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The efficacy of acetone leaf extracts of *Lespedeza cuneata* on egg hatching of *Haemonchus contortus*: *in vitro* studies

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INTRODUCTION



Infestation with gastrointestinal nematodes:

- **Severe production and animal losses worldwide**
- **Control backbone: Anthelmintic chemotherapy**

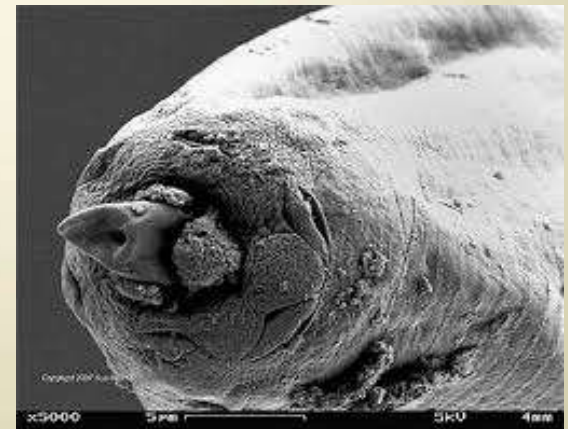
But

Increasing development of resistant strains of parasites

Increased public awareness for drug residues in animals products

Resulting

- **Search for sustainable alternatives**





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In the search for alternatives – Phytotherapy

Bio-active forages:

**Forages that contain secondary plant substances
and metabolites**

**.... beneficial for the animal health, not necessarily only
aimed to optimized nutrition
(Rahmann, 2004)**

**Added advantage of forages – already established
pasture with commercial seed available**



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But

Lack of verified implementation strategies to exploit these anthelmintic properties for use on farm level

- ***In vitro* experiments**
- ***In vivo* experiments**



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Lespedeza cuneata
(*Sericea lespedeza*,
Poormans lucerne)

Grazing value

+

Anthelmintic properties

- Dry land, perennial, low input summer legume pasture
- High tolerance – low pH and P (Sandy soils)
- Condensed tannins – anthelmintic properties



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***Lespedeza cuneata* research programme:**

- Yield and quality
- Feeding experiments:
 - Results indicated that *L. cuneata* hay can reduce the parasite infestation in sheep > 50%.
 - Less chemotherapy.
 - Less contamination of pastures.
- Condensed tannins (High>55 g CT kg⁻¹ DM)

