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Biological control of the Genus Hyalomma (*Hyalomma anatolicum*) by using entomopathogenic indigenous fungi (*Beauveria bassiana*).

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Abstract

A total of 1147 ticks were collected from 360 suspected cattle across three ecological zones to evaluate and compare the efficacy of biological and chemical methods against hard ticks (genus: Hyalomma), which are the highest infesting ticks on cattle in Sulaymaniyah, Iraq. Focused on three developmental stages-larvae, nymphs, and adults-they are tested with both synthetic (chemical) acaricides and entomopathogenic fungus (*Beauveria bassiana*). Biological control trials involved spraying *Beauveria bassiana* spores at three concentrations: 10^6, 10^7, and 10^8 spores/ml. The fungus preparation was applied directly to ticks at different life stages, and mortality rates were monitored. LT_{50} and LT_{90} values were calculated for each combination, showing higher mortality and faster lethal time at higher concentrations. Another test using commercial acaricides was conducted under the same experimental conditions, and the results showed that the chemical treatment at a concentration of 2.5% deltamethrin caused higher initial mortality, while the fungal treatment showed progressive and lasting effects, particularly at the adult stage and at higher spore concentrations. Overall, the study concludes that *Beauveria bassiana* is a promising biocontrol agent that, when properly formulated, can serve as an eco-friendly alternative to chemical acaricides in integrated tick management strategies in livestock systems.

Keywords: Cattle. Hard ticks. *Beauveria bassiana* fungus. Deltamethrin.Lethal time.

I. Introduction

Ticks are tiny, blood-feeding arachnids that parasitise amphibians, reptiles, birds, and mammals. also, members of the Ixodidae family, sometimes referred to as hard ticks **(Malik *et al.*, 2024)**. As blood-sucking ectoparasites of vertebrates, ticks are relatively large acarines; there are two families, Ixodidae and Argasidae, which can cause skin damage in ruminants **(Gashaw and Mersha, 2013)**. The tick life cycle consists of four stages: egg, larva, nymph, and adult, which need various hosts at every step of their life cycle **(Aboagye, 2024)**. live in wooded areas and exhibit "questing" behavior, scaling plants to cling to snagging hosts. Environmental factors, such as temperature and humidity, affect their spread and survival **(Zając *et al.*, 2023).** Ticks threaten all age categories of domestic ruminant groups, while nymphalid and larval stages infest domestic ruminant groups less than adult females and males **(Nasirian, 2022)**. Ticks play an important role in relapsing fever, which is caused by Borrelia *spp*. Also, able to transmit viruses and bacterial pathogens to humans **(Perumalsamy *et al.*, 2024)**. Ticks can resist acaricides through various strategies, including morphological changes resulting from amino acid substitutions, which lead to metabolic detoxification and resistance to acaricides **(Obaid *et al.*, 2022)**. Ivermectin and amitraz are ineffective at controlling ticks, while alpha-cypermethrin is a recognized successful alternative (**Makwarela *et al.*, 2025**). Carbaryl (85%) and diazinon (60%) insecticides were more effective than other acaricides (Mustafa and Faraj, 2013). Tick management primarily relies on the use of synthetic or traditional acaricides, which can lead to environmental and food product contamination, as well as acaricidal-resistant tick populations, in many developing nations **(Bhowmick and Han, 2020).** Entomopathogenic fungus, particularly *Beauveria bassiana*, is a potential biocontrol agent against ticks, providing a sustainable alternative to chemical

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acaricides. Temperature and humidity levels can affect the effectiveness of these fungi, which infect and kill various tick species **(Rajput *et al.*, 2024)**. They reduce the need for chemical acaricides by offering a natural, environmentally acceptable method of tick management **(Wadaan *et al.*, 2023)**. The mortality rate was greater for therapies with increased enzymatic activity. Also, depending on the isolate, there was a transient variation in the enzyme control potential **(Ferreira and Soares, 2023)**.

II. Materials and methods:

Study of the area

This study was conducted in 12 areas of Sulaymaniyah province from the beginning of May 2024 to April 2025 in three zones (zone Sulaymaniyah, zone Garmian, and zone Raparin), in Sulaymaniyah province, which is located in the northern part of Iraq, known as the Kurdistan region, and is neighbored by the countries Turkey, Iran, and Syria. It is the source of animals entering Iraq. The zones are divided into three climates, which are zone I, a moderate climate; zone II, a hot climate; and zone III, which is colder than both.

