Egypt. J. Vet. Sci. **Vol. 54,** No. 6, pp. 1073-1095 (2023)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Proteomics and Metabolic Patterns of *Hyalomma dromedarii* Ticks Treated with *Citrus sinensis* var *balady* Peels' Oil Extract

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THE CURRENT study evaluated the acaricidal effect of *Citrus sininses* var *balady* peels' extracted-oil on camels' ticks *Hyalomma dromedarii* (*H. dromedarii*). Mortality % in treated adult ticks is directely correlated to oil concentrations. LC50s results were 82.2% and 78% in dipping and physical contact methods of application, respectively. Phytochemical screening carried out by Gas Chromatography-Mass Spectrometry (GC-MS) detected: 2.8% β-Pinene, 43.334% Limonene, and 53.8426% D –Limonene. *Citrus* oils showed biolological chenges in *H. dromedarii* life cycle and its different instars along 2 *in vitro* generations. In comparison, *Citrus* oil treatments revealed high significant prolongation; than untreated group, in recorded periods by days of oviposition (17.8 > 6.8), hatching (17.6 > 7.4), larval-nymphal feeding (21 > 15.2), premolting (20 > 16), nymphal-adults feeding (19.4 > 6.2), otherwise, insignificantly affected prehatching and preoviposition periods in ticks of 1st generation. In 2nd generation, significantly increased oviposition (19 > 10) and molting periods (6.4 > 4.8), but reduced the prehatching (34 < 42), larval-nymphal feeding (17.8 < 25) periods, and sex ratio (12:1 > 3:1; male: female), yet insignificant effects on preoviposition, hatching, premolting periods, egg mass, and nymphal weights. Biochemically, total proteins contents quantitation, electrophoretic patterns, and enzymatic activities were significantly changed in treated than untreated and controls. SDS-PAGE patterns of treated larvae of 1st and 2nd generation and unfed females and males were separated into 17, 12, 6, and 10 bands within 240 to 31 kDa MWs, respectively. In addition, common bands with higher densitometry scanning phenotypic patterns were detected correlated to oil treatment and/or 2% DEMSO in positive controls. The appearance, disappearance, hyper, hypo -expressions of proteins bands indicated oil's efficacy on tick's faunas. Additionally, activities of antioxidant enzymes had been affected. Catalase increased in 1st but decreased in 2nd generation while glutathione reductase was significantly reduced in 1st and 2nd generations. In conclusion; *Citrus* peels' oil proved efficiency as an alternative eco-safe, biodegradable, and low cost acaricide useful in ticks control in the veterinary field.

Keywords: Acaricides, *H. dromedarii*, *Citrus* Peels oils, Biological Parameters, Catalase, Glutathione Reductase, SDS-PAGE, Camels, Egypt.

Introduction

The ticks' blood-sucking routine style enhances their roles as efficient transmitters and/or vectors of dangerous pathogenic agents causing various diseases in living beings worldwide [1, 2]. The identified 900 ticks' species have been classified within three families; the Ixodidae (hard ticks), the Argasidae (soft ticks), and the Nuttalliellidae [2]. *Hyalomma;* ixodid species infesting camels, is a genus of hard ticks having

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(Received 20/04/2023, accepted 22/07/2023)
DOI: 10.21608/EJVS.2023.206959.1493

more than 30 species [2], all are well-adapted to hot, humid and cold climates. Habitat diversity, vector ability, and emerging problem of acaricidal resistance in enzootic regions typify this genus in various countries around the world [3-5]. In Egypt, the most common hard ticks' species; *H. dromedarii,* was proved as vectors for Al-Khorma virus*, Borrelia* spp., *Ehrilichia* spp., *Anaplasma* spp., *Rickettsiae* spp., *Theileria* spp, *Babesia spp* and *Trypanosoma* spp. [6-16].

Tick's control was previously achieved by chemical-based acaricides that depended meanly on arsenicals, organophosphates, carbamates, formamidines and pyrethroids residues [5, 17, 18]. However, recent studies on the classical acaricides indicated their limitation. Hence, some issues related to public health hazards, acaricide residues in animal bi-products, destructive effect on ecology, and developing resistance to their synthetic material [19, 20]. Recently, there was a global trend to evaluate new alternative agents that are eco- safe, effective, and biodegradable with low resistance [5, 21]. Several studies indicated that plant extracts and essential oils represent alternative approaches to traditional chemical acaricides against ticks *in vitro* [17-24]. Many Essential oils had both repellent and acaricidal properties against all ticks' stages; adult, nymph, larva and egg, of economically important species with encouraging results; especially *Hyalomma* spp. [5, 17, 24]. Up to five main active substances in essential oils are typical in any given plant species, although their proportions vary depending on plant variety, geographic location and climate [22-24]. *Citrus sinensis* var *balady* (Orange) peels' oil extract was used in this study; genus *Citrus*- family Rutaceae, which includes about 140 genera and 1300 species. As a diverse tropical fruit, citrus includes species like; *Citrus sinensis* (Orange), *C. reticulata* (Mandarin), *C. aurantifolia* (Limes), *C. limon* (Lemon), *C. paradisi* (Grapefruit), *C. bergamia* (Bergamot), *C. junos* (Yuzu), and *C. japonica.* Their toxicity has been induced by immersion, physical contact with treated surfaces, and/or exposure to the vapor of the oils [5, 24]. On the other hand, the lack of standardization and consequent inconsistent efficacy has restricted their registration and use in control [18-20]. Botanical acaricides characterized by relative safety, lower cost, easily extractable, ecofriendly, and biodegradability. Their toxic effects have been studied intensively and proved by the biolological changes induced in ticks life cycle and its different instars through successive generations [5, 17, 21]. Hence, their toxicity affected the proteins synthesis and functionality in all living cells; processes of division, enzymes and hormones activities, controlling cell's metabolism [17, 22-24]. Most important is proteins' role in the synthesis of microsomal detoxifying enzymes which contribute in the toxicants detoxification that enter into the tick body [22-24].

The dromedary camel is extremely important in the economy and culture of Arabian countries and Middle East generally, these ruminants are commonly referred as "ships of the deserts" [4, 5]. They are able to adapt to tough environment, employed for a variety of tasks including; milk and meat production, racing, transportation, as well as, tourism [25, 26]. The camels' tick has cosmopolitan distribution closely associated with camels. In Egypt, *H. dromedarii* attacks camels as the main host [4, 5, 9]. This species is regarded as the most significant impediment to camel production. They induce decreasing in the productivity and quality of animals' byproducts, in addition to, anorexia, anemia, toxicosis, and general stress. Ticks had direct impact on human health through venomous bites, blood loss, and skin deterioration [27-29]. The use of acaricides has decreased the incidence of tick-borne diseases; however, ticks usually develop rapid resistance to acaricides. On the other hand, toxicity and environmental biohazards were recorded. Therefore, it is necessary to search continuously for eco-friendly acaricides. In fully integrated system, tick-control application uses a variety of viable strategies [5, 21]. This study aimed to evaluate the toxic effect of essential oil extract of *Citrus sinensis* var *balady* peels on camels' tick *H. dromedarii* under laboratory conditions. The analytical studies will investigate total protein quantity and quality of tick crude extracts. In addition, the enzymatic activities of both catalase and glutathione reductase within treated ticks' crude extracts will be examined.

Experimental Design

Ethical Approval

All experimental procedures were carried out in accordance with the ethical guidelines and approvals of scientific committees in the National Research Centre (NRC), Egypt (Approval No. NRC-16231).

Hyalomma dromedarii Camels' Ticks Collection:

Adult females of *H. dromedarii* (Koch 1818) were collected from Toukh city (35 km north of

Cairo; 30° 21′ 11.6″ N, 31° 11′ 31.5″ E) in Qalyubia Governorate, as well as, from Sinai Peninsula (29°30′N 33°50′E), Egypt as mentioned before [5]. Imported camels from Sudan (12.8628° N and 30.2176° E) and Somalia (2.855263, 45.185852); were also included during samples collection. The collected 400 fully engorged *H. dromedarii* ticks were identified according to the taxonomic keys [30, 31].

Citrus sinensis var balady Peels' Essential Oil Extraction:

The common orange *Citrus sinensis* var *balady* belonging to Rutacea Family [32] was selected for this study. Ripened orange fruits were collected from trees, orange peels were separated and dried at room temperature, then subjected to hydro-distillation for 3 successive hours using Clevenger-type apparatus; by boiling the peels with water, then distilling and collecting the distilled oil, during which volatile oils were carried over then condensed with the steam. The anhydrous Sodium sulphate was used for drying extracted oil, then kept in dark bottle in refrigerator till analyzed by GC-MS. Bioassay activities of extracted orange oil were studied as mentioned before on adult *H. dromedarii* tick [5].

