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The Nematicidal Activity of *Moringa Oleifera* Leaves Extract Against Gastro-Intestinal Strongyles of Small Goats

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ABSTRACT

Introduction: Gastrointestinal nematodes have a deleterious effect on animal health and production. In search of alternative to synthetic anthelminthics in the control of strongylosis among small ruminants, this study was conducted to evaluate the *in situ* adulticidal properties of *Moringa oleifera* on gastro-intestinal strongyles of goats.

Methods: Twenty (20) goats naturally infested with mixed gastrointestinal nematodes were distributed into 5 groups (n=4): a negative control group receiving Tween 80 at 2.8%, a positive control group treated with Albendazole at 5 mg/kg and tested groups treated with doses of extract (125, 250 and 500 mg/kg). *In situ* anthelminthic activity was assessed through determination of the faecal eggs count reduction, total worm count reduction, variation of packed cell volume (PCV) and body weight after treatment.

Results: Five species of nematodes were identified among experimental animals, with *Strongyloides papillorus* as the most prevalent (93.8%) followed by *Haemonchus contortus* (87.5%). At dose of 500 mg/kg, ethanolic extract of *M. oleifera* registered 76.4 % and 69.8 % eggs density reduction of gastro-intestinal strongyles eggs and parasitic load nineteen days post-treatment respectively while Albendazole induced 100 % reduction of these same parameters at the dose of 5 mg/kg. Moreover, the extract did not significantly affect PCV nor body weight of experimental goats.

Conclusion: This study validates scientifically the use of alcoholic leaves extract of *M. oleifera* in the treatment of gastro-intestinal strongyles. The standardization of this extract is however necessary for his use as a sustainable tool for controlling strongylosis in small ruminants.

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INTRODUCTION

In small ruminants breeding, gastro-intestinal strongylosis remains a dominant affection worldwide and particularly in tropical and sub-tropical region. This is due to the presence in digestive tract of sheep and goat of nematodes belonging to the Order Strongylidae with principal genus *Haemonchus* Cobb, 1898, *Teladorsagia* Stadelman, 1894, *Cooperia* Ranson, 1907, *Trichostrongylus* Looss 1905, *Nematodirus* Ranson, 1907, *Chabertia* Beldomenico PM, 2003 et Œsophagostomun Rudolphi, 1809, Bunostomum Raillet, 1902. Haemonchosis is one of the most significant parasitic diseases of livestock. It affects hundreds of millions of small ruminants and causes substantial losses to the livestock industry estimated at tens of billions of dollars per annum (Roeber et al., 2013).

Although a vaccine (Barbervax®) was recently released in Australia to support anthelmintic treatment programs against haemonchosis, the control of *H. contortus* and related nematodes relies largely on the use of anthelmintic drugs (Kumarasingha et al., 2016). In many countries, control of parasites is seriously compromised because of misused of these drugs which has led to widespread of resistance strain of nematodes to most classes of anthelmintics. Other limiting factors to the currently used anthelmintics include: unavailability to rural dwellers and problems of drug residues in animals intended for human consumption, toxicity and slow development of new drugs due to high cost of developing and licensing a new drug (Enejoh et al., 2015). This has led to increasing use of medicinal herbs by Africans and small holder livestock producers.

Natural compounds from plants provide an opportunity in the search for new, effective and safe anthelmintics. It is likely that many of these natural medicines may be acting on pathways in worms

KEYWORDS:
Gastro-intestinal strongyle; *Moringa oleifera*; Nematicidal activity.

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that differ from targets of currently used anthelmintic drugs and, therefore, might be able to kill nematodes that are resistant to one or more anthelmintics (Hrckova & Velebny, 2013). However, the lack of scientific evaluation of the efficacy, mode of action and identification of active components of the majority of such medicinal plants is a hindrance to the development of their potential and even a hindrance to the development of traditional medicine, as not all plants claimed to be active by naturopaths are always scientifically active.

