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Technical note

Ixodicidal effect of plant extracts of *Cinnamomum zeylanicum* and *Tagetes erecta* on *Rhipicephalus microplus* ticks

Perla Iris Miranda Reyes [a]

Francisco Martínez Ibañez [b]

Rodolfo Esteban Lagunes-Quintanilla [c*]

América Ivette Barrera Molina [a*]

[a] Universidad Autónoma del Estado de Morelos. C. Ixtaccíhuatl 100, Vista Hermosa, 62350, Cuernavaca, Mor. México.

[b] Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria. Centro Nacional de Servicios en Constatación en Salud Animal. Jiutepec 62550, Morelos, México.

[c] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad. Jiutepec 62550, Morelos, México.

*Corresponding author: america.barrera@uaem.mx

Abstract:

One of the main problems in cattle farming is infestations caused by *Rhipicephalus microplus* ticks, the most important parasitic species in the cattle industry. Its control is based mainly on the application of ixodicides. However, the excessive and inappropriate use of these products has generated resistant strains. As an alternative, biological control has been proposed as a promising method, as it prevents environmental contamination, promotes the safety of animal products, and contributes to sustainability. For this reason, the objective of the present study was to perform an *in vitro* evaluation of the ixodicidal effect of two plant extracts of *Cinnamomum zeylanicum* and *Tagetes erecta* on *R. microplus* ticks. The analysis was carried out using the larval immersion technique (LIT) and the adult female immersion technique (AIT), and the morphological damage to the cuticular structure of the ticks was

then determined by stereo microscopy. The most significant result was 100 % larval mortality (P<0.05) for the *C. zeylanicum* extract at a concentration of 6%, presenting evident morphological damage in the cuticular structure. In contrast, *T. erecta* extract showed no ixodicidal activity. Finally, it is concluded that the plant extract of *C. zeylanicum* shows efficacy in *in vitro* tests against *R. microplus* larvae and may prove useful as an economical and sustainable alternative for tick control.

Keywords: *Cinnamomum zeylanicum*, Biological control, Plant extracts, *Rhipicephalus microplus*, *Tagetes erecta*.

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Ticks are one of the most important ectoparasites in tropical and subtropical areas[1]. In Mexico, 82 species of ticks have been recorded in both wild and domestic animals, *Rhipicephalus microplus* being the most predominant one in livestock. This tick generates economic losses related to reduced production levels, disease transmission, mortality, and high control costs[1,2]. In recent years, the most widely used strategy for their control has been the application of chemicals known as ixodicides such as organophosphates, carbamates, formamides, synthetic pyrethroids, macrocyclic lactones, phenylpyrazolones, etc., which work adequately at recommended doses. However, the irrational use of these products has led to the selection of resistant tick populations, making control increasingly complex[3,4]. For this reason, the availability of alternative methods has become a necessity for the producers, as well as for the consumers who demand pesticide-free products made with environmentally safe technology, such as plant extracts with active ingredients that exhibit insecticidal and pest control effects, especially in ecological and organic production systems[5,6,7].

In view of this situation, the control of ticks with extracts such plants as garlic and Mexican oregano has been widely studied in some species of livestock importance, using various methods of obtainment and application[8-11]. On the other hand, commercial extracts from plants like cinnamon and marigold tend to have low toxicity to human health, slow development of resistance, and instability in the environment, and their active ingredients are rapidly metabolized by solar radiation and microclimatic humidity[12]. For this reason, the objective of this research was to evaluate the ixodicidal effect of the extracts of two plants —cinnamon (*Cinnamomum zeylanicum*) and marigold (*Tagetes erecta*)— on the mortality of *R. microplus* ticks using the larval package technique (LPT) and the adult female

immersion technique (AIT), and to determine the morphological damage caused at the microscopic level.

