ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 18, Issue, 08, pp.38035-38045, August, 2026
DOI: https://doi.org/10.24941/ijcr.50867.08.2026

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IN VITRO ACARICIDAL, REPELLENT AND REPRODUCTIVE EFFECTS OF *AZADIRACHTA INDICA* AND *LIPPIA MULTIFLORA* OILS ON CATTLE TICKS, GBÊKÊ REGION, CÔTE D'IVOIRE

Souleymane KANDÉ[1]*, Alain AZOKOU[2], Bienvenu Safiou ADEHAN[3], Ohoukou Marcel BOKA[1], Kolou Alla Serge Landry KOFFI[1], Zegauh Kouadio Jean Marc KONAN[1], Laciné Kalo BAMBA[4] and Emmanuel TIA[1]

[1]Centre for Medical and Veterinary Entomology (CEMV), Alassane OUATTARA University (UAO), BPV 18 Bouaké 01, Côte d'Ivoire; [2]Centre for Research in Ecology (CRE), Nangui ABROGOUA University (UNA), 02 BP 801 Abidjan 02, Côte d'Ivoire; [3]National Institute of Agricultural Research of Benin (INRAB), University of Abomey-Calavi (UAC), 01 BP 526 Cotonou, Benin; [4]Livestock Production Research Station (SRPEL), National Centre for Agricultural Research (CNRA), 01 BP 633 Bouaké 01, Côte d'Ivoire

ARTICLE INFO

Article History:
Received 17th May, 2026
Received in revised form
20th June, 2026
Accepted 27th July, 2026
Published online 31st August, 2026

Keywords:
Adult Immersion Test; Botanical Biopesticide; Oviposition; Integrated Tick Management; Cattle Ectoparasites.

**Corresponding author:*
Souleymane KANDÉ

ABSTRACT

Background: Resistance of ticks to conventional acaricides supports the evaluation of plant-based alternatives. Objective: This study assessed the in vitro acaricidal and repellent activities of *Azadirachta indica* vegetable oil, *Lippia multiflora* essential oil and their mixture, together with their effects on the reproduction of *Rhipicephalus microplus* and *Amblyomma variegatum*. Methods: The products were tested at 1, 5 and 9 mg/mL and undiluted. Monopropylene glycol and alphacypermethrin at 0.05 mg/mL served as negative and positive controls, respectively. Mortality was assessed at 24, 48 and 72 h after immersion. Oviposition and egg hatch were monitored in surviving engorged females, whereas repellency was evaluated in independent batches of unengorged females at 30, 45 and 60 min. Results: Mortality increased with concentration and time, with *A. variegatum* being more susceptible than *R. microplus*. At 9 mg/mL, the plant-based formulations caused very high, often complete mortality after 48–72 h. In *R. microplus*, no oviposition was observed from 5 mg/mL onward among the few surviving females, and no offspring were observed. Repellency increased with concentration and was more pronounced with *L. multiflora*. Concentration and tick species significantly affected repellency, whereas observation time and collection site did not. Conclusions: The formulations showed complementary acaricidal and repellent effects and potential for integrated tick management, subject to chemical characterisation, safety studies and in vivo validation.

Citation: **Souleymane KANDÉ, Alain AZOKOU, Bienvenu Safiou ADEHAN *et al. 2026.*** "In vitro acaricidal, repellent and reproductive effects of *Azadirachta Indica* and *Lippia multiflora* oils on cattle ticks, Gbêkê region, Côte d'Ivoire". ***International Journal of Current Research, 18, (08), 38035-38045.***

INTRODUCTION

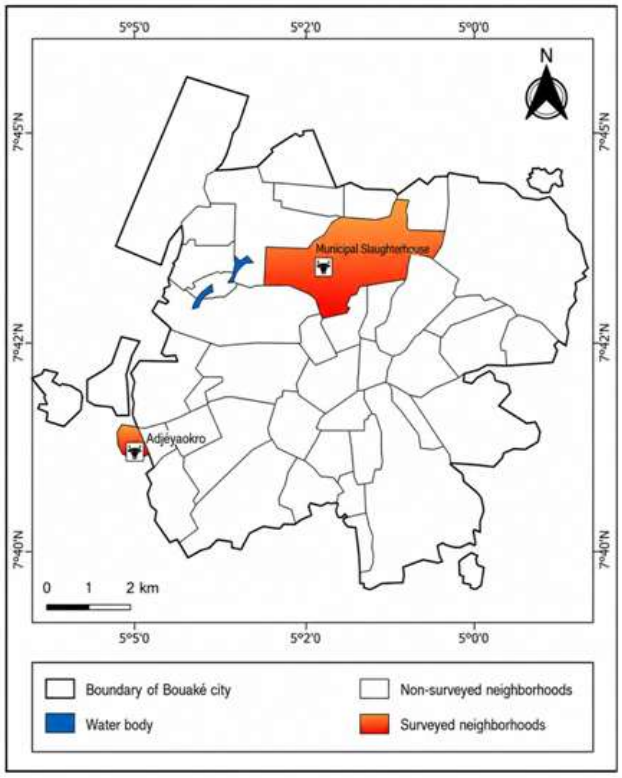
Ticks are major health and economic constraints on cattle production in tropical and subtropical regions. In addition to blood loss, skin lesions and reduced zootechnical performance, they transmit numerous pathogens responsible for important animal diseases [1], [2]. In sub-Saharan Africa, *Amblyomma variegatum* is among the major cattle ticks. This long-standing constraint has been compounded by the spread of *Rhipicephalus microplus*, an invasive species characterized by high reproductive capacity and a marked ability to develop resistance to several classes of acaricides[3],[4],[5]. In Côte d'Ivoire, chemical acaricides remain central to tick control, although resistance of *R. microplus* to alphacypermethrin, deltamethrin and amitraz has been documented [6].

Complementary and alternative control methods are therefore needed. Plant-derived products are of interest because their secondary metabolites can exert acaricidal, insecticidal, repellent or reproduction-disrupting effects [7]. In Côte d'Ivoire, Azokou *et al*. evaluated 22 extracts from 17 plant species against the eggs, larvae and adults of *R. microplus* and demonstrated acaricidal and fertility-inhibiting effects for several extracts [8]. *Azadirachta indica* A. Juss. (Meliaceae), commonly known as neem, contains limonoids such as azadirachtin that may interfere with arthropod feeding, development and reproduction [9]. Acaricidal effects of neem-based products have been reported against ticks [10]. *Lippia multiflora* Moldenke (Verbenaceae) is an aromatic plant from tropical Africa that produces an essential oil rich in volatile compounds, making it particularly interesting for repellent applications. In Côte d'Ivoire, comparative information

simultaneously addressing adult mortality, oviposition, egg hatch and repellency of these products against *R. microplus* and *A. variegatum* remains limited. The present study therefore evaluated the acaricidal and repellent activities of *A. indica* vegetable oil, *L. multiflora* essential oil and their mixture, as well as their effects on oviposition and egg hatch in engorged females.