Cattle examination:

Ticks were isolated and collected from 360 suspected cattle, which included 120 local breed, 120 Friesian breed, and 120 Simmental breed (30 cows at each season from each breed) at three zones: I, II, and III, as in Table 1.

Table 1. The numbers of suspected cattle were examined in three different zones.

Location	Location	No. of cows examined
Zone I	Sulaymaniyah, Penjwen, Halabja, and Saidsadq	120
Zone II	Kalar, Darbandikhan, Kfri, and Xanaqin	120
Zone III	Dukan, Ranya, Qaladza, and pshdar	120
Overall		360

Tick collection and identification:

A total of 1147 ticks were collected from 180 cattle as follows: 307, 494, and 346 ticks in different zones (I, II, III), respectively. They were placed into a clean plastic bottle with dimensions of (3cm x7cm) and covered with a gauze to provide ventilation, also the cover was bound with rubber bands to avoid losing the ticks, and sent to the laboratory for classification by using a dissecting microscope (Dissecting microscope, Moti-Education, China). This classification is described by **Walker *et al.* (2003) and Waldman *et al.* (2023).**

Tick incubation and control:

In vitro engorged females of *Hyalomma anatolicum* were collected in a sterile glass bottle and covered with gauze; some of them were separated to spray the fungal mixture, and mortality was computed in 3,5,7,10, and 14 days. While another engorged female was incubated at 28 ± 1 c°, kept in a desiccator with relative humidity (RH%) 85±1% (using saturated solution of potassium hydroxide (KOH) for control humidity, both humidity and temperature determined by Humidity-Temperature meter, Germany). Observation was made three times daily at morning, afternoon, and night hours until eggs were laid, during oviposition times of (21 ± 1) days after incubation. The eggs hatched into larvae with hatching times (22± 1) days., Larvae have three pairs of legs. They were kept in the incubator under the same conditions of temperature and humidity required for hatching within two weeks. The larvae (pre-feeding period) became sclerotized and hardened. The fungal mixture was spry on some of the larvae inside a petri dish and plastic box, as well as calculating mortality during 3,5,7,10, and 14 days, while some of them were allowed to feed on rabbit blood, which was put inside

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a small plate covered by plastic net to be like skin, after feeding larvae, molts into nymphs, which have four pairs of legs. As the same technique sprays a fugal mixture on nymphs and calculates mortality in 3,5,7,10, and 14 days. All procedures were carried out in the Department of Animal Science, College of Agriculture, Engineering Science, Bakrajo.

Biological control

The collected ticks were introduced into the laboratory, and a biological agent was used against ticks during different life stages, which is known as the Entomopathogenic fungus species (*Beauveria bassiana*). The spore (conidia) of the fungi is available, with concentration of $\mathbf{1}\times10^{10}$ conidia per ml and add this spore with three different volumes of distilled water (1gm spore to 999 ml water, 1gm spore to 99 ml water and 1 gm spore to 9 ml water) which got (10^7, 10^8 and 10^9 spore per ml) also used sunflower oil with emulsifier 1:1 as an adhesive and spreading material, got 20 ml sunflower oil with 20 ml emulsifier. At the end of preparation, 90% of the fungi mixture with 10% of emulsifier and sunflower oil, with the final concentration of the fungi mixture being (10^6, $10^{7,}$ and 10^8) by the following formula: C2=C1V1 / V1+V2. **(Rana *et al.*, 2016).** Once a mixture is ready, the process starts by spry the three different concentrations of the spore to Larvae, Nymphs, and Adults of the Genus: Hyalomma and calibrated after 3, 5, 7, 10, and 14 days, also calculating LT_{50}/ LT_{90}. *Beauveria bassiana* spores were counted in the laboratory within a loading hemocytometer slide by the following formula: (Spore concentration (spores per ml) = Average count per ml × Dilution factor × 10^4). **(Oliveira *et al*., 2015).**

Chemical control:

Also, the same strategy for deltamethrin 2.5% as a chemical compound for controlling, the preparation of the solution was added 1ml of Deltamethrin 2.5% to 1000 ml of distilled water and spry on the ticks during different stages of the life cycle (Larva, Nymph and Adult) inside a petri-dish and computing mortality percentage within different time frames.

Statistical analysis:

For the effect of the biological agent (*Beauveria bassiana*) on controlling the three tick species in terms of mortality, LT_{50}, and LT_{90}, the Chi-Square test of independence is used for testing the infection of associations between the cow's breed and the seasons, and the association between the cow breeds and the zones of the study. (To compare traded groups with the control).

