Evaluation of toxicity of *Eucalyptus cammadelulensis* essential oil in the hard tick *Hyalommaanatolicum* (Acari: Ixodidae) infested livestock in Basrah province, Iraq

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Abstract
The study was done in Basrah province, Southern Iraq, from January to December 2018. The first aim of this study was to determine the prevalence of *Hyalommaanatolicum* infestation in the livestock in Basrah province. The second was to evaluate the effect of *Eucalyptus cammadelulensis* essential oil on the mortality of the life cycle stages. The highest prevalence of *H. anatolicum* was recorded in cattle 34.66%, while the lowest infestation was recorded in buffalos 28.17%. The results also showed that the toxic effect of the eucalyptus oil was gradually reduced by the age of the tick; eggs were the most affected, followed by larvae and nymphs, whereas adults were more resistant. The rates of mortality of all stages increase with the rise of oil concentrations; the most mortality of all experiments was at the concentration 1%, compared with others especially 0.0625% which had the lowest rate of mortality. The fed individuals of larvae, nymphs and adults have recorded the highest rates of mortality compared with unfed individuals. Females were more sensitive than males in the treatment with eucalyptus oil. The LC50 values were varied according to the time of the exposure and feeding of the tick stage.

Key words: hard ticks, *Hyalommaanatolicum*, toxic activity, essential oil, *Eucalyptus*

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Introduction

The ticks (*Acari: Ixodidae*) are blood-feeding ectoparasites of domestic, wild and semi-aquatic animals (Guglielmone*et al.,* 2014). The hard ticks Ixodidae have hard plates; there are six major hard tick genera, named: *Amblyomma, Dermacentor, Haemaphysalis, Hyalomma*, *Ixodes* and *Rhipicephalus*. (Lindquist and Wu, 2016). Larvae of ixodids take 3-5 days to fully engorge with blood and nymphs 4-8 days, whereas adults 5-20 days (Guglielmone*et al.,* 2014). All ticks' stages feeding only on the blood of their host; they crawl onto the host and attach to the body by cutting mouthparts (Guglielmone*et al.,* 2014). Ticks are important in the health of animals and humans because they feed on blood and cause disease to their host. Taking blood may cause allergies and weakness and may sometimes cause poisoning (Walker, 2003).

Many diseases are caused when the ticks infected with pathogens. These microorganisms transmitted to animals and humans when the ticks feed and may cause lethal diseases to the host (Jongejan and Uilenberg, 1994). The hosts may be a single species or a group of similar species, most ticks have a predilection of the host to which they are adapted, and the activity of ticks' species is adapted to seasonal variations (Dhooria, 2016). When ticks feed with a large number on a wound is created in the skin, this reduces the gain in the live weight of cattle and loss of milk production (Nadolny*et al.,* 2014). Some ticks secrete toxins in their saliva; it causes several types of poisoning or toxicosis. For example, females of *Ixodesrubicundus* and *Rhipicephalusevertsi* may cause paralysis to cattle, sheep, and goats when they are feeding (Lindquist and Wu, 2016).

Many diseases caused by tick-borne pathogens, such as African swine fever, Crimean-Congo hemorrhagic fever, Anaplasmosis, Babesiosis, Borreliosis, Cowdriosis, Ehrlichiosis, Rickettsiosis, and Theileriosis (Aktas, 2014). There are some acaricides used to control ticks, such as coumaphos and chlorfenvinphos, propoxur, amitraz, deltamethrin, fenvalerate and flumethrin (Wall and Shearer, 2008). These acaricides have been used for several years and resort to having problems of toxicity on the animals and vegetation. When ticks are exposed to acaricides for a long time, some mutant strains of ticks will arise. These strains are able to survive at the normal dose of acaricide, this reduces effectiveness and acaricide resistance (Estrada-Peña, 2015).

