

Botanical Anthelmintics for Sustainable Sheep Production: In Vivo Efficacy of *Carica papaya* Seeds and *Moringa oleifera* Leaves Against *Haemonchus contortus* and *Moniezia* spp. in Ghana

PRINCE KYERE DWAAH

pkdwaah@aamusted.edu.gh

Veterinary Service Directorate <https://orcid.org/0009-0009-9515-3213>

Nana Yaa Awua-Boateng

Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development

Sylvia Afriyie Squire

Council for Scientific and Industrial Research

Issah Bagulo

University for Development Studies

Denis Yar Dekugmen

Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development

Hellen Djang-Fordjour

Sunyani Technical University

Helena Agyei Larrey

Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development

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Abstract

Gastrointestinal helminths, particularly *Haemonchus contortus* (*H. contortus*) and *Moniezia* spp., continue to constrain sheep productivity in sub-Saharan Africa. Increasing reports of reduced efficacy of conventional anthelmintics underline the need to evaluate sustainable alternative control options. This study assessed the in vivo anthelmintic efficacy of aqueous extracts of *Carica papaya* seeds (CPS) and *Moringa oleifera* leaves (MOL) in naturally infected sheep under field conditions in Ghana. Thirty-three sheep were allocated to four treatment groups: CPS, MOL, conventional dewormer, and distilled water. Treatments were administered orally on Day 0, and faecal egg counts (FEC) were determined on Days 0, 5, 10, and 30 using the McMaster technique. Anthelmintic efficacy was evaluated using the faecal egg count reduction test (FECRT), and body weight changes were monitored over the study period. By Day 30, MOL achieved a mean FECRT of 92.1% against *Moniezia* spp. and 60.9% against *H. contortus*, while CPS achieved reductions of 82.4% and 51.3%, respectively. The conventional dewormer showed lower efficacy ($\leq 50\%$) for both parasites. Sheep receiving MOL exhibited the greatest mean body weight gain (4.7 kg), although differences among treatments were not statistically significant. Egg counts increased markedly in the distilled water group. The findings indicate that MOL and CPS exhibit moderate to high anthelmintic activity under field conditions and may offer complementary options for helminth control in sheep production systems where resistance to conventional anthelmintics is of concern, which may contribute to integrated parasite management. Further studies with larger sample sizes and standardized extract characterization are warranted.

INTRODUCTION

Sheep production constitutes an important component of smallholder livestock systems across Africa, contributing to household income, food security, and livelihood resilience. In West Africa, sheep are commonly managed under extensive and semi-intensive systems, where productivity is closely linked to environmental conditions and animal health management practices (Food and Agriculture Organization {FAO}, 2021; Thornton & Herrero, 2021). Despite their adaptability, productivity in these systems is frequently constrained by parasitic diseases, with gastrointestinal helminths remaining among the most persistent challenges to sustainable sheep production.

Among the helminths affecting small ruminants, *Haemonchus contortus* (*H. contortus*) and *Moniezia* spp. are widely distributed in tropical and subtropical regions. *H. contortus* is regarded as one of the most pathogenic nematodes of sheep due to its hematophagous nature, high fecundity, and capacity for rapid population expansion under favourable climatic conditions, leading to anaemia, reduced growth, and increased mortality (Kaminsky et al. 2022; Papadopoulos et al. 2021). Although infections with *Moniezia* spp. are often considered of lower clinical importance, heavy or persistent burdens, particularly in young animals, may compromise feed efficiency and growth performance (Torres-Acosta & Hoste, 2023).

Control of gastrointestinal helminths has historically depended on the regular use of broad-spectrum anthelmintic drugs. While this approach initially resulted in substantial improvements in livestock productivity, increasing reports of reduced drug efficacy have emerged globally (Vineer et al. 2020). Anthelmintic resistance (AR) is now widely recognized as a major constraint to parasite control in small ruminants, particularly in low- and middle-income countries where treatment is often frequent, diagnostics are limited, and dosing practices may be imprecise (Levecke et al. 2022; Coles et al. 1992). Under such conditions, reliance on a narrow range of conventional anthelmintics has raised concerns about the long-term sustainability of conventional parasite control programmes.

In response to this challenge, attention has increasingly turned toward integrated parasite management approaches that combine strategic drug use with complementary control options. Ethnoveterinary medicine, which encompasses the use of locally available plant-based remedies, has gained renewed interest as a potential component of such strategies (Egualé 2021). Numerous medicinal plants have been reported to possess antiparasitic properties; however, their efficacy varies widely depending on plant species, preparation method, parasite species, and evaluation protocol (Belga et al. 2024; Jato et al. 2022). Moreover, many studies rely on *in vitro* assays or solvent-based extracts, limiting their applicability under field conditions typical of smallholder systems.

