



In Situ Evaluation of the Anthelmintic Effect of the Aqueous Extract of *Syzygium aromaticum* (L) Merr and Perry on Bovine Strongyles

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Abstract

Cattle farming in tropical and subtropical countries is negatively affected by unavailability of feed resources and high prevalence of gastrointestinal nematodes. The use of synthetic anthelmintics has led to the emergence of parasite strains resistant to most molecules. Research has shown that these pesticides, even when present in faecal matter, are toxic to the environment. Plant resources are alternatives to synthetic anthelmintics in tackling these parasites and are environmentally friendly. We studied anthelmintic properties by comparing quantities and numbers of health indicators (egg content of fecal matter, load reduction, treatment efficacy, average daily gain, haematological parameters). The anthelmintic effect of the aqueous extract of clove was thus demonstrated *in situ* on bovine strongyles with satisfactory results. In particular, a drop in egg load in fecal matter of bovine Gastro-Intestinal Parasites expressed as Eggs Per Gram of fecal matter (from 700.00 ± 86.60 to 100.00 ± 50.00 with 5mg/kg inclusion level; from 816.67 ± 57.74 to 300.00 ± 50.00 with 50 mg/Kg inclusion level and from 300.00 ± 86.60 to 116.67 ± 28.87 with 300 mg/kg inclusion level); resulting in; the reduction rate between 45 and 86%; the efficacy rate of 92.54% (5 mg/kg), 81.16% (50 mg/kg), and 69.07% (300 mg/kg); the average daily gain of; 2800g/d (5 mg/kg), 6800g/d (50 mg/kg), and 5800g/d (300 mg/kg); and restoration of physiological parameters (drop in white blood cells, increase in platelets, increase in red blood cells, and haemoglobin). The restoration of certain hematological parameters to normal levels and the reduction in the parasite egg load in dung demonstrated that *Syzygium aromaticum* cloves have anthelmintic properties.

Keywords: Anthelmintic; Cattle; Clove; *in situ*; *Syzygium aromaticum*

Introduction

Agropastoral development policies and research themes are increasingly oriented towards a sustainable

development. Even though intensive agropastoral practices have considerably increased productivity worldwide, more than 820 million people still suffer from hunger, particularly in Africa, Latin America and West Asia, and two

billion people are food insecure [1]. Faced with problems of inadequate animal nutrition and management practices, farmers resorted to conventional veterinary products and self-medication [2] to reduce cost and maximize profits, contributing to the development of anthelmintic resistance. These practices fuel a series of biological and chemical hazards on food, as well as on the environment, including pollution, the destruction of coprophagous insects and significant greenhouse gas emissions. By 2050, the world's population is expected to reach 9.8 billion, with food demand set to rise by over 50%, and even by more than 70% for animal products [3]. In order to meet this demand and reduce the harmful consequences, one alternative would be to develop a control method that is accessible to farmers and is environmentally friendly. As integral part of our dietary habits, spices have traditionally been used since many years ago to preserve food products, as they have antiseptic and disinfectant properties [4]. *Syzygium aromaticum* has long been used by traditional Ayurvedic healers in India to treat respiratory and digestive ailments [5,6]. Also considered a rich source of bioactive antimicrobial compounds [7], the effect of this spice on diseases and hematological parameters in cattle has not been the subject of any literature material to date, based on our findings. Given these rich and historic characteristics, this study aims to evaluate in situ the effect of *Syzygium aromaticum* on helminth parasites using hematological, weight and fecal parameters in cattle.

Materials and methods

Study site

The study was conducted at the Agricultural Research Centre in Wakwa, Ngaoundere, specifically at the Centre's farm and veterinary laboratory. The centre is located on the following coordinates: 7 ° 15'908 'North and 13 ° 32'912"

East at an Altitude of 1143 m in Ngaoundere I sub Division.

Plant material

Cloves purchased at the Ngaoundere urban market were cleaned and dried in an airy room away from dust and sunlight, then ground to powder, and stored in black jars until use.

Preparing the extract

100g of clove powder was mixed with 1000 mL of distilled water, boiled for 10 minutes and filtered through No.1 Wattman paper after cooling. Heating the filtrate in an oven at 40°C for 72 hours helped to remove any solvent before storing the extract obtained at 4°C. We weighed the extract prior to storing and calculated the yield using the

$$\text{formula } R = \frac{E}{MS} \times 100$$

Where;

R is the extraction yield;

E the quantity of extract;

MS the quantity of dry matter in the product.

