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Evaluation of the Activity of Plant Extracts in Boer Goats

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Abstract: Problem statement: The effect of extracts from Neem (*Azadirachta indica*), Wormwood (*Artemisia absinthium*) and Tobacco (*Nicotiana tabacum*) with added copper sulfate, on female Boer goats infected with gastrointestinal parasites (GIN) was evaluated. **Approach:** Following initial screening for infection, animals were artificially infected with a mix containing approximately 80% *Haemonchus contortus* and 20% *Trichostrongylus* spp. After 21 days, fecal samples were collected to determine the fecal egg count. Plant extracts (200 mg kg^{-1} body weight) in sterile distilled water were administered on day 28. Treatment I was a control without anthelmintic (distilled water); treatment II received Neem leaf extracts; treatment III received an extract prepared from Wormwood leaves, flowers and roots; treatment IV received Tobacco leaf extracts with copper sulphate. Body Weight (BW), Fecal Egg Count (FEC), Packed Cell Volume (PCV), Total White Blood Cell Counts (TWBCC) and White Blood Cell Differential Counts (WBCDC) were determined in goats once a week, for a 4-week period. **Results:** There was no difference in FEC among the groups. The results showed that the plant extracts at the tested concentration were not effective anthelmintics; The PCV of the control group was significantly higher than all treatment groups ($p \leq 0.05$). Groups treated with Wormwood and Tobacco copper sulphate had dramatic decreases in PCV which may be an indicator of toxicity from these treatments. Significant differences in BW were observed between the control group and that of the Tobacco-copper sulphate group ($p \leq 0.05$). Low concentrations of Tobacco-copper sulphate treatment enhanced body weight. The extracts tested were not effective in reducing the levels of TWBCC. Circulating eosinophil counts, TWBCC and FEC, were negatively correlated with PCV in goats in this study. **Conclusion:** Aqueous extracts containing water soluble proteins from Neem, wormwood and tobacco are not effective anthelmintics in goats. Further investigation on efficacy and toxicity of copper supplementation on animal health and performance in goats for use by producers is recommended. Genetic variability among goats may influence the response to plant extracts. These studies support the observation that the efficacy of plant based anthelmintics is influenced by the method of extraction and host genetics in goats.

Key words: Neem (*Azadirachta indica*), Wormwood (*Artemisia absinthium*), Tobacco (*Nicotiana tabacum*), copper sulfate, Boer goats, *Haemonchus contortus*, *Trichostrongylus*

INTRODUCTION

Gastrointestinal parasites pose the greatest challenge to goat health and production in humid areas[27,29]. The prevalence of anthelmintic resistance is a serious constraint to goat production globally[16,20,22]. The use of sustainable, integrated parasite control systems, using scientifically proven non-chemical methods and limited use of drugs is being considered to insure animal health and food safety[41].

Preparations derived from plants were the original therapeutic interventions used to control diseases [40]. A comprehensive natural alternative anthelmintic management program that includes the use of plants as a cheaper and sustainable alternative to synthetic drugs would result in beneficial health and economic impacts on the goat industry. Studies show that plant species can effectively reduce the degree of parasite infestation in livestock and are promising alternatives to conventional anthelmintics[2]. Further, feeding bioactive crops or "nutraceuticals" may be useful to prevent or

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cure disease[40]. The approaches used include the use of fresh or dried plants; plant parts or extracts; and plants or their extracts in combination with other compounds such as copper[2,30]. Scientific studies are needed to support ethnobotanic information on alternative antihelmintics. These studies should include measurements of animal performance, immunity and behavior[2,12,11].

Neem (*A. indica*), Wormwood (*A. absinthium*) and Tobacco (*N. tabacum*) are plants used in traditional medicine with anthelmintic properties[2,19,35]. The Neem tree, *A. indica*, is known for its medicinal properties and has been recommended for use against gastro-intestinal nematodes[3,32] and ecto-parasites[1,14,15]. Studies of the anthelmintic efficacy of parts of the Neem tree[6,7,10,13,30] and extracts[17,26,33] have been reported with varying results[2].

A. absinthium commonly called Wormwood has been used for medicinal purposes in folk medicine[36]. Recent studies in sheep suggest that *A. absinthium* extracts are a promising alternative to the commercially available anthelmintics for the treatment of GI nematodes[36]. The anthelmintic effects of the aqueous and methanol extracts of Tobacco (*N. tabacum*) have been reported in sheep[19]. Copper sulfate supplementation although used by producers alone[9] or in combination with Tobacco (www.sscp.org) has not be supported by scientific reports from studies in goats in the absence of tobacco[5].

