



Assessment of the larvicidal potential of *Momordica Charantia* and *Eucalyptus Globulus* against *Rhipicephalus Microplus* ticks using the larval packet test

Pratibha Jumade^{1*}, Kranti Kharkar¹, Alka Sawarkar¹, and Chandrakant Kale¹

¹Department of Veterinary Parasitology, Nagpur Veterinary College, MAFSU, Nagpur (M.S.)

Corresponding Author: Jumade Pratibha

DOI: 10.67224/ioasdjmps.2026.v03i04.007

Assistant Professor, Dept. of Veterinary Parasitology, Nagpur Veterinary College, MAFSU, Nagpur | Email: pratibhajumde@mafsu.ac.in

ABSTRACT	Original Research Article
The increasing development of acaricide resistance in <i>Rhipicephalus microplus</i> has created a need for alternative and environmentally sustainable approaches to tick control. Plant-derived products containing bioactive compounds may provide useful botanical alternatives to conventional acaricides. The present study evaluated the in vitro larvicidal activity of aqueous leaf extracts of <i>Momordica charantia</i> and <i>Eucalyptus globulus</i> against <i>R. microplus</i> larvae using the Larval Packet Test (LPT). Leaves of both plants were shade-dried, powdered and extracted with water. Approximately 14-day-old larvae obtained from eggs laid by laboratory-maintained engorged female ticks were exposed to extract concentrations of 12.5, 25, 50 and 100 mg/ml for 24 h. Deltamethrin was used as the reference acaricide and distilled water as the untreated control. Mortality increased with increasing extract concentration. <i>M. charantia</i> produced 34.6, 58.2, 84.7 and 100% mortality at 12.5, 25, 50 and 100 mg/ml, respectively, whereas <i>E. globulus</i> produced 26.4, 49.8, 76.5 and 95.8% mortality at the corresponding concentrations. The estimated LC₅₀ and LC₉₅ values for <i>M. charantia</i> were 33.84 and 57.42 mg/ml, respectively, while those for <i>E. globulus</i> were 29.67 and 62.95 mg/ml. Deltamethrin produced complete mortality, while no mortality occurred in the untreated control. Phytochemical screening indicated the presence of alkaloids, flavonoids, tannins, saponins, glycosides, steroids and triterpenoids. The findings demonstrate promising larvicidal activity of both botanical extracts, with <i>M. charantia</i> showing higher observed mortality at the tested concentrations. Further studies on active compounds, formulation, safety and field efficacy are warranted. Keywords: <i>Rhipicephalus microplus</i>, <i>Momordica charantia</i>, <i>Eucalyptus globulus</i>, larvicidal activity, botanical acaricide, Larval Packet Test, LC₅₀, LC₉₅.	Article History Received: 21-07-2026 Accepted: 21-09-2026 Published: 08-10-2026 Copyright © 2026 The Author(s): This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.
	

1. INTRODUCTION

Ticks are important ectoparasites of livestock and cause considerable economic losses through blood loss, irritation, reduced productivity, damage to hides and transmission of infectious diseases. *Rhipicephalus microplus* is one of the most important cattle ticks because of its widespread distribution and ability to transmit important haemoprotozoan pathogens. Chemical acaricides remain the major method of tick control; however, their repeated and indiscriminate use has resulted in the development of resistance in tick populations. Resistance to several acaricide groups has been documented in *R. microplus*, including populations in India (Shyma *et al.*, 2015; Gaur *et al.*, 2016).

The emergence of resistance, together with concerns regarding chemical residues and environmental contamination, has encouraged research into alternative

tick-control agents. Plants contain numerous secondary metabolites with insecticidal, acaricidal, repellent and antifeedant properties and therefore represent potential sources of botanical acaricides. The Larval Packet Test is a commonly used laboratory assay for evaluating acaricidal activity against *R. microplus* larvae and allows estimation of lethal concentrations through concentration–mortality analysis (Stone and Haydock, 1962).

Momordica charantia L. (Cucurbitaceae), commonly known as bitter gourd, contains several biologically active compounds, including flavonoids, saponins, alkaloids and triterpenoids. Previous investigations have reported pesticidal and acaricidal properties of this plant. *Eucalyptus globulus* Labill. (Myrtaceae) contains terpenoids, phenolic compounds, flavonoids, tannins and other secondary metabolites and

has also demonstrated activity against arthropod pests and ticks. Therefore, the present study was undertaken to evaluate the larvicidal activity of aqueous leaf extracts of *M. charantia* and *E. globulus* against *R. microplus* larvae using LPT.

2. MATERIALS AND METHODS

Leaves of *M. charantia* and *E. globulus* were collected from healthy plants, cleaned, shade-dried and powdered separately. Aqueous extracts were prepared from the powdered leaves and appropriate concentrations of 12.5, 25, 50 and 100 mg/ml were prepared for the bioassay.

Engorged female *R. microplus* maintained under laboratory conditions were allowed to oviposit. The eggs were incubated until hatching, and approximately 14-day-old unfed larvae were used for the experiment. The Larval Packet Test was conducted using approximately 100 larvae per treatment packet. Filter-paper packets treated with the respective extract concentrations were prepared and larvae were introduced into the packets. Deltamethrin was used as the positive control and distilled water as the untreated control. Following 24 h exposure, larval mortality was recorded. Larvae that failed to respond to gentle stimulation were considered dead.

Percentage mortality was calculated as:

Percentage mortality (%) = (Number of dead larvae / Total number of larvae exposed) × 100

The concentration–mortality data were subjected to probit analysis to estimate LC₅₀ and LC₉₅ values. Qualitative phytochemical screening of the extracts was also performed for detection of alkaloids, flavonoids, tannins, saponins, glycosides, steroids and triterpenoids.

