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Original Research Article

COMPARISON EFFICACY OF SYNTHETIC CHEMICALS AND PLANT EXTRACTS FOR TICK CONTROL

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Abstract: Ticks are considered as harmful and economically important ectoparasites because their infestation seriously affects the cattle worldwide. Tick control with synthetic acaricides is not only dangerous for animal and human health but also causes environmental pollution. The present study was designed to evaluate the plant extracts in comparison with synthetic acaricides to control *Hyalomma anatolicum*. Five different concentrations (50, 100, 250, 500 and 750 ppm) of methanolic plant extracts and acaricides, were employed to evaluate the mortality of ticks after 2, 4, 6, 12, 24 and 48 hrs. Mortality data was analyzed through Probit analysis to calculate the median lethal concentration (LC_{50}) and the median lethal time (LT_{50}). Methanolic extract from *Azadirachta indica* demonstrated the highest mortality (LC_{50} = 38.3 ppm) of ticks as compared to *Dalbergia sissoo* (LC_{50} = 58.76 ppm) and *Morus alba* (LC_{50} = 92.95 ppm). Amongst acaricides, fipronil exhibited highest mortality (LC_{50} = 35.01 ppm) when compared with emamectin (LC_{50} = 46.87 ppm) and cypermethrin (LC_{50} = 37.83 ppm). Higher concentration (750 ppm) of acaricides (fipronil, emamectin and cypermethrin) displayed quicker mortality (LT_{50} = 6.53-8.95 hrs) as compare to the plant extracts (LT_{50} = 8.49-29.17 hrs). Effects of these treatments were also studied on egg masses and reproductive index (RI) of the surviving ticks. The results revealed a significant, concentration-dependent variation among the egg masses treated with plant extracts and acaricides; and subsequently, their reproductive index values also decreased significantly. Phytochemical analysis of the tested plant extracts revealed presence of flavonoids, steroids, terpenoids, saponins, tannins and phenols in variable quantities. Conclusively, our results describe a significant scope of environment friendly plant extracts for ticks' management.

Key words: plant extracts; synthetic acaricides; tick mortality

Introduction

Ticks are blood sucking ectoparasites that act as vectors of diseases like rickettsiosis, anaplasmosis, tularemiosis, babesiosis and theileriosis in meat and dairy animals (1,2). The ectoparasites harm the hosts both directly (blood loss and reduction in weight gain) and indirectly (act as vectors for a wide range of viral, bacterial and protozoan pathogens to humans and domestic animals) (3,4). Their infestation leads to weaken the animals with poor growth and result in substantial economic loss (5,6). An estimated loss of 14-19 billion USD per year is reported because of tick borne diseases with a worldwide infection of 80% cattle population (7). The most important pathogen observed is Crimean Congo hemorrhagic fever virus usually associated with ticks of genus *Hyalomma*. Many outbreaks of this disease have been recorded from Pakistan (7).

Various chemical acaricides (chlorinated hydrocarbons, synthetic pyrethroids, organophosphates, formamidines and macrocyclic lactones) are used by pest exterminators to control ticks. But many problems are associated with these acaricides such as acaricidal resistance in ticks and long residual effects in milk and meat that cause health hazards for human beings. These acaricides also contaminate environment and water, so cause harmful effects to nontarget organisms (8). The chemical