The present research utilized 7- to 15-d-old *R. microplus* tick larvae and adult ticks 21 to 23 d collected from bovines infested artificially. The susceptible reference strain from Moyahua, Zacatecas, Mexico, was used, having been provided by the ectoparasites and diptera laboratory of the National Center for Animal Health Testing Services (Centro Nacional de Referencia en Parasitología Animal y Tecnología Analítica, CENAPA) of the National Service for Agri-Food Health, Safety and Quality (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria, SENASICA).

Two plant extracts formulated by oil-in-water emulsion of *C. zeylanicum* 15 % (equivalent to 151.80 g a.i./L) and *T. erecta* 90 % (equivalent to 831.60 g a.i./L) in weight of active compound were assessed and used mainly to control botanical pests such as red spider mites (*Tetranychus urticae*, *Oligonychus punicae*) or whiteflies (*Bemisia tabaci*). In order to evaluate the ixodicidal effect of each of the extracts, solutions were prepared using the commercial concentration recommended as a reference (1.5 %).

In order to evaluate the ixodicidal effect of the extracts of *C. zeylanicum* and *T. erecta* 90 %, a Probit analysis was performed, using five different concentrations —6 %, 3 %, 1.5 %, 0.75 % and 0.375 %— and a control group of 0.375 %. The bioassay was performed under the larval immersion methodology (LIT)[13]. A solution was prepared in distilled water (according to the manufacturer's instructions) with the highest concentration of the extracts (6 %); serial double dilutions were then made with a dilution factor of 0.5, established by the technique up to the lowest concentration (0.375 %). In glass Petri dishes of 15 cm in diameter, a 12.5 cm Whatman No. 1 paper filter was placed, and 10 ml of each solution were added to the respective dish. Subsequently, ~ 400 larvae distributed on the paper were placed with a brush and covered with another paper filter simulating a larval immersion for 10 min. After the exposure time had elapsed, ~100 larvae were taken and transferred to 7.5 x 8.5 cm filter paper packets, which were secured with a BACO™ Bulldog-type clip. Each package containing the treated larvae was identified and kept in an incubator at a temperature of 28 ± 2 ºC and at a relative humidity of 80-90 % for 24 h. Three replicates were performed for each concentration. Finally, package readings were taken by counting the number of live and dead larvae, following the methodology described by FAO in 2004[14].

On the other hand, the immersion test of *R. microplus* adult females (AIT) was performed using the methodology described above[15]. Only the commercial concentration recommended for *C. zeylanicum* and *T. erecta* according to the norm NOM-006-ZOO-1993 was used to evaluate their toxicity on female weight and inhibition of oviposition and hatching. Ticks were placed in beakers with 30 ml of 1.5 % solution of both extracts and kept in immersion for one minute while being subjected to circular movements. Subsequently,

they were removed in order to eliminate the excess product and placed in Petri dishes to be incubated at 28 ± 2 ºC and 80-90 % relative humidity. On d 14, the eggs were separated and weighed to determine the percentage of oviposition inhibition between the treated and control groups. Finally, 1 g vials of duly identified eggs were placed and incubated for 26 d until hatching. The shells and eggs within 3x3 quadrants were counted in order to determine the percentage of hatching.

At the end of the immersion test, a morphological analysis was performed using *R. microplus* tick larvae to determine potential morphological damage to the cuticular structure after exposure to *C. zeylanicum* and *T. erecta* extracts. Ten (10) larvae per treatment were selected from 6 %, 3 %, 1.5 % and 0.75 % concentrations and analyzed by stereo microscopy (Leica microscope) at the helminthology laboratory of the National Center for Disciplinary Research on Animal Health and Safety (Centro de Nacional de Investigación Disciplinaria en Salud Animal e Inocuidad, CENID-SAI, INIFAP).

Larval mortality parameters were obtained (% mortality = number of dead larvae x 100/number of total larvae)[16]. Data were subjected to analysis of variance and to the Kruskal-Wallis test in order to determine significant differences between treatments and controls. Additionally, the data generated in the bioassays from the Probit analyses were submitted to the Polo Plus program (LeOra Software, Petaluma, CA), with the purpose of calculating the lethal concentration 50 (LC_{50}) with a 95% confidence interval.