Moringa oleifera (Lam, 1785) is one of the most useful shrubs in the world as almost all its parts can be used as food or have beneficial properties. It is often referred to as a "miracle plant" because of its many virtues. *Moringa oleifera* has several curative and prophylactic properties. It has been used in traditional medicine for centuries in many cultures around the world against various ailments such as skin infections, anaemia, anxiety, asthma, blackheads, blood impurities, bronchitis, colds, cholera, conjunctivitis, coughs, diarrhoea, eye and ear infections, fever, headache, abnormal blood pressure, hysteria, joint pain, respiratory disorders, azoo and oligospermia, scurvy, angina, tuberculosis, sprain, intestinal worms and diabetes (Nikkon et al., 2003). Cameroonian traditional therapists advise taking leaf powder mixed with honey for the treatment of intestinal worms. The plant has been shown to possess antioxidant (Moya et al., 2012), antiepileptic (Amrutia et al., 2011), hepato-protective (Singh et al., 2012), antimicrobial (Devendra et al., 2011), anti-inflammatory (Chandrawathani et al., 1999), antidiabetic (Ndong et al., 2007), antiurolithiatic (Fahad et al., 2010), anti-asthmatic (Agrawal & Mehta, 2008), cardiovascular (Gilani et al., 1994), anti-fertility (Shukla et al., 1998) and anthelminthic properties (Mbogning tayo et al., 2014; Rastogi et al., 2009). Additionally, different types of active phytoconstituents with prooved anthelminthic activity are present in *M. oleifera* like alkaloids, protein, quinine, saponins, flavonoids, tannin, steroids, glycosides (Sharma et al., 2011).

Actually, an *In vitro* study was carried by Mbogning Tayo *et al*. (2014) to assess the activity of aqueous and ethanolic extract of *M. oleifera* on free-living stages (eggs and larva) of *Haemonchus contortus*. They revealed that ethanolic extract was the most active and by such could be used as disinfectant to inhibit eggs and larva development of strongyles in pasture. This bring us to pursue the study by evaluating the effectiveness of ethanolic extract of *M. oleifera In situ* on adults strongyles with the intention to scientifically demonstrate that ethanolic extract of *M. oleifera* can be used for preventive and curative purpose in the control of gastro-intestinal strongyles of small ruminants.

METHODOLOGY

Collection and Storage of Plant Material

Leaves from mature trees were collected at the teaching and research farm of the University of Dschang-Cameroon. A branch of leaves was taken to the National Herbarium of Cameroon where it was identified under the reference number 42885/HNC as leaves of *Moringa oleifera* Lam. The collected plant material was dried in shade, at ambient temperature for three weeks. Dried leaves were ground to powder and stored in airtight plastic bags in the Laboratory of Biology and Applied Ecology of the University of Dschang.

Ethanolic Extract Preparation

Ethanolic extract of *Moringa oleifera* was used because it was the most efficient during our previous *In vitro* trials on *Haemonchus contortus* (Mbogning Tayo et al., 2014). Extraction was done according to the procedure described by Wabo Pone *et al*. (2006 and 2010). Briefly, five hundred (500) grams of stored powder were macerated in 06 l of ethanol 95%. The mixture was daily stirred to permit better extraction of the active ingredients. Seventy-two (72) hours later, the solution was sieved and filtered through a cotton layer and a filter paper of pore size 2.5 µm. The filtrate was evaporated in a rota vapor at 82°C for 8 hours. The extract obtained was poured in a large Petri dish and allowed at room temperature for two days, at the end of which a completely dried ethanolic extract was obtained and stored in refrigerator (4°C) for further use.

Preparation of extract concentration

The following formula (1) was applied to calculate the concentration of solution to prepare corresponding to each dose.

$$C = \frac{DXM}{VX1000} \quad (1)$$

where C = **C**oncentration; **D** = **D**ose of extract to administer (125mg/kg, 250mg/kg and 500mg/kg); **M** = weight of the most heavy animal; **V** = **V**olume of extract solution to administer (3ml) (Mbogning Tayo et al., 2022).