Citrus sinensis var balady Oil Characterization by Gas Chromatography Mass Spectrometry (GC-MS):

The GC-MS analysis was done for the crude extracted oils of *Citrus sinensis* var *balady* using Agilent Technologies 7890B GC Systems combined with 5977A Mass Selective Detector. This was performed at the central laboratory at faculty of science, Ain Shams university, by injecting 1µl of examined extracted citrus oil into splitless mode at 290°, equipped with capillary column HP-5MS Capillary film (30.0 m × 0.25 mm ID × 0.25 µm) and the carrier gas was helium at 7.0 psi pressure with 1µl injection. The sample was analyzed with the column held initially for 3 min at 40°C after injection, then the temperature was increased to 300°C with 20°C/min heating ramp and 8.0 min hold. The MS operative parameters were as follows: scan range (*m*/*z*) 40–550 atomic mass units (AMU) under electron impact, and ionization (EI mode) at 70 eV [33, 34].

Preparation of Citrus sinensis var balady Oil Concentrations:

The research team prepared 1.5, 2, 2.5, 3, 4, 10, 20, 100 % concentrations of *C. sinensis* var *balady* extracted oil using 2% Dimethyl sulfoxide (DMSO) as solvent; ranging from 100 to 400 ppm. The dilution ratios were; 1:65, 1:50, 1:40, 1:30, 1:25, 1:10, 1:5 and 1:1; Oil: DMSO 2%. The treatment was applied by two methods; dipping in oil concentrations and physical contact as indicated by some investigators [5, 22].

Bioassays of Citrus sinensis var balady Peels' Oil on Hyalomma dromedarii Camels' Ticks:

Determination of the relative acaricidal strength of different concentrations of *Citrus* peels oil on *H. dromedarii* fed females ticks was determined [5].

Biological Studies on the effects of Citrus sinensis var balady against the Hyalomma dromedarii:

The effect of the extracted oil on the development of *H. dromedarii* ticks through *in vitro* two generations were evaluated using the LC50 (dilution 1:65; citrus oil: 2%DEMSO = 1.5%) by dipping method [5]. The different biological parameters evaluated including the observations results of pre-oviposition period, oviposition period, egg mass, pre-hatching period, hatching period, feeding period, pre-molting period, and molting period. The treated and control groups were 5 replicates each, and each replicate included five females. In treated group (positive control), engorged females were dipped for 30 seconds, for both concentrations-used and 2%DMSO then transmitted to filter paper. The females were separated in plastic cups (female/cup). In negative control group, 5 non-treated engorged females were placed in 7.5 cm× 2.5 cm plastic cup, covered by cotton wool and fixed with adhesive tape. Then all females were incubated at 26±2°C and 85%±2 humidity [5, 17]. The mortality rates were calculated daily. The survived females were observed daily till oviposition (egg laying) to estimate oviposition period for treated and control (positive and negative) females. Daily observations were made until all eggs start hatching, and then developed to larvae, and finally nymphs which then emerged to adults to estimate the following biological parameters.

First Generation

Preoviposition Period:

The preoviposition period of females was calculated from the date of detachment of feeding to the start of oviposition. The evaluation was done according to Alahmed and Khier [35] with some modifications related to the design of the present study and *in vitro* rearing environmental conditions.

Oviposition Period:

The oviposition period of treated and non-treated females was calculated as the intervals between the beginnings of eggs laying till the end of oviposition. It was calculated according to other researchers [34, 36, 37] with some modifications related to the design of the present study and *in vitro* rearing environmental conditions.

Egg Mass:

The egg mass was calculated by estimating the mean value of eggs batches weight for each group laid from females (treated and controls). It was calculated according to Rak and Ishii [38] with some modifications related to the design of the present study and *in vitro* rearing environmental conditions.

Prehatching Period:

The prehatching period was calculated as the intervals between the beginnings of eggs laying till the start of eggs hatching. It was calculated for all the treated and controls (positive and negative) females of *H. dromedarii*. It was calculated according to EL-Ghali and Hassan [39] with some modifications related to the design of the present study and *in vitro* rearing environmental conditions.

Hatching Period:

The hatching period was calculated as the intervals between the beginnings till the end of eggs' hatching. It was calculated for all the treated and controls (positive and negative) females of *H. dromedarii* tick. It was calculated according to Khalid et al. [34] and EL-Ghali and Hassan [39] with some modifications related to the design of the present study and *in vitro* rearing environmental conditions.

Feeding Period

The hatched larvae and emerged adults were fed on white hybrid rabbits (New Zealand and balady) about 2 kg weights. Each group had five white rabbits for larval-nymphal feeding period with approximately 100 larvae/rabbit [35, 36]. The engorged nymphs were collected, weighted and then the mean was determined. The larval-nymphal feeding period was calculated as the intervals between the attachments of unfed larvae on rabbit till the detachment of engorged nymphs [35, 36]. Moreover, the nymphal-adult feeding period was calculated as the intervals between the attachments of unfed adults till the detachment of fed adults from the rabbits' bodies [35]. All calculations were done for all the treated and controls (positive and negative) females of *H. dromedarii* tick.

Premolting Period:

After nymphs detached from rabbits' the engorged nymphs were collected and incubated at 26 ±2°C and 85±2% humidity for molting. The premolting period was counted from the date of incubation of engorged nymphs till starting of molting [35]. All calculations were done for all the treated and controls (positive and negative) of *H. dromedarii*.

Molting Period:

The molting period was calculated as the intervals between the beginnings of molting till the end of nymphs' molting [35, 39]. All calculations were done for all the treated and controls (positive and negative) females of *H. dromedarii*. Unfed adults (males and females) were obtained.

Second Generation

Three groups were designated for treatment and controls (positive and negative). Five rabbits were used for feeding the adults. Equal numbers/rabbit were placed of unfed adults of camel ticks *H. dromedarii* at 1:1 (female: male) sex ration on each rabbit. The engorged adults were collected after their detachment, weighed separately and the time taken to being engorged was recorded. Then each female incubated at 26±2°C and 85±2% humidity for oviposition. Daily observations were made till the emergence of larvae, nymphs then adult to estimate the previous biological parameters for the second generation obtained in the laboratory [35, 39, 40].

Preparation of Crude Extracts of Hyalomma dromedarii Ticks

The acaricidal effects of *Citrus* peels oil was additionally proved on treated ticks. The biochemical activities in treated females after incubation at 26°C and 85% humidity; as lab condition for tick rearing, were analyzed. Mortality rate was daily recorded for 7 days post treatments. Then, tick samples were weighed and homogenized at 1:5 ratio; 1gm tick body in 5ml deionized water, in Teflon-pestle homogenizer according to Laemmli [41] and Goldberg [42]. A 2% DMSO buffer was used as a positive control. Each concentration, as well as, control treatment was repeated 5 times. Each replicate included five ticks. Homogenates were centrifuged at 14000 rpm for 15 min, and then each supernatant was collected and stored at -5°C until used **[5]**.

Biochemical and Proteomic Patterns of Hyalomma dromedarii Crude Extracts Treated with Citrus sinensis var balady Oil:

Quantitative Determination of Total Proteins Contents:

Quantitative analysis of total protein contents was estimated for treated, negative and positive controls of larvae, nymphs, unfed adult's male and female from first generation, and larvae of second generation. The quantitation was first determined by the dye binding assay method of Bradford [43], using Bovine Serum Albumin (BSA) as a standard protein 1mg/ml (Sigma Aldrich), which is based on the binding of Coomassie Blue G250 dye to proteins. Total proteins contents were determined by two techniques using spectrophotometer (Quawell, Q9000 Series) at 100ug/ml-8mg/ml proteins concentration range at wavelength extended from 570 to 610 nm and 200 to 850 nm in Bradford and UV-Vis methods, respectively [44]. Sample volume ranged 200µl and 1-1.5µl in Bradford and UV-Vis methods, respectively [44].

Qualitative Determination of Total Proteins Contents by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE):

The proteins bands profiles and molecular weight were determined by 15% SDS-PAGE under reducing condition as described previously [45-47]. Samples used during this procedure were classified as: 1.5% *Citrus sinensis* var *balady* -treated groups (Larvae and unfed adults), DEMSO 2% -treated groups (Larvae), and untreated groups (Larvae and unfed adults; as negative controls) of the *H. dromedarii* collected from the field. In addition to the larvae collected from the *H. dromedarii* second generation. All samples were kept in sealed tubes under freezing conditions at -20°C till used. Broad range molecular weight marker BLUltra prestained protein ladder ranged 6.5 to 240 kDa was used (GeneDirex). Gel documentation and analysis was done by Gel Doc™XR+ with Image Lab™Software (BioRad).

Enzymatic Activities of Hyalomma dromedarii Crude Extracts:

Catalase Activity:

Catalase activity (CAT) determination was done on larvae, nymphs, unfed adults males and females ticks (treated, negative and positive control) from the first generation and larvae of the second generation (treated, negative and positive control) samples from *H. dromedarii*. The assay of catalase activity was carried out according to the method described by Aebi [48]. The reaction mixture of the assay contained 3 ml total volume of 0.02 M H_2O_2 in 50 mM phosphate buffer saline pH 7.0 (Adwic). The decomposition of H_2O_2 was followed as a decline in absorbance at 240 nm for 1 min at 25°C. One unit of CAT activity was defined as the calculated consumption of 1 µML of H_2O_2/min. The extension coefficient of H_2O_2 was taken to be 43.6 M^{-1} cm^{-1} (Eq. 2):

$$\text{Units/ml} = \frac{\Delta\,\text{A/min} \times 1000 \times \text{dilution} \times 3}{43.6 \times \text{vol. of the sample used}}$$

Where Δ A is the change in absorbance at 240 nm.