MATERIALS AND METHODS

Study area: The study was conducted from November 2024 to February 2025 in the Gbêkê region of central Côte d'Ivoire. Ticks used in the bioassays were collected from cattle at two sites: the Bouaké municipal slaughterhouse and the Adjéyaokro cattle farm (Supplementary Figure S1). The slaughterhouse was selected because cattle from different production areas arrive there regularly, providing access to large numbers of ticks. The Adjéyaokro farm complemented the sampling with specimens collected directly from naturally infested cattle under farming conditions. Laboratory experiments were conducted at the Acarology Laboratory of the Centre for Medical and Veterinary Entomology (CEMV).



Supplementary Figure S1. Location of the tick collection sites used for bioassays with plant-derived products

Tick collection, transport, identification and constitution of experimental groups: Adult ticks were manually collected between 7:00 and 9:00 a.m. from male and female zebu-type cattle while preserving their morphological integrity. At the Adjéyaokro farm, ticks were collected directly from naturally infested cattle, whereas at the Bouaké municipal slaughterhouse they were collected from slaughtered cattle. Collection was not restricted a priori to the two species investigated: all ticks encountered were collected, placed in labelled sampling containers and transported alive to the CEMV Acarology Laboratory. Morphological identification was performed between 9:00 and 11:00 a.m. under a stereomicroscope using the taxonomic keys of Walker *et al.* [11]. *Rhipicephalus microplus* and *Amblyomma variegatum* were then separated according to species, sex and engorgement status. Only live, active specimens with no visible morphological abnormalities were included. Engorged and unengorged females were used for mortality assessment; surviving engorged females were subsequently monitored for oviposition and egg hatch, whereas separate batches of unengorged adult females were used for repellency assays.

Origin of plant-derived products: *Azadirachta indica* vegetable oil and *L. multiflora* essential oil were supplied by PORO BIO SANTE, Korhogo, Côte d'Ivoire. According to the producer, *A. indica* oil was obtained from seeds by mechanical extraction followed by centrifugation to remove residual solids and impurities. *Lippia multiflora* essential oil was obtained from leaves by hydrodistillation. Both products were stored in tightly closed containers protected from light until use.

Preparation of formulations and concentrations: The two products were tested separately and as a 1:1 (v/v) mixture. Concentrations were selected with reference to the 1–5 mg/mL range used by Azokou *et al.* [8] and extended to 9 mg/mL in the present study. Monopropylene glycol (MPG) was used as the solvent. Three concentrations were prepared by incorporating 10, 50 and 90 mg of plant-derived product into 10 mL of MPG, corresponding to nominal concentrations of 1, 5 and 9 mg/mL, respectively. This procedure was applied separately to *A. indica* vegetable oil, *L. multiflora* essential oil and their mixture. The products were also tested undiluted. Monopropylene glycol alone served as the negative control, whereas alphacypermethrin at 0.05 mg/mL served as the positive control.

Adult immersion test and mortality assessment: Acaricidal activity was evaluated using an adaptation of the Adult Immersion Test described by Drummond *et al.* [12]. The assays involved engorged and unengorged adult females of *Rhipicephalus microplus* and *Amblyomma variegatum* collected from the Bouaké municipal slaughterhouse and the Adjéyaokro farm. After identification, live, active ticks with no visible morphological abnormalities were separated according to species, collection site and engorgement status, and then allocated to homogeneous groups of 10 individuals. Fourteen experimental treatments were evaluated: monopropylene glycol (MPG), used as the negative control; alphacypermethrin at 0.05 mg/mL, used as the positive control; four *A. indica* vegetable-oil treatments; four *L. multiflora* essential-oil treatments; and four treatments with the *A. indica* + *L. multiflora* mixture in equal proportions (1:1). For each of the three plant-derived products, concentrations of 1, 5 and 9 mg/mL and the undiluted formulation were tested. For each site × species × engorgement status × treatment combination, three independent replicates of 10 ticks were performed, corresponding to 30 ticks per experimental condition. One replicate therefore involved 140 ticks for a given engorgement status and species. The three replicates corresponded to 420 ticks per engorgement status and species at each site. Considering both engorged and unengorged females, 840 ticks were used per species and site, i.e., 1.680 ticks for both species at each site. The two sites together therefore involved 3.360 ticks for the assessment of acaricidal activity, including 1.680 engorged and 1.680 unengorged females; each species accounted for 1.680 individuals. For each group, 3 mL of the freshly prepared solution corresponding to the treatment was

poured into a previously labelled disposable cup. Ticks were completely immersed for 10 min to ensure uniform contact with the tested product. After immersion, they were gently removed, placed briefly on absorbent paper to remove excess product, and transferred to Petri dishes labelled according to site, species, engorgement status, treatment and replicate. The dishes, covered with mesh to allow air circulation, were maintained under uncontrolled ambient laboratory conditions. Mortality was assessed at 24, 48 and 72 h after immersion. The three observation times did not correspond to separate batches: the same ticks in each group were monitored successively at 24, 48 and 72 h. At each observation, individuals showing no spontaneous movement were gently stimulated on the legs or mouthparts; absence of a motor response after several successive stimulations led to classification as dead. Mortality rate (MR) was calculated as MR (%) = (Nm/Nt) × 100, where Nm is the number of dead ticks and Nt is the total number of ticks exposed under the condition considered. When mortality occurred in the negative control, corrected mortality was calculated using Abbott's formula [13]: CMR (%) = [(MRt − MR0)/(100 − MR0)] × 100, where CMR is corrected mortality, MRt is mortality observed in the treated group and MR0 is mortality observed in the negative control.

Assessment of oviposition: Oviposition was assessed in engorged females from the same groups used for mortality assessment. After mortality monitoring at 72 h, surviving engorged females were maintained individually to ensure traceability according to site, species, formulation, concentration and replicate. They were observed daily until the onset of oviposition or until death. Thus, no additional tick groups were established for the oviposition study. For each experimental condition, the initial number exposed was 30 engorged females (three replicates of 10 individuals), whereas the denominator used to calculate the oviposition rate was the number of females still alive at 72 h. The oviposition rate (OR) was calculated as OR (%) = (Np/Ns) × 100, where Np is the number of surviving females that oviposited and Ns is the number of engorged females surviving at 72 h.

Assessment of egg hatch: Egg hatch was assessed from egg masses produced by the same engorged females monitored after the immersion test. Egg masses from females that actually oviposited were kept separately according to site, species, formulation, concentration and replicate, and were examined regularly under a stereomicroscope until hatching was complete. The egg-hatch rate was visually estimated as the apparent proportion of hatched eggs within each egg mass; no systematic individual counting of eggs and larvae was performed. When no oviposition occurred, no egg mass was available for direct assessment of hatching; the zero value reported in the results therefore indicates the absence of observed offspring under the condition considered and should not be interpreted in isolation as a measure of a direct ovicidal effect. No additional groups of adult ticks were used for this step.

