IN VITRO AND IN VIVO ACARICIDAL ACTIVITY OF SOME INDIGENOUS PLANTS UNDER ORGANIZED AND FARMER FLOCK

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Summary

Present study has been evaluated *in vitro* and *in vivo* acaricidal activity of some indigenous plants under organized and farmer flock. The expeirment of *in vivo* and *in vitro* effect of adulticidal activity of crude extract of arand, yellow kaner and pudina in natural tick infestation have done. The results indicate that all these extracts had quite similar *in vivo* adulticidal activity on tick population. However, *in vitro* results revealed that the mortality percentage was higher as the concentration of extract increased and the time interval progresses. Statistical analysis of data further revealed that mortality was significantly different (p<0.5) at 4 and 24 hrs interval in case of crude extract of arand, yellow kaner and Pudina.

Key Words: Ticks, methanolicextract, Pudina, Arand, yellow kaner

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Introduction

Now a day's herbal preparation for disease and killing the pest, insect etc. are on its extreme due to their lack of side effect, low cost and target efficiency. However, this process is going on from ancient time by saints and Ayurvedacharya for curing against disease, mention in Ayurveda and many other books. Ectoparasites are major threat for human being as well as for animals which cause anemia, fever and carry some bacterial and viral diseases. Moreover in animals ticks and mites have an important place out of other ectoparasites. So many workers have been done studied with ticks and mites.

Pathak *et al.* (1) evaluated *in vivo* study with methanolic extracts of neem leaves and barks, nochi leaves, vashamber rhizome and punzu leaves against ixodid ticks in small ruminants, while Choudhary *et al.* (2) conducted *in vitro* study with *Nicotina tobaccum* against *R. haemaphysaloides*. Further, Vatsya *et al.* (3) reported *in vitro* tickicidal action of few herbal plants against *Boophilus microplus*. A few researchers have done with *in vivo* and *in vitro* studies also. Considering this fact present investigation have been designed to find out *in vitro* and *in vivo* acaricidal activity of some indigenous plants under organized and farmer flock.

Materials and Methods

Collection of plants

Three local plants *Mentha piperita* (Pudina), *Ricinis communis* (Arand) and *Thevetia peruviana* (yellow kaner) were selected on the basis of their medicinal properties against various parasites. These plants were identified and verified with taxonomical studies as reported by Kritiker *et al.* (4).

Preparation of plant material

Plant material was kept for drying for about 2 weeks, away from direct Sun light below 45°C (shade dried). The dried material was crushed in an electric grinder to coarse powder consistency. About 500 gm of the powder material was uniformly packed into a thimble of a soxhlet extractor. It was exhaustively extracted with methanol for a period of about 48 hrs or 22 cycles or till the solvent in the siphon tube of an extractor becomes colourless. The completion of the extraction was confirmed by taking the solvent from the thimble and evaporated to check the absence of residue. The extract was taken out, filtered and distilled to concentrate to get the syrupy consistency in rotary evaporator. The residue was dried over anhydrous sodium sulphate to remove traces of alcohol. The extracts were preserved in airtight container to avoid loss of volatile principles.

Collection of ticks

Tick population consists of specimens in active phase. The ticks was under taken from organized and farmer flock of goats to give a reliable estimate. The animals were examined monthly for the presence of ticks at various predilection sites of Jamuna pari, Barbari breed of organized flock and none descript goats maintained in village flocks. During each visit, 15 male and 15 female goats were surveyed. Animals were selected at random, depending upon their availability and locality. Ticks were collected from each selected site from the body by way of hand manipulation or with aid of blunt pointed forceps to avoid any harm to ticks and host. These collected ticks were put in vials and labeled with all details of animals such as sex, breed, age and date of collection. The vials with ticks were wrapped in cotton net cloth for oxygen supply and transported to laboratory were they were identify up to species level.