Probit analysis of the ixodicidal effect of the plant extracts applied using the larval immersion technique made it possible to determine a gradual mortality from the highest concentration (6 %) to the lowest (0.375 %), as shown in Table 1. The extract of *C. zeylanicum* produced a significant mortality ($P<0.05$) at concentrations of 6 % and 3 %, obtaining results of 100 and 97.8 %, respectively. On the other hand, the mortality resulting from the 1.5 % concentration was 64.2 %, which was not statistically significant, similarly to the lowest concentrations that exhibited 0 % mortality. In contrast, the extract of *T. erecta* showed no biological activity in any of the concentrations applied through the Probit methodology, yielding mortality percentages of 0 %, indicative of a null toxic effect on *R. microplus* ticks larvae.

Table 1: Mortality of *R. microplus* tick larvae treated with *C. zeylanicum* and *T. erecta* extracts by Probit analysis with five different concentrations

	Extract concentrations (%)				
Treatment	**6**	**3**	**1.5**	**0.75**	**0.375**
C. zeylanicum	100*	97.8*	64.2*	0	0
T. erecta	0	0	0	0	0
Control	0	0	0	0	0

* Statistical difference with respect to the Control group, $P<0.05$.

Table 2 shows the values obtained when analyzing the mortalities of each of the concentrations used in the Probit analysis. With the results of each concentration, an analysis was carried out using the Polo PC program to calculate the LC_{50} for each of the replicates. The total number of *R. microplus* tick larvae used in the first repetition was 1,413; it was 1,556 in the second repetition, and 1,529 in the third repetition. The LC_{50} ranges obtained in each repetition were a minimum of 1.37 and a maximum of 2.41, with a 95% confidence interval.

Table 2: Values obtained through the Polo PC program with the mortalities obtained from *C. zeylanicum* extract on *R. microplus* tick larvae

n	Slope	LC_{50}	CI (95 %)
1413	3.862 ± 0.185	2.41	1.92 - 3.09
1556	6.326 ± 0.338	1.43	1.29 - 1.58
1529	9.316 ± 0.766	1.37	1.24 - 1.48

n= number of *R. microplus* larvae; LC_{50}= lethal concentration; CI= confidence interval.

The results for the inhibition of oviposition and hatching indicate that there was no statistically significant difference for any of the extracts evaluated with respect to the control group (Table 3). The percentage of oviposition inhibition was 3.88 % and 0 % for *C. zeylanicum* and *T. erecta*, respectively. The percentage of hatching inhibition was 0 % for both extracts.

Table 3: Oviposition and hatching inhibition percentages of *C. zeylanicum* and *T. erecta* extracts on *R. microplus* ticks

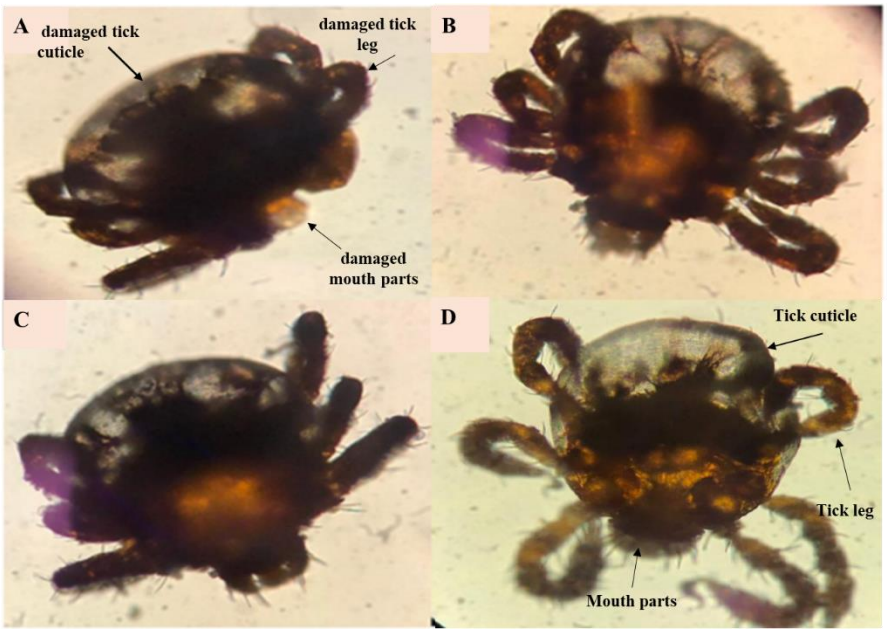
Extract	Concentration	Average female weight (g)	Average egg weight (g)	% O. I.	% H. I.
C. zeylanicum	1.5 %	3.69	2.02	3.88	0
T. erecta	1.5 %	3.75	2.10	0	0
Control	----	3.75	2.12	----	----

