RESEARCH ARTICLE

In Vitro Anthelmintic Efficacy of *Acacia nilotica* Pods on Eggs and Adult Worms of *Haemonchus contortus*

G. Zabre[1,2]*, B. Tindano[2], A. Dicko[3], B. Bayala[2], A. Kabore[3], A.M.G.Belem[4], H.H. Tamboura[3]

Abstract

The aim of the present study was to evaluate the *in vitro* anthelmintic efficacy of aqueous and acetone extracts of pods of *Acacia nilotica* on the eggs and adult worms of *Haemonchus contortus*. Eggs and worms were exposed to five increasing concentrations of *Acacia* nilotica pod extracts (1.25; 2.5; 5.0; 10.0 and 20.0 mg/mL). The results showed dose-dependent ovicidal ($p<0.001$) and vermicide ($p<0.05$) activity against *H. contortus*. For the egg hatch inhibition assay (EHA), the rate of inhibition ranged from 31 to 70% for aqueous extract and from 41 to 81% for acetone extract, while Benzal (positive control) showed the highest inhibition rate of 91%. For adult nematode mortality test (AMT), no mortality was recorded between 0-h and 2-h in any of the treatment groups as well as the two controls. Mortalities were recorded from the 4th and 6th hour of incubation. Concentrations of 10 and 20 mg/mL recorded the highest mortality rate (>80%) after 4-h of incubation. These results suggest that *A. nilotica* pods can be used in the control of gastrointestinal nematodes.

Key words: *Acacia nilotica*, Anthelmintic, Gastrointestinal nematodes, *Haemonchus contortus*.

Ind J Vet Sci and Biotech (2023): 10.48165/ijvsbt.19.6.18

Introduction

Gastro-intestinal nematode parasitic infection is one of the major health problems in the world. Nematodes are the most commonly encountered GI helminths, while Cestodes are the least. Genus Haemonchus is a well-known blood-sucking abomasal nematode that may be responsible for extensive losses in sheep and cattle especially in tropical area (Urquhart *et al.,* 1996).

Gastro-intestinal nematodiosis is one of the major problems which is responsible for serious economic losses due to weight loss, production losses, fertility disorders, morbidity and mortality of small ruminants (Hoste *et al.,* 2005; Andrea *et al.,* 2011; Fournier, 2020). The most economically significant endoparasitic diseases are GIT and respiratory nematodiosis. A wide range of either single or mixed nematode parasite infections is dominantly found in the abomasum, or small intestine, causing GIT nematodiasis. These include Haemonchus, Cooperia, Ostertagia, Bunostomum, Trichostrongylus, Oesophagostomum, and Nematodirus. *H. contortus,* also called the “red” strongyle of the abomasum of small ruminants because of its haem atophagous diet, is capable of causing severe anaemia that can lead to production losses and death in all categories of animals. The color and size allow the parasite to be easily recognized with the eye on the mucosa of the abomasum during autopsy (Eichstadt, 2017).

For many years, synthetic anthelmintics have been used to fight against GI nematodes. However, the inaccessibility of these anthelmintics to farmers together with the development of resistance to anthelmintics has prompted to the search for alternative such as the use of local plants. Previous work has shown that some local tannin-rich plants exhibit the effects as conventional anthelminthic, by (i) preventing egg hatch, (ii) killing adult worms, or (iii) immobilize infesting larvae (L3) (Hounzangbe-Adoté *et al.,* 2005a,b). Now-a-days the use of these plants is widely considered as an alternative to synthetic chemical anthelmintics in the control of gastrointestinal parasites in ruminants (Devendra *et al.,* 2011; Hoste *et al.,* 2015).

Acacia nilotica is recognized as bioactive plant for its many properties in traditional medicine. It is a plant used in the treatment of diseases of bacterial origin and parasitosis in ruminants. In Mauritania, the powder of the seeds of *A. nilotica* macerated with water, is used to treat diarrhoea. Likewise, the extracts of leaves, fruits and barks of *A. nilotica*

[1]Normal High School (ENS), Department of Science, Institute of Science and Technology, Koudougou, Burkina Faso.

