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# Efficacy of neem oil, *Zanthoxylum zanthoxyloides* and *Vernomia colorata* extracts on ticks and multi-resistant bacteria isolated from milk in the dairy value chain in Northern Côte d'Ivoire

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## ABSTRACT

Livestock diseases are a major constraint to farmers in sub-Saharan Africa. However, an inadequate use of acaracides and antibiotics for animal diseases control leads to multi-resistance. Research on medicinal plants can be an alternative. The aim of this study was to assess the efficacy of three plant extracts on cattle ticks and multi-resistant bacteria isolated in milk from the Korhogo dairy basin. A cross-sectional survey was conducted from March to August 2023 in 24 farms in northern Côte d'Ivoire. *Rhipicephalus (Boophilus) microplus* and *Amblyomma variegatum* female adult ticks were sampled. A questionnaire was administrated to farmers on their knowledge and practices on diseases control. Three plant extracts (*Azadirachta indica*, *Vernomia colorata* and *Zanthoxylum zanthoxyloides*) were screened for bio-acaricide and anti-bacterial activities using adult immersion test and disc-diffusion assay respectively. At 5000 parts per million (ppm), neem oil and hydroalcoholic extract of the stem bark of *Z. zanthoxyloides* were effective on *A. variegatum* while only neem oil at 5000 ppm was active on *R. (B) microplus*. The three plant extracts at 5000 ppm showed no antibacterial activity on multi-resistant isolates of *Escherichia coli* and *Staphylococcus aureus.* Neem oil can be used for plant-based bio-acaricide development to control the resistant cattle tick *R. (B) microplus* in Côte d'Ivoire.


**Keywords**: *Amblyomma variegatum*, bacteria, Côte d'Ivoire, Neem oil, *Rhipicephalus (Boophilus) microplus*

## INTRODUCTION

The national production of Côte d'Ivoire in animal proteins, covers 51% of the country's meat consumption and only 17% of milk and dairy products consumption (MIRAH-DPP, 2012). The north of the country is the main breeding area for domestic ruminants. Two-thirds of the national cattle

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herd were found in the Poro and Tchologo regions of the Savanna administrative district (Tanguy, 2004). Dairy production practices in this region, influenced by secular sociocultural habits and environmental conditions, were described as non-compliant with basic hygiene standards (Sanhoun et al., 2020). Fermented (62.8%) and unfermented (38.8%) milk were known to be predominant with *Streptococcus infantarius subsp. infantarius* associated with human and animal diseases (Sanhoun et al., 2020). A study conducted on quality of curdled milk consumed in Korhogo showed that this fermented milk contained less than 1 CFU/mL of *Escherichia coli* and $1.28 \pm 1.69 \times 10^3$ CFU/mL of *Staphylococcus aureus* (Coulibaly et al., 2022). Moreover, livestock farming in Poro region, in addition to hygiene problems, also faces several health constraints, including infectious diseases, ticks and tick-borne diseases (Babesiosis, Anaplasmosis, Theileriosis, Cowdriosis), which are confused by farmers with other diseases (Yéo et al., 2017). Important losses such as treatment costs, decreased productivity of milk and meat, reduced reproductive ability, and financial crisis to farmers could be caused by ticks and tick-borne diseases (Rajput et al., 2023). Indeed, each engorged female ingests 1.0 ml of blood during its parasitic phase on the bovine, resulting in a weight loss of approximately 1 g and a reduction in milk production of 8.9 ml. Taking into account production losses and processing costs, tick-borne diseases cost to farmers about US $ 7.30 / head / year (Jonsson, 2006; Yessinou et al., 2017). Ten species of ticks belonging to the genus *Rhipicephalus*, *Hyalomma* and *Ambyomma* were identified in Côte d'Ivoire of which *Rhipicephalus (Boophilus) microplus* has invaded the entire Ivorian territory followed by *Amblyomma variegatum* that still remains dominant in the North (Boka et al., 2017). *A. variegatum* was the dominant species in the country until the introduction of *R. (B) microplus,* discovered in 2007 (Madder et al., 2011), following the importation of dairy cows from Brazil.

To combat infections and tick-borne diseases, farmers used veterinary medicines of which, Oxytetracycline (41.39%) and Amitraz (82.88%) are the most commonly utilized with inappropriate dosing and dilutions (Yéo et al., 2017). Six acaricidal molecules are officially distributed in Côte d'Ivoire (Alphacypermethrin, Cypermethrin, Amitraz, Flumethrin, Deltamethrin and Fipronil) under various trade names (Boka et al., 2023). Despite the availability of a large range of acaricide products, cattle tick *R.(B) microplus* has become a common problem for cattle farmers (Achi et al., 2022). A cross-sectional survey conducted in 2016 in nine farms in the South, Centre and North of Côte d'Ivoire showed a high resistance of *R.(B) microplus* to amitraz and deltamethrin in all farms, whereas resistance of this tick to alphacypermethrin varied by farms from very susceptible to highly resistant (Achi et al., 2022). Farmers also complained about the decrease sensitivity of this tick to Amitraz (Azokou et al., 2016). Faced with recurrent treatment failures and complaints from breeders, a new range of acaricides is currently being offered to breeders based on flumethrin, fipronil, deltamethrin or cypermethrin associated with chlorpyrifos, butoxid and citronella (Boka et al., 2023)**.** A cross-sectional survey conducted in 2018 in the department of Azaguié (Southern Côte d'Ivoire) showed resistance of *R. (B.) microplus* tick to deltamethrin and flumethrin. However, an acceptable level of susceptibility expressed by *R. (B.) microplus* to the association of acaricides (cypermethrin-chlorpyrifos-piperonyl butoxid-citronella) was reported (Boka et al., 2023). The development of resistance means that there is a risk of therapeutic impasses in the treatment of livestock and humans who consume animal products (Zahar and Lesprit, 2014).

