2022, Scienceline Publication
World's Veterinary Journal
World Vet J, 12(1): 95-104, March 25, 2022
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2022.wvj13

In vitro Anthelmintic Efficacy of Nano-encapsulated Bromelain against Gastrointestinal Nematodes of Goats in Kenya

Ahmota Romain Daiba[1], John Maina Kagira[2*], Maina Ngotho[3], James Kimotho[4], and Naomi Maina[1,5]

[1] *Department of Molecular Biology and Biotechnology, Pan-African University of Institute of Basic Science, Technology and Innovation, Nairobi, Kenya*
[2] *Department of Animal Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*
[3] *Department of Clinical Studies, University of Nairobi, Nairobi, Kenya*
[4] *Innovation and Technology Transfer Division, Kenya Medical Research Institute, Nairobi, Kenya*
[5] *Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*

*Corresponding author's Email: jkagira@jkuat.ac.ke; ORCID: 0000-0001-5513-2113

ORIGINAL ARTICLE
pii: S232245682200013-12
Received: 21 January 2022
Accepted: 17 March 2022

ABSTRACT

Gastrointestinal nematodes (GIN) significantly affect goats' productivity, and thus farmers carry out regular deworming to manage the infections. The emergence of anthelmintic resistance and the high cost of current drugs call for the development of alternatives, including medicinal plant extracts. The current study aimed to assess the anthelmintic efficacy of chitosan encapsulated bromelain (EB) against a selected range of GIN affecting goats in Kenya. Bromelain was extracted using standard laboratory methods from peels of ripe pineapples and thereafter encapsulated with chitosan. The GIN eggs were isolated from goat feces using the flotation method and were then subjected to PCR to identify the species. Adult worms were collected from the gastrointestinal tract of goats slaughtered at the nearby Ruiru abattoir. The PCR showed the extracted strongyle eggs consisted of 7 species of nematodes, including *Haemonchus contortus*, *Oesophagostomum* spp., *Nematodirus filicollis*, *Ostertagia ostertagi*, *Trichostrongylus vitrinus*, *Trichostrongylus colubriformis*, and *Trichostrongylus axei*. The *in vitro* assays showed that chitosan EB had an IC_{50} of 0.184 mg/mL, 0.116 mg/mL, and 0.141 mg/mL for the egg hatch inhibition, larval, and adult mortality assays, respectively. In all the assays, EB indicated better activity than non-encapsulated bromelain. The EB affected the eggs and worms through softening and embrittling the cuticle and shell as well as damaging the blastomeres and causing the death of the growing embryo. According to the results of the current study, EB has high anthelmintic activity on a large range of GIN and has the potential to contribute to the management of these parasites of small ruminants.

Keywords: Anthelmintic activity, Bromelain, Chitosan, Goats, Nano-encapsulation, Nematodes

INTRODUCTION

In Africa, helminth infections in livestock are of major importance and a primary factor in the reducing productivity of goats (Nginyi et al., 2001; Maichomo et al., 2004). Among these helminths, gastrointestinal nematode (GIN) infections cause suppression of weight gain, reduction of reproductive efficiency, and high mortality, especially in kids (Lashari and Tasawar, 2011; Nsereko et al., 2016). In Kenya, the most common GIN affecting small ruminants are *Haemonchus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Ostertagia* spp., *Nematodirus* spp., and *Cooperia* spp. (Munyua et al., 1997; Maichomo et al., 2004; Waruru et al., 2005).

Consequently, the GIN of livestock is controlled mainly through aggressive anthelmintic treatment. Even with regular strategic control, treatments are expensive amongst resource-poor farmers and sometimes partially effective for some species of nematodes. In addition, the excessive and frequent usage of anthelmintics has resulted in the development of resistance amongst nematode populations, especially in the tropics (Roeber et al., 2013; Mickiewicz et al., 2021). Thus, there has been an increased focus on the development of new drugs with different modes of action (Buttle et al., 2011). The use of plant extracts has emerged as a possible sustainable, environmentally acceptable method of nematode control (Hunduza et al., 2020) because natural plant-derived products have long been known to possess anthelmintic properties (Gradé et al., 2008). One such group of potential anthelmintics is the cysteine proteinases found in fruits plants, such as pineapples (*Ananas comosus*) and papaya (Buttle et al., 2011; Misran et al., 2019).

In recent years, bromelain, a pineapple proteolytic enzyme, has been shown high activity against *Haemonchus contortus* (Amini et al., 2016; Hunduza et al., 2020; Wasso et al., 2020). However, its effect against other GIN infections, such as those caused by *Trichostrongylus* spp., *Oesophagostomum* spp., *Ostertagia* spp., and *Nematodirus* spp., has not been well investigated. One of the main challenges of oral bromelain is to maintain stability within the gastrointestinal system of animals. Recent studies have shown that the encapsulation of bromelain with chitosan can stabilize and maintain the activity of bromelain throughout the gut (Wasso et al., 2020). The objective of the present study was to evaluate the *in vitro* anthelmintics activity of encapsulated bromelain (EB) against multiple GIN affecting goats, with a view of developing a novel drug for the management of nematodes in goats.

To cite this paper: Daiba AR, Kagira JM, Ngotho M, Kimotho J, and Maina N (2022). *In vitro* Anthelmintic Efficacy of Nano-encapsulated Bromelain against Gastrointestinal Nematodes of Goats in Kenya. *World Vet. J.*, 12 (1): 95-104. DOI: https://dx.doi.org/10.54203/scil.2022.wvj13

MATERIALS AND METHODS

Ethical approval

Procedures involving sample collection from animals' rectum for this study were approved and conducted according to the guidelines of Institutional Research and Ethics Committee of Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.

Extraction and encapsulation of bromelain in chitosan

Bromelain was extracted from peels of pineapple (*Ananas comosus*) sold at the local Juja market in Kenya. The enzyme was extracted using the procedure described by Hunduza et al. (2020). Briefly, fresh ripe pineapple was grounded and blended in sodium acetate buffer (pH 7.4). Then, crude extract obtained was precipitated by adding 40% ammonium sulphate after sieved. The purification of extracted bromelain was done using dialysis membrane (12 kDa). The ionic gelation method was used to encapsulate bromelain into chitosan (Sigma Aldrich, USA). The pellet obtained after encapsulation was frozen at -60°C and placed in the freeze-dryer (MRC, Model FDL-10N-50-BA, equipment manufacturer, Israel). The freeze-drying was allowed to run until all the samples were completely dried. Successful conjugation of bromelain to the chitosan nanoparticles was confirmed by Fourier transform infrared spectrophotometer analysis.

