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Anthelminthic Activity of *Moringa oleifera* Leaf Extracts Evaluated *in Vitro* on Four Developmental Stages of *Haemonchus contortus* from Goats

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Abstract

Haemonchus contortus **is a blood-sucking abomasal helminth of small ruminants responsible for major economic losses to small farmers worldwide. Widespread resistance to synthetic anthelminthics has stimulated a need for alternative strategies of parasite control, among which is the use of medicinal plants with natural anthelminthic properties. This study assessed *in vitro* the efficacy of infused and macerated aqueous extract as well ethanolic extract of *Moringa oleifera* against fresh eggs, embryonated eggs, L_1 and L_2 larvae of *H. contortus*. For this purpose, five different concentrations (0.625, 1.25, 2.5, 3.75 and 5 mg/ml) were prepared from dry extracts via serial dilutions with distilled water. Fresh eggs obtained from artificially infected goat feces were exposed to these different concentrations for 48 hours, while embryonated eggs and larvae were exposed for 6 and 24 hours respectively. Distilled water and 1.5% DMSO were used as negative control. The results were expressed in terms of mean inhibition percentage of egg embryonation, mean inhibition percentage of egg hatch and mean percentage of larval mortality. An overview of results revealed that ethanolic leaf extract of *M. oleifera* was most efficient on eggs by inhibiting 60.3% ± 8.2% and 92.8% ± 6.2% eggs embryonation at 3.75 and 5 mg/ml respectively with a significant difference ($P < 0.05$), which contributed to obtaining the lowest LC_{50} value of 0.985 mg/ml. This extract also inhibited 99% ± 2% egg hatching of *H. contortus* at 5 mg/ml with an LC_{50} value of 1.7 mg/ml. Concerning activity on larvae, the ethanolic extract was also most potent against them by inducing 98.8% ± 2.5% and 100% ± 0% mortality of L_1 and L_2 larvae at 5 mg/ml respectively. Infused aqueous extract was more efficient on eggs than on larvae with an IC_{50} value less than 2 mg/ml and an LC_{50} value more than 3.5 mg/ml. Macerated aqueous extract showed good activity**

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against the four developmental stages with LC_{50} values ranging from 2.08 mg/ml for L_2 larvae to 2.92 mg/ml for L_1 larvae and 2.37 to 2.52 mg/ml for embryonated and fresh eggs respectively. The current study showed that all three extracts of *M. oleifera* tested possessed potential ovicidal and larvicidal activities against *H. contortus*. However, further *in vivo* studies are necessary to validate the anthelminthic property of this plant.

Keywords

***Moringa oleifera*, Ovicidal, Larvicidal, *Haemonchus contortus*, Goats, Cameroon**

1. Introduction

Livestock is an important prospective sector which may contribute in solving problems of small farmers and by such, help in poverty alleviation [1]. Small ruminants particularly goats have been considered the most important aspect of livestock throughout the world. In 2010, world caprine livestock counted 920 million animals, with more than 90% in Asia and Africa [2]. In Cameroon, Labonne *et al.* [3] registered 7 million goats, with half of this population concentrated in the Extreme-North Region. Parasitism by gastro-intestinal nematodes (GINs) is a major constraint in the production of goats in tropical countries. They can affect production through weight loss, diarrhea, anemia, reduction in milk and wool production, reproduction changes as well as mortality in the case of heavy infectations [4]. Actually, a majority of nematodes affecting the intestinal tract of small ruminants belong to the Order Strongylidae, with principal genera being *Haemonchus*, *Teladorsagia*, *Cooperia*, *Trichostrongylus*, *Nematodirus*, *Chabertia* and *Œsophagostomun* [5]. Compared to other nematodes, *H. contortus* is by far a highly pathogenic parasite of small ruminants, capable of causing acute disease and high mortality in all classes of livestock [6]. Up to now, a huge amount of money is spent annually worldwide to combat helminth parasites in livestock, the principal mode of control being based on the repeated use of commercial anthelminthic drugs such as levamisol, morantel, thiabendazole, mebendazole, albendazole, ivermectin and dormactin. However, nematodes have developed resistance against several families of anthelminthics. Moreover, toxicity due to inappropriate dose administration and risk of drug residues in animal products are other big problems associated with the use of synthetic drugs [7]. In most developing countries like Cameroon, small holder farmers have limited access to such drugs and veterinary services due to either high cost or unavailability. Therefore, most of the farmers rely on ethno-veterinary treatment. Medicinal plants are considered as an alternative source of compounds that are biodegradable into non-toxic products and sustainable methods readily adaptable to rural farming communities. *Moringa oleifera* is considered as one of the most useful trees as almost every part can be used for food or has some other beneficial properties. In the tropics, it is used as forage for livestock and in many countries; it is used to treat various ailments [8]. In Cameroon, *M. oleifera* is used to treat asthma, anemia, intestinal worms, cardio-vascular disorder, headache, skin infections and others (personal communication). Extracts from this plant have several pharmacological effects such as anthelminthic, anti-inflammatory, antimicrobial, anti-oxydant, hepato-protective, anti-glycemic and anti-dislipidemia [8]-[13]. As far as literature on this plant is concerned, there is no published work on *in vitro* anthelminthic activity of *Moringa oleifera* against *Haemonchus contortus*. The aim of the present study was thus to investigate the *in vitro* activities of aqueous and ethanolic leaf extracts of *M. oleifera* on four free-living stages of *H. contortus* from goats.

