UNIVERSITY OF SINDH JOURNAL OF ANIMAL SCIENCES
Vol. 3, Issue 4, Pp: (15-27), December, 2019
Email: editor .usjas@usindh.edu.pk
Website: http://usindh.edu.pk/index.php/USJAS
ISSN (P): 2521-8328
ISSN (E): 2523-6067
Published by University of Sindh, Jamshoro
(USJAS)

IN VITRO EVALUATION OF GINGER STEM EXTRACT AS REPELLENT AGAINST *HYALOMMA ANATOLICUM* TICK

Saleem Raza Solangi, Abdullah Arijo, Nasreen Akhtar, Bachal Bhutto

Department of Parasitology, Sindh Agriculture University, Tandojam

ARTICLE INFORMATION

Article History:
Received: 10 October 2019
Accepted: 30th July 2020
Published online: 1st September 2020

Author's contribution
SRS designed experimentation, AA edit data for final approval NA & BB analysis and complied results.

Key words:
In vitro, efficacy Ginger Stem Extract, *Hyalomma anatolicum*

ABSTRACT

The study was carried out during the year 2013-14 to evaluate the *in vitro* efficacy of Ginger Stem Extract as Repellent Against *Hyalomma anatolicum* Tick. For this purpose *Hyalomma anatolicum* engorged ticks were collected from cattle and buffalo at Livestock Experimental Farm, Sindh Agriculture University, Tandojam. The results shows that in experiment-1; *in vitro* repellency of ginger against *Hyalomma anatolicum* shows average 100%, 80%, 70% and 60% reppelency, in experiment-2; *in vivo* repellency of 90%, 70%, 60% and 50%, in experiment-3; *in vivo* repellency of 90%, 80%, 60% and 40%, in experiment-4; *in vivo* repellency of 80%, 70%, 60% and 40%, in experiment-5; *in vivo* repellency of 90%, 80%, 60% and 40% at 5%, 2.5%, 1% and 0.5% concentration of ginger extract. Overall, repellency was 90%, 76%, 62% and 46% under invitro conditions at 5%, 2.5%, 1% and 0.5% concentration of ginger extract. In experiment-1; *in vivo* efficacy of ginger against *Hyalomma anatolicum* shows average 90%, 90%, 90% and 60% mortality of ticks examined at 5%, 2.5%, 1% and 0.5% concentrations. In experiment-2;100%, 100%, 100% and 50% mortality of ticks under *in vitro* condition at 5%, 2.5%, 1% and 0.5% concentration. In experiment-3; average 70%, 60%, 80% and 40% mortality of ticks under *in vitro* condition at 5%, 2.5%, 1% and 0.5% concentration. In experiment-4; overall, 80%, 70%, 40% and 30% mortality of ticks under *in vitro* condition at 5%, 2.5%, 1% and 0.5% concentration. In experiment-5; overall, 90%, 70%, 50% and 60% mortality of ticks under *in vitro* condition at 5%, 2.5%, 1% and 0.5% concentration. Overall, 88%, 74%, 60% and 50% mortality of ticks under *in vitro* condition at 5%, 2.5%, 1% and 0.5% concentration. Zero mortality was seen in control group in all experiments. The calculations of LC 50 and LC 90 of ginger stem extract on *Hyallomma anatolicum* ticks shows that 33.75% ticks were died at lethal dose of 5% concentration and 32.25 % ticks were died at 2.5% concentration of ginger stem extract.From the present study it was concluded that *in vitro* efficacy of ginger extract against *Hyalomma anatolicum* ticks proved satisfactory results.

1. INTRODUCTION

Ticks are important ectoparasites of domestic animals and affect 800 million cattle and sheep around the world. They affect their hosts by damaging their hides and skins, reducing their growth rates and milk production and transmitting disease organisms (Sutherest and Wilson, 1986). Bites from ticks cause skin damage, and feeding adults predispose livestock to dermatophilosis (Hall, 1982). Ticks have been associated with wide variety of pathogenic organisms affecting both animals and human i.e. anaplasmosis,

Corresponding Author: abdullaharijo@gmail.com

brucellosis, anthrax and trypanosomiasis (Burgdorfer *et al.*, 1973).

Ticks cause great economic losses to livestock, and adversely affect livestock hosts in several ways. Loss of blood is a direct effect of ticks acting as potential vector for haemo-protozoa and helminth parasites. Blood sucking by large number of ticks causes reduction in live weight and anemia among domestic animals, while their bites also reduce the quality of hides (Khan, 2005). However, major losses caused by ticks are due to their ability to transmit protozoan, rickettsial and viral diseases of livestock, which are of great economic importance world-wide (Wilson and Bram, 2003). Botanical insecticides from plant based products have proved to be more effective and economical for the control of ticks as compared to synthetics (Chen and Xiao, 2007).

Common cattle ticks of genus Ixodidae are vectors of animal disease and can cause decreased animal productivity, especially in developing countries where control measures are not carried out regularly (Kilonzo, 1986). *Hyalomma* tick species are widespread and of great importance in various tropical and sub-tropical countries of Africa (Berkvens *et al.*, 1998). (Khan, 2005), Europe (Milutinovic *et al.*, 1997). In Pakistan, prevalence of *Hyalomma* species is significantly higher than *Haemaphysalis, Amblyomma* and *Boophlis* genera of hard ticks (Ali *et al.*, 2009).

Hyalomma anatolicum a three-host tick causes a major problem to veterinary health in various countries of the world as debilitating parasite or as a vector of a great variety of diseases or both (Kettle, 1995). It also causes a great problem to the dairy industry and beef industry inducing decrease in weight gain and milk production (Kettle, 1995).

There have been attempts to control ticks; however chemical control is reported to provoke many problems such as environmental pollution, development of resistant tick and more expensive costs (Dipeolu and Ndungu, 1991). The chemical preparations contain compounds like arsenicals, chlorinated hydrocarbons, amitraz and pyrethroids. Acaricide usage poses a health risk; there have been reports of increased residues in meat and the development of resistance (Natal *et al.*, 2005). In view of the above problems, there has been an increasing interest in searching for alternative sustainable control methods of tick control in recent years. These include biological control by means of pathogens and use of safe plant extracts (Kaaya *et al.*, 1995).