[2]University Joseph KI-ZERBO (UJKZ), Department of Animal Biology and Animal Physiology, Laboratory of Animal Physiology, Ouagadougou, Burkina Faso.

[3]National Center for Scientific and Technological Research (CNRST), Department of Animal Production, Ouagadougou, Burkina Faso.

[4]Nazi Boni University, Institute of Rural Development (IRD), Bobo-Dioulasso, Burkina Faso.

Corresponding Author: G. Zabre, Normal High School (ENS), Department of Science, Institute of Science and Technology, Koudougou, Burkina Faso, e-mail: gnvivezabr@yahoo.com (Z. Geneviève)

How to cite this article: Zabre, G., Tindano, B., Dicko, A., Bayala, B., Kabore, A., Belem, A. M. G., & Tamboura, H. H. (2023). *In Vitro* Anthelmintic Efficacy of *Acacia nilotica* Pods on Eggs and Adult Worms of *Haemonchus contortus*. Ind J Vet Sci and Biotech. 19(6), 90-94.

Source of support: Nil

Conflict of interest: The authors declare that they have no competing interest.

Submitted 20/07/2023 **Accepted** 02/08/2023 **Published** 10/11/2023

have also shown their anthelmintic efficacy *in vitro* and *in vivo* on different stages of development of gastrointestinal nematodes (Kahiya *et al.,* 2003 ; Bachaya *et al.,* 2009; Badar *et al.,* 2011). With this perspective the present work was initiated in order to evaluate the anthelmintic effect of *Acacia nilotica* pod extracts on eggs and female worms of *H. contortus.*

Materials and Methods

The pods of *A. nilotica var dansonii* were harvested in July 2022 in Ouagadougou and dried in the laboratory at room temperature without sunlight and dust and were crushed into powder. Eggs and adult worms of *H. contortus* were collected using ovine abomasum collected at the Saaba slaughterhouse.

Extracts Preparation

For aqueous extract, 150 g of powdered material of *A. nilotica* pods was macerated with 750 mL of distilled water for 24 h. Macerated extract was filtered over cotton wool and concentrated under reduced pressure in a rotary evaporator at 40-50°C and stored at 4°C until use. Likewise for acetonic extract, 150 g powder of *A. nilotica* pods was macerated with 750 mL acetone/water (70/30) (v/v) for 72 h. Macerated extract was filtered over Whatman paper before being concentrated under reduced pressure in a rotary evaporator at 40-50°C and stored at 4°C.

Eggs Collection

Egg collection was done according to Jabbar *et al.* (2006). The abomasums were collected at the Saaba slaughterhouse and kept in an ice box. In the laboratory, the abomasums were placed in Petri dishes, washed with water and then incised. The worms contained in the abomasum were emptied into a Petri dish, then the female *H. contortus* worms were identified, sorted and rinsed with PBS. These females were then placed in a porcelain mortar. Using a pestle, the worms were lightly crushed to release the eggs. The obtained ground solution/suspension was diluted with PBS and filtered through sieves of 1 mm and 100 µm. The released eggs were collected in a 38 µm sieve, rinsed several times with PBS before being recovered into a 15 mL tube. Ten (10) µL of the eggs solution/suspension was placed on a slide and observed under a microscope (x40) in order to count the number of eggs. The eggs solution was adjusted to approximately 200 eggs/mL solution/suspension.

Egg Hatching Inhibition Assay (EHA)

Egg hatch inhibition assay was performed according to Coles *et al.* (2006). 200 µL of egg suspensions were put in wells of a 24-well plate. Two hundred µL of aqueous extract of *A. nilotica* of increasing concentrations (1.25, 2.5, 5.0, 10.0 and 20.0 mg/mL) were added to each well. Two controls were used: PBS (Phosphate Buffer solution, pH: 7.2) as a negative control and Benzal as positive control. The plates were closed and placed in an incubator at 27°C for 48 h. After 48 h of incubation, three drops of formalin (10%) were placed in each well to stop eggs hatch. Three replicates were performed per concentration. The number of L1 larvae and the eggs was counted using microscope (x 10) then the hatching percentage was calculated according to the formula: Number of L1/(number of eggs +number of L1)*100