Repellency assay: The repellent activity of *A. indica* vegetable oil, *L. multiflora* essential oil and their mixture was evaluated at the Acarology Laboratory of the Centre for Medical and Veterinary Entomology (CEMV) using unengorged adult females of *Rhipicephalus microplus* and *Amblyomma variegatum* collected from the Bouaké municipal slaughterhouse and the Adjéyaokro farm. After identification and selection, ticks were allocated to groups of 10 individuals. Fourteen experimental treatments were considered: monopropylene glycol (MPG), used as the negative control; alphacypermethrin at 0.05 mg/mL, used as the positive control; four *A. indica* vegetable-oil treatments; four *L. multiflora* essential-oil treatments; and four *A. indica* + *L. multiflora* mixture treatments. For each of the three plant-derived products, concentrations of 1, 5 and 9 mg/mL and the undiluted formulation were evaluated. For each treatment, a Whatman filter-paper disc 3 cm in diameter (radius 1.5 cm) was impregnated with 3 mL of the corresponding preparation and then left exposed to air for 10 min. The impregnated disc was then placed in the centre of a disposable aluminum-foil-type plate 10 cm in diameter (radius 5 cm). The 10 ticks were placed near the edges, away from the impregnated zone. Three independent observation times were considered: 30, 45 and 60 min. Separate batches of ticks were used at each time; individuals evaluated at 30 min were therefore not reused at 45 or 60 min. For each site × species × treatment × observation-time combination, three independent replicates of 10 ticks were performed on separate days, with a one-day interval between successive replicates, corresponding to 30 ticks per experimental condition. At the end of the scheduled observation time, a tick was considered repelled when it was located outside the impregnated zone, whereas a tick present on the treated zone was considered non-repelled. For a given observation time and site, the three replicates involved 420 ticks per species, i.e., 840 ticks for both species. For the two sites combined, 1.680 ticks were therefore used at each observation time. Overall, the repellency assays involved 5.040 unengorged adult females, including 2.520 *R. microplus* and 2.520 *A. variegatum*. For each treatment and observation time, the number of repelled ticks among the 30 tested individuals was recorded. Repellency rate (RR) was calculated as RR (%) = (Nr/Nt) × 100, where Nr is the number of repelled ticks and Nt is the total number of ticks tested under the condition considered.

Statistical analysis: Data were entered into Microsoft Excel 2013 and analysed using R version 4.5.1 [14]. The variables examined included mortality at 24, 48 and 72 h, oviposition, egg hatch, and repellency at 30, 45 and 60 min. For mortality, because the same groups were followed successively at 24, 48 and 72 h, the values were interpreted as repeated observations over time. Comparisons among treatments presented in Table 1 were performed separately at each time point for each species × site × engorgement-status combination, using numbers of dead and live ticks and Fisher's exact test with Holm adjustment for multiple comparisons. For oviposition, the number of females that oviposited was related to the number of engorged females still alive at 72 h; when this number was zero, the oviposition rate was considered not calculable. Comparisons among evaluable treatments were performed within each species × site combination using the numbers of "ovipositing" and "non-ovipositing" females among survivors, with Fisher's exact test and Holm adjustment. For egg hatch, the available percentages were derived from visual estimation of egg masses, and interpretation remained descriptive because individual raw data for each egg mass were unavailable. For repellency, observations at 30, 45 and 60 min corresponded to independent experiments because separate batches were used at each time. Comparisons among treatments were performed separately at each time point for each species × site combination using Fisher's exact test with Holm adjustment. The overall repellency analysis examined the effects of concentration, species, site and observation time using a

generalized linear model for binomial data, with a quasi-binomial correction in the event of overdispersion. Statistical significance was set at 5%.

RESULTS

Acaricidal activity according to collection site, engorgement status and post-exposure time: Acaricidal activity was assessed in 3.360 adult ticks distributed across the two sites, two species and two engorgement statuses. Mortality generally increased with concentration and post-exposure time, whereas the two sites showed comparable profiles. In *R. microplus*, the lethal effect of *A. indica* vegetable oil became particularly marked from 5 mg/mL onward. At this concentration, mortality of engorged females from the Bouaké slaughterhouse reached 63.33%, 83.33% and 96.67% at 24, 48 and 72 h, respectively; the corresponding values at Adjéyaokro were 53.33%, 83.33% and 93.33%. *L. multiflora* produced lower mortality at 5 mg/mL, whereas the mixture generally showed an intermediate response. At 9 mg/mL, all three plant-based formulations caused very high mortality and reached 100% at 48–72 h under most conditions. Undiluted products caused complete mortality. *A. variegatum* was generally more susceptible: at 1 mg/mL, *A. indica* already caused 93.33% mortality at 48 h in engorged females from both sites and 100% at 72 h.
Comparisons performed separately for each species, site, engorgement status and observation time are indicated by the significance letters in Table 1.

Effect on oviposition: Engorged females exposed to monopropylene glycol descriptively showed the highest oviposition rates. In *R. microplus*, these rates were 44.83% at the slaughterhouse and 50.00% at Adjéyaokro. Alphacypermethrin yielded 25.93% and 23.08%, respectively. At 1 mg/mL, oviposition persisted with all three plant-based formulations. From 5 mg/mL onward in *R. microplus*, no oviposition was recorded among surviving females when any remained alive at 72 h. In *A. variegatum*, several plant-based treatments caused complete mortality at 72 h; under these conditions, the oviposition rate was not calculable. When at least one female survived but no oviposition occurred, the rate was reported as 0%. After comparison of oviposition proportions among survivors using Fisher's exact test with Holm adjustment, no significant difference was observed among evaluable treatments at the 5% level.

Effect on egg hatch: In *R. microplus*, the highest egg-hatch rates were observed in the monopropylene glycol controls (15% at the slaughterhouse and 13% at Adjéyaokro). Alphacypermethrin yielded 10% and 5%, respectively. At 1 mg/mL, *A. indica* produced egg-hatch rates of 5% and 2%, *L. multiflora* 7% and 9%, and the mixture 6% at both sites. No hatching was observed with the plant-based formulations from 5 mg/mL onward. In *A. variegatum*, no hatching was observed after plant-based treatments, consistent with the absence of oviposition.

Repellent activity: Repellent activity generally increased with concentration, with comparable profiles between the two collection sites. In the monopropylene glycol negative control, repellency remained low, ranging from 0 to 10% depending on species, site and observation time. Alphacypermethrin at 0.05 mg/mL produced repellency rates ranging from 20 to 40%. At 1 mg/mL, the three plant-based formulations showed low levels, ranging from 0 to 20%. At 5 mg/mL, rates ranged from 20 to 50% across all formulations. At 9 mg/mL, *Lippia multiflora* essential oil showed the highest values, ranging from 80 to 90% depending on species, site and time, whereas *A. indica* vegetable oil and the *A. indica* + *L. multiflora* mixture each produced rates ranging from 50 to 70%. When undiluted, *L. multiflora* produced 100% repellency in all species × site × time combinations, whereas *A. indica* and the mixture each produced rates of 60–90%. Values observed in *A. variegatum* were generally slightly higher than those recorded in *R. microplus*, consistent with the significant species effect. The overall analysis showed a highly significant effect of concentration ($p < 0.001$) and a significant effect of species ($p = 0.014$). In contrast, observation time ($p = 0.85$), collection site ($p = 0.96$) and the tested interactions were not significant.

