ANTHELMINTIC EFFICACY OF JACKFRUIT (*Artocarpus heterophyllus* L.) AND TAMARIND *(Tamarindus indica* L.) LEAVES DECOCTION AGAINST GASTROINTESTINAL NEMATODES OF GOATS

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ABSTRACT

Reports of increasing anthelmintic resistance to chemical dewormers used in goat farming necessitate research on alternative anthelmintics. The study was conducted to evaluate the efficacy of jackfruit and tamarind leaves decoction as alternative anthelmintics and to determine the mean effective dose (ED_{50}) of the different concentrations against larval development of gastrointestinal nematodes in goats. Fecal slurries with prepared decoctions at different concentrations were inoculated with 100 nematode eggs, then incubated at room temperature for seven days. Larvae were collected, enumerated and identified. Four genera of gastrointestinal nematodes were identified, namely, *Trichostrongylus* spp., *Oesophagostomum* spp., *Haemonchus* spp. and *Bunostomum* spp. Results showed that at increasing concentrationsof jackfruit and tamarind leaves decoctions, the number of larvae killed increased. Both jackfruit and tamarind decoctions showed high efficacy in killing nematode larvae at high concentrations. ED_{50} was at 40% concentration for both decoctions. The results suggest that decoctions of jackfruit and tamarind leaves can be used as anthelmintics in goats.

Keywords: anthelmintic, goat, jackfruit, nematode, tamarind

INTRODUCTION

Gastrointestinal nematodiasis causes deleterious effects mainly on the productivity of goats resulting in profit loss for goat raisers. Gastrointestinal parasites in ruminants cause a reduction in feed intake, alteration of gastrointestinal motility and digesta flow, causing inappetence and diarrhea (Holmes, 1987). Gastrointestinal parasitism also causes loss of proteins into the gastrointestinal tract; changes in host metabolism account for much of the reduced protein and

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energy retention by infected animals (Holmes, 1987). Mineral metabolism and water and electrolyte balance are also frequently disturbed (Holmes, 1987).

Chemical dewormers are being used to decrease these effects but there have been increasing reports of anthelmintic resistance to these chemical dewormers in Southeast Asia, including Malaysia, Thailand, Indonesia and the Philippines. In the Philippines, studies in different regions including Luzon, Visayas and Mindanao confirmed that resistance of different nematodes to different chemical dewormers is present among sheep and goats farms with possible widespread distribution (Venturina, 2003). In view of these reports, farmers resort to inexpensive alternative ways for controlling gastrointestinal parasitism in their farms. Jackfruit (*Artocarpus heterophyllus* L.) and tamarind (*Tamarindus indica* L.) leaves are being used as alternative anthelminthic in ruminants. However, their anthelmintic efficacy has not been extensively evaluated against nematodes in goats.

The objective of the study was to evaluate the anthelmintic efficacy of jackfruit and tamarind leaves decoction against gastrointestinal nematodes of goats in different concentrations using larval development assay (LDA).

MATERIALS AND METHODS

Gastrointestinal nematodes

Fresh feces of goats were collected from smallhold farms located at Los Baños, Laguna. The samples were processed using the method of Burgonio (1982). Fresh goat manure was added to 100 ml screw-top plastic bottle containing about ten 8 mm glass beads. The bottle was filled with water and shaken vigorously. Water was added to the fecal sample. The fecal sample was homogenized using an electric mixer. Homogenized fecal sample was made to pass through a 150 µm 20 cm diameter sieve. Filtrate was placed in containers and left for 30 min for sedimentation to occur. Water was then decanted. The sediments were placed in centrifuge tubes with added water. The tubes were centrifuged for 3 min, then the supernatant was poured out. The tubes were agitated to loosen the sediment. Sodium chloride was added until a meniscus formed above the tube. The top layer was pipetted and placed in test tube, which was filled with water and centrifuged for 3 min. Water was removed and eggs were resuspended. The number of eggs per ml were counted in 0.5 ml suspension. In this study, the suspension estimated to contain 100 eggs per 0.5 ml was inoculated into each of the different concentrations of the fecal slurry for alternative medium.

Preparation of fecal slurry for alternative medium

Fresh cattle dung was collected from the Dairy Training and Research Institute, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Baños, Laguna. After collection, it was autoclaved at 250^{o}F for two hours, then dried. Two (2) g of dried cattle dung was placed in a sterile container with cover. The amount of decoction to be added to form the fecal slurry was pre-determined by adding water.