Preparation of reference drug

The reference drug Albendazole 250mg used in this study was bought from a Veterinary Clinic in the Divisional Delegation of Livestock, Fisheries and Animal Industries in Dschang. This drug was chosen due to its broad spectrum activity to prevent and treat gastrointestinal nematodes of domestic animals. Dosage recommends a single intake of one tablet of Albendazole for an animal of 50kg. One tablet was weighed (7.25g) and the algorithm (2) was applied to calculate the mass of albendazole needed for each animal in the positive control group.

$$malb = 0.145\ mc \quad (2)$$

where: **malb** = mass of Albendazole and **mc** = body mass of the animal.

Once this mass was calculated, the fraction of the product was weighed and diluted in 3 ml of distilled water before being administered to the animal (Mbogning Tayo et al., 2022).

Treatment and grouping of experimental animals

For this study, Twenty (20) goats of both sexes were brought from local breeders while ensuring that they had not received any anthelminthic treatment for at least 4 weeks. Animals were kept for two weeks to get acclimatised with the environment before the start of the experiment. During that time, they were vaccinated against Small Ruminants Pest (SRP) using Capri-Pestovax. They also received a preventive antibiotic: phenoxyzone (1ml/10 kg) combined with Stress-Vita (1ml/10kg), a multivitamin anti-stress, for six days. After two

weeks, the experimental animals were divided into 05 groups (n=4 regardless gender) according to their body weight and the number of eggs per gram of faeces (EPG) as followed:

- Animals of group 01 received Tween 80 at 2.8%;
- Animals of group 02 reveived Albendazole at the dosage of 5mg/kg;
- Animals of group 03 received ethanolic extract of *M. oleifera* at the dosage of 125 mg/kg;
- Animals of group 04 received ethanolic extract of *M. oleifera* at the dosage of 250 mg/kg;
- Animals of group 05 received ethanolic extract of *M. oleifera* at the dosage of 500 mg/kg.

Each animal was weighed and treated with 3 ml of corresponding substance. The extract and Tween 80 at 2.8 % were administered twice daily (morning at 8 a.m and evening at 5 p.m) for three days, while the Albendazole treatment was a single dose. The substances were administered orally using a syringe. Throughout the experiment, the animals were kept indoor (Mbogning Tayo et al., 2022).

Identification of helminthes species found in experimental animals

Before evaluating the anthelmintic activity of the extracts, it was important to identify the different helminth species found in the faeces of the experimental animals. The identification of nematode eggs was done by differential diagnosis on the basis of morphological criteria such as size, shape, nature of the shell and number of blastomeres according to Thienpont *et al.*(1979) and Soulsby (1982).

Goat Faecal sample

Faecal samples were collected directly from the rectum of each goats before treatment (day 0) and after treatment (day 3, 7, 10, 14, 17 and 21). The specimens were transported to the laboratory for coprological analysis using the McMaster technique as describeb by Thienpont *et al*. (1986) to determine the number of egg per gram of faeces (EPG). The percentage of faecal egg count reduction (FECR) was calculated using the following formula **(3)** (Hounzangbe-Adote et al., 2001):

$$FECR = \frac{\text{initial EPG} - \text{EPG at x time}}{\text{initial EPG}} x100 \quad (3)$$

Worm recovery

On the twentieth day post treatment, the animals of each group were slaughtered and their abdominal cavity opened. The gastro-intestinal tract was sectioned, removed and placed in a 10 l container containing 5 l of water. This organ was opened longitudinally, the content was collected and inner wall was scratched to detach the worms. The solution obtained was homogenised then left to settle for 30 min. After three successive decantations, worms were collected and counted under a binocular magnifying glass. The percentage of total worm count reduction (TWCR) was calculated as follows (Enriquez, 1993):

$$TWCR = \frac{\text{total worm count in control group - total worm count in treated group}}{\text{total worm count in control group}} x100 \quad (3)$$

Packed Cell volume (PCV)

Blood samples were collected in EDTA coated tube from the jugular vein of animals in each group before treatment and eighty days after treatment. Blood of each tube were introduced into micro-haematocrit tubes and centrifuged using a micro-haematocrit centrifuge at 12,000 rpm for 6 min and the Packed Cell Volume (PCV) was determined using the following formula **(5)** (Mbogning Tayo et al., 2022)**:**

$$PCV = \frac{\text{heightofredbloodcell}}{\text{total height of blood}} x100 \quad (5)$$

Statistical analysis

Mean percentages of reduction in faecal egg density, haematocrit and animal body weight before and after the different treatments were compared using a two-factor analysis of variance (time and dose factors), supplemented by the Bonferroni post-test. Mean percentages of reduction in parasite load were compared using a one-factor analysis of variance followed by the Tukey multiple comparison post-test. Differences were considered significant at $P<0.001$, $P< 0.01$ and $P <0.05$.