O. I.= oviposition inhibition; H. I.= hatching inhibition.

Morphological analysis by stereo microscopy showed remarkable effects on *R. microplus* larvae after treatment with *C. zeylanicum* extract. It was observed that, as the concentration of the extract increased, the structural damage became more noticeable. In panel A of Figure 1, the larva exhibits evident morphological alterations in the gnathosome (mouthparts), characterized by a reduction in the size of the pedipalps and hypostome, a diminished amount of cuticle and a change in the coloration of the legs. In addition, the shield present in the gnathosome shows an orange coloration that differs from panel D or the control group, suggesting inadequate development and an affected gnathosome. Panel B and C show that the idiosome (body) is whiter and more transparent, indicating an adverse effect on the

intestinal caecum that is not adequately appreciated, possibly due to the toxicity of the extracts at the concentrations used. No morphological damage in panel D or the control group.

Figure 1: Morphological damage identified in *R. microplus* larvae treated with different concentrations with *C. zeylanicum* extract



A= 6 % concentration (the black arrows show structural damage). B= 3 % concentration. C= 1.5 % concentration; D= control larva (water).

In the present study, *C. zeylanicum* extract was observed to exhibit ixodicidal activity on *R. microplus* larvae. In particular, it was determined that the use of this extract at a 6 % concentration produced 100 % larval mortality. These findings are in agreement with previous studies where the efficacy of a chemotype derived from *C. verum* against *R. microplus* ticks was evaluated by LPT and AIT, observing that the use of essential oils with benzyl benzoate showed efficacy against *R. microplus* in its larval stage[17]. Likewise, previous findings[18], were similar to those obtained in the present investigation, where they evaluated the compounds (E)-cinnamaldehyde and α-bisabolol derived from cinnamon essential oils and obtained a 100 % larval mortality at concentrations of 2.5, 5 and 10 mg/ml, concluding that the essential oils and their compounds have high acaricidal activity; however, they report low toxicity in adult ticks filled with *R. microplus*.

On the other hand, it has been reported that the essential oils of *C. zeylanicum* act on adult ticks filled with *R. microplus* in pure form, reaching mortalities of 100 %, 97 %, and 62 % at concentrations of 10, 5, and 1 mg/ml, respectively[19]. However, in the present study, when evaluating the commercial concentration recommended of 1.5 %, no effect was observed on

the inhibition of oviposition and hatching of adult ticks. It is important to note that evaluations above the recommended concentration were not performed, since the norm NOM-006-ZOO-1993 states that, if this concentration does not have a 98 % mortality rate in adult ticks, the efficacy is considered negative.

However, it should be noted that the use of the commercial concentration of 1.5 % is for the control of phytophagous insect pests. In this work, a strategy of concentrations was standardized, considering that these arthropods are hematophagous and are at different stages of development. The 6 % concentration of *C. zeylanicum*, which is lethal to *R. microplus* tick larvae, proves that a higher amount is required than for the target species. In addition, no effectiveness was observed on adult ticks, which could indicate that this product does not have the potential to biologically affect the ectoparasite in the adult stage. However, previous studies assessed the combination of three essential oils, demonstrating that increasing the concentration of *C. zeylanicum* resulted in greater effectiveness in controlling adult stage ticks. These findings suggest that the use of higher concentrations of *C. zeylanicum* may be an effective strategy to combat *R. microplus* ticks at all life stages. However, further research is needed to determine the optimal concentrations and to evaluate their potential side effects, environmental effects, and economic feasibility[20].