Animal material (equipment and criteria)

The animals selected for this study had access to natural pasture (Figure 1a). They had not received any antiparasitic treatment six months prior to the experiment. Their general condition was good, with no signs of disease. All experimental and control animals lived in the same environment throughout the experimental period and were randomly divided into five batches of 3 animals each (Figure 1b).



Figure 1: images showing animals on natural pasture (a) and different batches in a crush (b)

Collecting faecal Samples

Faecal samples collected followed this procedure: the animal stood upright restrained by two shepherds, the collector stood behind the immobilized animal wearing gloves and lifted the animal's tail with one hand, then skilfully inserted fingers of the second hand in the shape of a cone into the animal's rectum collecting 50 to 100 g of dung into a plastic bag. Faecal sample labelled with identification numbers were packed in a cooler for transportation to the laboratory and stored at 4°C if analyses were not immediate.

Blood sampling

The collector applied compression by hand on the jugular vein, then inserted the needle perpendicularly to the animal to pierce the skin, and inclined at an angle of around 30° as it penetrated the vein until blood was collected to the desired volume (2-5 mL). Pressure applied with cotton wool against the punctured skin prevented bleeding [8,9].

Parasitological follow-up

An initial quantitative coproscopy was performed on 25 animals from the Wakwa Agricultural Research Center farm in order to select candidates. 24 hours before each treatment, we carried out; faeces and blood analyses and weighing to record eggs per gram of faecal matter, haematological parameters and weights to determine the corresponding concentration per subject. We determined the number of eggs per gram of faeces using Willis solution. In conjunction with quantitative pre- and post-treatment coproscopy [10,11], blood samples and weights recorded enabled various parameters to be evaluated.

Repairing the saturated NaCl solution

We dissolved 40% sodium chloride in pure water (40g of salt per 100g of water). A density meter was used to check the concentration of salt in the solution.

Effect of Clove Aqueous Extract on infestation intensity

This was evaluated based on the number of eggs per gram of dung used (OPG) as described by Thienpont et al. (10) using the following formula:

$$OPG = \frac{n1+n2}{2} \times 100$$

Where;

n1 is the number of eggs counted in cell 1

n2 the number of eggs counted in cell 2.

Since the McMaster slide has two compartments or cells, the total number of eggs is equal to the sum of the eggs in the two

cells divided by 2 and then multiplied by 100, or the sum of the eggs in two cells simply multiplied by 50.

The effect of Aqueous Clove Extract on reducing the parasite load between samples

At the start of the trial, the presence of strongyles eggs in the dung were noted in all batches. The reduction in the rate (R) of egg excretion in dung, made it possible to evaluate the variation in the level of parasite egg load at a given time (t) compared with the previous situation. This rate was calculated according to the following formula by Bauer et al. [12]:

$$R(\%) = \left[\frac{\text{Initial OPG} - \text{OPG at time t}}{\text{Initial OPG}} \right] \times 100$$

Where;

R (%): reducing the parasite load

OPG: number of eggs per gram

Treatment efficacy

Treatment efficacy (E%) was calculated using President's method [13], which considers the average Opg before and after treatment, according to the following formula:

$$E\% = \left[1 - \left(\frac{T1}{T2} \right) \left(\frac{C2}{C1} \right) \right] \times 100$$

Where;

E% = efficacy rate

T1 = Opg on nth day after treatment

T2 = initial Opg of treated batch

C1 = Opg on nth day after treatment of control batch

C2 = initial Opg of control batch.

Effect of Aqueous Clove Extract on average weight gain (AWG) after treatment

Weighing was carried out early in the morning before departure for grazing. Average daily gains were calculated using the formula proposed by Lhoste et al, [14].

$$GMQ = (Wf - Wi) \times \frac{1000}{\Delta T}$$

Where;

GMQ = average daily gain (g/d)

Wf = final live weight (kg)

Wi = initial live weight (kg)

ΔT = time between two weighings in days.

The effect of Aqueous Clove Extract on hematological parameters in cattle

An automatic counter (Mindray BC 20s flow cytometer, whose simplicity, speed, precision, and reliability are commendable), helped in analyzing several hematological

parameters, including white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular hemoglobin content, mean corpuscular hemoglobin concentration, mean corpuscular volume and platelets [15-17].

Statistical analysis

Data from hematological, fecal and weight were first processed in Excel version 2016 while the Statistical Package for Social Sciences (SPSS) was used to determine significance differences between treatments through analysis of variance

(ANOVA) and to do comparison tests.

Results and discussion

Yield

The aqueous extract of clove yielded around 20.50%. This percentage is considered high due to the polarity of the solvent used (water). These results are in line with those of Amina et al. [18], who found a yield of 19.8% using aqueous extraction.