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pesticides are much expensive products and are concerned with ecological threats. So, pesticide usage has forced scientists to find out less harmful and inexpensive chemicals. They have made great contributions to develop a substitute and found natural products as alternative source of synthetic acaricides (9). The naturally occurring plants are used as ethno-veterinary medicine. Botanical products when applied show insufficient adverse effects on non-target organisms as well as on the environment (10) as compared to the synthetic insecticides due to their low toxicity. Pesticidal products of plant origins have been found remarkably effective in the form of antifeedants, repellents, protectants and growth disrupting hormones as other biocides (11). Neem (*Azadirachta indica*) is extensively distributed in Africa, Asia and other tropical areas of the world. A variety of chemicals (azadirachtin, salannin, meliantriol, nimocinolide, isonimocinolide and triterpenoids) are present in neem extract. The neem seed extract usage was recognized for poor farmers as a potential source to control ticks particularly in cattle (12). *A. indica* is effective to be used for tick control in both dry and humid areas (13). *Dalbergia* genus has 300 species of which almost 25 species exist in India. Many species of *Dalbergia* are considered as vital timber trees, appreciated for their attractive and fragrant wood and are rich in aromatic oils (14). Bark of this tree is employed as antihelmintic, aphrodisiac, antipyretic, abortifacient, expectorant and is also used for treatment of blood diseases, dysentery and leukoderma, whereas seeds' oil is employed to treat scabies and the leaves extract has analgesic, antihelmintic and antipyretic properties (15). *Morus* contains over 150 species and among these, *Morus alba* L. is dominant and indigenous to Pakistan, Nepal, India, China and Japan (16). It is widely cultivated all over the plains of both Pakistan and India, and also on the mountains of Himalaya up to 3,300 m altitude for the purpose of its foliage, as a source of food for silkworms. Its extract represented a strong activity against gram-negative, gram-positive bacteria and fungi due to high pesticidal activity (17). Many reports provide information of different plant extracts possessing pesticidal properties and thus could be used against ticks. Plant extracts carry phytochemical constituents that have potential to control ticks population as effectively and equally as synthetic acaricides. Besides these, plant products (botanical pesticides) are considered environment friendly, safe to non- target organisms, and are inexpensive to be used by livestock owners and farmers. So, the present study was carried out to compare the efficacy of some selected synthetic acaricides and plant extracts.

Materials and methods

Collection of plant materials

Fresh leaves of *Azadirachta indica*, *Dalbergia sissoo*, and *Morus alba* were collected and identified by a botanist. The collected leaves were washed thoroughly with tap water and dried under shade for a span of one month. The dried leaves were then chopped and ground to powder form using an electric grinder (Anex Germany, TS-639).

Preparation of plants extract

The plant extracts were prepared by dissolving 500 grams of powdered material of each selected plants individually in methanol in a beaker at room temperature. The beaker was covered with aluminium foil and stirred daily for seven days. Afterwards, the material was filtered by Whatman No.1 filter paper and the solvent (methanol) was evaporated in rotary evaporator for 30 minutes at 60 ºC. After evaporation, the material was placed overnight in the incubator set at 40 ºC to evaporate remaining methanol.

Preparation of stock solution and dilutions

Stock solutions of each plant extract was prepared by dissolving 0.5 mg of the extracted material in a few drops of Dimethyl sulfoxide (DMSO) and then topped up with saline to make solutions of 0.25 mg/ml. The stock solution was then used for preparing different concentrations (50, 100, 250, 500 and 750 ppm) (5). Three different acaricides; emamectin (Tycon 1.9% EC, Four Brothers Group, Pakistan), fipronil (Regent 50 SC, Bayer Pakistan (Pvt.) Ltd.) and cypermethrin (Bulletin 10% EC, Ali Akbar Group, Pakistan) were purchased from the market and different concentrations (50, 100, 250, 500 and 750 ppm) were prepared for bioassay tests (18).

Collection and storage of ticks

The ticks were collected from rural area of Samundri (31°03′45″N 72°57′15″E), district Faisal-

abad from buffaloes with forceps having gloves on hands. Ticks were stored in sterile glass bottle with muslin cloth on the top (2). Ticks were then shifted in the department of Zoology, Government College University, Faisalabad for identification (19) and rearing. *Hyalomma anatolicum* were reared on rabbits for bioassay tests (8). For reproductive index calculation, a total of 20 *H. anatolicum* were weighed individually after washing with distilled water and drying with filter paper, and were placed in glass tubes covered with muslin cloth. Each glass tube, containing a single mated female *H. anatolicum* was kept in an incubator set at 28 ºC and 90% RH for oviposition. After 20 days, the eggs laid within the first 3-4 days were counted, weighed and transferred into 5-ml sterile glass tubes for hatching under same conditions (28 ºC and 90% RH). After 25-27 days, the hatched larvae were counted again (20).

