EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

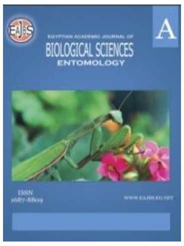
Vol. 14 No. 4 (2021)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 14(4) pp.145-158 (2021)*
DOI: 10.21608/EAJBSA.2021.207358

Egypt. Acad. J. Biolog. Sci., 14(4):145-158(2021)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



Comparative Studies on The Effect of Some Citrus Oils and Their Silver Nitrate Nanoparticles Formulation on Camels Tick, *Hyalomma dromedarii* (Acari: Ixodidae)

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ARTICLE INFO
Article History
Received:28/9/2021
Accepted:1/11/2021
Keywords:
Ticks, Essential oils, Nanotechnology, Adults, GC/MS, TEM, SEM, EDX.

ABSTRACT

Hyalomma dromedarii ticks are ectoparasite infesting camels. The acaricides used in controlling tick fauna developed resistance problems. Therefore, some citrus oils as well as their nano preparations were proposed as alternative agents during the present study. Fresh fruits peels were picked to extract oils by the Hydro-distillation method. Oil extracts were characterized by GC/MS technology. The phytochemical constitute present in *Citrus sininses var balady* were β-Pinene 2.8%, Limonene 97% while in *Citrus limon* were β-pinene 37.11%, α-Pinene 6.617% and Limonene 55.6%. Silver nitrate nanoparticles of citrus oils prepared were characterized by Electron microscope; TEM, SEM and EDX. The result showed Nano preparations were spherically shaped with homogeneous particle size. The toxicity of citrus oils was evaluated by dipping and physical contact methods in a wide range of concentrations. Despite that toxicity of *C. sinensis var. balady* and *C. limon* were similar in the dipping method, LC50 and LC90 values were 0.0024, 0.01473 and 0.00235, 0.14215%, respectively. On the other hand, during physical contact methodology, the toxicity of orange oils(C.sininses var balady) was higher than that of lemon(C.limon), hence, LC50 and LC90 recorded 0.00229, 1.995 and 0.00096, 0.10211% with *C. limon* and *C. sinessis var balady*, respectively. Recorded results showed a higher toxic effect of silver nitrate nanoparticles (AgNPS) from Citruspeels oils than those for citrus peels oil extract alone, where LC50 for *C. sinensis var. balady* were 0.009 and 0.0385 while were 0.013 and 0.19 for *C. limon*. Green nanoparticles of Citrus oils prepared during the present study proved their efficiency as eco-safe biodegradable acaricides that could be applied as medical treatments in the veterinary field.

INTRODUCTION

Ticks are blood-sucking arthropods that can be found in almost every part of the planet.Ticks are one of the most important ectoparasites of vertebrate animals and humans.Currently, 900 tick species have been identified within two families Ixodidae and Argasidae. Several tick species; *Ixodes ricinus* and *Ixodes persulcatus*, attack humans and numerous animal species **(** Mehlhorn, *et al*., 2012; Sonenshine, *et al*., 2014 and Abdel-Ghaffar, *et al.*, 2015). More than 20 ixodid species could infest camels. The most common is *Hyalomma dromedarii* which could act as vectors for *Theileria* spp, *Babesia spp* and *Anaplasma* spp. **(**Thorsell, *et al.,* 2006; Benelli, *et al*., 2016 and Centers for Disease Control and Prevention 2016).

Ticks are responsible for protozoa, bacteria, rickettsia and viruses' transmission (Da Fuente, 2008 and Sonenshine and Roe, 2013). Hence, most tick species are recognized as vectors of dangerous pathogenic agents which cause human and animal's diseases; including ehrlichiosis, Colorado tick fever, Rocky Mountain spotted fever, borreliosis, southern tick-associated rash, and tick-borne relapsing fever (Thorsell, *et al.*, 2006; Benelli, *et al*., 2016 and Centers for Disease Control and Prevention 2016). They induce decreasing in the productivity and quality of animals' byproducts, in addition to, anorexia, anemia, toxicosis, and general stress. Ticks had a direct impact on human health through venomous bites, blood loss, and skin deterioration resulting in reduced growth **(**Jabbar, *et al*., 2015**).**The tick's control was previously based on organophosphates, carbamates, amidines and pyrethroids **(**Mehlhorn, *et al*., 2012**;** Benelli, *et al*., 2018 and Benelli, *et al*.,2019). However, the number of acaricides now used has been limited due to legislative issues and the increasing resistance to their synthetic material **(**Mehlhorn, *et al*., 2012**;** Benelli, *et al*., 2018 and Benelli, *et al*.,2019). Recently, there was a global trend to evaluate new agents that are safe, effective, inexpensive, easily available, with low resistance and environmental contamination. Botanical acarticides represent alternative approaches for control, hence, their toxic effects have been studied intensively (Elles and Wall, 2014). High control efficacy has been achieved with essential oils. 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. Many essential oils have repellent and acaricidal effects against ticks *in vitro* (Habeeb *et al*., 2009). Their toxicity has been induced by immersion, physical contact with treated surfaces, and/or exposure to the vapor of the oils (Elles and Wall, 2014). On the other hand, the lack of standardisation and consequent inconsistent efficacy has restricted their registration and use in control (Mehlhorn, *et al*., 2012**;** Elles and Wall, 2014; Benelli, *et al*.,2018 and Benelli, *et al*.,2019).