Effect of extract on excretion,

OPG/EACG	Jo	J5	J10	J15	J20	J25
5 mg / kg	700.00±86.60 ^a	100.00±50.00 ^{ab}	316.67±28.87 ^{ab}	383.33±76.38 ^{abc}	166.67±76.38 ^{ac}	166.67±76.38 ^{ac}
50 mg / kg	816.67±57.74 ^a	300.00±50.00 ^{ab}	166.67±28.87 ^{ac}	850.00±50.00 ^{bc}	1016.67±76.38 ^{abc}	983.33±28.87 ^{abc}
300 mg / kg	300.00±86.60 ^a	316.67±76.38 ^b	250.00±50.00	116.67±28.87 ^{abc}	366.67±28.87 ^c	400.00±50.00 ^c
TN	200.00±50.00	383.33±57.74	216.67±28.87	250.00±50.00	133.33±28.87	616.67±28.87
TP	333.33±28.87	116.67±28.87	400.00±86.60	183.33±28.87	83.33±28.87	750.00±50.00

Table 1: Effects of aqueous clove extract on bovine Gastro-Intestinal Parasites egg load in dung .

Table values are presented as mean ± standard deviation. Small letters compare means on rows. Means containing the same letter indicate a significant difference between them at the 5 % threshold. OPG: Eggs per Gram; EACG: Aqueous Clove Extract; TN: Negative Control; TP: Positive Control.

Apart from the negative control, which did not induce a significant reduction in parasite egg load in dung, all three doses and the positive control in Table 1 induced a significant drop ($P < 0.5$) at different times during the trials. observations made 5 days after treatment with different doses revealed reduction in egg load as follows; 5mg/kg dose (700.00 ± 86.60 to 100.00 ± 50.00) and 50mg/Kg (816.67 ± 57.74 to 300.00 ± 50.00) This drop was brought below the threat threshold (< 200) with each dose.

Compared with the positive and negative control batches, the reduction in egg load of bovine Gastro-Intestinal Parasites in dung caused by the aqueous extract of clove was significant ($p < 0.05$) with doses of 5 and 50 mg/kg. The

desired effect, achieved with small doses, demonstrated the efficacy of *Syzygium aromaticum* in combatting gastro-intestinal parasites. These results concur with those of Hounzanbé-Adoté et al. [20] and Abladam et al. [21], who respectively showed that papaya seeds reduced infestation in sheep to a relatively low levels, and that the smaller doses (5 and 50 mg/kg versus 300 and 1000 mg/kg) induced a significant ($p < 0.05$) drop in egg density below 250 up to day 20. This work also corroborated the work of numerous authors who recognized clove's acaricidal [22], antifungal, antibacterial, antiviral and anesthetic properties [23,24].

The increase in egg load from D15 (50 mg/kg) and D20 (300 mg/kg) suggests the efficacy might drop with time. Hounzanbé-Adoté et al. [20], suggested that the reduction in egg load observed in vivo is due either to adult worm mortality or a drop in female worm prolificacy. Nfi et al. [25] also showed that the ineffectiveness of certain plants in the treatment of gastro-intestinal parasites of ruminants could be attributed to pastures constantly harbouring parasites.

Reduction in oviposition

Doses	J5	J10	J15	J20	J25
5 mg / kg	85,71	54,86	45,29	76,29	76,29
50 mg / kg	63,24	79,66	-4,17	-24,51	-20,47
300 mg / kg	-5,33	16,67	61,33	-22	-33,33
TP	65,17	-20,12	45,05	75,08	-125,23

The values in the table are presented as percentages. TP: Positive Control.

Table 2: Oviposition reduction rates of bovine gastrointestinal parasites after treatment with aqueous clove extract.

Table 2 shows a reduction rate over the whole trial period (ranged from 45 to 86% in the 5 mg/Kg dose and over two trials notably to 63 and 80% in the 50 mg/Kg dose, 5 and 10 days after treatment, respectively.

Oviposition reduction rates ranged from 45 to 86% and 63 and 80% respectively in 5 and 50 mg/Kg doses significantly ($p < 0.05$) demonstrated the effect of aqueous clove extract in reducing oviposition of bovine gastrointestinal parasites, even without repeated treatment. These results are comparatively more appreciable than those obtained with albendazole (75.08%) . in the work of Hounzanbé-Adoté et al. [20], according to which papaya seeds reduced the level of egg load of gastrointestinal parasites in sheep by up to 60% on D13. This is similar with the same doses of 5 and 50 mg/kg of the aqueous extract of *Carica papaya* seeds, which respectively gave 86.67 and 84.21% reduction in oviposition

of bovine gastro-intestinal parasites [21]. Nevertheless, these results, in agreement with those of many authors as stated above, are in contradiction with those of Nguessan et al. [26], according to whom the reduction rates from *Annona senegalensis* extracts during the first few days after treatment were lower than those of albendazole. This contradiction is also seen in the results of Githiori et al. [27], who found that herbal remedies in most cases had lower parasitism level reductions than synthetic anthelmintics in vivo control tests.