2. Materials and Methods

2.1. 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.

2.2. Plant Extracts Preparation

The infused and macerated aqueous extracts as well as ethanolic extract were prepared to compare their activities. Extraction was done according to the procedure described by Wabo Pone *et al*. [14] [15], at the end of which we obtained different dried extracts. Each dried extract was used to prepare a stock solution which was then diluted with distilled water to obtain five different solutions of concentrations 1.25, 2.5, 5, 7.5 and 10 mg/ml. The final tested concentrations were 0.625, 1.25, 2.5, 3.75 and 5 mg/ml.

2.3. Parasites Donor Goat

Goats' abomasums were obtained from the abattoir of the "Marché B" of Dschang town after necropsy of animals. Adult female *Haemonchus contortus* were recovered from abomasums. These female worms were crushed to liberate eggs. The eggs were then cultured *in vitro* in Petri dishes at room temperature for seven days. At the end of the 7^{th} day, infective larvae were harvested. About 2500 larvae were inoculated into a worm-free goat kept indoors in a separate house at the teaching and research farm of the University of Dschang throughout the study period. This goat served as *H. contortus* egg donor for subsequent *in vitro* trials.

2.4. Recovery of Nematode Eggs

Feces directly collected from the rectum of the donor goat mentioned above were used in recovering eggs according to the procedure carried out by Wabo Poné *et al*. [15].

2.5. Recovery of Nematode Larvae

L_1 and L_2 larvae were obtained from eggs recovered above according to Mbogning Tayo *et al*. [16].

2.6. Evaluation of Ovicidal and Larvicidal Activities

Egg embryonation assay using fresh eggs, egg hatch assay using embryonated eggs and larval mortality assay using L_1 and L_2 larvae were performed according to Wabo Poné *et al*. [17] [18], to evaluate the ovicidal and larvicidal efficacy of *M. oleifera* leaf extracts. Each test was repeated four times for each extract and control (distilled water and 1.5% DMSO).

3. Statistical Analysis

Comparison of the mean inhibition percentage of egg embryonation, mean inhibition percentage of egg hatch and mean percentage of larval mortality at different concentrations with control was performed by one-way analysis of variance (ANOVA). Statistical analysis was performed by using the software SPSS version 17.0. The post hoc statistical significance test employed was Duncan, differences between the means were considered significant at $P < 0.05$. The 50% inhibitory concentration (IC_{50}) and lethal concentration (LC_{50}), *i.e*., effective concentration to inhibit 50% of the eggs and to kill 50% of larvae were determined using the regression lines of the probit according to decimal logarithm of the concentrations.

4. Results

Efficacy of *M. oleifera* leaf extracts in inhibiting egg embryonation of *Haemonchus contortus* at different concentrations is presented in **Table 1**. From this table, we observed that negative controls (distilled water and 1.5% distilled water) had no effect on egg embryonation while *M. oleifera* extracts inhibited embryonation in a concentration dependent fashion. Ethanolic extract was efficient at 0.625 mg/ml by inhibiting 51.7% ± 20.7% egg embryonnation, reaching 92.8% ± 6.2% at 5 mg/ml with a significant difference ($P < 0.05$). Infused and macerated aqueous extracts also inhibited embryonation with mean efficacy of 69% ± 5.8% and 94.5% ± 4% at 5 mg/ml respectively. The IC_{50}s calculated from equations of regression lines of probit according to the decimal logarithm of concentrations (**Figure 1**) were 2.52, 1.52 and 0.985 mg/ml for macerated, infused aqueous extract and ethanolic extract respectively.

Table 2 shows activity of extracts in inhibiting egg hatch of *H. contortus* at different concentrations. Like on embryonation, distilled water and 1.5% DMSO did not affect egg hatch while extracts presented a concentration

Table 1. Mean inhibition percentage of egg embryonation ± standard deviation of *Moringa oleifera* leaf extracts at different concentrations against *Haemonchus contortus*.

Concentrations (mg/ml)	MIAE	MMAE	MEtE
	Mean ± sd	Mean ± sd	Mean ± sd
0.625	33.8 ±11.7^{b}	0.9 ± 1.9^{a}	51.7 ± 20.7^{b}
1.25	45.1 ± 23.9^{bc}	16.3 ± 14.5^{ab}	51.9 ± 6.8^{b}
2.50	62.7 ± 11.6^{cd}	28.9 ± 20.4^{b}	52.2 ± 12.4^{b}
3.75	66.3 ± 7.5^{d}	74.2 ± 6.5^{c}	60.3 ± 8.2^{b}
5.00	69.1 ± 5.8^{d}	94.5 ± 4^{d}	92.8 ± 6.2^{c}
DW	1.7 ± 1.9^{a}	1.7 ± 1.9^{a}	NA
1.5% DMSO	NA	NA	2 ± 2.3^{a}

Letters compare means in the columns. Different letters indicate significant difference ($P < 0.05$). **Legend**: MIAE = *Moringa oleifera* infused aqueous extract, MMAE = *Moringa oleifera* macerated aqueous extract, MEtE = *Moringa oleifera* ethanolic extract, sd = standard deviation, DW = Distilled water, DMSO = Dimethylsulfoxide, NA = Not applicable.

