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Original article

Prevalence of cattle ticks in various agro-ecological zones of Khyber Pakhtunkhwa, and evaluation of botanical extracts against *Hyalomma detritum*

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ABSTRACT

Background: The occurrence of infestation by ticks on livestock is a significant challenge in several semi-tropical and tropical countries including Pakistan. Polluted environment and adapted tolerance by ticks against acaricide are the limiting factors that restrict the management of ticks globally.
Methods: In this study, we evaluated the infestation by ticks on cattle from numerous livestock farms present in different districts and agroecological areas of Khyber Pakhtunkhwa, a province in Pakistan. Furthermore, we compared the anti-tick efficacy of different botanicals using ethanolic and aqueous extracts to control *Hyalomma detritum*.
Results: The prevalence rate was 1.78, 35.37, 36.23, 8.15, 15.50, 0.29 and 2.67 percent for *Amblyoma varigatum (A. varigatum), Hyalomma anatolicum (H. anatolicum), H. detritum, Hylomma rufipes (H. rufipes), Hyalomma truncatem (H. truncatum), Rhipicephalus microplus (R. microplus), and Hyalomma kashmirensis (H. kashmirensis)*, respectively. In terms of high mortality rate of ticks, extracts derived from *Calotropis procera* flower (93.33%), *Citrullus colocynthis* fruit (95.0%) and *Calotropis procera* flower (89.4%) showed significantly high efficancy (P < 0.05) than the extracts derived from other plants. In terms of their efficacy in causing mortality of *H. detritum* tick, *Calotropis procera* flower, *Citrullus colocynthis* fruit and *Calotropis procera* leaves extracts displayed non-significant variation.
Conclusion: This work revealed that the cattle tick *H. detritum* can be successfully controlled by employing both ethanolic and aqueous plant extracts. The intensive use of insecticides and chemical drugs are costly and may affect human health negatively due to residual effects in milk and animal meat.

1. Introduction

Pakistan is mostly an agricultural nation, and many of its citizens also raise livestock. In rural regions, the agriculture industry accounts for 43% of the labor force. There are enormous herds of cattle, buffalo, sheep, and goats in Pakistan. In 2020–21, total milk production was 61,690 metric tons and meat production was 4,708 metric tons; exports of meat and meat products were 53.4 metric tons, earning around 3.1% of total foreign currency and accounting for 11.7% of GDP (GOP, 2021). Dairy animals are raised in large numbers by rural small farmers, for whom livestock is the primary

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means of subsistence (Khan et al., 2013). In addition to other diseases and difficulties, ticks and tick-borne infections are the biggest restrictions on the production of dairy animals (Ghafar et al., 2021).

Tick infestation on livestock plays a pivotal role in the transmission of several pathogenic microorganisms responsible for tick-related diseases in human and animals (Ntondini et al., 2008) that instigate high mortality and heavy morbidity resulting in reduced productivity as well as financial losses to poor and small owners of livestock (Jabbar et al., 2015).

The traditional techniques used to control ticks rely on utilization of chemicals such as manufactured pyrethroids, amitraz, macrocyclic lactones and organophosphates (Reshma and Prakasan 2020). Such chemicals proved to be less effective, more expensive, contaminate surrounding environment and induced increased tolerance in ticks (Klafke et al., 2010, Fernández-Salas et al., 2012). Among crucial factors that hamper ticks control worldwide, are the environmental pollution and resistance development against acaricide in ticks (Fernández-Salas et al., 2012) that result in significant economic losses (Sajid et al., 2017). In the recent past, the utilization of acaricides observed a substantial decrease due to their role in causing polluted environment and retention of their residues in the meat and milk (Ribeiro et al., 2011). Additionally, the continues decline in support by the Khyber Pakhtunkhwa government to small-holding farmers, led to employ several customary veterinary exercises for the treatment of diseases and ticks' control.

Many studies have provided knowledge and understanding on the use of natural material in the form of traditional medicine, including both non-plants and plant treatments, to cure animal-related illnesses and diseases (Ndhlovu 2014). Poor livestock producers favor ethnoveterinary or traditional medicine over pricey and sophisticated animal health facilities because of limited and inadequate resources (Ghotge et al., 2002). The insecticidal properties of plant extracts provide a viable alternative that is safe for both people and the environment (Fernández-Salas et al., 2011). Because of their effectiveness against ticks, plant extracts have become a popular treatment option. Botanical extracts have been proposed as a practical method of dealing with ectoparasites like ticks (Ribeiro et al., 2011) since they are safe for animals, humans and environment (Rosado-Aguilar et al., 2017). To treat infection caused by the parasites, several ethnoveterinary materials derived from plants have successfully been utilized (Abbas et al., 2014). To control ectoparasites, around 55 species of botanical extracts belonging to different families of the plants were reported globally (Benelli et al., 2016). Although the ticks are the major ectoparasites that cause heavy financial losses to livestock especially the cattle, the information, about the volume of economic loss generated by ticks' infestation on cattle in the Khyber Pakhtunkhwa, is still lacking.

This study was carried out *in vitro* to evaluate the efficacy of targeted plant-derived extracts to control *H. detritum*, a cattle tick. It may lead to develop an alternative future acaricide source for tick population control and rescue livestock from diseases caused by ticks. In addition, such kind of acaricide must be eco-friendly, degrade quickly, less toxic to mammals and minimize the resistance development in the ticks against them.