Isolation and collection of gastrointestinal nematode eggs

Fecal samples were collected using sterile gloves from the rectum of 5 goats in a farm located in Juja Subcounty, Kenya. The feces were analyzed to determine the number of nematode eggs per gram (EPG) of feces using the method described by Coles et al. (1992). The eggs were harvested using the procedure described by Coles et al. (1992) with slight modification. Briefly, a 5 g feces sample was weighed and mixed in a mortar and pestle. The feces mixture was mixed in 50 mL of tap water and poured through a sieve into centrifuge tubes. The filtrate was centrifuged for 8 minutes at 1000 g. The resultant supernatant was gently discarded and the sediment suspended in saturated salt (NaCl) solution (specific gravity = 1.2 g) and allowed to stand for 10 minutes. The suspension was then centrifuged at 400 g for 6 minutes. Then, approximately 5 mL was sucked from the top and the eggs suspension was washed by centrifugation (at 1,000 × g for 8 minutes) in distilled water twice. The supernatant was discarded and the pellet containing eggs was suspended in Phosphate buffered saline (pH 7.4).

Polymerase chain reaction for identification of GIN eggs

To determine the species of the GIN isolated from the goats, DNA was extracted from the eggs, and polymerase chain reaction (PCR) was undertaken as previously described by Ammazzalorso et al. (2015) and Weier et al. (2019). Genomic DNA was extracted using the Quick-DNA^{TM} Tissue/Insect Miniprep Kit (Zymo Research Corp., Irvine, CA, USA). After elution, DNA was stored at -20°C until used. DNA yield and quality were assessed using a Nano-drop spectrophotometer (PCR Max, Lambda).

The PCR was optimized separately with the main GIN strongyles known to occur in Kenya goats (*Haemonchus contortus, Trichostrongylus vitrinus, Trichostrongylus axei, Trichostrongylus colubriformis, Teladorsagia circumcincta, Nematodirus battus, Nematodirus filicollis, Chabertia ovina, Oesophagostomum* spp., *and Ostertagia ostertagi).* The PCR mixture consisted of 2 µL of DNA template, 1 µL of each primer (Forward and Reverse, Table 1), 5 µL Tag reaction buffer, and 0.5 µL of Taq DNA polymerase (My Taq^{TM} DNA Polymerase, meridian BIOSCIENCE). The volume of the reaction was made up to 25 µL with DNA-free water. PCR was performed by Initial denaturation at 95^{o}C for one minute followed by 35 cycles each at 95^{o}C for 20 seconds, 53^{o}C for 20 seconds, and 72^{o}C for 30 seconds. This was followed by 2 minutes final extension at 72^{o}C. The trials were performed to define the optimal PCR conditions for each individual PCR assay. The amplicons were electrophoresed using 2% Tris-acetate-EDTA agarose gel (50 minutes, 70 V), after staining with ethidium bromide, and the DNA migration and resolution pattern were examined.

In vitro anthelmintic activity

Egg hatch assay

The egg hatch assay was performed according to the procedure described by Coles et al. (2006). For this, 1 mL of the egg suspension, adjusted to approximately 40 to 50 eggs per mL, was placed in 15 mL Eppendorf tubes. In each tube prepared, 1 mL of EB and non-encapsulated bromelain (NEB) of double-dilution concentrations (4, 2, 1, 0.5, 0.25, and 0.125 mg/mL) prepared in phosphate-buffered saline (PBS) was added. A negative (PBS) and a positive (Albendazole; Sigma Aldrich, USA) control were constituted. Then, the tubes were incubated at 28°C for 48 hours. The test was carried out in triplicate for each drug concentration. The percentage of egg hatch inhibition (EHI) for each of the drug concentrations was evaluated microscopically at 40× using the modified formula by Luoga (2013).

$$\text{Egg Hatch Inhibition percentage (\%)} = \frac{\text{Total number eggs - number hatched larvae}}{\text{Total number of eggs}} \times 100$$

To cite this paper: Daiba AR, Kagira JM, Ngotho M, Kimotho J, and Maina N (2022). *In vitro* Anthelmintic Efficacy of Nano-encapsulated Bromelain against Gastrointestinal Nematodes of Goats in Kenya. *World Vet. J.*, 12 (1): 95-104. DOI: https://dx.doi.org/10.54203/scil.2022.wvj13

Larval mortality assay

Larval mortality assay was carried out as described by Eguale et al. (2011). Briefly, 1 mL of the egg suspension in PBS, adjusted to approximately 30 eggs per mL, was placed in 15 mL Eppendorf tubes. Amphotericin B (5 µg/mL) was added to the egg suspension to control the growth of other contaminating bacteria. The tubes were incubated at 28°C for 48 h. Then, in each tube prepared, the hatched larvae were observed under the light microscope (OPTIKA, Italy) and 1 mL of EB and NEB of different concentrations (4, 2, 1, 0.5, 0.25, 0.125 mg/mL) prepared in PBS was added. A negative reference control (PBS) and a positive reference control (albendazole in PBS) were constituted. Each test was done in three times. The setup was further allowed to stand for 24h under the same conditions. The total number of larvae (dead and alive) was counted under a microscope (OPTIKA, Italy) and recorded. The percentage of mortality of larvae was calculated using the following formula (Luoga, 2013).

$$\text{Larval mortality (\%)} = \frac{\text{Number of dead larvaee}}{\text{Number of larvae in culture}} \times 100$$

Effect of bromelain on adult worms

Adult worm mortality assay was carried out as described by Eguale et al. (2007). Two common nematodes (*Haemonchus contortus* and *Oesophagostomum* spp.) in Kenya were collected from the abomasum of a goat slaughtered at the Ruiru slaughterhouse in Kenya. The collected parasites were transported to the laboratory in PBS. Once in the laboratory, the collected parasites were washed and the mobile worms were distributed in the petri dishes (10 worms per petri dish). Subsequently, 10 mL EB and NEB were used against the larvae at double-dilution concentrations (4, 2, 1, 0.5, 0.25, and 0.125 mg/mL) in PBS added. After 24 hours of incubation, the number of mobile (live) and immobile (dead) worms were counted. The mortality rate (%) was calculated as the number of dead worms divided by the total number of worms per petri dish. The test was done in triplicate for each drug concentration.

Preparation worms for scanning electron microscopy

The effect of bromelain on the worm's cuticle was examined under Scanning Electron Microscopy **(**SEM). The worms were prepared according to the procedure described by Ghahvei et al. (2020). Briefly, worms were fixed with 3% of glutaraldehyde and 1% of osmium tetroxide and dehydrated by sequentially placing in serial dilutions of ethanol (30, 50, 70, 90, and 100%). The specimens were dried in a laboratory oven and mounted onto aluminum stubs (15 × 5 mm). The specimens were viewed under a JEOL JCM-7000 Scanning Electron Microscopy.

Statistical analysis

The data obtained were entered into and analyzed using the Statistical Package for Social Sciences (SPSS) software version 28.0. The inhibition concentration 50 (IC_{50}) for eggs hatch, larval and adult mortality were calculated using the regression line of probit according to the Log_{10} of the extract concentration. The mean percentage at different concentrations and ratios were compared using paired sample t-test at $p < 0.05$ significant level.

