



Original Articles

## Efficacy of *Tithonia diversifolia* (Hemsl) A. Gray on the inhibition of larval development of *Haemonchus contortus*

Eficácia de *Tithonia diversifolia* (Hemsl) A. Gray na inibição do desenvolvimento larval de *Haemonchus contortus*

Eduardo Robson Duarte<sup>1\*</sup> , Pedro Paulo Dias David<sup>1</sup> , Katchuce Brito Amarante Oliveira<sup>2</sup> , Marcelo Dourado de Lima<sup>1</sup> , Fernando dos Santos Magaço<sup>2</sup> , Franciellen Morais-Costa<sup>1</sup> 

<sup>1</sup> Universidade Federal de Minas Gerais, Instituto de Ciências Agrárias, Campus Montes Claros, MG, Brazil

<sup>2</sup> Universidade Zambeze, Moçambique.

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### ABSTRACT

Sheep farming is an important economic activity from a commercial and subsistence perspective. However, helminth infections present a large sanitary problem for the production, especially haemonchosis caused by strains resistant to traditional anthelmintics. Here, we evaluated the effects of the stem and leaf of *Tithonia diversifolia* on the inhibition of *Haemonchus contortus* larval development. We performed a quantitative fecal culture test with feces of sheep inoculated with larvae tolerant to albendazole. Fecal cultures were treated with leaf or stem powder and were compared to the controls containing distilled water or levamisole. The *T. diversifolia* leaf and stem powders significantly reduced the production of *H. contortus* larvae. However, leaf powder was more effective, with 64.95% efficacy in the reduction of larvae production, representing a promising alternative in the integrated control of this nematode.

### RESUMO

A ovinocultura é uma importante atividade econômica em escala comercial e de subsistência. No entanto, um dos maiores desafios nessa atividade são as verminoses, destacando-se a haemoncose ocasionada por cepas resistentes a anti-helmínticos. Neste estudo objetivou-se avaliar o efeito do caule e folha de *Tithonia diversifolia* sob a inibição do desenvolvimento de larvas de *Haemonchus contortus*. Foram realizadas coproculturas quantitativas com fezes de ovinos inoculados com larvas do nematódeo tolerante ao albendazol. Foram avaliadas as produções de larvas infectantes em coproculturas tratadas: com pó da folha e do caule e com o extrato aquoso do caule da planta comparando-se com o tratamento controle contendo água destilada e levamisol. O pó da folha e do caule de *T. diversifolia* reduziram significativamente a produção de larvas infectantes de *H. contortus*. Entretanto, o pó das folhas foi mais efetivo, promovendo eficácia de 64,95% na redução de larvas em coproculturas, representando uma alternativa promissora no controle integrado do nematódeo.

### INTRODUCTION

Sheep farming is an important activity for small and large producers in different continents. However, gastrointestinal nematodes (GN) represent the main health problem, considerably reducing the production value of these ruminants (OLIVEIRA et al., 2017).

Among the parasites that infect small ruminants, *Haemonchus contortus* is the GN with the highest relevance due to the intensity of infection, pathogenicity, and high prevalence, related to its expressive biotic potential. Generally, chemical anthelmintics are used for GN control. However, the indiscriminate use of these products may favor the selection of GN resistant to different anthelmintic groups (SALGADO; SANTOS,

\* Corresponding author: [duartet@hotmail.com](mailto:duartet@hotmail.com)

2016). Reports of strains resistant to anthelmintics have occurred at high frequencies and in different continents, which has encouraged the search for alternative measures (BASTO et al. 2017, LAMB et al. 2017). Herbal medicines are one of these promising alternatives, since they are a natural resource with the capacity to control *Haemonchus* spp. resistant to conventional treatments, and show lower impacts on the environment and the host products (MORAIS COSTA et al. 2016, TARIQ et al. 2017).