2. Materials and Methods

2.1. Ticks sample collection

From different agroecological areas in Khyber Pakhtunkhwa's 13 districts, live ticks were collected from cattle infested naturally for period of two years between Jan. 2018 to Dec. 2019. A positive case for tick infestation was considered based on the one or more ticks infestation events on both female and/or male cattle. Blunt forceps were used to collect live ticks and supply of oxygen was maintained by placing them in the glass tubes which were covered with muslin cloth. Every single tube was properly labelled with all the information about collected sample.

2.2. Identification of ticks

The collected samples of ticks were moved to the Gomal University at the laboratory of Parasitology, Dera Ismail Khan, Pakistan. Tap water was used to rinse the collected samples of ticks and later distilled water treatment was done before drying using filter paper. For their identification based on the classification criterion for *H. detritum*, a stereomicroscope was used at 40X magnification scale (Miranpuri, 1979). After ticks' identification, the selected *H. detritum* were further used to evaluate the lethal effects of the Botanical extracts were evaluated on the targeted *H. detritum*.

2.3. Plant materials

To prepare botanical extracts, different tissues, and plant parts of various botanicals (Table 1) such as leaves, fruit, flower, peel, milk, fruit, fruit peel, seeds, bulbs and rhizome were gathered from the local herbal market and were identified at the facility of Gomal University, Dera Ismail Khan. To collect clean tissues of the plant samples collected, all the activities were performed with utmost care. Table 1 shows the volume or weight for every botanical collected.

2.4. Preparation of plant extracts

The plant-derived material obtained was air-dried under the shade and were crushed and converted in the form of a fine powder by grinding using a stainless-steel pestle mortar. An appropriate amount of individual botanical (Table 2) was socked for at least 24 h in 70 % ethanol or 2.0 L of distilled water and maceration method at room temperature was used to process for extraction. Once extraction process was completed, rotary vacuum evaporator at 20 rpm was utilized to dry the extracted products at 40 °C. The solution preparation for the ticks dipping, each dried extract was converted into a fine powder by softly grinding in a porcelain pestle mortar.

Table 1
Botanicals used for anti-tick activity.

No.	Botanical	Parts used	Weight/Volume
1.	*Alovera*	Leaves	1000 gm
2.	*Allium sativum*	Bulb	1000 gm
3.	*Azadirachta indica*	Leaves	1000 gm
4.	*Calotropis procera*	leaves	1000 gm
		Flower	1000 gm
		Milk	1000 ml
5.	*Citrullus colocynthis*	Fruit	1000 gm
6.	*Citrus sinensis*	Fruit peel	1000 gm
7.	*Datura alba*	Leaves	1000 gm
8.	*Eucalyptus camadulensis*	Leaves	1000 gm
9.	*Juglans regia*	Leaves	1000 gm
10.	*Melia azedarach*	Leaves	1000 gm
11.	*Mentha longifolia*	Leaves	1000 gm
12.	*Quercus berberidifolia*	Leaves	1000 gm
13.	*Peganum harmala*	Seed	1000 gm
14.	*Zingiber officinale*	Rhizome	1000 gm

Table 2
Dry weight of botanicals and volume of distilled water or ethanol used for extraction.

No.	Botanical	Dry Weight	The volume of aqua distilled	Volume ethanol
1.	*Aloe vera*	700 g	1000 ml	1000 ml
2.	*Allium sativum*	600 g	1000 ml	1000 ml
3.	*Azadirachta indica*	800 g	1000 ml	1000 ml
4.	*Calotropis procera*	800 g (leaves)	1000 ml	1000 ml
		700 g (flowers)	1000 ml	1000 ml
		1000 ml (milk)	1000 ml	1000 ml
5.	*Citrullus colocynthis*	600 g	1000 ml	1000 ml
6.	*Citrus sinensis*	700 g	1000 ml	1000 ml
7.	*Datura alba*	700 g	1000 ml	1000 ml
8.	*Eucalyptus camadulensis*	800 g	1000 ml	1000 ml
9.	*Juglans regia*	800 g	1000 ml	1000 ml
10.	*Melia azedarach*		1000 ml	1000 ml
11.	*Mentha longifolia*	700 g	1000 ml	1000 ml
12.	*Quercus berberidifolia*	800 g	1000 ml	1000 ml
13.	*Peganum harmala*	700 g	1000 ml	1000 ml
14.	*Zingiber officinale*	600 g	1000 ml	1000 ml

2.5. Preparation of dip/solution for adult immersion test (AIT)

For both aqueous and ethanolic extracts of 14 selected botanicals, 100 mg/ml concentration was prepared by dissolving 2.0 g of ethanolic and aqueous extract of every single botanical into 20 ml of distilled water (Table 3).

The number of dips and number of adult ticks (*H. detritum*) used were, no. of dip for *H. detritum* = no. of aqueous extract + no. of alcoholic extract = 16 + 16 = 32 (dip), the volume of distilled water for each dip = 20 ml, total volume used for preparation of dips for *H. detritum* = 20 $\times$ 32 = 640 ml, total no. of dips = 62, no. of *H. detritum* in each dip = 30, and total no. of *H. detritum* used = 3 2 $\times$ 30 = 960.

2.6. Adult immersion test (AIT)

Thirty adult ticks including *Hyalomma anatolicum* and *H. detritum* were separately dipped for five minutes in each aqueous and 70% ethanolic extract to determine efficacy of selected botanicals against ticks.