A major concern with the use of plant extracts is the possibility of toxicity[2] (www.sscp.org). *In vivo* studies of the efficacy and toxicology of these plants and extracts in goats lag behind that in sheep. Differences in gastro intestinal parasitism in goats and sheep are recognized[18]. Compared to sheep, goats seem to develop low immune response to nematodes of the gastrointestinal tract. Further, comparative pharmacokinetics of anthelmintic drugs in goat and sheep has clearly indicated differences between these species[18]. Thus the objectives of this study were to evaluate the effectiveness of water extracts from these three medicinal plants Neem (*A. indica*), Wormwood (*A. absinthium*) and Tobacco (*N. tabacum*) as alternative dewormers, for controlling gastrointestinal nematodes (GIN) in adult female Boer goats.

MATERIALS AND METHODS

Experimental design and study environment: Adult female Boer goats from the North Carolina Agricultural and Technical State University Farm were randomly chosen. Groups of goats were placed in pens in a barn with a sand rock floor. Wood shavings were applied over the ground as isolation material.

The animals were pre-adapted to the pen conditions prior to the start of the study. Water, hay and feed were provided regularly to all of the study animals. Each group was isolated from other groups and no physical contact was possible between animals from different treatment groups, each pen had its own feeder and watering system. The goats were fed a basal diet from Southern States SSC-31-911800, goat feed (17% crude protein). Clean water was available *ad libitum* to the animals at all times. Fecal samples were taken to determine the internal parasite burden.

Infection of animals: Twenty adult female Boer goats with an approximate weight of 40 kg were randomly selected. Before the start of the study, the animals were assessed for infection with GI parasites by fecal examination. Initial fecal egg counts for each goat were below 500 epg. To evaluate the effect of treatment post infection animals were artificially infected with approximately 30, 000 larvae containing 80% *H. contortus* and 20% *Trichostrongylus* spp. larvae in 100 mL of sterile distilled water. Twenty syringes were filled with a 5 mL aliquot of the internal parasites solution estimating a dose of 1500 larvae per animal. Each syringe was well mixed before drenching. The inoculums were administrated as far back in the goat's mouth as was possible to reduce expectoration of the solution.

Twenty one days after artificial infection fecal samples were taken to determine the fecal egg count. Animals were weighed and assigned to experimental groups for treatment.

Preparation of plant extracts: A local farmer provided dry Tobacco leaves for this experiment. The leaves were finely ground using a Warring commercial blender. Neem leaves were purchased from Neem Aura Naturals (Gainesville, Florida, US). A dry cut and sifted mix of Wormwood leaves, flower and roots were purchased from Ritchers Herb Specialists (Goodwood, Ontario, Canada).

A 25 g sample of each herb was mixed in 250 mL of distillated water for 5 min using a Fisher Thermix. Samples were then soaked over-night in an environmental incubator shaker at 37°C at a low speed shake. Following incubation, the herb-water mix was filtered using a porcelain Buchner funnel with a fixed perforated plate of 105 mm. To accelerate the filtration process, the filter was connected to a vacuum. Ordinary basket style coffee filters were used to filter the extract.

The herb extracts were then placed in a refrigerator at 4°C until use the following day.

The QuantiPro BCA Assay Kit (Sigma-Aldrich) was used to assess the amount of protein present in each herb extract. The concentration of each extract was adjusted to 5 g protein mL^{-1} sterile distilled water. To supplement the Tobacco extract Copper sulphate was added at a final concentration of 5 mg mL^{-1}.

Treatment of animals: Goats were randomly assigned to one of four treatment groups (5 animals each). Treatment I was a control without anthelmintic. Treatment II received Neem extracts. Treatment III received Wormwood extract and treatment IV was a drench of Tobacco extract with added-copper sulphate. Animals were drenched on day 28 after infection. Each goat was drenched with 40 mL plant extract. Thus each animal received 8 g of protein at a dose of approximately 200 mg of herb extract/pound body weight.