Table 2. Mean inhibition percentage of egg hatch ± standard deviation of *Moringa oleifera* leaf extracts at different concentrations against *Haemonchus contortus*.

Concentrations (mg/ml)	MIAE	MMAE	MEtE
	Mean ± sd	Mean ± sd	Mean ± sd
0.625	19.5 ± 4^{ab}	0 ± 0^{a}	6.8 ± 7.1^{a}
1.25	31.9 ± 19.6^{bc}	10.7 ± 9.2^{a}	26.8 ± 11.2^{b}
2.50	43.5 ± 14.5^{c}	69.8 ± 12.1^{b}	60.8 ± 17.9^{c}
3.75	69.2 ± 17.1^{d}	74.6 ± 15.7^{bc}	85.9 ± 3.2^{d}
5.00	97.9 ± 4.2^{e}	90.2 ± 8.4^{c}	99 ± 2^{d}
DW	2.5 ± 2.9^{a}	2.5 ± 2.9^{a}	NA
1.5% DMSO	NA	NA	10.1 ± 1.2^{a}

Letters compare means in the columns. Different letters indicate significant difference ($P < 0.05$). **Legend**: MIAE = *Moringa oleifera* infused aqueous extract, MMAE = *Moringa oleifera* macerated aqueous extract, MEtE = *Moringa oleifera* ethanolic extract, sd = standard deviation, DW = Distilled water, DMSO = Dimethylsulfoxide, NA = Not applicable.

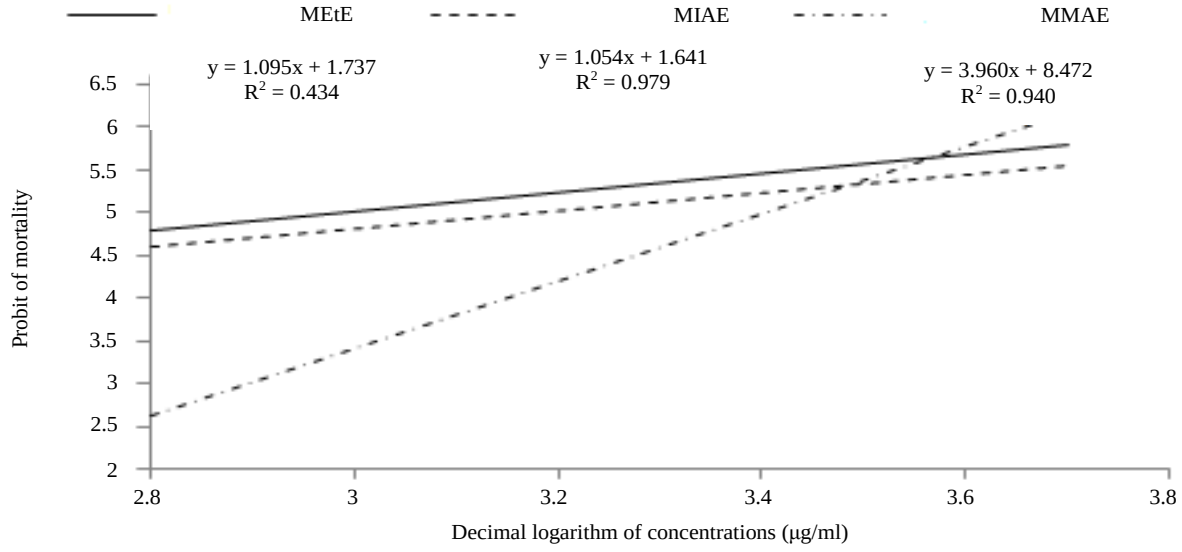

Figure 1. Evolution of probit of fresh egg mortality rate of *Haemonchus contortus* according to decimal logarithm of concentrations of *Moringa oleifera* extracts. Legend: MEtE = *Moringa oleifera* ethanolic extract, MIAE = *Moringa oleifera* infused aqueous extract, MMAE = *Moringa oleifera* macerated aqueous extract.

dependent activity. Macerated aqueous extract and ethanol extract inhibited more than 60% egg hatch at 2.5 mg/ml, reaching 90.2% ± 8.4% and 99% ± 2% at 5 mg/ml respectively. The infused aqueous extract inhibited 69.2% ± 17.1% and 97.9% ± 4.2% egg hatch at 3.75 and 5 mg/ml respectively with a significant difference ($P < 0.05$). IC_{50}s values of 2.37, 1.75 and 1.74 mg/ml were obtained for macerated, infused and ethanolic extracts respectively from equations of regression lines of the probit of egg hatch inhibition according to decimal logarithm of concentrations (**Figure 2**).