After immersion, ticks were transferred to the Petri plates which were covered by muslin cloth. The incubation temperature of Petri plates was 28 ± 2 °C with 85 ± 2% relative humidity. After incubating Petri plates for 48 h, another five minutes bath was given to the ticks using the same solution and once again transferred to Petri plates.

3. Results

3.1 Prevalence of cattle ticks in various agro-ecological zone/districts of Khyber Pakhtunkhwa. The rate of infestation by the ticks in the Southern Piedmont plains, Central valley Plains, Eastern wet mountains, Western dry mountains, Northern dry mountains of Khyber Pakhtunkhwa was 88.76%, 84.76%, 72.78%, 62.71% and 60.49% respectively (Table 4). According to the data, Southern Piedmont plains was the highest affected zone and Northern dry mountains was the lowest affected zone. With respect to the number of cattle infested with ticks, Dera Ismail Khan was the highly affected district whereas Swat was the lowest affected district.

The prevalence of *H. kashmirensis, R. microplus, H. truncatum, H. rufipes, H. detritum, H. anatolicum* and *A. varigatum* was 2.67%, 0.29%, 15.50%, 8.15%, 36.23%, 35.37% and 1.78% respectively. This data highlighted that the prevalence of *H. detritum* and *H. anatolicum* was in 36.23% and 35.37% cows respectively and cattle is the most preferred host for infestation as compared to other hosts.

Fig. 1 presents graphical data about number of the cattles infested by seven tick species in the five agroecological areas of Khyber Pakhtunkhwa. Fig. 2 presents graphically ticks prevalence in percentage at different agroecological zone/district of Khyber Pakhtunkhwa.

Table 3
Weight of botanical extract used for preparation of aqueous and ethanolic dips.

S.No.	Botanical	Dry Weight of extract	Volume of aqua distilled	Volume of 70% ethanol
1.	*Aloe vera*	2 g	20 ml	20 ml
2.	*Allium sativum*	2 g	20 ml	20 ml
3.	*Azadirachta indica*	2 g	20 ml	20 ml
4.	*Calotropis procera*	2 g Leaves	20 ml	20 ml
		2 g Flower	20 ml	20 ml
		100 ml (milk)	20 ml	20 ml
5.	*Citrullus colocynthis*	2 g	20 ml	20 ml
6.	*Citrus sinensis*	2 g	20 ml	20 ml
7.	*Datura alba*	2 g	20 ml	20 ml
8.	*Eucalyptus camadulensis*	2 g	20 ml	20 ml
9.	*Juglans regia*	2 g	20 ml	20 ml
10.	*Melia azedarach*	2 g	20 ml	20 ml
11.	*Mentha longifolia*	2 g	20 ml	20 ml
12.	*Quercus berberidifolia*	2 g	20 ml	20 ml
13.	*Peganum harmala*	2 g	20 ml	20 ml
14.	*Zingiber officinale*	2 g	20 ml	20 ml

3.1. In vitro efficacy of various botanical extracts against H. Detritum

Data in Table 5 highlights the number of ticks *H. detritum* killed because of immersion in ethanolic and the aqueous solutions having 100 mg/ml ethanolic and/or aqueous extract of plants i.e. *Allium sativum* bulb, *Aloe vera* leaves, *Calotropis procera* leaves, *Azadirachta indica* leaves, *Datura alba* leaves, *Calotropis procera* flowers, *Calotropis procera* milk, *Citrullus colocynthis* fruit, *Zingiber officinale* rhizome, *Melia Azedarach* leaves, *Juglans regia* leaves, *Quercus berberidifolia* leaves, *Peganum harmala* seed, *Mentha longifolia* leaves, *Peganum harmala* seed, *Eucalyptus camadulensis* leaves and *Citrus sinensis* peel.

The number of killed ticks *H. detritum* due to the aqueous solution derived from the aqueous extract of above mentioned plants were 27, 15, 78, 42, 51, 72, 18, 81, 17, 36, 33, 32, 46, 27, 30 and 45, and the efficacy was 30.0%, 16.66%, 86.66%, 46.66%, 56.66%, 80.0%, 19.0%, 90.0%, 20.0%, 43.33%, 36.66%, 36.66%, 56.66%, 30.66%, 33.33% and 50.0% respectively. Whereas, ticks killed in numbers due to 70% ethanolic solution derived from ethanolic extracts of the, *Zingiber officinale* rhizome, *Azadirachta indica* leaves, *Allium sativum* bulb, *Mentha longifolia* leaves, *Calotropis procera* milk, *Calotropis procera* flowers, *Citrus sinensis* peel, *Citrullus colocynthis* fruit, *Eucalyptus camadulensis* leaves, *Datura alba* leaves, *Melia Azedarach* leaves, *Juglans regia* leaves, *Calotropis procera* leaves, *Peganum harmala* seed, *Quercus berberidifolia* leaves and *Aloe vera* leaves were 30, 64, 39, 39, 24, 89, 60, 90, 48, 69, 57, 54, 90, 69, 48, and 21, and the efficacy of these botanicals was 33.33%, 71.11%, 43.33%, 43.33%, 26.67%, 98.89%, 66.67%, 100%, 53.33%, 76.67%, 63.33%, 60.0%, 100%, 76.67%, 53.33% and 23.33%, respectively, at incubation time of 120 h during two immersion at 0 and 48 h' time points of the experiment. No mortality of ticks was observed after both ethanolic and aqueous control immersions with no extracts of any botanicals. Hence, the tick's data generated by immersing in ethanolic, or aqueous extracts of selected plants was significantly different than in control dips.