In vitro study

In vitro effect of adulticidal activity of crude extracts of Mentha piperita (Pudina), Ricinis communis (Arand) and Thevetia peruviana (yellow kaner) was evaluated on ticks (Boophilus microplus) collected from natural infested goats. The collected ticks randomly divided into each groups containing 20 in each group. The pour on method was used as described by Vihan et al. (5). Each group of specimen was transferred in to Petri dish (6 cm diameter) in which Whatmen filter paper no. 1 (5.5 cm in diameter) was placed uniformly. The test extracts was dissolved in distilled water and four concentrations were prepared arithmetically viz. 12.5, 25.0, 50.0 and 100.0 mg/ml serial dilution. The perti dish containing each group of ticks was charged with test extracts. The control group was maintained with each set of experiment where the parasite was treated in a solution which contains distilled water. This Petri dish were kept at 25±2 °C and 80±5% RH in the desicator and transferred in to incubator. The mortality of ticks in all groups was recorded after 30 min, 1 hr, 2 hr, 4 hr, 6 hr respectively. Probit analysis was done on mortality data to calculate the LC₅₀ value (6).

In vivo study

In vivo effect of adulticidal activity of crude extract of *Mentha piperita* (Pudina), *Ricinis communis* (Arand) and *Thevetia peruviana* (yellow kaner) on naturally tick infested goats were evaluated. Study was conducted of goat flock of Jalal, village of Mathura. These goats were infested with *B. microplus*. The crude extract from these plants were used in 100, 50, 25 and 12.5 mg/ml concentrations. The extracts were dissolved in distilled water with 2 drops of tween-20. Four concentrations were sprayed on selected infested area with the help of spryer.

Total 30 animals taken for these experiments, comprising 6 animals in each group along with a control group of 6 animals in for evaluating the effect of three plant extracts. Each experiment was conducted during hot humid season, when the burden of parasites was very high in village condition.

The burden of ticks was calculated by summation of the total ticks counted by using the standard counting technique in 10x10 cm area as per standard method. After topical application of sample on the site (ventral abdomen and perennial region), mortality was recorded at 30 min, 1, 2, 4 and 24 hrs respectively. Efficacy percentage was calculated by comparing the number of ticks present before and after treatment groups in respect of reduction of ticks.

Percentage reduction=[1-(Ta/CaXCb/Tb)X100]

Where,

Ta= Infestation on treated animals after treatment.

Tb= Infestation on treated animals before treatment.

Ca= Infestation on untreated animals after treatment.

Cb= Infestation on untreated animals before treatment.

Results

The results of *in vitro* effect of acaricidal activity of crude extract of different plant extracts against tick have been partrayed in table 1, 2 and 3. The results revealed that the percentage mortality in tick was higher at 24 hrs interval. The mortality percentage of tick against crude extract of *Ricinus communis* at 4 hrs was 95.00±0.10, 40.00±0.21, 25.00±0.20 and 10.00±0.9 at 100, 50, 25 and 12.5 (mg/ml) concentrations respectively. However, the mortality percentage was 100.00±0.00, 85.00.±0.52, 80.00±0.50 and 65.00±0.44 at similar concentration after 24 hrs interval. The analysis of data revealed that there was significant difference (p<0.5) in tick mortality at 4 and 24 hrs interval in all the concentrations of extract, whereas significant difference in tick mortality was also observed at 30 min, 1 and 2 hrs at 100, 50 and 25% concentration.

In vitro adulticidal activity of crude extract of yellow kaner (*Thevetia peruviana*) against ticks showed 100.00±0.0, 90.00±0.67, 75.00±0.33 and 70.00±0.67 percentage mortality of ticks at 24 hrs intervals at 100, 50, 25 and 12.5 concentrations respectively. The extract of Pudina (*Mentha piperita*) was used at different concentrations. The percentage mortality of Pudina (*Mentha piperita*) against ticks at 24 hrs time intervals was 100.00±0.00, 90.00±0.74, 90.00±0.70 and 80.00±0.67 at 100, 50, 25 and 12.5 mg/ml concentration respectively.

The results revealed that the mortality percentage was higher as the concentration of extract increased and the time interval progresses. Statistical analysis of data further revealed that mortality was significantly different (p<0.5) at 4 and 24 hrs interval in case of crude extract of arand, vellow kaner and Pudina.

Comparative *in vitro* effect of adulticidal activity of different plants extract of *Ricinus communis*, *Thevetia peruviana* and *Mentha piperita* have been given in table 4. The results revealed that LC₅₀ values were lowest in case of *Mentha piperita* than *Thevetia peruviana* and *Ricinus communis*. It indicates that *Mentha piperita* crude extract was more efficious *in vitro* on ticks as compared to other two plants *Ricinus communis* and *Thevetia peruviana*.