Table 1. Sequences of genus-specific primers of gastrointestinal nematode parasites of goats and sheep

Nematode species	Primer sequences in 5'-3' direction	Fragment size in bp	Reference
Haemonchus contortus	F: GTTACAATTTCATAACATCACGT *R:* TTTACAGTTTGCAGAACTTA	321	Redman et al. (2008)
Generic ITS2	F: CACGAATTGCAGACGCTTAG R: GCTAAATGATATGCTTAAGTTCAGC	370-398	Bisset et al. (2014)
Trichostrongylus vitrinus	F: AGGAACATTAATGTCGTTACA R: CTGTTTGTCGAATGGTTATTA	104	Wimmer et al. (2004)
Trichostrongylus axei	F: AGGGATATTAATGTCGTTCA R: TGATAATTCCCATTTTAGTTT	67	Burgess et al. (2012)
Trichostrongylus colubriformis	F: CCCGTTAGAGCTCTGTATA R: TGCGTACTCAACCACCACTAT	165	Burgess et al. (2012)
Teladorsagia circumcincta	F: ATACCGCATGGTGTGTACGG R: CAGGAACGTTACGACGGTAAT	421	Burgess et al. (2012)
Nematodirus battus	F: CCGGTATACCCATTCAAGTAAGG R: CTACAGTCAGTTCCCCGTTG	148	Wimmer et al. (2004)
Nematodirus filicollis	F: CAGTCAATCCCATTCAAGTGAA R: GTTAAGAGCAGGTCCCCGATC	146	Wimmer et al. (2004)
Chabertia ovina	F: CATGTGTGATCCTCGTACTAGATAAGA R: ATGAACCGTACACCGTTGTCA	158	Wimmer et al. (2004)
Cooperia spp.	F: TATAGTAATATGAACATTTCTGAATGATATC R: CTATAACGGGATTTGTCAAAACAGA	177	Bisset et al. (2014)
Oesophagostomum spp.	F: CTTCAGCAGACGCCAATACA R: CGTCTGCAATTCGTGGTAAA	155	GenBank accession no: MW756992.1
Ostertagia ostertagi	F: CGAATTGCAGACGCTTAGA R: ATTAACAACCCTGAACCAGAC	88	GenBank accession no: KX92994.1

To cite this paper: Daiba AR, Kagira JM, Ngotho M, Kimotho J, and Maina N (2022). *In vitro* Anthelmintic Efficacy of Nano-encapsulated Bromelain against Gastrointestinal Nematodes of Goats in Kenya. *World Vet. J.*, 12 (1): 95-104. DOI: https://dx.doi.org/10.54203/scil.2022.wvj13

RESULTS

Molecular identification of gastrointestinal nematode eggs

The eggs isolated from the goats' feces were all strongyles. Out of 12 species-specific primers shown in Table 1 used to amplify DNA samples, 7 were amplified and yielded the expected product sizes of the following nematodes *Haemonchus contortus* (320 bp), *Trichostrongylus vitrinus* (104 bp*), Trichostrongylus axei* (67 bp), *Trichostrongylus colubriformis* (165 bp), *Nematodirus filicollis* (142 bp), *Oesophagostomum* spp. (155 bp*)* and *Ostertagia ostertagi* (88bp, figures 1 and 2). *Teladorsagia circumcincta*, *Nematodirus battus*, *Chabertia ovina* and *Cooperia* spp. DNA was not amplified and thus it was assumed these parasites were not present in the samples.

In vitro egg hatch assay of gastrointestinal nematode

The activity of the EB, Albendazole, and NEB in inhibiting the hatch of isolated eggs is summarized in Graph 1. The results showed that high extract/drug concentration caused higher egg hatch inhibition, highlighting direct proportionality between drug concentration and activity. The lowest concentration of EB (0.125 mg/mL) that was tested inhibited more than 45% of the eggs from hatching. The EB had higher ($p < 0.05$) activity than NEB. However, albendazole had significantly higher ($p < 0.05$) activity than encapsulated bromelain. The IC_{50} were 0.105 mg/mL, 0.184 mg/mL and 0.369 mg/mL for Albendazole, encapsulated bromelain and non-encapsulated bromelain, respectively (Table 2).

Effect of bromelain on eggs

Following the exposure of EB to GIN eggs the changes included degeneration of blastomere cells and internal layer wall, inhibition of embryo development, and death of growing embryo and larval forms were observed (Figure 3).

In vitro larval mortality assay

The activity of EB, Albendazole, and NEB on larval mortality are shown in Graph 2. The results showed that at the highest extract/drug concentration of albendazole and EB, there were no live larvae. The lowest concentration of EB (0.125 mg/ml) caused more than 60% of larval mortality. Albendazole had higher IC_{50} (0.090 mg/L) than EB (0.116 mg/mL) and NEB (0.313 mg/mL). There was a significant difference ($p < 0.05$) between the IC_{50} of albendazole and EB, and also between the IC_{50} of EB and NEB (Table 3).

Adult worm's mortality assay

Haemonchus contortus

The EB killed more worms than the NEB one at all concentrations tested ($p < 0.05$). The lowest concentration of EB tested induced more than 50% mortality with an IC_{50} = 0.136 mg/mL, while NEB induced only 20% at the same concentration with an IC_{50}=0.588 mg/mL (Graph 3 and Table 4).

Oesophagostomum spp.

Encapsulated bromelain at different concentrations induced significant mortality of adult *Oesophagostomum* spp. Even at the lowest concentration, EB caused more than 50% adult worm mortality (Graph 4). The IC_{50} observed were 0.069 mg/ml, 0.146 mg/mL and 0.408 mg/mL for Albendazole, EB and NEB, respectively (Table 5).

Damage of bromelain on worm's wall

The SEM results showed that exposure of worms to bromelain induced embrittlement of the wall, softening, and destruction of the cuticle (Figure 4).

Table 2. IC_{50} values of encapsulated bromelain, Albendazole, and non-encapsulated bromelain on egg hatch inhibition

Drug	Lower boundary (mg/mL)	Upper boundary (mg/mL)	Average (mg/mL)
Encapsulated bromelain	0.106	0.261	0.184[a]
Albendazole	0.007	0.151	0.105[b]
Non-encapsulated bromelain	0.254	0.502	0.369[c]

Values with the same superscript letter in the same column are not significantly different at $p \geq 0.05$.

To cite this paper: Daiba AR, Kagira JM, Ngotho M, Kimotho J, and Maina N (2022). *In vitro* Anthelmintic Efficacy of Nano-encapsulated Bromelain against Gastrointestinal Nematodes of Goats in Kenya. *World Vet. J.*, 12 (1): 95-104. DOI: https://dx.doi.org/10.54203/scil.2022.wvj13

Table 3. IC_{50} values of encapsulated bromelain, albendazole and non-encapsulated bromelain on larval mortality

Drug	Lower boundary (mg/mL)	Upper boundary (mg/mL)	Average (mg/mL)
Encapsulated bromelain	0.039	0.197	0.116[a]
Albendazole	0.069	0.127	0.090[b]
Non-encapsulated bromelain	0.235	0.397	0.313[c]

Values with the same superscript letter in the same column are not significantly different at $p \geq 0.05$.