International bodies (WHO, OIE and FAO) have been promoting research for preventive or alternative solutions for both humans and animals to deal with resistance and optimize the use of antibiotics (OIE, 2016; WHO, 2016). An alternative solution for the prevention or treatment of diseases could be the use of plants as antibiotics and bio-acaricides as they contain a very large number of molecules with pharmacological modes of action against bacteria or infected animals (Ducrot et al., 2017). Among plant-derived extracts, *Azadirachta indica* oil extract, commonly known as "Neem", showed an efficacity on *R. (B.) microplus* in Brazil (Giglioti et al., 2011) and on *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Fusobacterium nucleatuman* in Cameroon (Nokam et al., 2020). Ethanolic extracts of the leaves of *Zanthoxylum zanthoxyloides, Tephrosia vogelii, Zanthoxylum rubescens* showed activities on the different developmental stages (eggs, larvae and adults) of *R. (B.) microplus* in Côte d'Ivoire (Azokou et al., 2022). Ethyl acetate extracts from the leave of *Vernonia colorata* has inhibited *Micrococcus luteus, Klebsiella pneumoniae* and *Staphylococcus aureus* in South Africa (Reid et al., 2001).

To the best of our knowledge, there is very few information on studies already conducted on resistance of bacteria in milk and on the efficacity of *A. indica* oil and *V. colorata* on ticks and multi-resistant bacteria in dairy value chain of Korhogo. Moreover, no study has yet been conducted in the same area on the efficacity of *Z. zanthoxyloides* on multi-resistant bacteria. The purpose of the present study was thus two-fold. First, to assess the knowledge and practices of breeders belonging to the Union of Local Dairy Professionals of Korhogo on ticks and tick-borne diseases control. Second, to assess the efficacy of neem oil (*A. indica*), *Z. zanthoxyloides* and *V. colorata* extracts on female *R. (B.) microplus* and *A. variegatum* ticks and on multi-resistant bacteria isolated in the milk of Korhogo dairy basin.

## MATERIALS AND METHODS

### Study site

The department of Korhogo (9°27.4818" N; 5°37.7766" W) located in the Poro region (Northern Côte d'Ivoire) is a major dairy basin in the country. In Korhogo, milk is produced by local cattle breeders through an extensive production system. Since a couple of years, farmers are increasingly joining the Union of Local Dairy Professionals of Korhogo. Twenty-four (24) farms were sampled for this study. They are located in the peri-urban neighborhoods of the town of Korhogo and villages in the East of the Korhogo department. The peri-urban neighborhoods included Galbale (farms 2, 8, 19 and 20), Korkor (farm 10), Mongaha (farm 9), Cité Gon (farm 1), Natiokobadara (farms 6, 7, 21 and 23). Investigated villages included Ladiokaha (farm 12), Kafiokaha (farms 3 and 5), Larakaha (farm 14), Tarakaha (farm 15), Lakpolo (farm 11), Lablékaha (farm 13), Kaforo (farms 18, 22 and 24), Lagningèvogo (farms 16 and 17), Nonsorokaha (farm 4) (Figure 1).

### Choice of plants

The neem tree (*Azadirachta indica*), contains around 135 described compounds that can be active against arthropods, including the limonoids (tetranortriterpenoids). The fruits of this plant contain the highest amounts of azadirachtin. Its concentration can reach 40% in the extracted oil (Abdel-Shafy and Zayed, 2002). Neem oil obtained under cold mechanical pressing of neem seeds was purchased from the Wendpenga women's group in Kaya (Burkina Faso). *Vernonia amygdalina* (Asteraceae) and *Zanthoxylum zanthoxyloides* (Rutaceae) have been indicated by breeders as having acaricidal properties and the tree parts used are predominantly leaves and stem bark (Azokou et al., 2016).

### Preparation of hydroalcoholic extracts of *Z. zanthoxyloides* and *V. colorata*

Leaves of *V. colorata*, leaves and stem barks of *Z. zanthoxyloides* were collected in the departments of Dikodougou and Korhogo (Poro region in Northern Côte d'Ivoire). Leaves and stem barks were dried for two weeks in an air-conditioned room (22°C) and pounded by hand in a mortar. Ten (10) g of powder were mixed with 100 mL of 90 % ethanol. The different three mixtures were mechanically stirred for 24 h, filtered through Whatmann paper N° 1 and the filtrates obtained were concentrated using a rotary evaporator at 40 °C. The three concentrated extracts obtained were dried in an oven at 45°C and stored − 20◦C at the chemistry laboratory of *Centre Suisse de Recherches Scientifiques en Côte d'Ivoire* until use (Azokou et al., 2013).

**Preparation of chloroformic extracts of *Z. zanthoxyloides* and *V. colorata***

An ethanolic extract was prepared using 10-fold more ethanol (100 mL) than plant powder (10 g of powder for each plant), under mechanical stirring (150 rpm) during 14 h. The filtrate obtained was recovered in a separatory funnel in an equivalent volume of chloroform (v/v). The whole stirred vigorously was left to settle for the separation of the phases. The chloroform phase was recovered and washed with 100 ml of 1% NaCl in order to eliminate the tannins because they are toxic. The presence of tannins may further enhance the bioactivity of the extract. The chloroform phase, in principle free of tannins, was evaporated to obtain a dry extract which was conserved in refrigerator (Azokou et al., 2013).

**Survey with cattle breeders on their knowledge and practices on ticks and tick-borne diseases control**

A cross-sectional study was carried out from March to August 2023 in a major dairy basin covering most of the milk production farms that owners are members of the Union of Local Dairy Professionals of Korhogo. A structured questionnaire was administrated to 24 breeders affiliated to the Union to assess knowledge and practices of livestock owners around ticks and tick-borne diseases control. Subsequently, female ticks were collected on animals of those 24 farms previously mentioned in study site to assess the sensibility of ticks to plant extracts. Plant extracts were tested on ticks and multi-resistant bacteria isolated in the milk of Korhogo dairy basin.