Glutathione Reductase Activity:

Determination of glutathione reductase activity was measured according to Goldberg and Spooner [49]. The reactivities were done in larvae, nymphs, unfed adults' males and females (treated, negative and positive control) from the first generation and larvae of the second generation (treated, negative and positive control) samples from *H. dromedarii*. This method is based on the reduction of 5, 5`dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione to produce yellow compound. The reduced chromogenic substrate is directly proportional to glutathione concentration and its absorbance is measured at 405 nm (Stat Fax -2100, USA), (Eq. 3):

$$\textit{Glutathion in tick sample} = \frac{\text{A sample} \times 66.66}{\text{weight ot tick sample uesd in gm}}\ \text{mML/gm}$$

Statistical Analysis:

The obtained data were analyzed as factorial, using ANOVA in SAS [50] and all means were compared by LSD (*P*= 0.05 level) in the same program. The mortality percentage was calculated by Krishnaveni and Venkatalakshmi formula as the following (Eq. 4) [51]:

$$\textit{Mortality}\ \% = 100 \times \frac{\textit{Number of dead ticks}}{\textit{Total number of ticks}}$$

Then, the results were corrected according to Henderson –Tilton's formula (Eq. 5) [52]:

$$\text{Corrected Mortality \%} = 100 \times \left(1 - \frac{\textit{No.of Ticks C. before treatment} * \textit{No.of T.after treatment}}{\textit{No.of C.after treatment} * \textit{No. of T.before treatment}}\right)$$

Where: No. = tick population, T.= treated, C. =control.

Results

GC/MS Analysis of Citrus sinensis var balady Peels' Oil Extract:

The results of GC/MS revealed the presence of 3 main compounds (peaks) in *Citrus sinensis* var *balady* essential oil extract. The β-Pinene (6,6-dimethyl-2-methylidenebicyclo [3.1.1] heptane); $C_{10}H_{16,}$ which represented 2.8% average rate, 136.234 g/mol, with 7.7151 retention time. Limonene (1-methyl-4-(1-methylethenyl)-cyclohexene), $C_{10}H_{16}$ representing 43.334% average rate, 136.23g/mol, and 8.0648 retention time. D–Limonene; $C_{10}H_{16}$ representing 53.8426% average rate, 136.23g/mol and 7.9483 retention time.

Bioassay Toxicological Analysis of Citrus sinensis var balady Oil on Hyalomma dromedarii:

This study is the second part to be published of full characterization study on *Citrus sinensis* var *balady* peels' oil, as proposed to be alternative acaricidal preparation. Therefore, the first part included detailed results of *H. dromedarii* fed females tick's susceptibility, the relative acaricidal strength of different oil concentrations [1:65, 1:50, 1:40, 1:30, 1:25, 1:10, and 1:05]. The average positive and negative controls for all the tests were 10% & 4%, respectively. The mortality percentages; which increased with increasing oil concentrations, recorded [33.3, 42.2, 46.6, 51, 60, 68.9, 77.7, and 82.2%] by dipping method (Figure: 1), while recorded [20, 22.2, 33.3, 37.8, 44.4, 48.9, 60, and 68.9 %] on physical contact method (Figure: 2). The toxicity of *C. sinensis var balady* peels' oil was higher by dipping than physical contact method, hence, the LC50 and LC90 values were 0.0024%, 0.00096%, and 0.1473%, 0.10211 %, respectively, (Figures: 1 & 2) [5].

Biological Studies on Effects of Citrus sinensis var balady against Hyalomma dromedarii:

The biological aspects included the oviposition, hatching, molting, and feeding periods, adult longevity, and egg mass as shown in Tables (1 & 2).

Effects of Oil Treatment on the 1st Generation of Ticks:

Data recorded in Table (1) for females collected from the field. The mortality rate of female treated with LC50 concentration of *C. sinensis* var *balady* oil were 54%. The mean

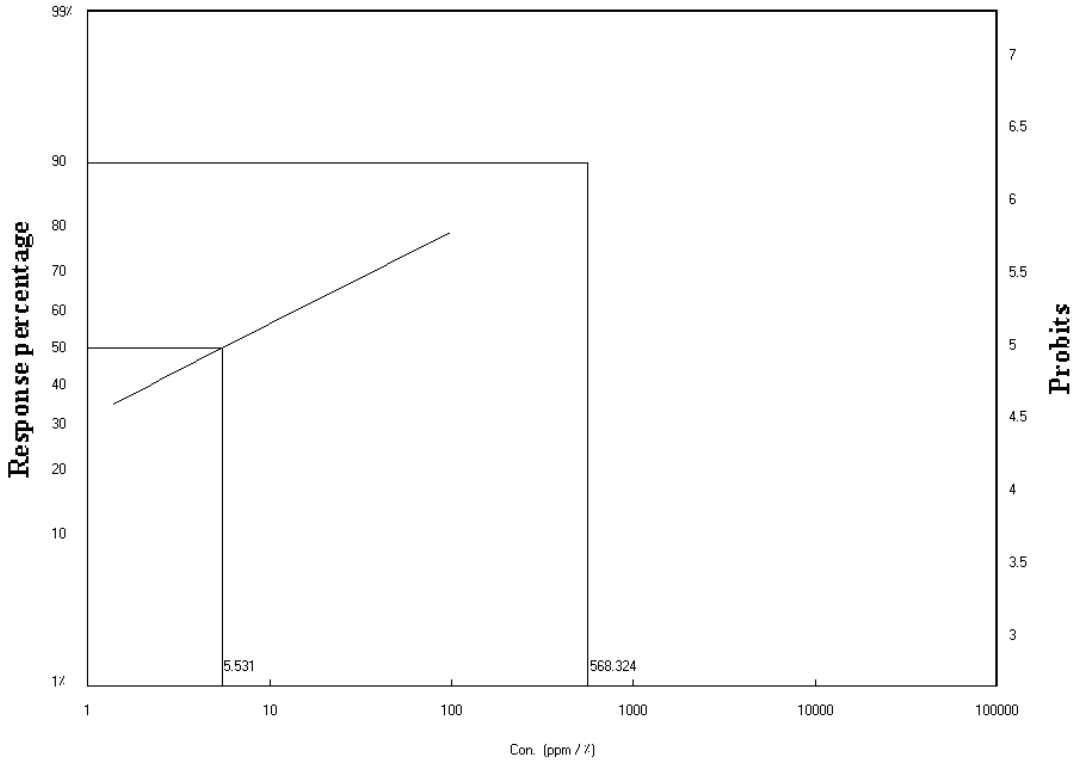

Fig. 1. Toxicity regression line of Hyalomma dromedarii treated with Citrus sienenses var balady by dipping method.

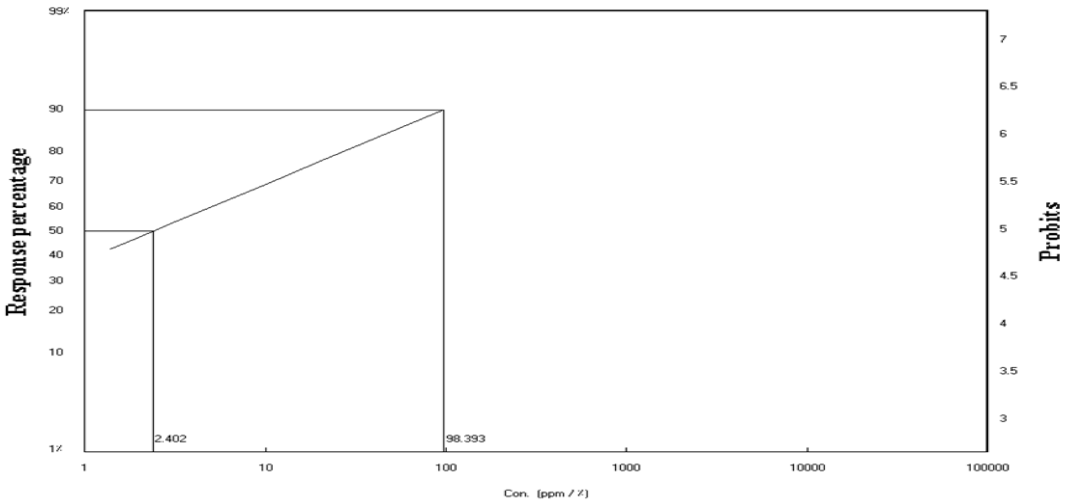

Fig. 2. Toxicity regression line of Hyalomma dromedarii treated with Citrus sinensis var balady by physical contact method.

preoviposition periods recorded for survival were 16.6 and 11.4 days for untreated and treated females, respectively. Therefore, treatment with *C. sinensis* var *balady* was with insignificant effect on the preoviposition period (Table: 1). On the other hand, high significant prolongation of oviposition period was recorded 17.8 and 6.8 days for treated and untreated females, respectively (Table: 1). The mean of prehatching period was 39.8 and 32.8 days in treated and untreated egg batches, respectively, therefore, treatment with citrus oil was with insignificant effect on the prehatching period (Table: 1). A highly significant increase in mean of the hatching period of treated egg batches than untreated ones was observed; 17.6 and 7.4 days were recorded in treated and untreated egg batches, respectively (Table: 1). Meanwhile, the larval-nymphal feeding period was significantly affected with the treatment with citrus oil. In the treated group was 21 days, while in the untreated larvae was 15.2 days (Table: 1). The mean weights of nymphs were insignificantly affected with the treatment with citrus oil when compared with untreated ones; were 0.020367 and 0.13535 gm in treated and untreated nymphs, respectively (Table: 1). The premolting period was significantly increased with treated nymphs when compared with untreated ones, the mean of premolting period was 20 and 16 days in treated and untreated nymphs, respectively (Table: 1). No significant change on molting periods in treated nymphs as compared to untreated ones. A highly significant prolongation was noted in the mean of adult feeding periods in treated adults up to 19.4 days when compared with 6.2 days in untreated adults (Table: 1).