Adult immersion test (Bioassay)

Adult immersion test (AIT) was performed as per the protocol described by Drummond et al. (21). Five replicates of each treatment were used for bioassay tests with 20 ticks in each treatment. Ticks were immersed in the solution (10 ml) at room temperature for two minutes in a 25 ml beaker with gentle agitation. Water was used as control treatment and the treated ticks were recovered from the solution, dried with absorbent paper and were placed in separate plastic specimen tubes (25 mm×50 mm). These tubes were incubated at 28±1°C and 85±5 per cent relative humidity in a biological oxygen demand (BOD) incubator. Ticks treated with different concentrations of the plant extracts and commercial acaricides were compared with the control ticks and the mortality of ticks was observed after 2, 4, 6, 12, 24 and 48 hrs period. The glass tubes with survivors were placed in an incubator (28 ºC and 90% RH) to determine the reproductive index of the ticks. After 20 days, the eggs laid within the first 3-4 days were counted, weighed and transferred into 5-ml sterile glass tubes for hatching under same conditions (28 ºC and 90% RH). After 25-27 days, the hatched larvae were counted again (20). The reproductive index was calculated by following formula according to FAO guidelines,

RI = (weight of eggs in milligrams/weight of female in milligrams) x % hatch

Phytochemical analysis

Phytochemical analysis of the three plant extracts viz., *Azadirachta indica* (Neem), *Dalbergia sissoo* (Sheesham) and *Morus alba* (White mulberry) was carried out for alkaloids, flavonoids, steroids, terpenoids, saponins, tannins and phenols by employing the methods as described by Rosenthaler (22). The powdered leaves were extracted using suitable solvent and necessary reagent added to the right quantity of the extract.

Statistical Analysis

The percent efficacy (mortality) was calculated by the formula explained by Holdsworth et al. (1);

$$\text{Efficacy (\%)} = N_0 - N / N_0 * 100$$

Where,

N_0 is the number of ticks before treatment.

N is the number of ticks after treatment.

Mortality data was analyzed through ANOVA followed by the post-hoc Tukey's test to significant factors and probit analysis was employed to calculate median lethal concentration (LC_{50}) and median Lethal time (LT_{50}) by using Minitab – 17 statistical software (23).

Results

The results of our bioassay experiments describe significant variation among the toxicity values (LC_{50}) of plant extracts and chemical acaricides (non-overlapping confidence intervals) against *H. anatolicum* and are displayed in table 1.

Table 1: LC_{50} of plant extracts and synthetic acaricides at different exposure periods against *H. anatolicum*