Ginger has been used by traditional Chinese and Indian medicine for over 25 centuries. Ginger was brought to Mexico by the Spaniards and later introduced to Jamaica, which is currently one of the world's foremost producers of this species. Ginger is used in Mexican traditional medicine, mainly for gastro intestinal complaints. In recent times, Ginger has been introduced into various tropical countries where diverse chemotypes have been developed. Many medicinal qualities found in the herb, Ginger (Ody, 2000).

Ginger has been written up in the many archives of the medicinal uses from the traditional Chinese and Ayurvedic Indian systems. Both systems clearly viewed Ginger as a healing gift from God. According to one of the most ancient Chinese pharmacopoeias, long-term usage of fresh ginger would put a person in contact with the spiritual effulgences. In ancient India Ginger was given the name vishwabhesaj, the universal medicine, and was viewed as an essential element in a majority of formulations (Sakai, 1988).

Ginger's principal enzyme action is called zingibain and one gram of zingibain can actually tenderize as much as twenty pounds of meat. Improved digestion would therefore be the most obvious impact. Besides improving digestion, Ginger's principal enzyme action also undoubtedly contributes to its combination of antibacterial, anthelmintic, and anti-inflammatory observed effects. Numerous studies have shown that, enzymes like zingibain can enhance the effectiveness of other antibacterial elements such as antibiotics by as much as 50 percent. To help eliminate parasites, an enzyme like zingibain can also aid the immune system by potentially digestion the parasite and its eggs (Baskanchiladze *et al.*, 1984).

The efficacy of ginger is attributed to its aromatic, carminative and absorbent properties. The medicinal properties of ginger include anti-arthritic, anti-migraine and hypo-cholesterolaemic, anti-thrombotic, anti-inflammatory, hypo-lipidaemic, hypo-cholesterolaemic, anti-nausea properties, anti-diabetic, antipyretic, antimicrobial, anti-schistosomal, antioxidant, hepato-protective, diuretic, hypotensive and gastrointestinal prokinetic activities (Govindarajan, 1982).

Many methods are currently being applied for the control of ticks, however, use of ethno-veterinary products are encouraged as they are cheap and safe. Ginger is known to have repellant property; that is why this study was proposed to check the repellent property of Ginger against *Hyalomma* ticks.

Limited research has been carried out on the search for natural repellent product. Zinger, *Zingiber officinale* (Zingiberaceae) is found to be repellent towards ticks *Hyaloma anatolicum*, *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Prajapati *et al.*, 2005).

2. MATERIALS AND METHODS

The study was carried out during the year 2013-14 to evaluate the *in vitro* and *in vivo* efficacy of Ginger Stem Extract as Repellent Against *Hyalomma anatolicum* Tick. The details regarding the study are given as under.

Collection and rearing of ticks

Hyalomma anatolicum engorged ticks were collected from cattle and buffalo at Livestock Experimental Station, Sindh Agriculture University, Tandojam by applying the technique of Durrani and Shakoori (2009) with some modifications. Ticks were reared on the host (rabbits). The rabbits were kept in small cage and provided with food and water. The cages were placed in Animal House of Sindh Agriculture University Tandojam.

Plate-1. *Hyalomma anatolicum* ticks identification

Tick Rearing

1. Total 30 male and equal number of females (nymph and adults) ticks (*Hyalomma anatolicum*) were collected from cattle and buffalo at Livestock Experimental Farm of Sindh Agriculture University Tandojam.
2. These ticks were brought at Animal House of Sindh Agriculture University Tandojam.
3. Ear of the Albino rabbit was shaved with electric shearer.
4. Ticks were attached on the ear of the rabbit.
5. Ear bags were fixed with bandage tightly that ticks should not skip away.
6. Neck collar was fixed on the neck of rabbit, in such a way that it could get feed and water easily.
7. The rabbit was transferred to cage.
8. Infested rabbit was kept under observation regularly.
9. Ticks were allowed to engorge.
10. Laid eggs were allowed to hatch and emerging nymph were fed on rabbits to keep the cycle going.

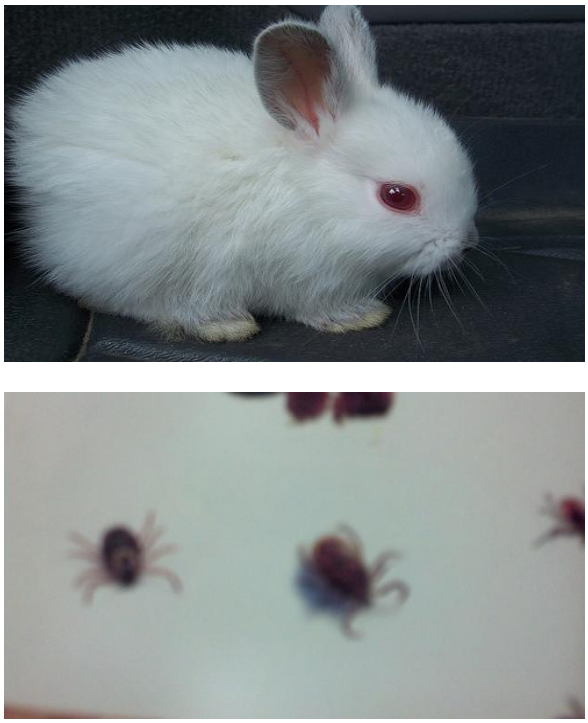

Plate-2. Rearing of *Hyalomma anatolicum* ticks in rabbit

Preparation of Ginger Stem Extract

1. Ginger stem was dried at room temperature for one week.
2. After drying, stem grounded in Pestel Marter.
3. Then sample was transferred to evaporator dish.
4. Total 50 grams of sample were kept in hot air oven at 105^0C for one hour for drying.
5. After drying the sample was placed in Desicator for half an hour (30 minutes) for cooling.
6. Again sample was placed in hot air oven at 105^0C for 30 minutes, then transferred to desicator for 30 minutes.
7. After cooling 5 grams of sample was placed on 2 Whatman filter papers. In each filter paper, a total of 10 grams of sample was used.
8. Filter papers containing sample were placed in Thimbles for extraction.
9. Thimbles were plugged with cotton.