Statistics of the available data showed significantly high efficacy ($P < 0.05$) of the extracts derived from *Citrullus colocynthis* fruit, *Calotropis procera* flower and *Calotropis procera* leaves, in high ticks mortality than the extracts derived from *Mentha longifolia* leaves, *Melia Azedarach* leaves, *Peganum harmala* seed, *Quercus berberidifolia* leaves, *Allium sativum, Alove vera, Calotropis procera* milk, *Azadirachta indica, Datura alba* leaves, *Citrus sinensis* peel, *Eucalyptus camadulensis* leaves, *Zingiber officinale* rhizome, and *Juglans regia* leaves. It was observed that there was a non-significant variation between *Calotropis procera* flower, *Citrullus colocynthis* fruit and *Calotropis procera* leaves extracts when it comes to their efficacy in causing mortality against ticks *H. detritum*.

Likewise, differences were non-significant between *Citrus sinensis* peel, *Azadirachta indica, Juglans regia* leaves, *Datura alba* leaves, and *Peganum harmala* seed, and *Melia Azedarach* leaves extracts regarding their efficacy in causing the mortality of the *H. detritum* ticks. *Calotropis procera* milk and *Aloe vera* leaves displayed lowest efficacy in ticks *H. detritum* mortality. When it comes to the mortality of the ticks *H. detritum*, there was non-significant variation between *Eucalyptus camadulensis* leaves, *Allium sativum* bulb, *Quercus berberidifolia* leaves, *Zingiber officinale* rhizome and *Mentha longifolia* leaves.

Fig. 3 represents graphically the effectiveness of ethanolic and aqueous solutions that contain 100 mg/ml of extract derived from selected botanicals in causing mortality of the ticks *H. detritum*. In the graph, moving average lines revealed the shift in ticks' mortality by ethanolic and aqueous extracts and the correlation among the ethanolic and aqueous extracts derived from various plants.

Table 4
Cattle ticks of various agro-ecological zone/districts of Khyber Pakhtunkhwa.

Agro-ecological zone/district	No. of cattle observed	Cattle infested (%)	Species of tick found						
			A. varigatum	*H. Anatolicum*	*H. detritum*	*H. rufipes*	*H. truncatum*	*R. microplus*	*H. kashmirensis*
Northern dry mountains	**2058**	**1245 (60.49)**	**24**	**535**	**357**	**109**	**220**		**–**
Dir	697	446 (63.99)	–	140	120	59	127	–	–
Swat	653	349 (53.45)	–	238	91	–	20		–
Shangla	708	450 (57.21)	24	157	146	50	73	–	–
Eastern wet mountains	**1933**	**1407 (72.78)**	**–**	**493**	**673**	**66**	**175**		**–**
Mansehra	559	405 (72.45)	–	177	153	–	75	–	–
Abbottabad	776	530 (68.29)	–	149	272	66	43	–	–
Haripur	598	472 (78.92)	–	167	248	–	57	–	–
Central valley Plains	**1799**	**1525 (84.76)**	**02**	**523**	**568**	**147**	**285**		**–**
Kohat	508	406 (79.92)	–	106	88	98	114	–	–
Peshawar	738	657 (89.02)	–	198	352	38	69	–	–
Charsadda	553	462 (83.54)	02	219	128	11	102	–	–
Southern Piedmont plains	**2679**	**2378 (88.76)**	**71**	**835**	**797**	**203**	**288**	**13**	**171**
Karak	556	475 (85.43)	71	109	124	57	101	13	–
Bannu	807	685 (84.88)	–	169	176	85	84	–	171
D.I. Khan	1316	1218 (92.55)	–	557	497	61	103	–	–
Western dry mountains	**480**	**301 (62.71)**	**25**	**39**	**89**	**34**	**95**	**07**	**12**
North Waziristan	480	301 (62.70)	25	39	89	34	95	07	12
Total	8949	6856 (76.6%)	122	2425	2484	559	1063	20	183

Amblyoma varigatum = A. varigatum, Hyalomma Anatolicum = H. anatolicum, Hyalomma detritum = H. detritum, Hyalomma rufipes = H. rufipes, Hyalomma truncatum = H. truncatum, Rhipicephalus microplus = R. microplus, Haemaphysalis kashmirensis = H kashmirensis.

