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Anuj Dixit
Department of Veterinary Parasitology, College of Veterinary & Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India

Hakim Manzer
Department of Veterinary Parasitology, College of Veterinary & Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India

Sanweer Khatoon
Department of Veterinary Parasitology, College of Veterinary & Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India

Anita Rathore
Department of Veterinary Pathology, College of Veterinary & Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India

Bhagat Singh Saini
Department of Veterinary Physiology and Biochemistry, College of Veterinary & Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India

Jitendra Kumar
Department of Veterinary Parasitology, College of Veterinary & Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India

Shyoji Ram Nagar
Department of Veterinary Parasitology, College of Veterinary & Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India

Corresponding Author
Hakim Manzer
Department of Veterinary Parasitology, College of Veterinary & Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India

In-vitro study on acaricidal efficacy of methanolic extracts of *Datura stramonium* against cattle tick (*Rhipicephalus* (*B.*) *microplus*) in Udaipur (Rajasthan)

Anuj Dixit, Hakim Manzer, Sanweer Khatoon, Anita Rathore, Bhagat Singh Saini, Jitendra Kumar and Shyoji Ram Nagar

Abstract
The present research was designed to evaluate acaricidal efficacy of methanolic extracts of *Datura Stramonium* against cattle tick *Rhipicephalus* (*Boophilus*) *microplus* in Udaipur (Rajasthan). Acaricidal efficacy was evaluated Larval packet Test (LPT) and Adult Immersion Test (AIT). Four concentrations of the *D. Stramonium* extract (12.5%, 25%, 50%, and 100%) and one control group with twice replications for each concentration were used in the bioassay. The Highest efficacy in both in-vitro tests (AIT and LPT) was recorded in 100% methanolic extracts of *D. stramonium.* The methanolic extracts of *D. stramonium* has highest IO% at 100% concentration which was 67.46% in AIT. The 100% concentrations of methanolic extracts showed minimum reproductive index (0.16). In AIT the decrease in reproductive index with increase in the percent inhibition of oviposition, was evident during the study. In LPT, methanolic extracts of *D. stramonium* has highest efficacy at 100% concentration which was 64%. Larval mortality increased with increasing concentrations of plant extracts.

Keywords: *D. stramonium*, ticks, larval packet test (LPT) and adult immersion test (AIT)

1. Introduction
India is predominantly an agricultural country where livestock and agriculture are closely associated with each other. For control of the economically important tick species, organophosphates, pyrethroids and macrocyclic lactones are used at frequent intervals leading to the development of resistance in ticks to most of these chemicals. In Indian subcontinent, the incidence of acaricide resistance has been widely reported in one host ticks of the genus Boophilus (Singh *et al.* 2012; Kumar *et al.* 2013; Shyma *et al.* 2013) [11, 7, 10]. In order to tackle the problem of resistance and other environmental issues linked with chemical control, efforts have been made to develop sustainable immunological means for controlling ticks and tick-borne diseases through alternative ecofriendly anti-tick natural products. Amongst the natural products, plant extracts and essential oils have been shown to have significant activity against economically important tick species like *Rhipicephalus* (*Boophilus*) *microplus*. (Kamaraj *et al*., 2010; de Souza Chagas *et al*., 2012; Juliet *et al*., 2012; Sunil *et al*., 2013) [7, 2, 6, 13]. The plant extracts can be made from easily available local plants which have history of some pesticidal or medicinal properties and traditionally used by the people. The plant like *Datura stramonium* was taken into consideration during this study. All parts of *Datura* plants contain high levels of the tropane alkaloids atropine, hyoscyamine and scopolamine which are classified as deliriants, or anticholinergics (Arnett., 1995) [1].

2. Materials and Methods
Plant materials were selected on the basis of available scientific literature and brought into the laboratory. After complete drying, the plant material was powdered in mortar pestle and grinder. The powder of *Dhatura Stramonium* parts was processed for extract preparation by using maceration methods (Shyma *et al.*, 2014) [9]. 100 gm powder from extracted using 400 ml methanol as 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 40 ˚C to remove the solvent. The semi-solid extract was kept under a ceiling fan to dry.

3. Collection of ticks

The ticks were collected from animals and the cattle sheds during early morning from Vallabhnagar tehsil of Udaipur district (Rajasthan). The ticks collected were preserved in 70% alcohol in clean, well-stopper glass vials and labeled properly. Permanent mounts of ticks were prepared as per standard keys. The damaged and discolored ticks were removed and remaining engorged female ticks were selected for AIT. The engorged female ticks collected from a particular area were labelled and kept individually in labeled glass tubes covered with muslin cloth. Ticks were put in desiccators maintained at room temperature and 85±5% relative humidity (RH) for oviposition. The eggs were collected after 7 days from commencement of incubation. Each tube containing the first week egg production was labeled to ensure the selection of more uniform batch of larvae for each LPT. The eggs laid were allowed to hatch under uniform conditions of incubation and 14- 21days old unfed larvae were utilized for the performance of Larval Packet Test (LPT).