10. Thimbles were placed in extractors under the condenser of the extraction apparatus.
11. Petrolum ether, 175 ml (90%) was placed in each solvent flask / round flask and was connected to the Soxhlet apparatus.
12. Heaters were turned on and water was allowed to run.
13. Extraction was performed at the rate of condensation at 3-4 drops / second.
14. After 8 hours, obtained ginger stem extract was left for 30 minutes for cooling.
15. Finally the extract was stored at 4^0C until used.

Acaricidal effect of ginger extract on ticks

Adult ticks were placed in petri dishes in four groups (A, B, C and D) with 10 ticks in each group. The bottom of Petri dishes in group A, B, C and D Petri dishes was covered with filter paper soaked in concentrations of ginger extract. Total stock solution was 600ml and doses divided into following four concentrations (0.5%, 2.5%, 1% and 0.5%). Group E served as control. Ticks were kept at various concentrations in Petri dishes for 24 hours. The mortality rate was checked with 8 hours interval. For result interpretation, adult and nymph ticks were observed for reduction in activity to outright death over a period of time.

Plate 3. *In vitro* applying ginger stem extract

Statistical analysis

The collected data were analyzed and tabulated by using MS-Stat-C Statistical Package (Gomez and Gomez, 1984). The LC50 and LC90 values were calculated using probit analysis as per standard formula given below:-

The percentage dead for 0 and 100 are corrected before the determination of probits as under:

Corrected % Formula for 0 and 100% mortality

For 0% dead: 100(0.25/n)

For 100% dead: 100(n-0.25/n)

The probit values were plotted against log-doses and then the dose corresponding to probit 5, i.e., 50%, were found out. In the present case the Log LD50/90 is 1.76 and LD50/90= 57.54 mg/kg. Calculation of Standard Error (SE) of LD50/90. The SE of LD50/90 was calculated from the following formula:

$$\text{Approx. } \underline{\text{SE of LD50= (Log LD84-Log LD16)}}{2N} \quad \dots (a)$$

Where N is number of animals in each group.

3. RESULTS

In vivo study was carried out to investigate the repellency effects of ginger extract on *Hyalomma anatolicum* ticks. The efficacy of ginger stem extracts at the concentration of 0.5%, 2.5%, 1% and 0.5% in groups A, B, C and D was evaluated against *Hyalomma anatolicum* and group E was kept as control. Moreover, LC 50 and LC 90 of ginger extract on adult and nymph tick was also calculated. The mortality of ticks was examined after 8, 16 and 24 hours of ginger extract application. The results covering all above aspects for ticks mortality are present in Table 1 to 10.

Repellency of ginger stems extract against *Hyalomma anatolicum* ticks (Exp-1)

The *in vitro* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks (Table-1) indicated that the repellency effect of 5% and 2.5% ginger extract was remarkable causing 30%, 30%, 40% and 20%, 30% and 30% ticks repellency when examined after 8, 16 and 24 hours of acaricide application. The repellency of ginger extract at 1% concentration causing 20%, 20% and 30% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 10%, 20% and 30% repellency of ticks were recorded after 8, 16 and 24 hours. No repellency was seen in control group. On average 100%, 80%, 70% and 60% repellency of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks. It was observed that *in vitro* repellency of ginger extract at 5% and 2.5% concentrations shows better repellency against *Hyalomma anatolicum* ticks.

Repellency of ginger stems extract against *Hyalomma anatolicum* ticks (Exp-2)

The *in vitro* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks (Table-2) indicated that the repellency effect of 5% and 2.5% ginger extract was remarkable causing 20%, 30%, 40% and 20%, 20% and 30% ticks repellency when examined after 8, 16 and 24 hours of acaricide

application. The repellency of ginger extract at 1% concentration causing 10%, 20% and 30% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 0%, 20% and 30% repellency of ticks were recorded after 8, 16 and 24 hours. No repellency was seen in control group. On average 90%, 70%, 60% and 50% repellency of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks. It was observed that *in vitro* repellency of ginger extract at 5% and 2.5% concentrations shows better repellency against *Hyalomma anatolicum* ticks.

Repellency of ginger stems extract against *Hyalomma anatolicum* ticks (Exp-3)

The *in vitro* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks (Table-3) indicated that the repellency effect of 5% and 2.5% ginger extract was remarkable causing 30%, 30%, 30% and 20%, 20% and 40% ticks repellency when examined after 8, 16 and 24 hours of acaricide application. The repellency of ginger extract at 1% concentration causing 10%, 20% and 30% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 0%, 20% and 20% repellency of ticks were recorded after 8, 16 and 24 hours. No repellency was seen in control group. On average 90%, 80%, 60% and 40% repellency of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks. It was observed that *in vitro* repellency of ginger extract at 5% and 2.5% concentrations shows better repellency against *Hyalomma anatolicum* ticks.

Repellency of ginger stems extract against *Hyalomma anatolicum* ticks (Exp-4)

The *in vitro* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks (Table-4) indicated that the repellency effect of 5% and 2.5% ginger extract was remarkable causing 20%, 20%, 40% and 20%, 20% and 30% ticks repellency when examined after 8, 16 and 24 hours of acaricide application. The repellency of ginger extract at 1% concentration causing 10%, 20% and 30% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 10%, 10% and 20% repellency of ticks were recorded after 8, 16 and 24 hours. No repellency was seen in control group. On average 80%, 70%, 60% and 40% repellency of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks. It was observed that *in vitro* repellency of ginger extract at 5% and 2.5% concentrations shows better repellency against *Hyalomma anatolicum* ticks.

Repellency of ginger stems extract against *Hyalomma anatolicum* ticks (Exp-5)

The *in vitro* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks (Table-5) indicated that the repellency effect of 5% and 2.5% ginger extract was remarkable causing 20%, 30%, 40% and 20%, 30% and 30% ticks repellency when examined after 8, 16 and 24 hours of acaricide application. The repellency of ginger extract at 1% concentration causing 10%, 20% and 30% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 0%, 10% and 30% repellency of ticks were recorded after 8, 16 and 24 hours. No repellency was seen in control group. On average 90%, 80%, 60% and 40% repellency of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks. It was observed that *in vitro* repellency of ginger extract at 5% and 2.5% concentrations shows better repellency against *Hyalomma anatolicum* ticks.