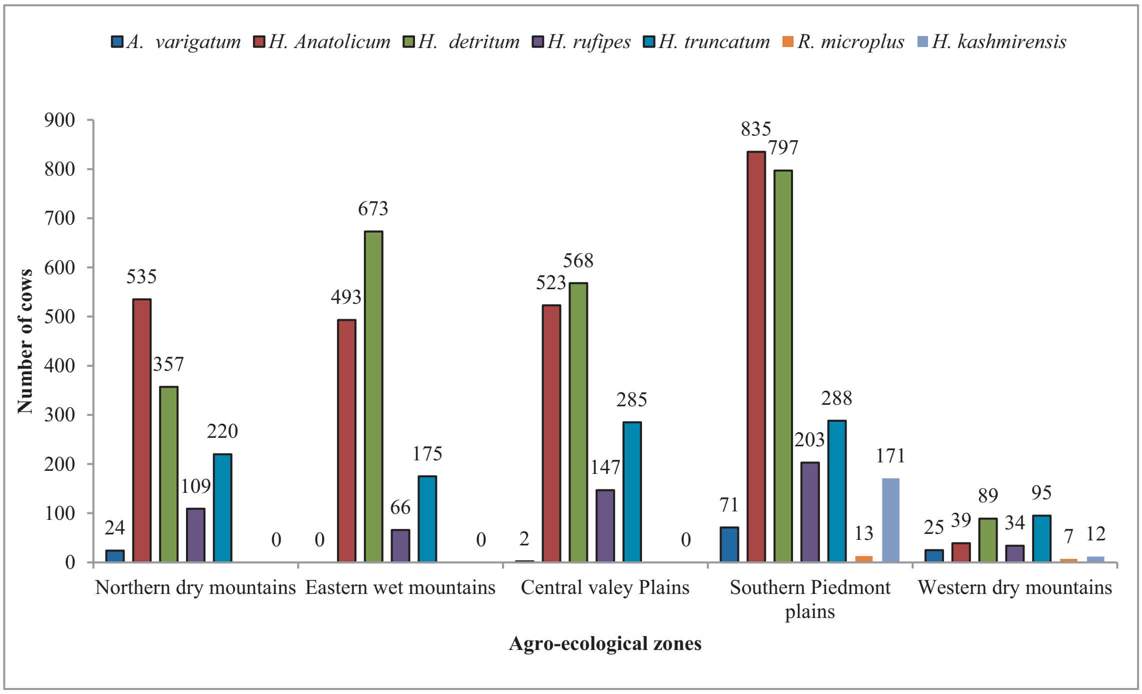

Fig. 1. Number of cattle infested by various species of tick in agro-ecological zones of KPK.

4. Discussion

4.1. Predominance of livestock ticks in agro-ecologically different regions of Khyber Pakhtunkhwa.

The current results are consistent with those of Rehman et al. (2017), who mentioned the existence of *Rhipicephalus turanicus*, *Hyalomma anatolicum*, *Hyalomma dromedarii* and *Rhipicephalus microplus* tick species in cattle farms in Pakistan's arid and semi-arid agro-ecological areas (Rehman 2017, Khan et al., 2022). Total proportion of tick-infested ruminants was 78.3%, with the occurrence of *H. anatolicum* in buffaloes being 81.4%, 60.0% in goats, 89.9% in cattle, and 11.1% in sheep. The current study identified seven species of the ticks which includes *H. rufipes, H. anatolicum, Haemaphysalis kashmirensis, H. truncatum, H. detritum, A. varigatum* and *R. microplus* from different regions of Khyber Pakhtunkhwa province and their occurrence was 2.67%, 35.37, 36.23, 8.15, 15.50, 0.29 and 1.78%, respectively. According to the available data,

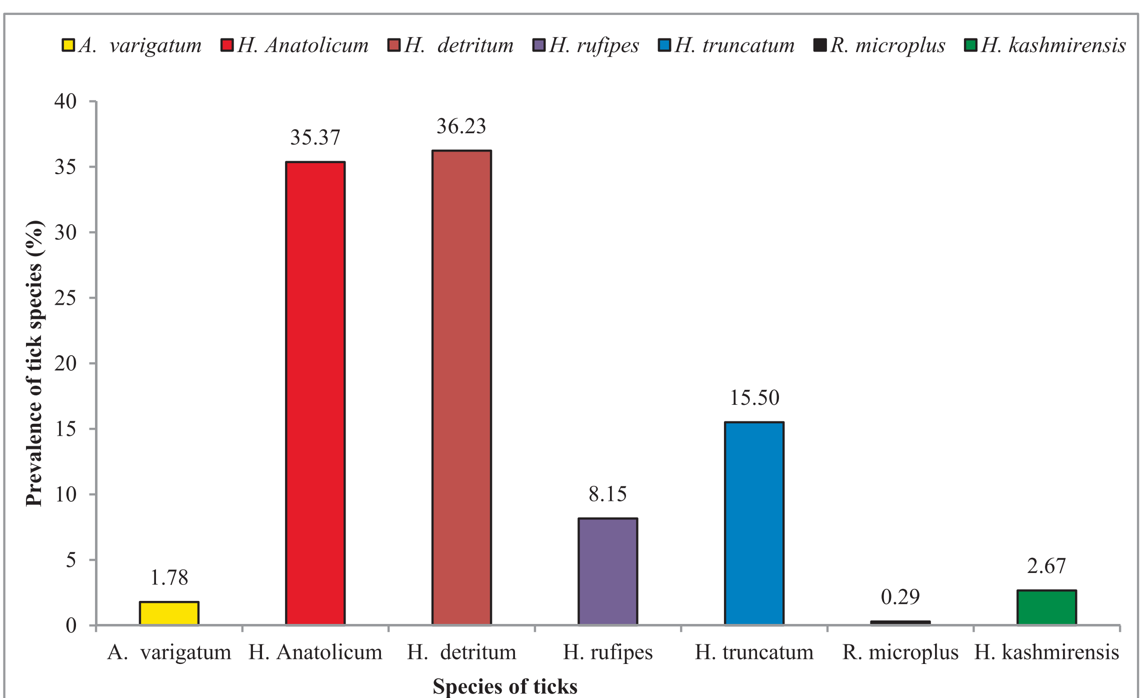

Fig. 2. Percent cattle infested by various tick species.

Table 5
In vitro efficacy of aqueous and ethanolic extracts (100 mg/ml concentration) of various botanicals in respect of killing the ticks *Hyalomma detritum*.