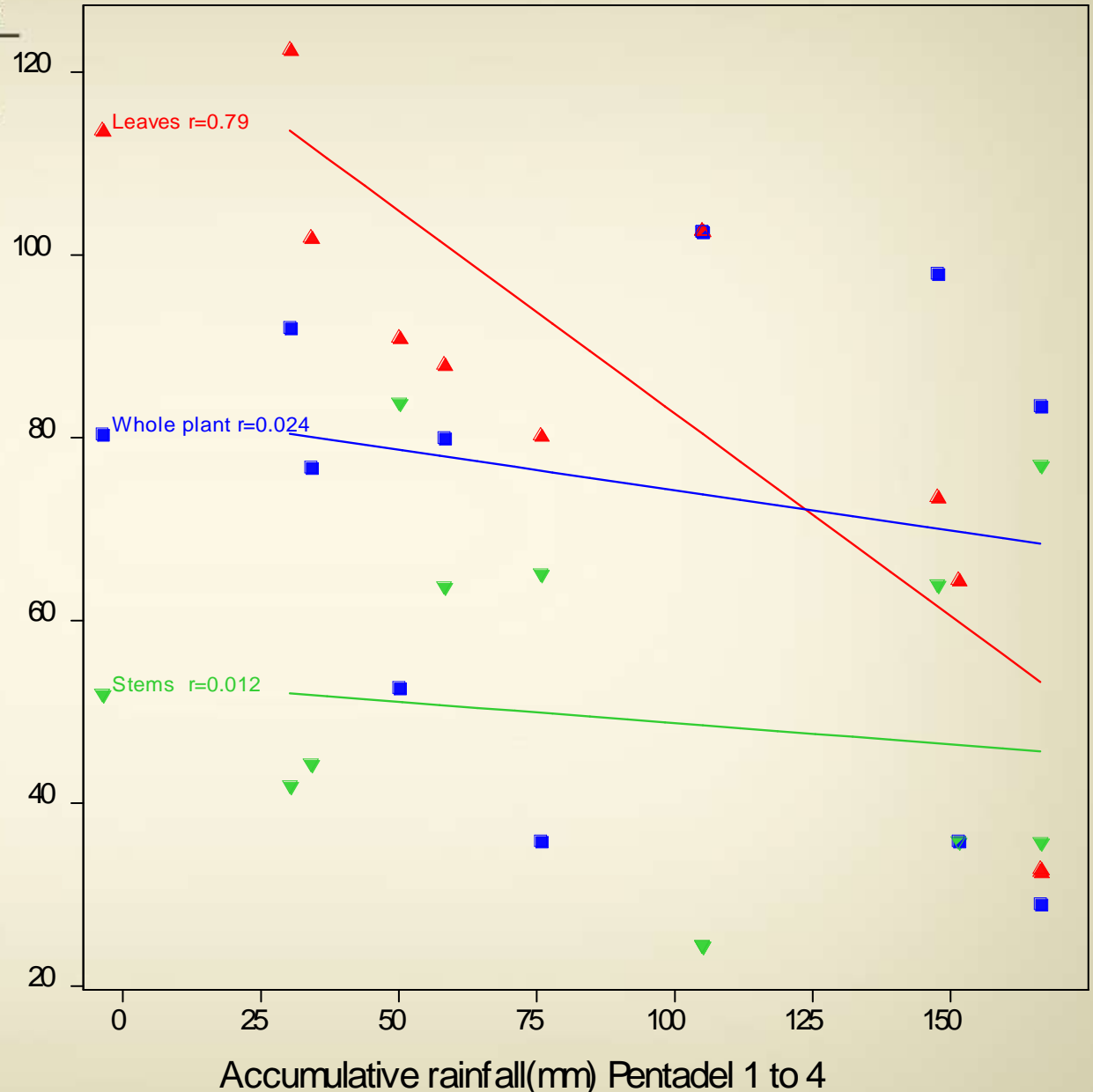


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**Withdraw
livestock from
grazing during
times of
moisture
stress, to curb
poor animal
production.**

**Graze during
wet
conditions –
Worms!**





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AIM OF THE RESEARCH

**To determine the efficacy of leaf extracts
of *Lespedeza cuneata*,
containing different Condensed tannin levels, on the egg
hatching of
Haemonchus contortus.**



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MATERIALS AND METHODS

1. Condensed tannin (CT) content analyses

Reed *et al.* (1982) and Waterman and Mole (1994).

CT extracted with aqueous acetone

**butanol-HCl and ferric reagent were added,
followed by the measuring of absorbance at 550 nm
with a spectrophotometer.**



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2. Plant Extractions

Eloff, 1998; Bizimenyera *et al.*, 2006; Kandu-Lelo, 2009; Ademola *et al.*, 2011; Van Wyk C (2012).

- **Leaf material with predetermined CT levels was used.**
- **Extracted active substances with Acetone (70%)**





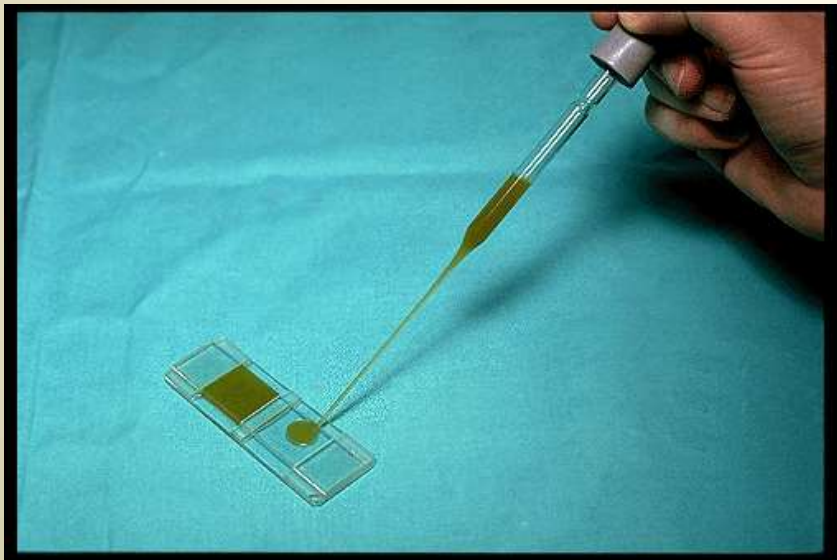
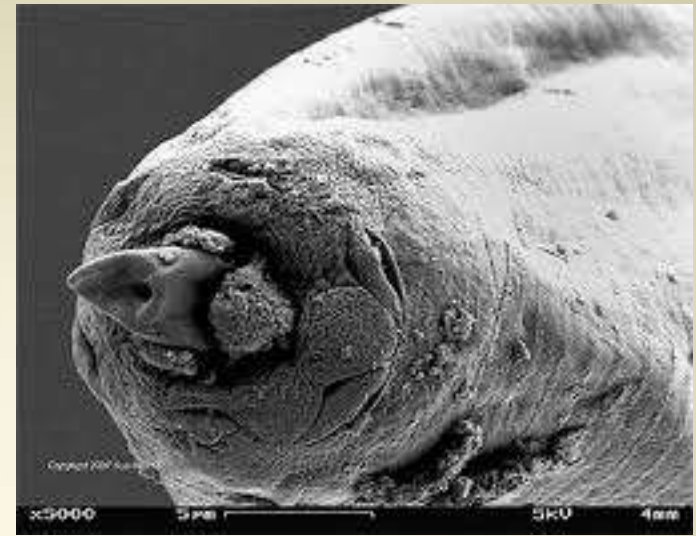
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3. Nematode egg recovery