**Collection and transportation of tick samples**

Ticks were searched on five predilection areas of the cattle body: (i) the inner and outer forelegs, hind legs and abdomen; (ii) tail and anal area; (iii) head and neck; (iv) lateral area and dorsal area from shoulders to tail base; (v) ears (Heylen et al., 2023). Selected cattle have not been treated with any acaricide for 12 days before the collection of ticks. Several studies have revealed that these ticks are resistant to most chemical acaricides (amitraz, deltamethrin flumethrin, etc.) present on the market (Achi et al., 2022; Boka et al., 2023). As it is the female ticks that transmit diseases by taking the blood meal to promote egg production, engorged female ticks were removed from the cattle body using forceps (Toure et al., 2012) and stored in specially prepared bottles. Bottles were clearly identified and placed on a tray containing a clean cloth soaked in water to maintain a moisture environment and thus allow ticks to survive. The lids of the vials and the trays were perforated and protected with a net and a fine-mesh screen to provide air for the ticks and avoid their escape (Achi et al., 2022). Morphological identification of ticks was carried out according to an identification key (Walker et al., 2023) at *Laboratoire National d'Appui au Développement Agricole* (LANADA), Korhogo station.

**Laboratory bioassays**

Biological tests were carried out at LANADA, the same day as the ticks were collected. Different concentrations of hydroalcoholic extracts from 1000 ppm (1 mg/mL) to 5000 ppm (5 mg/mL) were prepared by dissolving 10 mg, 20 mg, 30 mg, 40 mg and 50 mg of hydroalcoholic extract in 10 mL of distilled water, respectively. Similarly different concentrations of chloroform extracts from 1000 ppm to 5000 ppm were prepared by dissolving 10 mg, 20 mg, 30 mg, 40 mg and 50 mg of chloroform extract in 10 ml of Dimethylsulfoxide (DMSO) respectively. Different concentrations of neem oil from 1 mg/mL to 5 mg/mL were also prepared by dissolving 10 mL, 20 mL, 30 mL, 40 mL and 50 mL of neem oil in 10 mL of DMSO, respectively. The adult immersion test was used to evaluate the effectiveness of different concentrations of neem oil and extracts on ticks. Female ticks were separated into groups of 6, of homogeneous weight and species. Each batch of 6 ticks was immersed at room temperature and for 5 minutes in 10 mL of each of the different concentrations of neem oil and hydroalcoholic and chloroform extracts. Then, ticks from each batch were dried and placed in plastic tubes and incubated at 28°C. Two weeks after incubation, tick mortality was observed. Distilled water was used as a negative control for the tests carried out with the hydroalcoholic extracts and DMSO was used as a negative control for the tests carried out with neem oil and chloroform extracts. The adult immersion test was repeated three times for each concentration of neem oil and extracts tested as well as for controls (Coulibaly et al., 2020).

The percentage of mortality was calculated as follows:

**Percentage of mortality** = (Number of dead ticks /Total number of ticks) × 100

**Isolation and evaluation of antimicrobial susceptibility of bacteria from milk**

Twenty (20) bacteria, *E. coli* (n =10) and *S. aureus* (n =10) isolated from milk in the Korhogo dairy basin milk were used for the determination of antibiotic resistance. Rapid E. coli 2 agar was used for the detection and enumeration of *E. coli* according the AFNOR BRD-07/7-12/04(2) standard and Baird Parker agar was used for the enumeration of *S. aureus* as described in AFNOR NF 08-057. Six antibiotics belonging to three families (Beta-lactams, Cyclins, Macrolides and Aminosides) were used. The antibiotics were Spiramycyn (10 µg), (Macrolides), Penicillin (1 µg), (Beta-lactams), Ampicillin (30 µg), (Beta-lactams), Amoxicillin (30 µg), (Beta-lactams), Tetracycline (30 µg), (Cyclins) and Gentamicin (30 µg), (Aminosides). Prior to inoculation, the inoculum was prepared from a 24-hour culture of a pure strain obtained on nutrient agar. One or two colonies were emulsified in a tube containing 2 mL of 0.85% NaCl physiological water (BioMérieux, reference 08026E). The density of the mixture was then adjusted using a densimeter to obtain 0.5 Mac-Farland (108 CFU/mL).

Müeller Hinton agar poured into Petri dishes was inoculated using the flooding technique. After a contact time of 5 minutes, the excess inoculum was removed with a micropipette. Using a swab, excess inoculum was removed by pressing on the edges of the dish, tilting it at approximately 60°. The dish was dried at 37°C for 5 minutes (Velasco, 2022)

The antibiotic discs were applied to the agar surface with forceps, ensuring that the discs are 2 cm apart. Once applied, the antibiotic disc was not moved. The plates were incubated for 18 to 24 hours at 37°C for all strains. Critical diameters and interpretive reading rules were done according the French Society of Microbiology (SFM, 2020).

**Antibacterial activities of plant extracts**

The previous twenty (20) bacteria, *E. coli* (n =10) and *S. aureus* (n =10) isolated from milk in the Korhogo dairy basin milk were used to assess the efficacy of neem oil, *Z. zanthoxyloides* and *V. colorata* extracts. The antimicrobial activity was evaluated using the agar diffusion technique. Mueller-Hinton agar in Petri dishes (thickness = 4 mm) were soaked with an inoculum equivalent to 0.5 of McFarland. After drying, wells (diameter = 6 mm) were made in the agar using sterile Pasteur pipette. Only concentrations of neem oil and extracts that showed activity on ticks were retained for testing bacterial susceptibility. Thus, 80 µl of neem oil (5000 ppm), hydroalcoholic leaves extract of *V. colorata* (5 mg/ml) and hydroalcoholic stem bark extract of *Z. zanthoxyloides* (5 mg/ml) were poured in the wells. Gentamicin (30 µg) disc was applied to the agar surface with forceps. Once applied, the antibiotic disc could no longer be moved. The plates were incubated for 24 hours at 37°C. After incubation, the diameters (mm) of inhibition zones were measured (Koné et al., 2004).