DISCUSSION

The plant-derived products evaluated in this study affected several complementary biological components: adult mortality, oviposition capacity of survivors, fate of the eggs produced, and avoidance behaviour. This integrated interpretation is particularly relevant for botanical products, whose potential value does not rely solely on immediate lethality. Immersion, oviposition, egg-hatch and repellency bioassays are among the approaches commonly used to characterize the anti-tick activity of plant-derived products, although the diversity of protocols requires cautious comparison across studies [17]. In *R. microplus*, the acaricidal activity of *A. indica* became particularly marked from 5 mg/mL onward and increased with concentration. Differences among the three formulations were more apparent at 1 and 5 mg/mL. At 9 mg/mL, and especially when the products were used undiluted, mortality became high for several formulations, thereby reducing observable differences among them. These results primarily reflect a concentration-related effect and do not imply that intermediate concentrations were intrinsically more effective than higher concentrations. The absence of a significant collection-site effect and the broadly concordant trends observed at the Bouaké slaughterhouse and Adjéyaokro suggest that the main concentration-related profiles were not restricted to a single tick origin. Nevertheless, the study was not designed as a population-genetic comparison, and this result should not be interpreted as evidence that the two tick populations are biologically identical.

Amblyomma variegatum was generally more susceptible than *R. microplus* under the conditions tested. Comparison with alphacypermethrin highlights an interesting contrast between the plant-based formulations and the reference chemical acaricide. Whereas alphacypermethrin at 0.05 mg/mL caused relatively low mortality in *R. microplus*, formulations based on *A. indica*, *L. multiflora* and their mixture showed greater acaricidal activity, particularly at higher concentrations. However, this observation does not mean that the plant-derived products are intrinsically more potent than alphacypermethrin, because they were tested at much higher nominal concentrations (1, 5 and 9 mg/mL, as well as undiluted). It nevertheless shows that these natural products can exert substantial acaricidal activity under the conditions of this study.

Table 1. Mortality of engorged and unengorged females of *R. microplus* and *A. variegatum* at 24, 48 and 72 h according to collection site, treatment and concentration

Tick species	Collection site	Treatment	Concentration (mg/mL)	Mortality of engorged females at 24 h (%)	Mortality of engorged females at 48 h (%)	Mortality of engorged females at 72 h (%)	Mortality of unengorged females at 24 h (%)	Mortality of unengorged females at 48 h (%)	Mortality of unengorged females at 72 h (%)
R. microplus	**Bouaké slaughterhouse**	Monopropylene glycol	0	0.00 (0/30)e	3.33 (1/30)e	3.33 (1/30)f	0.00 (0/30)e	3.33 (1/30)e	3.33 (1/30)d
		Alphacypermethrin	0.05	3.33 (1/30)e	10.00 (3/30)de	10.00 (3/30)ef	3.33 (1/30)e	3.33 (1/30)e	6.67 (2/30)d
		A. indica	1	26.67 (8/30)cde	50.00 (15/30)bcd	66.67 (20/30)bcd	16.67 (5/30)cde	40.00 (12/30)d	53.33 (16/30)bc
			5	63.33 (19/30)bcd	83.33 (25/30)ab	96.67 (29/30)ab	43.33 (13/30)bcd	66.67 (20/30)bcd	86.67 (26/30)ab
			9	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
		L. multiflora	1	20.00 (6/30)de	33.33 (10/30)cde	46.67 (14/30)de	13.33 (4/30)de	26.67 (8/30)de	36.67 (11/30)cd
			5	40.00 (12/30)cd	63.33 (19/30)bc	76.67 (23/30)abcd	26.67 (8/30)bcde	53.33 (16/30)d	66.67 (20/30)bc
			9	70.00 (21/30)abc	100.00 (30/30)a	100.00 (30/30)a	60.00 (18/30)bc	93.33 (28/30)abc	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
		A. indica + *L. multiflora*	1	23.33 (7/30)de	43.33 (13/30)bcd	56.67 (17/30)cd	16.67 (5/30)cde	36.67 (11/30)de	46.67 (14/30)bc
			5	46.67 (14/30)bcd	70.00 (21/30)abc	90.00 (27/30)abc	30.00 (9/30)bcde	60.00 (18/30)cd	76.67 (23/30)abc
			9	86.67 (26/30)ab	100.00 (30/30)a	100.00 (30/30)a	70.00 (21/30)ab	96.67 (29/30)ab	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
	Adjéyaokro	Monopropylene glycol	0	3.33 (1/30)f	6.67 (2/30)e	6.67 (2/30)f	0.00 (0/30)d	3.33 (1/30)e	3.33 (1/30)f
		Alphacypermethrin	0.05	6.67 (2/30)ef	6.67 (2/30)e	13.33 (4/30)ef	10.00 (3/30)cd	10.00 (3/30)e	10.00 (3/30)ef
		A. indica	1	26.67 (8/30)cdef	50.00 (15/30)bcd	60.00 (18/30)bcd	10.00 (3/30)cd	33.33 (10/30)cde	50.00 (15/30)bcde
			5	53.33 (16/30)bcd	83.33 (25/30)ab	93.33 (28/30)ab	43.33 (13/30)bc	70.00 (21/30)abc	86.67 (26/30)ab
			9	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
		L. multiflora	1	16.67 (5/30)def	30.00 (9/30)de	40.00 (12/30)def	10.00 (3/30)cd	20.00 (6/30)de	30.00 (9/30)def
			5	40.00 (12/30)cde	70.00 (21/30)abcd	80.00 (24/30)abcd	30.00 (9/30)bcd	53.33 (16/30)cd	66.67 (20/30)bcd
			9	70.00 (21/30)abc	100.00 (30/30)a	100.00 (30/30)a	60.00 (18/30)b	93.33 (28/30)ab	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
		A. indica + *L. multiflora*	1	16.67 (5/30)def	36.67 (11/30)cde	50.00 (15/30)cde	10.00 (3/30)cd	30.00 (9/30)cde	40.00 (12/30)cde
			5	50.00 (15/30)bcd	76.67 (23/30)abc	90.00 (27/30)abc	30.00 (9/30)bcd	56.67 (17/30)bcd	76.67 (23/30)abc
			9	90.00 (27/30)ab	100.00 (30/30)a	100.00 (30/30)a	66.67 (20/30)b	96.67 (29/30)a	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a

Continue ….