Hubert and Kerboeuf (1992)

- ***H. contortus* eggs were recovered from faeces of specific infected donor sheep.**





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4. Egg hatch assay – (dose response trial)

Ademola and Eloff (2011), originally by Coles *et al.* (1992)

- **An egg suspension was allocated in each well of a 24-flat-bottomed microtitre plate.**
- **Add extracts, in six serial dilutions (at concentrations of 0.63 to 20 mg ml⁻¹)**
- **Eggs were incubated in this mixture for 48 h at 27°C and 70% relative humidity.**
- **After incubation, the eggs and first-stage larvae (L₁) in each plate were counted under an inverted microscope.**



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Statistical analysis

**Egg hatch assay: Repeated Measures Analysis of Variance.
Fisher's test of least significant differences (LSD) was
conducted at a 5% significance level.**



RESULTS AND DISCUSSIONS

Table 1: Condensed tannin (CT) level (g CT kg⁻¹ DM) of samples of *L. cuneata*

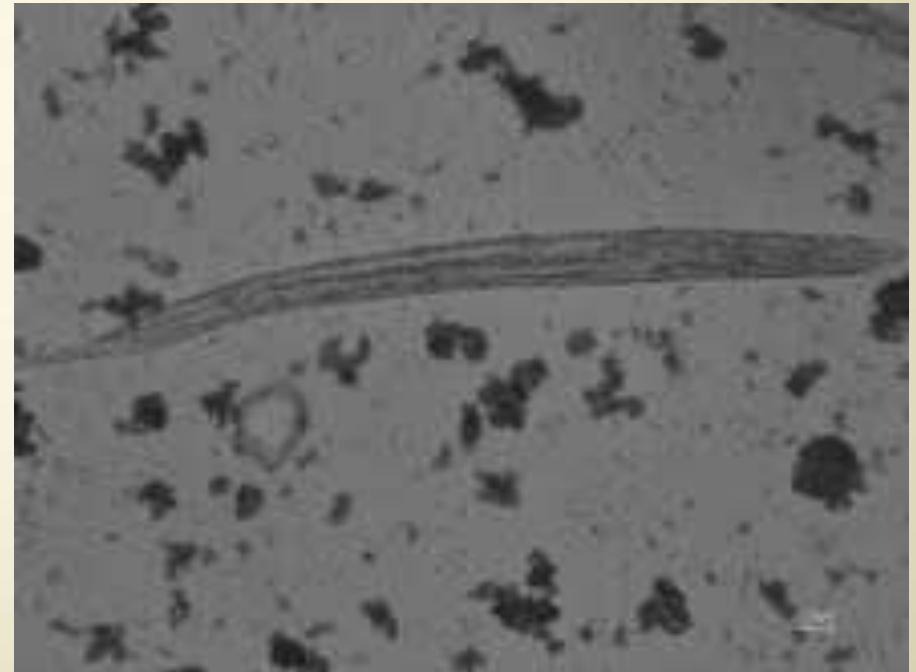
Extracts	CT Levels (g CT kg⁻¹ DM)
Ultra high	122
High	102
Medium	88
Low	74