Sample collection: Initial fecal egg counts, blood samples and bodyweights were determined prior to drenching. Following drenching, body weight, fecal and blood samples were collected once a week, for a 4 week period. Fecal samples were collected from the rectum of the animals. Aliquots of two grams of the fecal sample from each goat were placed in a zip lock plastic bag for laboratory analysis. Blood samples from each goat were collected from the jugular vein in test tubes containing 0.1 mL of acid citrate dextrose to prevent blood coagulation.

Fecal examination: Fecal samples were analyzed on the day of collection for eggs per gram of feces. Fecal aliquots were weighed to two grams fresh. The fecal samples were analyzed using a modified McMaster technique (Paracount-EPG^{TM}, quantitative fecal analysis kit, Olympic Equine Products, Issaquah, WA). Slides were read using an Olympus B 201 microscope (Optical Elements Corporation) using 10 × magnification. The egg count was focused to determine the presence of *Trichostrongyle* type eggs (round worms) especially *H. contortus*, *Trichostrogylus* spp. and *Ostertagia* spp. The number of eggs counted on the McMaster slide was multiplied by 50 to obtain the parasite eggs per gram of feces for each animal. All animals were treated with a commercial dewormer at the end of the study.

Packed cell volume assay, total white blood cells count and differential white blood cell count: An aliquot of blood with anticoagulant from each goat was collected in micro-capillary tubes and then centrifuged for 10 min at 14 000 rpm in an IEC MB Micro Hematocrit centrifuge (Damon/IEC Division). After centrifugation, samples were analyzed for PCV using a micro-capillary reader (Damon/IEC Division). Total white blood cells were determined using a Coulter Z series particle counter and size analyzer (Beckman coulter) following the protocol described in the Beckman Counter User Manual 9914591-C. Differential white blood cell counts were performed on blood smears stained using the SureStain Wright CS-432 stain as described by the manufacturer (Fisher Scientific). White blood cell differential counts were performed using an Olympus B 201 microscope (optical elements corporation) using 100x magnification.

Statistical analysis: Two-way analysis (treatment and date) of variance test using SAS statistical analysis software (SAS Institute Inc., 1985) was used to analyze BW, FEC, PCV, TWBCC and WBCDC. Statistical significance was determined using $p<0.05$. Dunnett's method was used with the GLM procedure to compare between the control (I) and each treatment effect (II-IV) to determine the response to plant anthelmintics. The correlations amongst PCV, BW, TWBCC, WBCDC and FEC were also evaluated using CORR procedure.

RESULTS

The three plant aqueous extracts tested did not have any anthelmintic effect in female Boer goats infected with internal parasites. No significant differences were observed in fecal egg count between treatment groups ($p>0.05$; Table 1). The highest number of eggs per gram of feces was observed in week two. The highest FEC were observed in untreated animals.

The effect of plant extracts on Packed Cell Volume (PCV) of goats is shown in Table 2. In this study the PCV of untreated animals, varied between 24 and 48% ($p\leq0.05$), which is within the normal range[4,21,28]. Treated animals had lower PCVs although they were not significantly different from controls ($p>0.05$). A serious decline in PCV was observed following the second week of treatment in the groups treated with Wormwood and Tobacco-copper sulfate. Consequently, animals with a low PCV were treated immediately with a drug cocktail of Levamisol, Albendazol and Ivermectin for three days. Overall, the Neem group maintained fairly stable PCV values throughout the experimental period. Studies in sheep have reported increases in PCV following treatment with aqueous extracts of Neem[23].

Table 1: The effect of plant extracts on fecal egg count of goats infected with gastrointestinal parasites

Treatment	Week 1	Week 2	Week 3	Week 4
Control	740.0±476.1	4,175.0±3494.8	1,713.5±699.2	2,866.7±938.5
Neem	790.0±596.2	1,920.0±1244.7	1,710.0±1169.6	2,260.0±2065.0
Wormwood	640.0±379.8	3,420.0±2099.5	700.0±353.5	1,450.0±70.7
Tobacco	900.0±828.8	7,010.0±7068.8	2,050.0±1126.9	1,225.0±106.1

The result are expressed as mean ± SE of n = 5. Result presented in egg per gram of feces

Table 2: Effect of plant extracts on packed cell volume of goats infected with gastrointestinal parasites