Overall comparison of *in vitro* repellency of ginger stems extract against *Hyalomma anatolicum* ticks in all experiments

The *in vitro* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks are presented in Table-6, which indicated that the acaricidal effect of 5% and 2.5% ginger extract was remarkable causing 24%, 28%, 38% and 20%, 24%, 32% tick repellent when examined after 8, 16 and 24 hours of acaricide application respectively. The efficacy of ginger extract at 1% concentration causing 12%, 20% and 30% tick repellent when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 4%, 16% and 26% repellency of ticks were recorded after 8, 16 and 24 hours. No repellency was seen in control group. Overall, 90%, 76%, 31% and 46% repellency of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vitro* conditions.

Mortality of ginger stems extract against *Hyalomma anatolicum* ticks (Exp-1)

The *in vitro* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks are presented in Table-7, which indicated that the acaricidal effect of 0.5% and 2.5% ginger extract was remarkable causing 20%, 20%, 50% and 20%, 20% and 40% tick mortality when examined after 8, 16 and 24 hours of acaricide application. The efficacy of ginger extract at 1% concentration causing 20%, 20%

and 30% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 0%, 20% and 40% mortality of ticks were recorded after 8, 16 and 24 hours. No mortality was seen in control group. On average 90%, 80%, 70% and 60% mortality of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vitro* conditions. It was observed that *in vitro* efficacy of ginger extract at 5% and 2.5% concentrations shows better repellency against *Hyalomma anatolicum* ticks.

Mortality of ginger stems extract against *Hyalomma anatolicum* ticks (Exp-2)

The *in vitro* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks are presented in Table-8, which indicated that the acaricidal effect of 5%, 2.5% and 1% ginger extract was remarkable causing 20%, 30%, 40% and 10%, 20%, 40% tick mortality when examined after 8, 16 and 24 hours of acaricide application respectively. The efficacy of ginger extract at 0.5% concentration causing 10%, 20% and 30% tick mortality when examined after 8, 16 and 24 hours. No mortality was seen in control group. On average 90%, 70%, 60% and 50% mortality of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vitro* conditions. It was observed that *in vitro* efficacy of ginger extract at 5%, 2.5% and 1% level showed significantly better repellency against *Hyalomma anatolicum* ticks.

Mortality of ginger stems extract against *Hyalomma anatolicum* ticks (Exp-3)

The*in vitro* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks are presented inn Table-9, which indicated that the acaricidal effect of 5% and 1% ginger extract was remarkable causing 30%, 20%, 40% and 20%, 20%, 40% tick mortality when examined after 8, 16 and 24 hours of acaricide application respectively. The efficacy of ginger extract at 2.5%concentration causing 30%, 10% and 30% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5%concentration of ginger extract 20%, 20% and 30% mortality of ticks were recorded after 8, 16 and 24 hours. No mortality was seen in control group. On average 90%, 80%, 70% and 60% mortality of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vitro* conditions. It was observed that *in vitro* efficacy of ginger extract at 5% and 2.5% concentrations shows significant better repellency against *Hyalomma anatolicum* ticks.

Mortality of ginger stems extract against *Hyalomma anatolicum* ticks (Exp-4)

The *in vitro* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks are presented inn Table-10, which indicated that the acaricidal effect of 5% and 2.5% ginger extract was remarkable causing 20%, 20%, 40% and 20%, 20%, 30% tick mortality when examined after 8, 16 and 24 hours of acaricide application respectively. The efficacy of ginger extract at 1%concentration causing 10%, 10% and 20% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 00%, 10% and 20% mortality of ticks were recorded after 8, 16 and 24 hours. No mortality was seen in control group. Overall, 80%, 70%, 40% and 30% mortality of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vitro* conditions. It was observed that *in vitro* efficacy of ginger extract at 5% and 2.5% concentrations proved excellent repellency effects against *Hyalomma anatolicum* ticks.

Mortality of ginger stems extract against *Hyalomma anatolicum* ticks (Exp-5)

The *in vitro* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks are presented inn Table-6, which indicated that the acaricidal effect of 5% and 2.5% ginger extract was remarkable causing 20%, 30%, 40% and 20%, 20%, 30% tick mortality when examined after 8, 16 and 24 hours of acaricide application respectively. The efficacy of ginger extract at 1% concentration causing 10%, 20% and 30% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 10%, 20% and 20% mortality of ticks were recorded after 8, 16 and 24 hours. No mortality was seen in control group. Overall, 90%, 70%, 60% and 50% mortality of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vitro* conditions. It was observed that *in vitro* efficacy of ginger extract at 5% and 2.5% concentrations show exceptional repellency effects against *Hyalomma anatolicum* ticks.

Overall comparison of *in vitro* efficacy of ginger stems extract against *Hyalomma anatolicum* ticks in all experiments

The *in vitro* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks are presented in Table-12, which indicated that the acaricidal effect of 5% and 2.5% ginger extract was remarkable causing 22%, 24%, 42% and 18%, 20%, 36% tick mortality when examined after 8, 16 and 24 hours of acaricide application respectively. The

efficacy of ginger extract at 1% concentration causing 16%, 16% and 28% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 06%, 18% and 26% mortality of ticks were recorded after 8, 16 and 24 hours. No mortality was seen in control group. Overall, 88%, 74%, 60% and 50% mortality of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vitro* conditions. The calculations of LC 50 and LC 90 of ginger stem extract on *Hyallomma anatolicum* ticks shows that 33.75% ticks died at lethal dose of 5% concentration and 32.25 % ticks died at 2.5% concentration of ginger stem extract. This shows that remarkable decrease in populations from each subsequent period after treatment by probit analysis and significant increases in mortality occurred from one lower concentration to the next higher concentrations. There was considerable increase in the LC 90 when concentration of 5% ginger stem extract concentration was compared to lower concentrations while serial concentrations of ginger stem extract were applied knock down concentration (LC 50) was obtained.