Nanotechnology provides important new tools expected to have the most impact on many areas in medical sciences. The so-called, "green pesticides" are currently proposed as one of the helpful tools for controlling ectoparasites (Benelli, *et al*., 2015). Silver nanoparticles (AgNPs) have been recognized for their useful biomedical properties, e.g., antibacterial (Sharma, et *al*., 2009 and Dutta1, *et al*., 2020), antiviral (Trefry and Wooley, 2012**),** insecticidal (Habeeb *et al*., 2009 and Shater *et al*., 2020), and larvicidal activities (Govindarajan, *et al*., 2016**)**. As well as, their agricultural and pharmaceutical applications because they are non-toxic to humans and possess a wide array of biological activities (Skiba and Vorobyova, 2019 and Al Shater *et al*. ,2020).

In Egypt, *Hyalomma dromedarii* attacks camels as the main host (Abdel-Shafy *et al*., 2012**).** This species is regarded as the most significant impediment to camel production. 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 (Al-Rajhy *et al*., 2003). In a fully integrated system, a tick-control application uses a variety of viable strategies. Our study aimed to evaluate the effect of essential oils of *Citrus sinensis var. balady* and *Citrus Limon* peels and their silver nitrate nanoparticles on camel tick *Hyalomma dromedarii*.

MATERIALS AND METHODS

Collection of Ticks:

About 400 fully engorged females of *Hyalomma dromedarii* (Koch 1818) were collected from the ground of camel pens, as well as, from camel bodies in the back yards of the quarantine in the slaughterhouse of Toukh city (35 km north of Cairo; 30° 21′ 11.6″ N, 31° 11′ 31.5″ E), in Qalyubia Governorate, Egypt. Camels were originally imported from Sudan (12.8628° N and 30.2176° E.) and Somalia (2.855263, 45.185852); also, they were brought from Sinai Peninsula **(**29°30′N 33°50′E**).** *Hyalomma dromedarii* ticks were identified according to taxonomic keys (Hoskins, 1991 and Walker, 2014).

Extraction of the Essential Oil:

Citrus sinensis var. balady (orange) from Rutacea Family and *Citrus limon* (lemon) were collected at the ripening stage from trees. Fresh orange and lemon peels were collected and dried at room temperature, then were subjected to hydro-distillation using Clevenger-type apparatus for three hours (Clevenger, 1928). This is carried out by boiling each orange and lemon peels with water in a suitable vessel, then distilling and collecting the distilled oil. The volatile oils were carried over then condense with the steam. The extracted oils were dried using anhydrous Sodium sulphate and kept in a dark bottle in a refrigerator till analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Bioassay activities of each essential oil were studied on adult *Hyalomma dromedarii* tick (Salido, *et al*.2004**).**

Synthesis of Silver Nitrate Nanoparticles of Citrus Oils:

To prepare silver nitrate nanoparticles of the two tested citrus oils, 1ml *Citrus sinensis var. balady* or *Citrus limon* Oil was dissolved in 2 ml DMSO and 1ml tween 80, silver nitrate ($AgNO_3$, 0.017 gm., 0.01 mmol.) in 46 ml deionized water then continuous stirring. The mixture was warmed at 60°C for 1h; for shaping in-situ silver nanoparticles. The change to an earthy shade of the subsequent arrangement because of the development of silver nanoparticles was affirmed and portrayed by Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and EDEX (EL-Sayed *et al.*, 2016 and Rajaganesh, *et al.*, 2016).

The Component of *Citrus sinensis var balady* and *Citrus limon* Oils Analysis by Gas Chromatography-Mass Spectrometry (GC-MS):

Chromatographic analysis using GC–MS was performed (Agilent Technologies 7890B GC Systems combined with 5977A Mass Selective Detector) according to **(**Adams, 2007; Habeeb *et al*. 2009 and Bekley *et al*. 2014).

Testing and Characterization of *Citrus sinensis var balady* and *Citrus limon* Extract in-situ Silver Nanoparticles (*Limon*-AgNPs) and (*Orang*-AgNPs):

1-Transmission Electron Microscopy (TEM):

The shape and size of silver nitrate nanoparticles of citrus oils (*Limon*-AgNPs and *Orang*-AgNPs) were practically obtained using High-Resolution Transmission Electron Microscopy (HRTEM) JEOL (JEM-2100 TEM). Samples were prepared as previously described (El-Sayed *et al*. 2020).

2-Scanning Electron Microscopy (SEM):

The surface morphology of silver nitrate nanoparticles of citrus oils samples was examined using a Philips XL30 scanning electron microscope (SEM) connected to a LaB6 electron gun (Philips-EDAX/DX4) energy-dispersive spectroscope (EDX). Samples were prepared as previously described (Khan, *et al*., 2019 and El-Sayed *et al.* ,2020).