Jackfruit and tamarind leaves decoctions

Mature leaves from jackfruit and tamarind trees were obtained from orchards at the University of the Philippines Los Baños, Laguna. The leaves were washed before decoction. One kilogram of leaves was chopped into pieces 0.5 cm to 1.0 cm in size and allowed to boil in two liters of distilled water for 15-20 min from the time the water started to boil or until the original volume was reduced to half. The mixture was allowed to cool, then strained using cheesecloth and placed in a container. Jackfruit and tamarind leaves decoction were diluted to concentrations of 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%. At 0% concentration, 9 ml of water was used as a control and at 100% concentration, pure leaves decoctions were used.

Experimental design

Nine (9) ml of the decoction was added to 2 g of cattle dung to form the fecal slurry. For the control, 9 ml of water was used. For the treatment groups, 0.5 ml of the egg suspension was added to the fecal slurry with different concentrations of jackfruit and tamarind leaves decoctions in a completely randomized design and were replicated 5 times.

Incubation, enumeration and identification of nematodes

Eggs were incubated at room temperature with slight aeration for 7 days. After incubation, the covers were removed and the sterile containers were filled with water. With Petri dishes firmly held over the mouth of the sterile containers, the containers were turned upside down. Water was added on the Petri dishes until the water surrounded the mouth of the sterile containers (Burgonio and Manuel, 1982). Standing for 3 h allowed the larvae to move down from the sample to the water in the Petri-dishes. The water in the Petri dish containing the infected larvae was then pipetted. One (1) ml of 10% formalin was added to preserve the larvae collected. The larvae collected were then counted and identified with Lugol's Iodine under the microscope with magnification of 10x and 40x. The parasites were identified using a standard reference for infective nematode larvae of sheep and goats (Whitlock, 1960).

Estimation of effective dose (ED_{50})

Since the control manifested a high percent reduction during the experiment, correction for control mortality was done using the Abbot formula: Pt = (Po-Pc/ 100 – Pc) x 100. where Pt = corrected mortality, Po = observed mortality and Pc = control mortality (all in %).

Statistical analysis

Data were analyzed using Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD). They were tested at 5% level of significance. Means were compared using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Identification of the nematodes

Four genera of gastrointestinal nematode larvae were identified from the fecal slurries, namely, *Trichostrongylus* spp., *Oesophagostomum* spp., *Haemonchus* spp. and *Bunostomum* spp. (Figure 1).

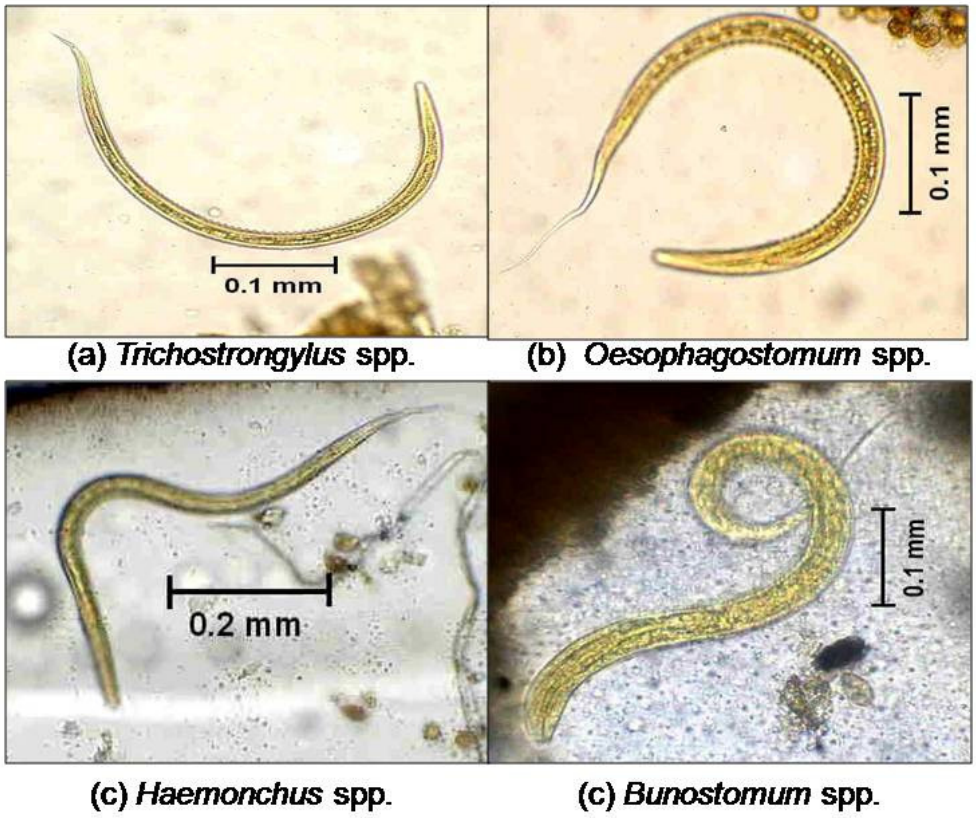

Figure 1. Microphotographs of gastrointestinal larvae (L3) of the different nematodes identified in the study.