Effects of Oil Treatment on the 2nd Generation of Ticks:

Data reported in Table (2), illustrated that the preoviposition period were insignificantly affected with treatment. on the other hand, high significant prolongation of oviposition period of treated females was reported. The oviposition period mean was 19 and 10 days in treated and untreated females, respectively (Table: 2). Treatment with citrus oil had significantly reduced the prehatching period as compared with untreated. Hence, the premolting period were 34 and 42 days in treated and untreated egg batches, respectively (Table: 2). A highly significant reduction in the larval-nymphal feeding period was observed with treated larvae when compared with untreated ones; reported 17 and 25 days in treated and untreated groups, respectively. There was significant increase in molting period in treated nymphs when compared with untreated ones. Since molting periods were 6.4 and 4.8 days in treated and untreated nymphs, respectively (Table: 2). No significant changes were observed in the mean hatching period days, egg mass, nymphal weight and premolting periods in treatment groups with citrus oil when compared with the untreated ones (Table: 2). A highly significant reduction in the percentage of adult female emergence were observed in the

TABLE 1. Effect of citrus peels' oil on different biological parameters of Hyalomma dromedarii of 1st generation.

Biological Parameters	Preoviposition Period	Oviposition Period	Prehatching Period	Hatching Period	Larval-Nymphal Feeding Period	Nymph Weight (gm ±SE)	Premolting Period	Molting Period	Nymphal -Adult Feeding Period
Un treated	16.60± 1.70[a]	6.81[b]	32.80± 1.20[a]	7.40± 1.15[b]	15.20 ± 0± 0.50[b]	0.16± 0.70[a]	16.00± 1.30[b]	4.40± 0.45[a]	6.20± 0.59[b]
Treated with LC50 of Citrus peels oil	11.40± 0.40[a]	17.80± 0.70[a]	39.80± 2.80[a]	17.60± 0.80[a]	21.00± 1.60[a]	0.02± 0.006[a]	20.00± 0.90[a]	4.60± 0.45[a]	19.40± 0.66[a]
Treated with DEMSO	10.00± 0.80[a]	7.40± 0.90[b]	26.00± 1.10[a]	9.00± 0.90[b]	14.00± 0.90[b]	0.92± 0.20[a]	13.00± 1.00[c]	3.90± 0.30[a]	11.60± 0.66[c]
F value	2.48	555	3.26	41.29	8.76	0.79	13.04	0.08	147.24
Least Significant Difference value	7.6203	3.4048	7.9214	3.6607	4.5188	0.2189	3.5846	1.6629	2.3060

Means with same letters are not significantly different.
Each value represents the mean of 5 replicates ±SE (Stander error).

treated group when compared with the percentage of adult male. Hence, sex ratio was 12: 1; male: female. It worth to be mentioned that the reduction in sex ratio (female < male), and the increased mortality rate of unfed adults caused the failure of the nymphal-adult feeding period, so the start of the next generation was prohibited (Table: 2).

Biochemical Analysis of Hyalomma dromedarii Crude Homogenates Treated with Citrus sinensis var balady Oil:

The biochemical changes in the total proteins' profiles, catalase and glutathione reductase activities are recorded in tables (3, 4, 5 & 6) and figure (3) in the whole-body tissues of larvae, nymph and adult of *H. dromedarii* after treatment with LC50 (1.5%) of *C. sinensis* var *balady* oil.

Quantitative Analysis of Total Proteins Contents of Hyalomma dromedarii Crude Extracts:

From the obtained data by the two quantitation methods, it was noticed that the proteins contents were highly significantly increased in treated samples than those untreated and controls ones (Tables: 3). However, minor deviations were also noticed in some of the developmental stages; nymph of 1st generation and fed adults, hence the untreated were higher than those treated samples (Tables: 3). In addition, the data showed that the total contents of protein were significantly different between larvae of the two generations (Table: 3). It is noticed that proteins contents increased in the first than second generation (Tables: 3). In addition, they increased in treated than untreated larvae in both the first and second generations (Table: 3).

Qualitative Analysis of Total Proteins Contents of Hyalomma dromedarii Crude Extract:

SDS-PAGE exhibited the electrophoretic proteomics phenotypic patterns of the following samples; *citrus* oil treated, positive (DEMSO 2%) and negative controls larvae of first generation, also unfed treated and untreated adults, and larvae of second generation of *H. dromedarii*. The expressed proteins were separated into bands according to their molecular weights (MWs) in relation to marker's bands. The color density of each Coomassie blue G250-stained-band considered as phenotypic indication of its expression rhythm, subsequently, its concentration within crude protein mixture in relation to treatment type (hypo- or hyper- expression), as shown in Figure (3).

TABLE 2. Effect of citrus peels' oil on different biological parameters of Hyalomma dromedarii of 2nd generation.

Biological Parameters	Preoviposition Period	Oviposition Period	Prehatching Period	Hatching Period	Egg Mass (gm.± SE)	Larval-Nymphal Feeding Period	Nymph Weight (gm ±SE)	Premolting Period	Molting Period	Mortality Rate of unfed Adults	Sex Ratio of Adult Male: Female
Untreated	8.00± 0.60^{ab}	10.00± 0.30^{b}	42.00± 0.80^{a}	6.00± 0.30^{a}	1.30± 0.1^{a}	25.00± 0.30^{a}	0.040± 0.005^{a}	20.4± 0.70^{a}	4.80± 0.30^{b}	41%	3:1
Treated with citrus peels oil	9.00± 0.40 ^{a}	19.00± 0.60^{a}	34.00± 0.30^{b}	5.00± 0.60^{a}	1.40 ± 0.03^{a}	17.80± 0.80 ^{b}	0.030± 0.003^{a}	20.0± 0.60^{a}	6.40± 0.50^{a}	57%	12:1
Treated with 2%DEMSO	7.00± 0.30^{b}	11.00± 0.60^{b}	35.00± 1.10^{b}	3.00± 0.30^{b}	1.30± 0.08^{a}	100% mortality of larvae*	------	------	------	-------	
F value	3.75	66.36	22.34	10.00	0.80	61.71	0.79	0.14	6.40	2.07	-
Least Significant Difference value	1.591	1.866	2.934	1.488	0.219	2.114	0.219	2.483	1.458	30.541	-

* No feeding, therefore, 100% mortality of larvae.
Means with same letters are not significantly different.
Each value represents the mean of 5 replicates ±SE (Stander error).

TABLE 3. Effects of LC50 Citrus sinensis var balady peels' oil on total proteins contents, catalase, and glutathione reductase enzymes activities in Hyalomma dromedarii ticks' crude homogenates.