**Statistical analysis**

Data were entered into a Microsoft Excel 2020 extension and were then imported into R software version 4.2.1 (R foundation team, 2022). The Z-test was performed to compare percentage of mortality of adult female ticks according to plant extracts. A p-value of less than 0.05 was considered to indicate statistical significance.

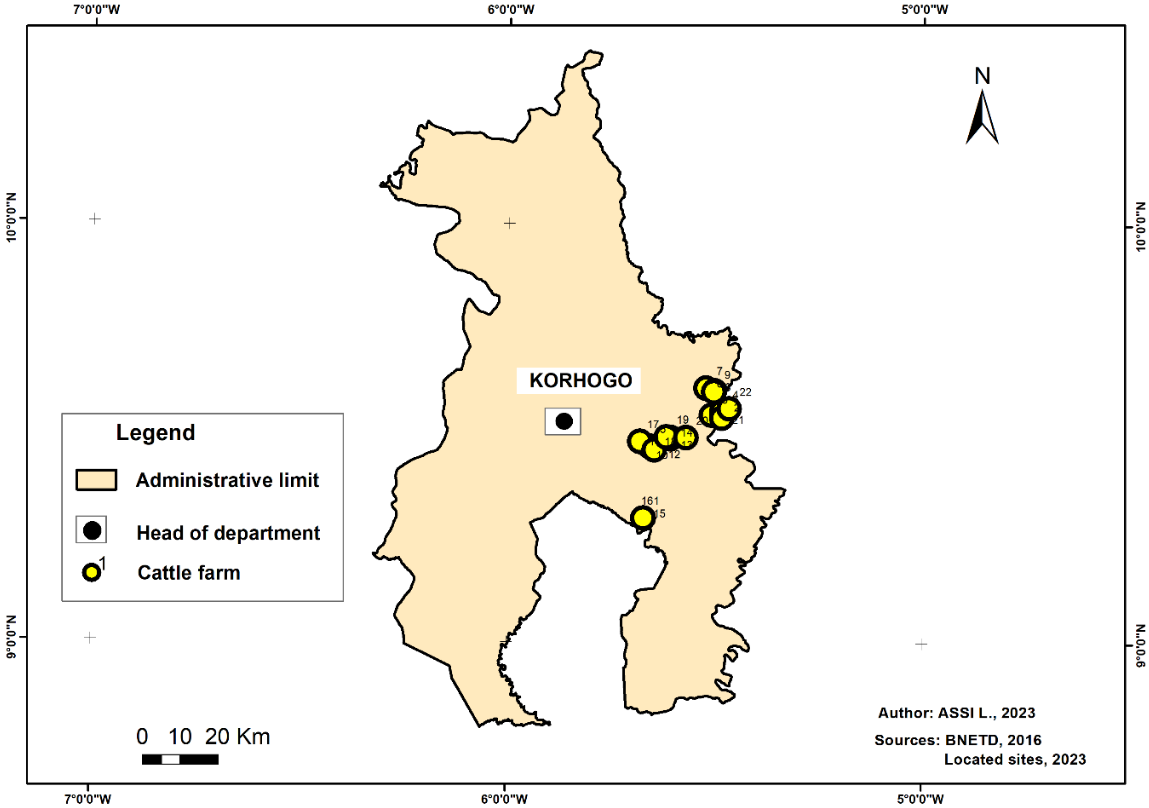


**Figure 1:** Study sites in the Department of Korhogo, North Côte d'Ivoire.

## RESULTS

### Typology of farms and socio-demographic characteristics of farmers

Farmers affiliated to the Union of local dairy professionals of Korhogo raise mainly local cattle breeds including Zebu, Ndama and Goudali. In the sampled farms, the size of herd ranged between 14 and 400 heads with a mean of 91.04 heads (89.64-92.44). Milk cows ranged between 1 and 41 with a mean of 14.13 cows (13.98-14.28). The mean quantity of milk produced per day and per farm was 6.45 L (6.41-6.51).

Table 1 shows that most cattle breeders practiced extensive farming. In two-third of farms (67%), the main objective of cattle production was mixed (milk production and fattening). In 79% of farms, the main purpose of the production was for sale. Out of 24 respondents involved in the study, 9 (37%) had no formal education. All of them were men. Among these farmers, 8 (33%) were over 60 years old, while 23 (96%) were Muslim and 22 (92%) were married.

### Knowledge and practices about ticks and tick-borne diseases

All of 24 (100%) breeders had knowledge on ticks and their ability to transmit diseases to cow. However, 15 (63%) did not know where ticks come from. For 14 (58%), 9 (38%) and 1 (4%) farmers, ticks were insects, microbe and parasite respectively. According to 15 (63%) breeders, the most common disease transmitted by ticks was Dermatophilosis ("*Kroukrouba*" in Malinké). For 8 (33%) farmers, the most common disease was Foot-and-mouth disease ("*Safa*" in Malinké) and for 1 breeder (4%), Scabies ("*Kroukrou*" in Malinké) was the most common disease. To deal with tick-borne diseases, 18 (75%) of breeders treated animals themselves by injecting cows with antibiotics, while 4 (17%) called the veterinary and 2 (8%) treated animals themselves and also refered to the veterinary. Concerning the use of antibiotics and acaricides, 7 (29.2%) farmers confirmed not respecting the latency time between the milking and the milk sale after veterinary drugs use. Most of farmers, 22 (92%) used antibiotics sold in the market while, 2 (8%) did not use them. Even though resistance to antibiotics is a public health concern, 23 (95.8%) breeders thought there was no need to reduce the use of antibiotics and 1 (4.2%) used medicinal plants as alternative to the use of antibiotics and acaricides (Table 2).