III. Results

Table 2. Show the infestation number of different breeds of cattle from three distinct zones, also illustrating those ticks that most infest the local breed. At the same time, Simmental is intermediate, while Friesian is less. Overall, 50% of the cattle were infected with hard ticks; most infestations occurred in zones 2, 1, and 3, respectively. Moreover, the local breed is more susceptible to infection than other breeds; this may be related to the cow's resistance or acclimation, and the Simmental was moderated in all zones. The results are statistically highly significant at a significance level of 0.01 according to the chi-square over the others

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Table 2. The prevalence of cattle breeds across different zones with hard ticks.

Location	Total exam	Cattle breeds examined in each zone /infested %						Total P (+)	Chi-Square
		Local cattle		Friesian cattle		Simmental cattle			
		No. exam	No. P (+) %	No. exam	No. P (+) %	No. exam	No. P (+) %		
Zone I	120	40	24 (43)	40	14(35)	40	17(42.5)	55(45.8)	41.01**
Zone II	120	40	30(75)	40	18(45)	40	24(60)	72(60)	
Zone III	120	40	22(55)	40	14(35)	40	17(42.5)	53(44.2)	
Over all	360	120	76(63.33)	120	46(38.33)	120	58(48.33)	180(50)	

A total of 1147 ticks were collected from three different zones and different parts of the body. The highest number of hard ticks collected was in zone II, which was 480 ticks. As follows, Zone III had 357 ticks, while the lowest was in Zone I, which had 309. *Hyalomma anatolicum* has the highest rate in all zones. These results are shown in Table 3.

Table 3. Tick species collected according to the side of the body infested.

Types of ticks identified	No. of ticks collected			Total No. of ticks collected/ %	Place of infection
	Zone I	**Zone II**	**Zone III**		
Hyalomma anatolicum,	172	237	183	592(51.6)	Udder, Tail, Neck, Ears, legs.
Hyalomma marginatum	89	176	92	357(31.1)	
Rhipicephalus, Boophilus	48	67	83	198(17.3)	
Overall	309	480	358	1147	

The number of suspected cattle was experimentally examined, which detected that 50% of them were infested, and ticks were collected from infested cattle in three different zones were equivalent to 1147 ticks, with an average of 6.35 ticks per cow, as shown in Table 4.

Table 4. The ratio of ticks collected on the cattle's body.

Location	Cattle breed	Cattle infested %	No. of ticks collected	Ticks/ cow
Zone I	120	55 (45.8)	309	5.61
Zone II	120	72 (60)	480	6.66

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Zone III	120	53 (44.2)	358	6.75
Total	**360**	**180** (50)	**1147**	**~ 6.35**

This result shows the distribution of tick infections in association with the cattle breeds (Local, Friesian, and Simmental) across different seasons in three study Zones. Chi-square value indicates no statistically significant differences in infected prevalence among breeds during seasons in all zones, as shown in Table 5.

Table 5. The prevalence of *Hyalomma anatolicum* infestation in cattle breeds/ different seasons.

Seasons/Breed	Local breed- 120 Cattle	Friesian breed-120 cattle	Simmental breed-/ 120 cattle	Chi-Square
Zone -I				
Spring	9	5	6	0.14 n. s
Summer	9	6	7	
Autumn	6	3	4	
Winter	0	0	0	
Total	**24**	**14**	**17**	
Zone -II				
Spring	10	6	8	0.15 n. s
Summer	10	7	9	
Autumn	8	5	7	
Winter	2	0	0	
Total	**30**	**18**	**24**	
Zone -III				
Spring	8	5	6	0.03 n. s
Summer	9	6	7	
Autumn	5	3	4	
Winter	0	0	0	
Total	**22**	**14**	**17**	

In Table 6. Shows the Effect of *Beauveria bassiana* within three concentrations on larvae of the genus Hyalomma after 14 days of treatment. As shown, the larvae require less time and concentrations because it is smaller and not completely molted. Therefore, it can be controlled by a low dosage of spores, while increasing the dosage of the spores can significantly control the genus Hyalomma. Got non-significant differences in the level of 0.05 and 0.01 when using the chi-square test.

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Table 6. Mortality rate of *Hyalomma anatolicum* larvae, with the ability of different concentrations of *Beauveria bassiana* fungi.