Tick sample	Total Protein (mg\ml)	Catalase Activity (U\ ml)	Specific Activity U/ mg	Glutathione Reductase Activity mg\ gm	Specific Activity U/mg
Treated larvae 1st generation	88.78±0.80^{a}	522.90±0.48^{a}	5.84±0.05^{a}	194.16±0.35^{b}	2.18±0.02^{c}
Untreated larvae of 1st generation	31.90±0.56^{c}	91.50±0.64^{c}	2.86±0.03^{b}	192.65±0.30^{c}	6.03±0.14^{a}
Positive control larvae 1st generation	44.38±0.59^{b}	261.50±0.18^{b}	6.41±0.48^{a}	240.40±0.30^{a}	5.42±0.07^{b}
Treated nymph 1st generation	30.60±0.26^{a}	490.60±0.18^{b}	16.30±0.08^{b}	134.73±0.07^{c}	4.40±0.05^{c}
Untreated nymph1st generation	31.83±0.37^{a}	467.90±0.41^{c}	14.70±0.18^{b}	179.86±0.68^{b}	5.65±0.07^{b}
Positive control nymph 1st generation	17.08±0.40^{b}	598.50±0.63^{a}	35.04±0.79^{a}	197.51±0.30^{a}	11.6±0.3^{a}
Treated unfed adult female 1st generation	20.58±0.42^{a}	121.30±0.38^{b}	5.90±0.11^{c}	162.92±0.40^{b}	7.9±0.13^{c}
Treated unfed adult male 1st generation	11.90±0.35^{a}	247.70±0.43^{a}	20.81±0.60^{a}	99.90±0.40^{c}	8.40±0.30^{b}
Untreated unfed adult's 1st generation	2.67±0.01^{b}	31.00±0.49^{c}	11.58±0.22^{b}	319.46±0.40^{a}	119.00±0.60^{a}
Treated fed female 1st generation	20.60±0.20^{b}	144.50±0.31^{b}	6.93±0.16^{b}	843.00±0.70^{a}	40.33±0.60^{a}
Untreated fed female 1st generation	41.83±0.12^{a}	473.40± 0.50^{a}	11.31±0.02^{a}	73.64±0.20^{b}	1.76 ±0.03^{b}
Treated larvae 2nd generation	29.70±0.33^{a}	185.80±0.22^{a}	6.25±0.070^{b}	97.46±0.40^{c}	3.26±0.08^{c}
Untreated larvae 2nd generation	20.20±0.41^{b}	375.80±0.30^{b}	17.71±0.37^{a}	194.40±0.50^{a}	9.60±0.30^{a}
Positive control 2nd generation	22.56±0.28^{c}	144.40±0.24^{c}	6.40±0.07^{b}	135.76±0.40^{b}	5.98±0.14^{b}

Means with the same later are not significantly different.
Each value represents Mean of (5 replicates) ± Stander error.
Results presented as Means (5 replicates) ± Stander error

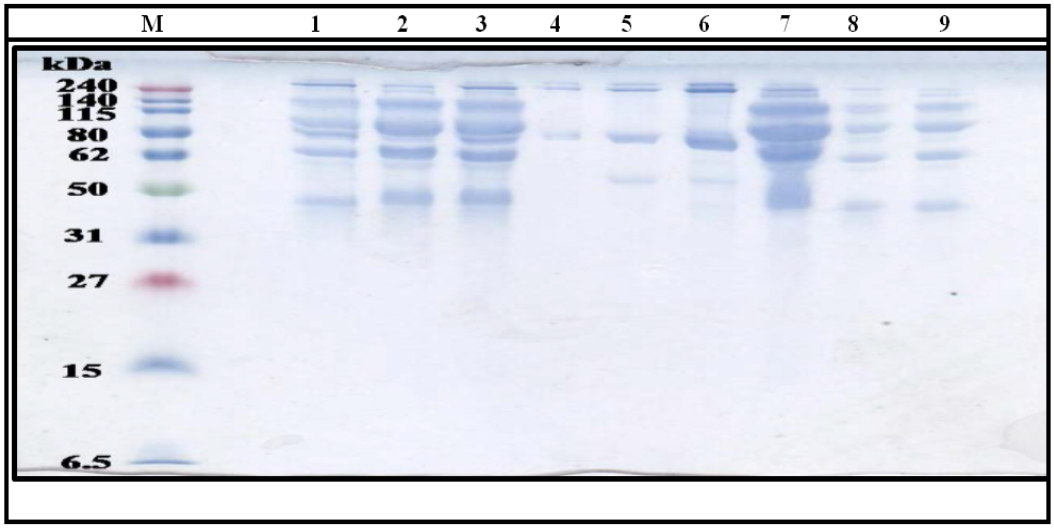

Fig. 3. SDS–PAGE Electrophoretic Profiles for Total Proteins of Hyalomma dromedarii Crude Homogenates

Proteomic Patterns in Hyalomma dromedarii Treated with Citrus sinensis var balady Peels' Oil Extract:

Larvae in 1st Generation:

In Table (4), the larvae proteins contents electrophoretic profile; due to citrus oil treated and controls in the 1st generation were separated into 17, 14, and 14 protein bands, respectively. They had molecular weights ranged from 240 to 33.2 kDa. The percentage amount of the expressed proteins ranged from 0.3 to 23.1% while the relative quantity ranged from 0.11 to 19.33 mg/ml. Common bands with molecular weights; 240, 136-133.1-131.3, 86.9-86.4-85.8, 71.4-70.4-68.1, 61.8-61.3-60.5, 55.4-52.1-51, and 48.5-46 kDa were shared with treated, and positive and negative controls larvae. The bands with molecular weights; 136-133.1-131.3, 86.9-86.4-85.8, 71.4-70.4-68.1, 61.8-61.3-60.5, 55.4-52.1-51, and 48.5-46 kDa were considered common homogenous bands and the 1-3 kDa differences in molecular weights could be correlated to the effect of treatment with the citrus oil and/or 2% DEMSO (positive control). In addition, hyper expression was characteristic in the 136, 133.1, and 131.3 kDa protein bands as indicated from the difference in band % and relative quantities between negative and positive controls in comparison to treated larvae group; 0.3%, 0.5% and 1.4%, and 0.11 mg/ml, 0.4 mg/ml, and 0.52 mg/ml, respectively, (Table: 4). The bands; 86.9, 86.4, 85.8, 71.4, 70.4, 68.4, 61.1, 61.3, 60.5, 55.4, 52.1, 51, 48.5, 46 kDa revealed hypo expression behavior due to treatments (*citrus* oil and 2% DEMSO) in treated larvae than negative control (Table: 4). On the other hand, disappearance of four bands with molecular weights; 119.3, 96.8, 92.2, 67.2 kDa was noted in oil-treated samples. Nonetheless, the appearance of six protein bands with molecular weights; 161.9, 112.1, 98.7, 58.8, 56.7, and 35.7 kDa with band % and relative quantity; 2.3%, 17%, 1.3%, 10.2%, 11.6%, 3%, and 0.85 mg/ml, 6.35 mg/ml, 0.48 mg/ml, 3.82 mg/ml, 4.33 mg/ml, 1.14 mg/ml, respectively, which were not found in untreated larvae group (Table: 4). Densitometric scanning of proteomics phenotypic patterns of the citrus oil treated samples in 1st generation showed that the 240 kDa band in 2% DEMSO and citrus oil treated larvae had higher density than the other bands with 23.1% and 12.2% band percentage and 19.33mg/ml and 4.56mg/ml relative quantity, respectively, (Figure 2 & Table 3). Similarly, the 61.3 kDa band in the untreated larvae group had higher density than the other bands with 20.3% and 7.44mg/ml; band % and relative quantity, respectively, (Figure 3 & Table 4).

Adults in 1st Generation:

In Table (5), the electrophoretic profile of proteins contents of adults' ticks was separated into 5, 6, and 10 protein bands in untreated adult, treated unfed female, and treated unfed males of *H. dromedarii*, respectively. These bands had molecular weights ranged from 240 to 41.9 kDa, while their percentage amount ranged between 0.9 to 44.8 (Table: 5). Common band with MW 240 kDa was shared between treated unfed adult's

TABLE 4. Proteomics phenotypic patterns of citrus oil treated Hyalomma dromedarii tick larvae of 1st generation.

Detected bands	Untreated		Treated		Positive control		Specificity
	Band%	Rel. Quantity	Band %	Rel. Quantity	Band %	Rel. Quantity	
Band number	14		17		14		
Protein conc.	31.90mg/ml		88.78mg/ml		44.38mg/ml		
240	7.40	2.70	0.70	0.25	23.10	19.33	
240	10.10	3.67	12.20	4.56	10.60	8.92	Common
240	10.50	3.84	9.60	3.61	10.70	9.01	
230.3					18.30	15.33	Specific to DEMSO treatment
161.9			2.30	0.85			Specific to oil treatment
136					0.50	0.40	
133.1			1.40	0.52			Common
131.1	0.30	0.11					
119.3	4.50	1.66					Absent in oil treatment
118.4					6.10	5.15	
112.1			17.00	6.35			Specific to oil treatment
98.7			1.30	0.48			
96.8	0.80	0.30					Specific to untreated
92.2	1.30	0.49					
86.9			2.50	0.92			
86.4	4.90	1.81					Common
85.8					2.10	1.74	
78.5	5.10	1.86			2.60	2.14	Absence in oil treatment
71.4	14.40	5.28					
70.4			5.20	1.93			Common
68.1					3.00	2.56	
67.2	3.00	1.11					Specific in untreated
61.8			6.60	2.74			
61.3	20.30	7.44					Common
60.5					12.10	10.11	
58.8			10.20	3.82			Specific in oil treatment
56.7			11.60	4.33			
55.4	4.80	1.75					
52.1					3.00	2.47	
51			3.50	1.30			Common
48.5	12.40	4.54			0.40	0.31	
46			3.10	1.15			
39.3			6.40	2.41			Absence in untreated
39.1					2.80	2.32	
35.7			3.00	1.14			Specific in oil treatment
33.4			3.50	1.31			Absence in untreated
33.2					4.80	4.02	

(male and female) and untreated unfed adults. In unfed male and female treated with citrus peels' oil two protein bands with MWs ranged (53.9-53.4) and (42.6-41.9) kDa had emerged when compared to untreated unfed adults (Figure: 3 & Table: 5). The bands% and relative quantities were 28.6%,16.1% and 8.7mg/ml, 4.9mg/ml, respectively, in females. While in treated unfed male were 6.3%, 5.5% and 3.16mg/ml, 2.74mg/ml, respectively. Meanwhile, the disappearance of one of the three 240 kDa protein bands was noted in the treated unfed female when compared with both the untreated negative control, as well as, treated unfed males (Figure: 3 & Table: 5). On contrast, oil treatments led to appearance of five protein bands in treated unfed male with MWs; 136, 101.3, 86.4, 74.3, and 61.3 kDa, with band percent 0.9%, 17.2%, 2.3%, 1.1% and 0.9%, and relative quantity 0.44mg/ml, 8.63mg/ml, 1.14mg/ml, 0.58mg/ml, and 0.45mg/ml, respectively, in comparison to untreated negative controls. Densitometric scanning of proteomics phenotypic patterns in 1st generation adults *H. dromedarii* tick showed that the 240 kDa band in both untreated and treated male adults had higher densities than the other bands, with band percentage 44.8%, 25% and relative quantity 9.61mg/ml, 12.5mg/ml, respectively. Similarly, in the treated unfed adult's female 53.4 kDa band had higher density than others, with band percentage 28.6% and relative quantity 8.71mg/ml (Figure: 3 & Table: 5).