RESULTS

Prevalence of different species of gastrointestinal helminthe identified in experimental animals

Five species of nematodes were identified (Figure 1) namely *Strongyloides papillorus*, *Haemonchus contortus*, *Trichostrongylus sp*, *Bunostomum sp* *Cooperia sp*, *Trichuris ovis* and *Ascaris sp*. In addition to these nematodes, one cestode species was identified: *Monezia expansa*. Their prevalence is reported in Table 1.

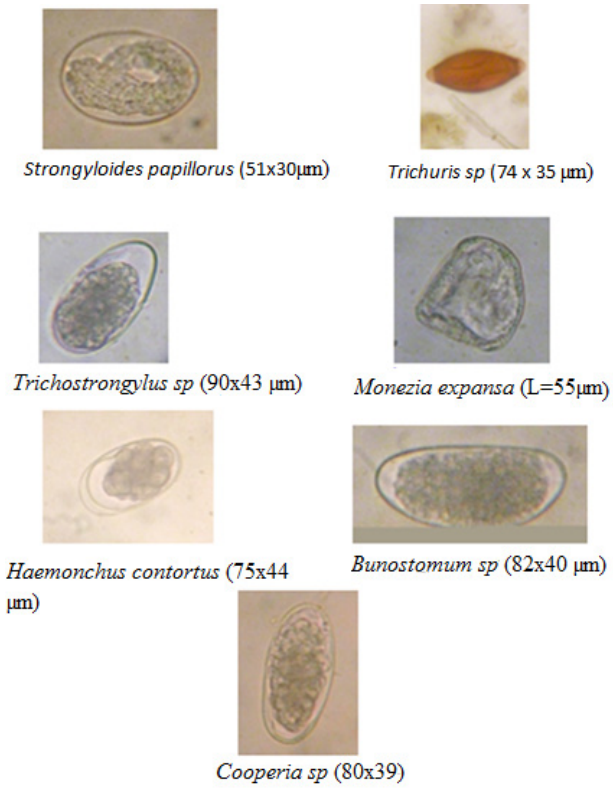

Fig. 1: Eggs of helminthes found on experimental animals

Table 1, *Strongyloides papillorus* was the most prevalent (93.8%), followed by *Haemonchus contortus* (87.5%).

Effect of ethanolic extract of *Moringa oleifera* on fecal eggs excretion

From Table 2, it can be seen that the ethanol extract of *Moringa oleifera* reduced the faecal egg count in animals treated with 250 and 500 mg/kg. This activity began to be felt on day 7 with the 500 mg/kg dose and was more marked on day 21 with a reduction rate of 76.4% with the same dose. The reference product, Albendazole, completely stopped the excretion of digestive strongyles eggs at D14. Tween 80 at 2.8% did not affect the reproduction of strongyles, resulting in negative or less than zero reduction percentages.

Table 1: Prevalence of gastro-intestinal (GI) helminthes identified among experimental goats

GI helminthes	*Prevalence (%)*
Strongyloides papillorus	93.8
Haemonchus contortus	87.5
Trichostrongylus sp	53.1
Bunostumum sp	28.1
Cooperia sp	12.5
Trichuris ovis	9.4

Table 2: Mean eggs density variation of strongyle and mean percent reduction in fecal egg load before and after administration of different treatments[A, B, C] indicate significant differences within a single column at P <0.05; P <0.01 and P <0.001, respectively. (n = 4).