Tick spp.	Conidia/ ml	Mortality % / 14 day	LT_{50}	LT_{90}
Hyalomma	10^6	52.00	9.50	14.00
Hyalomma	10^7	85.00	6.20	10.00
Hyalomma	10^8	97.00	4.20	7.20
LSD 0.05		4.59	0.30	NS
LSD 0.01		6.32	0.41	NS

The Effect of *Beauveria bassiana* within three concentrations on nymphs of the *genus Hyalomma* after 14 days of treatment. Based on this research, nymphs are more resistant to *Beauveria bassiana;* this may be due to the nymphs' resistance or body development. Showed significant differences at the level 0.05, while non-significant differences at the level 0.01 when using the chi-square test. This data is described in Table 7.

Table 7. Mortality rate of the nymphal stage of *Hyalomma anatolicum* by Beauveria bassiana fungi.

Tick spp.	Conidia ml	Mortality%/ 14 days	LT_{50}	LT_{90}
Hyalomma	10^6	50.00	10.00	14.00
Hyalomma	10^7	80.00	7.50	12.00
Hyalomma	10^8	95.00	5.00	9.00
LSD 0.05		3.21	0.31	1.05
LSD 0.01		4.43	0.43	NS

Table 8. Shows the effect of *Beauveria bassiana* within three concentrations on adults of the genus Hyalomma after 14 days of treatment. The result shows that whenever ticks are mature, they increase their tolerance to biological agents or acaricides, indicating significant differences at both levels 0.05 and 0.01.

Table 8. Mortality rate of the adult stage of *Hyalomma anatolicum* by using *Beauveria bassiana* fungi.

Tick spp.	Conidia ml	Mortality %/ 14	LT_{50}	LT_{90}
Hyalomma	10^6	45.00	10.00	14.00
Hyalomma	10^7	75.00	8.50	13.00
Hyalomma	10^8	93.00	6.20	10.50
LSD 0.05		3.00	0.23	0.54
LSD 0.01		4.13	0.32	0.75

Effect of chemical acaricide (Deltamethrin 2.5%) on mortality of the genus: Hyalomma in all life stages (larva, nymph, and adult), which resulted in a non-significant difference in mortality percentage according to Chi-Square. Because the acaricide can control all life stages of the hard tick, while noting the time taken to control three different stages of Hyalomma anatolicum with (1.5, 2.5, and 3) days by using Deltamethrin 2.5% resulted in a significant difference according to the Chi-Square at level 0.01. Notably, the acaricide can control all life stages at different times because

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larvae are smaller and their cuticle is thinner compared to nymphs and adults, or due to tick resistance or acaricide concentration, these results are described in Table 9.

Table 9. Mortality rate on different life stages of Hyalomma *species* by using Deltamethrin 2.5%.

Ixodidae/ Genera	Life Stage/ Mortality rate %/ days						Chi-Square
	Larva		Nymph		Adult		0.01^{s}
	MR%	days	MR%	days	MR%	days	
Hyalomma	90	1.5	80	2.5	72.5	3	