Table 4. IC_{50} values of encapsulated bromelain, albendazole and non-encapsulated bromelain on adult *Haemonchus contortus* mortality

Drug	Lower boundary (mg/mL)	Upper boundary (mg/mL)	Average (mg/mL)
Encapsulated bromelain	0.050	0.276	0.136[a]
Albendazole	0.040	0.190	0.096[b]
Non-encapsulated bromelain	0.312	1.053	0.588[c]

Values with the same superscript letter in the same column are not significantly different at $p \geq 0.05$.

Table 5. IC_{50} values of encapsulated bromelain, albendazole and non-encapsulated bromelain on mortality of adult *Oesophagostomum* spp.

Drug	Lower boundary (mg/mL)	Upper boundary (mg/mL)	Average (mg/mL)
Encapsulated bromelain	0.023	0.273	0.146[a]
Albendazole	0.006	0.172	0.069[b]
Non-encapsulated bromelain	0.177	0.725	0.408[c]

Values with the same superscript letter in the same column are not significantly different at $p \geq 0.05$.

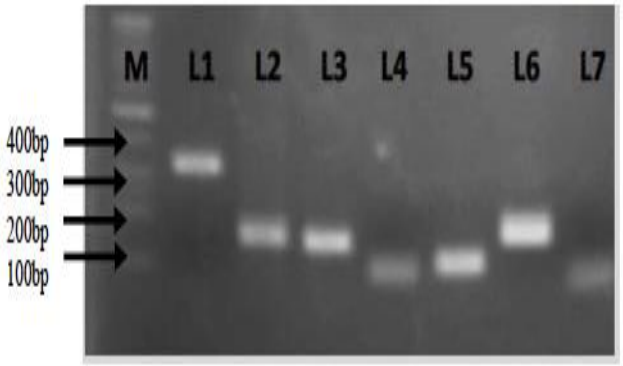


Figure 1. Analysis of PCR products using DNA isolated from gastrointestinal nematode egg isolated from faecal samples of goats in Kenya. **M:** 100 bp ladder; **L1:** Amplification of *Haemonchus contortus* DNA (320 bp), **L2:** Amplification of *Oesophagostomum* spp. (155 bp), **L3:** Amplification of *Nematodirus filicollis* (142 bp), **L4:** Amplification of *Ostertagia ostertagi* (88 bp), **L5:** Amplification of *Trichostrongylus vitrines* (104 bp), **L6:** Amplification of *Trichostrongylus colubriformis* (165 bp) and **L7:** Amplification of *Trichostrongylus axei* (67 bp).

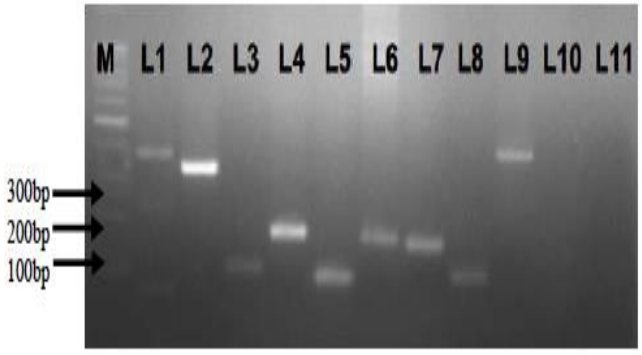


Figure 2. Analysis of PCR products using DNA isolated from gastrointestinal nematode egg separated from faecal sample of goats in Kenya. **M:** 100 bp ladder; **L1:** Amplification of Generic ITS2 (380 bp), **L2:** Amplification of *Haemonchus contortus* DNA (320 bp), **L3:** Amplification of *Trichostrongylus vitrines* (104 bp), **L4:** Amplification of *Trichostrongylus colubriformis* (165 bp), **L5:** Amplification of *Trichostrongylus axei* (67 bp), **L6:** Amplification of *Oesophagostomum* spp. (155 bp), **L7:** Amplification of *Nematodirus filicollis* (142 bp), **L8:** Amplification of *Ostertagia ostertagi* (88 bp), **L9:** Amplification of Generic ITS2 (380 bp), L10 and L11: Negative control.

To cite this paper: Daiba AR, Kagira JM, Ngotho M, Kimotho J, and Maina N (2022). *In vitro* Anthelmintic Efficacy of Nano-encapsulated Bromelain against Gastrointestinal Nematodes of Goats in Kenya. *World Vet. J.*, 12 (1): 95-104. DOI: https://dx.doi.org/10.54203/scil.2022.wvj13

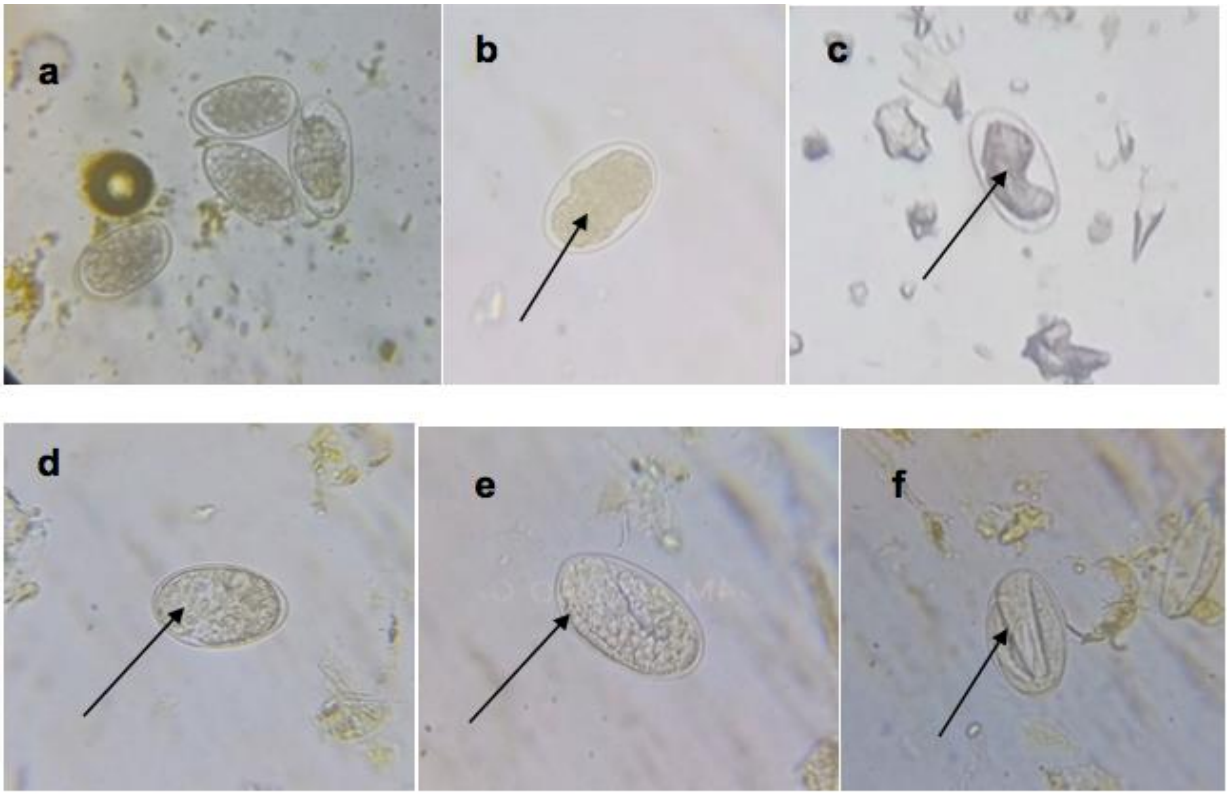


Figure 3. Damages of bromelain on eggs. **a**: Normal eggs containing blastomere; **b**: Egg with degeneration of blastomere cells; **c**: egg with degeneration and distortion of blastomere cells; **d**: Eggs with death embryo; **e** and **f**: Eggs with death larval forms.