Concerning larvicidal activity, **Table 3** and **Table 4** present the efficacy of *M. oleifera* extracts in inducing L_1 and L_2 larva mortality at different concentrations respectively. From **Table 3**, infused aqueous extract showed weak activity on L_1 larvae, inducing only 50.5% ± 8.8% mortality at 5 mg/ml. However, macerated aqueous extract induced 89.6% ± 8.8% at 5 mg/ml while at the same concentration ethanolic extract registered 98.8% ± 2.5% L_1 larva mortality. LC_{50}s values of L_1 larvae calculated from equations of regression lines (illustrated on **Figure 3**) were 7.83, 2.92 and 1.89 mg/ml for infused, macerated aqueous extracts and ethanolic extract respectively.

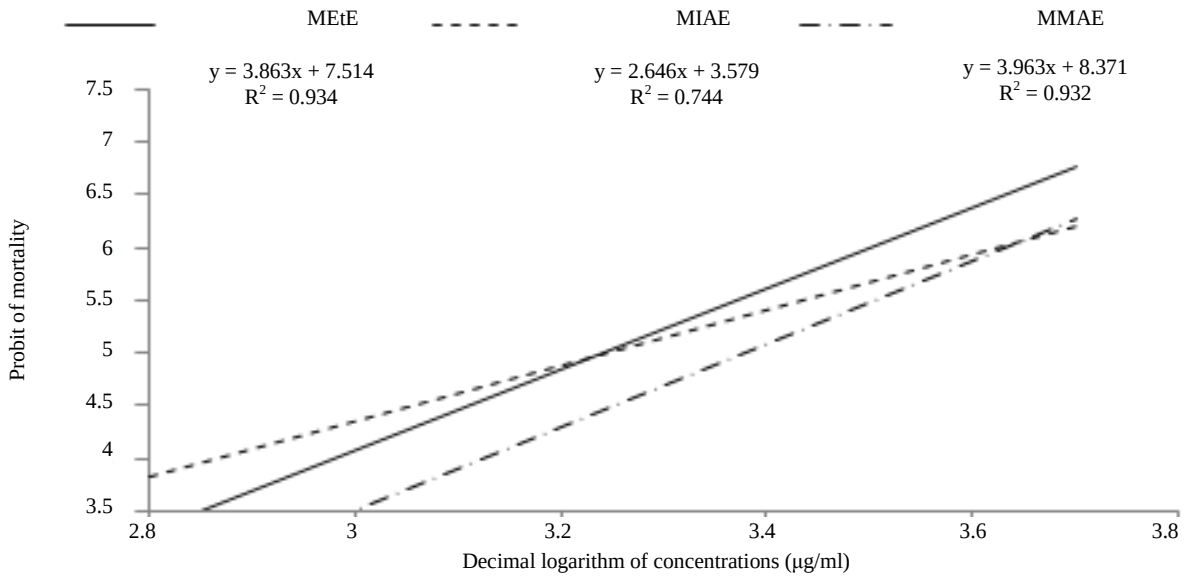

Figure 2. Evolution of probit of embryonated egg mortality rate of *Haemonchus contortus* according to decimal logarithm of concentrations of *Moringa oleifera* extracts. Legend: MEtE = *Moringa oleifera* ethanolic extract, MIAE = *Moringa oleifera* infused aqueous extract, MMAE = *Moringa oleifera* macerated aqueous extract.

Table 3. Mean mortality percentage of L_1 larvae ± standard deviation of *Moringa oleifera* leaf extracts at different concentrations against *Haemonchus contortus*.

Concentrations (mg/ml)	MIAE	MMAE	MEtE
	Mean ± sd	Mean ± sd	Mean ± sd
0.625	4.8 ± 5.5^{a}	1.9 ± 3.8^{a}	9.6 ± 19.6^{a}
1.25	8.5 ± 6.8^{a}	3.8 ± 7.7^{a}	12.7 ± 12.9^{a}
2.50	11.5 ± 10.1^{a}	23.7 ± 10.5^{b}	55.8 ± 27.9^{b}
3.75	28.5 ± 7.4^{b}	71.9 ± 21.6^{c}	80.6 ± 18.2^{bc}
5.00	50.5 ± 8.8^{c}	89.6 ± 11.5^{d}	98.8 ± 2.5^{c}
DW	0 ± 0^{a}	0 ± 0^{a}	NA
1.5% DMSO	NA	NA	5 ± 5.8^{a}

Letters compare means in the columns. Different letters indicate significant difference ($P < 0.05$). **Legend**: MIAE = *Moringa oleifera* infused aqueous extract, MMAE = *Moringa oleifera* macerated aqueous extract, MEtE = *Moringa oleifera* ethanolic extract, sd = standard deviation, DW = Distilled water, DMSO = Dimethylsulfoxide, NA = Not applicable.