MR: Mortality rate

IV. Discussion:

Isolated 360 suspected cattle were experimented in all zones, the infestation rate in zones were 180 (50%), also, prevalence rate of infestation in zone 1 was 55 (45.83%), zone 2 was 72 (60%), and zone 3 was 53 (44.17%), the percentage of cattle infected by ticks in the province of Thi-Qar from January 2002 to December 2004 was 48.2% **(Tuama *et al.*, 2007)**. Also, the infestation rate in the local breed was higher than in other breeds, with the local breed having 76 (63.33%), while the Friesian recorded the lowest, 46 (38.33%), and Simmental had 58 (48.33%). A high rate of Hyalomma *spp* was recorded in Duhok, 88.6%, while the lowest rate was 49.94% in Nineveh **(Al-Zubaidei *et al.*, 2023)**. Infestation wasn't detected from November to February because of cold weather; however high in March, April, and May. The prevalence rate was higher in March, April, May, and July, while from November to February, no infestation was observed in three zones in Sulaymaniyah governorate, Iraq **(Kadir *et al.*, 2012)**. Seasonal variations were observed in ticks, specifically, *Hyalomma anatolicum*, was appeared in cold regions. Also, seasons affect ticks' in vitro feeding **(González *et al.*, 2017).** In this study, four species of ticks were detected: *Hyalomma anatolicum*, *Hyalomma marginatum*, *Rhipicephalus*, and *Boophilus*. Five species of hard ticks were observed to be members of the genera Hyalomma and Rhipicephalus **(Shanan *et al.*, 2017).** *Hyalomma anatolicum*, *H. marginatum*, and *Rhipicephalus* were detected in the Sulaymaniyah governorate, Iraq **(Kadir *et al.*, 2012)**. The first most common genus of hard tick is Hyalomma, while the second is Rhipicephalus in Iraq **(Al-Zubaidei *et al.*, 2023)**. Genus Boophilus was observed in October in zone 2, which is hot weather in a rare case. Identified all tick species that morphologically depend on (**Walker *et al*., 2003). Waldman *et al.* (2023).** A total of 1147 ticks were collected from 180 (50%) cattle in all zones. 947 cattle were observed that were infected with one or more tick species, which equals 47.51%, the cattle infestation rate was 47% while sheep was 46.9% in the middle and south of Iraq **(Shubber *et al*., 2014).** The infestation occurred in different parts of the body, like the neck, leg, udder, tail, and ears. Most infestation was found in the tail 14 (29.17%) in zone one and 13 (27.08%) in zone three; while infestations occurred in the neck 15.5 (26.72%) and the tail 13.5 (23.28%) in zone two. Engorged ticks were collected to apply both biological agent and chemical acaricide, while some of engorged ticks were reared an incubated invitro in the temperature of 27 and relative humidity (RH 85%) to lay eggs and hatch larva then molts to nymph and adult, ticks was collected and incubated at 27±1.6C° with relative humidity (RH 85 ± 1.4%) **(Mustafa, 2012).** After having all life stages of ticks, the fungi mixture and emulsifier with sunflower oil were applied to larvae, nymphs, and adults with three concentrations: 10^{6}, 10^{7}, and 10^{8}. The usage of an emulsifier with sunflower oil in the adhesive and spreading conidia on the tick's cuticle. The study results illustrated that the first treatment can control al life stages of genus: Hyalomma but not defectively while by increasing the concentration of spore per ml from 10^{6} to 10^{7} can control ticks better, however, the last treatment is picked the top at controlling all of larva, nymph and adult in less time frame compare to other treats with fewer side effects and negative feedback. At a concentration of 10^{9} conidia/ml, Beauveria bassiana B. bAT17 caused lethal time LT50 and LT90 of 7.14 and 9.33 days, respectively, against engorged *R. (B.) microplus* females **(Sun *et al*. 2013)**. Using *Beauveria bassiana* at 10^{12} conidia/ml, computed 100%

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efficacy **(Marzouk and Ali, 2023).** There is no effect of the Iranian strain *Beauveria bassiana* 5791, which is used to control *Hyalomma anatolicum anatolicum* and *Hyalomma marginatum* by the dipping technique, while *Beauveria bassiana* Evin shows acute phase signs of paralysis in the test group after dipping ticks in suspension made from the Evin strain of *B. bassiana*. In addition, the test groups were totally died out after four months, but the control groups survived for six months **(Abdigoudarzi *et al.*, 2009)**. On the other side, control by chemical acaricide (deltamethrin 2.5%) gives a very quick response in controlling all life stages of Hyalomma just in 1-4 days; also, the use of lambada-cyhalothrin is effective and broad-spectrum in controlling 3 species of hard ticks **(Lu *et al.*, 2015).** At 7 days post treatment, Diazinon showed 100% effectiveness, and the efficacy of deltamethrin was increased gradually until controlling at 3^{rd} d.p.t **(El-Bahy *et al.*, 2015).** A good suggested option dosage of acaricide cypermethrin was (10%), and ivermectin was (1%) **(Mustafa and Faraj, 2013).** While the biological agent control within 10^8 conidia/ ml, effectiveness also increases with increasing dosages.

V. Conclusion:

Based on our scientific results of this study, *Beauveria bassiana* causes significant mortality in all tested ticks, especially the Hyalomma species. Adults were the most resistant, while Larvae and Nymphs generally showed higher mortality and lower LT_{50}/ LT_{90} values. Spore concentrations significantly affect mortality rates by increasing conidia/ml; also, oil and emulsifier improve efficacy. As shown in the results high concentration of spores is required to control ticks, which does not make ticks resistant or environmental pollution, also no side effects on the meat and dairy products to be consumed directly after treatment. Species-specific and stage-specific strategies are needed for effective control.

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