Treatment	Week 1	Week 2	Week 3	Week 4
Control	27.2±4.4*	27.4±6.5*	24.1±4.2*	23.5±3.8*
Neem	22.7±3.3	23.2±2.1	21.0±2.2	20.1±2.3
Wormwood	25.8±4.6	16.2±1.3	17.3±1.7	18.3±1.1
Tobacco	22.7±4.8	17.3±6.9	21.6±5.5	20.0±4.0

The result are expressed as mean ± SE of n = 5. Result presented in the %. *: p≤0.05, compared with the treated groups

Table 3: Effect of plant extracts on white blood cell counts of goats

Treatment	Week 1	Week 2	Week 3	Week 4
Control	17.94±1.69	31.90±9.61	56.85±11.55	38.59±20.16
Neem	15.35±3.82	51.70±16.85	29.10±22.16	47.69±11.88
Wormwood	15.33±4.04	43.20±15.50	29.57±32.40	26.07±28.57
Tobacco	10.29±6.20	43.91±20.90	41.04±31.34	32.39±23.99

The result are expressed as mean ± SE of n = 5. Result presented in millions mL^{-1}

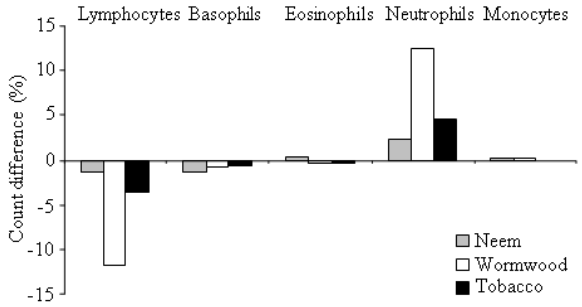

Fig. 1: Relative changes in white blood cell differential counts when compared to counts from untreated goats. Positive values indicate a relative increase and negative values a relative decrease

The TWBCC increased for all treatment groups following infection (Table 3). No statistically significant differences were observed among treatment groups (p>0.05). The relative changes in white blood cell differential counts when compared to counts from untreated goats are shown in Fig. 1. No significant differences were observed between groups at the 5% level.

There was a significant negative correlation between neutrophil and lymphocyte counts (r = -0.920; p<0.0001). Plant extracts tested did not have a significant effect on body weight in goats (Table 4). Weekly body weight measurements were not statistically different over time. Plant extracts tested did not have a significant effect on body weight in week 1.

Table 4: Effect of plant extracts on body weight of goats infected with gastrointestinal parasites

Treatment	Week 1	Week 2	Week 3	Week 4
Control	36.4±2.9	36.0±4.3	38.6±6.1	41.7±5.3
Neem	39.9±3.5	41.3±3.4	41.5±4.0	41.5±4.1
Wormwood	39.6±4.8	40.0±5.1	41.3±5.4	41.0±5.1
Tobacco	42.0±8.1*	43.7±7.8*	42.9±7.8*	42.8±8.4*

The result are expressed as mean ± SE of n = 5. Result presented in Kg. *: p≤0.05, compared with the control group

Increased body weight was observed following treatment with Tobacco-copper sulphate in the remaining weeks of the study (p<0.05).

DISCUSSION

The high fecal egg count indicates elevated presence of adult parasites in reproductive status in the host digestive system[8,25]. All animals were treated with a commercial dewormer at the end of the study.

All three plants have been reported to have anthelmintic properties in traditional medicine and in controlled scientific studies[2,3,32,35]. The anthelmintic properties reported have varied as a result of variations in source of plant material, season and environment variability, extraction process and host species[2,7,10]. Controlled tests are indispensable to confirm anthelmintic activity. Further, concern over dosage levels and associated margins of safety have been reported especially in the use of nicotine sulfate for control of parasites[39].

Studies of the anthelmintic efficacy of parts of the Neem tree[3,6,10,30] and extracts[10,17,26,33] have been reported with varying results. In goats the ethanol extract of Neem, at the dose of 100 mg kg^{-1} showed a 100% killing of parasites *in vitro*[33]. Chagas and Vieira[6] reported no anthelmintic of Neem at a dosage of 30 g of dried leaves per goat/day given for 5 days. Alcohol and aqueous extracts of *A. absinthum* reduced fecal egg counts and fecundity of the parasites in sheep[19,36]. In sheep the alcoholic extracts of *Artemisia brevifolia* showed better anthelmintic activity against *H. contortus* as compared to aqueous extracts[19,37].