Plants	Time (hrs)	LC_{50} (95.0% Fiducial Cl)	SE	$\mathbf{x}^2$ (df=4)	p-value
Azadirachta indica	2	431.42(398.01–515.02)	0.909	2.123	0.000
	4	320.75(299.19–438.02)	0.863	2.596	0.000
	6	252.20(199.26–304.26)	0.727	5.477	0.000
	12	128.55(80.25–192.80)	0.569	0.161	0.038
	24	93.98(0.051–230.92)	0.567	0.235	0.068
	48	38.30(0.39–94.08)	0.585	0.454	0.068
Dalbergia sissoo	2	492.5(385.24–552.5)	1.242	0.537	0.001
	4	343.69(373.43–451.5)	1.168	0.772	0.000
	6	293.80(170.32–352.5)	0.848	2.699	0.000
	12	192.50(120.51–292.5)	0.590	0.201	0.010
	24	164.68 (103.8–209.5)	0.565	0.202	0.098
	48	58.76(20.5–79.2)	0.565	0.184	0.218
Morus alba	2	501.50(402.79–592.2)	6.209	0.177	0.116
	4	395.90 (299.8 – 435.2)	2.256	0.339	0.009
	6	339.80(279.8–435.2)	0.909	2.123	0.000
	12	230.94(192.8–301.5)	0.616	0.148	0.014
	24	198.50 (113.5 – 259.5)	0.572	0.018	0.111
	48	92.95(25.60 –135.5)	0.561	0.020	0.260
Fipronil	2	289.12(201.78–368.51)	0.900	0.166	0.000
	4	201.109(172.2–290.4)	0.637	0.150	0.008
	6	131.68(82.01–159.02)	0.584	0.070	0.021
	12	82.54(50.44–102.11)	0.569	0.299	0.024
	24	38.30(0.39–94.08)	0.585	0.454	0.068
	48	35.01(10.84–59.02)	0.757	1.472	0.002
Emamectin	2	302.79(207.6–389.9)	0.888	0.061	0.001
	4	231.78(190.76–298.5)	0.687	0.174	0.003
	6	150.72(88.62–201.76)	0.599	0.293	0.007
	12	98.12(50.71–150.45)	0.575	0.219	0.005
	24	81.85(12.98–156.55)	0.576	0.031	0.011
	48	46.87(17.73–75.60)	0.684	3.377	0.000
Cypermethrin	2	299.14(211.78–392.9)	1.242	0.537	0.001
	4	219.80(162.8–307.57)	0.706	0.062	0.001
	6	142.82(102.8–216.8)	0.607	0.077	0.008
	12	95.74(49.9–122.54)	0.572	0.313	0.028
	24	81.85(12.98–156.55)	0.568	0.544	0.088
	48	37.83(06.14–74.15)	0.625	0.487	0.010

At minimum exposure time (2hrs), LC_{50} values of *A. indica, D. sissoo* and *M. alba* were 431.42, 492.50, 501.5 ppm and that of fipronil, emamectin and cypermethrin were 289.12, 302.79, 299.14 ppm while after maximum exposure time (48hrs), plant extracts showed 38.30, 58.76, 92.95 ppm and synthetic acaricides showed 35.01, 46.87, 37.83 ppm respectively. The LC_{50} value 38.30 ppm shown by *A. indica* after exposure period of 48h was significantly very close to the LC_{50} values of synthetic acaricides that caused significant mortality of *H. anatolicum* as compared to *D. sissoo* and *M. alba* as shown in table 1.

Table 2: LT_{50} of plant extracts and synthetic acaricides at various concentrations against *H. anatolicum*

Plants	Concentration (ppm)	LT_{50} (95.0% Fiducial Cl)	SE	$\mathbf{x}^2$ (df=4)	p-value
A. indica	50	32.31(21.57-63.99)	0.3022	3.644	0.000
	100	19.71(12.92-38.10)	0.2489	0.920	0.001
	250	16.20(10.61-29.69)	0.2416	0.657	0.000
	500	10.91(7.36-16.85)	0.2378	0.602	0.000
	750	8.49(5.76-12.30)	0.2365	0.308	0.001
D. sissoo	50	44.44(29.17-94.92)	0.3614	2.199	0.000
	100	30.24(20.60- 56.25)	0.3000	0.815	0.0001
	250	25.84(17.38-48.82)	0.2736	0.541	0.000
	500	20.76(13.95-38.07)	0.2565	0.880	0.000
	750	16.97(11.30-30.46)	0.2450	0.401	0.000
M. alba	50	49.50(32.67-106.49)	0.4119	3.019	0.000
	100	44.44(29.17-94.92)	0.3614	2.199	0.000
	250	38.90(25.73-80.23)	0.3312	2.210	0.000
	500	34.85(22.58-75.00)	0.2950	0.923	0.000
	750	29.17(18.88-61.94)	0.2721	0.733	0.000
Fipronil	50	22.43(15.67-38.15)	0.2767	1.522	0.0001
	100	15.28(11.10-22.74)	0.2634	1.056	0.000
	250	11.60(8.68-16.01)	0.2610	0.793	0.000
	500	8.31(6.37-10.80)	0.2640	1.659	0.000
	750	6.53(4.96-8.42)	0.2599	2.584	0.0001
Emamectin	50	31.01(20.56-62.13)	0.2901	1.546	0.000
	100	21.27(14.82-36.10)	0.2694	0.375	0.000
	250	14.03(10.19-20.65)	0.2581	0.302	0.0001
	500	9.37(7.16-12.35)	0.2629	0.794	0.000
	750	7.37(5.65-9.50)	0.2636	3.703	0.000
Cypermethrin	50	29.17(20.31-51.52)	0.3106	2.639	0.000
	100	20.66(14.93-32.39)	0.2845	1.420	0.0001
	250	15.96(11.67-23.70)	0.2684	1.205	0.000
	500	11.60(8.68-16.01)	0.2610	0.793	0.000
	750	8.95(6.77-11.85)	0.2591	0.781	0.000

