



## IN VIVO 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

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#### Key words:

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### ABSTRACT

The study was carried out during the year 2013-14 to evaluate the *in vivo* 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 vivo* repellency of ginger against *Hyalomma anatolicum* shows average 100%, 80%, 70% and 60% repellency, 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 76%, 64%, 50% and 32% under *in vivo* conditions at 5%, 2.5%, 1% and 0.5% concentration of ginger extract. In experiment-1; 70%, 70%, 50% and 30% mortality of ticks was examined under *in vivo* conditions. In experiment-2; 50%, 100%, 70% and 30% mortality of ticks under *in vivo* conditions at 5%, 2.5%, 1% and 0.5% concentration. In experiment-3; average 40%, 40%, 50% and 40% mortality of ticks under *in vivo* conditions at 5%, 2.5%, 1% and 0.5% concentration. In experiment-4; overall, 40%, 40%, 30% and 20% mortality of ticks under *in vivo* condition at 5%, 2.5%, 1% and 0.5% concentration. In experiment-5; overall, 40%, 40%, 30% and 20% mortality of ticks under *in vivo* condition at 5%, 2.5%, 1% and 0.5% concentration. Overall, 66%, 52%, 42% and 26% mortality of ticks under *in vivo* 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 *Hyalomma 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 vivo* efficacy of ginger extract against *Hyalomma anatolicum* ticks was agreeable.

## 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, 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

Corresponding Author: [riazsodaywa@gmail.com](mailto:riazsodaywa@gmail.com)

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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).

*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).

Ginger has been written up in themany 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).

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 repellent 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). In view of the facts stated above; the present study was designed to evaluate the *in vivo* efficacy of ginger extract against *Hyalomma anatolicum* ticks with following objectives.

## 2. MATERIALS AND METHODS

The study was carried out during the year 2013-14 to evaluate the *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.

## *In vivo* evaluation of ginger stem against tick

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<sup>0</sup>C 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<sup>0</sup>C 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. Petroleum 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<sup>0</sup>C until used.

### *In vivo* evaluation of Ginger Stem Extract

30 cattle and equal number of buffalo infested with *Hyalomma anatolicum* ticks at Livestock Experimental Farm, Department of Livestock Management, were subjected to spray of ginger stem extract in various concentrations.

30 cattle and equal number of buffalo were divided into 5 groups, A, B, C and D. Group A, B, C were sprayed with ginger stem extract as used in vitro trials, whereas group E served as control. Prior to applying spray, ticks were counted and visuals were taken. After spray, the host animals were checked at 8 hrs, 16 hrs and 24 hrs for tick presence. The ticks at each time point were re-counted and visuals were taken.



**Plate-4. In vivo applying of 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:

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

where N is number of animals in each group.

### **3. RESULTS**

An *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 vivo* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks indicated that the repellency effect of 5% and 2.5% ginger extract was remarkable causing 20%, 30%, 30% 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 20%, 20% and 20% 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 80%, 70%, 60% and 30% 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 vivo* efficacy of ginger extract against *Hyalomma anatolicum* ticks was agreeable.

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

The *in vivo* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks indicated that the repellency effect of 5% and 2.5% ginger extract was remarkable causing 20%, 20%, 30% and 10%, 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 20% 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 70%, 60%, 50% 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 vivo* efficacy of ginger extract against *Hyalomma anatolicum* ticks was agreeable.

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

The *in vivo* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks indicated that the repellency effect of 5% and 2.5% ginger extract was remarkable causing 20%, 20%, 40% and 10%, 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 20% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 0%, 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%, 50% and 30% 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 vivo* efficacy of ginger extract against *Hyalomma anatolicum* ticks was agreeable.

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

The *in vivo* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks indicated that the repellency effect of 5% and 2.5% ginger extract was remarkable causing 10%, 20%, 40% and 10%, 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 20% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 10%, 10% and 10% repellency of ticks were recorded after 8, 16 and 24 hours. No repellency was seen in control group. On average 70%, 60%, 50% and 30% 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 vivo* efficacy of ginger extract against *Hyalomma anatolicum* ticks was agreeable.

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

The *in vivo* repellency of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks indicated that the repellency effect of 5% and 2.5% ginger extract was remarkable causing 20%, 20%, 40% and 10%, 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%, 10% and 20% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 0%, 10% and 20% repellency of ticks were recorded after 8, 16 and 24 hours. No repellency was seen in control group. On average 80%, 60%, 40% and 30% 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*

*in vivo* efficacy of ginger extract against *Hyalomma anatolicum* ticks was agreeable.