Hyalommaanatolicum is important over widely distributed in the Tropical and Afrotropical regions, especially from North Africa to India. It is a parasite on cattle, sheep, goats, camels, horses, and donkeys (Latrofa*et al.,* 2014). This species is a vector of the tropical theileriosis in cattle, and ovine theileriosis in equines, and Trypanosomiasis in bovines (Wall and Shearer, 2008). The present study was conducted to determine the distribution and prevalence of *Hyalommaanatolicum* among economic mammals in Basrah province. In addition to this, the current study was aimed to evaluate the acaricidal effects of *Eucalyptus cammadelulensis* essential oils on the eggs, larvae, nymphs and adults' mortality.

Materials and Methods

Collection and identification of ticks' samples

This study has been carried out in Basrah Province, Southern Iraq, between January to December 2018. Many locations as farmlands and animal husbandry stations were visited. A total of 500 animals were examined as follows: cattle, sheep, goats, and buffaloes. Samples of ticks were collected from the body using a fine forceps, cotton, and ethanol. Samples of *Hyalommaanatolicum* were identified based on morphological characteristics, using the following taxonomic keys: (Wall and Shearer, 2008), (Abdigoudarzi et al., 2011), (Guglielmone et al., 2014) and (Walker, 2003). The diagnosis was performed by several external features, which are coloration, capitulum, scutum, legs, spiracles, anal plates, and festoons. Prevalence, mean intensity and relative density of *H. anatolicum* in livestock were recorded.

Study the toxic activity ofEucalyptus cammadelulensisessential oil

Leaves of *E. cammadelulensis* (family: Myrtaceae) were collected from the gardens in Basrah City, kept and dried in a light place. The essential oils prepared by the hydrodistillation method (Périno-Issartier*et al.,* 2010). A stock solution of the *E. cammadelulensis* oil was prepared in methanol. Prior to each experiment, the stock solution was diluted in distilled water. The final concentration of methanol was 0.1% (v/v). In order to study the toxic activity of oil on ticks, four concentrations were prepared (0.0625%, 0.125%, 0.25%, 0.5%, 1% (v/v)). The acaricidal activity of ticks was studied according to (de FreitasFernandes and Freitas, 2007). This method was carried out by fitting the impregnated filter paper to the bottom of the glass petri dish which contains 20 ml of the extract for 1 minute only. 30 of eggs or 10 of larvae, nymph and adult ticks were immersed in the oil, and then placed on filter paper in a big petri dish. The mortality rate of ticks calculated after 24 and 48 hours. One control packet was prepared for each method by impregnated it with distilled water only. This procedure was repeated 3 times. Control experiments were done with distills water containing 0.1% (v/v) methanol. All tests were carried out at laboratory conditions.

Statistical analysis

Statistical analyses were performed using the IBM SPSS statistics version 25. The mortality rates were calculated according to the modified Abbot formula. The data of mortalities were analyzed using a one-way analysis of variance (ANOVA). This followed by Tukey's post hoc multiple tests. Significance differences were considered when the p-value <0.05. The lethal concentration required to kill 50% (LC50) of tick life cycle stages was calculated using Graph Pad Prism 7.

Results

The presence of the hard tick Hyalommaanatolicum between livestock inBasrah province

The results of the survey of *H. anatolicum* are shown in table 1, which recorded that 164 of 500 animals were infested with this species in Basrah province from January to December 2018. The total prevalence rate of all infestations was 32.80%. Table1 also reveals that cattle were recorded the highest prevalence with 34.66%, followed by sheep 33.33%. Buffaloes recorded the lowest prevalence during study periods with 28.17%. The statistical analysis of data was not shown significant differences between the prevalence of ticks' infestation in the examined animals. Figure1 shows some variations in the mean intensity of *H. anatolicum* between the economic mammals. The highest mean intensity was being recorded in sheep with 5. 42, followed by cattle, while the lowest mean intensity 3.00 was recorded in buffaloes. Some differences in relative densities of *H. anatolicum* are also shown in Figure1. The highest relative density 1.75 was recorded in sheep whereas the lowest relative density 1.21 was recorded in buffaloes.

Table 1. The presence of *H.anatolicum* infested the livestock in Basrah province during the study period.