The results *in vivo* effect of crude extract of these plants have been depicted in table 5, 6, 7 and 8. The highest mortality of ticks was observed at 4 hrs and 24 hrs in 100% concentration of *Ricinus communis* extract. Increasing the concentration of crude plant extract (50, 25 and 12.5) was causing higher tick mortality. The tick mortality was 100.00±0.24, 95.00±0.80,

40.00±0.12 and 20.00±0.04 at 100, 50, 25 and 12.5% concentration of extract at 24 hrs respectively. Statistical analysis of results revealed significantly higher (p<0.5) mortality of tick at 2, 4 and 24 hrs periods against 100, 50, 25 and 12.5 concentration. Similarly, *in vivo* effect of yellow kaner (*Thevetia peruviana*) on ticks revealed highest mortality at 4 and 24 hrs in 100 and 50% extract concentration. Analysis of results showed significant difference in mortality percentage at 4 and 24 hrs against different concentration.

In vivo effect of crude extract of Pudina (Mentha piperita) revealed highest mortality of ticks at 4 and 24 hrs against 100 and 50% concentration of extract. The mortality of ticks was ranging from 15.00±0.12 to 100.00±0.90 at 24 hrs against 12.5 to 100% concentration. Analysis of data revealed a significant higher (p<0.5) percentage mortality of ticks after 4 and 24 hrs at 100, 50, 25 and 12.5% concentration. Significantly difference (p<0.5) was also observed at 1 and 2 hrs time interval between 100 and 50% concentrations.

Results of *in vivo* effect of adulticidal activity of crude extract of arand, yellow kaner and pudina in natural tick infestation was depicted in table 8. Comparative effect of various plant extract showed higest mortality (63.75±0.34) of ticks in arand extract at 24 hrs. Similarly, extract of yellow kaner and pudina showed slightly low mortality (62.50±0.24) and (60.00±0.43) at 24 hrs respectively. In control group, there was no mortality till 24 hrs. The tick mortality in these plants at 30 min, 1 hr and 2 hrs were very low as compared to 4 and 24 hrs. The results indicate that all these extracts had quite similar *in vivo* adulticidal activity on tick population.

Table 1. *In vitro* effect of adulticidal activity of crude extract of Arand (*Ricinis communis*) on tick collected from natural infested village flock of non-descript breed of goats

Extract concentration (mg/ml)	No. of	Percent mortality (Mean ± S.E.)						
	ticks	30min	1hr	2hr	4hr	24hr		
100	20	10.00° ±0.05	20.00 a ±0.08	30.00 a ±0.09	95.00 a ±0.10	100.00 a ±0.0		
50	20	0.00 b ±0.00	5.00 b ±0.01	10.00 b ±0.10	40.00 b ±0.21	85.00 b ±0.52		
25	20	0.00 b ±0.00	0.00° ±0.00	5.00° ±0.01	25.00° ±0.20	80.00° ±0.50		
12.5	20	0.00 b ±0.00	0.00° ±0.00	0.00 d ±0.00	10.00 ^d ±0.09	65.00 d ±0.44		
D. Water (control)	20	0.00 b ±0.00	0.00° ±0.00	0.00 d ±0.00	0.00 ° ±0.00	0.00 ° ±0.00		

Table 2. In vitro effect of adulticidal activity of crude extract of Yellow Kaner (Thevetia peruviana) on tick collected from natural infested village flock non-descript breed of goats

Extract concentration	No. of	Percent mortality (Mean ± S.E.)						
(mg/ml)	ticks	30min	1hr	2hr	4hr	24hr		
100	20	5.00 a ±0.02	10.00 a ±0.02	20.00 a ±0.05	45.00 a ±0.25	100.00 a ±0.0		
50	20	0.00 b ±0.00	5.00 b ±0.08	15.00 b ±0.20	40.00 b ±0.22	90.00 b ±0.67		
25	20	0.00 b ±0.00	0.00 ° ±0.00	10.00 ° ±0.09	25.00 ° ±0.10	75.00 ° ±0.33		
12.5	20	0.00 b ±0.00	0.00 ° ±0.00	0.00 d ±0.00	15.00 d ±0.17	70.00 d ±0.67		
D. Water (control)	20	0.00 b ±0.00	0.00 ° ±0.00	0.00 d ±0.00	0.00 e ±0.00	0.00 e ±0.00		