Botanicals	Ticks treated by each treatment (n = 90)				Mean efficacy (%)
	Aqueous Extract		Alcoholic Extract		
	No. of Ticks killed	Efficacy (%)	No. of Ticks killed	Efficacy (%)	
Aloe vera	15	16.66	21	23.33	19.99[f]
Allium sativum	27	30	39	43.33	36.66[def]
Azadirachta indica	42	46.66	64	71.11	58.9[bc]
Calotropis procera leaves	78	86.66	90	100.00	93.33[a]
Calotropis procera flower	72	80	89	98.89	89.44[a]
Calotropis procera milk	18	19	24	26.67	22.84[f]
Citrullus colocynthis fruit	81	90	90	100.00	95.00[a]
Citrus sinensis peel	45	50	60	66.67	58.34[bc]
Datura alba leaves	51	56.66	69	76.67	66.7[b]
Eucalyptus camadulensis leaves	30	33.33	48	53.33	43.3[cde]
Juglans regia leaves	33	36.66	54	60.00	48.3[b]
Melia azedarach leaves	36	43.33	57	63.33	53.3[bcd]
Mentha longifolia leaves	27	30.66	39	43.33	36.99[def]
Quercus berberidifolia leaves	32	36.66	48	53.33	44.99[cde]
Peganum harmala seed	46	56.66	69	76.67	66.7[b]
Zingiber officinale rhizome	17	20.0	30	33.33	26.66[ef]
Total ticks killed	650	45.14	891	61.88	53.51

Figures that do not share a letter are significantly different within a column while different letters indicate significant differences at p value < 0.05.

H. detritum and *H. anatolicum* were observed in 36.23% and 35.37% of cows, respectively, and prefer cattle as a host for infestation over other hosts. Wanzala (2017) also stated that Hyalomma was one among the most important ticks in tropical countries. Singh and Rath (2013) conducted a study in East Punjab and found highest percentage occurrence of *H. anatolicum* being 58.08%, while 11.34% for *Hyalomma anatolicum*, 50.16% for *R. microplus*, *Ixodid* ticks and mixed infestation being, 58.06% and 3.45% respectively. It was also stated that the prevalence of *H. anatolicum* was 79.36% in the sub-mountain undulating region, highlighting its preference for moderately arid region and the overall occurrence of Ixodid ticks was maximum during the monsoon (83.74%), preceded by summer (69.01%) and lowest during winters (31.64%).

Hyalomma species are the most frequently observed ticks on domestic animals in Pakistan (Ali et al., 2022). The most commonly found species are *H. dromedarii, H. marginatum rufipes, H. anatolicum, H. truncatum*, and *H. detritum*. The first one take food from camels while the last four species feed on domesticated animals. *Hyalomma* spp. can inhabit locations with a varied range of temperature fluctuations and precipitation (Rehman 2017). Hyalomma

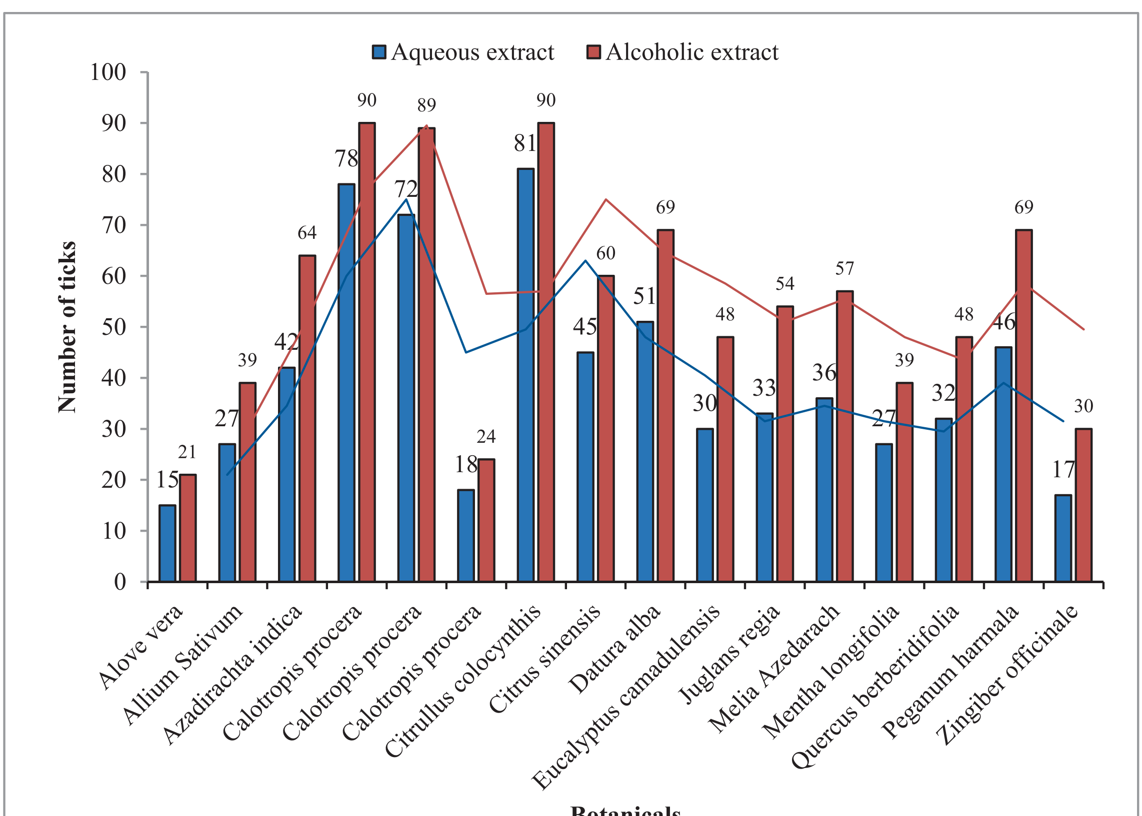

Fig. 3. The mortality of *Hyalomma detritum* during *in vitro* application of aqueous and alcoholic extract of various botanicals.