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Egg hatching counts





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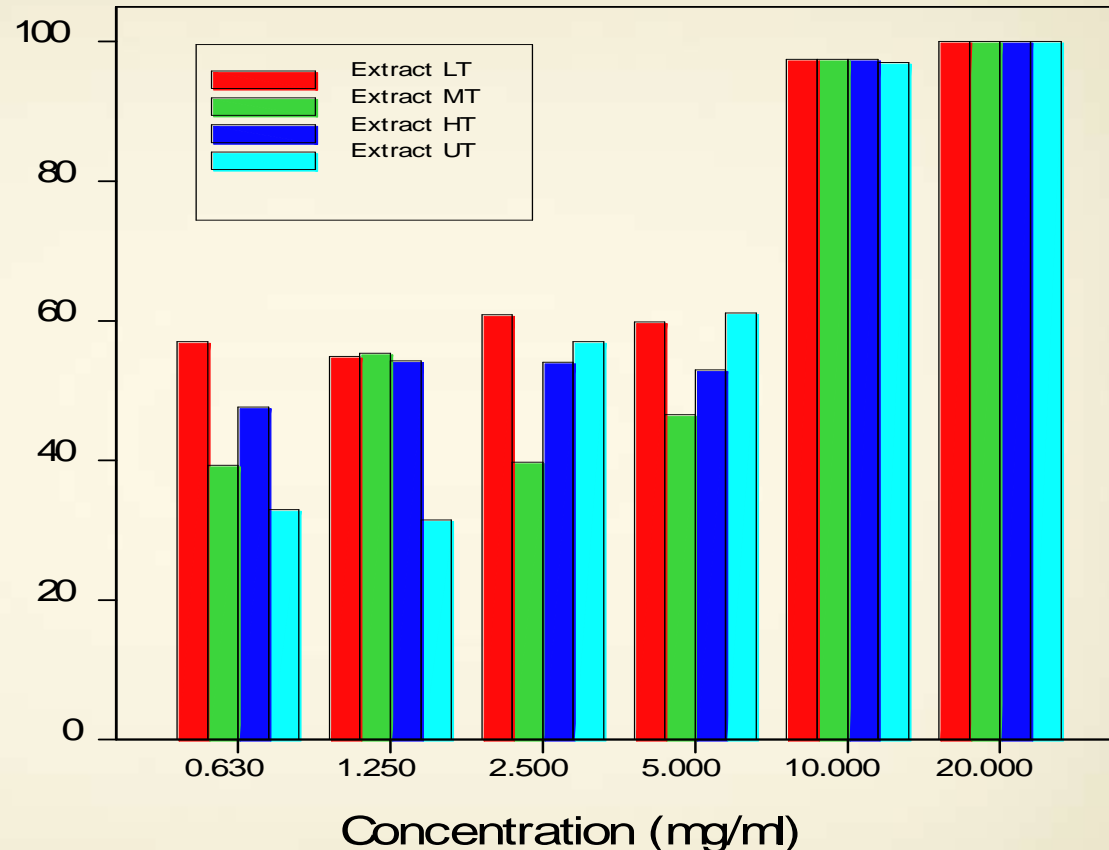


Figure 1: Mean inhibition of *H. contortus* eggs hatching (%) by four different Condensed tannin containing leaf extracts in different concentrations (mg ml⁻¹) of *L. cuneata*

(UT: Ultra high CT; HT: High CT; MT: Medium CT and LT: Low CT)



Table 2: Egg hatch assay of different Condensed tannin level containing extracts of *L. cuneata* against *H. contortus*.

Concentration (C) (mg ml ⁻¹)	EHI% of extracts of <i>L. cuneata</i> (E)				Means of C
	UT	HT	MT	LT	
0.63	33.0	47.7	39.3	57.1	44.3 ^a
1.25	31.5	54.3	55.3	54.9	49.0 ^{ab}
2.5	57.0	54.1	39.7	60.9	52.9 ^b
5.0	61.1	53.0	46.6	59.8	55.1 ^b
10	97.0	97.4	97.4	97.4	97.3 ^c
20	100.0	100.0	100.0	100.0	100.0 ^c
Means of E	63.3 ^a	67.7 ^a	63.1 ^{ab}	71.7 ^b	
CV %					11.8 %
LSD:	Concentration (C):		6.453 (P < 0.001)		
	Extracts (E):		5.269 (P < 0.05)		
	C x E interaction:		12.906 (P < 0.05)		



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CONCLUSION

- **Extracts of *L. cuneata* had a substantial inhibitory effect on *H. contortus* egg hatching, at concentrations of 20 and 10 mg ml⁻¹.**
- **Even at the lowest concentration, still resulted in an average of 44.3 % EHI.**
- **In practise – lower egg hatching, lower pasture contamination, lower infection.**
- **Influence on adult worms? Yes**
- **On farm: Reduced need for anthelmintics. Part of holistic parasite control programme**



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Thank you