Adult Worms Mortality Assay (AMA)

Mortality test was performed according to Jackson and Hoste (2010). The test was carried out using Petri dishes containing 3 adult worms per concentration. In each Petri dish, worms were brought in contact with increasing concentrations of *A. nilotica* (0.625, 1.25, 2.5, 5.0 and 10.0 mg/mL) and two control including PBS and Benzal used. Dishes were incubated at room temperature and observed at 0h, 2h, 4h and 6h to enumerate dead and alive worms according to the method of Skantar *et al.* (2005). Nematodes were considered dead when they did not show any movement and as alive when there were at least some tails, head or pharyngeal movements during 10s of observation.

Statistical Analysis

The statistical data concerning the mean percentages of eggs hatch inhibition assay (EHA) and adult worms mortality assay (AMA) were analyzed with the software GraphPad Prism 8.4. The results were expressed as mean ± standard error of mean. The variations were considered as significant when $p < 0.05$.

Results and Discussion

The use of plants with anthelmintic properties is one of the approaches that could reduce the development of parasite resistance (Kahiya *et al.,* 2003 ; Kaboré *et al.,* 2009). The *in vitro* tests carried out with *A. nilotica* pods extracts revealed ovicidal and vermicidal activities.

Egg Hatch Inhibition Assay (EHA)

The aqueous and acetone extracts of *A. nilotica* pods showed inhibition of egg hatching as compared to the control (PBS). The inhibition of egg hatching increased with increasing concentrations of *A. nilotica* pods. Only the concentration of 1.25 and 2.5 mg/mL for aqueous extract and 1.25 mg/mL for acetone extract was recorded, for which there was no significant increase compared to control (PBS). Benzal exhibited the highest inhibition rate of 91% (Fig. 1).

The results showed that both extracts of *A. nilotica* pods at all five concentrations had inhibitory action against egg hatch of *H. contortus* compared to the control group (PBS). Acetone extract was more effective than aqueous extract. The doses of 5 mg/mL recorded more than 60% of egg hatch inhibition rate for both extracts. On the contrary Zabré *et al.* (2017) recorded 21% of egg hatch inhibition rate at 5 mg/mL with aqueous extract of *A. nilotica* leaves. However, Boly *et al.* (2018) recorded higher ovicidal activity

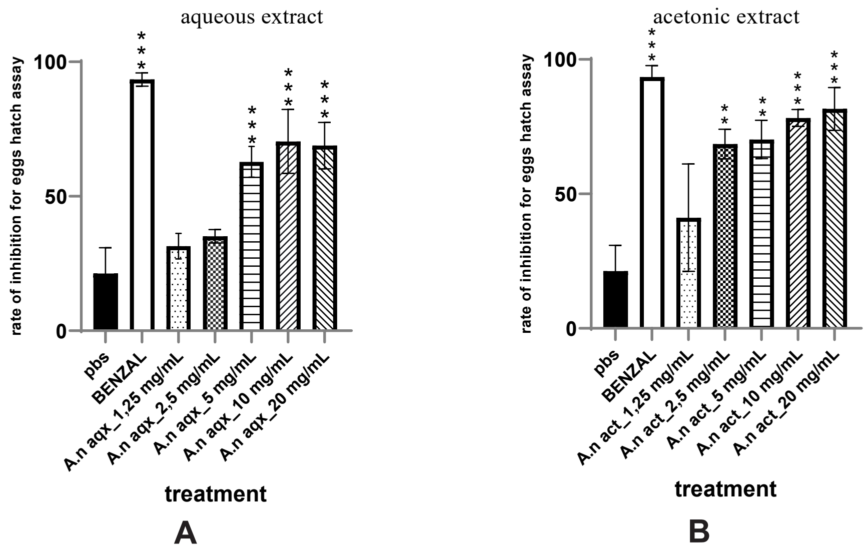

Fig. 1: Egg hatch inhibition assay of H. *contortus* for aqueous (A) and acetonic (B) extracts of *A. nilotica* ***= p<0.0001 ; **= p<0.05 ; A.n act = acetonic extract ; A.n aqx = aqueous extract

(93% at 0.1 mg/mL and 99% at 15 mg/mL) with aqueous extract of *A. nilotica* bark. Similarly, Eguale *et al.* (2006) obtained a rate of 50% of egg hatch inhibition at 0.87 mg/mL with aqueous extracts of *A. nilotica*.