Table 2 represented LT_{50} values of plant extracts and synthetic acaricides at various concentrations against *H. anatolicum*. At minimum concentration (50 ppm), LT_{50} values of plant extracts (*A. indica, D. sissoo* and *M. alba*) were 32.31, 44.44, 49.5 hrs and that of acaricides (fipronil, emamectin and cypermethrin) were 22.43, 31.01, 29.17 hrs while at maximum concentration (750 ppm), LT_{50} values of plant extracts were 8.49, 16.97, 29.17 hrs and that of acaricides were 6.53, 7.37, 8.95 hrs respectively. Results also revealed that *A. indica* could kill *H. anatolicum* in minimum duration among the employed plant extracts that was statistically similar to the synthetic chemicals.

To check the statistical significance of the plants, chemical acaricides, different time intervals (2, 4, 6, 12, 24 and 48 hrs) and concentrations (50, 100, 250, 500 and 750 ppm), the analysis of variance (ANOVA) was applied.

Table 3: Analysis of variance (ANOVA) results

Source	df	Sum of Squares	Mean Square	F	p-value
Time	5	5198.921	1039.784	938.619	<0.001
Chemicals	2	166.980	83.49	78.16	<0.001
Plant	2	185.311	92.655	75.041	<0.001
Concentration	5	8710.566	1742.113	1618.86	<0.001
Error	735	953.949	1.298		
Total	749	15215.727			

Table 4: Reproductive parameters of control and treated groups of *Hyalomma anatolicum*

Treatments	Conc. ppm	Tick wt. (mg) (mean ± SD)	Egg mass (mg) (mean ± SD)	Fecundity = Egg mass/tick wt.	% Hatch	RI = Fecundity x % hatch
Control	dH_2O	198.8±9.06	75.8±10.6	0.38	80	30.4
A. indica	50	196.6±10.93	59±8.9	0.300	54	16.2
	100	195.4±9.74	40±7.5	0.204	43	8.77
	250	187.9±8.96	38±7.1	0.202	32	6.46
	500	202.3±11.07	32±7.1	0.158	24	3.79
	750	194.6±9.86	25±6.4	0.128	10	1.28
D. sissoo	50	196.6±10.71	63±9.9	0.320	56	17.92
	100	195.4±9.76	42±7.4	0.214	43	9.202
	250	193.3±8.67	40±7.2	0.206	33	6.80
	500	197.1±9.75	38±6.5	0.192	27	5.18
	750	194.8±9.58	37±6.2	0.189	13	2.46
M. alba	50	202.8±9.45	65±6.5	0.320	59	18.88
	100	197.3±8.51	46±6.6	0.233	46	10.72
	250	195.4±9.85	44±6.5	0.225	32	7.2
	500	201.1±11.09	43±6.1	0.213	30	6.39
	750	196.1±8.91	40±5.8	0.204	14	2.856
Fipronil	50	198.3±10.94	21±8.9	0.105	25	2.63
	100	197.7±9.76	16±7.5	0.080	17	1.36
	250	188.6±8.96	9±7.1	0.047	8	0.376
	500	201±10.97	0±7.1	0	0	0
	750	196.4±9.95	0±6.4	0	0	0
Emamectin	50	197.8±10.95	29±9.9	0.146	29	4.23
	100	199.6±8.77	18±7.4	0.090	18	0.162
	250	189.9±8.65	12±7.2	0.063	4	0.252
	500	198.3±10.91	0±6.5	0	0	0
	750	198.9±9.55	0±6.2	0	0	0
Cypermethrin	50	202.9±10.48	45±6.5	0.221	29	6.409
	100	193.9±9.67	32±6.6	0.165	17	2.805
	250	192.5±9.73	23±6.5	0.119	8	0.952
	500	201.7±10.44	10±6.1	0.049	3	0.147
	750	197.6±9.86	0±5.8	0	0	0