Moringa oleifera (*M. oleifera*) and *Carica papaya* (*C. papaya*) are widely distributed in Sub-Saharan regions and commonly used for nutritional and medicinal purposes. Experimental studies suggest that extracts from *M. oleifera* leaves and *C. papaya* seeds contain bioactive compounds capable of disrupting helminth development and survival (Páez-León et al. 2022; Cabral et al. 2019). Nevertheless, *in vivo* evidence derived from naturally infected sheep, particularly using aqueous preparations representative of practical on-farm use in West Africa, remains limited.

The present study therefore, assessed the anthelmintic activity of aqueous extracts of *M. oleifera* leaves and *C. papaya* seeds in sheep naturally infected with *H. contortus* and *Moniezia* spp. under field conditions in Ghana. By comparing their effects with a commonly used synthetic anthelmintic and an untreated control, the study seeks to generate applied evidence relevant to sustainable parasite control in tropical small ruminant production systems.

METHODOLOGY

Study Area

The study was conducted at the Disease Investigation Farm (DIF) in Techiman, Bono East Region, Ghana, an area known for its high prevalence of gastrointestinal parasitic infections in small ruminants, to investigate conventional dewormers by the Veterinary Directorate Service (VSD) of Ghana (VSD, 2019; MoFA, 2014). The site lies within the transitional ecological zone (7.59529° N, -1.93331° W), characterized by Guinea savannah and semi-deciduous vegetation. Techiman experiences a tropical

savannah climate with annual rainfall of 1,200-1,500 mm and temperatures ranging from 22°C to 34°C during the study period (September-October 2024).

Plant Materials and Preparation of Extracts

Papaya seeds and moringa leaves were selected based on prior ethnoveterinary evidence and unpublished work by the research team. Mature papaya fruits and fresh moringa leaves were harvested from organically maintained plants at DIF.

Plant materials were shade-dried at ambient temperature (25–30°C) for 10–14 days, ground, and sieved (1 mm mesh). Fifty grams of each powdered sample were macerated in 500 mL of distilled water (1:10 w/v) for 12 hours, filtered through sterile muslin, and stored at 4°C for not more than 72 hours, following established protocols (Belga et al. 2024; Kedir, 2024; Jato et al. 2022; Castangna et al. 2021; Bessell et. al. 2021).

Determination of Safe Dosage

A pilot dose-ranging trial was performed using 1–10 mL/kg BW of each extract, observing for toxicity or behavioural changes over 72 hours. No adverse effects were recorded; therefore, 10 mL/kg BW was selected as the therapeutic dose for the main trial.

Experimental Design

A randomized controlled design was used involving 33 naturally infected sheep. Sample size determination followed the statistical framework of (Leveck et al. 2022; Coles et al. 1992). Animals were randomly allocated into four treatment groups as shown in Table 1

Table 1
Experimental groups, treatments, and administered dosages in naturally infected sheep.

Group	Treatments	Dosage
1	Distilled water (negative control)	10 mL/kg BW
2	Papaya seed extract	10 mL/kg BW
3	Moringa leaf extract	10 mL/kg BW
4	Albendazole (positive control)	10 mg/kg BW
Footnote:		

1. BW = body weight.

2. All treatments were administered orally on Day 0. Faecal samples were collected on Days 0, 5, 10, and 30 for FEC analysis.

Faecal Collection and Parasitological Examination

Faecal samples were obtained directly from the rectum using standard operating procedures (Denwood et. al 2023). Quantitative FECs were performed using the McMaster technique (Mohammedsalih et. al, 2025; Charles Sturt University, 2024; Playford & Besier, 2024; Coles et. al, 1992). Three grams of faeces were homogenized in 60 mL of saturated NaCl solution, sieved, and examined microscopically (×10 objective) for helminth eggs within the grid area. Laboratory personnel were blinded to treatment allocation to minimize bias.

The Faecal Egg Count Reduction Test (FECRT) was adopted from (Denwood et. al 2024; Coles et. al. 1992):
$$\text{FECRT (\%)} = \frac{(\text{Mean FEC before treatment}) - (\text{Mean FEC after treatment})}{(\text{Mean FEC before treatment})} \times 100$$
, using pre-treatment (Day 0) and post-treatment (Day 30) values for *Haemonchus contortus* and *Moniezia spp.*

Statistical Analysis

Descriptive statistics (mean ± SD) were computed using Microsoft Excel 2019. Data normality was assessed with the Shapiro-Wilk test. One-way ANOVA was used to compare mean FECs among groups, with Tukey's Honest Significant Difference (HSD) post hoc test for pairwise comparisons ($p < 0.05$). Two-way ANOVA evaluated treatment × time interaction effects, and paired t-tests compared pre- and post-treatment values within groups. Post hoc power analysis ($\alpha = 0.05$) confirmed sample adequacy (> 0.8).