Figures 2 and 3 show that the presence of *Trichostrongylus* spp. is greater than the other 3 genera of gastrointestinal nematodes found in any of the concentrations. This implies that *Trichostrongylus* spp. predominated and was relatively more resistant than any of the genera that were present. Data further showed that both decoctions were efficient in the reduction of the nematode larvae at high concentrations. At 100% jackfruit leaves decoction, 95.2% reduction of *Trichostrongylus* spp was observed while 100% reduction was observed at 80% to 100% concentration of tamarind leaves decoction. Several studies showed that *Haemonchus* sp. and *Trichostrongylus* sp. are predominant nematodes occurring in goats. According to Venturina *et al.* (2003), species belonging to *Haemonchus* sp.

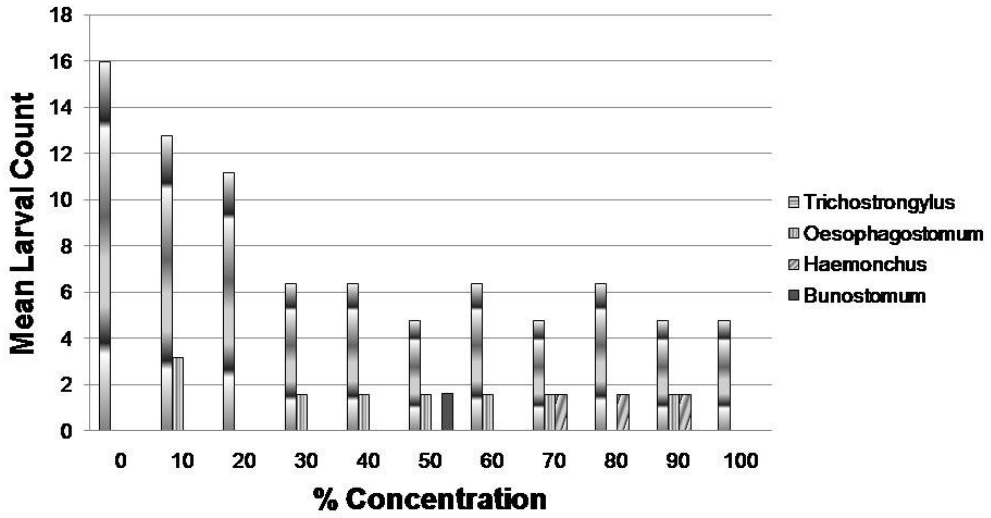

Figure 2. Nematode larval count in fecal slurries with jackfruit leaves decoction.

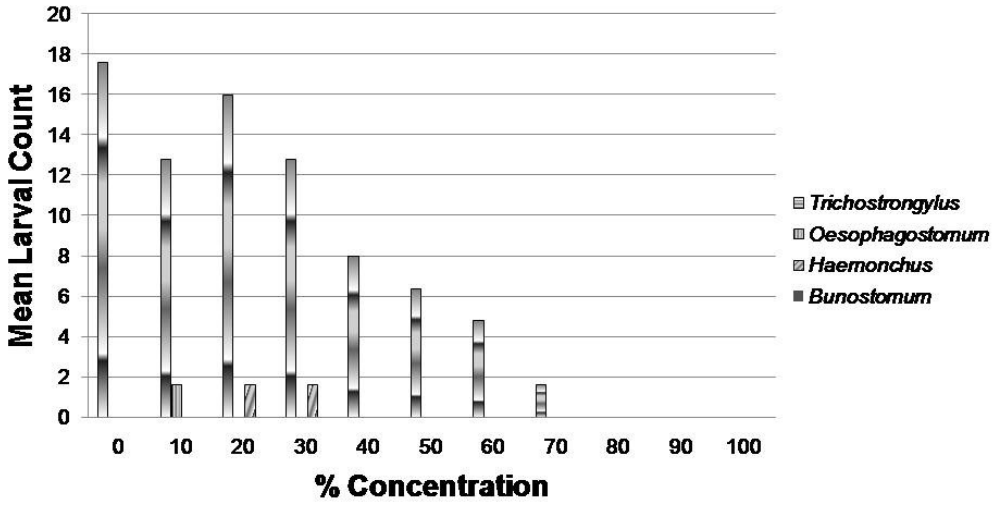

Figure 3. Nematode larval count in fecal slurries with tamarind leaves decoction.