species of tick serve as a vector for the transmission of many bacterial, viral, and parasitic diseases throughout the world, attempting to make them significant ixodids from economic point of view (Mehlhorn, 2012). The infestation rate of the ticks within the Eastern wet mountains, Western dry mountains, Southern Piedmont plains, Central valley Plains, and Northern dry mountainous range of Khyber Pakhtunkhwa was, 72.78%, 62.71%, 88.76%, 84.76%, and 60.49%, respectively. Following the data, the Southern Piedmont plains were the most affected region, while the Northern dry mountains were the least affected. In terms of the total number of ticks infested cattle, Dera Ismail Khan was the most affected region, while Swat was the least affected. A recent study performed in temporal regions of Khyber Pakhtunkhwa, identified the occurrence of ticks being 10.33%, 0.42%, 10.08%, 78.50%, and 0.67% for Heamaphysalis, Amblyomma, Hyalomma, Rhepicephalus and Dermacenter respectively (Farooqi et al., 2017).

The current study's findings are consistent with Singh and Rath (2013), who mentioned that lower rainfall and higher temperatures promote the growth of *H. anatolicum*. Previously, *H. anatolicum* was found to be the most common cattle tick throughout the Punjab. The decline in predominance may be attributed to adaptation under improved farm animal management system, which seems to be a threat to the longevity of multi-host ticks. Furthermore, macro- and micro-climate factors may influence tick prevalence, causing diverse epidemiological patterns in different agro-climatic regions. (Singh and Rath 2013). The outcomes of this research are consistent with those of Rehman et al. (2016), who reported that the total tick prevalence in domesticated animals in Pakistan was 78.3%. (Rehman 2017).

In Pakistan, two reports showed varied tick occurrence as 31% and 85% (Iqbal et al., 2013) in comparison to India and Iran where it was recorded to be 58% and 77%, respectively. (Singh and Rath 2013). Varied tick predominance is primarily due to differences in the study areas' geographical and climatic conditions, target populations, study seasons and husbandry practices. Tick prevalence in ruminants seems to be much higher in Asia and Africa than in any other continent (Ali et al., 2022).

4.2. In vitro efficiency of different plant derived extracts against H. detritum

Amidines, organophosphates, pyrethroids, and macrocyclic lactones are common chemical acaricides that were effective in reducing the number of hard ticks (Aguilar-Tipacamu et al., 2011). Due to the extensive usage of new chemical acaricides over a long period of time, tick populations have developed a variety of resistances (Rodriguez-Vivas et al., 2011), particularly in the usage region (Aguilar-Tipacamu et al., 2011). Hence, it is essential to explore other products to restrict tick population. It has been demonstrated that plants containing toxicological molecules impact ticks in killing or repelling them from animal bodies. Consequently, plant extracts have the potency to be used as alternatives to presently used acaricides (Magadum et al., 2009). Besides this, when compared to synthetic acaricides, plant extracts get the benefits of being used in organic cattle farming because they are linked to fewer food and environmental contaminations, reduced toxicity to humans and animals as well as slower resistance development. Several plant-based extracts have revealed promising outcomes, but most of them have not been examined on animals to verify and confirm their use. Yet, owing to internal and external variables that affect their chemical composition and a dearth of information regarding active acaricide chemicals, it is difficult to produce their standard formulations. Moreover, since crude extracts sometimes include numerous active chemicals with distinct modes of action, using a variety of potent plant-based extracts may result in a gradual emergence of resistance (Borges et al., 2011).

Farmers used the plants to manage ticks with the most widely mentioned plants used by them are *Citrullus colocynthis, Azadirachta indica, Calotropis procera, Eruca vesicaria, Brassica rapa, Peganum harmala, Capsicum annuum, Eucalyptus camaldulensis, Nicotiana tabacum, Juglans regia, Melia azedarach, Prunus persica, Quercus berberidifolia, Trigonella foenumgraecum* and *Pinus gerardiana*. Numerous parts of the plant, such as seed rhizome leaves, resin of the plants and fruits are introduced to prepare plant derived solution against tick infestation. Traditionally, different plant parts were utilized to make powders, pastes, or decoctions. The decoctions have been used for spraying, washing, or bathing, powder for sprinkling, and the paste was used for creams or ointments. (Babar et al., 2012). It has been mentioned that ethanol based extracts of Annona squamosal (Sitaphal) and *Azadirachta indica* (Neem) are efficacious against the developmental phases of Hyalomma and Boophilus ticks. (Rehman 2017).

The present study findings do not agree with the findings of Shyma et al. (2014) where the mortality rate for the adult tick *Boophilus* was found to be 80% during 2 weeks after their treatment by crude methanolic extract derived from *Allium sativum* (Shyma et al., 2014). During this experiment, *H. detritum* displayed 30% and 39% mortality after their treatment with ethanolic and aqueous extracts derived from *A. sativum* at the concentration of 100 mg/ml, respectively. It was reported that the tick prevalence can be inhibited by utilizing extract derived from *Allium sativum* (-garlic bulb) as it contains potential repellents for ticks including dithiane and thiophene (Nchu et al., 2016). Furthermore, it was reported that *A. sativum* has the insecticidal, fumigant, larvicidal and the acaricidal features (Niroumand et al., 2016). Aboelhadid *et al.* (2013) confirmed that *A. sativum* has acaricidal activity against *Boophilus annulatus* during whole life cycle. To control *Spodoptera littoralis*, the extracts derived from the leaves of *A. sativum* and bulb agglutinins displayed insecticidal properties (Sadeghi et al., 2008).