RESULTS

Helminth Load before Treatment

At baseline, 60.6% (20/33) of sheep were co-infected with *H. contortus* and *Moniezia spp.*, while 30.3% (10/33) were infected with *H. contortus* alone and 9.1% (3/33) with *Moniezia spp.* alone (Table 2). Parasite burdens ranged from moderate to high according to Veterinary Services Directorate classification criteria. Rams showed a higher mean FEC than females and younger animals, although these differences were not subjected to statistical testing due to sample size constraints. There were no significant differences in baseline FECs among treatment groups for either *H. contortus* ($p = 0.72$) or *Moniezia spp.* ($p = 0.68$), indicating comparability across groups before treatment.

Table 2

Pre-treatment distribution of *H. contortus* and *Moniezia* spp. infections by sex and age category (n = 33 sheep).

Sex and Age	No. Sheep	Type of infection		
		<i>H. contortus</i> only	<i>Moniezia</i> spp. only	Both helminths
Ram	4	-	-	4
Ewe	21	8	2	11
Young Ram	4	2	-	2
Young Ewe	4	-	1	3
n (%)	33 (100)	10 (30.3)	3 (9.1)	20 (60.6)
Footnote: Both <i>M. expansa</i> and <i>M. benedeni</i> were detected and are reported collectively as <i>Moniezia</i> spp.				

FECRT of Aqueous *Moringa oleifera* Leaves and *Carica papaya* Seeds against *H. contortus*

Reductions in *H. contortus* FEC differed among treatment groups over time (Fig. 1). From Fig. 2, by Day 30 post-treatment, sheep receiving aqueous *M. oleifera* leaf extract showed a mean FECRT of 60.9% (95% CI: 52.2–69.5), while those treated with aqueous *C. papaya* seed extract achieved a reduction of 51.3% (95% CI: 43.1–59.4). Conventional dewormer treatment resulted in a mean FECRT of 43.9% (95% CI: 35.4–52.1). In contrast, mean FEC in the untreated control group increased over the same period.

One-way ANOVA indicated significant differences in mean FECs between treated groups and the untreated control at Day 30 ($p < 0.05$). However, when evaluated using two-way ANOVA, neither the main effects of treatment ($p = 0.219$) nor time ($p = 0.117$) nor their interaction ($p = 0.995$) were statistically significant.

FECRT of aqueous *oleifera* leaves and aqueous *C. papaya* seeds against *Moniezia* spp.

A progressive reduction in *Moniezia* spp. FEC was observed in sheep treated with both plant extracts (Fig. 3). From Fig. 4, by Day 10, aqueous *M. oleifera* leaf extract achieved a FECRT of $52.6\% \pm 6.7$ (95% CI: 40.2–65.1), which increased to $92.1\% \pm 4.8$ (95% CI: 83.6–100.0) by Day 30. Sheep treated with aqueous *C. papaya* seed extract showed mean FECRTs of $35.3\% \pm 5.5$ (95% CI: 24.1–46.3) at Day 10 and $82.4\% \pm 6.2$ (95% CI: 70.1–94.6) at Day 30.

Conventional dewormer treatment resulted in a mean FECRT of $50.0\% \pm 7.3$ (95% CI: 33.5–66.5) by Day 30. In contrast, the untreated control group exhibited a mean increase in egg counts of $111.8\% \pm 9.2\%$ over the study period.

Effect of Treatment Type and Time on Anthelmintic Efficacy

Two-way ANOVA was used to assess the effects of treatment, time, and their interaction on FECRT% (Table 3). No statistically significant main effects of treatment or time were detected, and no considerable treatment × time interaction was observed ($p > 0.05$ for all comparisons). These results indicate substantial within-group variability and suggest that treatment-associated changes over time were not consistent across all animals.

Exploratory post hoc comparisons suggested within-group reductions in egg counts over time for sheep receiving *M. oleifera*. Still, these findings should be interpreted cautiously, given the absence of significant overall interaction effects.

Table 3
Two-way ANOVA assessing the effects of treatment, time, and their interaction on faecal egg count reduction (%).

Source of Variation	Sum of Squares	df	F-Statistic	p-Value
Treatment Type	2276.78	2	1.8	0.219
Time	3468.11	2	2.75	0.117
Treatment × Time (Interaction)	115.22	4	0.046	0.995
Residual (Error)	5677.5	9		

Interpretation

Lack of significant interaction ($p = 0.995$) implies consistent treatment effect over time.