Evaluation of Toxicity Effects of *Citrus sinensis var balady and Citrus limon* Peel Oils and Silver Nanoparticles (*Limon*-AgNPs) and (*Orang*-AgNPs) against Engorged Females of *Hyalomma dromedarii* tick:

Concentrations of the essential oils, *C. sinensis var. balady* and *Citrus limon* were prepared using 2% DMSO (Dimethyl sulfoxide) as solvent. *The concentrations were 1.5, 2, 2.5, 3, 4, 10, 20, 100 %*. Since the dilution ratios were; 1:65,1:50, 1:40, 1:30, 1:25, 1:10, 1:5 and 1:1; Oil: DMSO 2%) for each oil (Habeeb *et al*., 2009).

Comparative Studies on Toxic Effect of Citrus Oils and Their Nanoparticles:

Different concentrations ranging from 100 to 400 ppm were prepared for each oil and its nanoparticles. Application methods were by dipping method only.

2% DMSO was used as a positive control treatment. Each concentration and control treatment was replicated 5 times and the replicates included five females. The treatment to evaluate toxicity effects was applied by two methods (by dipping on oil concentration and physical contact treated surfaces method, applied on treated filter paper). During the dipping method; dipping females for 30 seconds in each concentration or DMSO; in case of control treatments, and then transmitted to filter paper. The females were separated into plastic cups (female/cup). In the negative control, 5 non-treated engorged females/cups were used. The females were incubated at 26^0 C and 85% humidity (the suitable lab condition for tick rearing). The mortality rate was recorded daily for 7 days. Calculated mortality percentages of females were based on the females with brown-black color. LC50 and LC90 values for each concentration from *Citrus sinensis var. balady* or *Citrus limon* were calculated **(**Finney, 1971**)**.

Statistical Analysis:

The obtained data were analyzed as factorial, using Proc ANOVA in SAS **(**Anonymous, 2003) and means were compared by LSD (*P*= 0.05 level) in the same program. The mortality percentage was calculated by Krishnaveni and Venkatalakshmi formula as follows:

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

The result was corrected according to Henderson –Tilton's formula:

Corrected Mortality % =

$$100 \times (1 - \frac{No.of\ Ticks\ C.\ before\ treatment * No.of\ T.after\ treatment}{No.of\ C.after\ treatment * No.\ of\ T.before\ treatment})$$

Where:

No.= tick population

T.= treated

C. =control

RESULTS

GC/MS Analysis of the *Citrus sinensis var balady* and *Citrus limon*:

The results presented in table (1) & fig. (1) revealed the presence of two compounds in *Citrus sinensis* var *balady*. B-Pinene (6,6-dimethyl-2-methylidenebicyclo [3.1.1] heptane) 2.8% and limonene (1-methyl-4-(1-methylethenyl)-cyclohexene) 97.1% (monoterpenoid hydrocarbon compounds) were found in essential oil extracted from *Citrus sinensis var balady peels.* While *C. limon* reveled B-Pinene37.11%, α-Pinene (1*S*,5*S*)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene ((−)-α-Pinene) 6.6127%, and Limonene 55.8%. (Table 2 & fig. 2).

Table 1. GC-Ms analysis of *Citrus sinensis var balady* peel oil.

Peak	Retention time (RT)	Area% (Average rate)	Name	Molecular Formula
1	8.0648	43.334%	Limonene	$C_{10}H_{16}$
2	7.7151	2.8234%	B-Pinene	$C_{10}H_{16}$
3	7.9483	53.8426	D –Limonene	$C_{10}H_{16}$

Table 2. GC-Ms analysis of *Citrus limon* peel oil.

Peak	Retention time (RT)	Area% (Average rate)	Name	Molecular Formula
1	6.8288	6.6127%	α-Pinene	$C_{10}H_{16}$
2	7.7322	0.3435%	B-Pinene	$C_{10}H_{16}$
3	7.2251	36.767%	B-Pinene	$C_{10}H_{16}$
4	7.8605	42.59%	Limonene	$C_{10}H_{16}$
5	8.3093	1.5612%	D- Limonen	$C_{10}H_{16}$
6	8.3675	11.652%	D- Limonen	$C_{10}H_{16}$

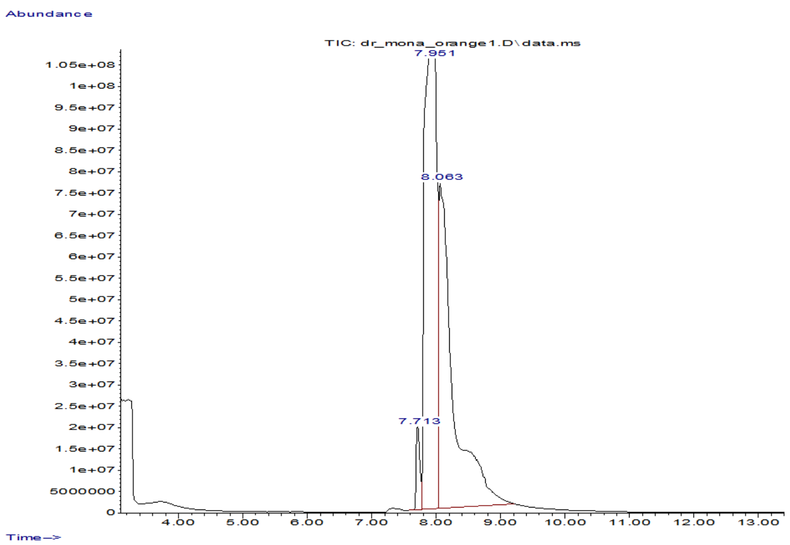


Fig. 1. GC/MAS analysis of *Citrus sinensis var balady* peel oil.