The results presented in Table 3 revealed that all the three plants, three synthetic acaricides, times and concentrations were significantly different (p-values <0.001). Then we applied Tukey's test to see which plant and acaricide provide highest mortality. The results revealed that *A. indica* was statistically significant (p-value = 0.04) from *M. alba* and *D. sissoo* and it provided highest mortality. Tukey's test revealed that fipronil was statistically insignificant (p-value = 0.99) from cypermethrin and significant (p-value = 0.02) differences were noted from emamectin. Tukey's test was also applied to time intervals, the mortality was found to be statistically significant (p-value = 0.02). For the concentrations with the control, Dunnet's test was applied. The results of Dunnet's test showed that the mortality in all the concentrations (50, 100, 250, 500 and 750 ppm) was significantly higher than the control group (water only).

The reproductive parameters of ticks treated with different concentrations of plant extracts and synthetic acaricides were shown in table 4. This table showed that all plant extracts and synthetic acaricides showed excellent results in lowering reproductive index at high concentrations (250, 500 and 750 ppm). All three acaricides even with low concentrations were as effective in tick mortality as higher concentrations, however plant extracts were not very effective at lower concentrations (50 and 100 ppm) as shown in table 4.

Table 5: Phytochemical analysis of plants extracts tested against *H. anatolicum*

Phytochemical	Plant extracts		
	Azadirachta indica	*Dalbergia sissoo*	*Morus alba*
Alkaloids	_	_	_
Flavonoids	+++	+	+++
Steroids	++	_	++
Terpenoids	+	++	++
Saponins	+++	+	+
Tannins	++	++	++
Phenols	_	+++	++

(-) Not detected, (+) Low in concentration, (++) Moderate, (+++) High in concentration

The phytochemical analysis of plant extracts used in our bioassay experiments revealed that saponins and flavonoids showed highest scoring in neem extract while tannins and steroids indicated moderate scores (Table 5). Phenols represented highest scores in *Dalbergia sissoo* extract, while tannins and terpenoids showed moderate scoring. Phytochemical analysis revealed highest scoring of flavonoids and moderate scoring of steroids, terpenoids, tannins and phenols. Alkaloids were not detected in the extracts of selected plants.

Discussion

In the present study methanolic extracts of three locally existing plants *Azadirachta indica* (Neem), *Dalbergia sissoo* (Sheesham), *Morus alba* (Shehtoot) and three synthetic acaricides fipronil, emamectin and cypermethrin were employed to evaluate the mortality of *H. anatolicum* under laboratory conditions. Maximum mortality of *H. anatolicum* was observed after exposing these ticks for a period of 48h at 750 ppm concentration and minimum mortality was recorded at least time duration (2h) and concentration (50 ppm).