Further studies using different concentrations on adult ticks are needed, as, in this case, only the commercial concentration recommended was used. These additional studies will make it possible to evaluate the efficacy of the product at different doses and to determine whether there are more effective concentrations for the control of adult ticks. Thus, it will be possible to obtain a better understanding of the response of ticks to different concentrations and to establish more precise guidelines for their control.

Furthermore, in the present study, no distillation or chromatography was performed to separate the compounds from the evaluated extract. However, it has been reported that the main biocomponent of *C. zeylanicum* is eugenol, which could be involved in its toxicity against *R. microplus* tick larvae[21]. In addition, the plant *C. zeylanicum* is known to possess certain properties, notably its insecticidal and acaricidal activity[6,18,20], which suggests that it is an alternative method for the biological control of ticks. In contrast, assessment of the *T. erecta* extract showed no toxicity on *R. microplus* larvae or adults. In this regard, no acaricidal activity of *T. erecta* extract against ticks has been reported. To date, there are only studies where the ixodicidal effect of *T. minuta* essential oil against *R. microplus* tick infestation was evaluated, and statistically significant results were obtained in the productive parameters (number and weight of the ticks, oviposition, and viability of larvae), showing an efficacy of 99.9 % at a concentration of 20 %[22]. Therefore, future research is needed to isolate, identify and characterize the compounds of *T. erecta* extract in order to determine whether it has ixodicidal properties against *R. microplus* ticks.

The efficacy shown by the plant extract of *C. zeylanicum* for the control of *R. microplus* tick larvae highlights the potential use of this natural product as a biological control method and as an economical and sustainable alternative for tick control. However, additional studies are needed to evaluate different methods of plant extraction; *in vivo* tests and toxicity studies must yet be performed in order to elucidate its effect on adult *R. microplus* ticks and the possible risk of using it on domestic animals.

Conflict of interest

The authors declare that they have no conflict of interest in relation to the preparation, revision, and publication of this work.

Literature cited:

1. Tabor AE, Ali A, Rehman G, Rocha Garcia G, Zangirolamo AF, Malardo T, *et al*. Cattle tick *Rhipicephalus microplus*-host interface: a review of resistant and susceptible host responses. Front Cell Infect Microbiol 2017;(7):506.

2. Estrada-Peña A, Mallón AR, Bermúdez S, de la Fuente J, Domingos A, García MPE, *et al*. One health approach to identify research needs on *Rhipicephalus microplus* ticks in the Americas. Pathogens 2022;(11):1180.

3. Yessinou RE, Akpo Y, Adoligbe C, Adinci J, Assogba MN, Koutinhouin B. *et al*. Resistance of tick *Rhipicephalus microplus* to acaricides and control strategies. J Entomol Zool Stud 2016;(4):408-414.

4. Rojas MC, Loza RE, Rodríguez CSD, Figueroa MJV, Aguilar RF, Lagunes QRE, *et al*. Antecedentes y perspectivas de algunas enfermedades prioritarias que afectan a la ganadería bovina en México. Rev Mex Cienc Pecu 2021;(12):111-148.

5. Soto GA. Manejo alternativo de plagas de ácaros. Rev Cie Agr 2013;(2):34-44.

6. Chungsamarnyart N, Jiwajinda S. Rattanagrithaku C, Jansawan W. Practical extraction of sugar apple seeds against tropical cattle ticks. Kasetsart J 1991;(25):101-105.

7. Williams LAD. Adverse effects of extracts of *Artocarpus altilis* Park, and *Azadirachta indica* (A. Juss) on the reproductive physiology of the adult female tick, *Boophilus microplus* (Canest). Invertebr Reprod Dev 1993;(23):159-164.

