60	90.60	91.53	81.70	91.06	82.64	93.58
85.45	39.99	81.70	92.79	78.89	80.76	50.70	81.70	80.76	80.76	50.70	83.57	51.13	82.64
78.89	34.69	77.02	81.70	50.27	52.67	46.84	74.21	51.13	51.56	46.84	66.72	46.84	51.56
50.49	31.42	50.49	51.13	45.13	46.84	42.99	51.34	46.41	46.63	42.56	51.13	42.99	48.13
46.20	26.59	44.91	47.27	41.49	42.99	36.99	46.41	41.70	42.34	27.66	47.06	37.63	44.70
41.91	23.45	40.84	42.77	26.92	37.84	33.94	42.34	36.13	38.06	25.59	42.56	27.74	39.13
29.66	21.21	27.50	27.25	21.46	34.19		36.34	34.19	33.94	22.45	38.27	25.92	34.69
23.45	17.95	24.77	22.45	13.21	27.91		34.57	27.91	27.66	16.29	34.32	22.45	33.18
17.48		22.37	15.58		23.36		27.49	25.10	25.84		28.65	16.77	28.40
		17.72			17.95		25.18	22.12	24.35		27.66		25.76
		13.44					24.52	18.66	18.90		26.01		23.78
							22.95				23.11		19.61
							18.19				18.90		

Treat. = Treatment Cont. = Control

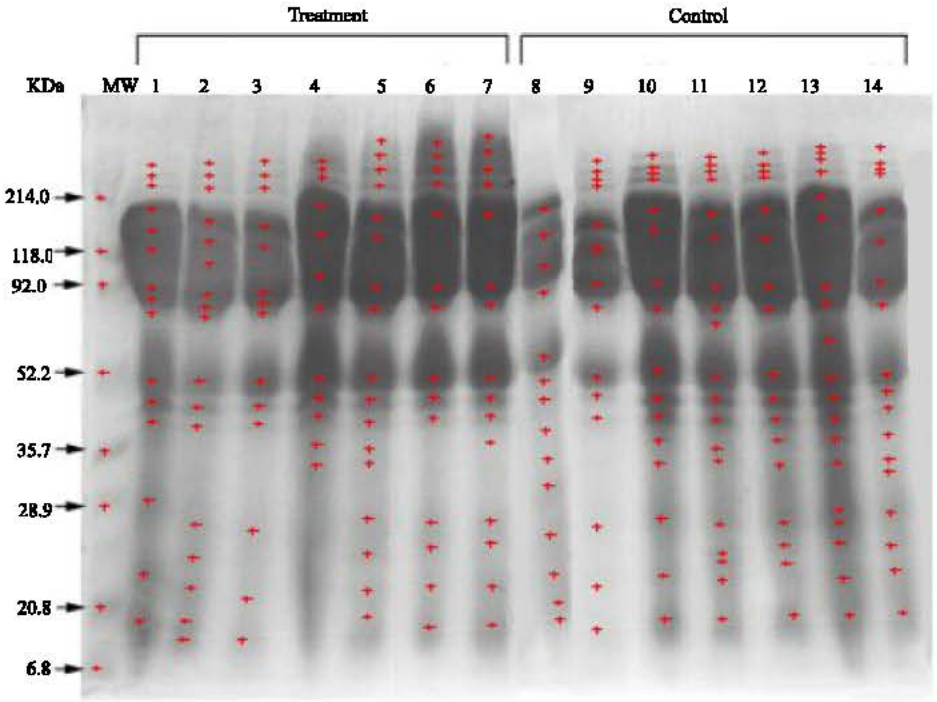

Fig. 10: SDS-PAGE analysis of treated egg extracts of *H. dromedarii* with essential oil, *C. sinensis* (lane 1-7) and untreated egg extracts or control (lane 8-14); MW, molecular weight marker, Lane 1 and 8 for 10 day, lane 2 and 9 for 11 day, lane 3 and 10 for 12 day, lane 4 and 11 for 13 day, lane 5 and 12 for 14 day, lane 6 and 13 for 15 day and lane 7 and 14 for 17 day

pedipalp (p) lobes are on the other side. The proctodeum (proct) lies in the ventral midline of the germ band in sections of control (Fig. 5A). On the other side, the sections of treated eggs showed that the embryo did not develop or the organelles of the embryo did not develop and the cells gathered as a small masses and finger shape accurred (Fig. 5B-D).

Effect of essential oil on egg shell: SEM of untreated egg shells of *H. dromedarii* (control) showed that the eggs have circular structure with dorsoventral curvature and the chorion or shell has heavy small bulging wrinkles (Fig. 6A-C). Treatment with the essential oil, *C. sinensis* showed marked changes in the outer surface of chorion and the egg shape. The later became elongated with a smaller in size chorion and bulging wrinkles changed to large size at 11 day POP (2 day PTM) (Fig. 7A-D). Wrinkles disappear and egg surface became smooth and probably became hollow at 15 and 17 POP (6 and 8 day PTM), (Fig. 8A-D and 9A-D).

