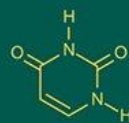


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Jumade Pratibha

Assistant Professor,
Department of Veterinary
Parasitology, Nagpur
Veterinary College, MAFSU,
Nagpur Maharashtra, India

Manmod Pranita

M.V.Sc. Scholar, Department
of Veterinary Parasitology,
Nagpur Veterinary College,
MAFSU, Nagpur
Maharashtra, India

Sawarkar Alka

Assistant Professor,
Department of Pharmacology
and Toxicology, Nagpur
Veterinary College, MAFSU,
Nagpur Maharashtra, India

Dhurve Devika

M.V.Sc. Scholar, Department
of Veterinary Parasitology,
Nagpur Veterinary College,
MAFSU, Nagpur
Maharashtra, India

Corresponding Author:**Jumade Pratibha**

Assistant Professor,
Department of Veterinary
Parasitology, Nagpur
Veterinary College, MAFSU,
Nagpur Maharashtra, India

Evaluation of *in vitro* acaricidal efficacy of *Ricinus communis* against tick infestation in cattle

Jumade Pratibha, Manmod Pranita, Sawarkar Alka and Dhurve Devika

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Abstract

Tick and tick-borne diseases are a global problem that impact the health and production of animals, resulting in financial losses for livestock owners. Traditional synthetic acaricides used to control ticks have been demonstrated to produce acaricidal resistance and environmental degradation. To overcome this, a study was designed to assess the *in vitro* acaricidal efficacy of methanolic leaf extract of *Ricinus communis* using five graded concentrations of 12.5, 25, 50, 75 and 100 mg/ml of distilled water against tick infestation in cattle. The mean percent mortality of engorged female ticks at the concentration of 75 and 100mg/ml of *Ricinus communis* was observed as 78.50 and 88.70 percent respectively on 15 days post treatment. The inhibition of oviposition of treated female ticks was 72.88 and 83.89 percent respectively at the same concentrations. The reduction of egg hatch assay was noted as 30.34 and 18.98 at the concentration of 75 and 100 mg/ml respectively showing significant efficacy of *Ricinus communis* at higher concentrations. The efficacy of *Ricinus communis* was observed as concentration dependant showing minimal efficacy at lower concentrations. The herbal extract of *Ricinus communis* has been shown to have considerable acaricidal efficacy in terms of mortality, egg laying capability and egg hatchability of ticks infesting cattle and can be an alternative to synthetic acaricides.

Keywords: Cattle, tick, acaricide, resistance, efficacy, leaf, extract

Introduction

Tick infestation in animals is one of the largest global problem pertaining to veterinarians (Rodriguez *et al.*, 2011) [18]. Around the world, 80% of cattle are afflicted with ticks with the biggest economic impact (Benavides and Romero, 2001) [4] in regards to production and well-being of the animals, either directly through their bites, bloodsucking tendencies or indirectly through the infectious agents they spread, which include bacteria, viruses, rickettsiae, and protozoa. (Eskezia and Desta, 2016) [8].

In India, *Rhipicephalus microplus* is the most prevalent and harmful species amongst ticks that infects livestock, zoo, and wild animals. (Ghosh *et al.*, 2007) [10]. The tick control is mainly based on use of chemical acaricides. The exposure of *R. microplus* to a range of acaricides has acquired an amazing resistance to group of chemicals used to manage them (Singh *et al.*, 2014; Kumar *et al.*, 2019) [24, 14]. The frequent application of acaricides, has led to issues of contamination of milk and meat, environmental pollution and the emergence of resistance that raises the expense of tick control. (Pirali-Kheirabadi and da Silva, 2011) [16]. Acaricide resistance has been extensively documented in the Indian subcontinent in one host ticks *Rhipicephalus (Boophilus) microplus* (Singh *et al.* 2012; Kumar *et al.* 2013; Shyma *et al.* 2013) [23, 15, 22]. This may be due to high tick population throughout the year in tropics and indiscriminate and haphazard use of chemicals for tick control. India has a big heritage for medicinal plants used in human and animal practices since an ancient time. *Ricinus communis* is a tropical plant belongs to family-Euphorbiaceae also known as Castor oil or castor bean plant in English; in Hindi: arand, that is distributed widely across the world. The plant is native of India and cultivated throughout the country and also grows wild in waste places (Rana *et al.* 2012) [17]. *Ricinus communis* has pharmacological activities and ethnomedicinal uses with the chemical constituents showed the presence of flavonoids, phenolic compounds, fatty acids, amino acids, terpenoids, phytosterol etc. The current study was aimed to introduce an inexpensive and environmentally beneficial phytoformulation of *Ricinus communis* leaf extract for tick management program to combat acaricide resistance and environmental contamination.