Table 4. Mean mortality percentage of L_2 larvae ± standard deviation of *Moringa oleifera* leaf extracts at different concentrations against *Haemonchus contortus*.

Concentrations (mg/ml)	MIAE	MMAE	MEtE
	Mean ± sd	Mean ± sd	Mean ± sd
0.625	2.3 ± 4.5^{a}	5.9 ± 6.8^{a}	9.9 ± 9.7^{a}
1.25	5 ± 10^{a}	20 ± 7.3^{a}	16.1 ± 7.9^{a}
2.50	33.9 ± 26.7^{b}	69.8 ± 16.5^{b}	54.2 ± 23.1^{b}
3.75	55.3 ± 5.2^{c}	79.8 ± 13.6^{cd}	98.8 ± 3.1^{c}
5.00	70.1 ± 7.3^{c}	86.1 ± 7.9^{d}	100 ± 0^{c}
DW	0 ± 0^{a}	0 ± 0^{a}	NA
1.5% DMSO	NA	NA	4.2 ± 4.8^{a}

Letters compare means in the columns. Different letters indicate significant difference ($P < 0.05$). **Legend**: MIAE = *Moringa oleifera* infused aqueous extract, MMAE = *Moringa oleifera* macerated aqueous extract, MEtE = *Moringa oleifera* ethanolic extract, sd = standard deviation, DW = Distilled water, DMSO = Dimethylsulfoxide, NA = Not applicable.

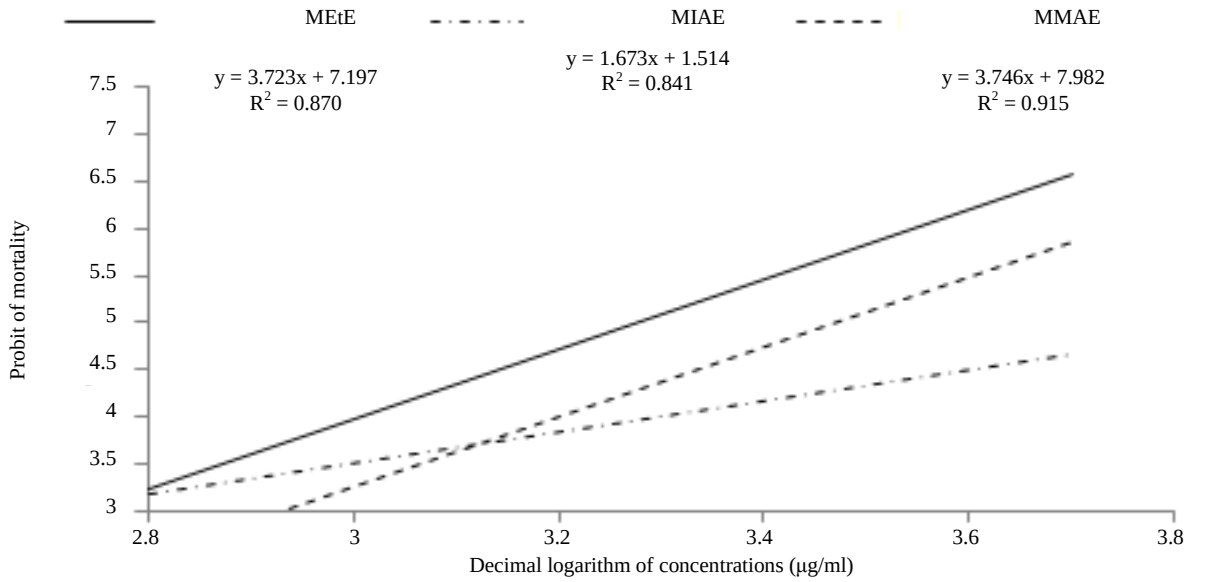

Figure 3. Evolution of probit of L_1 larva mortality rate of *Haemonchus contortus* according to decimal logarithm of concentrations of *Moringa oleifera* extracts. Legend: MEtE = *Moringa oleifera* ethanolic extract, MIAE = *Moringa oleifera* infused aqueous extract, MMAE = *Moringa oleifera* macerated aqueous extract.

As seen from **Table 4** the ethanolic extract was most efficient in inducing 98.8% ± 3.1% and 100% ± 0% L_2 larva mortality at 3.75 and 5 mg/ml respectively, with no significant difference ($P > 0.05$). At these same concentrations, aqueous extracts also induced L_2 mortality even though the mean mortality rates were lower than those obtained with ethanolic extract. LC_{50}s values of L_2 larvae calculated from equations of regression lines (illustrated on **Figure 4**) were 3.57, 2.08 and 1.5 mg/ml for infused, macerated aqueous extract and ethanolic extract respectively. Like on eggs, distilled water and 1.5% DMSO had no effect on larvae while *M. oleifera* extracts were active in a concentration dependent manner.