Effect of ethanolic extract of *Moringa oleifera* leaves on digestive strongyles parasite load

Figure 2 shows the variation of reduction percentage in parasite load after 19 days of Albendazole administration and doses of ethanol extract of *M. oleifera*. It was found that the ethanol extract of *M. oleifera* induced the reduction of the parasite load of strongyles. A reduction rate of 69.8% was obtained at the dose of 500 mg/kg while Albendazole induced a 100% reduction in strongyles.

Effect of ethanolic extract of *Moringa oleifera* on packed cell volume

Figure 3 shows the packed cell volume (PCV) values in animals before and 19 days after administration of ethanolic extracts of Albendazole and *M. oleifera*. In general, the different treatments did not significantly affect the PCV of the animals. However, an increase was observed with Albendazole and the 500 mg/kg dose of the ethanol extract of *M. oleifera*.

Table 2: Mean eggs density variation of strongyle and mean percent reduction in fecal egg load before and after administration of different treatments

Products	*Doses*	*Pre-treatment*	*Post-traitment*					
	(mg/kg)	*D0*	*D3*	*D7*	*D10*	*D14*	*D17*	*D21*
Tween 80 à 2.8 %	/	3400 ± 430.1	3625 ± 497.5 (-8.1)	4475 ± 466.5 (-35.3)	4313 ± 621 (-26.7)	4288 ± 494 (-28.2)	4813 ± 356.8 (-45.4)	5200 ± 837.7 (-55.2)
Albendazole	5	6425 ± 556	1250 ± 341.6 (80.3)˚C	100 ± 115,5 (98.5)˚C	50 ± 57.7 (99.2)˚C	0 ± 0 (100)˚C	0 ± 0˚c (100)˚C	0 ± 0 (100)˚C
Moringa oleifera	125	4475 ± 330.4	4575 ± 670.2 (0.8)	4350 ± 544.7 (-7.0)B	3925 ± 206.2 (-3.9)A	3725 ± 221.7 (4.7)˚C	3375 ± 499.2 (3.3)˚C	3350 ± 57.7 (1.6)˚C
	250	4475 ± 330.4	4575 ± 670.2 (-3.2)	4350 ± 544.7 (2.4)˚C	3925 ± 206.2 (12.0)˚C	3725 ± 221.7 (16.2)˚C	3375 ± 499.2 (24.8)˚C	3350 ± 57.7 (24.8)˚C
	500	9375 ± 1292	6275 ± 359.4 (32.2)˚C	4275 ± 618.5 (54.3)˚C	4400 ± 529.2 (52.9)˚C	3550 ± 387.3 (61.8)˚C	3125 ± 713.6 (66.7)˚C	2200 ± 182.6 (76.4)˚C

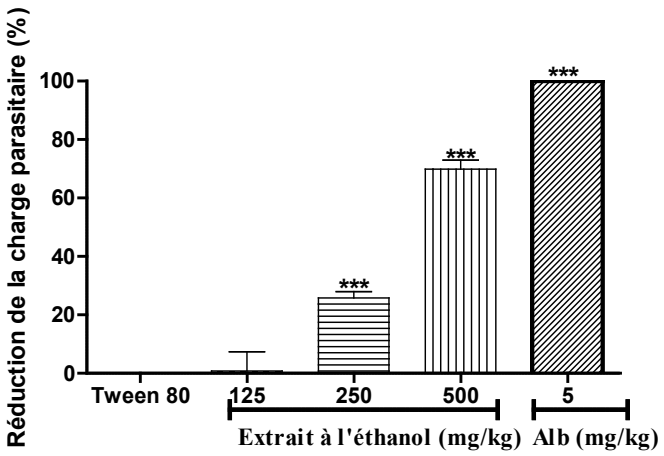

Fig. 2: Variation of reduction percentage in parasite load 19 days after administration of Albendazole and doses of ethanolic extracts of *Moringa oleifera*. ***P <0,001. Each bar represents the mean S.E.M. (n = 4).