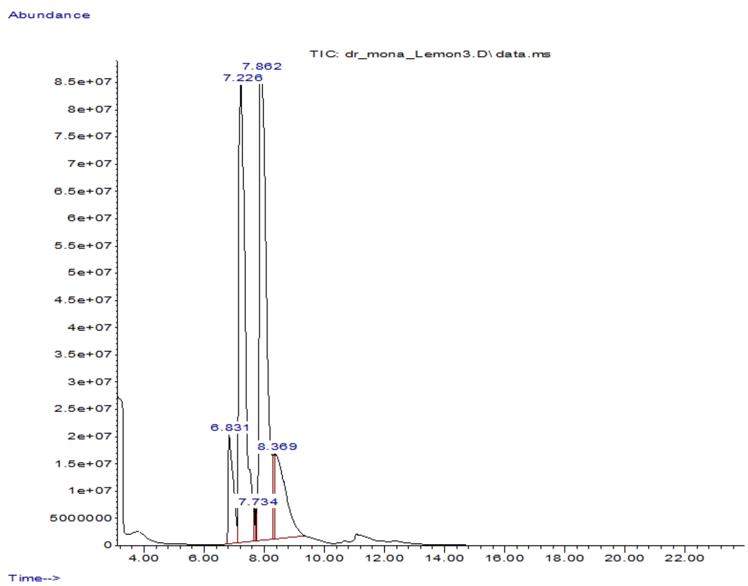


Fig. 2. GC/MAS analysis of *Citrus limon* peel oil.

Characterization of *Citrus sinensis var balady* and *Citrus limon* Extract in-situ Silver Nanoparticles:

Transmission Electron Microscopy (TEM):

Citrus sinensis var. balady peel oil and *Citrus limon* nanoparticles has been confirmed by TEM as shown in figures 3 & 4 the synthesized silver nitrate nanoparticles (AgNPS) obtained have a relatively spherical shape with an average size of about 8.1- 15.8 nm in *Citrus sinensis var. balady* (orange AgNPS) and about 5.1 -7.5 in *Citrus limon* AgNPS.

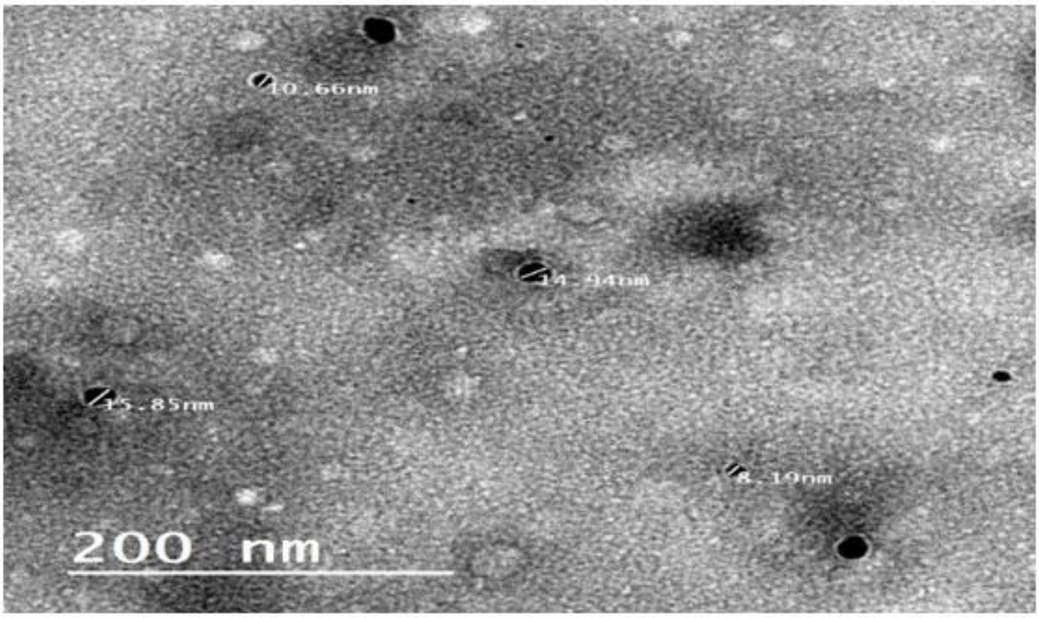


Fig. 3. TEM photomicrograph of *Citrus sinensis var. balady* silver nitrate nanoparticles.

