The Pharma Innovation Journal 2022; SP-11(7): 731-734

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 731-734

www.thepharmajournal.com
Received: 15-04-2022
Accepted: 21-05-2022

Vikram Punia
Department of Veterinary Parasitology CVAS, Navania, Udaipur, Rajasthan, India

Hakim Manzer
Assistant Professor, Department of Veterinary Parasitology CVAS, Navania, Udaipur, Rajasthan, India

Jitendra Kumar
Department of Veterinary Parasitology CVAS, Navania, Udaipur, Rajasthan, India

Anita Rathore
Assistant Professor, Department of Veterinary Pathology, CVAS, Navania, Udaipur, Rajasthan, India

Pravin Bano
Department of Veterinary Pathology, CVAS, Bikaner, Rajasthan, India

Corresponding Author
Vikram Punia
Department of Veterinary Parasitology CVAS, Navania, Udaipur, Rajasthan, India

In vitro study on acaricidal efficacy of *Azadirachta indica* (Neem) methanol extracts against *Rhipicephalus* (B.) *microplus* in Udaipur (Rajasthan)

Vikram Punia, Hakim Manzer, Jitendra Kumar, Anita Rathore and Pravin Bano

Abstract
Tick and tick-borne pathogens affect 80% of the world's cattle population and are widely distributed throughout the world, particularly in the arid and semi-arid area, majorly affecting livestock production and productivity. The present research was designed to evaluate acaricidal efficacy of leaves, seeds and bark of Azadirachta indica (Neem) in methanol extracts against cattle tick *Rhipicephalus (B.) microplus* in Udaipur (Rajasthan). Four concentrations of the A. indica extract (10%, 25%, 50%, and 70%) and one control group with twice replications for each concentration were used in the bioassay. The Highest efficacy in both *in vitro* test (LPT and AIT) was recorded in methanolic extract of A. indica seeds. The A. indica seeds, leaves and bark in methanoilc extracts has highest efficacy at 70% concentration which was 84%, 63% and 50.5% respectively in LPT after 24 hrs of treatment. Minimum mortality was at 10 mg/ml (12.5%, 8% and 7.5%) respectively. In LPT, increasing mortality of larvae with increasing concentrations of Neem methanol extracts was observed. In AIT methanolic seed, leaves and bark extracts, the percentage inhibition of oviposition was 84.91%, 54.41% and 52.15% respectively at 70 mg/ml concentration. Minimum inhibition of oviposition (IO%) 40.21%, 22.62% and 9.38% in seed, leaves and bark extracts respectively at 10 mg/ml.

Keywords: *Azadirachta indica*, acaricidal activity, *Rhipicephalus* (*Boophilus*) *microplus*, Adult Immersion Test (AIT) and Larval Packet Test (LPT)

1. Introduction
Neem (*Azadirachta indica*) is one of commonly grown indigenous plants in domestic backyards in India, neem tree is mostly found in Rajasthan regions. The tree contains bioactive ingredients and more than 100 chemical compounds have been identified so far, but the most effective bio active compounds are azadiractin and salamin. The bioactive components are generally extracted in organic solvents particularly methanol. The insecticidal effect of neem has been proved to be efficient to control several insect importance including more than 540 insect species belonging to Arachnida, Diptera, Coleoptera, Homoptera and Hemiptera.

Parasitic diseases are a global problem and considered as a major obstacle in the health and product performance of animals. Amongst many parasites infesting livestock; ticks are obligate, blood-feeding ectoparasites of vertebrates belonging to the class Arachnida, Globally the ticks are second to mosquitoes as vector of infectious pathogens to humans and animals. They transmit diseases like babesiosis, theileriosis, anaplasmosis etc. In the past two decades, the incidence of ticks borne diseases has increased and poses major public and animal health problems that essentially require the strategic tick control methods. These conventionally used acaricidal and larvicidal drugs are responsible for drug resistance after prolonged use. In the past years, research studies on plants have been carried out to prospect bioactive molecules with acaricidal properties because they are rapidly degraded, decrease poisoning of human applicators and non-target organisms, reduce environmental contamination, and decrease the development of resistance to these substances. The herbal acaricdes also provide cost effective alternative to chemical acaricidals. Several herbal agents are well known for their larvicidal and acaricidal potency. Neem is one of them. The current study was carried out to evaluate potency of neem against cattle tick *Rhipicephalus (Boophilus) microplus* in Vallabhnagar tehsil of Udaipur district in Southern Rajasthan.