TABLE 5. Proteomics phenotypic patterns of citrus oil treated Hyalomma dromedarii male and female and untreated unfed adults' ticks of 1st generation.

Detected Bands	Untreated unfed adult		Treated unfed female		Treated unfed male		Specificity
	Band %	Rel. Quantity	Band %	Rel. Quantity	Band %	Rel. Quantity	
Band number	5		6		10		
Protein conc.	2.67mg/ml		20.58mg/ml		11.90mg/ml		
240	3.40	0.73	16.60	5.06	24.20	12.15	Common
240	18.80	4.03	25.00	7.60	16.70	8.36	
240	44.80	9.61			25.00	12.58	Absent in female
136					0.90	0.44	Specific to unfed male oil treated
101.3					17.20	8.63	
91.4	17.00	3.66	11.10	3.39			Absent in male
86.4					2.30	1.14	Specific in male
78.9	15.90	3.42	2.50	0.77			Absent in male
74.3					1.10	0.58	Specific in male
61.3					0.90	0.45	
53.9					6.30	3.16	
53.4			28.60	8.71			Appeared in adult oil treated
42.6					5.50	2.74	
41.9			16.10	4.89			

Larvae in 2nd Generation:

In Table (6), the electrophoretic profile of proteins contents in larvae of the 2nd generation were separated into 12, 10, and 10 bands in oil treated and both negative and positive controls larvae, respectively, with MWs ranged from 240 to 39.3 kDa, amount percentage ranged from 29.3 to 1.3%, and relative quantity ranged from 10.2 to 0.42mg/ml. Common bands with MWs; 240 kDa, 94.4 -93.8 kDa, 65.3-65 kDa, 52- 50.6 -50 kDa, and 43- 39.3 kDa were shared between oil treated, positive and negative control larvae. Novel two protein bands were recorded in oil treated larvae of the 2nd generation with MWs 152.1 kDa and 54.5 kDa, with band percentage 2.1% and 6.9%, and relative quantity 0.79mg/ml and 2.6mg/ml, respectively, (Figure 3 & Table 6). However, five protein bands had disappeared of MWs 168.7, 125.5, 110.7, 81, and 45.9 kDa in oil treated when compared with untreated larvae group. Densitometric scanning of proteins phenotypic profile in the 2nd generation larvae of *H. dromedarii* tick showed 43 kDa band in oil treated group with higher density than the other bands; band percentage 27.2% and relative quantity 10.2mg/ml. Similarly, the bands ranged 82.1-81 kDa MWs had higher densities than the other bands in untreated and 2% DEMSO treated larvae, with band percentage 18.1- 29.3% and relative quantity 4.23-10.62mg/ml, respectively, (Figure 3 & Table 6).

Enzymatic and Metabolic Patterns of Hyalomma dromedarii treated with Citrus sinensis var balady Oil against Acaricidal Effects:

Catalase Enzyme Activity:

The LC50 *C. sinensis* var *balady* peels' oil affected on catalase activity in crude homogenates of larvae, nymphs, unfed and fed adults of *H. dromedarii* ticks (Table 3). Data revealed that the specific activity was significantly increased in treated more than untreated larvae; 4.83 and 2.86 units/mg protein, respectively, in the 1st generation . Whereas, in the 2nd generation the specific activity of catalase enzyme was significantly reduced in treated compared with untreated larvae which recorded 6.249 and 17.71 units/mg protein . In addition, it was increased in the treated compared with untreated nymphs recorded 16.3 and 14.699 units/mg protein (Table: 3). Moreover, the activity was significantly decreased in the treated unfed female to 5.89 units/mg protein, while increased to 20.81 units/mg protein in treated unfed males, when compared with untreated unfed adults which recorded 11.59 units/mg protein . In treated fed adult the specific activity of catalase enzyme was significantly decreased more than untreated fed adult recording 6.91 and 11.31 unite/mg protein, respectively (Table 3).

TABLE 6. Proteomics phenotypic patterns of citrus oil treated Hyalomma dromedarii tick larvae of 2nd generation.

Detected Bands	Treated		Untreated		Positive Control		Specify
	Band %	Rel. Quantity	Band %	Rel. Quantity	Band %	Rel. Quantity	
Bands number	12		10		10		
Protein conc.	29.70mg/ml		20.20mg/ml		22.56mg/ml		
240	14.80	5.57	7.00	1.64	2.70	0.98	Common
240	6.30	2.38			5.10	1.87	Appeared in oil treatment
240	5.20	1.96			6.10	2.21	
168.7			8.20	1.91	6.70	2.42	Disappeared in oil treatment
152.1	2.10	0.79					Specific to oil treatment
125.5			13.50	3.15			Disappeared in oil treatment
110.7			3.80	0.88			Specific to untreated
107.9	7.10	2.65					Appeared in treatment
106.5					1.40	0.51	
94.4	3.30	1.23					Common
93.8			9.50	2.22	19.50	7.08	
82.1					29.30	10.62	Disappeared in oil treatment
81.0			18.10	4.23			
72.1	4.10	1.54					Appeared in oil treatment
69.8					1.30	0.49	
65.3	4.00	1.51	6.40	1.50			Common
65					6.70	2.44	
62.3	15.80	5.93					Disappeared in DEMSO treatment
60.0			8.60	2.02			
54.5	6.90	2.60					Specific to oil treatment
52.0	3.20	1.18					
50.6					4.20	0.70	Common
50.			7.90	1.84			
46.3					6.30	1.10	Absent in oil treatment
45.9			15.10	3.51			
43	27.20	10.20					Common
39.3			1.80	0.42	10.60	3.87	

Glutathione Reductase Enzyme Activity:

The effect of LC50 *C. sinensis* var *balady* peels' oil on glutathione reductase activity in crude homogenates of larvae, nymphs, unfed and fed adults of *H. dromedarii* ticks were summarized in Table (3). The specific activities of glutathione reductase were significantly reduced in treated larvae of 1st and 2nd generations, nymphs, and unfed adults when compared with untreated *H. dromedarii*. Additionally, it was noted that glutathione reductase concentration in the 1st generation treated fed adult exceeded the untreated fed adults (73.64 mg/ml), which generally exceeded the linearity limits of the reaction (120 mg/ml) even after dilutions, recording 843 mg/ml; these odd results were excluded from the comparison, hence, it was affected by host blood meals(Table 3).

Discussion

Several studies indicated that plant extracts and essential oils represented alternative approaches to traditional chemical acaricides against ticks [17-20, 24, 51, 52]. Many Essential oils had both repellent and acaricidal properties against all ticks' stages; adult, nymph, larva and egg, of economically important species with encouraging results; especially *Hyalomma* spp. [53-58].

In the present investigation, the chemical analysis of locally prepared *C. sinensis* var *balady* peels' oil was identified by GC-MS where β-Pinene (oxygenated compounds) and limonene (hydrocarbon compound) were detected. The natural toxicity of citrus oil in this study was attributed to Limonin; the most dominant component with high average rate. Similar pattern of results was obtained by many

other authors [17-22] who found that limonene was identified as monoterpenoid hydrocarbon compounds that had insecticidal activity on many pests such as: house fly, cockroach and Mosquitos. In addition, limonene is a naturally occurring monoterpene (tetracyclic triterpenoid compound) which derived from plants of *Rutaceae* and *Meliaceae*, including citrus fruits peels [53-64], moreover, the insecticidal effects included: repellency, acute toxicity, fumigant activity, reproductive toxicity, and neurotoxicity. These proposed activities, each caused by an interaction of monoterpenoid at an active site in the insect [53-59]. Similarly, Vinturelle et al. [65] found that essential oils of citrus with limonene as the major compound, caused 100% mortality in engorged females *Rhipicephalus (Boophilus) microplus* at a concentration of 10%. Analogous study was carried out by Armugam et al. [66] who reported that the toxic effect of orange peels' extracts obtained from the genus *Citrus sinensis* against the dog tick *(Rhipicephalus sanguineus*) induced viable and safe tick repellant.