A. variegatum	**Bouaké slaughterhouse**	Monopropylene glycol	0	3.33 (1/30)d	6.67 (2/30)b	6.67 (2/30)b	3.33 (1/30)c	3.33 (1/30)b	3.33 (1/30)b
		Alphacypermethrin	0.05	86.67 (26/30)ab	100.00 (30/30)a	100.00 (30/30)a	73.33 (22/30)ab	100.00 (30/30)a	100.00 (30/30)a
		A. indica	1	60.00 (18/30)bc	93.33 (28/30)a	100.00 (30/30)a	53.33 (16/30)b	80.00 (24/30)a	100.00 (30/30)a
			5	90.00 (27/30)ab	100.00 (30/30)a	100.00 (30/30)a	76.67 (23/30)ab	100.00 (30/30)a	100.00 (30/30)a
			9	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
		L. multiflora	1	40.00 (12/30)cd	73.33 (22/30)a	96.67 (29/30)a	36.67 (11/30)bc	73.33 (22/30)a	93.33 (28/30)a
			5	56.67 (17/30)bc	83.33 (25/30)a	100.00 (30/30)a	50.00 (15/30)b	73.33 (22/30)a	100.00 (30/30)a
			9	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
		A. indica + *L. multiflora*	1	53.33 (16/30)bc	83.33 (25/30)a	100.00 (30/30)a	50.00 (15/30)b	90.00 (27/30)a	100.00 (30/30)a
			5	73.33 (22/30)abc	83.33 (25/30)a	100.00 (30/30)a	60.00 (18/30)b	80.00 (24/30)a	100.00 (30/30)a
			9	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
	Adjéyaokro	Monopropylene glycol	0	0.00 (0/30)d	3.33 (1/30)b	3.33 (1/30)b	0.00 (0/30)c	0.00 (0/30)b	3.33 (1/30)b
		Alphacypermethrin	0.05	80.00 (24/30)abc	100.00 (30/30)a	100.00 (30/30)a	83.33 (25/30)ab	100.00 (30/30)a	100.00 (30/30)a
		A. indica	1	60.00 (18/30)bc	93.33 (28/30)a	100.00 (30/30)a	50.00 (15/30)b	83.33 (25/30)a	100.00 (30/30)a
			5	86.67 (26/30)ab	100.00 (30/30)a	100.00 (30/30)a	76.67 (23/30)ab	100.00 (30/30)a	100.00 (30/30)a
			9	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
		L. multiflora	1	40.00 (12/30)c	70.00 (21/30)a	93.33 (28/30)a	40.00 (12/30)b	70.00 (21/30)a	100.00 (30/30)a
			5	63.33 (19/30)bc	83.33 (25/30)a	100.00 (30/30)a	70.00 (21/30)ab	90.00 (27/30)a	100.00 (30/30)a
			9	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
		A. indica + *L. multiflora*	1	50.00 (15/30)bc	80.00 (24/30)a	100.00 (30/30)a	50.00 (15/30)b	90.00 (27/30)a	100.00 (30/30)a
			5	76.67 (23/30)abc	90.00 (27/30)a	100.00 (30/30)a	70.00 (21/30)ab	90.00 (27/30)a	100.00 (30/30)a
			9	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a
			Undiluted	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a	100.00 (30/30)a

Mortality is expressed as a percentage of the ticks initially exposed. "Undiluted" indicates a plant-derived product tested without dilution in monopropylene glycol. For the same species, site, engorgement status and observation time, values sharing at least one letter are not significantly different at the 5% level. Comparisons among treatments were performed on the numbers of dead and live ticks using Fisher's exact test, with Holm adjustment for multiple comparisons. Letters should be interpreted separately at 24, 48 and 72 h and separately for engorged and unengorged females.

Table 2. Effect of treatments on oviposition of engorged *R. microplus* and *A. variegatum* females according to collection site and concentration

Tick species	Collection site	Treatment	Concentration (mg/mL)	Females initially tested	Females surviving at 72 h	Females that oviposited	Oviposition rate (%)
R. microplus	**Bouaké slaughterhouse**	Monopropylene glycol	0	30	29	13	44.83a
		Alphacypermethrin	0.05	30	27	7	25.93a
		A. indica	1	30	10	3	30.00a
			5	30	1	0	0.00a
			9	30	0	0	—
			Undiluted	30	0	0	—
		L. multiflora	1	30	16	5	31.25a
			5	30	7	0	0.00a
			9	30	0	0	—
			Undiluted	30	0	0	—
		A. indica + *L. multiflora*	1	30	13	2	15.38a
			5	30	3	0	0.00a
			9	30	0	0	—
			Undiluted	30	0	0	—
	Adjéyaokro	Monopropylene glycol	0	30	28	14	50.00a
		Alphacypermethrin	0.05	30	26	6	23.08a
		A. indica	1	30	12	3	25.00a
			5	30	2	0	0.00a
			9	30	0	0	—
			Undiluted	30	0	0	—
		L. multiflora	1	30	18	7	38.89a
			5	30	6	0	0.00a
			9	30	0	0	—
			Undiluted	30	0	0	—
		A. indica + *L. multiflora*	1	30	15	3	20.00a
			5	30	3	0	0.00a
			9	30	0	0	—
			Undiluted	30	0	0	—
A. variegatum	**Bouaké slaughterhouse**	Monopropylene glycol	0	30	28	9	32.14a
		Alphacypermethrin	0.05	30	0	0	—
		A. indica	1	30	0	0	—
			5	30	0	0	—
			9	30	0	0	—
			Undiluted	30	0	0	—
		L. multiflora	1	30	1	0	0.00a
			5	30	0	0	—
			9	30	0	0	—
			Undiluted	30	0	0	—
		A. indica + *L. multiflora*	1	30	0	0	—
			5	30	0	0	—
			9	30	0	0	—
			Undiluted	30	0	0	—
	Adjéyaokro	Monopropylene glycol	0	30	29	8	27.59a
		Alphacypermethrin	0.05	30	0	0	—
		A. indica	1	30	0	0	—
			5	30	0	0	—
			9	30	0	0	—
			Undiluted	30	0	0	—
		L. multiflora	1	30	2	0	0.00a
			5	30	0	0	—
			9	30	0	0	—
			Undiluted	30	0	0	—
		A. indica + *L. multiflora*	1	30	0	0	—
			5	30	0	0	—
			9	30	0	0	—
			Undiluted	30	0	0	—

The oviposition rate corresponds to the proportion of engorged females still alive at 72 h that subsequently oviposited. When no female remained alive at 72 h (Ns = 0), the oviposition rate was not calculable and is indicated by "—"; no significance letter was assigned to these treatments. For evaluable treatments (Ns > 0), comparisons of the numbers of females that did or did not oviposit among survivors were performed, for the same species and site, using Fisher's exact test with Holm adjustment for multiple comparisons. Values bearing the same superscript letter are not significantly different at the 5% level. After adjustment for multiple comparisons, no significant difference was observed among evaluable treatments; all therefore belong to the same homogeneous group "a". "Undiluted" indicates a plant-derived product tested without dilution in monopropylene glycol.

Moreover, the low response to alphacypermethrin is particularly relevant in the Ivorian context, where resistance of *R. microplus* to several chemical acaricides, including alphacypermethrin, deltamethrin and amitraz, has already been reported 6. Plant-based formulations could therefore represent a complementary option to conventional acaricides, particularly where susceptibility to certain chemical compounds has declined. This possibility should, however, be confirmed through comparative dose–response assays and specific resistance studies.