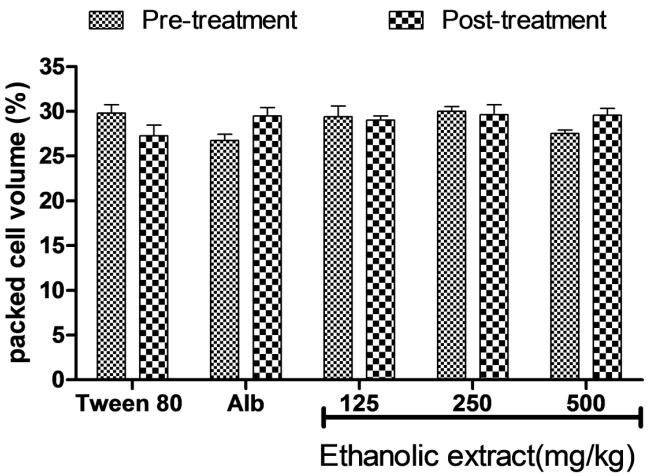

Fig. 3: Packed cell volume in animals before treatment and 19 days after administration of 2.8% Tween 80, albendazole (Alb), and the three doses of ethanol extracts of *Moringa oleifera*. Each bar represents the mean ± S.E.M. (n = 4).

Effect of ethanolic extract of *Moringa oleifera* on body weight

Like PCV, the different substances did not significantly influence the body weight of the animals 19 days after their administration (**Figure 4**).

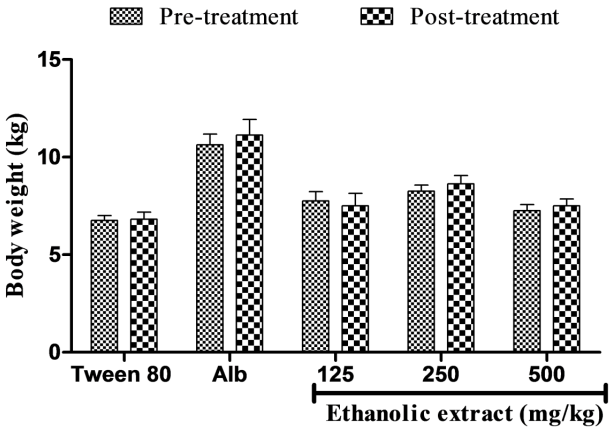

Fig. 4: Body weight of animals before and 19 days after administration of 2.8% Tween 80, albendazole (Alb), and the three doses of ethanol extracts of Moringa oleifera. Each bar represents the mean ± S.E.M. (n = 4).

DISCUSSION

During this study the doses of extract used were 125, 250 and 500 mg/kg. One of the common challenges in this area of research is the use of high doses for achieving *in situ* efficacy (Chagas, 2015). A plausible explanation would be the nature of the target here the nematode which is a living organism that can develop various escape mechanisms to the administered product. Mbafor *et al*. (2014) achieved 77.6% efficacy in reducing fecal egg excretion and 73.5% reduction in parasite load 14 days after administration of a double dose of 500 mg/kg of the methanol extract of *Terminalia glaucescens* in naturally infested sheep. In other situations, even high doses produce low activity. The methanol extract of *Ferula costata* for example, administered at doses of 2 and 3 g/kg produced respective strongyles egg fecal excretion reduction rates of 36.2% and 4.9% in naturally infested sheep (Siraj et al., 2013). The highest dose of 500 mg/kg of ethanolic extract of *M. oleifera* significantly affected the reproduction and survival of digestive strongyles in naturally infested goats. Percentage of reductions in faecal egg density and adult worm population of 76.4% and 69.8% were obtained respectively. According to Kerboeuf (1981), there is very little correlation between the number of eggs excreted and the number of worms actually presents in the digestive tract because some species are not very prolific and lay only a small number of eggs. Furthermore, the laying also depends on physiological status, time of day and host resistance. However, the work of Cabaret (1996) shows a real relationship between egg excretion and the number of parasites actually present in the digestive tract. The anthelmintic activity of *M. oleifera* remained significantly low compared to that of Albendazole (100% reduction in fecal egg density and parasite load on day 14 of treatment), which is quite normal since Albendazole is a pure substance unlike the extract which contains impurities. However, optimization and standardization of the extract could enhance the activity of the active compounds (Tariq et al., 2008).