Weight Gain Change

Mean body weight increased over time in all treated groups (Table 4). Sheep treated with aqueous *M. oleifera* leaf extract exhibited the greatest numerical increase in mean body weight over the 30 days (mean gain: 4.7 kg), followed by those treated with aqueous *C. papaya* seed extract and Conventional dewormer. Sheep in the untreated control group showed minimal change in body weight over the same period. One-way ANOVA did not detect statistically significant differences in body weight among treatment groups at any sampling point ($p > 0.05$).

Table 4
Mean body weight (kg, mean $\pm$ SD) of sheep by treatment group at each sampling time.

Treatment	Days				
	0	5	10	30	P-value
Conventional Dewormer	24.13 $\pm$ 2.5	25.06 $\pm$ 2.5	26.13 $\pm$ 2.4	27.63 $\pm$ 2.4	0.770
Aqueous moringa leaves	24.5 $\pm$ 2.9	25.63 $\pm$ 3.0	27.06 $\pm$ 3.1	29.19 $\pm$ 3.1	0.720
Aqueous papaya seeds	26 $\pm$ 2.9	27.06 $\pm$ 2.8	28.06 $\pm$ 2.8	29.06 $\pm$ 2.9	0.886
Distilled Water	26 $\pm$ 2.6	25.44 $\pm$ 2.5	26.22 $\pm$ 2.7	26.39 $\pm$ 2.8	0.995
Footnote: No significant differences in body weight were detected among treatment groups (one-way ANOVA; $p > 0.05$).					

Post Hoc Analysis

Although the overall treatment $\times$ time interaction was not significant, exploratory pairwise comparisons indicated within-group changes over time, as shown in Table 5. These findings should be interpreted cautiously, given the absence of statistically significant main effects.

Table 5
Tukey's HSD pairwise comparisons of faecal egg count reduction (%) across sampling days.

Group 1	Group 2	Mean Diff	p-Value	95% CI (LP)	Significant
C.D-Day 0	C.D-Day10	30	0.939	-69.36 to 129.36	No
C.D-Day 0	C.D-Day5	17	0.998	-82.36 to 116.36	No
C.D-Day10	ACPS-Day 10	-20.0	0.994	-119.36 to 79.36	No
ACPS-Day 0	AMOL- Day 10	57.5	0.43	-41.86 to 156.86	No
ACPS-Day 5	AMOL-Day 5	21	0.992	-78.36 to 120.36	No
Footnote: i. CD is Conventional Dewormer, ACPS is Aqueous <i>Carica Papaya</i> seeds, and AMOL is Aqueous <i>Moringa oleifera</i> leaves					

ii. Pairwise comparisons are exploratory and should be interpreted in the context of the non-significant overall ANOVA.

DISCUSSION

This study assessed the in vivo efficacy of two ethnoveterinary plant extracts against gastrointestinal helminths in sheep under natural grazing conditions. Both aqueous *M. oleifera* leaf and *C. papaya* seed extracts were associated with reductions in FEC, while the conventional dewormer demonstrated comparatively lower efficacy by Day 30. These findings are consistent with increasing reports of reduced

benzimidazole effectiveness in small ruminant production systems in sub-Saharan Africa, where frequent use and limited drug rotation are common (Papadopoulos et al., 2021; Vineer et al., 2020).

According to World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines, full anthelmintic efficacy requires a $\geq 95\%$ reduction in FEC with a lower confidence limit above 90% (Levecke et al., 2022; Coles et al., 1992). In this present study, *M. oleifera* approached this threshold against *Moniezia* spp. but achieved only moderate reductions against *H. contortus*, while *C. papaya* exhibited a similar pattern with greater activity against cestodes than nematodes. These results indicate parasite-specific responses and suggest that the tested botanicals are unlikely to fully replace conventional anthelmintics but may contribute to integrated parasite control strategies (Torres-Acosta and Hoste, 2023; Eguale, 2021).

The low FEC reductions observed for conventional dewormers are consistent with suspected reduced efficacy under field conditions characterized by repeated drug exposure, suboptimal dosing, and limited resistance surveillance (Kaminsky et al., 2022; Wondimu and Bayu, 2022). Although formal resistance confirmation was not undertaken, the magnitude of reduction observed aligns with earlier reports of compromised albendazole performance in small ruminants in sub-Saharan Africa (Eguale et al., 2009; Afful et al., 2010).