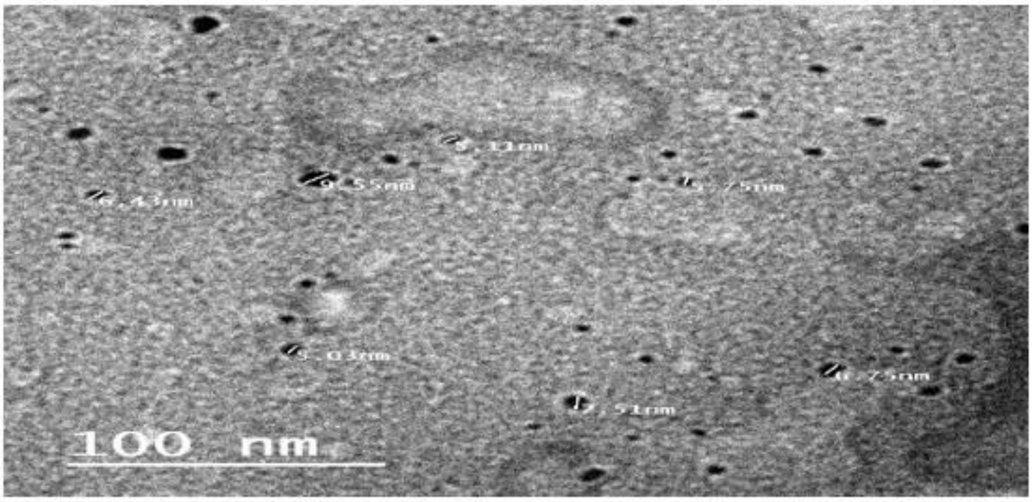


Fig. 4. TEM photomicrograph of *Citrus limon* silver nitrate nanoparticles**.**

Scanning Electron Microscope and EDX:

As can be seen from the figures (5a, 5b & 6), the synthesized silver nanoparticle using *Citrus sinensis var. balady* and *Citrus limon* had spherical shapes with homogeneous-sized particles. The EDX result as shown in figures (7 & 8) indicated the presence of silver and other main elements present in both citrus oils silver nitrate nanoparticles.

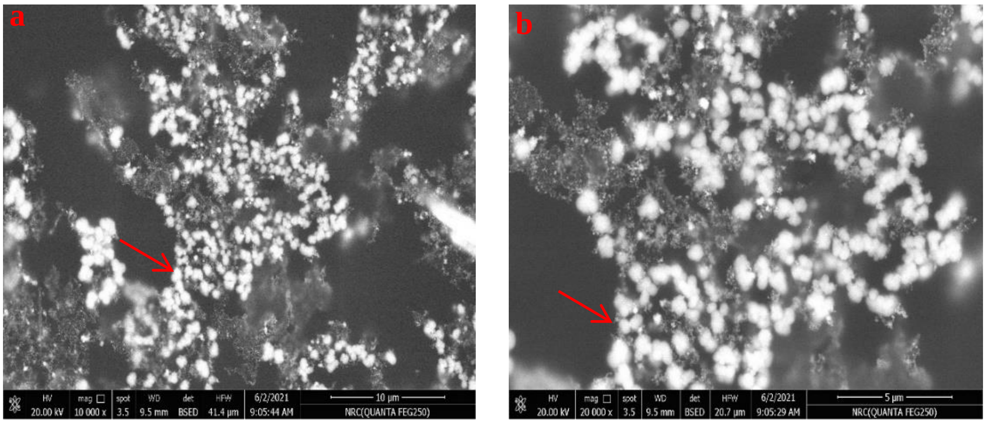


Fig. 5. SEM photomicrograph of *Citrus sinensis var balady* silver nitrate nanoparticles.

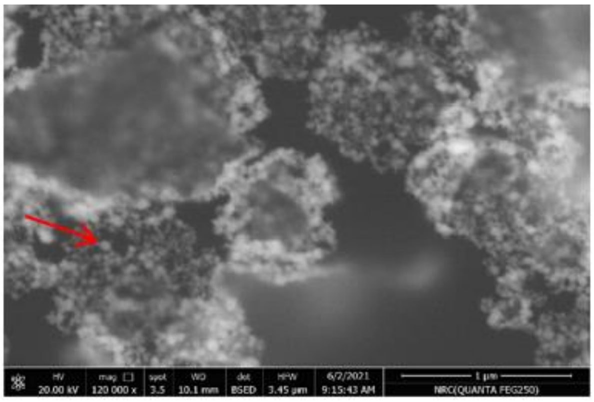
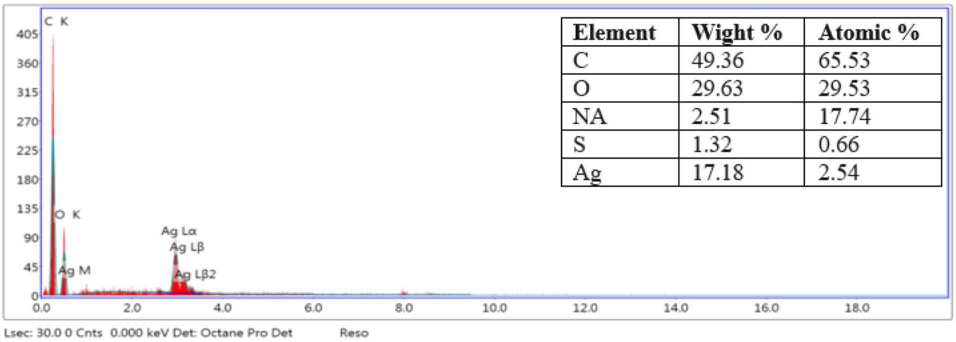
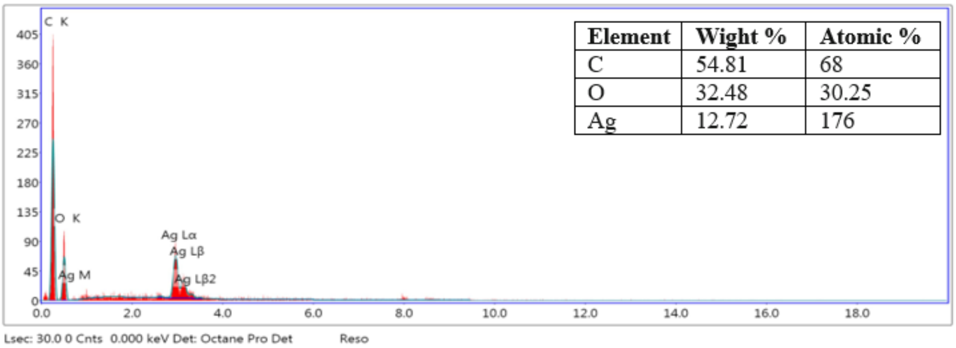