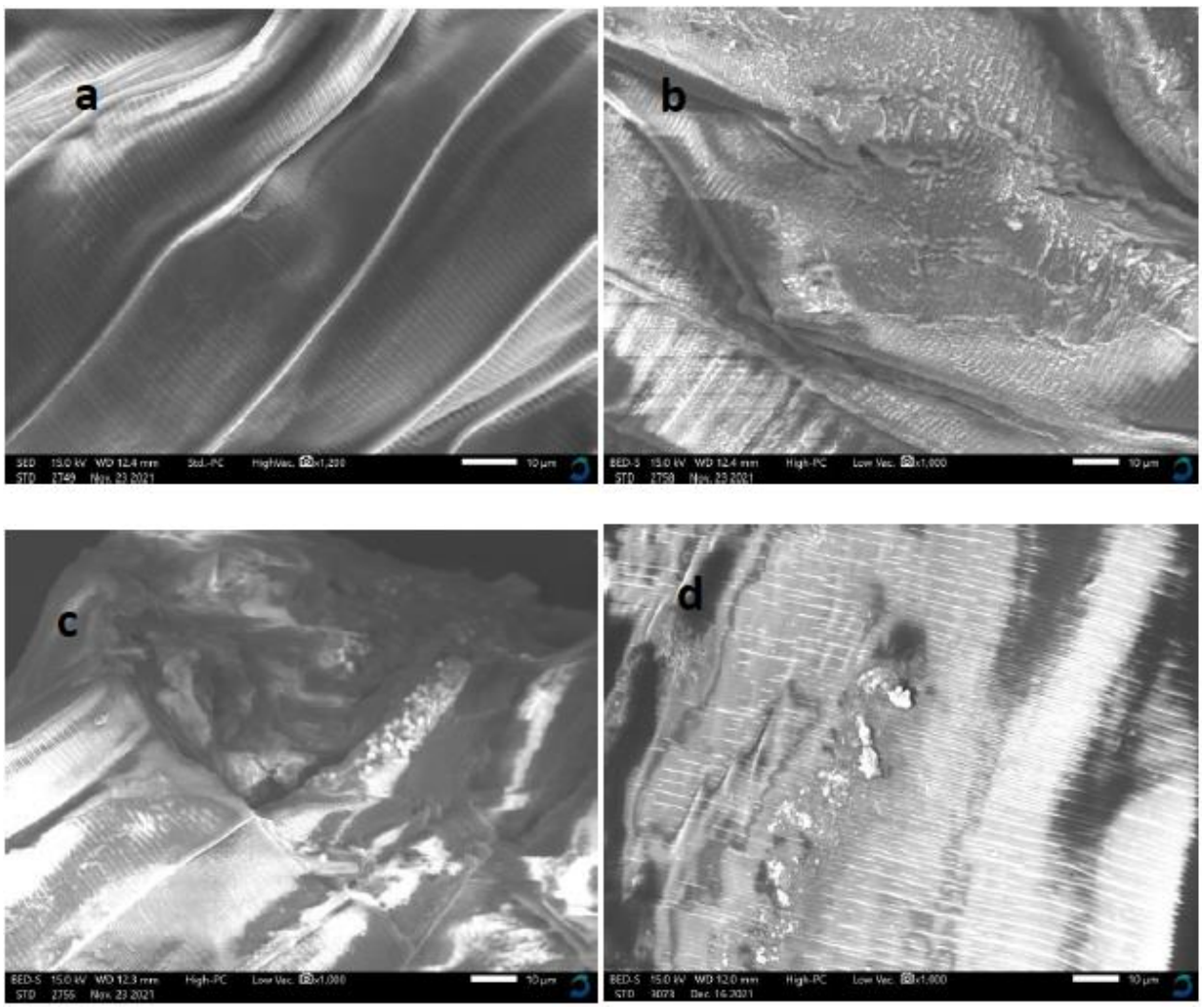
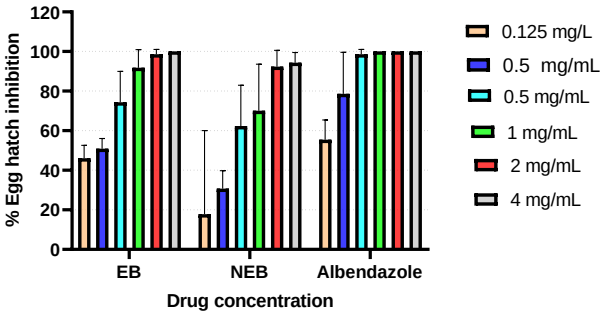
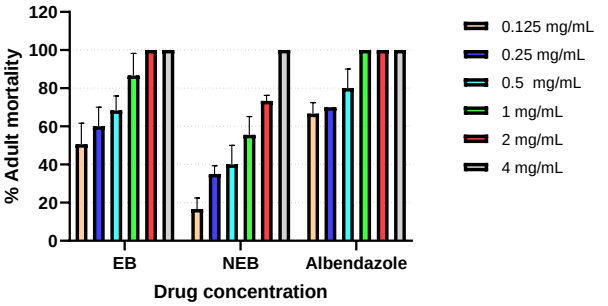


Figure 4. Damages of bromelain on *Haemonchus contortus* worm's wall. **a**: Normal cuticle surface, **b**, **c** and **d**: Degraded cuticle surface.