Magadum et al. (24) evaluated the efficacy of *Azadirachta indica* and *Annona squamosa* extracts against *Rhipicephalus* (syn. *Boophilus*) *microplus* in India. They observed 71 % efficacy with *A. squamosa* extracts against the *R. microplus* by in vivo but in vitro methods showed more efficacy of *A. indica* extracts than the extracts of *A. squamosa*. These results are related to the present in vitro outcomes in which *A. indica* was highly effective against *H. anatolicum* than *D. sissoo and M. alba.*

In our study, *A. indica* showed lethal effects against *H. anatolicum* and these results are related to the investigations of Zaman et al. (25) who evaluated the anti-tick efficacy of combined aqueous herbal extracts of *A. indica* leaves, *Nicotiana tabacum* leaves, *Calotropis procera* flowers and *Trachispermum ammi* seeds against the *Rhipicephalus* (*Boophilus*) *microplus* using adult immersion, larval packet and ear bag method. They

stated that the extract exhibited lethal effects on egg laying, hatching and total larval mortality.

Parte et al. (26) screened the acaricidal activity of aqueous extracts of *Azadirachta indica, Mangifera indica, Polyalthia longifolia, Annona squamosa* and *Ficus benghalensis* against the *Rhipicephalus* (*Boophilus*) *microplus*. They observed that the combination of five plant extracts showed 100 percent mortality as compared to individual plant extracts. Furthermore, they concluded that extended exposure of the target pest to individual plant extract is required to obtain 100 percent mortality. Increased mortality of ticks was also observed in present studies with the increase of exposure time at the same concentration of employed plant extract.

Results of *M. alba* leaves extract exhibited a moderate acaricidal activity against *H. anatolicum*. Percentage mortality of the test ticks evaluated for a concentration of 750 ppm after periods of 24h and 48h were 50% and 66.67%, respectively. Data represented that mortality of ticks was time and concentration dependent. Mortality increased with the increase of concentration and time of exposure. The results obtained in this study are supported by Dantas et al. (27) who studied the acaricidal activity of crude ethanolic extract and fractions from the leaves of *Morus nigra* on female cattle ticks *Rhipicephalus microplus*, using the adult immersion test. The mortality and fertility of females exposed to different concentrations of hexane, chloroform and ethyl acetate fractions, as well as ethanolic extract of *M. nigra.* The chloroform extract of leaves of *M. nigra* (25 mg/mL) showed the best results, obtaining 62.6% of inhibition of oviposition, 39.3% of eggs eclosion average and 65.4% of effectiveness.

D. sissoo leaves extract showed mean mortality of ticks after 24h and 48h periods as 48.67% and 58.67% respectively. Highest mean mortality of ticks (42.22%) was observed at 750 ppm concentration. Mortality of ticks was found to be dependent on exposure time and concentration of extract applied. These results are in line with those obtained by Singh et al. (28). They evaluated mortality and fecundity of *Rhipicephalus* (*Boophilus*) *microplus* exposed to Sheesham leaf aqueous (SLA) and ethanolic (SLE) extracts. Higher acaricidal activity was recorded in SLA with a lower LC_{50} (95% CL) value of 1.58% than SLE (5.25%).

Synthetic acaricide, cypermethrin showed a remarkable efficacy against *H. anatolicum* and the mortality was increased with increase in acaricides concentration and exposure periods. The mean mortality of ticks observed after 24h period was 58.00% and mortality recorded after 2 days (48h) was 73.33%. Similar investigation was performed by Khalaf-Allah (29), who reported 100% effectiveness of cypermethrin against *R. annulatus* up to 7 weeks of post-treatment after which the efficacy was dropped to 98 %. Sajid et al. (30) also investigated the pour-on preparations of cypermethrin which showed a higher in vivo efficacy compared to ivermectin against *Hyalomma anatolicum anatolicum* at 15 days post-treatment interval.

Burridge et al. (31) employed eight acaricides (amitraz, carbaryl, chlorpyrifos, cyfluthrin, fipronil, permethrin, pyrethrin, and selamectin) for their efficacy in the rapid killing of *Rhipicephalus sanguineus* (Acari: Ixodidae). *R. sanguineus* was most sensitive to fipronil, carbaryl and cyfluthrin. Our findings also suggest that fipronil possesses greater potential to control ticks and has caused 84% mortality of *H. anatolicum* when compared with emamectin and cypermethrin.