The effects of *C. sinensis* var *balady* peels' oil on the biological parameters of *H. dromedarii* tick through two generations revealed changes in the different developmental stages. The obtained data showed that treatment of engorged females induced highly significant prolongation in the oviposition period in both first and second generation, which could be attributed to the inhibition of egg development caused by citrus oil. In this study the data showed that the mean preoviposition period was insignificantly different between treated and un treated females. The effect of *C. sinensis* var *balady* peels' oil on different biological parameters revealed high significant prolongation in recorded periods of time. Where, it was recoded as the following; in comparison to untreated group, in oviposition (17.8 d than untreated 6.8 d) hatching (17.6 d than untreated 7.4 d), larval-nymphal feeding (21 d than untreated 15.2 d), premolting (20 d than untreated 16 d), and nymphal-adults feeding (19.4 d than untreated 6.2 d) periods of *H. dromedarii* ticks of 1st generation. On the other hand, it was with insignificantly effect on prehatching and preoviposition periods. While, in 2nd generation treatment by citrus oil, highly significantly increased the oviposition (19 d than untreated 10 d) and molting periods (6.4 d than untreated 4.8 d), but reduced the prehatching (34 d than untreated 42 d), larval-nymphal feeding (17.8 d than untreated 25 d) periods and percentage of adult female's emergence sex ratio (12:1 male: female than untreated 3:1). There were insignificant effects on preoviposition, hatching, premolting periods, egg mass, and nymphal weights. Sharma et al. [67] and Senthil-Nathan [68] found that the terpenoid compounds inhibited the egg development of *Locusta migratoria* adult females. Hadi and Adventini [69] found that the longer egg-laying period allowed the ticks to produce eggs in larger numbers. This period is associated with the weight of the tick itself. Treatment with citrus oil insignificantly affected prehatching period in first generation but in second generation was significantly reduced. These results were in agreement with EL-Ghali and Hassan [39]. They found that the mean prehatching period of *H. dromedarii* tick were significantly increased in the shad and sun. The treatment with citrus oil caused a significant prolongation on hatching period which was attributed to slower development of embryogenesis of eggs to larvae as a direct effects of citrus oil application. The obtained data supposed that treatment with citrus oil significantly increased the larval-nymphal feeding period in first generation as compared with untreated and significantly reduced in second generation. Similar results were described by Alahmed and Khier [35] who studied the larval-namphal feeding period of *H. dromedarii* tick on rabbits which were altered to 14 and 13 at 25 and 32ºC, respectively. Moreover, premolting period insignificantly affected with treatment with *C. sinensis* var *balady* peels' oil as compared with untreated nymphs in first and second generation, respectively. In contrary to Al-Rajhy et al. [70] recorded significant reduction in feeding activity of larvae, prolonged the period for molting to nymphal stage, and caused 60% reduction in molting ability on larvae and adult stages of camel tick *H. dromedarii* treated with cardiac glycoside, Azadirachtin, and neem oil. On the other hand, EL-Ghali and Hassan [39] recorded that the premolting period of *H. dromedarii* nymphs ranged from 7.8 to 20.7 days in shade and from 10.5 to 14.4 days in the sun. The data also showed prolongation in feeding period of treated adults. The obtained data showed the sex ratio in the emerged adults in the second generation were 1:12 (female:male) in treated adults. While, in the untreated were 1:3 (female:male). The reduction in the percentage of female's emergence in *H. dromedarii* tick in second generation may be due to the latent effect of treatment with the tested *C. sinensis* var *balady* peels' oil which was similar to data obtained by Farag and Emam [71].

Only few studies have been done on the effect of citrus oils against biological parameters on *H. dromedarii* ticks. Flores-Fernández et al. [72] found that essential oils extracted from Mexican oregano leaves (*Lippia graveolens* H.B.K.) had altered oviposition and hatching activities of cattle tick *R. microplus* by inhibiting the oviposition and hatching percentage. Alahmed and Khier [35] found that the mean preoviposition period of female of H. dromedraii tick at 25ºC were 6.6 days while 4.3 at 32ºC. In addition, they found that incubation period (from initiation of oviposition until the eclosion of the first larva) of H. dromedarii tick were 62.0 days at 25 ºC and 42 days at 32ºC. EL-Ghali and Hassan [39] found that the mean preoviposition periods ranged between 9.8 and 11.7 days in the shade but longer in the sun in December (14.7 days). Parallel results were observed for its closely related species *Hyalomma rufipes.* Chen et al. [73] found that the mean preoviposition and oviposition periods were 12.2 and 39.7 days, respectively, in *H. rufipes*. Similarly, Khater and Hendawy [40] found that the treatment of *H. dromedarii* engorged females' ticks with low concentrations of rose bengle and ivermectin induced reduction in the number of survived and ovipositing females, eggs per female, ticks laid hatched eggs, and hatched eggs. Correspondingly, Hadi et al. [69] found that the mean of preoviposition and oviposition periods of brown dog tick, *R. sanguineus* were 4.9 and 14.3 days, respectively. On the other hand, Habeeb et al. [22] studied the reproduction failure in ticks treated with *C. sinensis* var *balady* due to strong toxic effects on *H. dromedarii* eggs especially during early embryonic development. In addition, the sublethal concentration of Ivermectin (0.02%) induced significant ($P \leq 0.05$) reduction in the number of survived and ovipositing females, eggs per female, ticks laying viable eggs, and egg hatchability recorded 26.53%, 86.67%, 7661.27±377%, 87.80%, and 89.40 %, respectively [40].

Proteins are present in all living cells and they are important to the process of cell division, enzymes and hormones functionality, and controlling chemical reactions in the cell's metabolism [71]. Wilkinson [74] stated that proteins had role in the synthesis of microsomal detoxifying enzymes which contribute in the detoxification of toxicants that enter into the tick's body. The determination of total proteins contents; during the present study, was carried out in crude samples of larvae, nymphs, fed and unfed adults of *H. dromedarii* ticks by Bradford and UV-vis method. Similarly, Wilkinson [74], and Ibrahim et al. [75-77] determined the proteins contents of nymph, larvae, and adults of *H. dromedarii* ticks by using the dye-binding protein assay method of Bradford [43] using bovine serum albumin as a standard protein. The treatment with *C. sinensis* var *balady* peels' oil increased the total proteins contents significantly in larvae of both first and second generation and unfed adults, but significantly reduced their concentration in treated fed adult. Hence, it had hyperactivity effect through; cell division, hormones functionality, metabolism cascades, and synthesis of microsomal detoxifying enzymes which contributed in the deactivation of toxicants that enter into the tick body [22-24, 71-74]. The reduction in proteins content in crude proteins of treated fed adults; in the present work, might be due to proteins binding with foreign compounds of tested extracts. Otherwise, mobilization of amino acids to meet the energy demand stress, or might be due to the destructive effects on some of the cerebral neurosecretory cells of the brain responsible for secretion of the proteins in the treated adult females. Similar findings were obtained by Wu et al. [78] who noticed that total proteins levels in male moth *Sitotroga cerealella* treated with garlic essential oil were significantly increased. As well, Senthilkumar et al. [79] noticed that proteins in *Anophilus stephensi* larvae treated with some plant extracts were reduced and resumed that it was the result of interference of the extract with normal proteins synthesis mechanism. While, Valizadeh et al. [80] reported the reduction in total proteins contents of Elm Leaf Beetle (*Xanthogaleruca luteola*) after treatment with essential oils extracted from six medicinal plants including; *Artemisia annua* L., *Lavandula angustifolia Mill*., *Origanum vulgare L*., *Rosmarinus officinalis Spenn*., *Satureja hortensis L*., and *Thymus vulgaris L*. On the other hand, Elhadek et al. [81] found a significant increase of total proteins contents for cotton leafworm *Spodoptera littoralis* treated with essential oil from *Trigonella foenum graecum* but a significant decrease within larvae treated with *Nigella sativa.* In the present study, the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) exhibited the proteomics phenotypic patterns (electrophoretic profile) of proteins contents of citrus oil treated, positive and negative controls larvae of 1st generation. Their proteins contents were separated into 17, 14, and 14 protein bands,

respectively. However, revealed 5, 6 and 10 protein bands in the untreated and treated unfed adult female and males, respectively. Whereas, in the larvae of 2nd generation; proteins were separated into 12, 10, and 10 bands in treated and both negative and positive controls, respectively. Our results were in agreement with Friesen [82]. While, Abdullah et al. [83] found that crude extracted of *H. dromedarii tick* released seven proteins bands. Nevertheless, Habeeb et al. [17] studied the electrophoretic profiles of midgut, ovary and hemolymph of the engorged female *H. dromedarii* within 7 days after injection with ivermectin and *C. limon* oil. SDS-PAGE revealed different bands numbers and MWs in the controls and the treated females. Ivermectin adversely affected the proteins inducing the formation of new protein bands.