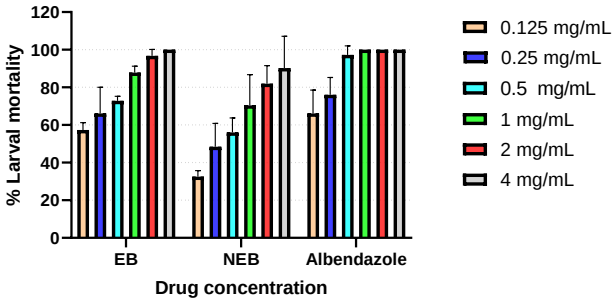
To cite this paper: Daiba AR, Kagira JM, Ngotho M, Kimotho J, and Maina N (2022). *In vitro* Anthelmintic Efficacy of Nano-encapsulated Bromelain against Gastrointestinal Nematodes of Goats in Kenya. *World Vet. J.*, 12 (1): 95-104. DOI: https://dx.doi.org/10.54203/scil.2022.wvj13



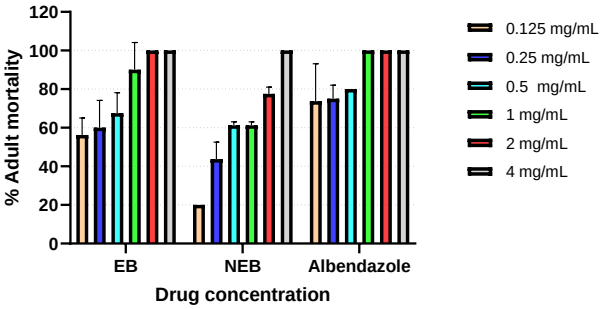
Graph 1. The effect of encapsulated bromelain, Albendazole, and non-encapsulated bromelain on GIN egg hatch inhibition



Graph 3. The effect of encapsulated bromelain, Albendazole, and non-encapsulated bromelain on adult *Haemonchus contortus* mortality percentage



Graph 2. The effect of encapsulated bromelain, albendazole and plain bromelain on GIN larval mortality percentage



Graph 4. The effect of encapsulated bromelain, albendazole, and non-encapsulated bromelain on adult *Oesophagostomum* spp. mortality percentage

DISCUSSION

The current study was targeted towards a further investigation of the efficacy of bromelain on GIN affecting ruminants. Previous studies had only determined the effect of EB on *H. contortus* while the current study investigated the activity of EB on a broader number of GIN affecting goats (Hunduza et al., 2020; Wasso et al., 2020). Since it is cumbersome to reliably distinguish in egg and larval stages of gastrointestinal strongyles, molecular identification through PCR was used as a sensitive and rapid alternative test for differentiating species of GIN in samples (Lin et al., 2008; Bandyopadhyay et al., 2009). The PCR results showed the goats were excreting eggs of most trichostrongylids species previously reported in Kenya (Munyua et al., 1997 ; Maichomo et al., 2004 ; Waruru et al., 2005). Present study indicated presence of *H. contortus*, *Oesophagostomum* spp., *N. filicollis*, *O. ostertagi*, *T. vitrinus*, *T. colubriformis* and *T. axei* in the fecal samples of the goats. Similar diversity of trichostrongylids has been reported in other African countries (Munyua et al., 1997; Waruru et al., 2005).

The results of the present study showed that encapsulated bromelain has anthelmintic activity against eggs, larvae, and adult worms of goat GIN. The results showed that EB has higher activity than that NEB and this could be due to the fact that encapsulation of bromelain in chitosan would stabilize the activity of cysteine protease (bromelain) (Bhatnagar et al., 2014). The present findings are in line with the Hunduza et al. (2020) and Wasso et al. (2020) observations with the same bromelain extract on *H. contortus* egg, larvae, and adult. Hunduza et al. (2020) observed anthelmintic activities of EB on egg hatch inhibition and larvae mortality on *H. contortus* were more than 50% in low concentration (0.125mg/mL). The result of the current study about the IC_{50} (0.141mg/mL) was similar to the findings of Hunduza et al. (2020) and Wasso et al. (2020), respectively 0.140 and 0.151mg/mL for adult mortality. Moreover, the present study observed better eggs hatch and larval mortality assay IC_{50} (0.184 and 0.116 mg/mL, respectively) on a broader range of GIN eggs than that of Hunduza et al. (2020), who only used *H. contortus*.

Other studies have shown some plant extracts have activities against nematodes (Thuo et al., 2017; Sambodo et al., 2018; Yongwa et al., 2020). It was reported a moderate level of anthelmintic activity of *Albizia gummifera* (root bark) and *Zanthoxylum usambarense* (stem bark) against nematodes eggs in sheep with a concentration of IC_{50} = 219±94 mg/mL and 297±122 mg/mL, respectively (Thuo et al., 2017). Castagna et al. (2020) observed more than 82% of unhatched GIN sheep eggs after exposition to aqueous pomegranate extract (*Punica granatum* L.). Yongwa et al. (2020) obtained an inhibition of *H. contortus* egg hatching with LC_{50} of 0.69 mg/mL and 0.48 mg/mL, respectively with

To cite this paper: Daiba AR, Kagira JM, Ngotho M, Kimotho J, and Maina N (2022). *In vitro* Anthelmintic Efficacy of Nano-encapsulated Bromelain against Gastrointestinal Nematodes of Goats in Kenya. *World Vet. J.*, 12 (1): 95-104. DOI: https://dx.doi.org/10.54203/scil.2022.wvj13

aqueous and ethanolic extract of *Senna italica* (caesalpiniaceae). These different studies show that some plant extracts have the potential of anthelmintic activity depending on their species and could play a decisive role against gastrointestinal helminths (Thuo et al., 2017).

This study showed that the activity of NEB on nematodes eggs was mainly due to degeneration and distortion of blastomere cells and cessation of growth in embryos. As previously observed, it is possible that bromelain extracts inhibit embryo development by inactivating endogenous enzymes responsible for the development process (André et al., 2017; Lalthanpuii and Lalchhandama, 2020). The diffusion of the enzyme could be transcuticular, a common route of entry into helminths for non-nutritive and non-electrolytic substances in nematodes. This transcutile route has been proven to be a predominant route for the absorption of the main anthelmintics (such as Albendazole, Ivermectin) in various parasitic helminths (Eguale et al., 2007; André et al., 2017). In the present study, the encapsulated bromelain would have affected the wall layers of the egg, softening it and damaging the central cytoplasmic mass (blastomere) as evidenced by the abnormal observation of the contents of the eggs exposed to various concentrations. The exposure of worms to bromelain also resulted in embrittlement of the wall, softening, and destruction of the shell tissue. This often results in weakening of the cuticle of the larva and adult worm and eventual bursting of worm and release of the contents (Stepek et al., 2006). Present results confirm the observations reported by Njom et al. (2021) and Sambodo et al. (2018) who show the effects of plant cysteine proteinases on the nematode cuticle. Njom et al. (2021) reported papain caused damage and disruption of nematode cuticle. The disruption of cuticle by plant cysteine proteinase would cause by the destruction of nematode cuticle structural proteins due to the dislocation of covalent tyrosine cross-links (Njom et al., 2021). The presence of wrinkles and the destruction of the worm cuticle after exposure to plant extract was also described by Sambodo et al. (2018).

CONCLUSION

The current study shows that bromelain encapsulated in chitosan has high activity against GIN affecting goats mainly through affecting the shell and cuticle of the eggs and worms. Accordingly, this formulation of bromelain can contribute to controlling GINs of small ruminants. Further studies should be undertaken to evaluate the *in vivo* effects of EB against the broad range of GINs.

DECLARATIONS

Acknowledgments

The authors acknowledge Pan-African University of Institute of Basic Science, Technology and Innovation (PAUSTI), Japan International Cooperation Agency (JICA, Africa-ai-Japan) and Jomo Kenyatta University of Agriculture and Technology (JKUAT) for financially sponsoring this research and providing a technical and infrastructural support used during the project.

Competing interests

No competing interests.