Both plant extracts demonstrated progressive reductions in egg counts beyond Day 14 post-treatment. This delayed response contrasts with the rapid parasite clearance typically observed following administration of synthetic anthelmintics and may reflect cumulative or indirect modes of action, such as reduced worm fecundity or gradual disruption of parasite physiology (Belga et al., 2024; Hoste et al., 2006). Consequently, evaluation windows designed for synthetic compounds may underestimate the biological activity of botanical treatments, although confirmation in larger and more controlled studies is required (Levecke et al., 2022).

The observed antiparasitic effects may be attributed to phytochemical constituents previously reported in *M. oleifera* leaves, including condensed tannins, flavonoids, alkaloids, and saponins, and in *C. papaya* seeds, such as benzyl isothiocyanate and proteolytic enzymes (Elghandour et al., 2023; Jato et al., 2022; Páez-León et al., 2022). These compounds have been associated with impaired larval development, altered metabolism, and reduced egg viability in gastrointestinal helminths. However, the absence of extract standardization and phytochemical profiling in the present study limits mechanistic interpretation and reproducibility across ecological settings.

Sheep treated with *M. oleifera* showed the greatest numerical increase in body weight, although differences among treatment groups were not statistically significant. This trend may reflect partial parasite control combined with the nutritional properties of *M. oleifera* leaves, which are known to be rich in protein, minerals, and antioxidants (Pareek et al., 2023). Similar improvements in animal performance have been reported in studies involving tannin-containing forages and nutraceutical plant supplements in small ruminants (Bessell et al., 2018; Hoste et al., 2006).

Several limitations warrant consideration. The modest sample size limited statistical power, particularly for detecting treatment × time interactions, while the single-site, single-season design restricts broader extrapolation. In addition, extract preparation was not chemically standardized, and long-term safety was not assessed. Despite these constraints, the study provides field-based evidence that locally available botanical resources may have a role in parasite management where conventional anthelmintic efficacy is reduced. Future research should prioritize extract standardization, dose optimisation, multi-site evaluation, and economic feasibility to better define the practical applicability of plant-based anthelmintics in small ruminant production systems (Egualé, 2021; Torres-Acosta and Hoste, 2023).

CONCLUSION

This study evaluated the anthelmintic activity of aqueous *C. papaya* seed and *M. oleifera* leaf extracts in sheep naturally infected with gastrointestinal helminths under field conditions in Ghana. Both plant extracts were associated with reductions in faecal egg counts of *H. contortus* and *Moniezia* spp., with *M. oleifera* showing higher and more consistent reductions by Day 30. The conventional dewormer exhibited low FECRT%, consistent with reduced efficacy under the prevailing management conditions.

Although statistical significance across treatment groups was limited, the observed biological trends suggest that these botanicals may offer complementary value within integrated parasite management programmes, particularly in settings where conventional anthelmintic efficacy is compromised. Given the small sample size, single-site design, and lack of extract standardization, the findings should be interpreted cautiously. Further research involving larger populations, standardized preparations, and extended monitoring is required before recommendations for routine field use can be made.

Declarations

Ethical Approval

Permission was sought from the Veterinary Service Directorate. All procedures were approved by the AAMUSTED Institutional Ethics Review Committee (Approval No. AAMUSTED/IERC/2024/031) and conducted according to the Three R's principles (Replacement, Reduction, Refinement).

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Conflict of Interest Statement: The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

P.K.D.: conceptualized the study, coordinated fieldwork and laboratory analyses, collected data, and drafted the manuscript.

N.Y.A.-B.: contributed to study design, ethical approval procedures, and critical revision of the manuscript.

I.B. & D.D.Y.: contributed to interpretation of results and manuscript revision.

H.D.-F.: contributed to study design and manuscript preparation.

S.A.S. & H.A.L.: performed statistical analysis, contributed to data interpretation, and revised the manuscript.

All authors reviewed and approved the final version of the manuscript.