and *Trichostrongylus* were the most numerous genera present in the fecal samples of goats. Tongson (1981) examined fecal samples of 1,230 goats of varying ages located in 15 selected provinces in the Philippines and found 90% were infected with *Trichostrongylus sp.,* 87 % were infected with *Haemonchus contortus* , 85%

with Oe*sophagostomum* sp.*,* 47% with *Strongylus sp.* and 8.7% *Monieza* sp. and *Cooperia* sp.*. Bunostomum* infections were found to be rare in goats. On the other hand, Eduardo (1986) showed that in 30 goats examined, 9 helminths parasites were found, including *Oesophagostomum* spp. *(O. columbianum, O. venulosum, O. asperum, O cervi), Trichostrongylus* spp. *(T. axei and T. Longispcularis), Haemonchus* spp*. (H. contortus and H. similes*), *Cooperia punctata* and *Capillaria bilobata.* Ducusin and Faylon (1996) enumerated the gastrointestinal nematodes reported in goats in the Philippines, and these were *Bunostomum sp., Cooperia curticei, Haemonchus placei, Oesophagostomum venulosum, Strongyloides papillosus, Strongyloides sp., Trichostrongylus axei, Trichostrongylus capricola* and *Trichostrongylus colubriformis.*

Efficacy of jackfruit and tamarind leaves decoctions

Figure 4 reveals an increasing trend in the number of nematode larvae killed as the concentration of decoctions increased. It further shows that any given concentration can kill nematode larvae. The highest number of the larvae that was killed was observed at 100% tamarind leaves decoction.

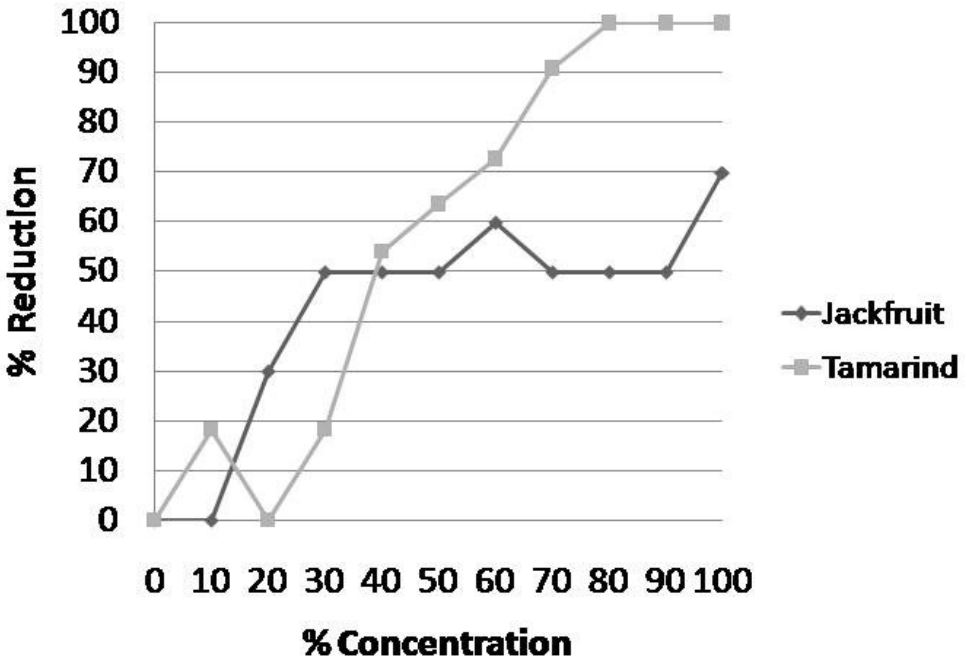

Figure 4. Percent reduction of nematode larvae in fecal slurries with jackfruit and tamarind leaves decoctions at different concentrations.

Table shows that increasing the concentration of the jackfruit and tamarind leaves decoctions resulted in decrease in the number of live nematode larvae. Furthermore, data on jackfruit leaves decoction indicate that there was no significant difference between the 0% until the concentration of the jackfruit leaves reached

Table. Nematode larval count and percent reduction of gastrointestinal larvae in jackfruit and tamarind leaves decoctions (1 kilogram leaves per 1 liter of water).