Local name	Scientific name	Examined ticks	Infested ticks	Collected ticks
cattle	*Bostaurus*	176	61	271
sheep	*Ovisaries*	165	55	288
goats	*Capra aegagrus*	88	28	133
buffalos	*Bubalisbubalus*	71	20	86
total		500	164	778

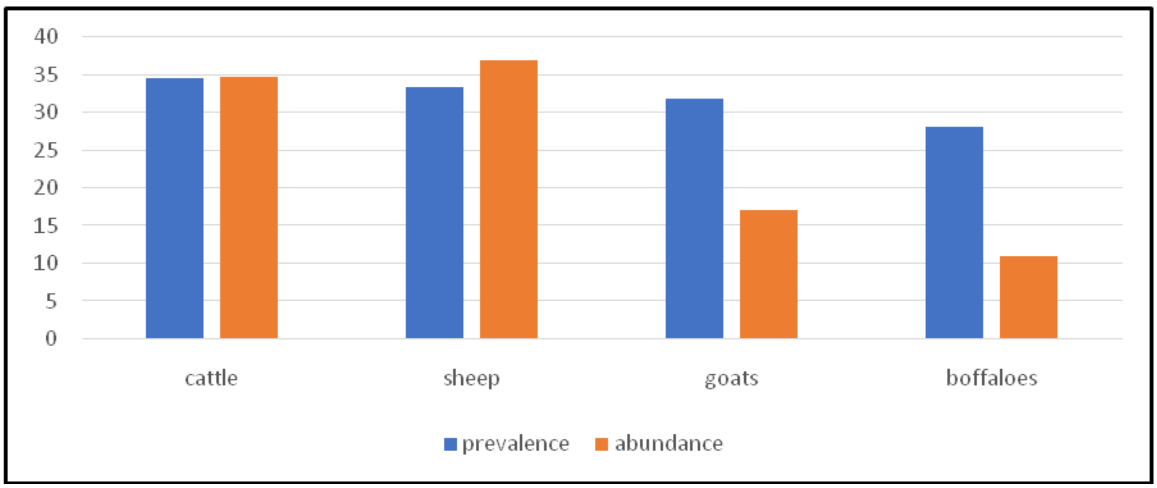

Figure 1: The prevalence and abundance of *H. anatolicum* infested the livestock in Basrah province during the study period.

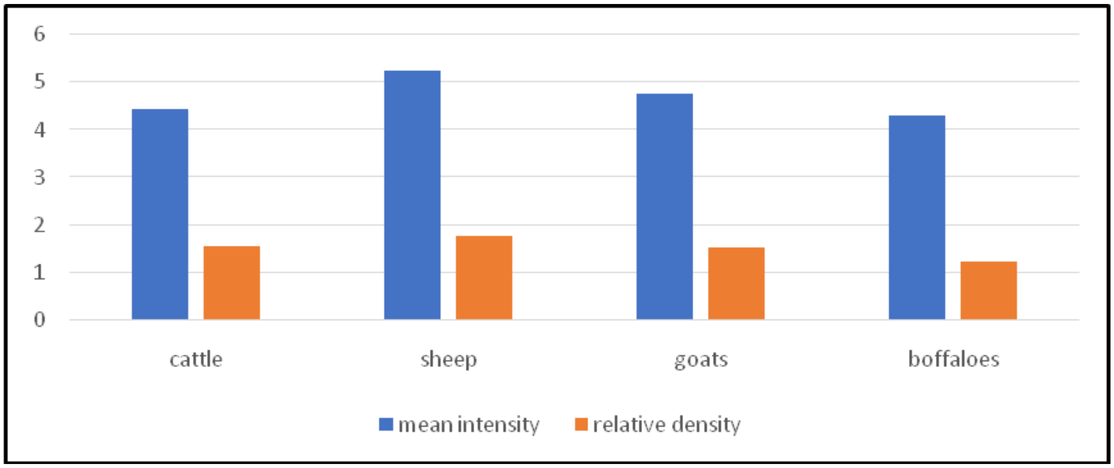

Figure 2: The mean intensity and relative density of *H. anatolicum* infested the livestock in Basrah province during the study period.