Materials and Methods

Preparation of methanolic extract of plant material

The methanolic leaf extract of *Ricinus communis* was prepared as per the protocol described by Dharajiya *et al.*, 2017^[5] with some modifications. The plant material (Leaves) was collected from shrubs which were grown abundantly in nearby areas of Seminary hills of Nagpur district of Maharashtra state. The collected leaves were adequately washed in water, dried in the shed, powdered and subjected to methanolic extraction for 72 hrs at room temperature. The suspension was filtered, evaporated in petri plates and the extract plates were stored at 4 °C for further use.

The four concentrations of methanolic extracts of *Ricinus communis* viz. 12.5, 25, 50, and 100 mg/ml of distilled water were prepared. The control group treated with distilled water and standard treatment group treated with deltamethrin @ 25 ppm was maintained for comparative study.

Collection of ticks

Ticks from different unorganized farms with the history of relapse of tick infestation after treatment with synthetic pyrethroids were collected from the body of cattle and also from the ground, cracks, crevices and under stones of cattle shade in collection bottles. The ticks were then treated with 1% Sodium hypochlorite solution for surface antisepsis (Angelo *et al.* 2010 and Drummond *et al.* 1973)^[2, 7]. The identification of ticks under a zoom stereoscopic microscope was carried out using morphological keys described by Soulsby (1982)^[25] and Walker (2003)^[26]. The female ticks with approximately similar weight were used for experiment.

Adult Immersion Test

The engorged female ticks of approximately similar weights were grouped into six groups each containing six female ticks. The ticks were immersed in each concentration for 2 minutes with gentle agitation, dried on filter paper and transferred to a sterile glass test tube, covered with muslin cloth and kept in a BOD incubator at 28 °C and 85±5% RH. (FAO, 2004; Gonçalves, *et al.*, 2007)^[9, 12]. The ticks were observed daily for oviposition and mortality up to 15 days by observing their pedal reflexes. The egg mass of treated female ticks was weighed to assess reproductive index. The eggs of treated female ticks were visually observed for larval hatchability.

The reproductive index and percent inhibition of oviposition was calculated using the following formulas

$$\text{Reproductive Index (RI)} = \frac{\text{Weight of eggs laid (mg)}}{\text{Engorged female tick weight}}$$

$$\text{Percentage inhibition of oviposition (IO\%)} = \frac{\text{RI (control)} - \text{RI (treated)}}{\text{RI (control)}} \times 100$$

Results and Discussion

The mean percent mortality of engorged female ticks against 12.5, 25, 50, 75 and 100 mg/ml concentration of methanolic leaf extract of *Ricinus communis* was observed as 10.00, 24.60, 58.50, 78.50 and 88.70 percent respectively. Standard treatment group treated with deltamethrin at recommended doses tends to have no mortality showing drug resistance. However control group treated with distilled water showed

no adult mortality at 15 days post treatment. The reproductive index of treated female ticks was noted as 0.109, 0.108, 0.052, 0.032 and 0.019 at the concentration of 12.5, 25, 50, 75 and 100 mg/ml respectively. Inhibition of oviposition in regards to egg laying capacity of treated female ticks showed reduction of 72.88 percent at 75 mg concentration however at the concentration of 100 mg the significant values of 83.89 percent reduction in oviposition was observed. The percent hatchability of larvae by visual method showed 92.22, 91.65, 60.34, 30.34 and 18.98 percent larval hatch at 12.5, 25, 50, 75 and 100 ml concentrations of extract respectively. However the larval hatchability treated with deltamethrin was observed as 84.53 and in control group was 97.32 percent. From the results obtained in present study it was observed that the methanolic leaf extract of *Ricinus communis* at the higher concentration of 75 and 100 mg/ml of distilled water showed significant results in regards to mortality of engorged female ticks, reduction in oviposition and egg hatchability of *R. microplus* ticks infesting cattle. Though it is a herbal, eco friendly, easy to use and low price product, it is recommended to incorporate *Ricinus communis* as an acaricide for tick control programme.