In general, extracts acted in a dose-dependent manner. The dose-dependent effect has been obtained by many authors with aqueous and methanolic extract of *B. aegyptiaca* fruit (Ashenafi *et al.,* 2017), aqueous and acetone extract of *A. nilotica* and *A. raddiana* leaves (Zabré *et al.,* 2017). The *in vitro* anthelmintic efficacy observed would be due to the different chemical groups contained in the pods. Indeed, previous work has shown that the pods of *A. nilotica* are rich in tannin (Seremé *et al.,* 2008; Koné and Dahafolo, 2009), steroid-terpene (Chaibou *et al.,* 2020); saponins and flavonoids (Keita *et al.,* 2021). As per the littérature, these compounds may be involved in killing the parasites.

The work of Barrau *et al.* (2005) and Ayers *et al.* (2008) showed that flavonoïds could play an essential role in the anthelmintic activity of plants. Likewise, saponins would have the ability to increase membrane permeability and the formation of pores thus inhibiting the development of the parasite (Husori *et al.,* 2018) and damage the mucopolysaccharide membrane of worms to cause the death of the nematode (Husori *et al.,* 2018).

Adult Worms Mortality Assay (AMA)

The AMA assay showed, compared to the control (PBS), a significant increase (p<0.05) of female worms mortality. In all treated groups and the two controls, no mortality was recorded between 0h and 2h after the treatments. Mortalities were recorded from the 4th and 6th hour of incubation. Concentrations of 10 and 20 mg/mL recorded the highest mortality rates (>80%) after 4 h of incubation (Table 1).

Table 1: Rate of mortality of adult worms of *H. contortus* using aqueous extracts (A) and acetonic extracts (B) of *A. nilotica*

Treatments (mg/mL)	(A)			
	0h	2h	4h	6h
	Rate of mortality (%)			
pbs	0 ± 0	0 ± 0	0 ± 0	11.11± 14.8
benzal	0 ± 0	0 ± 0	100 ± 0**	100 ± 0***
1,25	0 ± 0	0 ± 0	22.22± 14.8	22.22 ± 14.8
2,5	0 ± 0	0 ± 0	22.22 ± 14.8	33.33 ± 0
5	0 ± 0	0 ± 0	55.55 ± 29.6**	66.66 ± 22.2**
10	0 ± 0	0 ± 0	77.78 ± 29.6**	88.89 ± 14.8**
20	0 ± 0	0 ± 0	77.78 ± 14.8**	88.89 ± 14.8**

Treatments (mg/mL)	(B)			
	0h	2h	4h	6h
	Rate of mortality (%)			
pbs	0 ± 0	0 ± 0	0 ± 0	11.11
benzal	0 ± 0	0 ± 0	100 ± 0***	100 ± 0***
1,25	0 ± 0	0 ± 0	33.33 ± 22.2	33.33 ± 22.2
2,5	0 ± 0	0 ± 0	44.44 ± 14.8	55.55 ± 14.8
5	0 ± 0	0 ± 0	66.67 ± 22.2**	77.78 ± 14.8**
10	0 ± 0	0 ± 0	83.33 ± 22.2**	88.88 ± 14.8**
20	0 ± 0	0 ± 0	88.89 ± 14.8**	88.88 ± 14.8**

***= p<0.0001 ; **= p<0.05

The mortalities of female worms were recorded after 4th hour of incubation with the two extracts and those in all treated groups. Our results concurred those of Segda (2020), who recorded mortality after 4th hour with aqueous extract of *B. aegyptiaca* fruit. By comparing the two controls,