**Yield of plant extracts**

The yield of hydroalcoholic extract from 10 g of *Z. zanthoxyloides* leaves powder, 10 g of *Z. zanthoxyloides* stem bark powder and 10 g of *V. colorata* leaves powder were 9.67%, 9.07% and 11.67% respectively.

The yield of chloroformic extracts from 10 g of plant powder were 0.17%, 0.87% and 2.77% respectively (Table 3).

**Efficacy of neem oil and hydroalcoholic extract of *Z. zanthoxyloides* and *V. colorata* on ticks**

Figure 2 shows that at 4000 ppm, only neem oil caused 100% mortality of adult *A. variegatum* ticks while the hydroalcoholic extracts of *V. colorata* and *Z. zanthoxyloides* leaves caused 33% mortality. A statistically significant difference was observed at 4000 ppm between the percentage mortality of adult females of *A. variegatum* immersed in the hydroalcoholic extracts of the leaves of *V. colorata* and *Z. zanthoxyloides* and the percentage observed with neem oil ($p < 0.0001$).

At 5000 ppm, the neem oil and the hydroalcoholic extracts of the stem bark of *Z. zanthoxyloides* caused respectively 100% of female adult *A. variegatum* mortality while the extracts of *V. colorata* and *Z. zanthoxyloides* leaves caused 50% mortality. A statistically significant difference was observed at 5000 ppm between the percentage mortality of adult females of *A. variegatum* immersed in the leaves hydroalcoholic extracts of *V. colorata* and *Z. zanthoxyloides* and the percentage observed with neem oil ($p = 0.005$).

The acaricidal tests carried out on female adult of *R. (B.) microplus* using hydroalcoholic extracts and neem oil showed that only neem oil caused 100% mortality at 5000 ppm and 4000 ppm (Figure 3).

**Efficacy of chloroform extracts of *Z. zanthoxyloides* and *V. colorata* on ticks**

Tables 4 shows the effect of chloroform extracts from *Z. zanthoxyloides* stem bark, *V. colorata* leaves and *Z. zanthoxyloides* leaves. At 4000 ppm, these extracts had no effect on adult *A. variegatum* and *R. (B.) microplus* ticks**.** At 5000 ppm, the chloroform extracts from *Z. zanthoxyloides* stem bark caused 33% mortality of adult *A. variegatum* ticks.

**Antimicrobial resistance profiles of *E. coli* and *S. aureus* isolated from milk**

All the ten isolates of *E. coli* were resistant to Penicillin and Ampicillin and sensitive to Gentamicin. Of the ten (10) isolates of *E. coli*, eight (80%) were resistant to Spiramycyn (Macrolides), 5 (50%) were resistant to Tetracycline (Cyclins), all were resistant to Penicillin (Beta-lactams) and Ampicillin (Beta-lactams) (Table 5).

All the ten isolates of *S. aureus* were resistant to Amoxicillin and sensitive to Gentamicin and Spiramycyn. Of the (10) isolates of *S. aureus*, eight (80%) were resistant to Ampicillin (Beta-lactams) and Tetracycline (Cyclins) and nine (90%) were resistant to Penicillin (Beta-lactams) (Table 5).

**Efficacy of neem oil, and hydroalcoholic extracts of *Z. zanthoxyloides* and *V. colorata* on multi-resistant bacteria**

All twenty (20) isolates of bacteria, *E. coli* (n =10) and *S. aureus* (n =10) studied were resistant to neem oil and hydroalcoholic extracts of *Z. zanthoxyloides* and *V. colorata* at 5000 ppm. However, Gentamycin gave inhibition diameter ranging from 16 to 25 mm.

**Table 1:** Socio-demographic characteristics of farmers (n = 24).

| **Socio-demographic characteristics of farmers** | | **Number (%)** |
|---|---|---|
| **Gender** | Men | 24 (100) |
| | Women | 0 (0) |
| **Age group** | 30-40 | 4 (17) |
| | 40-50 | 6 (25) |
| | 50-60 | 6 (25) |
| | ≥ 60 | 8 (33) |
| **Origin** | Autochthonous | 11 (45) |
| | Allochthonous | 2 (8) |
| | Foreigner | 11 (45) |
| **Religion** | Muslim | 23 (96) |
| | Christian | 0 (0) |
| | Animist | 1 (4) |
| **Education** | None | 9 (37) |
| | Koranic | 6 (25) |
| | Primary | 4 (17) |
| | Secondary | 4 (17) |
| | Superior | 1 (4) |
| **Marital status** | Single | 1 (4) |
| | Married | 22 (92) |
| | Divorced/Separated | 1 (4) |
| **Number of children in the household** | ≤ 2 | 2 (8) |
| | 3-5 | 7 (29) |
| | ≥ 6 | 15 (62) |
| | | |
| **Farm characteristics** | | |
| Type of farming | Extensive | 14 (58) |
| | Semi-extensive | 10 (42) |
| Objective of cattle production | Milk production | 7 (29) |
| | Fattening | 1 (4) |
| | Mixed | 16 (67) |
| Purpose of cattle production | Sale | 19 (79) |
| | Self-consumption and sale | 5 (21) |

