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

Abstract

The present research was done to evaluate acaricidal efficacy of *Datura stramonium* aqueous extracts. Acaricidal efficacy was evaluated against cattle tick *Rhipicephalus (Boophilus) microplus* in Udaipur (Rajasthan). The study was done by 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 two replications for each concentration. The highest acaricidal efficacy was recorded in 100% aqueous extracts of *D. stramonium* in both in-vitro tests (AIT and LPT). The aqueous extracts of *D. stramonium* showed highest IO% at 100% concentration (67.46%) in AIT. The 100% concentrations of aqueous extracts showed minimum reproductive index of 0.16. In AIT the decrease in reproductive index with increase in the percent inhibition of oviposition, was evident during the study. In LPT, aqueous extracts of *D. stramonium* has highest acaricidal efficacy at 100% concentration which was 64%. Larval mortality showed increasing trend with increasing concentrations of plant extracts.

Keywords: Tick, Datura, Larval packet test (LPT), Adult immersion test (AIT)

1. Introduction

India is a prominant agricultural country where livestock and agriculture are closely associated with each other. The main methods used for control of economically important tick species includes chemical compounds like organophosphates, pyrethroids and macrocyclic lactones which have been used for long time leading to the development of resistance in ticks.. In Indian sub continent, the acaricide resistance has been reported in one host ticks of the genus Boophilus (Shyma *et al.*, 2013). To tackle the problem of resistance and other environmental issues linked with chemical control, major efforts have been done to develop immunological means for controlling ticks and tick-borne diseases through alternative ecofriendly anti-tick natural products. Plant extracts and essential oils have showed significant activity against ticks species like *Rhipicephalus (Boophilus) microplus*

(Sunil *et al*., 2013). The plant extract are made from local plants which have history of some pesticidal or medicinal properties and have been traditionally used. Plants like *Datura stramonium* was selected for this study. All parts of Datura plants have high levels of the tropane alkaloids atropine, hyoscyamine and scopolamine which are deliriants or anticholinergics (Arnett, 1995).

2. Materials and Methods

Plants were selected based on available scientific literature. Plant materials were brought to the laboratory and subsequently dried in room temperature for 8-10 days. The materials were dried completely. After drying, plant materials were powdered in mortar pestle and grinder. The powder of *Datura stramonium* parts was then processed for extract preparation by using maceration methods (Shyma *et al.,* 2014), 100 gm powder were extracted using 400ml aqueous as solvents. The extracts 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 was present indicating that no more extraction from the plant material was possible. The extracts were then subjected to water bath evaporation at 40℃ to for removing solvent. The semi-solid extracts were dried under a ceiling fan.

3. Collection of ticks

The ticks were collected from different animals during early morning from Vallabhnagar. The ticks collected were preserved in70% alcohol in clean, well-stopper glass vials and labeled properly. Permanent mounts of ticks were prepared as per standard keys and only engorged female ticks were selected for AIT. Ticks collected from a particular area were labelled and then kept individually in labeled glass tubes covered by muslin cloth. oviposition of ticks was done in desiccators which were maintained at room temperature and 85±5% relative humidity (RH). After 7 days eggs were collected 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 were allowed to hatch by providing uniform conditions of incubation and the larvae unfed for 14- 21days were only utilized for Larval Packet Test (LPT).

Adult Immersuion Test (AIT)

The AIT was done according to FAO, (2004) protocols. The engorged female ticks

were thoroughly washed thrice using distilled water. Ticks were then kept to dry on filter paper. The experiment was then done by immersing ticks in each crude extract of plant materials made for 5 min. In control group only distilled water was used. The ticks were then placed on Petri dishes over Whatman filter paper no.1. The Petri dishes with treated ticks were kept at room temperature for 24 hours. After 24 hours The ticks were then transferred after 24 hrs to glass vials which were covered with muslin cloth. They were 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. Percent adult tick mortality was evaluated and the weight of the eggs laid by the treated ticks were recorded and compared with control group. The experiment was conducted in two replicates in each treatment group and the mean of two was estimated. Control groups were run singly. The eggs were incubated in similar conditions. The percentage of hatched eggs is estimated visually.

$$\text{Reproductive index} = \frac{\text{Weight of egg laid (mg)}}{\text{RI (control group)}}$$

$$\text{Inhibition of oviposition (IO\%)} = \frac{\text{RI (control group)} - \text{RI (treated group)}}{\text{Weight of adult females (mg)}} \times 100$$

Larval Packet Test (LPT)

The larval packet test was conducted according to FAO, (2004) to determine the *In-vitro* acaricidal activity. Engorged female ticks were collected from the cattle from the study area. They were identified, cleaned, stored in a petri dish. They were maintained at 85-92% RH. and 27.0 ±1.0 ºC. dily examination of female ticks was done until oviposition. The eggs were separated and allowed to hatch in glass vials with cotton plug and kept in optimal conditions. The seed ticks obtained were maintained at 27.0±1.0ºC and 85-92% RH for 14-21 days. The larvae aged between 14 to 21 days were then subjected to larval packet test. Packets made of Whatman filter paper No. 1 (12 cm x 18 cm) was impregnated with 3ml of respective compounds and dried at room temperature for two hours. The total of 100 larvae are kept on acaricide impregnated filter paper packet. The top of the packet was sealed by white tape. All close packets were then incubated at 27.0 ± 1.0 ºC and 85-92% R.H for 24 hours., Mortality was observed and measured by counting the dead and live larvae after 24 hours. The non-motile tick larvae were presumed to be dead and were not counted.

$$\text{Percent mortality} = \frac{\text{Totao number of dead larvae}}{\text{Total number of larvae}} \times 100$$

$$\text{Corrected percent mortality} = \frac{\%\ \text{Test Mortality} - \%\ \text{Control Mortality}}{100 - \%\ \text{Control Mortality}}$$

3.1. Preparation of working solutions of plant extracts

The working solutions of all plant extracts were prepared using standard protocols. The dried powder of plant materials were kept at room temperature for 15-20 minutes. The quantity of extracts required were weighed and then dissolved in distilled. Four different dilutions were made at the rate of 12.5 mg/ml, 25 mg/ml, 50mg/ml and 100 mg/ml.