4. DISCUSSION

The use of natural products mainly the botanical acaricides for the control of ticks has been the focus of research in many countries, principally to withstand the noticeable increasing frequency of acaricides resistant-tick strains. The spread of such tick population shall spoil the imperative efforts of improving livestock and animal intensive industry due to impact of ticks and tick-borne diseases. Acaricides resistance has been documented in the country as a result of continuous uses or abuses of acaricides. This situation has encouraged efforts that should be undertaken to address the emergence of acaricides resistant-ticks of veterinary importance. Hence the present study was conducted to test the efficacy of ginger stem extract as repellent against *Hyalomma anatolicum* infesting cattle and buffalo.

Many methods are currently being applied for the control of ticks, however, use of ethno-veterinary products are encouraged as they are cheap and safe. Ginger is known to have repellant property; that is why this studywas proposed to check the repellent property of Ginger against *Hyalomma* ticks. The efficacy of ginger is attributed to its aromatic, carminative and absorbent properties. The medicinal properties of ginger include anti-arthritic, anti-migraine and hypo-cholesterolaemic, anti-thrombotic, anti-inflammatory, hypo-lipidaemic, hypo-cholesterolaemic, anti-nausea properties, anti-diabetic, antipyretic, antimicrobial, anti-schistosomal, antioxidant, hepato-protective, diuretic, hypotensive and gastrointestinal prokinetic activities (Govindarajan, 1982). Limited research has been carried out on the search for natural repellent products zinger, *Zingiber officinale* (Zingiberaceae) found to be repellent towards ticks *Hyaloma anatolicum*, *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Prajapati *et al.,* 2005).

The present study concluded that *in vitro* efficacy of ginger extract against *Hyalomma anatolicum*ticks proved satisfactory results in all 10 experiments. Whereas; *invivo* efficacy of ginger extract against *Hyalomma anatolicum* ticks was agreeable in all 10 experiments. The similar findings are supported by Zafar *et al.,* (2006) they shows that ginger possesses in vivo anthelmintic activity in sheep. Furthermore, the present study are in agreement with Azima *et al.,* (2012)theyshowed the plant extracts have various degrees of repellency against the larval mites and the repellency increased with increasing concentrations of the extracts.Tawatsin *et al.,* (2009) theyreveals the potential of the development of repellent products derived from ginger and black pepper essential oil against mosquitoes, black flies, biting midges and land leeches and the larvicidal products, especially the tablet formulation derived from the spray-dry extract of black pepper and Indian longer pepper against *A. aegypti* larvae. Bullitta *et al.,* (2007) reported thatidentification of plant species traditionally used for veterinary practices from the local flora could be also potentially useful for the isolation of natural extracts of phytotherapic interest to increase animal welfare and quality of animal productions in organic farming.Kaaya, (2003) reported that the conventional method of tick control is the application of chemical acaricides, but it is associated with a number of problems including environmental pollution, chemical residues in meat and milk products as well as in wool, development of tick resistance and high cost. Likewise, Madhumitha *et al.,* (2012) reported that despite the fact that there are many reports on the acaricidal activity of ginger stem extracts yet scanty research has been carried out on toxicity and potency of the leaves extract on ticks. The fruit aqueous extract of *A. squamosa* exhibited larvicidal activity against the cattle tick *Rhipicephalus* (Boophilus) *microplus*. The ethanolic extract *of A. squamosa* at the highest concentrations exhibited adulticidal activity against *H. anatolicum*. Variation of mortality rate observed might be depending on the concentration of the wet crude extract that contact and entered the tick's skin. Ilham *et al.,* (2014) used the *A. squamosa* leaves extract at

the highest concentrations induced 100% larval mortality which decreased gradually with concentration declining; the result is similar to that reported by Chungsamarnyart (1991) who examined *Rhipicephalus* (Boophilus) microplus. The death of the tested ticks using LIT suggesting that a contact toxic of larvicidal nature is present in the *A. squamosa* leaves ethanolic extract. This finding is in accordance with results recorded using ethanolic crude extracts of Ambrosia maritime, Guiera senegalensis (Ilham, 2009; Osman *et al.,* 2014) against larvae of *Hyalomma anatolicum* and Neem Azal extracts against larvae of *H. anatolicum excavatum* (Abdel-Shafy and Zayed, 2002). Various plant extracts such as Chamomile flower (Khodadad *et al.,* 2007); Calea serrata (Ribeiro *et al.,* 2008); Petiveria alliacea (Rosado-Aguilar *et al.,* 2010), were found to be toxic to ticks. In the search of developing herbal acaricides, eight medicinal plants were screened for their efficacy against ticks. Of these only the extracts prepared from the *A. squamosa* seed showed high level of efficacy against *Boophilus microplus* (Magadum *et al*., 2009) reported that the fruit aqueous extract of *A. squamosa* was efficient against adult ticks of *Haemaphysales bispinosa*. *Azadirachta indica* is one of the commonly grown indigenous plant in farmer‟s fields and backyards in Pakistan. It is an extensively studied medicinal plant throughout the world (Steinhauer, 1994; Subapriya and Nagini, 2005). It possesses a wide range of biologically active compounds and have been evaluated for acaricidal (Landau *et al*., 2009), insecticidal (Kilonzo *et al*., 2001). Weekly spraying with neem seed extracts decreased the number of ticks on cattle (Webb and David, 2002). Abdel-Shafy and Zayed (2002) recorded 100% death of unfed larvae of Hyalomma anatolicum excavatum with neem seed oil on day 3 post-exposure. Shrivastava and Das (2003) have reported highest reduction in the number of ticks 72 hours post treatment in cattle with oils of Sapindus trifoliatus (78.58%) 168 followed by Pongamia pinnata (62.0%), Azadirachta indica (52.46%), Terminalia arjuna (45.77%), Cucurbita maxima (39.22%) and Ricinus communis (2222.45%).

5. CONCLUSION

From the present study it was concluded that *in vitro* repellency and efficacy of ginger extract against *Hyalomma anatolicum* ticks proved satisfactory results. It is recommended that ginger extract may be preferably used for controlling *Hyalomma anatolicum.*

6. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interest regarding publication of this article.