Authors' contribution

All authors contributed to the conception of the experiments. Ahmota Romain Daiba developed the study design, collected the samples, undertook the laboratory analyses and wrote the draft of the manuscript. John Maina Kagira developed the study design, undertook the parasitological analysis, and participated in the writing of the first draft of the manuscript. James Kimotho undertook the extraction and encapsulation of bromelain, data analyses and participated in the writing of the first draft of the manuscript. Maina Ngotho participated in molecular analyses and participated in the writing of the first draft of the manuscript. Naomi Maina participated in encapsulation of bromelain, molecular assays and data analyses. All authors read and approved the final manuscript.

Ethical considerations

Ethical issues (Including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

REFERENCES

Amini A, Masoumi-Moghaddam S, and Morris DL (2016). Utility of bromelain and N-Acetylcysteine in treatment of peritoneal dissemination of gastrointestinal mucin- Producing Malignancies. Springer International Publishing Switzerland, p. 229. DOI: https://www.doi.org/10.1007/978-3-319-28570-2

Ammazzalorso AD, Zolnik CP, Daniels TJ, and Kolokotronis SO (2015). To beat or not to beat a tick: Comparison of DNA extraction methods for ticks (*Ixodes scapularis*). Peer Journal, (8) : 1-14. DOI : https://www.doi.org/10.7717/peerj.1147

André WPP, Cavalcante GS, Ribeiro WLC, Dos Santos JML, Macedo ITF, De Paula HCB, De Morais S M, De Melo JV, and Bevilaqua CM (2017). Anthelmintic effect of thymol and thymol acetate on sheep gastrointestinal nematodes and their toxicity in

mice. Revista Brasileira de Parasitologia Veterinaria, 26(3): 323-330. DOI: https://www.doi.org/10.1590/S1984-29612017056

Bandyopadhyay S, Naskar S, Devi P, Bera AK, De S, Pan D, Rana T, Das S, Sikdar S, and Bhattacharya D (2009). Multiplex PCR for identification of parasitic eggs of *Oesophagostomum* spp. isolated from sheep and goat. Proceedings of the Zoological Society, 62(2): 125-129. DOI: https://www.doi.org/10.1007/s12595-009-0014-4

Bhatnagar P, Patnaik S, Srivastava AK, Mudiam MKR, Shukla Y, Panda AK, Pant AB, Kumar P, and Gupta KC (2014). Anti-cancer activity of bromelain nanoparticles by oral administration. Journal of Biomedical Nanotechnology, 10(12): 3558-3575. DOI: https://www.doi.org/10.1166/jbn.2014.1997

Bisset SA, Knight JS, and Bouchet CLG (2014). A multiplex PCR-based method to identify strongylid parasite larvae recovered from ovine faecal cultures and/or pasture samples. Veterinary Parasitology, 200: 117-127. DOI: https://www.doi.org/10.1016/j.vetpar.2013.12.002

Burgess CGS, Bartley Y, Redman E, Skuce PJ, Nath M, Whitelaw F, Tait A, Gilleard JS, and Jackson F (2012). A survey of the trichostrongylid nematode species present on UK sheep farms and associated anthelmintic control practices. Veterinary Parasitology, 189: 299-307. DOI: https://www.doi.org/10.1016/j.vetpar.2012.04.009

Buttle DJ, Behnke JM, Bartley Y, Elsheikha HM, Bartley DJ, Garnett MC, Donnan AA, Jackson F, Lowe A, and Duce IR (2011). Oral dosing with papaya latex is an effective anthelmintic treatment for sheep infected with *Haemonchus contortus*. Parasites and Vectors, 4(1): 1-11. DOI: https://www.doi.org/10.1186/1756-3305-4-36

Castagna F, Britti D, Oliverio M, Bosco A, Bonacci S, Iriti G, Ragusa M, Musolino V, Rinaldi L, Palma E, and Musella V (2020). *In vitro* anthelminthic efficacy of aqueous pomegranate (*Punica granatum* l.) extracts against gastrointestinal nematodes of sheep. Pathogens, 9(12): 1-11. DOI: https://www.doi.org/10.3390/pathogens9121063

Coles GC, Bauer C, Borgsteede FHM, Geerts S, Klei TR, Taylor MA, and Waller PJ (1992). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology, 44: 35-44. DOI: https://www.doi.org/10.1016/0304-4017(92)90141-U

Coles GC, Jackson F, Pomroy WE, Prichard RK, Von Samson-Himmelstjerna G, Silvestre A, Taylor MA, and Vercruysse J (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology, 136: 167-185. DOI: https://www.doi.org/10.1016/j.vetpar.2005.11.019

Eguale T, Tadesse D, and Giday M (2011). *In vitro* anthelmintic activity of crude extracts of five medicinal plants against egg-hatching and larval development of *Haemonchus contortus*. Journal of Ethnopharmacology, 137(1): 108-113. DOI: https://www.doi.org/10.1016/j.jep.2011.04.063

Eguale T, Tilahun G, Debella A, Feleke A, and Makonnen E (2007). *Haemonchus contortus*: *In vitro* and *in vivo* anthelmintic activity of aqueous and hydro-alcoholic extracts of Hedera helix. Experimental Parasitology, 116(4): 340-345. DOI: https://www.doi.org/10.1016/j.exppara.2007.01.019

Ghahvei Y, Mirzaei M, Hashemnia S, Golchin M, Kheirandish R, Uni S, Mendoza-Roldan JA, Otranto D, and Sazmand A (2020). Scanning electron microscopy of *Onchocerca fasciata* (Filarioidea: Onchocercidae) adults, microfilariae and eggs with notes on histopathological findings in camels. Parasites and Vectors, 13(1): 1-10. DOI: https://www.doi.org/10.1186/s13071-020-04123-0

Gradé JT, Arble BL, Weladji RB, and Van Damme P (2008). Anthelmintic efficacy and dose determination of *Albizia anthelmintica* against gastrointestinal nematodes in naturally infected Ugandan sheep. Veterinary Parasitology, 157: 267-274. DOI: https://www.doi.org/10.1016/j.vetpar.2008.07.021

Hunduza A, Kagira J, Maina N, Andala D, Cheruiyot K, and Kahiro S (2020). *In vitro* anthelmintic activity of chitosan encapsulated bromelain against eggs, larval and adult stages of *Haemonchus contortus*. Journal of Applied Life Sciences International, 23(3): 28-38. DOI: https://www.doi.org/10.9734/jalsi/2020/v23i330151

Lalthanpuii PB, and Lalchhandama K (2020). Phytochemical analysis and *in vitro* anthelmintic activity of *Imperata cylindrica* underground parts. BMC Complementary Medicine and Therapies, 20(1): 1-9. DOI: https://www.doi.org/10.1186/s12906-020-03125-w

Lashari MH, and Tasawar Z (2011). Prevalence of some gastrointestinal parasites in sheep, Southern Punjab, Pakistan. Pakistan Veterinary Journal 31(4): 295-298. Available at: http://www.pvj.com.pk/pdf-files/31_4/295-298.pdf