Fig. 6. SEM photomicrograph of *Citrus limon* silver nitrate nanoparticles



Element	Wight %	Atomic %
C	49.36	65.53
O	29.63	29.53
NA	2.51	17.74
S	1.32	0.66
Ag	17.18	2.54

Fig. 7. EDX of *Citrus sinensis var balady* silver nitrate nanoparticles



Element	Wight %	Atomic %
C	54.81	68
O	32.48	30.25
Ag	12.72	176

Fig. 8. EDX of *Citrus limon* silver nitrate nanoparticles.

Bioassay:

1-Toxicological Studies of *Citrus sinensis var balady* and *Citrus limon* against *Hyalomma dromedarii* Tick:

The susceptibility effect of citrus oil against adult's camel tick, *Hyalomma dromedrii* investigated in tables (3 & 4). In the case of *C. sinensis var balady* (Table 3), the mortality increased with increasing oil concentrations. The mortalities were recorded 33.3 ,42.2 , 46.6 , 51 ,60 68.9 , 77.7, and 82.2%, at dilution 1:65, 1: 50, 1:40 ,1:30 ,1: 25, 1:10 , and 1:05 by dipping method, respectively. While the mortality percent recorded 20, 22.2, 33.3, 37.8, 44.4, 48.9, 60, and 68.9 % on physical contact method at the same dilution ratio mentioned above, respectively. While in case of *C. limon* (Table 4), the dilution 1:65, 1:50, 1:40 ,1:30 ,1: 25, 1:10, and 1:05 recorded 22, 24, 36, 38, 49, 51, 60, and 78 % mortality percentages by dipping method, respectively. While 11.1, 18, 22, 24, 36, 38, 49, and 56 % mortality percentages were recorded on physical contact application. The average positive control and negative control for all the tests were 10% & 4%, respectively. Therefore, the toxicity of *C. sinensis var balady* and *C. limon* was similar in the dipping method. LC50 and LC 90 values were 0.0024, 0.01473 and 0.00235, 0.14215%, respectively, but in the physical contact method, the toxicity of *C. sinensis var balady oil* was higher than that of *C. limon*. The LC50 and LC90 in *C. limon* and *C. sinessis var balady* by physical contact method were 0.00229, 1.995 and 0.00096,0.10211 %, respectively.

2-Comparison Study Between the Toxic Effects of Citrus Oils and Their Nanoparticles (*Limon*-AgNPs) and (*Orange*-AgNPs):

In tables (5 & 6) the comparison between the toxic effects of citrus oils alone and their silver nanoparticles was presented. LC50s of *C. sinessis var balady* and its Orange-AgNPS were 0.385 and 0.009 % respectively, while LC90s were 8.16 and 2.491 %, respectively (Table. 5). While Lc50s of *C. limon* and its Limon-AgNPS were 0.19 and 0.013 %, respectively. The LC90s were 47.91 and 28.6%, respectively (Table. 6). From the above-mentioned results, it was noticed the highly toxic effect of both AgNPS than their citrus oils alone.

Table.3. Toxicity effect of *C. sinensis var. balady* on *Hyalomma dromedarii* tick by different methods of application.

Concentration of *C. sinensis var balady*		**Corrected Mortality %**	
Conc. %	**Dilution Ratio (Oil: DEMSO)**	**Dipping**	**Physical Contact**
1.5	1 : 65	33.33 ± 9.9 ^{d}	20 ± 10.77^{c}
2	1:50	42.22 ± 11.3 ^{cd}	22.22 ± 7.02^{c}
2.5	1:40	46.67 ±11.3 ^{cd}	33.3 ± 11.3b^{c}
3	1:30	51 ± 8.3^{bcd}	37.78 ± 12.9^{abc}
4	1:25	60 ± 12.5^{abcd}	44.4 ±13.1^{abc}
10	1:10	69 ± 8.8 ^{abc}	48.89± 12.9^{abc}
20	1:5	77.8 ± 7.0^{ab}	60 ± 12.9^{ab}
100		82.22 ±12.95^{a}	68.9 ±15^{a}
Average of positive control mortality rate 10%			
Average of negative control mortality rate 4%			
F		2.07	2.01
P		0.0247	0.0843
LSD		30.541	35.42
LC50		0.0024	0.0009565
LC90		0.1473	0.10211

SE=Stander Error

LSD= Lest significant difference

Corrected Mortality % = Mean ± SE

Values (means ± SE) followed by similar letter within the same column do not differ significantly (P< 0.05).