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

The *in vivo* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks indicated that the acaricidal effect of 0.5% and 2.5% ginger extract was remarkable causing 18%, 22%, 36% and 12%, 22% and 30% tick repellent when examined after 8, 16 and 24 hours of acaricide application respectively. The efficacy of ginger extract at 1% and 0.5% concentration causing 12%, 16%, 20% and 2%, 12%, 18% tick repellent when examined after 8, 16 and 24 hours respectively. No mortality was seen in control group. Overall, 76%, 64%, 50% and 32% repellency of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vivo* conditions. It was observed that *in vivo* efficacy at same concentration of ginger extract against *Hyalomma anatolicum* ticks was reasonable.

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

The *in vivo* efficacy of ginger extract on *Hyalomma anatolicum* ticks indicated that the acaricidal effect of 5% and 2.5% ginger extract was remarkable causing 20%, 20%, 30% and 10%, 10% and 40% tick mortality when examined after 8, 16 and 24 hours of acaricide application. The efficacy of ginger extract at 1% concentration causing 10%, 20% and 20% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 0%, 10% and 20% mortality of ticks were recorded after 8, 16 and 24 hours. No mortality was seen in control group. On average 70%, 60%, 50% 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 vivo* conditions. It was observed that *in vivo* efficacy of ginger extract against *Hyalomma anatolicum* ticks was agreeable.

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

The *in vivo* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks indicated that the acaricidal effect of 2.5% and 1% ginger extract was remarkable causing 20%, 30%, 30% and 10%, 20% and 30% tick mortality when examined after 8, 16 and 24 hours of acaricide

application respectively. The efficacy of ginger extract at 5% concentration causing 10%, 20% and 20% tick mortality when examined after 8, 16 and 24 hours. Moreover at 0.5% concentration of ginger extract 0%, 10% and 20% mortality of ticks were recorded after 8, 16 and 24 hours. No mortality was seen in control group. On average 80%, 60%, 50% 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 vivo* conditions. It was observed that *in vivo* efficacy of ginger extract against *Hyalomma anatolicum* ticks was sensible.

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

The *in vivo* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks indicated that the acaricidal effect of 5% and 2.5% ginger extract was remarkable causing 20%, 20%, 40% and 10%, 20%, 30% tick mortality when examined after 8, 16 and 24 hours of acaricide application. The efficacy of ginger extract at 1% and 0.5% concentration causing 20%, 10%, 20 and 10%, 10%, 20% tick mortality when examined after 8, 16 and 24 hours respectively. No mortality was seen in control group. On average 80%, 60%, 50% and 40% mortality of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vivo* conditions. It was observed that *in vivo* 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-5)**

The *in vivo* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks indicated that the acaricidal effect of 5% and 2.5% ginger extract was remarkable causing 10%, 10%, 30% and 10%, 10% and 20% tick mortality when examined after 8, 16 and 24 hours of acaricide application respectively. The efficacy of ginger extract at 1% and 0.5% concentration causing 10%, 10%, 10% and 00%, 10%, 10% tick mortality when examined after 8, 16 and 24 hours respectively. No mortality was seen in control group. Overall, 50%, 40%, 30% and 20% mortality of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vivo* conditions. It was observed that *in vivo* efficacy

at same concentration of ginger extract against *Hyalomma anatolicum* ticks was adequate.

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

The *in vivo* efficacy of ginger extract at 5%, 2.5%, 1% and 0.5% on *Hyalomma anatolicum* ticks indicated that the acaricidal effect of 0.5% and 2.5% ginger extract was remarkable causing 16%, 20%, 30% and 10%, 14% and 28% tick mortality when examined after 8, 16 and 24 hours of acaricide application respectively. The efficacy of ginger extract at 1% and 0.5% concentration causing 12%, 14%, 16% and 02%, 10%, 14% tick mortality when examined after 8, 16 and 24 hours respectively. No mortality was seen in control group. Overall, 66%, 52%, 42% and 26% mortality of ticks was examined at 5%, 2.5%, 1% and 0.5% concentration of ginger extract against *Hyalomma anatolicum* ticks under *in vivo* conditions. It was observed that *in vivo* efficacy at same concentration of ginger extract against *Hyalomma anatolicum* ticks was reasonable.