Reproductive observations should be interpreted in continuity with mortality. In *R. microplus*, oviposition persisted at 1 mg/mL with all three plant-based formulations, whereas no oviposition was recorded from 5 mg/mL onward among females still alive at 72 h. However, comparisons of oviposition proportions among survivors were not significant after Holm adjustment. This lack of statistical significance does not imply an absence of biological effect: the most lethal treatments left very few females available for oviposition assessment, greatly reducing the power of comparisons

Table 3. Effect of treatments on egg hatch in *R. microplus* and *A. variegatum* according to collection site and concentration

Tick species	Collection site	Treatment	Concentration (mg/mL)	Females initially tested	Females that oviposited	Egg-hatch rate (%)
R. microplus	Bouaké slaughterhouse	Monopropylene glycol	0	30	13	15
		Alphacypermethrin	0.05	30	7	10
		A. indica	1	30	3	5
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
		L. multiflora	1	30	5	7
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
		A. indica + *L. multiflora*	1	30	2	6
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
	Adjéyaokro	Monopropylene glycol	0	30	14	13
		Alphacypermethrin	0.05	30	6	5
		A. indica	1	30	3	2
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
		L. multiflora	1	30	7	9
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
		A. indica + *L. multiflora*	1	30	3	6
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
A. variegatum	Bouaké slaughterhouse	Monopropylene glycol	0	30	9	13
		Alphacypermethrin	0.05	30	0	0
		A. indica	1	30	0	0
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
		L. multiflora	1	30	0	0
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
		A. indica + *L. multiflora*	1	30	0	0
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
	Adjéyaokro	Monopropylene glycol	0	30	8	11
		Alphacypermethrin	0.05	30	0	0
		A. indica	1	30	0	0
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
		L. multiflora	1	30	0	0
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0
		A. indica + *L. multiflora*	1	30	0	0
			5	30	0	0
			9	30	0	0
			Undiluted	30	0	0

The egg-hatch rate corresponds to the apparent proportion of hatched eggs, visually estimated in egg masses produced by females that oviposited. The eggs originated from the same engorged females initially subjected to the immersion test and subsequently monitored for mortality and oviposition. When no oviposition was observed, the value 0% indicates the absence of observed offspring and does not, by itself, constitute a direct measure of an ovicidal effect. No significance letters are assigned to egg hatch because the available values were aggregated visual estimates without individual egg counts or raw data for each egg mass that would allow rigorous recalculation of comparisons. "Undiluted" indicates a plant-derived product tested without dilution in monopropylene glycol.

conditional on survival. In *A. variegatum*, several treatments caused complete mortality at 72 h; in these situations, the oviposition rate was logically not calculable rather than zero. It is therefore essential to distinguish absence of oviposition among survivors from absence of reproduction secondary to complete mortality. The findings obtained with *A. indica* vegetable oil remain biologically consistent with those of Giglioti *et al*., who reported reduced oviposition and egg hatch in *R. microplus* after exposure to *A. indica* seed extracts [18].

Egg hatch provides complementary information, but its interpretation is more limited in the present study. In *R. microplus*, low levels of hatching persisted at 1 mg/mL, whereas no offspring were observed from 5 mg/mL onward. However, when females did not oviposit, the absence of hatching cannot be equated with a direct ovicidal effect because no eggs were available for viability testing. In addition, because hatching was visually estimated without systematic individual counting and without raw data for each

Table 4. Repellent activity of treatments against unengorged *R. microplus* and *A. variegatum* females at 30, 45 and 60 min according to collection site and concentration

Tick species	Collection site	Treatment	Concentration (mg/mL)	Repellency at 30 min (%)	Repellency at 45 min (%)	Repellency at 60 min (%)
R. microplus	**Bouaké slaughterhouse**	Monopropylene glycol	0	0.0 (0/30)g	0.0 (0/30)f	10.0 (3/30)e
		Alphacypermethrin	0.05	20.0 (6/30)efg	30.0 (9/30)def	30.0 (9/30)cde
		A. indica	1	0.0 (0/30)g	10.0 (3/30)ef	10.0 (3/30)e
			5	30.0 (9/30)defg	20.0 (6/30)def	20.0 (6/30)de
			9	60.0 (18/30)bcde	50.0 (15/30)bcde	60.0 (18/30)bcd
			Undiluted	70.0 (21/30)abcd	80.0 (24/30)abc	60.0 (18/30)bcd
		L. multiflora	1	20.0 (6/30)efg	10.0 (3/30)ef	10.0 (3/30)e
			5	40.0 (12/30)cdef	50.0 (15/30)bcde	40.0 (12/30)cde
			9	90.0 (27/30)ab	80.0 (24/30)abc	90.0 (27/30)ab
			Undiluted	100.0 (30/30)a	100.0 (30/30)a	100.0 (30/30)a
		A. indica + *L. multiflora*	1	10.0 (3/30)fg	0.0 (0/30)f	10.0 (3/30)e
			5	40.0 (12/30)cdef	40.0 (12/30)cde	40.0 (12/30)cde
			9	70.0 (21/30)abcd	60.0 (18/30)bcd	60.0 (18/30)bcd
			Undiluted	80.0 (24/30)abc	90.0 (27/30)ab	70.0 (21/30)abc
	Adjéyaokro	Monopropylene glycol	0	0.0 (0/30)f	0.0 (0/30)e	0.0 (0/30)d
		Alphacypermethrin	0.05	20.0 (6/30)def	40.0 (12/30)bcd	30.0 (9/30)cd
		A. indica	1	0.0 (0/30)f	10.0 (3/30)de	0.0 (0/30)d
			5	30.0 (9/30)cdef	20.0 (6/30)cde	40.0 (12/30)c
			9	50.0 (15/30)bcde	60.0 (18/30)bc	50.0 (15/30)bc
			Undiluted	70.0 (21/30)abc	60.0 (18/30)bc	70.0 (21/30)abc
		L. multiflora	1	10.0 (3/30)ef	20.0 (6/30)cde	0.0 (0/30)d
			5	30.0 (9/30)cdef	40.0 (12/30)bcd	50.0 (15/30)bc
			9	80.0 (24/30)ab	80.0 (24/30)ab	90.0 (27/30)ab
			Undiluted	100.0 (30/30)a	100.0 (30/30)a	100.0 (30/30)a
		A. indica + *L. multiflora*	1	10.0 (3/30)ef	0.0 (0/30)e	0.0 (0/30)d
			5	40.0 (12/30)bcde	30.0 (9/30)cde	40.0 (12/30)c
			9	60.0 (18/30)bcd	50.0 (15/30)bcd	60.0 (18/30)bc
			Undiluted	80.0 (24/30)ab	80.0 (24/30)ab	60.0 (18/30)bc
A. variegatum	**Bouaké slaughterhouse**	Monopropylene glycol	0	0.0 (0/30)e	10.0 (3/30)e	0.0 (0/30)g
		Alphacypermethrin	0.05	30.0 (9/30)cde	40.0 (12/30)cde	20.0 (6/30)efg
		A. indica	1	10.0 (3/30)de	10.0 (3/30)e	0.0 (0/30)g
			5	40.0 (12/30)bcd	40.0 (12/30)cde	30.0 (9/30)defg
			9	70.0 (21/30)abc	70.0 (21/30)abc	60.0 (18/30)bcde
			Undiluted	80.0 (24/30)ab	90.0 (27/30)ab	80.0 (24/30)abc
		L. multiflora	1	10.0 (3/30)de	20.0 (6/30)de	10.0 (3/30)fg
			5	40.0 (12/30)bcd	40.0 (12/30)cde	50.0 (15/30)bcdef
			9	90.0 (27/30)a	90.0 (27/30)ab	90.0 (27/30)ab
			Undiluted	100.0 (30/30)a	100.0 (30/30)a	100.0 (30/30)a
		A. indica + *L. multiflora*	1	10.0 (3/30)de	20.0 (6/30)de	0.0 (0/30)g
			5	30.0 (9/30)cde	40.0 (12/30)cde	40.0 (12/30)cdef
			9	70.0 (21/30)abc	60.0 (18/30)bcd	70.0 (21/30)abcd
			Undiluted	90.0 (27/30)a	80.0 (24/30)abc	80.0 (24/30)abc
	Adjéyaokro	Monopropylene glycol	0	10.0 (3/30)fg	0.0 (0/30)f	0.0 (0/30)f
		Alphacypermethrin	0.05	30.0 (9/30)defg	20.0 (6/30)def	30.0 (9/30)def
		A. indica	1	0.0 (0/30)g	10.0 (3/30)ef	10.0 (3/30)ef
			5	40.0 (12/30)cdef	30.0 (9/30)cdef	30.0 (9/30)def
			9	70.0 (21/30)abcd	60.0 (18/30)bcd	60.0 (18/30)bcd
			Undiluted	70.0 (21/30)abcd	80.0 (24/30)ab	80.0 (24/30)abc
		L. multiflora	1	20.0 (6/30)efg	10.0 (3/30)ef	10.0 (3/30)ef
			5	30.0 (9/30)defg	40.0 (12/30)bcde	40.0 (12/30)cde
			9	90.0 (27/30)ab	80.0 (24/30)ab	90.0 (27/30)ab
			Undiluted	100.0 (30/30)a	100.0 (30/30)a	100.0 (30/30)a
		A. indica + *L. multiflora*	1	0.0 (0/30)g	20.0 (6/30)def	10.0 (3/30)ef
			5	40.0 (12/30)cdef	40.0 (12/30)bcde	30.0 (9/30)def
			9	60.0 (18/30)bcde	70.0 (21/30)abc	60.0 (18/30)bcd
			Undiluted	80.0 (24/30)abc	80.0 (24/30)ab	80.0 (24/30)abc