The toxic effect of *E. cammadelulensis* oil onthe egg stage of *H. anatolicum*

The results of toxic activity showed high percentages of *H. anatolicum*egg mortality after the treatment with the essential oil of *E. cammadelulensis* compared with the control. Figure 3 shows the results after the immersion test using eucalyptus oil. The mortality percentage of eggs was ranged between from 43.3% in 0.0625% eucalyptus oil concentration to 100% in the concentrations 1% and 0.5% after 24 hours of the immersion test. The mortality percentages of eggs were recorded 100% in all concentrations after 48 hours of the treatment. Some significant differences were recorded between mortality percentages in the concentrations and the time period. The LC50 values were 0.08% after 24 hours, and 0.04% after 48 hours of the exposure.

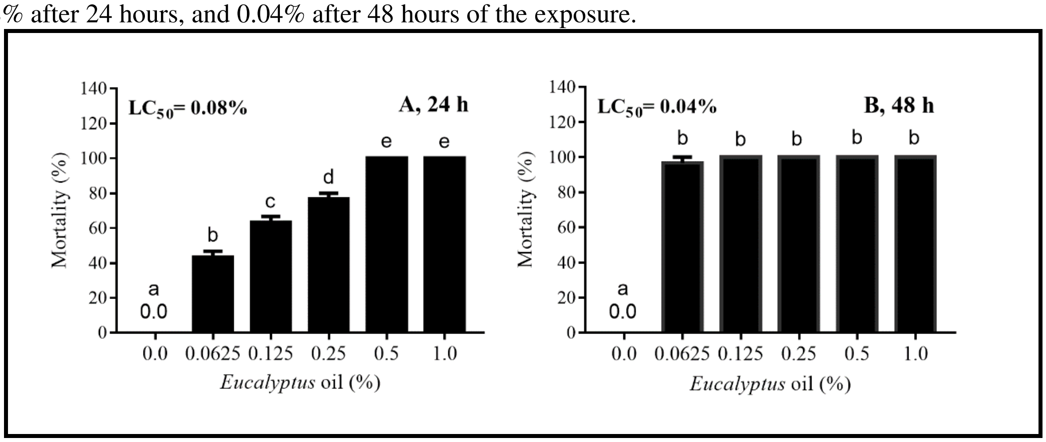

Figure3. The mortality percentage of *H.anatolicum* eggs by treatment with *E.cammadelulensis*oil. The data are the mean ± standard error of the mean (n=3). Significant differences among the treatment concentrations are indicated by different letters ($p \leq 0.5$).

The toxic effect of *E. cammadelulensis*onthe larval stage of*H. anatolicum*

The efficacy of *E.cammadelulensis*oil against *H. anatolicum*larvae was assessed by estimating the percentage of mortality compared with control treatment. Figure4 showsthe mortality percentage caused by the eucalyptus oil varied according to the different concentrations from 26.6% to 83.3% when the unfed larvaetested at concentrations ranging from 0.0625% to 1% after 24 hours of the treatment. However, after 48 hours, the percentages rangedbetween from43.3% to 100% to the same concentrations. The mortality percentages mortalities of fed larvae were higher than unfed with significant differences. The mortality percentageswere ranged between 43.3% in the concentrations 0.0625% and 93.3% in 1% after 24 hours of the immersion test, whereasthey were ranged from 46.6% to100% to the same concentrations after 48 hours of the treatment.The LC_{50} values of unfed larvae were 0.13% after 24 hours, and 0.07% after 48 hours of the exposure. Similarly, the values of LC_{50} of fed larvae were 0.1% and 0.07 after 24 and 48 hours respectively.