3. RESULTS

Both aqueous leaf extracts exhibited a clear concentration-dependent larvicidal effect against *Rhipicephalus microplus* larvae, with mortality increasing progressively as the extract concentration increased. The response was more pronounced with *Momordica charantia* than with *Eucalyptus globulus* at the tested concentrations.

The *M. charantia* extract produced 34.6% mortality at 12.5 mg/ml, which increased to 58.2% at 25 mg/ml and 84.7% at 50 mg/ml. At the highest concentration of 100 mg/ml, complete mortality (100%) was recorded. In comparison, *E. globulus* caused 26.4, 49.8, 76.5 and 95.8% mortality at 12.5, 25, 50 and 100 mg/ml, respectively. Thus, *M. charantia* showed higher observed larval mortality than *E. globulus* at each concentration tested. The difference between the two extracts was particularly evident at the lower

concentrations, although both exhibited substantial activity at 50 and 100 mg/ml.

Probit analysis was used to estimate the lethal concentration values. The LC₅₀ and LC₉₅ of *M. charantia* were 33.84 and 57.42 mg/ml, respectively. For *E. globulus*, the corresponding values were 29.67 and 62.95 mg/ml, respectively. Although *E. globulus* showed a lower LC₅₀ value, its higher LC₉₅ compared with *M. charantia* indicates that a greater concentration of the extract was required to achieve 95% larval mortality. The LC estimates further demonstrate the larvicidal potential of both plant extracts under the experimental conditions.

The reference acaricide deltamethrin produced 100% larval mortality, confirming its strong acaricidal activity in the assay. In contrast, no mortality was recorded in the untreated control, indicating that the observed mortality in the treatment groups was associated with exposure to the respective plant extracts rather than the experimental conditions.

Preliminary phytochemical screening revealed the presence of several classes of secondary metabolites in the plant extracts, including alkaloids, flavonoids, tannins, saponins, glycosides, steroids and triterpenoids. The presence of these bioactive phytochemical groups may contribute to the larvicidal activity observed in the present study. Overall, the results demonstrate that both *M. charantia* and *E. globulus* possess promising in vitro activity against *R. microplus* larvae, with *M. charantia* exhibiting comparatively higher observed mortality across the tested concentrations.

4. DISCUSSION

Both *Momordica charantia* and *Eucalyptus globulus* showed concentration-dependent larvicidal activity against *Rhipicephalus microplus*. *M. charantia* produced 34.6–100% mortality, while *E. globulus* produced 26.4–95.8% mortality across 12.5–100 mg/ml. The LC₅₀ and LC₉₅ values were 33.84 and 57.42 mg/ml for *M. charantia* and 29.67 and 62.95 mg/ml for *E. globulus*, respectively. The activity may be associated with the alkaloids, flavonoids, tannins, saponins, glycosides, steroids and triterpenoids detected in the extracts (Jia *et al.*, 2017; Dhakad *et al.*, 2018; Adenubi *et al.*, 2020). Plant-derived compounds may therefore provide useful alternatives to conventional acaricides, particularly in view of increasing acaricide resistance in *R. microplus* (Shyma *et al.*, 2015; Gaur *et al.*, 2016). Deltamethrin caused complete mortality, whereas the untreated control showed no mortality, confirming the validity of the assay. The Larval Packet Test is a useful method for evaluating acaricidal activity against *R. microplus* larvae (Stone and Haydock, 1962). Further studies are required to identify active compounds, assess safety, develop standardized formulations and validate efficacy under field conditions.

5. CONCLUSION

Aqueous leaf extracts of *Momordica charantia* and *Eucalyptus globulus* showed concentration-dependent larvicidal activity against *Rhipicephalus microplus*, with *M. charantia* showing comparatively higher efficacy. The findings support their potential as botanical acaricides for integrated tick management, warranting further studies on active compounds, formulation, safety and field efficacy.

REFERENCES

- Adenubi OT, Fasina FO, McGaw LJ, Eloff JN, Naidoo V. (2020). Botanical acaricides and repellents in tick control: Current status and future prospects. *Ticks Tick Borne Dis.* 2020.
- Dhakad AK, Pandey VV, Beg S, Rawat JM, Singh A. (2018). Biological, medicinal and toxicological significance of *Eucalyptus* leaf essential oil: A review. *J Sci Food Agric.* 98:833–848.
- Gaur RS, Sangwan AK, Sangwan N, Kumar S. (2016). Acaricide resistance in *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* collected from Haryana and Rajasthan states of India. *Exp Appl Acarol.* 69:487–500.
- Jia, S., Shen, M., Zhang, F., & Xie, J. (2017). Recent advances in *Momordica charantia*: functional components and biological activities. *International journal of molecular sciences*, 18(12), 2555.
- Shala AY and Gururani MA. (2021). Phytochemical properties and diverse beneficial roles of *Eucalyptus globulus* Labill.: A review. *Horticulturae.* 7:450.
- Shyma KP, Gupta JP, Singh V, Patel KK. (2015). In vitro detection of acaricidal resistance status of *Rhipicephalus (Boophilus) microplus* against commercial preparations of deltamethrin, flumethrin and fipronil from North Gujarat, India. *J Parasitol Res.* 506586.
- Stone BF, Haydock KP. (1962). A method for measuring the acaricide-susceptibility of the cattle tick *Boophilus microplus* (Canestrini). *Bull Entomol Res.* 53:563–578.