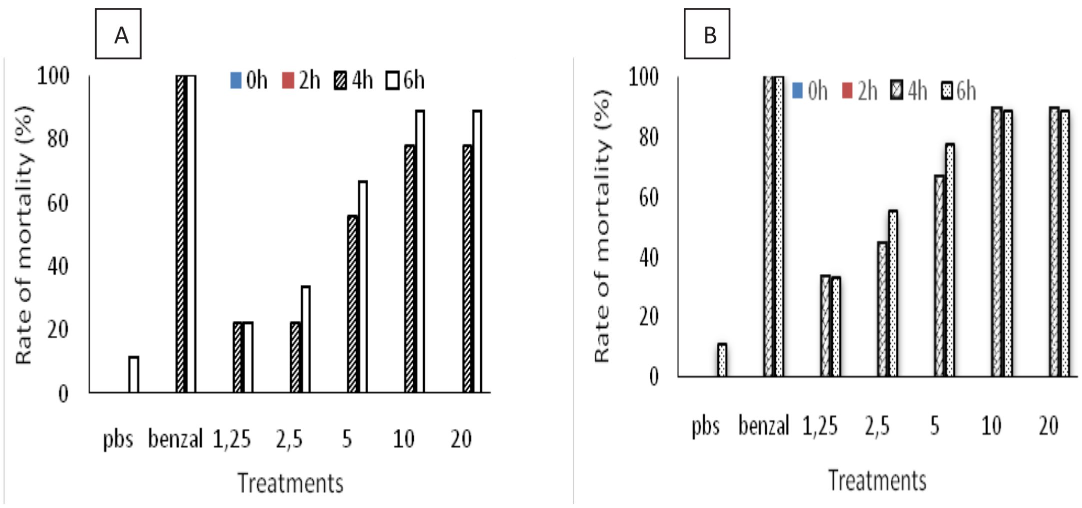

Fig. 2: Rate of mortality of adult *worms of H. contortus* using different concentration of aqueous (A) and acetonic (B) extracts of *A. nilotica*

it appears that Benzal showed 100% mortality from the 4th hour of incubation as compared to control (PBS). The efficacy of this molecule could be due to it's mode of action on parasites. Indeed, previous studies showed that Benzimidazol (BZs) binds to the tubuline of helminths, indirectly preventing metabolic deficits such as microtubule polymerization. Similarly, they act on protein mobilization by interfering with cell division and glucose uptake (Prichard, 1994). The active molecule of Benzal is albendazole and the latter acts by blocking the uptake of glucose by larvae and adult worms, thus depleting their glycogen reserves by decreasing the formation of ATP (Martin, 1997).

Conclusion

The study carried with two extracts of *A. nilotica* pods showed ovicidal and vermicidal activities on eggs and female worms of *H. contortus,* respectively. Thus, the use of *A. nilotica* pods by livestock breeders would be a good approach to fight against gastrointestinal nematodes in small ruminants. However, it would be wise to carry out toxicity study and *in vivo* tests on sheep or goats.

References

Andrea, B., Doeschl-Wilson., Davidson, R., Conington, J., Rugueux, T., Hutchings, M., Villanueva, B. (2011). Implications of host genetic variation on the risk and prevalence of infectious diseases transmitted through the environment. *Journal of Genetics, 188*(3), 683-693.

Ashenafi, A., Ysehak, K., Taye, T., Assefa, K., & Eshetu, S. (2017). Anthelmintic effects of indigenous multipurpose fodder tree extracts against *Haemonchus contortus*. *Tropical Animal Health and Production, 50,* 727-732.

Ayers, S., Zink, D.L., Mohn, K., Powell, J.S., Brown, C.M., Murphy, T., Brand, R., Pretorius, S., Stevenson, D., Thompson, D., & Singh, S.B. (2008). Flavones from *Struthiola argentea* with anthelmintic activity *in vitro*. *Phytochemistry, 69*(2), 541-545.