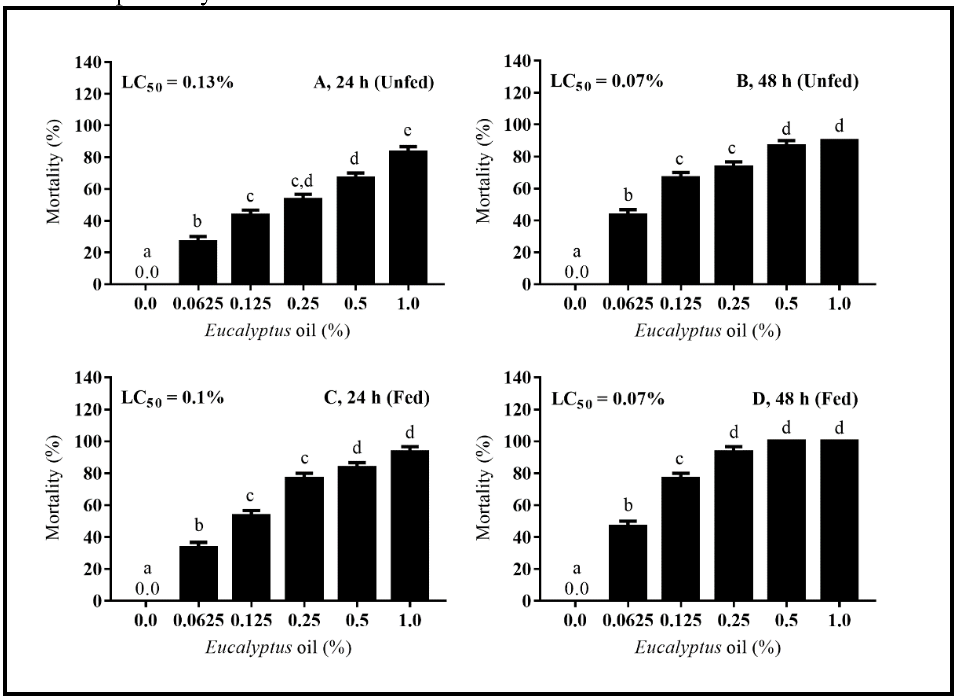

Figure4. The mortality percentage of *H.anatolicum* larvae by treatment with *E. cammadelulensis*oil. The data are the mean ±standard error of the mean (n=3). Significant differences among the treatment concentrations are indicated by different letters (*p*<0.5)

The toxic effect *E. cammadelulensis* oil on the nymph stage of *H. anatolicum*

Figure 5 reveals the mortality percentage of unfed nymphs of *H. anatolicum* were ranged between 16.6% in the concentration 0.0625% and 76.6% at the concentration 1% after 24 hours of the immersion test with *E. cammadelulensis*. After 48 hours of the treatment with various concentrations of the oil, the mortality percentages increased at all concentration and they ranged between 23.3% and 100% in the concentrations 0.0625% and 1%, respectively. The mortality percentages of fed nymphs were higher than unfed nymphs with significant differences. They were ranged between 23.3% in the concentration of 0.0625% to 86.6% at 1% after 24 hours of the immersion test. While they were ranged between from 43.3% to 100% in the same concentrations after 48 hours of the treatment with significant differences between mortality percentages in the concentrations and the time period. The LC50 values for unfed nymphs not varied after 24 and 48 hours of the exposure and recorded 0.16%. Whereas the values of LC50 of fed nymph were 0.13% after 24 hours and 0.09% after 48 hours.