4. Statistical Analysis

The enumerated data from the experiment was statistically evaluated analysis using SPSS, version 20.0. Analysis of variance (Snedecor and Cochran, 1980) was done. The means were compared by Duncan's multiple range test (Duncan, 1995) at 5% level of significance ($P<0.5$).

5. Results and Discussion

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

The plant extracts kept in refrigerator were collected and kept in room temperature for 15-20 minutes. The quantity of extracts required were weighed and dissolved in distilled water. Four different dilutions at the rate of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml were prepared. The peak mortality (42.67%) was observed at a concentration of 100 mg/ml. Four treatment groups and one control group were used for experiment. Control group showed no larval mortality. A significant larval mortality was recorded by application of extracts of 50 mg/ml, 25 mg/ml and 12.5 mg/ml which were 31.67%, 14.33% and 7% respectively. Percent mortality rate increased with the increase in concentration level as shown in (Table. 1).

Table 1: Efficacy of different concentrations of aqueous extracts of *Datura stramonium* against *Rhipicephalus (Boophilus) microplus* larvae by LPT

Concentration of extract (mg/ml)	Live larvae	SE	Dead larvae	SE	% of Larval mortality	SE
Control	100.000[e]	0.000	0.000[a]	0.000	0.000[a]	0.000

100	57.330[a]	1.202	42.670[e]	1.202	42.670[e]	1.202
50	68.330[b]	3.283	31.670[d]	3.283	31.670[d]	3.283
25	83.670[c]	0.882	16.330[c]	0.882	14.330[c]	2.333
12.5	93.000[d]	1.528	7.000[b]	1.528	7.000[b]	1.528

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

5.2 Efficacy of aqueous extracts of *Datura stramonium* in against *Rhipicephalus (Boophilus) microplus* in Adult Immersion Test (IO %)

AIT was done in present study to evaluate the acaricidal activity against *Rhipicephalus (Boophilus) microplus.* Different concentrations of aqueous extracts of *Datura stramonium* were prepared as per FAO, (2004). The plant extracts kept in refrigerator were taken out and kept in room temperature for 15-20 minutes. The quantity of extracts required were weighed and dissolved in distilled water. Four different dilutions at the rate of 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml were prepared. In AIT the decrease in reproductive index and increase in inhibition of oviposition was recorded from concentration 12.5 to 100 mg/ml. A significant percentage inhibition of oviposition (IO%) at 100, 50, 25 and 12.5 mg/ml the extracts were measured to be 48.33, 38.07, 27.80 and 18.81 % respectively. Mortality of ticks observed at different concentrations is shown (Table. 2).

Table 2: Acaricidal efficacy of aqueous extracts of *Datura stramonium* on *Rhipicephalus (Boophilus) microplus* by AIT using different concentrations

Concentration of extract (mg/ml)	weight of Live ticks (gm) (Mean)	(SE)	Weight of eggs (gm) (Mean)	(SE)	Repro-duction Index (RI) (Mean)	(SE)	%IO (Mean)	(SE)
Control	.725[cd]	.001	.365[e]	.002	.503[e]	.002	.000[a]	.000
100	.738[d]	.005	.192[a]	.002	.260[a]	.004	48.333[e]	1.149
50	.699[a]	.001	.218[b]	.001	.312[b]	.001	38.079[d]	.008
25	.704[ab]	.009	.256[c]	.001	.363[c]	.004	27.803[c]	1.199
12.5	.717[bc]	.001	.294[d]	.003	.408[d]	.004	18.812[b]	.488

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

Shyma *et al*., (2014) reported similar findings from acaricidal activities of methanolic *D. stramonium* extracts against *R (Boophilus) microplus.* The inhibition of oviposition of 77.17% and larval mortality of 71% at highest concentration of 100 mg/ml was observed. The inhibition of oviposition of 75.52% and larval mortality of 65% at highest concentration of 50 mg/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. These findings are similar with the present findings. Ghosh *et al.,* (2015) observed acaricidal efficacy of 95% ethanolic extracts of *S. anacardium* fruits and *D. stramonium* leaves. The results showed of 50 and 20%, respectively, within 72h, while 50% hydro-ethanolic extracts exhibited no acaricidal activity. 95% ethanolic extracts of *D. metel* caused mortality of 65.0% at 10% concentration within 72 hours of application. The probit regression analysis of the extracts of *D. metel* showed significant increase in the mortality rate of treated ticks with significant inhibition in reproduction with the increase in the dose of extracts. These findinds correspond to our findings.

6. Conclusion

The plant extracts under consideration showed varying degree of acaricidal efficacy against *Rhipicephalus (Boophilus) microplus*. *D. stramonium* showed highest activity based on their larval mortality, reproductive index and inhibition of fecundity. The results infer that these can be used as alternatives to commercially available synthetic acaricides. However, further investigations need to be done on different tick species. The actual In-vivo application also needs to be done to determine potentiality of plant extracts.

7. References

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