REFRENCES

[1] Abdel-Shafy, S. and A.A. Zayed. 2002. In vitro acaricidal effect of plant extract of neem seed oil (Azadirachta indica) on egg, immature, and adult stages of Hyalomma anatolicum excavatum (Ixodoidea: Ixodidae). Vet Parasitol 106: 89-96

[2] Ali, Z., A. Maqbool, K. Muhammad, I. Altaf, M. S. Khan, M. Rabbani, A. Hanif and H. J. Iqbal. 2009. Antibody Response of Buffalo Calves to Montanide Based *Hyalomma* Tick Homogenate Vaccine. Pakistan Journal of Zoology, (9) : 167-172.

[3] Azima, L.H., H.T. Ming, V.V. Narainasamy and A.T. Yusoff. 2012. Laboratory evaluation of six crude plant extracts as repellents against larval Leptotrombidium deliense (Acari: Trombiculidae). Journal of Veterinary Parasitology. 45 (3) : 315-320.

[4] Baskanchiladze, G.S., L.A. Khurtsilava, I.A. Gelovani, M.V. Asatiani and V.I. Rossinskii. 1984. In combination with papain in experimental septicemia. Antibiotiki. 29 (1): 33-35.

[5] Berkvens, D.L., D.M. Geysen, G. Chaka, M. Mader and G.R.A. Brand. 1998. A survey of the ixodid ticks parasitizing cattle in the eastern province of Zambia. Medicine Veterinary Entomology. 12 : 234.240.

[6] Bullitta, S., G. Piluzza and L. Viegi. 2007. Plant resources used for traditional ethnoveterinary phytotherapy in Sardinia (Italy). Journal of Biomedical and Life Sciences. 54 (7): 1447-1467.

[7] Burgdorfer, W., M.L. Scmidth and H. Hoogstral. 1973. Detection of *Trypanosoma theileri* in Ethiopian cattle, Acta Tropica. 30: 340-346.

[8] Castleman, M. 2001. The New Healing Herbs 2nd Ed. Emmaus, Pennsylvania: Rodale Press;.

[9] Chen, W. and C. Xiao. 2007. Importance of ticks and their chemical and immunological control in livestock. Chinese Journal of Parasitology. 34 (3/4) : 651-656.

[10] Chungsamarnyart, N., S. Jiwajinda, C. Rattanakreetakul and W. Jansawan. 1991. Practical extraction of Sugar Apple Seeds against Tropical Cattle Ticks. Kasetsart J Nat Sci 25: 101-105.

[11] Dipeolu, O.O. and J.N. Ndungu. 1991. Acaricidal activity of "*Kupetaba"* a ground mixture of natural products against *Rhipicephalus appendiculatus.* Veterinary Parasitology. 38 : 327-338.

[12] Du, Y.H., R.Y. Jia, Z.Q. Yin, Z.H. Pu, J. Chen, F. Yang, Y.Q. Zhang and Y. Lu. 2008. Acaricidal activity of extracts of neem (Azadirachta indica) oil against the larvae of the rabbit mite Sarcoptes scabiei var. cuniculi in vitro. Journal of Veterinary Parasitology. 20 (2) : 144-148.

[13] Durrani, A.Z. and A.R. Shakoori. 2009. Study on ecological growth conditions of cattle *Halomma* ticks in Punjab, Pakistan. Irannian Journal of Parasitology. 4 (1): 19-25.

[14] Govindarajan, V.S., 1982. Ginger-chemistry technology and quality evaluation: part 1. Critical reviews in Food Science Nutrition, 17: 1-96.

[15] Ilham, M.O. 2009. Comparative effects of some botanical and synthetic pesticides on ticks and Goats. PhD Thesis Sudan Academy of Sciences (SAS), Khartoum/Sudan.

[16] Ilham, M.O., A.A.A. Razzig, M.T. Elhaj and Y.O. Mohammed. 2014. Acaricidal Activity of Crude Extract of Annona Squamosa against Hyalomma Anatolicum (Ixodoidea: Ixodidae). Altern Integr Med. 3 (4) : 38-40.

[17] Kaaya, G.P., E.N. Mwangi and M.M. Malanza. 1995. Acaricidal activity of *Margaritaria discoedea* plant extracts against the ticks *Rhipicephalus appendiculaltus* and *Hyalomma verigatum.* International Journalof Acarology. 21: 123-129.

[18] Kaaya, P. G. 2003. Prospects for innovative tick control methods in Africa. International Journal of Tropical Insect Science. 23 (3): 210-225.

[19] Kettle, D.S. 1995. Medical and Veterinary Entomology. CAB International Wallingford, UK.

[20] Khan, J.A. 2005. Prevalence of ecto parasites and comparative efficacy of different drugs against tick infestation in cattle. The Journal of Animal and Plant Sciences (Pakistan). 13 (1/2) : 25-28.

[21] Khodadad, Pirali-Kheirabadi, and Mehdi Razzaghi Abyaneh. 2007. Biological activities of chamomile (Matricaria chamomile) flower's extract against the survival and egg laying of the cattle fever tick. J. Zhejiang University Science B 8: 693-696.

[22] Kilonzo, B. S., A. J. Ngomuo, C. A. Sabuni and G. F. Mgode. 2001. Effects of azadirachta indica (neem) extract on livestock fleas in Morogoro district, Tanzania. Insect Science Application. 21, (1) : 89-92.

[23] Kilonzo, B.S. 1986. Flea infestation of farm animals as a potential limiting factor of animal productivity in Tanzania. Proc. of TVA Science Conference Arusha, 264-271.

[24] Kilonzo, B.S., A.J. Ngomuo, C.A. Sabuni and G.F. Mgode. 2001. Effects of Azadirachta indica (Neem) extract on livestock fleas in Morogoro District, Tanzania. Insect Sci. Appl. 21, 322 89–92.

[25] Landau, S.Y., F.D. Provenza, D.R. Gardner, J.A. Pfister, E.L. Knoppel, C. Peterson, D. Kababya, G.R. Needham and J.J. Villalba. 2009. Neem-tree (Azadirachta indica Juss.) extract as a feed additive against the American dog tick (Dermacentor variabilis) in sheep (Ovis aries). Vet. Parasitol. 165, 311-317.

[26] Langner, E., S. Greifenberg and J. Gruenwald. 1998. Ginger: history and use. Advance Theology. 15(1):25-44.