Chemical analysis of *A. absinthium* has shown that its volatile oil is rich in thujone, which has been earlier reported as an anthelmintic[24]. Azadirachtin-A, the

substance believed to act against parasites, is present in Neem seeds at a concentration of approximately 24.85 mg/100 g, while the leaves only contain 0.59 mg/100 g[34]. The extraction procedure may have an impact on the level of active substance. In traditional medicine plant preparations are extracted using water[13]. In the present study we used aqueous extracts to reflect the use of these plants in traditional medicine. In our studies the aqueous extracts containing water soluble protein components of these three plants were not effective in reducing fecal egg counts. These studies support the observation that the aqueous extraction procedure may not release the active substance and further optimization and characterization of extracts is needed[36].

In scientific studies in sheep the aqueous extract was not as effective as the alcohol extract[10,19,33,37]. Aqueous and ethanol extracts of Tobacco did not show a strong anthelmintic effect in goats[33]. In sheep aqueous and methanol extracts of *Nicotiana tabacum* exhibited dose-dependent anthelmintic activity both *in vitro* and *in vivo*, thus justifying its use in traditional medicine[19]. However, the efficacy was much lower than the standard antihelmitic. In the present study copper sulphate supplementation of Tobacco had no effect on fecal egg counts. This is similar to reports[5] that Copper sulfate supplementation in feed did not influence fecal egg counts in goats. Packed cell volume values are directly related to anemia, correlated with high FEC and parasite burdens as a hall mark of infection with *H. contortus*[25,38]. The need for species specific *in vivo* studies and toxicological evaluation is recognized. Few studies report the toxicological effect of plant extracts in goats. The observed PCVs in the Tobacco-copper sulphate group are consistent with observations of decreased PCV in goats receiving oral doses of copper sulfate[31]. However more recently, copper sulphate supplementation in goats did not show an impact on PCV. It has been observed that although the risk of copper toxicity is not high in goats, extended feeding of copper sulphate in combination with Tobacco may result in toxicity due to interactions[5]. Studies on the anthelmintic properties of Tobacco and Wormwood have not reported on the effects of plant anthelmintics on PCV, TWBCC and RBCC[10,19,39]. Khalid *et al.*[23] report significant reduction in Total Leukocyte Count (TLC) in sheep treated with aqueous extracts of Neem.

In the current study the mean leukocyte count for goats in all treatment groups exceeded the normal upper limit reported for goats[4,21]. Considerable research has been reported on the effect of anthelmintics in sheep. Goats and sheep differ in their response to internal parasites and to control measures to control parasitism[18]. Thus, the results of this study may reflect differences in the response of goats compared to reports in sheep. Reports in sheep observed significant increases in body weight following treatment with Neem extract[23].

The increase in TWBCC is attributable to the animals' immune response to infection or sensitization. Circulating eosinophil counts and TWBCC were negatively correlated with PCV in contrast to reports by Chiejina *et al.*[8]. This relationship and the wide individual variability may reflect the effect of genetics in the response to treatment among goats and needs to be considered in studies of the efficacy of plant extracts and other treatment interventions.

A dose effect has been proposed for the role of copper as a growth stimulant in goats[24,31]. In the current study goats received a dose of 2 mg of copper sulphate (5 µg kg^{-1} body weight). Copper sulfate contains 25.4% copper. Thus our results support observations that lower doses of copper may be beneficial to animal performance[31]. At higher concentrations copper sulfate supplementation did not influence parasite levels and may reduced weight gains in goats[5].

CONCLUSION

At the concentrations tested extracts of Neem, Wormwood and Tobacco did not have anthelmintic properties. Aqueous extracts of these plants may not be effective anthelmintics in goats. Toxic effects of Wormwood and Tobacco copper sulphate were manifested as sever anemia and leukocytosis. Supplementation with copper sulphate was associated with increases in body weight. Further studies on the effect of copper concentrations are needed to elucidate the role of copper in goats and its interaction with different plants in goat feed or medication. The extracts tested were not effective in reducing the levels of TWBCC. A relationship was observed between circulating eosinophil counts and the FEC, PCV and TWBCC in goats in this study. Genetic variability in susceptibility to internal parasites may contribute to the level of toxicity and effectiveness of extracts of these plants in goats. These studies support the observation that the efficacy of plant based anthelmintics is influenced by the method of extraction and host genetics.

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