8. Panella NA, Karchesy J, Maupin GO, Malan J, Piesman J. Susceptibility of immature *Ixodes scapularis* (Acaris: Ixocidae) to plants derived acaricides. J Med Entomol 1997;(34):340-345.

9. Vatsya S, Yadav C, Kumar R, Banerjee P. *In vitro* acaricidal effect of some medical plants against *Boophilus microplus.* J Vet Parasitol 2006;(20):141-143.

10. Álvarez V, Loaiza J, Bonilla R, Barrios M. Control *in vitro* de garrapatas (*Boophilus microplus*, Acari: Ixodidae) mediante extractos vegetales. Rev Biol Trop 2008;(56):291-302.

11. Bravo M, Coronado A, Henríquez H. Eficacia *in vitro* del amitraz sobre poblaciones de *Boophilus microplus* provenientes de explotaciones lecheras del estado de Lara, Venezuela. Zoot Trop 2008;(26):35-40.

12. Broglio-Micheletti SMF, Neves-Valente EC, Alves de Souza L, Silva-Dias ND, Girón-Pérez K, Prédes-Trindade RC. Control de *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) con extractos vegetales. Rev Colomb Entomol 2009;(35):145-149.

13. Shaw RD. Culture of an organophosphorus-resistant strain of *Boophilus microplus* (Can.) and an assessment of its resistance spectrum. Bull Entomol Res 1966;(56):389-405.

14. FAO. Acaricide resistance: diagnosis, management and prevention. guidelines resistance management and integrated parasite control in ruminants, animal production and health division, agriculture department. Food and Agriculture Organization of the United Nations. Rome. 2004.

15. Drummond RO, Ernst SE, Trevino JL, Gladney WJ, Graham OH. *Boophilus annulatus* and *B. microplus*: laboratory tests of insecticides. J Econ Entomol 1973;(66):130-133.

16. Reyes-Domínguez IJ, Arieta-Román RJ, Fernández-Figueroa JA, Romero-Figueroa MZ, Peniche-Cardeña AJE. Resistencia de *Rhipicephalus* (*Boophilus*) *microplus* a ixodicidas en ranchos bovinos del municipio Evangelista, Veracruz, México. Rev Electron de Vet 2013;(14):1-6.

17. Monteiro IN, Monteiro ODS, Costa-Junior LM, da Silva Lima A, Andrade EHA, Maia JGS, *et al.* Chemical composition and acaricide activity of an essential oil from a rare chemotype of *Cinnamomum verum* Presl on *Rhipicephalus microplus* (Acari: Ixodidae). Vet Parasitol 2017;(238):54-57.

18. Dos Santos DS, Boito JP, Santos RCV, Quatrin PM, Ourique AF, Dos Reis JH, *et al.* Nanostructured cinnamon oil has the potential to control *Rhipicephalus microplus* ticks on cattle. Exp Appl Acarol 2017;(73):129-138.

19. Marchesini P, Oliveira DR, Gomes GA, Rodrigues THS, Maturano R, Fidelis QC, *et al.* Acaricidal activity of essential oils of *Cinnamomum zeylanicum* and *Eremanthus erythropappus*, major compounds and cinnamyl acetate in *Rhipicephalus microplus*. Rev Bras Parasitol Vet 2021;(30):e009221.

20. Lazcano E, Padilla E, Castillo G, Estarrón M. Development of essential oil-based phyto formulations to control the cattle tick *Rhipicephalus microplus* using a mixture desing approach. Exp Paratitol 2019;(30):26-33.

21. Gende LB, Floris I, Fritz R, Eguaras, MJ. Antimicrobial activity of cinnamon (*Cinnamomum zeylanicum*) essential oil and its main components against *Paenibacillus larvae* from Argentine. Bull Insectology 2008;(61):1-4.

22. Andreotti R, Garcia MV, Cunha RC, Barros JC. Protective action of *Tagetes minuta* (Asteraceae) essential oil in the control of *Rhipicephalus microplus* (Canestrini, 1887) (Acari: Ixodidae) in a cattle pen trial. Vet Parasitol 2013;197(1-2):341-345.