Among the plants with phytotherapeutic potential, *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae), "Mexican sunflower," has biological properties related to the presence of phenolic and alkaloid compounds (ABE et al., 2015, TAGNE et al. 2018). It is a tropical forage crop with high protein content, and contains essential oils with antibacterial and antioxidant activity (AJÃO; MOTEETEE, 2017). However, the anthelmintic potential of this plant in *H. contortus* strains is unknown. In this study, we evaluated the effects of the stem and leaves of *T. diversifolia* on the inhibition of *Haemonchus contortus* larvae development.

## MATERIALS AND METHODS

All experimental procedures were performed and approved in accordance with the Ethics Committee on Animal Experimentation of the Universidade Federal de Minas Gerais (CETEA-UFMG) under 42/2008 protocol.

When the *T. diversifolia* plant was approximately 2.5 meters high and prior to flowering, stems and leaves were collected, and materials with some type of deterioration or injury were discarded. Subsequently, the material was dehydrated in an oven with forced air circulation at 40±5°C for 72 hours, and subsequently crushed. The powder obtained was stored in a location with good air circulation and protected from sunlight. Sub-samples of leaves and stem powder were submitted to dry matter (MS) determination in an oven at 105°C to calculate the tested concentrations.

For the anthelmintic efficacy test on coprocultures, feces were collected directly from the rectal ampulla of approximately five-month-old Dorper x Santa Inês lambs. The animals were raised in stalls and had no elimination of nematode eggs in the feces. Three examinations of fecal egg counts (FEC) were performed to confirm that the lambs were free of nematode infection. The animals were inoculated with approximately 1000 *H. contortus* larvae tolerant to albendazole to produce eggs for coproculture. The nematode strain was derived from the adult females of *H. contortus* collected from the abomasum of a lamb in a herd, in which the effectiveness of albendazole was less than 15% (DUARTE et al., 2012). The eggs were removed from the uteri of the *H. contortus* females and were incubated in coprocultures to produce infective larvae.

Feces were collected 25 days after inoculation with larvae and stored in plastic bags at approximately 5°C for

up to two hours until use. The FEC were performed in a McMaster counting chamber, following the methodology proposed by Gordon and Whitlock (1939), appointing an average of more than 1000 eggs per gram of feces.

Subsequently, the quantitative coproculture technique was performed (BORGES 2003; MORAIS-COSTA et al., 2016, VIEIRA et al., 2017). Two experiments were carried out to evaluate the efficacy of *T. diversifolia* stem powder and leaf powder. In both experiments, a positive control of levamisole phosphate at 0.03 mg/g of fecal culture and a negative control of sterile distilled water was included. The plant parts were tested at five final concentrations (40.83, 81.66, 163.33, 244.99, and 326.66 mg per gram of coproculture). Vermiculite completed the reduction of these concentrations. All procedures were repeated five times (VIEIRA et al., 2017).

The coprocultures were kept moist and incubated at 28°C for seven days to obtain the infective larvae. After this period, distilled water was added to the edge of each cup. The cups were covered with petri dishes and abruptly turned. 20 mL of distilled water at 38°C was added to enable the parasite larvae to migrate to cleaner water. Three hours after coprocultures were turned, the larvae were collected and stored in test tubes containing 1 mL of formalin (10%) and kept refrigerated between 3 and 8°C until counting. Larvae were quantified using an optical microscope at 10x magnification. The total number of larvae observed was divided by two, and the result expressed as the number of larvae developed per gram of feces (LPGF).

The percentage of efficacy in larval development inhibition was determined by the below modified equation (BORGES, 2003).

$$\text{Efficacy (\%)} = 100 - [(\text{treated group LPGF} / \text{negative control group LPGF}) \times 100].$$

The data assigned to LPGF values were previously transformed into Log (x + 10) and submitted to an analysis of variance and means compared by the Scott-Knott test at 5% significance. For the probity regression analysis, the inhibitory concentration that could inhibit 90% of the larval production (IC<sub>90</sub>) was estimated, when possible. The SAEG® 9.1 statistical package was used.