2. Materials and Methods

2.1 Study methodology

Ticks (Male and female) were collected from cattle randomly during morning hours from Vallabhnagar tehsil of Udaipur District Rajasthan. In the laboratory the ticks were subjected to mounting and morphological identification. Engorged female tick *R. (B.) microplus* was subjected to AIT and larval hatching for LPT.

2.2 Collection, Identification and Processing of Plant Materials

2.2.1 Collections and processing of plant material

Plant materials were selected on the basis of available scientific literature and their traditional use with the villagers. The parts of plant like leaves bark and seeds of *A. indica* (Neem) were collected from the fields, road side and gardens in and around Vallabhnagar tehsil of Udaipur (Rajasthan).

2.2.2 Preparation of organic solvent extract

The leaves, seeds and bark of *Azadirachta indica* (Neem) were brought into the laboratory washed with distilled water and plant materials were shade-dried and crushed in grinder to make a powdery consistency The powder was sieved through a mesh (2mm size) and store in a tight zip poly bag. The powder prepared from different part of plant were labelled properly.

The powder of *Azadirachta indica* (Neem) seeds, leaves and bark parts was processed for extract preparation by using maceration methods (Shyma *et al.*, 2014) [4]. 50 gm powder from extracted using 400 ml methanol solvents. The mixtures were kept for 2 days in tightly sealed vessels at room temperature and stirred several times daily with a sterile glass rod. These mixtures were filtered through muslin cloth. Further extraction of residue was done repeating 3-5 times until a clear colorless supernatant extraction liquid was present indicating that no more extraction from the plant material was possible. The extracted liquid was subjected to water bath evaporation at 45˚C to remove the solvent. The semi-solid extract was kept under a ceiling fan to dry. The extracts were collected in beakers. The beakers were sealed with perforated aluminum. The extract residues were individually marked, kept in airtight glass beakers in the cool and dark place at 4ºC (refrigerator) for further use. Different concentrations (10%, 25%, 50% and 70%) of each extract residues (Leaves, Bark and Seeds) were prepared in methanolic solvent of *Azadirachta indica* (Neem) plant extract. Controls were run side by side for each solvants.

2.3 Larval Packet Test

The larval packet test was performed as per FAO, (2004) [1] to determine the *In vitro* acaricidal activity of the test compounds. Engorged female ticks were obtained from the cattle in the study area, identified, cleaned, stored in a Petri dish and maintained at 85-92% RH. and 27.0 ±1.0 ºC. The female ticks were examined daily until oviposition. The eggs were separated and allowed to hatch in glass vials with cotton plug and kept in optimal conditions. The obtained seed ticks were maintained at 27.0 ±1.0 ºC and 85-92% RH. for 14-21 days. The larvae aged between 14 to 21 days were subjected to larval packet test. Packets made of Whatman filter paper No. 1 (12 cm x 18 cm) was impregnated Different concentrations (10%, 25%, 50% and 70%) with control group and dried at room temperature for two hours. About 100 larvae are placed in acaricide impregnated filter paper packet and the top of the packet was sealed with white tape. The close packets were incubated at 27.0 ± 1.0 ºC and 85-92% R.H for 24 hours. After 24 hours, observations for mortality were made by counting the dead and live larvae. All non-motile tick larvae were counted to be dead. The data obtained was calculated as percentage mortality at each concentration of acaricide.

The percentage mortality in all of the experimental batches of larvae will be corrected by applying Abbott's formula.

$$\text{Percent mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \text{x}100$$

2.4 Adult immersion test (AIT)

The AIT was conducted according to FAO, (2004) [1] guidelines. The engorged female ticks from the field was washed thoroughly thrice with distilled water and kept for drying on filter paper and weighed. The ticks were immersed in each crude extract of plant material for 5 min. The control group was immersed in water. The ticks were then placed on Petri dishes over Whatman filter paper no.1. All the Petri dishes with treated ticks were kept at room temperature for 24 h. After 24 h, ticks were transferred to glass vials covered with muslin cloth and kept in desiccators having 85±2 % relative humidity and placed in BOD incubator at 28±2 °C. These ticks were observed for oviposition and mortality. The percent adult tick mortality and the weight of the eggs laid by the treated ticks were recorded in comparison with the control. The eggs incubated at the same condition, and the percentage of hatched eggs is estimated visually. The index of egg laying and percentage inhibition of fecundity was calculated.

$$\text{Reproductive index} = \frac{\text{Weight of egg laid (mg)}}{\text{Weight of adult females (mg)}}$$

$$\text{Inhibition of oviposition (IO \%)} = \frac{\text{RI (control group)} - \text{RI (treated group)}}{\text{RI (control group)}} \text{x}100$$

2.5 Statistical analysis

The collected data from the experiment was subjected to statistical analysis using SPSS, version 20.0 for analysis of variance (Snedecor and Cochran, 1980) [5]. The treatment means were compared by Duncan's multiple range test at 5% level of significance ($P<.05$).