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Figures

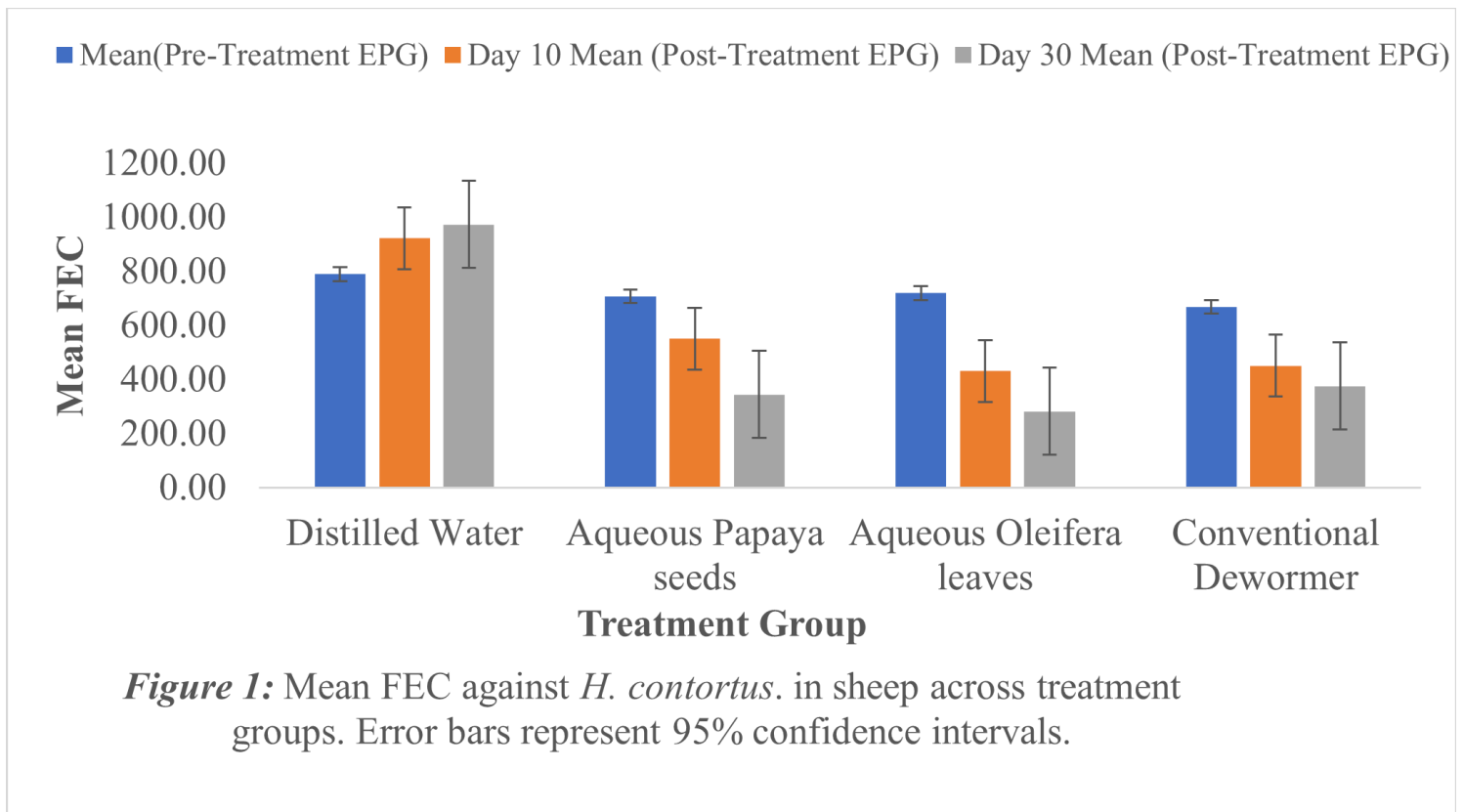


Figure 1

See image above for figure legend