## RESULTS AND DISCUSSION

The best anthelmintic result was observed in treatments with *T. diversifolia* leaf powder at a concentration of 326.66 mg per gram of fecal culture, with an efficacy rate of 64.95% for larval development inhibition (LDI) (Table 1), representing a significant reduction in the LPGF, as compared to the control with distilled water (p < 0.05). The efficacy of *T. diversifolia* leaf powder to LDI was dose-dependent (Figure 1) and the estimated IC<sub>90</sub> was 676 mg/g (confidence interval from 515 to 716 mg/g).

Treatment with *T. diversifolia* stem powder showed better results when used at a concentration of 326.66 mg

of stem powder per gram of coproculture, which resulted in an LDI efficacy of 31.0%, presenting a significant

reduction in the LPGF mean number produced, as compared to the control with distilled water (Table 1).

Table 1. Efficacy of *Tithonia diversifolia* (Hemsl) leaf and stem powder at different concentrations (mg/g of coproculture) on the inhibition of larval development in *Haemonchus contortus*

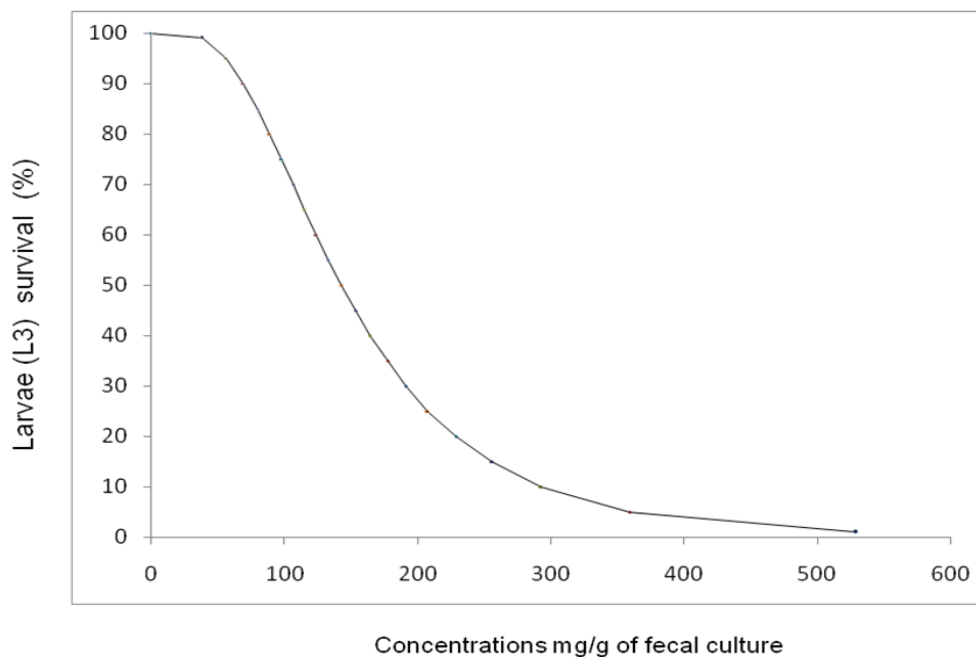
Experimental Groups	Leaf powder		Stem powder	
	LPGF*	Efficacy (%)	LPGF*	Efficacy (%)
326.66 mg/g	239 <sup>e</sup>	64.95	1365 <sup>c</sup>	31.06
244.99 mg/g	368 <sup>d</sup>	46.04	1935 <sup>b</sup>	2.27
163.33 mg/g	520 <sup>c</sup>	23.75	2085 <sup>b</sup>	--
81.66 mg/g	621 <sup>b</sup>	8.94	2250 <sup>a</sup>	--
40.83 mg/g	764 <sup>a</sup>	--	2415 <sup>a</sup>	--
Distilled water	682 <sup>b</sup>	--	1980 <sup>b</sup>	--
Levamisol phosphate (0.03 mg/g)	0 <sup>f</sup>	100.0	0 <sup>d</sup>	100.0

Different letters indicate significant difference ( $P < 0.05$ ), by the Scott-Knott test. Coefficient of variation: 1.35%.