[27] Madhumitha, G., G. Rajakumar, S.M. Roopan, A.A. Rahuman and K.M. Priya. 2012. Acaricidal, insecticidal, and larvicidal efficacy of fruit peel aqueous extract of Annona squamosa and its compounds against bloodfeeding parasites. Parasitol. Res. 111 : 2189-2199.

[28] Magadum, S., D.B. Mondal and S. Ghosh. 2009. Comparative efficacy of Annona squamosa and Azadirachta indica extracts against Boophilus microplus Izatnagar isolate. Parasitol. Res. 105 : 1085-1091.

[29] Makeri, H. K., V. A. Maikai and J. A. Nok. 2008. Effect of topical application of neem seed (Azadiracta indica) extract on sheep infested with Amblyomma variegatum. African Journal of Biotechnology. 20 (6) : 122-128.

[30] Natal, A.J., A.D. Agyei and B. Awumbila. 2005. Susceptibility of *Ambylomma varigatum* ticks to acaricides in Ghana. Experimental Applied Acarrology. 35 (3): 259-268.

[31] Ody, P. 2000. Complete Guide to Medicinal Herbs 2nd Ed. London: Dorling- Kinderesly;.

[32] Osman, I.M., A.S. Mohammed and A.B. Abdalla. 2014. Acaricidal properties of two extracts from Guiera senegalensis J.F. Gmel. (Combrataceae) against Hyalomma anatolicum (Acari: Ixodidae). Vet Parasitol 199: 201-205.

[33] Prajapati V., A.K. Tripathi, K.K. Aggarwal and S.P. Khanuja. 2005. Insecticidal, repellent and oviposition-detergent activity of selected essential oils against *Hyalomma anatolicum*, *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus.* Biores Technolology. 96 : 1749-1757.

[34] Rajput, Z.I., S.H. Hu, W.J. Chen, A.G. Arijo and C.W. Xiao. 2006.Importance of ticks and their chemical and immunological control in livestock. Journal of Animal Science. 7 (4) : 912-921.

[35] Ramzan, M., M. S. Khan, M. Avais, J. A. Khan, K. Pervez and W. Shahzad. 2008.Prevalence of ecto parasites and comparative efficacy of different drugs against tick infestation in cattle. 18 (1) : 208-210.

[36] Ribeiro, V.L., C. Avancini, K. Gonçalves, E. Toigo and G. Von Poser. 2008. Acaricidal activity of Calea serrata (Asteraceae) on Boophilus microplus and Rhipicephalus sanguineus. Vet. Parasitol. 151: 351-354.

[37] Rosado-Aguilar, J.A., A. Aguilar-Caballero, R.I. Rodriguez-Vivas, R. BorgesArgaez and Z. Garcica-Vazquez. 2010. Acaricidal activity of extracts from Petiveria alliacea (Phytolaccaceae) against the cattle tick, Rhipicephalus (Boophilus) microplus (Acari: ixodidae). Vet. Parasitol. 168 : 299-303.

[38] Sakai, Y. 1988. Effects of medicinal plant extracts from Chinese herbal medicines on the mutagenic activity of benzo(a)pyrene. Mutation Research. 206 (3): 327-34.

[39] Shrivastava, S.K. and K. Das. 2003. Effectiveness of some plant extracts on tick *Boophilus microplus* (Canestrini) infestation in cattle. J. Appl. Zool. Res. 14, 198-199.

[40] Soll, M.D. J.A. d'Assonville and C.J. Smith. 1992. Efficacy of topically applied ivermectin against sarcoptic mange (Sarcoptes scabiei var. bovis) of cattle. Parasitology Resesearch. 78 (2): 120-122.

[41] Srivastava, K.C. 1984. Effects of aqueous extracts of onion, garlic, and ginger on platelet aggregation and metabolism of arachidonic acid in the blood vascular system: In vetro study. Journal of Animal Sciences. 2 (3): 227-35.

[42] Steinhauer, B., 1994. Antifungal compounds from Azadirachta indica. Practice oriented results on use and production of neem ingredients and pheromones. 3rd workshop held at Wetzlar, Germany, 22–25 November, 1993, pp. 93–97.

[43] Subapriya, R. and S. Nagini. 2005. Medicinal properties of neem leaves: a review. Curr. Med. Chem. Anticancer Agents. 5, 149–146.

[44] Sutherest, R.W. and L.J. Wilson. 1986. Tropical legumes and their ability to immobilize and kill cattle tick, pp. 185-194. In B.E. Juniper and T.R.E. South Wood (eds.). Insect and Plant Surface. Edaward and Arnold, London.

[45] Tawatsin, A., U. Thavara, P. Siriyasatien and S. Lamlertthon. 2009. Biological activities of plant extracts derived from ginger, black pepper and Indian long pepper against mosquitoes, black flies, biting midges and land leeches. Journal of Veterinary Parasitology. 12 (1) : 147-150.

[46] Webb, E.C. and M. David. 2002. The efficacy of neem seed extract (Azadirachta indica) to control tick infestation in Tswana, Simmentaler and Brahman cattle. South African J. Anim. Sci. 32, 1–6.

[47] Wilson, D.D. and R.Z. Bram. 2003. Foreign pests and vectors of Arthropod-Borne Diseases. In Foreign Animal Diseases. Richmand VA United States Animal Health Association. Pp 225-239.

[48] Zafar, I., M. Lateef, M. S. Akhtar, M. N. Ghayur and A. H. Gilani. 2006. In vivo anthelmintic activity of ginger against gastrointestinal nematodes of sheep. Journal of Ethnopharmacology. 106 (2) : 285-287.

Table-1 Comparison of *in vitro* repellency of ginger stems extract against *Hyalomma anatolicum* ticks (Experiment-1).

Doses	Total ticks	Repellency (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	3 (30%)	3 (30%)	4 (40%)	10 (100%)
Group B=2.5%	10	2 (20%)	3 (30%)	3 (30%)	8 (80%)
Group C=1%	10	2 (20%)	2 (20%)	3 (30%)	7 (70%)
Group D = 0.5%	10	1 (10%)	2 (20%)	3 (30%)	6 (60%)
Group E= Control	10	-	-	-	-

Table-2 Comparison of *in vitro* repellency of ginger stems extract against *Hyalomma anatolicum* ticks (Experiment-2).