**Table 2:** Knowledge and practices on ticks and tick-borne diseases.

| **Variables** | **Effectif (%)** |
|---|---|
| **Knowledge of ticks** | |
| Yes | 24 (100) |
| No | 0 (0) |
| **Representation of ticks for the farmer** | |
| Insect | 14 (58) |
| Microbe | 9 (38) |
| Parasite | 1 (4) |
| **Knowing where ticks come from** | |

| | |
|---|---|
| Yes | 9 (37) |
| No | 15 (63) |
| **Knowledge of ability of ticks to transmit disease** | |
| Yes | 24 (100) |
| No | 0 (0) |
| **Knowledge of most common diseases transmitted by ticks** | |
| Dermatophilosis | 15 (63) |
| Foot-and-mouth disease | 8 (33) |
| Scabies | 1 (4) |
| **Person responsible for animal treatment** | |
| Breeder | 18 (75) |
| Veterinary | 4 (17) |
| Mixed | 2 (8) |
| **Opinion about need to reduce the use of antibiotics** | |
| Yes | 1 (4.2) |
| No | 23 (95.8) |
| **Use of antibiotics to treat animals** | |
| Yes | 22 (92) |
| No | 2 (8) |
| **Alternatives to the use of antibiotics** | |
| Vaccination of animals | 23 (95.8) |
| Use of medicinal plants | 1 (4.2) |
| **Respect of latency time between treatment of animals and sale of milk** | |
| No | 7 (29.2) |
| Yes | 10 (41.7) |
| Not always | 7 (29.2) |

**Table 3:** Yield of extraction.

| **Plant extracts** | Mass of plant powder (g) | Mass of extracts after drying | Yield (%) |
|---|---|---|---|
| **Hydroalcoholic extract** | | | |
| Leaves of *Z. zanthoxyloides* | 10 | 0.967 | 9.67 |
| Stem bark of *Z. zanthoxyloides* | 10 | 0.907 | 9.07 |
| Leaves of *V. colorata* | 10 | 1.167 | 11.67 |
| **Chloroformic extracts** | | | |
| Leaves of *Z. zanthoxyloides* | 10 | 0.017 | 0.17 |
| Stem bark of *Z. zanthoxyloides* | 10 | 0.087 | 0.87 |
| Leaves of *V. colorata* | 10 | 0.277 | 2.77 |

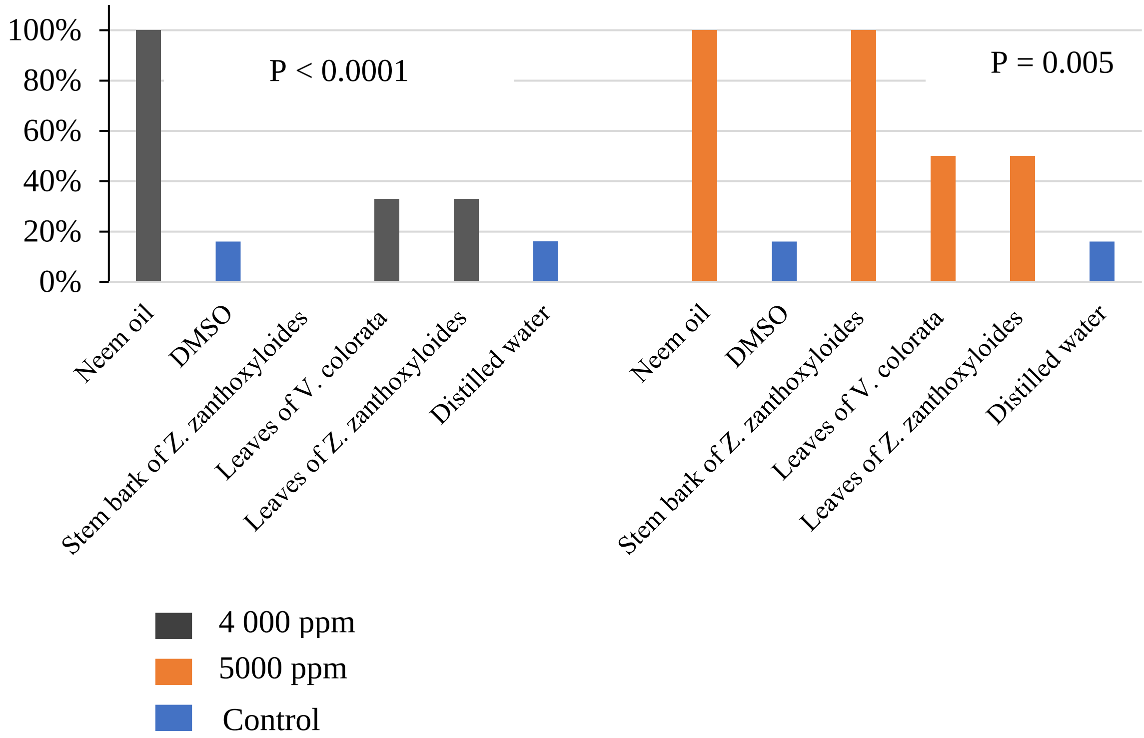


**Figure 2:** Mortality percentage of adult female *A. variegatum* ticks using hydroalcoholic plant extracts and neem oil.