\* LPGF number of larvae (L3) per gram of feces

Efficacy: % efficacy =  $100 \times (1 - \text{LPGF of treated group} / \text{LPGF of control group})$

Figure 1. Inhibition of the larval development of *Haemonchus contortus* at different concentrations of *Tithonia diversifolia* leaf powder.



Because of the high tolerance to anthelmintics reported in different regions, mainly for albendazole (BASTOS 2017, DUARTE et al. 2012), the search for alternatives for the integrated control of this nematode has been persistent. The results of this study present the use of *T. diversifolia* leaves as an important measure for the control of resistant nematode strains. Extracts of *T. diversifolia* have already been reported to be effective for parasite control (KEREBBA et al, 2019). Additionally, the plant could contribute to anti-inflammatory, analgesic, and antimicrobial activities (GALLON et al, 2019a), which could support the treatment of animals with haemonchosis.

In this study, we used quantitative coproculture methodology, adding the plant's powder directly to feces inoculated with albendazole-tolerant nematode eggs. This methodology has often been employed because it mimics a condition closer to that verified in the biological cycle of the parasite (MOARIS-COSTA et al. 2016). In another study, the quantitative coproculture technique was performed to evaluate the anthelmintic efficacy of aqueous extracts (AE) of the leaf, stem, and heart of the banana tree. Extracts of  $\geq 75$  mg/mL significantly decreased *H. contortus* larval development with an efficacy above 96.9% (OLIVEIRA et al. 2009).

In studies with Cerrado species, the bark AE of *Caryocar brasiliense* (Pequi) at 200 mg/mL inhibited 94.8% of *H. contortus* larval development in fecal cultures (NOGUEIRA et al., 2012). *Ximena americana* administered at 333.3 mg/g of fecal culture showed 99.8% LDI (MORAIS-COSTA et al., 2015). When evaluating *Pipadenia viridiflora* (Surucucu), the IC<sub>90</sub> of the leaf AE for LDI of *H. contortus* was 13.6 mg (MORAIS-COSTA et al., 2016). These studies all used concentrated plant extracts, and had higher LDI than those observed in this study. However, in this study, *T. diversifolia* stems and leaves were evaluated in powder form, containing metabolites in proportions that would naturally be ingested by the animals, in paste form or as chopped fodder and supplied in the trough.

External and environmental factors in the cultivation of *T. diversifolia* could influence its performance in herbal medicine, which should be elucidated for its anthelmintic effect in future studies. When the plant is submitted to stress due to soil moisture, soil and air contaminants, temperature, excessive radiation, and/or seasonal variation, qualitative and quantitative changes occur, interfering in the production of primary and secondary metabolites due to deviations of biosynthetic routes (ZEINELDIN et al., 2018). The stress caused to *T. diversifolia* by lack or excess of water, frost, or parasitism alters the biosynthesis of the plant and the quality of its biological potential, highlighting the need to use a controlled environment for this crop to reduce variations in metabolite concentrations that may cause errors during the experiments (ZEINELDIN et al., 2018).

The use of extracts and essential oils of *T. diversifolia* containing high concentrations of metabolites, and further research on their mechanisms of action, could contribute to increasingly effective control of nematodes in the gastrointestinal tract of ruminants. Future *in vivo* studies should evaluate the efficacy of plant leaves used in the form of hay or direct pasture, and analyze the possible health benefits to animals with haemonchosis, considering the already known anti-inflammatory effects of this plant (GALLON et al., 2019b; TAGNE et al., 2018).

## CONCLUSIONS

The leaves and stem of *T. diversifolia* show anthelmintic activity by *in vitro* inhibiting of the development of *H. contortus* larvae. The leaf powder is more effective and could represent an alternative in integrated nematode control, reducing the population of albendazole-tolerant infective larvae in the environment.

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