3.1. Preparation of working solutions of plant extracts

The working solutions of plant extracts were prepared as per standard protocol. Dried powder was kept in room temperature for 15-20 minutes. Required quantity of extracts were weighed and dissolved in methanol for making four different dilutions at the rate of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml. In control group only distilled water was used.

4. 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) [12]. The treatment means were compared by Duncan's multiple range test (Duncan, 1995) [3] at 5% level of significance ($P<.05$).

5. Results and Discussion

5.1. Efficacy of methanolic extracts of *Datura stramonium* in Larval Packet Test (LPT)

The concentrations of methanolic extracts of seeds of *Datura stramonium* of varied from 12.5 to 100 mg/ml. A total of four treatment groups with three replications and one control group were used in experiment. In each replication 100 larvae were used for experiment and the average of the three was calculated. The peak mortality (64%) was recorded at a concentration of 100 mg/ml. A significant larval mortality produced by application of extracts of 50 mg/ml, 25 mg/ml and 12.5 mg/ml were 56.33%, 44.33% and 18.67% respectively (Table 1). With the increase in concentration level the percent mortality rate also increased.

Table 1: A significant larval mortality produced by application of extracts of 50 mg/ml, 25 mg/ml and 12.5 mg/ml were 56.33%, 44.33% and 18.67% respectively

Concentration of extract (mg/ml)	Live larvae	SE	Dead larvae	SE	Percent of Larval mortality	SE
Control	100.000^{e}	0.000	0.000^{a}	0.000	0.000^{a}	0.000
100	36.000^{a}	2.646	64.000^{e}	2.646	64.000^{e}	2.646
50	43.670^{b}	1.453	56.330^{d}	1.453	56.330^{d}	1.453
25	55.670^{c}	1.202	44.330^{c}	1.202	44.330^{c}	1.202
12.5	81.330^{d}	1.453	18.670^{b}	1.453	18.670^{b}	1.453

Means bearing different superscript in the same column differ significantly $P<0.05$

5.2 Results in Adult Immersion Test

AIT was used in present study to determine the acaricidal activity against *Boophilus microplus* with various concentration of *Datura Stramonium* of methanolic extract; FAO, (2004) [4]. The different concentration used were same as that in LPT. A significant percentage inhibition of oviposition (IO %) at 100, 50, 25 and 12.5 mg/ml the extracts were 67.46, 61.26, 56.89 and 49.41% respectively (Table 2). In AIT the dose dependent decrease in reproductive index and increase in inhibition of oviposition was observed from concentration 12.5 to 100 mg/ml. No mortality of ticks was observed at any concentration.

Table 2: A significant percentage inhibition of oviposition (IO %) at 100, 50, 25 and 12.5 mg/ml the extracts were 67.46, 61.26, 56.89 and 49.41% respectively

Conc. of extract (mg/ml)	Live ticks weight (gm) (Mean)	(SE)	Weight of eggs (gm) (Mean)	(SE)	Repro- duction Index (RI)(Mean)	(SE)	%IO (Mean)	(SE)
Control	.725^{d}	.001	.365^{e}	.002	.503^{e}	.002	.000^{a}	.000
100	.709^{bc}	.007	.117^{a}	.004	.164^{a}	.008	67.469^{e}	1.696
50	.698^{b}	.002	.136^{b}	.003	.195^{b}	.004	61.264^{d}	.687
25	.682^{a}	.002	.148^{c}	.003	.217^{c}	.003	56.890^{c}	.3768
12.5	.715^{cd}	.006	.182^{d}	.003	.255^{d}	.006	49.411^{b}	.9425

Means bearing different superscript in the same column differ significantly $P<0.05$

Shyma *et al*., (2014) [9] reported significant acaricidal activities of methanolic *D. stramonium* extracts against *R* (*Boophilus*) *microplus* by observing inhibition of oviposition of 77.17% and larval mortality of 71% at highest concentration of 100 mg/ml, followed by 75.52% of inhibition of oviposition and 65% larval mortality at 50mg/ml, followed by 73.15% inhibition of ovipostion and 60.2% of larval mortality at 25mg/ml, and 70.61% inhibition of oviposition and 22.6% larval mortality which are similar with the present findings. Ghosh *et al*., (2015) [5] in acaricidal screening observed 95% ethanolic extracts of *S. anacardium fruits* and *D. stramonium* leaves showed acaricidal efficacy of 50 and 20%, respectively, within 72h, while 50% hydro-ethanolic extracts exhibited no acaricidal activity while as 95% ethanolic extracts of *D. metel* caused significant mortality of 65.0% at 10% concentration within 72 hours of application. On analyzing the acaricidal activities of *D. metel* the probit regression analysis of the extracts showed that with the increase in the dose of extracts, a significant increase in the mortality rate of treated ticks with significant inhibition in reproduction which are similar to our findings.

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