Effect of essential oil on polypeptides: The electrophoretic profile of control and treated egg proteins at 10th, 11th, 12th, 13th, 14th, 15th and 17th day old is shown in Table 3 and Fig. 10. Protein of egg control at 10th day old resolved under reducing conditions on SDS PAGE into 14 molecular entities with molecular weights ranging from 195.54-17.95 kDa. The protein bands of treated eggs were 15 molecular entities with molecular weights ranging from 273.08-17.48 kda. Fractionated control egg extracts of *H. dromedarii* at 11th, 12th, 13th, 14th, 15th and 17th day old revealed 15, 16, 19, 17, 19 and 18 polypeptides in each extract, respectively. The polypeptides molecular weight ranged from 280.46-15.58, 289.69-17.95, 286.00-18.19, 295.23-18.90, 306.31-18.90 and 304.46-19.61 KDa at 11th, 12th, 13th, 14th, 15th and 17th day old, respectively. The protein of treated egg at 11th, 12th, 13th, 14th, 15th and 17th day old resolved under reducing condition into several bands. Protein bands were 17, 14, 12, 17, 14 and 15 molecular entities with molecular weight ranging from 276.77-13.44, 278.62-13.21, 280.46-33.94, 315.54-18.66, 311.85-16.29 and 322.92-16.77 kDa at 11th, 12th, 13th, 14th, 15th and 17th day old, respectively.

DISCUSSION

The essential oil of *C. sinensis* had insecticidal effects against larvae of *Spodoptera littoralis* (Omer *et al*., 1997), larvae and adults of *Culex pipiens* and *Musca domestica* (Shalaby *et al*., 1998) and fourth-instar

larvae of *Culex pipiens* (Traboulsi *et al*., 2005). It also had fungicidal effects against *Aspergillus flavus*, *Penicillium italicum* and *Alternaria alternata* (Shukla *et al*., 2000; Rao *et al*., 2000; Patra *et al*., 2003; Raina, 2004). Additionally, the activity of the oil, *C. sinensis* did not expire even up to 48 months of storage and persisted up to a temperature of 80°C (Patra *et al*., 2003). Therefore, our study used for the first time this essential oil against egg stage of the common camel tick, *Hyalomma dromedarii* in Egypt. However, it was tested only on larvae and engorged females of the cattle tick *Boophilus microplus* (Chungsamarnyart and Jansawan, 1996).

This study revealed that the main compounds of the essential oil *C. sinensis* were limonene (83.28%) as a hydrocarbon compound and linalool (3.97) as an oxygenated compound in agreement with that reported by Njoroge *et al*., 2005. Whereas, Omer *et al*. (1997) detected only 78.36% limonene in fruit peels of *balady* orange *C. sinensis*. Trozzi *et al*. (1999) reported that limonene of *C. sinensis* (L.) Osbrck cv. Maltese was 92.6%. The toxicity of the oil attribute to these two main compounds. Liu *et al*. (1990) found that limonene extracted from the seeds of grape fruit caused a significant reduction in feeding and delay in development of 4th instar larvae of the chrysomelid *Leptinotarsa decemlineatai*. Perruci *et al*. (1994) and (1996) showed that linalool extracted from *Lavandula angustifolia* had a high potential powerful miticidal activity against *Psoroptes cuniculi*. Chungsamarnyart and Jansawan (1996) found that the peel oils of *Citrus reticulate* and *C. maxima* showed higher acaricidal activity 2 times than that of (+) – limonene against engorged females of *B. microplus*. They added that *C. sinensis* and *C. maxima* oils exhibited a higher larvicidal activity 1.5 times stronger than that of (+) – limonene.

In the toxicity test, dead eggs of different treated egg-ages were counted. LC_{50} was calculated based on changing of egg color from shiny brown to dark-brown and the egg becoming corrugated in shape. The other eggs were considered healthy (with normal color and shape) and left to the end of experiment to observe hatchability. Surprisingly, all healthy eggs under all treated egg-ages did not hatch except those under 20 day old treated eggs. This indicated that the embryonic development occurred inside these healthy eggs but did not reach to larval stage. Whereas, under 20 day old eggs treatment organelles of larval stage may have developed completely before treatment with oil. To ensure this explanation, HE, SEM and SDS-PAGE were performed.

HE confirmed that embryonic development occurred at both control and treated eggs. In control, it was exactly as described by El-Kammah *et al*. (1982) but in treated eggs, it was observed that embryonic cell division occurred but it was abnormal. Distinctive organelles did not form on treated 17 day old-egg but they formed in control at the same age. Therefore, hatchability failed at egg ages; 2, 4, 6, 9, 11, 13, 16 and 18 day and succeeded only at 20 day old-egg. This finding may be attributed to the fact that embryonic cells was under division or differentiation conditions and the oil caused disruption in formation of organelles. On the other hand, change in treated egg shell observed by SEM may led to egg-shell became more vulnerable to be penetrated by oil to reach the embryo of egg. SDS-PAGE revealed that there were differences in band numbers and their molecular weights in both control and treated eggs. This result means that an embryonic development occurred under control and treated cases. It was concluded that egg of *H. dromedarii* was more sensitive to the essential oil of *C. sinenesis* var. *balady* especially in earlier embryonic development.

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