Lin RQ, Ai L, Zou FC, Verweij JJ, Jiang Q, Li MW, Song HQ, and Zhu XQ (2008). A multiplex PCR tool for the specific identification of *Oesophagostomum* spp. from pigs. Parasitology Research, 103(4): 993-997. DOI: https://www.doi.org/10.1007/s00436-008-1070-9

Luoga W (2013). Factors affecting the anthelmintic efficacy of cysteine proteinases against GI nematodes and their formulation for use in ruminants. University of Nottingham. Thesis. Life Sciences, p. 265. Available at: http://eprints.nottingham.ac.uk/12954/1/Thesis_final_version_after_viva_IRM%5B1%5D.pdf

Maichomo MW, Kagira JM, and Walker T (2004). The point prevalence of gastro-intestinal parasites in calves, sheep and goats in Magadi division, South-Western Kenya. Onderstepoort Journal of Veterinary Research, 71(4): 257-261. DOI: https://www.doi.org/10.4102/ojvr.v71i4.229

Mickiewicz M, Czopowicz M, Moroz A, Potărniche AV, Szaluś-Jordanow O, Spinu M, Górski P, Markowska-Daniel I, Várady M, and Kaba J (2021). Prevalence of anthelmintic resistance of gastrointestinal nematodes in Polish goat herds assessed by the larval development test. BMC Veterinary Research, 17(1): 1-12. DOI: https://www.doi.org/10.1186/s12917-020-02721-9

Misran E, Idris A, Mat Sarip SH, and Ya'akob H (2019). Properties of bromelain extract from different parts of the pineapple variety Morris. Biocatalysis and Agricultural Biotechnology, 18: 101095 DOI: https://www.doi.org/10.1016/j.bcab.2019.101095

Munyua WK, Githigia SM, Mwangi DM, Kimoro CO, and Ayuya JM (1997). The effects of a controlled-release albendazole capsule (Profril-Captec) on parasitism in grazing Corriedale ewes in Nyandarua District of Kenya. Veterinary Research Communications, 21: 85-99. DOI: https://www.doi.org/10.1023/a:1005705131222

Nginyi JM, Duncan JL, Mellor DJ, Stear MJ, Wanyangu SW, Bain RK, and Gatongi PM (2001). Epidemiology of parasitic gastrointestinal nematode infections of ruminants on smallholder farms in central Kenya. Research in Veterinary Science, 70(1): 33-39. DOI: https://www.doi.org/10.1053/rvsc.2000.0438

To cite this paper: Daiba AR, Kagira JM, Ngotho M, Kimotho J, and Maina N (2022). *In vitro* Anthelmintic Efficacy of Nano-encapsulated Bromelain against Gastrointestinal Nematodes of Goats in Kenya. *World Vet. J.*, 12 (1): 95-104. DOI: https://dx.doi.org/10.54203/scil.2022.wvj13

Njom VS, Winks T, Diallo O, Lowe A, Behnke J, Dickman MJ, Duce I, Johnstone I, and Buttle DJ (2021). The effects of plant cysteine proteinases on the nematode cuticle. Parasites and Vectors, 14(1): 1-11. DOI: https://www.doi.org/10.1186/s13071-021-04800-8

Nsereko G, Emudong P, Mulindwa H, and Okwee-Acai J (2016). Prevalence of common gastro-intestinal nematode infections in commercial goat farms in Central Uganda. Uganda Journal of Agricultural Sciences, 16(1): 99. DOI: https://www.doi.org/10.4314/ujas.v16i1.8

Redman E, Packard E, Grillo V, Smith J, Jackson F, and Gilleard JS (2008). Microsatellite analysis reveals marked genetic differentiation between Haemonchus contortus laboratory isolates and provides a rapid system of genetic fingerprinting. International Journal for Parasitology, 38(1): 111-122. DOI: https://www.doi.org/10.1016/j.ijpara.2007.06.008

Roeber F, Jex AR, and Gasser RB (2013). Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance - An Australian perspective. Parasites and Vectors, 6(1): 1-13. DOI: https://www.doi.org/10.1186/1756-3305-6-153

Sambodo P, Prastowo J, Kurniasih K, and Indarjulianto S (2018). *In vitro* potential anthelmintic activity of *Biophytum petersianum* on *Haemonchus contortus.* Veterinary World, 11(1): 1-4. DOI: https://www.doi.org/10.14202/vetworld.2018.1-4

Stepek G, Buttle DJ, Duce IR, and Behnke JM (2006). Human gastrointestinal nematode infections: Are new control methods required? International Journal of Experimental Pathology, 87(5): 325-341. DOI: https://www.doi.org/10.1111/j.1365-2613.2006.00495.x

Thuo BM, Thoithi GN, Maingi N, Ndwigah SN, Gitari RN, Githinji RW, and Otieno RO (2017). *In vitro* anthelmintic activity of Albizia gummifera , Crotalaria axillaris , Manilkara discolor , Teclea trichocarpa and Zanthoxylum usambarense using sheep nematodes. African Journal of Pharmacology and Therapeutics, 6(1): 38-42. Available at: http://www.uonbi.ac.ke/journals/kesobap/

Waruru RM, Mutune MN, and Otieno RO (2005). Gastrointestinal parasite infections of sheep and goats in a semi-arid area of Machakos District, Kenya. Bulletin of Animal Health and Production in Africa, 53: DOI: https://www.doi.org/10.4314/bahpa.v53i1.32686

Wasso S, Maina N, and Kagira J (2020). Toxicity and Anthelmintic Efficacy of Chitosan Encapsulated Bromelain against Gastrointestinal Strongyles in Small East African Goats in Kenya. Veterinary World, 13(1): 177-183. DOI: https://www.doi.org/10.14202/vetworld.2020.177-183

Weier SM, Moodley Y, Fraser MF, Linden VMG, Grass I, Tscharntke T, and Taylor PJ (2019). Insect pest consumption by bats in macadamia orchards established by molecular diet analyses. Global Ecology and Conservation, 18: e00626. DOI: https://www.doi.org/10.1016/j.gecco.2019.e00626

Wimmer B, Craig BH, Pilkington JG, and Pemberton JM (2004). Non-invasive assessment of parasitic nematode species diversity in wild Soay sheep using molecular markers. International Journal for Parasitology, 34(5): 625-631. DOI: https://www.doi.org/10.1016/j.ijpara.2003.11.022

Yongwa G, Ngnoda BFNF, Ndjonka D, and Saotoing P (2020). *In vitro* anthelmintic activity of aqueous and ethanolic extract of *Senna italica* (Caesalpiniaceae) on Three-stages of *Haemonchus contortus*. Journal of Pharmaceutical Research International, 32(3): 25-34. DOI: https://www.doi.org/10.9734/jpri/2020/v32i330411

To cite this paper: Daiba AR, Kagira JM, Ngotho M, Kimotho J, and Maina N (2022). *In vitro* Anthelmintic Efficacy of Nano-encapsulated Bromelain against Gastrointestinal Nematodes of Goats in Kenya. *World Vet. J.*, 12 (1): 95-104. DOI: https://dx.doi.org/10.54203/scil.2022.wvj13