Table. 4. Toxic effect of *C. limon* oil by two different applications against engorged female *Hyalomma dromedarii*

Concentration of *Citrus limon*		Corrected Mortality %	
Conc. %	Dilution Ratio	Dipping	Physical Contact
1.5	1:65	22 ±7 [b]	11.1±9 [c]
2	1:50	24 ± 15 [b]	18 ± 7 [bc]
2.5	1:40	36 ±19 [b]	22 ±10 [bc]
3	1:30	38 ± 13 [ab]	24 ±5 [abc]
4	1:25	49 ± 22 [ab]	36 ±12 [abc]
10	1:10	51 ± 18 [ab]	38± 11 [bc]
20	1:5	60 ± 13 [ab]	49 ± 16 [ab]
100		78 ± 9.9 [a]	56 ± 14 [a]
Average of positive control mortality rate 10%			
Average of negative control mortality rate 4%			
F		1.53	2.03
P		0.1924	0.0812
LSD		43.643	31.446
LC50		0.00058	0.000229
LC90		0.14215	1.995

SE=Stander Error
LSD= Lest significant difference
Corrected mortality = Mean ±SE.
Values (means ± SE) followed by similar letter within the same column do not differ significantly ($P< 0.05$)

Table 5. The effect of silver nitrate nanoparticles of *Citrus sinensis var balady* on engorged female *Hyalomma dromedarii*

Concentration ppm	*C. sinensis var. balady* oil Corrected Mortality %	*C. sinensis var. balady* oil AgNPS Corrected Mortality%
20000	78.3 ± 0 [a]	83.6 ±10.4 [a]
400	56.5 ± 15.3 [ab]	67.3± 14 [ab]
300	51 ±18.7 [ab]	58 ± 15.5 [ab]
200	35 ±0 [b]	51 ±5.4 [ab]
100	32 ± 17 [b]	34.3 ±17.1[b]
LC50	0.0385	0.009
LC90	8.16	2.491
F	2.02	2.15
P	0.1431	0.1247
LSD	39.87	39.7
Average of positive control 8%		
Average of negative control 4%		

SE=Stander Error
LSD= Lest significant difference
Corrected mortality = Mean ±SE.
Values (means ± SE) followed by similar letter within the same column do not differ significantly ($P< 0.05$)

Table 6. The effect of *Citrus limon* oils and their silver nitrate nano formation on engorged female *Hyalomma dromedarii*

Concentration ppm	*C.limon* oil Corrected Mortality %	*C. limon* AgNPS Corrected Mortality%
20000	6 7.4±14 [a]	72.8 ±5.4 [a]
400	45.65 ±20.8 [a]	62 ±16.3 [ab]
300	40.2 ±13.6 [a]	42 ±16.7 [ab]
200	34.8 ±0 [a]	40.2 ±10.4 [ab]
100	26 ±12.4 [a]	34.8 ±8.9 [b]
LC50	0.19	0.013
LC90	47.91	28.6
F	1.24	1.72
P	0.336	0.1975
LSD	42.02	37.185
Positive control average mortality rate 8%		
Negative control average rate 4%		

SE=Stander Error
LSD= Lest significant difference
Corrected mortality = Mean ±SE.
Values (means ± SE) followed by similar letter within the same column don't differ significantly (P< 0.05).

DISCUSSION

According to the presented results, the analysis of the chemical composition of citrus oil *(Citrus sinensis var balady* and *Citrus limon*) by GC/MS showed that B-Pinene 2.8% and limonene 97.1% (monoterpenoid hydrocarbon compounds) in the essential oil extracted from *Citrus sinensis var balady* peels and β-Pinene (37.11%), α-Pinene (6.6127%), and Limonene (55.8%) in *Citrus limon* peel essential oil were the major compounds. A similar pattern of results was obtained by Habeeb *et al*. (2009), they found three main compounds, Limonene (45. 99%), Myrcenol (21. 85%), Cis Ocimene (15. 49%) in fresh fruit peel of *Citrus limon* by using GC/MS. While Habeeb *et al.* (2007) when analysed the essential oil of fresh fruit peel of *Citrus sinensis var balady,* by GC/MAS to identify its components, they found the main natural toxic component is Limonene (83.28%) as a hydrocarbon compound and Linalool (3.97%) as oxygenated compounds. Vinturelle *et al.* (2017) studied the chemical composition of *Citrus limonum* essential oil by Gas Chromatography/Mass Spectrometry (GC-MS). They found limonene (50.3%), β-pinene (14.4%), and γ-terpinene (11.7%) as the major components.

In our study, we used silver nitrate of citrus oil that had an acaricidal effect on *Hyalomma dromedrii* tick. Analytical characterization by TEM, SEM and EDX showed that AgNPS of citrus oil had a spherical shape with homogeneous particle size. A similar pattern of results was obtained by Sivakumar *et al*. (2008) who studied the analytical characterization of silver nitrate nanoparticles by TEM, DLS, XRD and FTIR supported the structure, size, crystallinity and reduction mechanism of the synthesized nanoparticles. EL-Sayed *et al*. (2020) studied the characterization of the AgNPs of Moringa by different methods such as UV–Vis spectra and TEM and indicated that synthesized nanoparticles have a spherical shape with an average diameter of 1–17 nm.