3. Results

In the present study larvae were used for performing "Larval Packet Test" (LPT). The viability of 100 larvae was examined against 12 treatment groups as compared to control group. The results for LPT showed methanolic extract of *Azadirachta indica* (Neem) seeds, leaves and bark were used in four different concentration for LPT (70%, 50%, 25% and 10%). with twice replications for each concentration were used in the bioassay. Then Acaricidal efficacy of plant extracts was estimated by using Adult Immersion Test (AIT) treatment and control groups.

3.1 Larval Packet Test (LPT)

A modified version of FAO, (2004) [1] Larval Packet Test (LPT) was used to determine the acaricidal activity against *R.* (*B.*) *microplus* with various concentrations of methanolic

extracts of seed, leaves and bark of *Azadirachta indica*. The concentrations of methanolic extracts of seed, leaves and bark of *A. indica* of varied from 10 to 70 mg/ml. Methanolic seed, leaves and bark extracts showed higher mortality seed (84%), followed by leaves (63%) and bark (50.5%) respectively at 70mg/ml concentration among the three extracts of *A. indica* under evaluation. Minimum mortality was at 10 mg/ml (12.5%, 8% and 7.5%) respectively. The LPT showed that there was increasing mortality of larvae with increasing concentrations of plant extracts where no mortality in the control group could be observed. (Table.1).

Srivastava *et al*., 2008 [6] observed that out of eight plant extract, *A. indica* seed extract was most effective (80%) after 5 h of treatment. The extract from seed showed higher larvicidal and acaricidal potency because highest levels of the active ingredients that can be used as bio insecticides. The larvicidal and acaricidal effect of neem seed are probably due to azadirachtin and triterpenoids, salannin, nimbin. Among the different active components, azadirachtin is the most important active principle.

Table 1: The results larval packet test (LPT) to methanol extracts *Azadirachta indica* (Seed, leaves and bark) against *R. (B.) microplus*

Product	Concentration of extract (mg/ml)	Live larvae	SE	Dead larvae	SE	% of Larval mortality	SE
A. indica (Seed) extract	Control	100^{e}	0	0^{a}	0	0^{a}	0
	70	16^{a}	2	84^{e}	2	84^{e}	2
	50	40^{b}	1	60^{d}	1	60^{d}	1
	25	68^{c}	2	32^{c}	2	32^{c}	2
	10	87.5^{d}	0.5	12.5^{b}	0.5	12.5^{b}	0.5
A. indica (leaves) Extract	Control	100^{e}	0	0^{a}	0	0^{a}	0
	70	37^{a}	1	63^{e}	1	63^{e}	1
	50	54.5^{b}	0.5	45.5^{d}	0.5	45.5^{d}	0.5
	25	75.5^{c}	1.5	24.5^{c}	1.5	24.5^{c}	1.5
	10	92^{d}	1	8^{b}	1	8^{b}	1
A. indica (bark) Extract	Control	100^{e}	0	0^{a}	0	0^{a}	0
	70	49.5^{a}	0.5	50.5^{e}	0.5	50.5^{e}	0.5
	50	62.5^{b}	1.5	37.5^{d}	1.5	37.5^{d}	1
	25	81.5^{c}	0.5	18.5^{c}	0.5	18.5^{c}	0.5
	10	92.5^{d}	0.5	7.5^{b}	0.5	7.5^{b}	0.5

*Means bearing different superscript in the same column differ significantly $P<.05$

3.2 Adult Immersion Test

AIT was used in present study to determine the acaricidal activity against *R. (B.) microplus* with various concentration of *Azadirachta indica* (seed, leaves and bark) of methanolic extract; FAO, (2004) [1]. The different concentrations of methanolic extract various from 10 to 70 mg/ml. In AIT the dependent decrease in reproductive index and increase in inhibition of oviposition was observed from concentration 10 to 70 mg/ml. A significant percentage inhibition of oviposition (IO%) at 70%, 50%, 25% and 10% mg/ml the extracts were seed (84.91, 77.28, 64.18 and 40.12), leaves (54.41, 48.95, 39.14 and 22.62) and bark (52.15, 37.08, 18.56 and 9.38) % respectively. No mortality of ticks was observed at any concentration, as shown in (Table. 2).