At the same observation time, for the same species and collection site, values sharing at least one letter are not significantly different at the 5% level. Comparisons were performed on the numbers of repelled and non-repelled ticks using Fisher's exact test, with Holm adjustment for multiple comparisons. Letters should be interpreted separately at 30, 45 and 60 min.

egg mass available for reanalysis, these results should remain descriptive. This caution is important, even though the literature shows that *A. indica*-based products can indeed reduce egg hatch in *R. microplus*[18]. Repellency followed a different pattern from mortality. *L. multiflora* was generally the most repellent formulation, particularly at 9 mg/mL and when undiluted, whereas *A. indica* was distinguished more by its lethal activity. This pattern is biologically plausible because essential oils are rich in volatile compounds that can affect orientation and host-seeking behaviour in arthropods[15],[16].

Recent work further supports the potential of *L. multiflora* for controlling *R. microplus*: Coulibaly *et al*. showed in 2026 that a combination of essential oils including *L. multiflora* could inhibit oviposition and egg hatch and produce high acaricidal efficacy against *R. microplus*[19]. However, the concentrations and combinations used in that study differed substantially from ours, so direct quantitative comparison would not be justified. In the present study, the significant concentration effect confirms the dose-dependent nature of repellency, whereas the species effect indicates an overall difference in response

between *A. variegatum* and *R. microplus*. In contrast, the absence of a significant observation-time effect ($p = 0.85$) indicates that no systematic temporal trend was detected between 30, 45 and 60 min. Because separate batches were used at each time point, the observed fluctuations represent variation among independent experiments rather than changes in the behaviour of the same individuals over time.

The *A. indica* + *L. multiflora* mixture was active but was not consistently superior to the products used separately; the data therefore do not support a claim of synergy. This distinction is important because demonstrating synergy requires an analysis specifically designed to quantify interactions between components. For comparison, Coulibaly *et al*. used combination indices to demonstrate synergistic interactions among several essential oils, including *L. multiflora*, against *R. microplus*[19]. Because no equivalent approach was applied here, our results support only the hypothesis of functional complementarity between the acaricidal profile of *A. indica* and the more pronounced repellent profile of *L. multiflora*.

This study has several limitations that define the scope of its conclusions. First, the plant-derived products were not chemically characterized; their exact composition and variability therefore cannot be linked to the observed effects. Second, egg hatch was visually estimated, which warrants descriptive interpretation. Third, the high mortality associated with some treatments reduced the number of females available for conditional analysis of oviposition and limited the statistical power for this parameter. Fourth, the experimental design did not allow estimation of dose–response parameters such as LC50 or LC90. Finally, in vitro bioassays do not reproduce exposure on the animal or constraints related to persistence, skin tolerance and safety. The literature also emphasizes that differences in methods, solvents, substrates and experimental conditions complicate comparisons among studies of botanical acaricides [17]. Future work should therefore combine chemical characterisation, replication of reproductive measurements at the individual level, dose–response modeling, stability and safety studies, and subsequent in vivo validation before any recommendation for use on cattle.

CONCLUSION

Azadirachta indica vegetable oil, *Lippia multiflora* essential oil and their mixture showed complementary in vitro effects against *R. microplus* and *A. variegatum*. Mortality generally increased with concentration and post-exposure time, with *A. variegatum* appearing more susceptible than *R. microplus* under the tested conditions. In *R. microplus*, the lethal effect of *A. indica* became particularly marked from 5 mg/mL onward, whereas *L. multiflora* was distinguished more by its repellent activity. Reproductive observations complemented these findings but could not be separated from mortality: in *R. microplus*, no oviposition was observed from 5 mg/mL onward among the few survivors, but comparisons of oviposition among evaluable treatments were not significant after Holm adjustment; in *A. variegatum*, several treatments caused complete mortality, making the oviposition rate not calculable. Egg hatch, estimated visually, should be regarded as a descriptive outcome. The mixture was active but showed no consistent superiority, and no synergy can be claimed without a specific analysis of interactions. The relatively low mortality of *R. microplus* under alphacypermethrin at 0.05 mg/mL contrasts with the activity observed for some plant-based formulations, but differences in tested concentrations preclude any conclusion of intrinsic superiority. In the Ivorian context of previously documented resistance to several conventional acaricides, these plant-derived products should primarily be viewed as complementary options rather than demonstrated substitutes for chemical acaricides. These findings support further evaluation of *A. indica* and *L. multiflora* as potential components of integrated cattle tick management, with chemical characterisation, dose–response analyses, safety studies and in vivo validation required before practical use.