Obtained results indicated that the toxic effect of *citrus sinensis var balady* and *Citrus limon* against engorged females of *Hyalomma dromedarii* were nearly similar by dipping but in the physical contact method it was higher in *citrus sinensis var balady*. In contrast to the above, Habeeb *et al*. (2009) evaluated two essential oils obtained from two plant species, *Citrus sinensis var balady*, *Citrus limon* and avermectin as control agents against the engorged female of *Hyalomma dromedarii*. They found that the toxicity of *C. limon* was higher than that of *Citrus sinensis var balady*. The toxicity of citrus oils may be attributed to the presence of the main component Limonen, where it was 97.1% in *citrus sinensis var balady* and 55.8% in *Citrus limon*.

In the present study, the research team evaluated the acaricidal effect of citrus oil (*Citrus sinensis var balady* and *Citrus limon*) in its pure and nanostructured (silver nitrate nanoparticles) on camel tick (*Hyalomma dromedarii*) females. It was noticed the higher toxic effect of silver nitrate nanoparticles of both citrus oils than the pure citrus oils. Similarly Pazinato *et al*. (2014) demonstrated acaricidel effect of tea tree oil (TTO) (*Melaleuca alternifolia)* in its pure and nanostructured (TTO nanoparticles) forms on the reproduction of female cattle tick, *Rhipicephalus microplus*, since both forms affected tick reproduction by inhibiting egg-laying and hatching percentages. Nanoparticles potentiated the inhibitor effect of pure TTO on the reproduction of *Rhipicephalus microplus*. Former findings supported previous publication; hence, green silver nitrate nanoparticles of citrus oils had accelerated the acaricidal effect on *Hyalomma dromedrii* tick than pure citrus oils. A similar conclusion was reached by Abdel-Ghany *et al*. (2021) on green nickel oxide *Melia azedarch* nanoparticles with acaricidal effect on *Hyalomma dromedrii* tick.

Conclusion

Green nanoparticles of Citrus oils prepared during the present study proved their efficiency as eco-safe biodegradable acaricides that could be applied as medical treatment and pest control in the veterinary field.

Acknowledgment:

The authors would like to thank the field investigators who helped in collecting specimens in the investigated locality. The authors would like to thank both the Entomology Department, Faculty of Science, Ain Shams University, as much as, National Research Centre- Dokki, for supporting the study within 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, Cairo, Egypt for his help during natural oils extraction.

Funding Statement:

This study was funded by the National Research Centre (NRC), 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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ARABIC SUMMARY

دراسات مقارنة حول تأثير بعض زيوت الموالح وتركيباتها النانوية من نترات الفضة على قراد الإبل

منى أبوبكرعاشور[1]- سريا الطنطاوى حافظ[1] –سلوى محمود حبيب[2]- أحمد على السيد[3]-نسرين علام طنطاوى علام[2]

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يعد قرا د الابل واحد من الطفيليات الخارجيه التى تصيب الابل وعند مقاوته باستخدام المواد الكميائيه ينتج عن ذالك مقاومه من القراد ضد هذه المبيدات , لذالك تستهدف هذه الدراسه الى استخدام الزيوت المستخلصه من قشور الموالح كما هى وفى صوره مركبات نانونيه ايضا لمقاومه قراد الابل .الزيوت المستخلصه يتم تحليلها لمعرفه المركبات الكميائيه المكونه لها باستخدام كتله المطياف الغازى وقد نتج عن هذا التحليل وجود مركبات الليمونين بنسبه 97%ومركب البيتا بينين بنسبه 2.8% فى الزيت المستخلص من قشور البرتقال البلدى بينما الزيوت المستخله من قشور الليمون يوجد الليمونين بنسبه 55,6% وبيتا بنين بنسبه 37,11% والفا بنين بنسبه 6,617% وتم تحليل المركبات التانومتريه من زيوت قشور الموالح باستخدام المجهر الالكترونى النافذ والمجهر الالكترونى الماسح والتى اوضح ان المركبات النانويه لزيوت الموالح وجدت ذات شكل كروى متماثله الحجم. و قد تم تطبيق الزيوت المستخلصه بطرقتين اما بغمس عينات القراد فى التركيزات المختلفه من الزيوت او بطريقه التلامس باسطح مشبعه بالزيوت . اظهرت النتائج ان سميه الزيوت المستخلصه من البرتقال والليمون كانت متشابه فى حاله الغمس حيث كانت قيم الجرعه المميته ل50% ول90% من مجموعات القراد المطبق عليها المعالجات لزيت البرتقال 0.0024, 0.01473 ولزيت اليمون 0.00235, 0.14215%,على التوالى. بينما فى طريقه التلامس الفزيائى فان سميه الزيت المستخلص من قشور البرتقال اعلى من سميه الزيت المستخلص من قشور الليمون . أظهرت النتائج المسجلة وجود تأثير سمي أعلى للمركبات النانويه لزيوت الموالح عن الزيوت المستخلصه فقط. أثبتت الجسيمات النانوية الخضراء لزيوت اامولح التي تم تحضيرها خلال هذه الدراسة كفاءتها لمقاومه قراد الابل وانها قابلة للتحلل البيولوجي وآمنة للبيئة لذا يمكن استخدامها كعلاجات طبية في المجال البيطري.