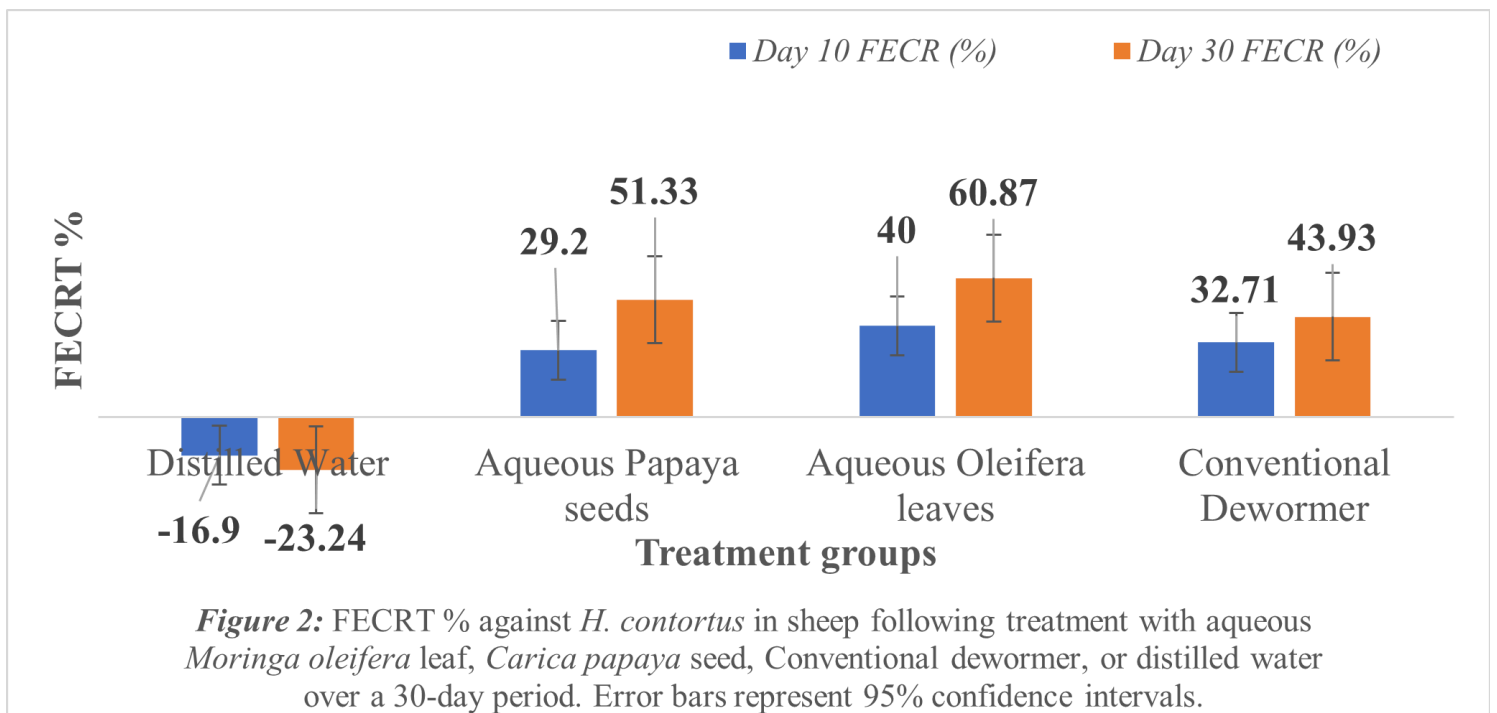


Figure 2

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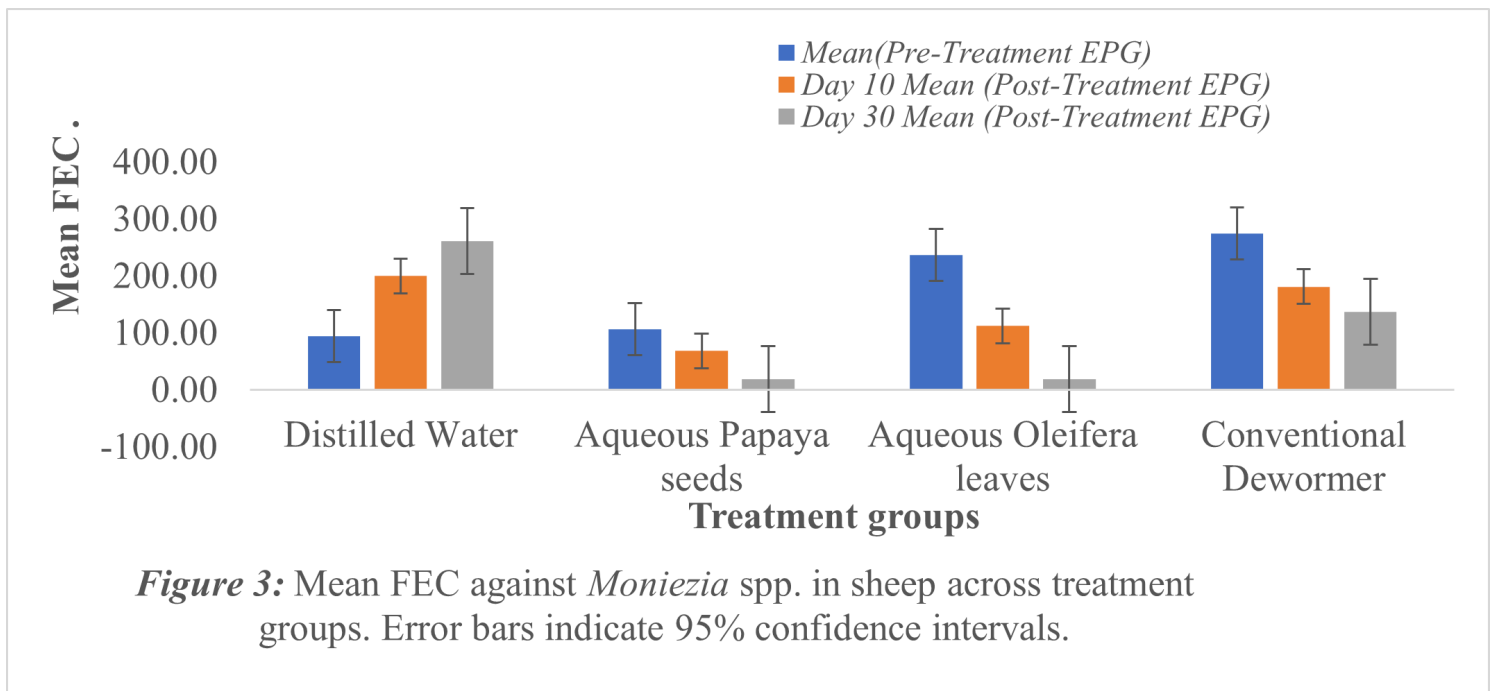