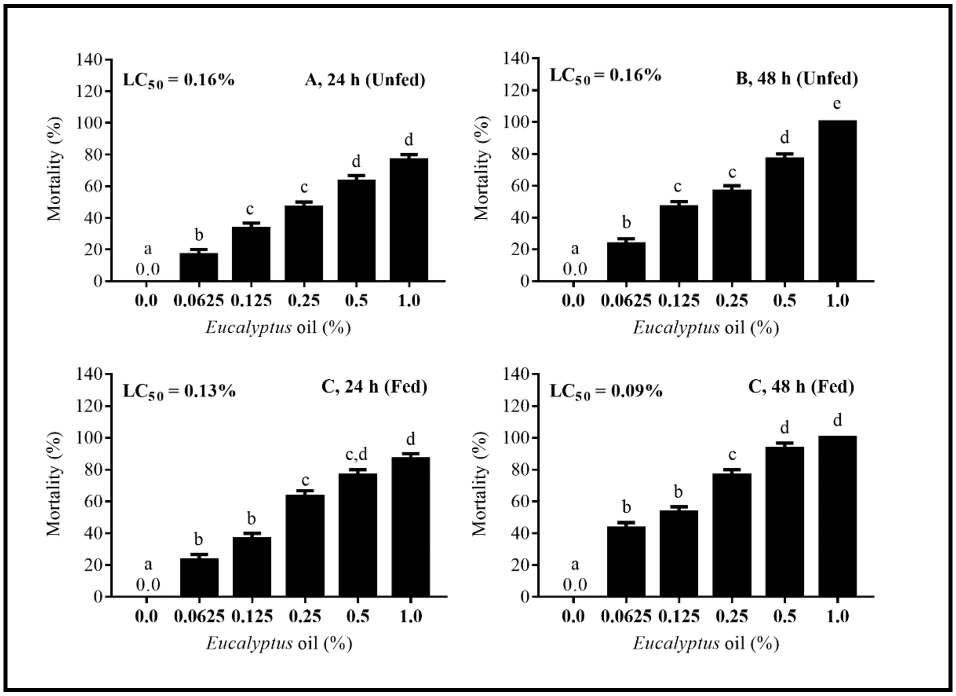

Figure5. The mortality percentage of *H.anatolicum* nymphs by treatment with *E. cammadelulensis*oil. The data are the mean ±standard error of the mean (n=3). Significant differences among the treatment concentrations are indicated by different letters (p<0.5).

The toxic effect of*E. cammadelulensis* oil on the adult males' stage of *H. anatolicum*

The results of *H. anatolicum* adult males' immersion test with the oil of *E. cammadelulensis* were shown in Figure 6. High mortality percentages were observed in unfed males treated with oil compared to the control after exposure 24 hours. These percentages ranged from 13.3% to 76.6 % at the concentrations 0.0625% to 1% with significant differences. The mortality percentages increased significantly in all concentrations after 48 hours of the treatment. They ranged between 23.3% from 93.3%. Mortality percentages in fed adult males recorded were higher than for unfed adult males. They were ranged between 16.6% in the concentration of 0.0625% and 83.3% at 1% after 24 hours of the immersion test. These mortality percentages increased after 48 hours, ranging between from 26.6% to 96.6 at the same concentrations with significant differences. The LC50 values of unfed adult males were 0.2% after 24 hours, and 0.11% after 48 hours of the exposure. While the LC50 values for fed males were 0.17% and 0.1% after 24 hours and 48 hours, respectively.

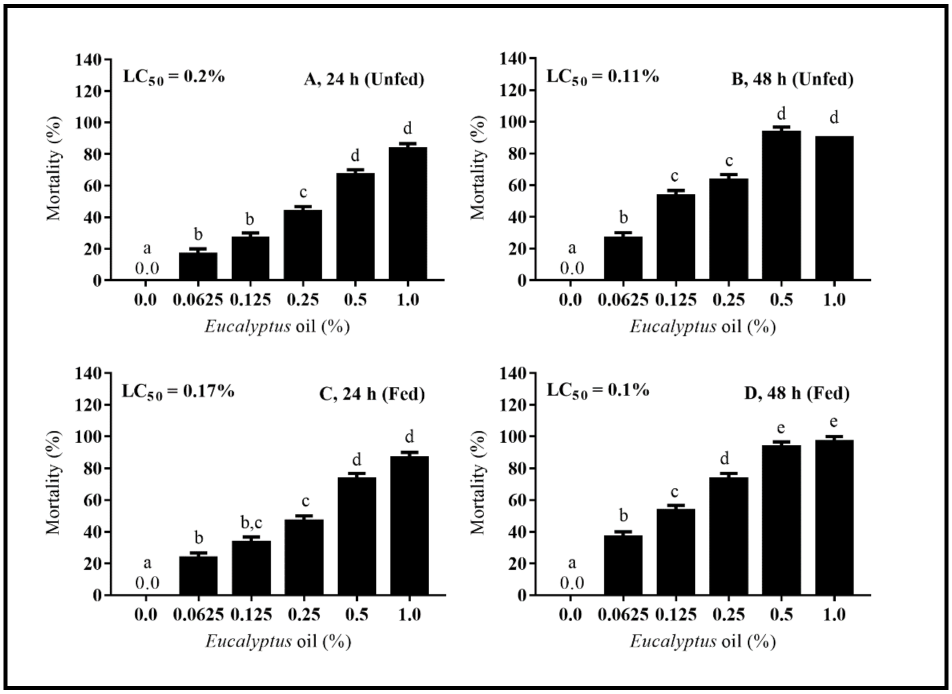

Figure6. The mortality percentage of *H.anatolicum* adult males by treatment with *E. cammadelulensis* oil. The data are the mean ±standard error of the mean (n=3). Significant differences among the treatment concentrations are indicated by different letters (p<0.5).