Doses	Total ticks	Repellency (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	3 (30%)	4 (40%)	9 (90%)
Group B=2.5%	10	2 (20%)	2 (20%)	3 (30%)	7 (70%)
Group C=1%	10	1 (10%)	2 (20%)	3 (30%)	6 (60%)
Group D = 0.5%	10	-	2 (20%)	3 (30%)	5 (50%)
Group E= Control	10	-	-	-	-

Table-3 Comparison of *in vitro* repellency of ginger stems extract against *Hyalomma anatolicum* ticks (Experiment-3).

Doses	Total ticks	Repellency (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	3 (30%)	3 (30%)	3 (30%)	9 (90%)
Group B=2.5%	10	2 (20%)	2 (20%)	4 (40%)	8 (80%)
Group C=1%	10	1 (10%)	2 (20%)	3 (30%)	6 (60%)
Group D = 0.5%	10	-	2 (20%)	2 (20%)	4 (40%)
Group E= Control	10	-	-	-	-

Table-4 Comparison of *in vitro* repellency of ginger stems extract against *Hyalomma anatolicum* ticks (Experiment-4).

Doses	Total ticks	Repellency (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	2 (20%)	4 (40%)	8 (80%)
Group B=2.5%	10	2 (20%)	2 (20%)	3 (30%)	7 (70%)
Group C=1%	10	1 (10%)	2 (20%)	3 (30%)	6 (60%)
Group D = 0.5%	10	1 (10%)	1 (10%)	2 (20%)	4 (40%)
Group E= Control	10	-	-	-	-

Table-5 Comparison of *in vitro* repellency of ginger stems extract against *Hyalomma anatolicum* ticks (Experiment-5).

Doses	Total ticks	Repellency (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	3 (30%)	4 (40%)	9 (90%)
Group B=2.5%	10	2 (20%)	3 (30%)	3 (30%)	8 (80%)
Group C=1%	10	1 (10%)	2 (20%)	3 (30%)	6 (60%)
Group D = 0.5%	10	-	1 (10%)	3 (30%)	4 (40%)
Group E= Control	10	-	-	-	-

Table-6 Overall comparison of *in vitro* repellency of ginger stems extract against *Hyalomma anatolicum* ticks.

Doses	Total ticks	Repellency (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	12 (24%)	14 (28%)	19 (38%)	45 (90%)
Group B=2.5%	10	10 (20%)	12 (24%)	16 (32%)	38 (76%)
Group C=1%	10	6 (12%)	10 (20%)	15 (30%)	31 (62%)
Group D = 0.5%	10	2 (4%)	8 (16%)	13 (26%)	23 (46%)
Group E= Control	10	-	-	-	-

Table-7 Comparison of *in vitro* efficacy of ginger stems extract against *Hyalomma anatolicum* ticks (Experiment-1).

Doses	Total ticks	Mortality (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	2 (20%)	5 (50%)	9 (90%)
Group B=2.5%	10	2 (20%)	2 (20%)	4 (40%)	8 (80%)
Group C=1%	10	2 (20%)	3 (30%)	3 (30%)	7 (70%)
Group D = 0.5%	10	-	2 (20%)	4 (40%)	6 (60%)
Group E= Control	10	-	-	-	-

Table-8 Comparison of *in vitro* efficacy of ginger stems extract against *Hyalomma anatolicum* ticks (Experiment-2).

Doses	Total ticks	Mortality (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	3 (30%)	4 (40%)	9 (90%)
Group B=2.5%	10	1 (10%)	2 (20%)	4 (40%)	7 (70%)
Group C=1%	10	1 (10%)	2 (20%)	3 (30%)	6 (60%)
Group D = 0.5%	10	-	2 (20%)	3 (30%)	5 (50%)
Group E= Control	10	-	-	-	-

Table-9 Comparison of *in vitro* efficacy of ginger stems extract against *Hyalomma anatolicum* ticks (Experiment-3).

Doses	Total ticks	Mortality (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	3 (30%)	2 (20%)	4 (40%)	9 (90%)
Group B=2.5%	10	2 (20%)	2 (20%)	4 (40%)	8 (80%)
Group C=1%	10	3 (30%)	1 (10%)	3 (30%)	7 (70%)
Group D = 0.5%	10	2 (40%)	2 (20%)	2 (20%)	6 (60%)
Group E= Control	10	-	-	-	-

Table-10 Comparison of *in vitro* efficacy of ginger stems extract against *Hyalomma anatolicum* ticks (Experiment-4).

Doses	Total ticks	Mortality (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	2 (20%)	4 (40%)	8 (80%)
Group B=2.5%	10	2 (20%)	2 (20%)	3 (30%)	7 (70%)
Group C=1%	10	1 (10%)	1 (10%)	2 (20%)	4 (40%)
Group D = 0.5%	10	-	1 (10%)	2 (20%)	3 (30%)
Group E= Control	10	-	-	-	-

Table-11 Comparison of *in vitro* efficacy of ginger stems extract against *Hyalomma anatolicum* ticks (Experiment-5).

Doses	Total ticks	Mortality (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	3 (30%)	4 (40%)	9 (90%)
Group B=2.5%	10	2 (20%)	2 (20%)	3 (30%)	7 (70%)
Group C=1%	10	1 (10%)	2 (20%)	3 (30%)	6 (60%)
Group D = 0.5%	10	1 (10%)	2 (20%)	2 (20%)	5 (50%)
Group E= Control	10	-	-	-	-

Table-12 Comparison of *in vitro* efficacy of ginger stems extract against *Hyalomma anatolicum* ticks in all experiments.

Doses	Total ticks	Mortality (%)			
		In vitro			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	100	11 (22%)	12 (24%)	21 (42%)	44 (88%)
Group B=2.5%	100	9 (18%)	10 (20%)	18 (36%)	37 (74%)
Group C=1%	100	8 (16%)	8 (16%)	14 (28%)	30 (60%)
Group D = 0.5%	100	3 (6%)	9 (18%)	13 (26%)	25 (50%)
Group E= Control	100	-	-	-	-