ETHICAL CONSIDERATIONS

No experimental treatment was administered to cattle as part of this study. The vertebrate-animal component was limited to collection of naturally occurring ectoparasitic ticks; all experimental exposures described in this manuscript were performed on collected ticks in the laboratory.

ACKNOWLEDGEMENT

The authors sincerely thank the Centre for Medical and Veterinary Entomology (CEMV) of Alassane OUATTARA University for the scientific, technical and logistical support provided during this study. They also thank the management and staff of the Bouaké municipal slaughterhouse and the Adjéyaokro farm for facilitating access to cattle and tick collection. The authors are grateful to PORO BIO SANTE, Korhogo, for supplying the *Azadirachta indica* vegetable oil and *Lippia multiflora* essential oil used in the experiments, as well as to the livestock farmers, technicians and all those who contributed to tick collection and identification, bioassays and laboratory monitoring.

CONFLICT OF INTEREST: None declared.

FUNDING: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

KEY POINTS

- Plant-derived formulations produced concentration-dependent acaricidal activity against *Rhipicephalus microplus* and *Amblyomma variegatum*.
- *Lippia multiflora* essential oil showed the strongest repellent activity, particularly at 9 mg/mL and when undiluted.
- *Azadirachta indica* vegetable oil showed marked lethal activity against *R. microplus* from 5 mg/mL onward under the tested conditions.
- The findings support further chemical characterisation, safety assessment and in vivo validation before field use in integrated tick management.
- Reduced reliance on ineffective conventional acaricides may have animal-health and One Health relevance where acaricide resistance is established.

GLOSSARY OF ABBREVIATIONS

AIT – Adult Immersion Test
CEMV – Centre for Medical and Veterinary Entomology
CMR – Corrected mortality rate
MPG – Monopropylene glycol

MR – Mortality rate
OR – Oviposition rate
RR – Repellency rate

REFERENCES

Jongejan F. and G. Uilenberg, The global importance of ticks, *Parasitology*, 129, suppl. S1, S3–S14, 2004. doi:10.1017/S0031182004005967.

Ghosh, S. P. Azhahianambi, and M. P. Yadav, Upcoming and future strategies of tick control: A review, *Journal of Vector Borne Diseases*, 44, 2, 79–89, 2007.

Madder, M. E. Thys, D. Geysen, C. Baudoux, and I. Horak, *Boophilus microplus* ticks found in West Africa, *Experimental and Applied Acarology*, 43, 3, 233–234, 2007. doi:10.1007/s10493-007-9110-1.

Guerrero, F. D. L. Lovis, and J. R. Martins, Acaricide resistance mechanisms in *Rhipicephalus (Boophilus) microplus*, *Revista Brasileira de Parasitologia Veterinária*, 21, 1, 1–6, 2012. doi:10.1590/S1984-29612012000100002.

Abbas, R. Z. M. A. Zaman, D. D. Colwell, J. Gilleard, and Z. Iqbal, Acaricide resistance in cattle ticks and approaches to its management: The state of play, *Veterinary Parasitology*, 203, 1–2, 6–20, 2014. doi:10.1016/j.vetpar.2014.03.006.

Achi, L. Y. M. Boka, A. Biguezoton, P. K. Yao, H. Adakal, S. Kande, A. Azokou, L. S. Koffi, P. R. Akoto, and M. Kone, Resistance of the cattle tick *Rhipicephalus microplus* to alphacypermethrin, deltamethrin and amitraz in Côte d'Ivoire, *International Journal of Biological and Chemical Sciences*, 16, 3, 910–922, 2022. doi:10.4314/ijbcs.v16i3.2.

Isman, M. B. Botanical insecticides in the twenty-first century—Fulfilling their promise? *Annual Review of Entomology*, 65, 233–249, 2020. doi:10.1146/annurev-ento-011019-025010.

Azokou, A. F. H. Coulibaly, L. Achi, A. C. A. Diaha-Kouamé, and M. W. Koné, Plants used in Côte d'Ivoire (West Africa) against ticks: Evaluation for acaricidal activity against *Rhipicephalus (Boophilus) microplus*, *Veterinary Parasitology: Regional Studies and Reports*, 35, 100780, 2022. doi:10.1016/j.vprsr.2022.100780.

Mordue (Luntz) A. J. and A. J. Nisbet, Azadirachtin from the neem tree *Azadirachta indica*: Its action against insects, *Anais da Sociedade Entomológica do Brasil*, 29, 4, 615–632, 2000.

Abdel-Shafy S. and A. A. Zayed, In vitro acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature, and adult stages of *Hyalomma anatolicum excavatum*, *Veterinary Parasitology*, 106, 1, 89–96, 2002. doi:10.1016/S0304-4017(02)00023-7.

Walker A. R. *et al*., Ticks of Domestic Animals in Africa: A Guide to Identification of Species. Edinburgh, UK: Bioscience Reports, 2003.

Drummond, R. O. S. E. Ernst, J. L. Trevino, W. J. Gladney, and O. H. Graham, Boophilus annulatus and *Boophilus microplus*: Laboratory tests of insecticides, *Journal of Economic Entomology*, 66, 1, 130–133, 1973. doi:10.1093/jee/66.1.130.

Abbott, W. S. A method of computing the effectiveness of an insecticide, *Journal of Economic Entomology*, 18, 2, 265–267, 1925. doi:10.1093/jee/18.2.265a.

Core Team, R R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, 2025.

Nerio, L. S. J. Olivero-Verbel, and E. Stashenko, Repellent activity of essential oils: A review, Bioresource Technology, 101, 1, 372–378, 2010. doi:10.1016/j.biortech.2009.07.048.

Ellse L. and R. Wall, The use of essential oils in veterinary ectoparasite control: A review, *Medical and Veterinary Entomology*, 28, 3, 233–243, 2014. doi:10.1111/mve.12033.

Adenubi, O. T. L. J. McGaw, J. N. Eloff, and V. Naidoo, In vitro bioassays used in evaluating plant extracts for tick repellent and acaricidal properties: A critical review, *Veterinary Parasitology*, 254, 160–171, 2018. doi:10.1016/j.vetpar.2018.03.008.

Giglioti, R. M. R. Forim, H. N. Oliveira, A. C. S. Chagas, J. Ferrezini, L. G. Brito, T. O. R. S. Falcoski, L. G. Albuquerque, and M. C. S. Oliveira, In vitro acaricidal activity of neem (*Azadirachta indica*) seed extracts with known azadirachtin concentrations against *Rhipicephalus microplus*, *Veterinary Parasitology*, 181, 2–4, 309–315, 2011. doi:10.1016/j.vetpar.2011.03.053.

Coulibaly, A. B. J. Delma, A. S. Biguezoton, F. F. Dah, A. Kaboré, M. Kiendrebeogo, and R. C. H. Nébié, Combination of essential oils from Ocimum americanum L., Ocimum gratissimum L., and *Lippia multiflora* Mold.: a potential solution for controlling the cattle tick *Rhipicephalus microplus* (Acari: Ixodidae), *BMC Veterinary Research*, 22, 447, 2026. doi:10.1186/s12917-026-05563-z.