The toxic effect of*E. cammadelulensis* oil on the adult females' stage of *H. anatolicum*

Figure 7 shows the mortality percentage of unfed adult females of *H. anatolicum* which ranged between 83.3% at the concentration 1% and 16.6% at the concentration 0.0625% after 24 hours of the immersion test with *E. cammadelulensis* oil. The mortality percentage increased at all concentrations after 48 hours of the treatment. They recorded 26.6% and 96.6% in 0.0625% and 1% concentrations of oil, respectively. The mortality percentages of fed adult females (engorged) were higher than unfed with significant differences. These mortality percentages were ranged between 23.3% at the concentration 0.0625% to 86.6% at the concentration 1% after 24 hours of the immersion test. While after 48 hours of exposure, they were ranged between from 36.6% to 100% at the same concentrations with some significant differences. The LC50 values for unfed adult females were 0.16% after 24 hours, and 48 hours of exposure. While the values of LC50 for fed were 0.13% after 24 hours and 0.09% after 48 hours, respectively.

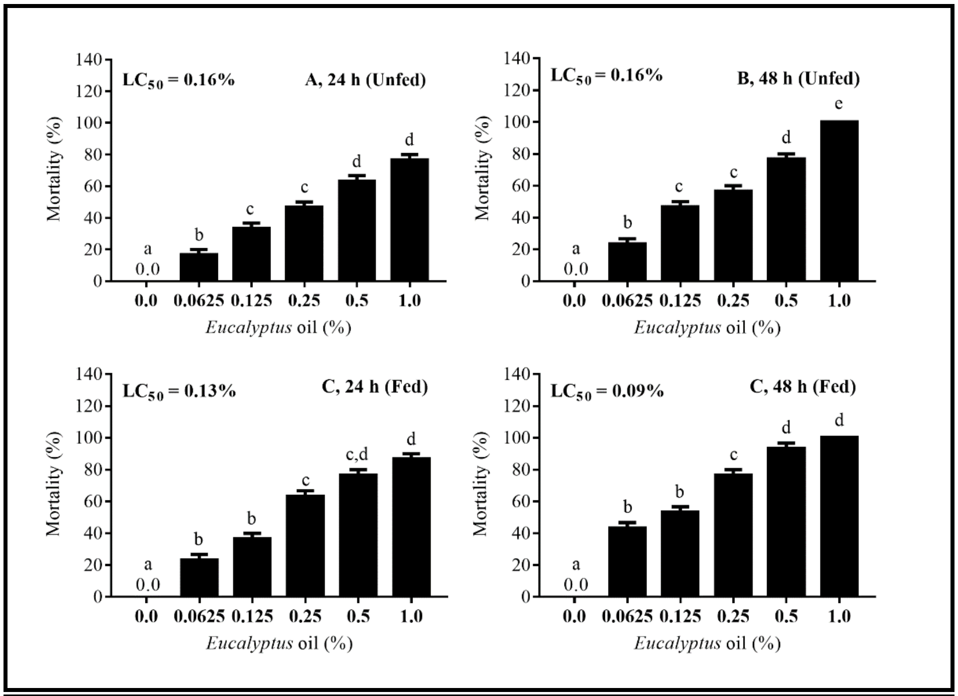

Figure7. The mortality percentage of *H.anatolicum* adult females by treatment with *E. cammadelulensis* oil. The data are the mean ±standard error of the mean (n=3). Significant differences among the treatment concentrations are indicated by different letters (p<0.5).