Treatment efficacy

Table 3 shows a treatment efficacy of 81.81% for the control (albendazole) and 92.54% (5mg/kg), 81.16% (50mg/kg) and 69.07% (300mg/kg) for the experimental units.

Doses	J5	J10	J15	J20	J25
5 mg / kg	92,54	58,20	56,23	64,34	92,31
50 mg / kg	80,80	81,16	16,67	-87,23	60,89
300 mg / kg	44,99	22,84	69,07	-83,46	56,71
TP	81,81	-11,22	56,04	62,52	26,88

The values in the table are presented as percentages. TP: Positive Control

Table 3: Treatment efficacy with aqueous clove extract

Compared with the efficacy of albendazole (81.81%) found, Table 3 shows a higher efficacy of treatment with aqueous clove extract at the lowest dose of 5 mg/kg (92.54%). This higher rate with the lowest dose is in line with that obtained by Abladam et al. [21] showing that the aqueous extract of clove eliminates gastrointestinal parasites

in cattle even in small quantities. On the other hand, these results disagree with those of Yonwa et al. [28], who showed that the efficacy of treatment with *Senna italica* extract on *Haemonchus contortus* is concentration-dependent in vitro, implying that efficacy increases with concentration.

Weight gain

GMQ (g / j) EACG	J5	J10	J15	J20	J25
5 mg / kg	2800	-1400	-3400	600	2000
50 mg / kg	-1600	200	-2800	-2600	6800
300 mg / kg	3000	-3000	-6800	400	5800
TP	2400	800	2800	-5800	1000
TN	4200	-400	1400	-5600	2200

Table values are given in g/d. ADG: Average Daily Gain; AECG: Aqueous Clove Extract

Table 4: Average daily weight gain of cattle after treatment .

With the exception of the 50 mg/kg animals, which showed gains from D10 onwards, all animals in the other batches (positive control, negative control, 5 and 300 mg/kg) showed average daily gains ranging from 2400 to 4200g/d from D5 onwards.

The average daily gain after treatment with aqueous clove extract showed significant temporal variability ($p < 0.05$) in results both between individuals in the same batch (5 mg/kg: 2800g/d at D5, 600g/d at D20 and 2000g/d at D25) and between batches (6800g/d with 50 mg/kg at D25 and

5800g/d with 300 mg/kg at D25). This variability in average daily weight gain was also observed by Yonwa et al. [33] in their work evaluating in vivo the anthelmintic activity of

Callistemon rigidus on *Haemonchus contortus*. This gain can be attributed to the low parasite load caused by the aqueous extract, helping animals to maximise nutrients.

Effect of extract on haematological parameters

	5 mg / kg					
	Jo	J5	J10	J15	J20	J25
GB (10 ⁹ /l)	10,70	10,33	9,23	7,20	6,90	7,63
Gran (10 ³ /μL)	4,57	3,47	3,13	1,23	1,70	2,10
Gran (%)	43,57	33,37	33,80	31,10	24,70	27,03
GR (10 ⁶ /μL)	4,89	4,90	5,63	4,26	4,32	4,69
HGB (g/dL)	10,90	10,60	11,50	9,60	8,90	9,17
HCT (%)	28,73	27,33	31,87	28,17	26,63	29,50
VGM (μ ³)	58,00	55,73	56,83	42,80	62,53	63,60
TCMH (pg)	22,33	21,57	20,17	21,33	21,33	19,83
CCMH (g/dL)	38,87	38,87	35,77	34,23	34,03	31,23
PLT (10 ³ /μL)	165,33	157,33	187,33	125,33	280,67	370,67
	50 mg / kg					
	Jo	J5	J10	J15	J20	J25
GB (10 ⁹ /l)	11,40	13,50	11,53	13,30	9,17	10,63
Gran (10 ³ /μL)	4,43	4,60	3,23	3,47	2,83	2,93
Gran (%)	39,00	35,23	27,03	30,73	31,13	27,77
GR (10 ⁶ /μL)	4,70	4,43	4,54	4,77	4,47	4,88
HGB (g/dL)	10,27	9,43	8,80	9,47	9,03	9,17
HCT (%)	27,27	24,03	26,00	28,00	25,80	28,80
VGM (μ ³)	60,27	54,37	56,93	58,50	57,40	58,60
TCMH (pg)	21,77	21,30	19,23	19,77	20,37	18,70
CCMH (g/dL)	36,877	39,33	33,93	33,87	35,67	32,07
PLT (10 ³ /μL)	177,33	147,00	174,00	280,00	760,33	107,33
	300 mg / kg					
	Jo	J5	J10	J15	J20	J25
GB (10 ⁹ /l)	13,23	12,47	11,00	10,10	8,53	8,00
Gran (10 ³ /μL)	5,93	4,10	3,77	3,27	3,07	2,17
Gran (%)	44,57	33,40	34,37	32,27	34,93	28,57
GR (10 ⁶ /μL)	5,88	4,89	5,57	5,20	5,18	5,37
HGB (g/dL)	13,07	10,73	11,33	10,57	10,87	10,37
HCT (%)	33,53	27,30	32,77	30,37	30,60	31,63
VGM (μ ³)	55,40	55,83	58,57	58,20	58,70	58,97
TCMH (pg)	22,03	21,90	20,30	20,33	21,13	19,30
CCMH (g/dL)	38,77	39,37	34,87	35,13	36,40	32,83
PLT (10 ³ /μL)	164,00	178,00	386,33	214,67	428,33	155,33