Only few reports had explored the effect of acaracides on catalase and glutathione reductase activity of *H. dromedarii* tick spp. Catalase is an antioxidant hydro peroxidase enzyme that protects the cellular environment from harmful effects of H_2O_2 by facilitating its degradation to oxygen and water [84]. Glutathione reductase is responsible for maintaining the supply of reduced glutathione; one of the most abundant reducing thiols in the majority of cells. In its reduced form, glutathione plays key roles in the cellular control of reactive oxygen species [85]. During the present study, the treatment with citrus oil increased the specific activity of catalase significantly in treated more than untreated larvae. Results reported 4.83 and 2.86 units/mg protein, respectively, in the larvae of the first generation, while in the second generation the specific activity of catalase was significantly reduced to 6.249 units/mg protein in treated larvae compared with untreated larvae which recorded 17.71 units/mg protein. The specific activity of catalase (unit/mg) enzyme was increased in the treated nymphs to 16.3 units/mg protein, when compared with untreated nymphs which recorded 14.699 units/mg protein. Similarly, Ibrahim et al. *[75] found that catalase activity of the larval crude e*xtract was 25.9 units/mg protein. On the other hand, the specific activities of glutathione reductase were significantly reduced in treated larvae of 1st and 2nd generations, nymphs, and unfed adults when compared with untreated *H. dromedarii* ticks during the present study. Similar reports were released previously [76, 86-93] which tested the insecticidal effect of essential oils on *P. polyxenes, Plodia interpuncletta*, flour beetle, *H. dromedarii*, and *Cx. pipiens*, respectively. These records were in agreement with da Silva Vaz Jr et al. [86] who reported that acaricides such as amitraz, chlorpyrifos, DDT, cypermethrin, diazinon, ivermectin, deltamethrin and flumethrin had inhibition effect on the enzyme activity of *R. microplus* recombinant glutathione S-transferase (rGST). Nonetheless, were in contrast to Abdelaal et al. [89] results. It was formerly reported the role of the previous enzymes (catalase and glutathione reductase) during native immune reaction against the toxic effects exhibited by acaricides; antioxidants [84-85]. The suppression is indicative of disfunction (male= no role) during essential oil toxicity so increasing the tick's susceptibility to essential oil, while the hyper expression is a prove of detoxification effect against acaricidal toxicity induced by *C. sinensis* var *balady* peels' oil on *H. dromedarii* ticks.

Finally, from the presented study it could be concluded that tested extracted oil from *C. sinensis* var *balady* peels showed acaricidal activities against *H. dromedarii* ticks by two applications methods. Therefore, they could be used in tick control and management strategies as alternatives to traditional acaricides to decrease costs and impact of chemicals in the environment, and positively affect animal and human health. The use of acaricides has decreased the incidence of tick-borne diseases; however, ticks usually develop rapid resistance to chemicals, in addition to, toxicity and environmental biohazards records. Therefore, it is necessary to search continuously for eco-friendly acaricides. Tick-control via *C. sinensis var balady* peels' oil had promising results as viable alternative.

Conclusions

It could be concluded that tested extracted oil from *C. sinensis* var *balady* peels showed acaricidal activities against *H. dromedarii* ticks by two application methods. Therefore, it might be used in tick control and management strategies as safe alternatives to traditional acaricides to decrease costs and impact of chemicals in the environment, and positively affect animal and human health. Hence, it is necessary to continuously search for eco-friendly acaricides.

Acknowledgment

The authors would like to thank the field investigators who helped in collecting specimens within the investigated localities. The authors would like to thank both the Entomology Department, Faculty of Science, Ain Shams University, as much as, Parasitology and Animal Diseases Department, Veterinary Research Institute, National Research Centre (NRC), Cairo, Egypt for supporting the study with the infrastructure and facilities to carry out the research plan. In addition, the authors are thankful for Prof. Dr. Ahmed E. El-Goharya at Medicinal and Aromatic Plants Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre (NRC), Cairo, Egypt for his help during natural oils extraction.

Conflicts of interest

The authors declare no competing interests relevant to the content of this article. The authors have no relevant financial or non-financial interests to disclose.

Funding statement

This study was funded by the National Research Centre (NRC), Cairo, Egypt, under a research grant [No. 11040301]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript

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سمات البروتينات والأنماط الأيضية في قراد الجمال نوع هيالوما دروميدارى المعالج بخلاصة زيت قشور الموالح من نوع البرتقال البلدي.

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استهدفت الدراسه تقييم فاعليه الزيت المستخلص من قشور البرتقال لمكافحه الاصابه بالقراد فى الابل. حيث قيمت هذه الدراسة تأثير الزيت المستخلص على قراد الجمال نوع *Hyalomma dromedarii*. وكانت نسبة الوفيات (نتائج LC50s) مرتبطة ارتباطا مباشرا بتركيزات الزيت. حيث كان التأثير القاتل بنسب ٨٢,٢٪ و ٧٨٪ في كلا طريقتى التطبيق: طريقه الغمس والتلامس الجسدي الفيزيائى على التوالي (الجرعات الممميته لنسبه ٥٠٪ من القراد المعامل بالمستخلص الزيتى). من خلال التوصيف الكيميائى والكشف عن المحتوى باستخدام جهاز كتله المطياف الغازى (GC-MS) وجد ٣ مركبات اساسيه: بيتا-بينين بمعدل متوسط ٢,٨٪، الليمونين بمعدل متوسط ٤٣,٣٣٤٪، و دى- ليمونين بمعدل متوسط ٥٣,٨٤٢٦٪ .

أظهرت المعاملات بزيت البرتقال البلدى تاثيرا واضحا على العوامل البيولوجية في دورة حياة قراد الابل وأطوارها المختلفة خلال جيلين تم تربيتهم فى ظروف المعمل، حيث كان لها تأثير معنوى على المجموعات المعالجه مقارنه بتلك التى لم تتلقى اى معامله و ذلك باطاله مده كلا من وضع البيض (١٧,٨> ٦,٨)، والفقس (١٧,٦> ٧,٤)، و تغذية اليرقات الحورية (٢١> ١٥,٢)، التسخين المبكر (٢٠> ١٦)، تغذية البالغين الحوريات (١٩,٤> ٦,٢)، خلاف ذلك، تتأثر بشكل ضئيل فترات ما قبل التفرز و ما قبل وضع البيض في القراد من الجيل الأول. في الجيل الثاني زاد بشكل ملحوظ وضع البيض (١٩> ١٠)، وفترات الريش (٦,٤> ٤,٨)، لكنه قلل من فترة الفقس (٣٤ >٤٢)، وتغذية اليرقات الحورية (١٧,٨ >٢٥)، ونسبة الجنس (١٢:١ > ١:٣ ♀ أنثى: ذكر)، ولكن ليس لها تأثير يذكر على ما قبل وضع البيض، و ماقبل الانسلاخ، وفترات الإنزال المبكر، وكتلة البيض، وأوزان الحورية.

من الناحية الكيميائية الحيوية ، تم تغيير كمية محتويات البروتينات الكلية وأنماط الرحلان الكهربي والأنشطة الأنزيمية بشكل كبير في المجموعات المعالجة مقارنة بالضوابط غير المعالجة. تم فصل محتوى أنماط البروتينات SDS-PAGE لليرقات المعالجة للجيل الأول والثاني والإناث والذكور غير المكسوة إلى ١٧ و ١٢ و ٦ و ١٠ حزم بروتينيه على التوالي، مع وزن جزيئي يتراوح من ٢٤٠ إلى ٣١ KDa. بالإضافة إلى ذلك، تم الكشف أيضًا عن نطاقات شائعة ذات أنماط مظهرية أعلى لقياس الكثافة والتي ترتبط بتأثير المعالجة بكل من زيت البرتقال البلدى و ٢ ٪ DEMSO في الضوابط الإيجابية. تم تغيير أنماط كمية البروتين والرحلان الكهربي بشكل كبير في العينات المعالجة أكثر من غير المعالجة والضوابط؛ فمما لاشك فيه ان الظهور، والاختفاء، والتعبيرات المفرطة، ونقص التعبير لبعض الحزم البروتينيه على جل البولى اكرالاميد SDS-PAGE يفسر إلى فعالية تاثيرالزيت على القراد خلال جيلين الدراسه المعمليه. بالإضافة إلى ذلك، تأثرت الأنشطة الكيميائية الحيوية والأنزيمية للأنزيمات المضادة للأكسدة فى مجموعات الدراسه خلال جيلين حيث زاد انزيم الكاتاليز في الجيل الأول ولكنه انخفض في الجيل الثاني، كما انخفض اختزال الجلوتاثيون بشكل كبير في الجيلين الأول والثاني.

ختاما: أثبت زيت قشور البرتقال البلدى كفاءته كمبيد بديل جديد آمن بيئيا وقابل للتحلل البيولوجي ومنخفض التكلفة مما يدلل على انه مفيد في مكافحة القراد في المجال البيطري.

الكلمات الدالة: قراد - زيوت قشور الموالح - GC/MS - اختبار حيوى - عوامل بيولوجيه - أنماط بروتينه - glutathione reductase -catalase -SDS-PAGE - الجمال - مصر.