Table 3. *In vitro* effect of adulticidal activity of crude extract of Pudina (*Mentha piperita*) on tick collected from natural infested village flock of non-descript breed of goats

Extract concentrati	No. of	Percent mortality (Mean ± S.E.)						
on (mg/ml)	ticks	30min	1hr	2hr	4hr	24hr		
100	20	5.00 a ±0.05	10.00 a ±0.33	25.00 a ±0.50	90.00 a ±0.67	100.00 a ±0.00		
50	20	0.00 b ±0.00	0.00 b ±0.00	15.0 b ±0.20	35.00 b ±0.50	90.00 b ±0.74		
25	20	0.00 b ±0.00	0.00 b ±0.00	10.00 ° ±0.10	30.00° ±0.12	90.00 ° ±0.70		
12.5	20	0.00 b ±0.00	0.00 b ±0.00	0.00 d ±0.00	0.00 d ±0.00	80.00 d ±0.67		
D. Water (control)	20	0.00 b ±0.00	0.00 b ±0.00	0.00 d ±0.00	0.00 d ±0.00	0.00 e ±0.00		

Means with similar superscripts did not differs significantly (P<0.05)

Table 4. *In vitro* effect of adulticidal activity of crude extracts on ticks collected from natural infested village flock of non-descript breed of goats

Botanical name of Plants	Hindi	Part			Mor	tality %	6 (24 h	rs)	
	name	used	LC 50	Control	Concentration of extr				
					12.5	25	50	100	
Mentha Piperita	Pudina	Leaves	2.49	0	80	90	90	100	
Ricinis communis	Arand	Leaves	8.65	0	65	80	85	100	
Thevetia peruviana	Kaner	Leaves	7.75	0	70	75	90	100	

Table 5. *In vivo* effect of crude extract of *Ricinis communis (Arand)* in natural tick infestation in non-descript breed of goats of village flock

Percent	No. of	No. of ticks	Percent mortality (Mean ± S.E.)					
extract concentrations	goats	count in selected area (10x10cm ²)	30min.	1hr	2hr	4hr	24hr	
100	6	126	15.00 ° ±0.02	30.00 ° ±0.05	35.00 ^a ±0.09	100.00 a ±0.24	100.00 ^a ±0.24	
50	6	193	5.00° ±0.01	10.00 a ±0.03	20.00 b ±0.01	60.00 ^b ±0.98	95.00 b ±0.80	
25	6	85	0.00 b ±0.00	0.00 b ±0.00	15.00° ±0.09	35.00° ±0.12	40.00 ° ±0.12	
12.5	6	108	0.00 b ±0.00	0.00 ^b ±0.00	5.00 ^d ±0.01	10.00 ^d ±0.04	20.00 ^d ±0.04	
D. Water (control)	6	113	0.00 b ±0.00	0.00 b ±0.00	0.00 e ±0.00	0.00 e ±0.00	0.00 e ±0.00	

Table 6. In vivo effect of crude extract of Thevetia peruviana (Yellow Kaner) in natural ticks infestation in non-descript breed of goats of village flock

Percent	No.	No. of ticks		Perce	Percent mortality (Mean ± S.E.)				
extract concentra tions	of goats	count in selected area (10x10cm²)	30min	1hr	2hr	4hr	24hr		
100	6	124	10.00 a ±0.20	15.00 a ±0.02	25.00 a ±0.08	100.00 a ±0.56	$100.00^{a} \pm 0.56$		
50	6	88	5.00 b ±0.01	7.00 b ±0.01	20.00 b ±0.05	45.00 ^b ±0.09	80.00 ^b ±0.20		
25	6	97	0.00 ° ±0.00	0.00 ° ±0.00	16.00 ° ±0.08	35.00 ° ±0.10	55.00° ±0.16		
12.5	6	104	0.00° ±0.00	$0.00^{\text{ c}} \pm 0.00$	0.00 d ±0.00	5.00 ^d ±0.01	15.0 ^d ±0.04		
D. Water (Control)	6	94	0.00° ±0.00	0.00° ±0.00	0.00 ^d ±0.00	0.0 e ±0 0	0.00° ±0.0 0		