Bachaya, H.A., Iqbal, Z., Khan, MN., Sindhu, Z.U.D., & Jabbar, A. (2009). Anthelmintic activity of *Ziziphus nummularia* (bark) and *Acacia nilotica* (fruit) against Trichostrongylid nematodes of sheep. *Journal of Ethnopharmacology, 123*(2), 325-329.

Badar, N., Iqbal, Z., Khan, M.N., & Akhtar, M.S. (2011). *In vitro* and *in vivo* anthelmintic activity of *Acacia nilotica* (L.) wild. ex delile bark and leaves. *Pakistan Veterinary Journal, 31*(3), 185-191.

Barrau, E., Fabre, N., Fouraste, I., & Hoste, H. (2005). Effect of bioactive compounds from sainfoin (*Onobrychis viciifolia Scop*.) on the *in vitro* larval migration of *Haemonchus contortus*: Role of tannins and flavonol glycosides. *Parasitology, 131*(4), 531-538.

Boly, A.G.I., Belemlilga, M.B., Traore, A., Ouedraogo, S., & Guissou, E.T.I.P. (2018). Phytochemical study and *in vitro* anthelminthic properties of the trunk barks aqueous extract from *Acacia Nilotica Var.* Adansonii (Guill & Perr). *O. Ktze* (Mimosaceae). *International Journal of Pharmacognosy and Phytochememical Research, 10*, 5-10. doi: 10.25258/phyto.10.1.2

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., Samson-Himmelstjerna, G.V., Silvestre, A., Taylor, M.A., & Vercruysse, J. (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology, 136*(3-4), 167-185.

Chaibou, M., Abdoul, N.M.B., Idrissa, M., Amadou, Tidjani, I., & Ikhiri, K. (2020). Etude Bibliographique et Phytochimique de Quelques Plantes Médicinales Utilisées Pour Le Traitement de Certaines Maladies par les Tradipraticiens de la Zone de l'Azawagh au Niger. *Faculté des Sciences et Techniques,* Université Abdou Moumouni (27 pp). doi:10.19044/esj.2020.v16n6p126.

Devendra, B.N., Srinivas, N., Prasad-Talluri, V.S.S.L., & Swarna Latha, P. (2011). Antimicrobial activity of *Moringa oleifera* Lan, leaf extract, against selected bacterial and fungal strains. *International Journal of Pharma and Bio Sciences, 2,* 13-18.

Eguale, T., Tilahun, G., Gidey, M., & Mekonnen, Y. (2006). *In vitro* anthemintic activities of four ethiopian medicinal plants against *Haemonchus contortus*. *Phamacology Online, 3*, 153-165.

Eichstadt, M. (2017). Evaluation de la résistance des strongles gastro-intestinaux aux anthelminthiques dans quatre élevages ovins allaitants de Corrèze. *Ecole Nationale Vétérinaire de Toulouse* - ENVT.

Fournier, A. (2020). Etude de la résistance aux anthelminthiques des nématodes gastro-intestinaux chez le mouton en Wallonie. *Master en médecine vétérinaire*, Université de Liège, Belgique. (49 pp).

Hounzangbe-Adote, M.S., Paolini, V., Fouraste, I., Moutairou, K., & Hoste, H. (2005a). *In vitro* effects of four tropical plants on three life-cycle stages of the parasitic nematode, *Haemonchus contortus*. *Researchin Veterinary Science, 78* (2), 155-160.

Hounzangbe-Adote, M.S., Moutairou, K., & Hoste, H. (2005b). *In vitro* effects of four tropical plants on three stages of the parasitic nematodes, *Trichostrongylus colubriformis*. *Journal of Helminthologie, 79*(1), 29-33.

Hoste, H., Torres-Acosta, J.F.J., Paolini, V., Aguilar-Caballero, A., Etter, E., Lefrileux, Y., Chartier, C., & Broqua, C. (2005). Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. *Small Ruminant Research, 60*(1-2), 141-151.

Hoste, H., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Mueller-Harvey, I., Sotiraki, S., Louvandini, H., Thamsborg, S.M., & Terrill, T.H. (2015). Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock. *Veterinary Parasitology, 212* (1-2), 5-17.