5. Discussion and Conclusion

In developing countries, the identification of a plant with anthelminthic property may help to build an integrated and sustainable approach for the control of gastro-intestinal nematodes in small ruminants. The objective of this study was to evaluate *in vitro* ovicidal and larvicidal efficacy of aqueous and ethanolic leaf extracts of

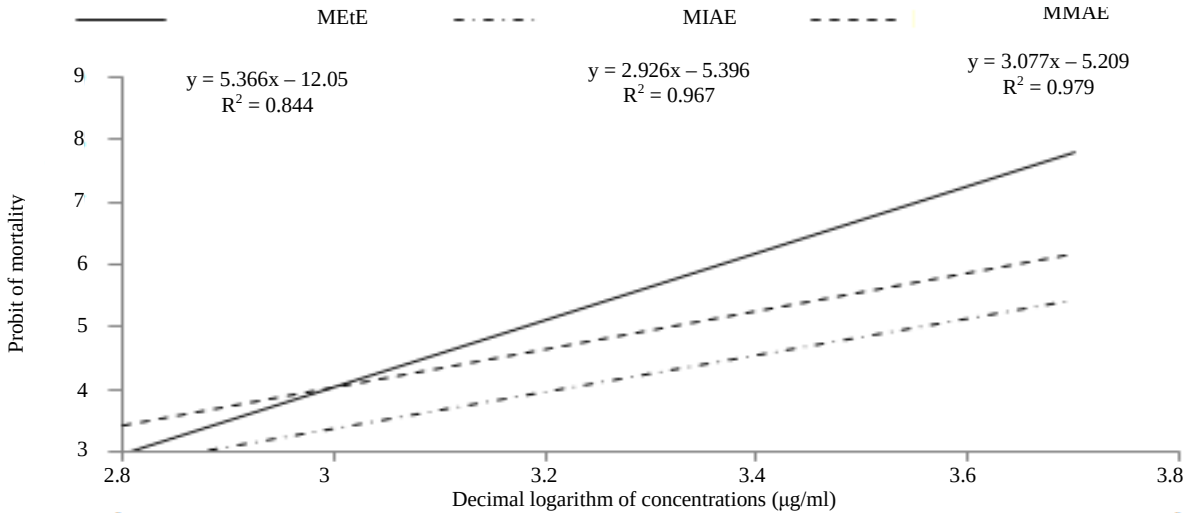

Figure 4. Evolution of probit of L_2 larvae mortality rate of *Haemonchus contortus* according to decimal logarithm of concentrations of *Moringa oleifera* extracts. Legend: MEtE = *Moringa oleifera* ethanolic extract, MIAE = *Moringa oleifera* infused aqueous extract, MMAE = *Moringa oleifera* macerated aqueous extract.

M. oleifera against *H. contortus*. *In vitro* bioassay provides means to rapidly screen for plant extracts and to analyze the possible mechanisms involved in the interactions between active compounds and parasites [19]. Asase *et al*. [20] reported that *in vitro* tests using free living stages of parasitic nematodes as is the case in the present study are considered the best means of screening the anthelminthic activity of new plant compounds. In this study, *M. oleifera* leaf extracts presented a concentration-dependent activity against the four different free-living stages of *H. contortus*, suggesting that increase in concentration of plant extract is followed by a supplementary input of different active compounds. Infused aqueous extract and ethanolic extract of *M. oleifera* presented comparable activity on eggs. The first extract (infused) inhibited 69% ± 1% and 97.9% ± 4.2% of egg embryonation and egg hatch at 5 mg/ml with IC_{50}s values of 1.52 and 1.75 mg/ml respectively. While the latter (ethanolic) inhibited 92.8% ± 6.2% and 99% ± 2% of egg embryonation and egg hatch at 5 mg/ml, with IC_{50}s values of 0.985 and 1.74 mg/ml respectively. Tatik and Dwatmadji [21] recorded the same observation between crude aqueous and ethanolic extracts of *Melastoma malabatricum* on eggs of *H. contortus*. They also found out that there was no statistically significant difference in the activity of aqueous and hydro-alcoholic extracts of *Hedera helix* after evaluation of *in vitro* anthelminthic activity against *H. contortus*. The observed uniform activity of infused aqueous extract and ethanolic extract of *M. oleifera* on eggs could be due to the presence of similar or related chemicals having ovicidal property in nearly equivalent proportions. Based on LC_{50}s values, ethanolic extracts presented the highest larvicidal activity since it registered the lowest values of 1.89 and 1.5 mg/ml on L_1 and L_2 larvae respectively. These LC_{50}s values revealed that L_1 larvae were more susceptible to ethanolic extract than L_2 larvae, confirming the literature findings of Soulby [22] and could be explained by the fact that since L_2 are just from the process of molting, they are still weak and more vulnerable to active compounds [23]. However, Wabo Poné *et al*. [24] found opposite results when evaluating *in vitro* activities of acetonic extracts of leaves of three forage legumes on *H. contortus*, since they obtained LC_{50}s values below 0.9 and above 1 mg/ml for L_1 and L_2 larvae respectively. Infused aqueous extract generally exhibited higher activity on eggs than on larvae. Macerated aqueous extract showed a good activity on almost all the four stages of parasites. The probable reason for the minor differences between aqueous and ethanolic extracts could be due to variation in solubility of the active compounds in the solvent. The ovicidal and larvidal activities observed in this study with different extracts may be attributed to the presence of saponins, steroids, carbohydrates, alkaloids, tannins, flavonoids which were previously reported to be present in leaves of *M. oleifera* after preliminary phytochemical screening [25]. These secondary metabolites may take the two ways of anthelminthic drugs that are diffused through egg shells or cuticle of larvae or diffusion into intestinal cells to exert their inhibition or mortality action on eggs and larvae of *H. contortus*.