Figure 3

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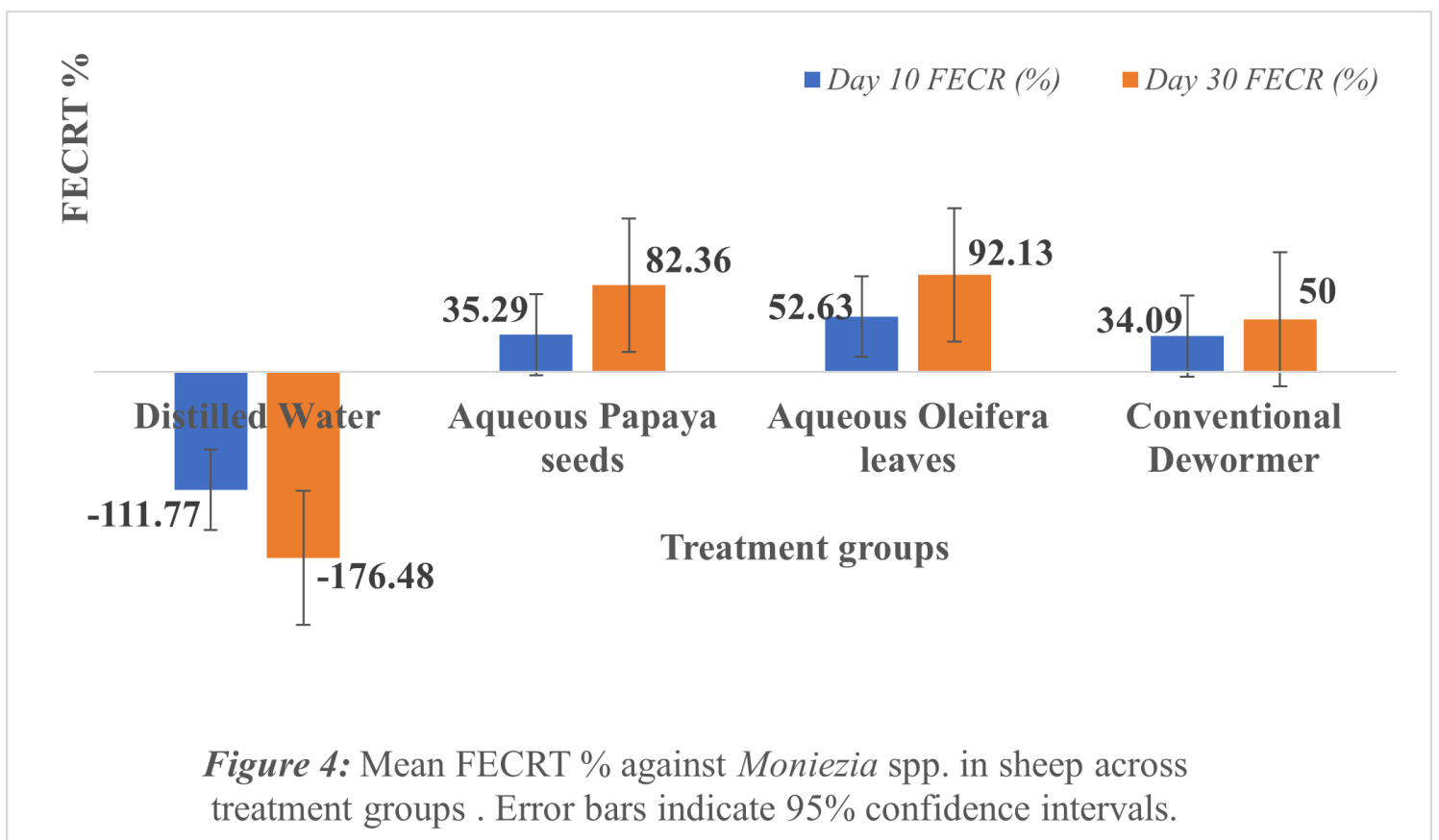


Figure 4

See image above for figure legend