Discussion

The *Ricinus communis* extract has a strong dose-dependent effect on tick mortality rates in *in vitro* assays, ranging from 35.0±5.0 to 95.0±5.0% against multi-acaricide resistant ticks. It also has an influence on tick reproductive index by preventing 36.4-63.1% of oviposition. (Ghosh *et al.* 2013)^[11]. The *in-vitro* efficacy of 3% methanolic leaf extract of verenda (*Ricinus communis*) against ticks infesting cattle confers highest efficacy against ticks. and could be used alternatively as acaricides. (Islam *et al.* 2018). The effects of ricinoleic acid esters from *Ricinus communis* castor oil on the vitellogenesis of *Rhipicephalus sanguineus* ticks attached to hosts showed cytoplasmic changes that inhibited the development of oocytes in addition to preventing the maturation of oocytes. In addition, sperm was not observed in ampoules confirms the acaricide potential of ricinoleic acid esters. Field evaluation of the acaricidal activity of castor bean oils (*Ricinus communis*) against the nymphs and adults of the two-spotted spider mite, *Tetranychus urticae* showed the reduction of 77.32% for adults and 82.91% for nymphs. The study revealed that the essential oil of *R. communis* exhibited potent acaricidal activity which may be attributed to the presence of different bioactive compounds which may affect alone or in synergism. The efficacy of aqueous plant extract of *Ricinus communis* against *Hyalomma dromedarii* ticks was assessed against semi-fed males, using seven concentrations (10, 20, 40, 90, 110, 130, and 170 mg/ml) by adult immersion test based on their mortality percent, 15 days post-treatment showing higher efficacy of 96 percent with 170 mg/ml concentration of extract. The phytochemical studies of *R. communis* revealed presence of Phenolic, tanninic, and flavonoid substances recommended for tick control. The acaricidal efficacy of ethanolic and aqueous extracts of leaves of *Ricinus communis* (Euphorbiaceae) was conducted with *R. (B.) microplus* on larvae and engorged females at concentrations ranging from 25 to 400 mg/ml in the laboratory. At the concentrations of 200 and 400 mg/ml, the ethanolic extracts were very active with a mortality rate of 100% of the larvae. At 400 mg/ml concentration, the extract of *R. communis* was more active on the hatch with a low hatching rate of eggs (25.06%). (Diaha *et al.* 2017)^[6].

Table 1: *In vitro* evaluation of acaricidal efficacy of methanolic leaf extract of *Ricinus communis* against *Rhipicephalus microplus* ticks

| Concentrations Mg/ml | Mean percent mortality | Female wt.(mg) N=6 | Egg wt. (mg) | RI | IO | Hatching % (Visual) (N=100) |
|--|------------------------|--------------------|--------------|-------|-------|-----------------------------|
| 12.5 | 10.0 | 448.62±0.005 | 48.12±0.001 | 0.109 | 7.62 | 92.22 |
| 25 | 24.60 | 427.62±0.006 | 46.5±0.003 | 0.108 | 8.47 | 91.65 |
| 50 | 58.50 | 491.95±0.006 | 25.95±0.001 | 0.052 | 55.93 | 60.34 |
| 75 | 78.50 | 491.05±0.006 | 15.95±0.001 | 0.032 | 72.88 | 30.34 |
| 100 | 88.70 | 442.07±0.008 | 8.66±0.002 | 0.019 | 83.89 | 18.98 |
| Deltamethrin | 10.00 | 473.29±0.003 | 46.33±0.006 | 0.097 | 17.79 | 84.53 |
| Control | 0 | 460.87±0.005 | 54.66±0.001 | 0.118 | 0.00 | 97.32 |
| Coefficient of Variation = 276.700 | | | | | | |
| Treatments found Significant at 1% and 5% level of significance; CD (0.01) = 37.958 CD (0.05) = 28.311 | | | | | | |

Conclusion

The methanolic leaf extract of *Ricinus communis* is found to have an acaricidal efficacy in respect to mortality, egg laying capacity of female ticks and egg hatchability of treated ticks. The herbal plant extract is easily assessable, environment-friendly, non-toxic, non-accumulating ethno medicine having strong acaricidal efficacy and can be used as an alternative to synthetic acaricides to combat resistance and environmental pollution.

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