The calculations of LC 50 and LC 90 of ginger stem extract on *Hyalomma 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 repellent property; that is why this study was 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; *in vivo* 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) they showed 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) they reveals 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 that identification of plant species traditionally used for veterinary practices from the local flora could be also potentially useful for the isolation of natural extracts of phytotherapeutic 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 ticks 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* (22.45%).

## 5. CONCLUSION

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

## CONFLICT OF INTEREST

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

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

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

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

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

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

Doses	Total ticks	Repellency (%)			
		<i>In vivo</i>			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	2 (20%)	4 (40%)	8 (80%)
Group B=2.5%	10	1 (10%)	3 (30%)	3 (30%)	7 (70%)
Group C=1%	10	1 (10%)	2 (20%)	2 (20%)	5 (50%)
Group D = 0.5%	10	-	1(10%)	2 (20%)	3 (30%)
Group E= Control	10	-	-	-	-

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

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

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

Doses	Total ticks	Repellency (%)			
		<i>In vivo</i>			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	2 (20%)	4 (40%)	8 (80%)
Group B=2.5%	10	1 (10%)	2 (20%)	3 (30%)	6 (60%)
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-6 Overall comparison of *in vivo* repellency of ginger stems extract against *Hyalomma anatolicum* ticks.**

Doses	Total ticks	Repellency (%)			
		<i>In vivo</i>			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	9 (18%)	11 (22%)	18 (36%)	38 (76%)
Group B=2.5%	10	6 (12%)	11 (22%)	15 (30%)	32 (64%)
Group C=1%	10	6 (12%)	8 (16%)	10 (20%)	25 (50%)
Group D = 0.5%	10	1 (2%)	6 (12%)	9 (18%)	16 (32%)
Group E= Control	10	-	-	-	-

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

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

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

Doses	Total ticks	Repellency (%)			
		<i>In vivo</i>			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	3 (30%)	3 (30%)	8 (80%)
Group B=2.5%	10	1 (10%)	2 (20%)	3 (30%)	6 (60%)
Group C=1%	10	1 (10%)	2 (20%)	2 (20%)	5 (50%)
Group D = 0.5%	10	-	1 (10%)	2 (20%)	3(30%)
Group E= Control	10	-	-	-	-

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

Doses	Total ticks	Repellency (%)			
		<i>In vivo</i>			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	2 (20%)	2 (20%)	4 (40%)	8 (80%)
Group B=2.5%	10	1 (10%)	2 (20%)	3 (30%)	6 (60%)
Group C=1%	10	2 (20%)	1 (10%)	2 (20%)	5 (50%)
Group D = 0.5%	10	1 (10%)	1 (10%)	2 (20%)	4 (40%)
Group E= Control	10	-	-	-	-

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

Doses	Total ticks	Repellency (%)			
		<i>In vivo</i>			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	1 (10%)	2 (20%)	2 (20%)	5 (50%)
Group B=2.5%	10	1 (10%)	1 (10%)	2 (20%)	4 (40%)
Group C=1%	10	1 (10%)	1 (10%)	1 (10%)	3 (30%)
Group D = 0.5%	10	-	1 (10%)	1 (10%)	2 (20%)
Group E= Control	10	-	-	-	-

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

Doses	Total ticks	Repellency (%)			
		<i>In vivo</i>			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	10	1 (10%)	1 (10%)	3 (30%)	5 (50%)
Group B=2.5%	10	1 (10%)	1 (10%)	2 (20%)	4 (40%)
Group C=1%	10	1 (10%)	1 (10%)	1 (10%)	3 (30%)
Group D = 0.5%	10	-	1 (10%)	1 (10%)	2 (20%)
Group E= Control	10	-	-	-	-

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

Doses	Total ticks	Repellency (%)			
		<i>In vivo</i>			
		8 hrs	16 hrs	24 hrs	Total
Group A= 5%	100	8 (16%)	10 (20%)	15 (30%)	33 (66%)
Group B=2.5%	100	5 (10%)	7 (14%)	14 (28%)	26 (52%)
Group C=1%	100	6 (12%)	7 (14%)	8 (16%)	21 (42%)
Group D = 0.5%	100	1 (2%)	5 (10%)	7 (14%)	13(26%)
Group E= Control	100	-	-	-	-