Table 5: Effect of aqueous clove extract on bovine complete blood count.

Table 5 shows, on the one hand, a decrease in white blood cell count with each of the three doses 5 mg/kg (43.57 to 24.70%), 50 mg/kg (39.00 to 27.03%) and 300 mg/kg (39.00 to 27.03%). Platelet counts increased from 165.33 to 370.67x10³/μL at 5 mg/kg, from 177.33 to 760.33x10³/μL at 50 mg/kg and from 164.00 to 428.33x10³/μL at 300 mg/kg. Similarly, an increase in red blood cell count from 4.89 (D0) to 5.63 (D10) and haemoglobin from 10.90 (D0) to 11.50 (D10) was observed at the 5mg/kg dose.

The significant downgrading ($p < 0.05$) of the leukocyte count from D0 (43.57%, 39.00% and 44.57%) to almost half (24.70%, 27.03% and 28.57%) in the three doses respectively, would result from low levels of chemotactic factors that usually trigger their production during an infestation.

When the cause of their production fades, following the possible elimination of the infestation by the aqueous extract of clove, and as their half-life is only about ten hours, the white blood cells decrease (neutropenia and eosinopenia) through apoptosis. These observations are in line with those of Drieu [34] and Abladam et al. [21], who respectively demonstrated that when granulocytes perform their function, they can disappear in a controlled, programmed manner, and granulocyte levels drop significantly after the anthelmintic action of the aqueous extract of papaya seeds. Nevertheless, all these observations stand in contrast to those of Kouadio et al. [35], according to whom *Ficus sycomorus* leaf extracts revealed a significant increase in the white blood cell and platelet levels of treated rats.

Crucial for characterizing circulating erythrocytes in the blood, Wintrobe erythrocyte indices reveal macrocytic anemia from D20 (5 mg/kg) and normocytic anemia from D5 to the end of the experiment (50 mg/kg). The normocytic anemia in the batch receiving 50 mg/kg throughout the test period could be due to the balance of the aqueous clove extract.

The aqueous extract of clove restored normochromic anemia, with values for Mean Corpuscular Hemoglobin Concentration returned to the reference range from D5 (5 mg/kg) and D10 (50 and 300 mg/kg), as well as values for Mean Corpuscular Hemoglobin Content from D5 in all treated batches.

Despite the absence of data highlighting the effect of plants on bovine hematology, the results confirm the anthelmintic activity of this plant species in restoring the animal's physiological equilibrium, and corroborate those of Amina et al. [18], in which the nematotoxic effect of aqueous and hydroethanolic extracts of *Calotropis procera* and *Faidherbia albida* leaves, bark and roots were evaluated in vitro on *Onchocerca ochengi* (microfilariae and adults) and

the resistant free-living nematode *Caenorhabditis elegans* with LC50s of 0.13 and 0.18 mg/mL.

Conclusion

Clove has proven to be an attractive alternative in the fight against strongyles. It restores blood parameters to normal levels for cattle, reduces the excretion of parasite eggs in feces and promotes weight gain. The buccal administration of its aqueous extract yields promising results in the elimination of digestive strongyles from cattle. Further trials involving incorporation of the extract into cattle feed would determine in vivo alicament quantities relative to animal masses.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

ADE: technical manipulations, data collection and analysis, drafting of article; DNE: technical manipulations, data collection and analysis; MHF: article writing; ND: study conception and article writing.

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