Our results indicated that all plant extracts inhibit oviposition and reduce hatching percentage depending on concentration of extracts. These results are in line with those of Rawani et al. (32) who also noted these deterrent properties in *Carica papaya* against ticks. Roobakkumar et al. (33) used garlic extract and noted more than 70% mortality in ticks with reduced oviposition and hatching percentage of surviving parents. These results are different from Kalakumar et al. (34) and Borges et al. (35) who noted mortality (more than 60%) but failed to record the inhibition of oviposition and reduction in hatching percentage of ticks with neem extract treatment. Our results are also at par with the results of Shyma et al. (36) who noted 0 to 50% reduction in hatching with neem, calotropis, datura, garlic and papaya plant extracts.

Conclusion

Plants presented a significant mortality of *H. anatolicum* but less than that of synthetic chemicals. From above observations it has been concluded that plant extracts could effectively control ticks population when applied on house hold animals as well as farm animals. As synthetic acaricides cause toxicity in environment, affect animal health and may develop resistance in ticks against these chemicals. It is recommended

to encourage the use of plant extracts instead of synthetic chemicals to control ticks population on animals. These botanical pesticides could efficiently control ticks population without posing any health risk to the animals.

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PRIMERJAVA UČINKOVITOSTI SINTETIČNIH KEMIKALIJ IN RASTLINSKIH EKSTRAKTOV ZA NADZOR NAD KLOPI

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Izvleček: Klopi veljajo za škodljive in ekonomsko pomembne ektoparazite, kajti njihova okužba po vsem svetu hudo prizadane govedo na paši. Zatiranje klopov s sintetičnimi akaricidi ni nevarno samo za zdravje živali in ljudi, temveč povzroča tudi onesnaževanje okolja. Študija je bila zasnovana z namenom ovrednotenja rastlinskih izvlečkov v primerjavi s sintetičnimi akaricidi za nadzor nad *Hyalomma anatolicum*. Za oceno umrljivosti klopov po 2, 4, 6, 12, 24 in 48 urah je bilo uporabljenih pet različnih koncentracij (50, 100, 250, 500 in 750 ppm) metanolnih rastlinskih izvlečkov in akaricidov. Podatki o smrtnosti so bili analizirani z analizo Probit za izračun srednje smrtne doze (LC_{50}) in srednjega časa smrti (LT_{50}). Metanolni ekstrakt iz *Azadirachta indica* je pokazal najvišjo umrljivost (LC_{50}=38,3 ppm) klopov v primerjavi z *Dalbergia sissoo* (LC_{50} =58,76 ppm) in *Morus alba* (LC_{50}=92,95 ppm). Med akaricidi je imel fipronil največji učinek smrtnosti (LC_{50}=35,01 ppm) v primerjavi z emamektinom (LC_{50}=46,87 ppm) in cipermetrinom (LC_{50}=37,83 ppm). Višja koncentracija (750 ppm) akaricidov (fipronil, emamektin in cipermetrin) je pokazala hitrejšo smrtnost (LT_{50}=6,53-8,95 ur) v primerjavi z rastlinskimi ekstrakti (LT_{50}=8,49-29,17 ur). Učinke zdravljenj so preučevali tudi na jajčnih masah in obravnavali reproduktivni indeks (RI) preživelih klopov. Rezultati so pokazali pomembno, koncentracijsko odvisno variacijo med jajčnimi masami, obdelanimi z rastlinskimi izvlečki in akaricidi. Posledično so se vrednosti njihovega reproduktivnega indeksa znatno zmanjšale. Fitokemijska analiza preizkušenih rastlinskih izvlečkov je razkrila prisotnost flavonoidov, steroidov, terpenoidov, saponinov, taninov in fenolov v spremenljivih količinah. Rezultati opravljene raziskave opisujejo pomembne lastnosti okolju prijaznih rastlinskih izvlečkov pri preprečevanju napadov klopov.

Ključne besede: rastlinski izvlečki; sintetični akaricidi; smrtnost klopov