Although the mechanisms of action of biological systems are very difficult to establish they are widely used in herbal medicine (Gauthier et al., 2009). The pharmacological basis for treatments of helminthe infestations usually involves interference with the integrity of the parasite's cells, neuromuscular coordination and energy metabolism, protective mechanisms against host immunity that leads to starvation, paralysis and expulsion of the parasite (Veerakumari, 2015). However, searching the mechanisms of action of anthelminthics can help to know the properties of the products needed to attack nematodes. The use of plants against gastrointestinal helminthes in small ruminants is well documented, but the mechanism by which these plants or their extracts can affect viability, mortality, and fertility *in vitro* and *in situ* is still largely unknown (Veerakumari, 2015). Nonetheless, hypotheses are being formulated. Thus, the anthelmintic activity of a plant would be due to their direct action on the parasite or to the indirect effect on changing the gut environment favoring low fecundity and worm expulsion. For some plants, it has been suggested that their consumption may be associated with an increase in the host immune response against the parasite (Athanasiadou et al., 2007). As already reported, we cannot rule out the potential role of secondary metabolites (tannins, steroidal flavonoids, alkaloids, glycosides and even cysteine proteinases) in the observed anthelminthic effects (Hoste et al., 2006; Oliveira et al., 2009 and Piyush et al., 2013). Two types of mechanisms are envisaged for tannins. A direct mechanism by disrupting the integrity of the parasite cuticle, binding to proteins normally used by the worms for their nutritional and reproductive functions and finally by disrupting the functioning of the genital tract of female strongyles, thus causing a decrease in their fertilization and a reduction in the fecal excretion of eggs by the animals (Athanasiadou et al., 2001; Paolini et al., 2003). Indirectly, tannins would prevent the degradation of proteins by ruminal flora and thus a greater intestinal availability allowing an additional supply of digestible proteins in the intestine, which would stimulate the local immune response of the host and reinforce its resistance to parasitism (Athanasiadou et al., 2000).

To estimate the effect of the extract on host resilience, we measured packed cell volume (PCV) and body mass before and after treatment. It was found that ethanolic extracts of *M. oleifera* as well as Albendazole did not cause any significant changes in these parameters. PCV values remained normal and almost stable. This result corroborates those of Houngzangbe *et al*. (2001) and Okombé (2013) obtained with *Carica papaya* powder and *Vitex thomasii* powder respectively. PCV is directly related to anemia, correlated with a high parasite load of hematophagous strongyles especially *H. contortus* (Chagas et al., 2008). Thus, extracts of *M. oleifera* would help animals to withstand the effects of parasitism, especially the anemic effect of hematophagous nematodes. The absence of anemia in animals treated with the dose 500 mg/kg of the ethanol extract of *M. oleifera* could confirm its anthelmintic activity, particularly against *H. contortus* the main hematophagous strongle identified in experimental goats.

CONCLUSION

This work contributes to the scientific knowledge on the nematicidal activity of *Moringa oleifera* against strongyles

of small ruminants. The discovery of a potent medecine from plant origin will be a great advancement in anthelmintic therapies. In the present study, the ethanolic extract of *Moringa oleifera* was effective at 500 mg/kg by inducing 76.4 % and 69.8 % strongyle eggs density reduction and parasitic load respectively. These reductions have important consequences in terms of the epidemiology of parasitic infestations, as they reduce infesting larvae in pastures in a sustainable way, as well as the risks of economic losses and clinical diseases. However, the plant extract assessed in the present study requires phytochemical analysis to elucidate the compounds responsible for the nematicidal activity and understand their mechanism of action. Morever, purification of this plant extract is necessary for his use as a sustainable tool for controlling strongylosis in small ruminants and other species of economic interest in the livestock industry.

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Ethical approval

This work was carried out in accordance with the Animal Ethical Committee of the Animal Biology Department of the University of Dschang, Cameroon.

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Conflict of interest: The authors declare that there are no conflicts of interest.

ConsentL Not applicable

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