In conclusion, the activity from this study shows the potential value of *Moringa oleifera* leaf extracts in the management of haemonchosis, since inhibition of egg embryonation, egg hatch and mortality of L_1 and L_2 larvae

are important in reducing pasture contamination thereby helping in the overall helminth control programme. However, in order to use this plant for better beneficial purpose in the fight against gastro-intestinal nematodes, further *in vivo* and toxicity studies are necessary.

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References

[1] Sujon, M.A., Mostoja, M., Jahan, M.S., Das, A.R. and Rob, S. (2008) Studies on Medicinal Plants against Gastrointestinal Nematodes of Goats. *Bangladesh Journal of Veterinary Medicine*, **6**, 179-183.

[2] Sabater, M. (2012) Détermination d'une dose efficace et d'une dose toxique de tanins condensés dans le contrôle des strongyloses digestives chez les Caprins. Thèse d'exercice, Médecine vétérinaire, Ecole National Vétérinaire de Toulouse—ENVT, Toulouse, 135p.

[3] Labonne, M., Magrong, P. and Oustalet, Y. (2002) Le secteur de l'élévage au Cameroun et dans les provinces du grand Nord: Situation actuelle, contraintes, enjeux et défis. Acte du colloque, Garoua, 27-31 Mai 2002.

[4] Botura, M.B., Silva, G.D., Lima, H.G., Oliveira, J.V.A., Souza, T.S., Santos, J.D.G., Branco, A., Moreira, E.L.T., Almeida, M.A.O. and Batatinha, M.J.M. (2011) *In Vivo* Anthelminthic Activity of an Aqueous Extract from Sisal Waste (*Agave sisalana* Perr.) against Gastro-Intestinal Nematodes in Goats. *Veterinary Parasitology*, **177**, 104-110. http://dx.doi.org/10.1016/j.vetpar.2010.11.039

[5] Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M. and Jennings, F.W. (1996) Veterinary Parasitology. 2nd Edition, Blackwell Science Ltd., Oxford, 224-234.

[6] Kamaraj, C. and Abdul Rahuman, A. (2011) Efficacy of Anthelmintic Properties of Medicinal Plant Extracts against *Haemonchus contortus. Research on Veterinary Science*, **91**, 400-404. http://dx.doi.org/10.1016/j.rvsc.2010.09.018

[7] Hafiz, A.B., Zafar, I., Muhammad, N.K., Zia-ud-Din, S. and Abdul, J. (2009) Anthelmintic Activity of *Ziziphus nummularia* (Back) and *Acacia nilotica* (fruit) against Trichostrongylid Nematodes of Sheep. *Journal of Ethnopharmacology*, **123**, 325-329. http://dx.doi.org/10.1016/j.jep.2009.02.043

[8] Devendra, B.N., Srinivas, N., Talluri, P.S.S.L.P. and Latha, P.S. (2011) Antimicrobial Activity of *Moringa oleifera* Lam., Leaf Extract, against Selected Bacterial and Fungal Strains. *International Journal of Pharma and Bio Sciences*, **2**, 13-18.

[9] Wasswa, P and Deogracious, O. (2005) The *in Vitro* Ascaridal Activity of Selected Indigenous Medicinal Plants Used in Ethno-Veterinary Practices in Uganda. *African Journal of Traditional, Complementary and Alternative Medicines*, **3**, 94-103.

[10] Chandrashekar, K.S., Thakur, A. and Prasanna, K.S. (2010) Anti-Inflammatory Activity of *Moringa oleifera* Stem Back Extracts against Carrageenen Induced Rat Paw Edema. *Journal of Chemical and Pharmaceutical Research*, **2**, 179-181.

[11] Moya, B., Oyedemi, S., Masika, P.J. and Muchenje, V. (2012) Polyphenolic Content and Antioxydant Properties of *Moringa oleifera* Leaf Extracts and Enzymatic Activity of Liver from Goats Supplemented with *Moringa oleifera* Leaves/Sun Flower Seed Cake. *Meat Science*, **91**, 441-447. http://dx.doi.org/10.1016/j.meatsci.2012.02.029

[12] Singh, N., Verna, V.K., Saxena, P. and Singh, R. (2012) Anti-Ulcer and Antioxidant Activity of *Moringa oleifera* (Lam) Leaves against Aspirin and Ethanol Induced Gastric Ulcer in Rats. *International Research Journal of Pharmaceuticals*, **2**, 46-57.