Discussion

There were few studies about the diversity, biology, and control of ticks in Iraq. The species Hyalommaanatolicum is one of the most common ticks' species in Iraq and the Middle East. The results of the current survey showed that *H. anatolicum* is the most common species of tick in Basrah province and parasitize on most economic mammals, but to varying degrees. Latrofa*et al.,* (2014) showed that *Hyalommaanatolicum* is distributed widely in the Tropical and Afrotropical regions, especially from North Africa to India. Wall and Shearer (2008) reported this species is infested with many domestic and wild mammals in tropical regions, such as cattle, sheep, goats, horses, big, camels. The results of the present study are consistent with some previous studies. Al-Mayah and Hatem (2018) showed *H. anatolicum* is the highest species infested buffaloes. Hatem*et al.,* (2018) recorded *H. anatolicum* infested many wild mammals in the South of Iraq.

In the present study, the essential oil of *Eucalyptuscammadelulensis*revealed good results in the mortality of tick stages.Previous studies have been shown that the monoterpenes 1,8-cineole and pinene are the main components in most *Eucalyptus*species, ranges from 10-90% *(Dellacassa et al.,* 1990). Thus,the high mortalityeffect of *Eucalyptus* oil on ticks may be attributed due to the presence of monoterpenes, in particular 1,8-cineole and pinene, in the structure of the oil. The main components in essential oils from leaves varied between different *Eucalyptus*species,they are monoterpenes and sesquiterpenes(Zrira *et al.*, 2004).

The chemical structure of Eucalyptus was contained a-pinene, b-pinene, a- phellandrene, d-limonene, 1,8-cineole Camphor, and Sabinene (Wang *et al.,* 2014). Essential oils are a complex mixture and they possess higher activities than their individual components (Dellacassa*et al.,* 1990). The total bioactivity of essential oils may be due to the combined effects of many minor components or the synergistic effect of the components (Abdossi et al., 2015). Batish*et al.,* (2008) explained that Eucalyptus oils as a natural pesticide against bacteria, fungi, nematodes, insects, and mites. Kumar *et al.,* (2012) focused on the insecticidal activity of *E. globulus* oil against housefly

Muscadomestica. Benelli and Pavela (2018) showed the use of plant compounds as active ingredients, due to the high effectiveness efficiency of some compounds, with special reference to the essential oils.

The high mortality rates in eggs may be attributed to the penetration of toxic compounds present in the oil to the egg and the killing of the embryo during formation, or they cause hardening of the shell and prevent gas exchange (Abdel-Shafy and Zayed, 2002).For larvae, nymphs and adults, high mortality may be caused by toxic substances that affect in the central nervous system or damage to cellular enzymes(Muro Castrejón *et al.,* 2003).The toxic effect of the extract was higher on fed individuals than unfed (Abdel-Shafy and Zayed, 2002); this may be due to the opening of the dorsal plates and the expansion of the body, thus, toxic compounds penetrate into the body coelom(Ghosh *et al.,* 2015;Al-Shareefi*et al.,* 2019).

In the previous studies of the acaricidal potential of some plants, de FreitasFernandes and Freitas (2007) studied the toxic activity of *Sapindussaponaria* on the larvae of the cattle tick *Boophilusmicroplus*. Abass et al. (2011) showed the effect of volatile oils of some medicinal plants in *Hyalomma sp*. Gazim*et al.,* (2011) referred to the activity of the essential oils from *TetradeniaRiparia* on the cattle tick *Rhipicephalusmicroplus*. Pazinato*et al.,* (2016) studied in vitro the effect of seven essential oils on the reproduction of the cattle tick *R. microplus*. Costa-Júnior*et al.,* (2016) report the acaricidal potential of *Lippiagracilis* oil and its phytochemicals against organophosphate-resistant and susceptible strains of *R. microplus*.

In Conclusion, the species *Hyalommaanatolicum* is the most common ticks' species in Basrah province, and parasitize on most economic mammals. Thus, it may be an important vector for a number of tick-borne diseases. The essential oil extract of *Eucalyptus cammadelulensis* which used in this study showed high potential in killing life cycle stages of ticks. Plant oils can be used in tick control programs as alternative methods for acaricides that cause environmental damage. The tests showed that tick resistance to the control increased with age and decreased exposure period.

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