Maske *et al*., 1995 [2] reported herbal preparation containing extract of *A. indica* resulted in 100%, 80% and 70% efficacy against all stages (larvae, nymph and adult) of *R. microplus*. Micheletti *et al.*, (2009) [3] reported a mortality of 65% on use of neem leaves. Shyma *et al.*, (2014) [4] reported 33.33 % mortality and 20.73 inhibition of oviposition at the highest concentration of *A. indica* extract-treated ticks.

Table 2: Acaricidal efficacy of different concentrations methanolic extracts of *Azadirachta indica* (seed, leaves and bark) on *R. (B.) microplus*

Product	Conc. of extract (mg/ml)	Live ticks weight (gm) (Mean)	(SE)	Weight of eggs laid (gm) (mean)	(SE)	Repro-duction Index (RI) (Mean)	(SE)	%IO (Mean)	(SE)
A. indica (seed) extract	Control	0.76^{b}	0.040	0.355^{c}	0.025	0.46^{d}	0.010	0^{a}	0.000
	70	0.485^{a}	0.015	0.035^{c}	0.015	0.07^{a}	0.030	84.915^{d}	6.195
	50	0.465^{a}	0.025	0.05^{a}	0.010	0.105^{ab}	0.025	77.28^{cd}	4.940
	25	0.39^{a}	0.010	0.065^{a}	0.005	0.165^{b}	0.015	64.18^{c}	2.480
	10	0.47^{a}	0.030	0.13^{b}	0.010	0.275^{c}	0.005	40.21^{b}	0.210
A. indica (leaves) extract	Control	0.76^{ab}	0.040	0.355^{c}	0.0250	0.46^{c}	0.010	0^{a}	0.000
	70	0.55^{a}	0.050	0.1145^{a}	0.0005	0.21^{a}	0.020	54.415^{c}	3.355
	50	0.735^{ab}	0.065	0.1735^{a}	0.0265	0.235^{a}	0.015	48.955^{c}	2.155
	25	0.91^{bc}	0.060	0.2535^{b}	0.0075	0.28^{a}	0.010	39.145^{c}	0.855
	10	1.15^{c}	0.100	0.4045^{c}	0.0045	0.355^{b}	0.035	22.62^{b}	9.290
A. indica (Bark) extract	Control	0.7600	0.040	0.355^{d}	0.0250	0.46^{c}	0.0100	0^{a}	0.000
	70	0.7550	0.0250	0.1665^{a}	0.0065	0.22^{a}	0.0010	52.15^{e}	0.820
	50	0.7400	0.060	0.215^{a}	0.0250	0.2895^{a}	0.0105	37.085^{d}	0.915
	25	0.7350	0.035	0.2755^{bc}	0.0165	0.3745^{b}	0.0045	18.565^{c}	0.795
	10	0.8050	0.015	0.336^{cd}	0.0200	0.417^{bc}	0.0170	9.38^{b}	1.730

*Means bearing different superscript in the same column differ significantly $P<.05$

4. References

1. FAO. Resistance management and integrated parasite control in ruminants: Guidelines Animal production and health division, Food and agriculture organization of the united nation Rome, 2004, 25-77.
2. Maske DK, Bhilegaonka NG, Jangde CR. Treatment of

tick infestation in cattle. Indian J. Indigenous Med. 1995;17(2): 81-83.
3. Micheletti SM, Valente EC, de Souza LA, Dias NS, Araujo M. Plant extracts in control of Rhipicephalus (Boophilus) microplus (Acari: Ixodidae) in laboratory. Rev. Bras. Parasitol. Vet. 2009;18(4):44-8.
4. Shyma KP, Gupta JP, Ghosh S, Patel KK, Singh V. Acaricidal effect of herbal extracts against cattle tick *R. (B.) microplus* using *in vitro* studies. Parasitol. Res. 2014;113(5):1919-1926.
5. Snedecor GB, Cochran WG. Statistical methods. 7th edition, lowa state university press, Ames, 1980.
6. Srivastava R, Ghosh S, Mandal DB, Azhahianambi P, Singhal PS, Pandey NN, *et al*. Efficacy of *A. indica* extracts against *B. microplus*. Parasitol. Res. 2008;104(1):149-153.