[13] Mbikay, M. (2012) Therapeutic Potential of *Moringa oleifera* Leaves in Chronic Hyper-Glycemicemia and Dyslipidemia: A Review. *Frontiers in Pharmacology*, **3**, 1-37.

[14] Wabo Poné, J., Bilong Bilong, C.F., Mpoame, M., Catherine, F.N. and Coles, C.C. (2006) *In Vitro* Activity of Ethanol, Cold and Hot Water Extracts of the Stem Back of *canthium mannii* (Rubiaceae) on *Ancylostoma caninum* Eggs. *East and Central African Journal of Pharmaceutical Sciences*, **9**, 14-18.

[15] Wabo Poné, J., Bilong Bilong, C.F. and Mpoame, M. (2010) *In Vitro* Nematicidal Activity of Extracts of *Canthium mannii* (Rubiaceae) on Different Life Cycle of *Heligmosomoides polygyrus* (Nematoda, Heligmosomatidae). *Journal of Helminthology*, **84**, 156-165. http://dx.doi.org/10.1017/S0022149X09990435

[16] Mbogning Tayo, G., Payne, V.K., Wabo Poné, J., Komtangi, M.C., Yondo, J., Ngangout, A.M., Mpoame, M. and Bi-

long Bilong, C.F. (2012) *In Vitro* Ovicidal and Larvicidal Activities of Aqueous and Ethanolic Extracts of the Leaves of *Bidens pilosa* (Asteraceae) on *Heligmosomoides bakeri* (Nematoda: Heligmosomatidae). *Medicinal Plants*, **4**, 121-125.

[17] Wabo Poné, J., Olivia Fossi, T., Yondo, J., Komtangi, C.M., Mpoame, M. and Bilong Bilong, C.F. (2011) The *in Vitro* Effects of Aqueous and Ethanolic Extracts of the Leaves of *Ageratum conyzoides* (Asteraceae) on Three Life Cycle Stages of the Parasitic Nematode *Heligmosomoides bakeri*, (Nematoda: Heligmosomatidae). *Veterinary Medicine International*, **2011**, Article ID: 140293.

[18] Wabo Poné, J., Ngankam, N.J.D., Bilong Bilong, C.F. and Mpoame, M. (2011) A Comparative Study of the Ovicidal and Larvicidal Activities of Aqueous and Ethanolic Extracts of Pawpaw Seed *Carica papaya* (Caricaceae) on *Heligmosomoides bakeri*. *Asian Pacific Journal of Tropical Medicine*, **4**, 447-450.

[19] Katiki, L.M., Chagas, A.C.S., Bizzo, H.R., Ferreira, J.F.S. and Amarante, A.F.T. (2011) Anthelmintic Activity of *Cymbopogon martini*, *Cymbopogon schoenanthus* and *Mentha piperita* Essential Oils Evaluated in Four Different *in Vitro* Tests. *Veterinary Parasitology*, **183**, 103-106. http://dx.doi.org/10.1016/j.vetpar.2011.07.001

[20] Asase, A., Oting-Yeboah, A.A., Odamtten, G.T. and Simmonds, M.S.J. (2005) Ethnobotanical Study of Some Ghanaian Anti-Malarial Plants. *Journal of Ethnopharmacology*, **99**, 273-279. http://dx.doi.org/10.1016/j.jep.2005.02.020

[21] Tatik, S. and Dwatmadji (2011) Anthelmintic Activity of *Melastoma malabatricum* Extract on *Haemonchus con- tortus* Activity *in Vitro*. *Asian Journal of Pharmaceutical and Clinical Research*, **4**, 68-70.

[22] Soulsby, E.J.L. (1982) Helminths, Arthropods and Protozoa of Domesticated Animals. 7th Edition, Bailler, Tindall, London.

[23] Wabo Poné, J., Payne, V.K., Mbogning, T.G., Komtangi, M.C., Yondo, J., Ngangout, A.M., Mbida, M. and Bilong Bilong, C.F. (2013) *In Vitro* Anthelminthic Efficacy of *Dichrocephala integrifolia* (Asteraceae) Extracts on the Gastro-Intestinal Nematode Parasite of Mice: *Heligmosomoïdes bakeri* (Nematoda, Heligmosomatidae). *Asian Pacific Journal of Tropical Biomedicine*, **3**, 100-104. http://dx.doi.org/10.1016/S2221-1691(13)60032-5

[24] Wabo Poné, J., Kenné Tameli, F., Mpoame, M., Pamo, T.E. and Bilong Bilong, C.F. (2011d) *In Vitro* Activities of Acetonic Extracts from Leaves of Three Forage Legumes (*Calliandra calotyrsus*, *Gliricidia sepium* and *Leucaena diversifolia)* on *Haemonchus contortus*. *Asian Pacific Journal of Tropical Medicine*, **4**, 125-128.

[25] Rastogi, T., Bhutda, V., Moon, K., Aswar, P.B. and Khadabadi, S.S. (2009) Comparative Studies on Anthelmintic Activity of *Moringa oleifera* and *Vitex negundo*. *Asian Journal of Research in Chemistry*, **2**, 181-182.