Leaves	% concentration (ml decoction/9ml water)	Mean larval count	% Reduction
Jackfruit	0[1]	16.0[a]	0.0
	10	16.0[a]	0.0
	20	11.2[ab]	30.0
	30	8.0[ab]	50.0
	40	8.0[ab]	50.0
	50	8.0[ab]	50.0
	60	6.4[ab]	60.0
	70	8.0[ab]	50.0
	80	8.0[ab]	50.0
	90	6.4[b]	50.0
	100[2]	4.8[b]	70.0
Tamarind	0[1]	17.6[a]	0.0
	10	14.4[a]	18.2
	20	17.6[a]	0.0
	30	14.4[a]	18.2
	40	8.0[b]	54.0
	50	6.4[bc]	63.6
	60	4.8[bc]	72.7
	70	1.6[c]	90.9
	80	0.0[c]	100.0
	90	0.0[c]	100.0
	100[2]	0.0[c]	100.0

[1]water only; [2]9 ml of pure jackfruit and tamarind leaves decoction.

80% except for the deviant result at 60%. The same can be observed between 20% and until it reached 100% concentration of jackfruit leaves decoction. However, there was a distinct difference in the treatment effect between 10% and 100% concentration of jackfruit leaves decoction, implying that the death of the nematode larvae is more efficient at higher concentrations ($P<0.05$). The effect of the tamarind leaves decoction on survival of the nematode larvae at 10-30% concentration was significantly different from 40-60% concentration and 70-100% concentration of tamarind leaves decoction. This further proves the high efficacy of higher concentrations of the decoctions in killing nematodes.

Active ingredients found in plants responsible for anthelmintic activities are quinines, alkaloids, glycosides, acids, phenols and tannins (Puri *et al*., 2011). Direct anthelmintic effects of tannin-containing plants have already been shown in sheep and goat gastrointestinal nematodes. These anthelmintic properties are mainly associated with condensed tannins (Novobilský *et al*., 2011). Several studies showed several possible mechanisms on the direct and indirect anthelmintic effects of tannins on gastrointestinal nematodes. Direct anthelmintic-like effects have been demonstrated in *in vitro* assays, which have shown that incubation in crude condensed tannins extracts reduced the development, viability, motility, and migratory ability of parasite larvae (Athanasiadou *et al*., 2001; Butter *et al*., 2001; Molan *et al*., 2002). Condensed tannins in forages may have acted similarly by binding to fecal egg proteins, inhibiting egg hatching and larval development (Min *et al*., 2004). The direct action of condensed tannins in most *in vitro* studies could be defined as strong, dose related and with efficacy levels approaching 100%. Direct anthelmintic-like effects have also been demonstrated in *in vivo* studies, where the addition of condensed tannins to high quality foods has resulted in significantly reduced worm burdens and nematode egg counts with 50% or more (Athanasiadou *et al*., 2000; Butter *et al*., 2001).

Other possible mechanisms of action of tannins are interfering with energy generation by uncoupling oxidative phosphorylation or interfering with glycoprotein of cell surface (Harekrishna Roy *et al*., 2010). An indirect antiparasitic effect is likely due to stable CT-protein complexes that form at pH 4.0–7.0 in the rumen and that subsequently dissociate in the acidic conditions of the abomasums (Barry and Manley, 1986). This increases the supply of digestible protein to the host (Barry and McNabb, 1999; Min *et al*., 2004) and the improved protein nutrition may enhance host immune response to gastrointestinal nematodes. The leaves used in the study are being fed to goats by farmers as an alternative feed source and anthelminthics. It was found that jackfruit and tamarind leaves have tannins. Jackfruit leaves are widely used as a feed source despite their apparently low protein digestibility thought to be due to the high tannin content (Brenda *et al*., 1997). Phytochemical analysis of the juice of jackfruit leaves revealed the presence of tannins (Mute *et al*., 2009).

Mean effective dose (ED_{50}) of jackfruit and tamarind leaves decoctions

The observed larval reduction in the control group could have been from natural causes or from those causes unconnected with the treatment used. To determine the mean effective dose in jackfruit and tamarind leaves decoction at different concentrations, Abbott's formula was used to correct for the mortality

observed in the control. Table shows that at 40% to 90% concentration of jackfruit leaves decoction, the development of larvae of gastrointestinal nematodes was generally reduced by 50% with the exception at 60% concentration. In the tamarind leaves decoction, the concentration that exhibited closest to 50% reduction was at 40%. Thus, it can be said that ED_{50} using either jackfruit and tamarind leaves decoction was at 40% concentration.

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