Table 7. *In vivo* effect of crude extract of *Mentha piperita* (*Pudina*) in natural ticks infestation on non-descript breed of goats of village flock

Percent extract	No. of No. of Percent mortality (Mean goats ticks						
concentra tions	guats	count in selected area (10x10c m²)	30min	1hr	2hr	4hr	24hr
100	6	188	8.00 a ±0.02	15.00^{a} ± 0.03	30.00 ^a ±0.10	100.00 ^a ±0.90	$100.00^{a} \pm 0.90$
50	6	99	5.00 b ±0.02	10.00 ^b ±0.08	20.00 ^b ±0.04	40.00 b ±0.09	90.00 b ±0.50
25	6	113	0.00 ° ±0.00	0.00 ° ±0.00	18.00° ±0.06	30.00° ±0.20	35.00° ±0.20
12.5	6	126	0.00 ° ±0.00	0.00 ° ±0.00	0.00 ^d ±0.00	8.00° ±0.20	15.00 ^d ±0.12
D. Water (Control)	6	86	0.00 ° ±0.00	0.00 ° ±0.00	0.00 ^d ±0.00	0.00 ° ±0.00	0.00 e ±0.00

Table 8. In vivo effect of adulticidal effect of crude extracts of different plants in natural tick infestation in non-descript breed of goat of village flock

Botanical name of	Hindi Name	Percent Mortality (Mean ±S.E.)						
Plants	Ivallie	30 min.	1 hr.	2 hr.	4 hr.	24 hr.		
Ricinis communis	Arand	4.00±0.01	10.00±0.02	18.75±0.05	51.25±0.34	63.75±0.34		
Thevetia peruviana	Yellow Kaner	3.75±0.05	5.50±0.00	15.25±0.04	46.25±0.16	62.50±0.24		
Mentha piperita	Pudina	3.25±0.01	6.25±0.020	17.00±0.05	44.50±0.34	60.00±0.43		
D.Water (Control)		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00		

Discussion

Control of ticks with chemical acaricides was become difficult due to resistance development. Toxicity and resistance problems of insecticides (7) have directly reach towards alternate control using plants as acaricides. The chemical also cause environmental pollution due to prolonged time for biodegradability and health hazards owing to residue in milk and meat. Pesticides are known to exert side effects in host including immunotoxicity and delayed neuropathy at low doses.

In vitro effect

In vitro activity of crude methanolic extract of pudina (Mentha peperita), arand (Ricinus commuins) and kaner (Thevetia peruviana) was evaluated on ticks (Boophilus microplus) collected from natural infested goat of village flock. At earlier studies Vihan et al. (5) reported highest acaricidal property of Annona squamosa extract against ticks in vitro as well as in vivo experiment. Further, Pathak et al. (1) studied plant extracts of neem (Azardiachta indica) leaves and bark, nochi (Vitex nugundo) leaves, vashambu (Acorus calamus) rhizome and Pungu (Pongamia pinnata) leaves against ixodid ticks of small ruminants. Bisen et al. (8) conducted in vitro efficacy trial against Boophilus microplus of neem seed oil, karanj seed oil and found that karanj seed oil exhibit highest efficacy (70%). Meshram et al. (9), Khudrathulla and Jagannath (10), Kumar et al. (11) evaluated various extract in vitro against ectoparasites.

In vivo effect

Tick infestation affects the health and productivity of animals through direct injury and transmission of serious diseases. Tick infestation has traditionally been death with a wide variety of chemical compounds. The efficacy *in vivo* of different plant extracts against tick were carried out by various workers in India and elsewhere Kumar *et al.* (11), Muraleedhran *et al.* (12), Mulraleedhran and Shadev (13), John *et al.* (14), Fajimi *et al.* (15) and Variation in percentage reduction was depending on the stage of tick harbored by the animals at the time of treatment. Compare to synthetic durg pyrethroids, the herbal extract in present study were comparatively effective in eliminating the adult ticks *in vivo*. Therefore, it is concluded that plant extracts evaluated in present study such as Arand, Pudina and yellow kaner proved a better therapy in eliminating tick infestation in goats in comparison of synthetic drugs.

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