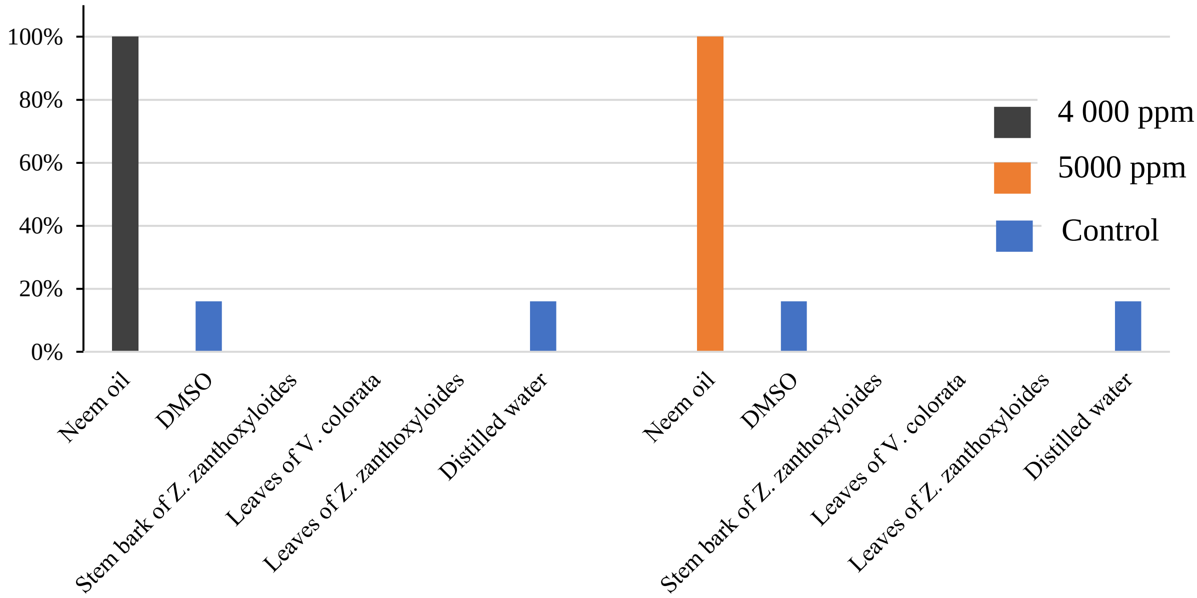


**Figure 3:** Mortality percentage of adult female *R. (B.) microplus* ticks using hydroalcoholic plant extracts and neem oil.

**Table 4**: Percentage of adult female *Amblyomma variegatum* and *Rhipicephalus (Boophilus) microplus* tick mortality using chloroform extracts.

| **Plant extracts** | **Concentrations** | | |
|---|---|---|---|
| | 5000 ppm | 4000 ppm | Control |
| | | **Adult female *A. variegatum* ticks** | |
| *Z. zanthoxyloides* stem bark | 33% | 0% | 16% |
| *V. colorata* leaves | 16% | 0% | 16% |
| *Z. zanthoxyloides* leaves | 16% | 0% | 0% |
| | | **Adult female *R. (B.) microplus* ticks** | |
| *Z. zanthoxyloides* stem bark | 0% | 0% | 0% |
| *V. colorata* leaves | 16% | 0% | 0% |
| *Z. zanthoxyloides* leaves | 0% | 0% | 0% |

**Table 5:** Antimicrobial Resistance Profiles of *E. coli* and *S. aureus* isolated from milk.

| | **Spiramycyn** | | **Penicillin** | | **Ampicillin** | | **Amoxicillin** | | **Tetracycline** | | **Gentamicin** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Strains** | **Inhibition Diameter (mm)** | | | | | | | | | | | |
| 1 (*E. coli*) | **R** | 0 | R | 0 | **R** | 0 | R | 13 | S | 20 | S | 25 |
| 2 (*E. coli)* | **R** | 14 | R | 0 | **R** | 0 | S | 20 | S | 19 | S | 17 |
| 3 (*E. coli)* | **R** | 13 | R | 0 | **R** | 0 | I | 16 | S | 20 | S | 19 |
| 4 (*E. coli)* | **R** | 12 | **R** | 0 | **R** | 0 | I | 16 | **R** | 0 | S | 20 |
| 5 (*E. coli)* | **R** | 0 | **R** | 0 | **R** | 0 | S | 19 | **R** | 0 | S | 18 |
| 6 (*E. coli)* | I | 15 | **R** | 0 | **R** | 0 | S | 18 | S | 21 | S | 19 |
| 7 (*E. coli)* | **R** | 6 | **R** | 0 | **R** | 0 | S | 21 | **R** | 0 | S | 18 |
| 8 (*E. coli)* | I | 16 | **R** | 0 | **R** | 0 | I | 17 | **R** | 0 | S | 20 |
| 9 (*E. coli)* | **R** | 7 | **R** | 0 | **R** | 0 | S | 20 | I | 18 | S | 19 |
| 10 (*E. coli)* | **R** | 8 | **R** | 0 | **R** | 0 | S | 20 | **R** | 0 | S | 16 |
| 11 *(S. aureus)* | **S** | 21 | **R** | 0 | **R** | 10 | **R** | 0 | **R** | 10 | S | 22 |
| 12 *(S. aureus)* | **S** | 23 | **R** | 0 | **R** | 0 | **R** | 0 | **R** | 12 | S | 25 |
| 13 *(S. aureus)* | **S** | 20 | **R** | 0 | **R** | 0 | **R** | 0 | **R** | 5 | S | 30 |
| 14 *(S. aureus)* | **S** | 22 | **R** | 0 | **R** | 0 | **R** | 0 | **R** | 10 | S | 23 |
| 15 *(S. aureus)* | **S** | 20 | S | 20 | S | 19 | **R** | 5 | **S** | 23 | S | 20 |
| 16 *(S. aureus)* | **S** | 21 | **R** | 0 | S | 0 | **R** | 0 | **R** | 3 | S | 23 |
| 17 *(S. aureus)* | **S** | 22 | **R** | 0 | **R** | 0 | **R** | 0 | **S** | 12 | S | 25 |
| 18 *(S. aureus)* | **S** | 20 | **R** | 0 | **R** | 0 | **R** | 0 | **R** | 10 | S | 25 |
| 19 *(S. aureus)* | **S** | 22 | **R** | 0 | **R** | 10 | **R** | 0 | **R** | 10 | S | 22 |
| 20 *(S. aureus)* | **S** | 22 | **R** | 10 | **R** | 10 | **R** | 0 | **R** | 10 | S | 28 |