Husori, D.I., Sumardi., Tarigan, H., Gemasih, S., & Ningsih, S.R. (2018). *In vitro* anthelmintic activity of *Acanthus ilicifolius* leaves extracts on *Ascaridia galli* and *Pheretima posthuma*. *Journal of Applied Pharmaceutical Science, 8*(2), 164-167.

Jabbar, A., Iqbal, Z., & Nisar Khan, M. (2006). *In vitro* anthelmintic activity of *Trachyspermum ammi* seeds. *Pharmacognosy Magasine, 6,* 126-129.

Jackson, F., & Hoste, H. (2010). *In vitro* screening of plant resources for extra-nutritional attributes in ruminants: Nuclear and related methodologies. Eds. Vercoe P. E., Makkar H. P. S., Schlink A. FAO/IAEA.

Kaboré, A., Belem, A.M.G., Tamboura, H.H., Traoré, A., & Sawadogo, L. (2009). *In vitro* anthelmintic effect of two medicinal plants (*Anogeissus leiocarpus* and *Daniellia oliveri*) on *Haemonchus contortus*, an abomasal nematode of sheep in Burkina Faso. *African Journal of Biotechnology, 8*(18), 4690-4695.

Kahiya, C., Mukaratirwa, S., & Thamsborg, S.M. (2003). Effects of *Acacia nilotica* and *Acacia karoo* diets on *Haemonchus contortus* infection in goats. *Veterinary Parasitology, 115*(3), 265-274.

Koné, M., & Dahafolo. (2009). Etude de la phytochimie et des activités larvicide, anticholinesterasique et antioxydante des extraits de quatre plantes du Mali : Acacia nilotica Guill. et Perr. (Mimosaceae), Calotropis procera (Ait.) Ait.f. (Asclepiadaceae), Euphorbia sudanica A. Chev (Euphorbiaceae) et Hyptis suaveolens (L.) Poit (Lamiaceae). *Thèse de doctorat*, Republique du Mali. (123 pp).

Keita, S., Yaya, B., Méminata, D., Lassana, S., Moriba, D., & Mamado W. (2021). Étude phytochimique et activité antibactérienne des extraits de fruits de *Acacia nilotica* Var. (Guill et Per.) sur des souches cliniques des infections urinaires à Bamako au Mali. *African Science Journal, 18*, 260-272.

Martin, J. (1997). Modes of action of anthelmintic drugs. *Veterinary Journal, 154*(1), 11-34.

Prichard, R. (1994). Anthelmintic resistance. *Veterinary Parasitology, 54*(1-3), 259-268.

Segda, R. (2020). Efficacité anthelminthique *in vitro* de *Balanites aegyptiaca* sur trois stades de développement de *Haemonchus contortus,* parasite nématode de la caillette des petits ruminants. *Mémoire de master*, Université Joseph KI-ZERBO. (43 pp).

Seremé, A., Millogo-Rasolodimby, J., Guinko, S., & Nacro, M. (2008). Propriétés thérapeutiques des plantes à tanins du Burkina Faso. *Pharmacopée et Médecine traditionnelle Africaines, 15,* 41-49.

Skantar, A.M., Agama, K., Meyer, S.L.F., Carta, L.K., & Vinyard, B.T. (2005). Effects of geldanamycin on hatching and juvenile mortality in *Caenorhabditis elegans* and *Heterodera glycines*. *Journal of Chemical Ecology, 31,* 2481-2491.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., & Jennings, F.W. (1996). *Veterinary Parasitology.* 2nd edn. The University of Glasgow. Black well Science. Scotland. Pp. 3- 34.

Zabré, G., Kaboré, A., Bayala, B., Katiki, L.M., Costa-Júnior, L.M., Tamboura, H.H., Belem, A.M.G., Abdalla, A.L., Niderkorn, V., Hoste, H., & Louvandini, H. (2017). Comparison of the *in vitro* anthelmintic effects of *Acacia nilotica* and *Acacia raddiana*. *Parasite, 24*, 44.