The current study yielded the results which are partly supporting the findings of Shyma et al. (2014) who found mortality rate of adult ticks as 33.33% for 2 weeks when crude methanolic extract derived from *A. indica* was used to treat them. But, during this study, when *H. detritum*, the adult ticks, were given treatment with the solutions ethanolic and aqueous that contain concentration of 100 mg/ml of extract derived from the leaves of *Azadirachta indica*, 46.66% and 71.11% mortality rate was acheived, respectively. This mortality rate was attained by immersing for 120 h at time points of 0 and 48 h while incubating at 28 °C with relative humidity of 85%.

The present study produced the results which are closely related to the findings of Shyma et al. (2014) who found the mortality rate of adult ticks as 66.67% during 2 weeks of their treatment using crude methanolic extract derived from *Datura stramonium*. However, the current study findings are different from Al-Hasnawi and Wathah (2019) where phenolic, alkaloids and terpenoids extracts obtained from *Datura metel* leaves showed no effects in terms of the mortality of adult female and male *Hyalomma schulzei* ticks (Al-Hasnawi and Wathah 2019). Later on, after performing bioassay, it was demonstrated that 90% mortality of the eggs and larvae, whereas 74.21% and 64.41% mortality of starved and fed nymphs, respectively, was caused by crude phenolic compounds, alkaloids and terpenoids derived from leaves of *Datura metel*. That alkaloid extracts derived from *Datura metel* leaves displayed no effect on adult female and male ticks but may have a pivotal role in controlling different stages of *Hyalomma schulzei* life cycle. This study revealed 56.66% and 76.67% *in vitro* efficacy against *H. detritum*, for ethanolic and aqueous extracts derived from the leaves of *Datura alba*, respectively. The results variation can be attributed to differences in plant varieties. Lower efficacy was reported for methanolic or aqueous extracts derived from *Citrullus colocynthis* mortality of adult females of *hyalomma dromedarii* (Mahran et al., 2020). Whereas this study revealed 90% efficacy of both ethanolic and aqeuous extract of *Citrullus colocynthis* whole fruit (Peal and seed) for *H. detritum*. Such findings are exactly in line with the results reported by Ullah *et al.* (2015) where it was demonstrated that ethanolic and aqeuous extracts derived from *Citrullus colocynthis* fruit, seeds of *Peganum harmala*, and Curcuma longa in combination found to be 100% efficient in terms of mortality of *Rhipicephalus microplus* larvae. In addition, minimum mortality was shown for the extract derived from *P. harmala* against larvae. To control ectoparasites of animals, the utilization of plant-derived crude extracts has been recommended. The fruit and seed of *Citrullus colocynthis* plant produce secondary metabolites having activities against aphids (Soam et al., 2013).

The essential oils derived from several botanicals such as *Cymbopogon martinii* (palmarosa), *Coriandrum sativum* (coriander, Chinese parsley, cilantro), *Laurus nobilis (laurel), Geranium* spp (cranesbills, several species)*, Thymus vulgaris* (thyme) and *Rosa* spp (Roses several species), contains geraniol which is a natural alcohol and a monoterpenoid. The palmarosa oil derived from *Cymbopogon martini* contains upto 70–85% geraniol and being used in several cosmetics, perfumes, industrial production of citronella, the tobacco industry, citronellol and food products. It is also found to act as a repellent and insecticide for mosquitoes, fleas, ticks and lice (Marsin et al., 2020).

Available literature demonstrates that evaluation of essential oils and various plant-derived extracts for acaricidal activity have been perfomed (Borges et al., 2011, Rodriguez-Vivas et al., 2018). The acaricide activity is attributed to the compounds identified such as acids, alcohols, aldehydes, sulfurated compounds, terpenes coumarins, geraniol, and stilbenes, especially to counter genera such as Dermacentor, Ixodes, Argas, Amblyomma, *Rhipicephalus* and Hyalomma. Ethanolic extract derived from *A. indica* leaves found to be less efficient (30%) as compared to the extract derived from *A. indica* seeds (80%) against adult *R. microplus* (Srivastava et al., 2008).

5. Conclusion

It is evident from history that the key factor for passing down ethnoveterinary knowledge was inheritance and for the treatment of animal diseases, various locally available botanicals were utilized. With the advent of high-tech medicine and modern facilities, trend to treat sick animal treatment is changed. Among advanced nations, excessive use of modern medicine in humans and animals resulted in the resistance development against the drugs used. Therefore, to escape or minimize the resistance development, plant-derived martials can be employed for better health and fitness practices as well as producing medicine. Further research and evaluation can be expedited by conserving the ethnoveterinary knowledge in the shape of national documents. To prepare standard extracts for the control of ticks, more investigations are required to better understand pharmacokinetics of plant-derived extracts examined in this experiment. It is pivotal to maintain the proportions of active compounds in the extracts and more efforts are needed to keep homogenous plants by gaining better understanding about the climate, soil and cultivation-related protocols. In addition, for the identification of the risk factors involved for animal and human health, toxicological studies are required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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