Sensible (S), Resistant (R), Intermediate (I)

## DISCUSSION

The present study shows that, according to 15 (63%) breeders, the most common disease is Dermatophilosis. To handle tick-borne diseases, 18 (75%) of breeders treated animals themselves by injecting cows with antibiotics, while 4 (17%) called the veterinary and 2 (8%) treated animals themselves and also referred to the veterinary. Only 1 (4.2%) farmer uses medicinal plants as alternative to antibiotics and acaricides. The results obtained in this study are different from those found in a study conducted on traditional methods for the control of cattle ticks in Côte d'Ivoire (Azokou et al., 2016). In this study, diseases or symptoms frequently observed following tick bites were anemia (36.7%), fever (23.6%), dermatoses (20.1%) and weight loss (19.6%). To handle ticks, breeders used synthetic acaricides (68.1%). The other practices in the fight against ticks were manual lifting or manual diptank (42.2%), use of waste or engine oil (33.3%) and plants (11.1%).

Yield of extraction was more important and the hydroalcoholic extracts of plants showed the most efficiency compared to chloroformic extracts. This finding could be explained by the fact that water and alcohol contain compounds such as terpenoids responsible for the activity of hydroalcoholic extracts of plants.

The acaricidal tests carried out on ticks using hydroalcoholic extracts of leaves and stem bark of *Z. zanthoxyloides* showed no effect on female adult *R. (B.) microplus* at 4000 and 5000 ppm. Findings in this study are different from the conclusions of another study conducted on evaluation for acaricidal activity against *R. (B.) microplus*. The study published in 2022, reported that the extracts of leaves and stem bark of *Z. zanthoxyloides* caused mortality of 66.66% and 50% of tick strains from the localities of Normandia and Tomassé, respectively. For stem bark of *Z. zanthoxyloides*, the mortality percentage was 16.67% for the Normandia strain and 0% for Tomassé strain (Azokou et al., 2022).

Neem oil at 4000 and 5000 ppm caused 100% mortality of adult female of *A. variegatum* and *R. (B.) microplus*. This finding could be explained by the fact that the neem oil contains compounds such as azadirachtin (tetranortriterpenoids) that can be active against arthropods (Mordue and Nisbet, 2000). Indeed, in the study carried out on *in vitro* acaricidal activity of neem *(A. indica)* seed extracts with known azadirachtin concentrations against *R. microplus*, the product effectiveness (PE) calculations for all the solutions tested showed that the AZA solution at 10,000 ppm was the most effective (Giglioti et al., 2011).

Neem oil and hydroalcoholic extract of stem barks of *Z. zanthoxyloides* at 5000 ppm caused mortality of 100% on *A. variegatum* adult female while only neem oil at 5000 and 4000 ppm caused 100% mortality of the adult female *R. (B) microplus*. This finding indicates that neem oil was more effective compared to hydroalcoholic extract of stem bark of *Z. zanthoxyloides.*

Moreover, the evaluation of antimicrobial susceptibility of bacteria isolated form milk showed that the isolates of *E. coli* and *S. aureus* from milk are multi-resistant because they are resistant to three families of antibiotics (Beta-lactams, Cyclins and Macrolides). The multi-resistance of the isolates can be explained by the fact that in bovine cow, bacteria are exposed to antibiotics, whose overuse has led to increased cases of drug resistance (Sombie et al., 2022). Our finding is different from the one of a study conducted in northwest of Iran on milk samples collected from different retail sellers (Hassani et al., 2022). In that study, all *E. coli* and *S. aureus* isolated showed high rates of resistance to amoxicillin, penicillin, and cefalexin (Hassani et al., 2022).

However, neem oil at 5000 ppm did not show any antibacterial activity on isolates of *E. coli* and *S. aureus* isolated from milk in the Korhogo dairy basin. This result can be explained by the fact that neem oil does not possess chemical constituents such as alkaloids, tannins, and flavonoids responsible for antimicrobial activities. A study carried out in Bangladesh on antibacterial activity of ethanolic extract of *Camellia sinensis* (green tea) and neem leaves on Methicillin-Resistant *S. aureus* (MRSA) and Shiga-Toxigenic *E. coli* (STEC) showed different results. In the study mentioned, an inhibition zone of neem leaves extract was observed against MRSA only, while STEC was not affected by the used extracts (Zihadi et al., 2019).

One limitation of this study is inherent to the use of neem oil and hydroalcoholic extracts of *Z. zanthoxyloides* and *V. colorata* at only 5000 ppm to access antibacterial activities of extracts on multi-resistant isolates of *E. coli* and *S. aureus.* All concentrations of neem oil and hydroalcoholic extracts of *Z. zanthoxyloides* and *V. colorata* should have been used to access antibacterial activities of extracts on multi-resistant isolates of *E. coli* and *S. aureus*. Despite this shortcoming, this study sheds new light on the efficacy of neem oil in the control of ticks in Côte d'Ivoire.

## Conclusion

Findings from this study strongly suggest that Neem oil exhibits *in vitro* acaricide activity against both R. *(B.)* microplus and *A. variegatum* female adult ticks at 5000 ppm and 4000 ppm. Neem oil can be used for plant-based bio-acaricide development to control *R. (B) microplus*, one of most resistant cattle tick known nowadays.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

VK, SGT, AA and GF designed the research project. SGT is responsible for the overall coordination and implementation of the project in the field. ABK involved in data collection. ABK and AA involved in ticks samples analysed in lab. TS and KJN involved in antibacterial analysed in lab. SGT, MOS, AA and AD cleaned, analysed the data. SGT wrote the first draft of the paper. VK, AA, AD, GF, KJN, ABK, MOS, ARCA, MBG, MC and MKC revised the paper. All authors read and approved the final version of the manuscript prior to submission.

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