

# Efficacy of *Allium Sativum* (Garlic) in Controlling Nematode Parasites in Sheep

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## ABSTRACT

This study evaluated the efficacy of *A. Sativum* in controlling nematode parasites in Blackhead Persian ewes in third parity in Zimbabwe. A completely randomized design was used in the experiment. There were four treatment groups with three animals in each treatment under four varying inclusion levels of *A. Sativum*. The fifth group constituted the control where animals were dosed with the conventional dewormer, Valbazen, as per the manufacturer's recommendations. The trials were carried out from January to March 2008 during the rain season, and subsequently replicated five times. Faecal samples were collected from treated animals and analysed using the McMaster technique. The results obtained were recorded as egg per gram (EPG) of faecal samples. The percentage mean faecal egg counts (FEC) reductions were used for the analysis of the data using SPSS. It was observed that *A. Sativum* had the ability to reduce the FCEs of the two most prominent parasites, the Strongyles and Trychostrongylus species. There were no significant differences between the treatments and the control ( $P < 0.005$ ), indicating

that *A. Sativum* is as effective as the conventional dewormer.

## INTRODUCTION

Gastrointestinal parasitism continues to be a major constraint on profitable livestock production, especially in sheep (Esyker and Ploeger, 2000). An unmanageable load of endoparasites is associated with lowered outputs of animal products and by-products, thus contributing to production and productivity losses (Preston and Allonby, 1979). Helminthiasis caused by helminths is the commonest and severest form of all gastrointestinal diseases. Nematodes (nematodes) have since proved to be greatest challenge for conventional sheep producers. Sheep graze closer to the ground and directly over their manure, which is in pellet form. This increases their susceptibility to the nematodes more than other class of livestock (Padgham, 2000).

Since the 1960s, nematode control has relied heavily on the use of synthetic anthelmintics such as ivermectins, Levamisoles, and Thiabendazole (Padgham, 2000). Ready access to anthelmintics and the ease by which they can be applied, coupled with the immense progress made in the knowledge of the epidemiology of parasites,

has led to a period of success in the control of gastrointestinal parasites, particularly in the commercial livestock production (Orr, 1998).

However, even when properly administered, the long-term regular use of drugs and chemicals such as anthelmintics leads to the loss of an animal's natural resistance. If for whatever reason the anthelmintics are suddenly unavailable, the animal becomes susceptible to the worst effects of the parasites which those drugs were keeping under control (Norval, 1983). According to Fielding (1997), the continued and inappropriate uses of synthetic anthelmintic have led to the development of anthelmintic resistance. As a result, this has compromised the efficiency of current and future nematode control programs.

The availability of expensive synthetic drugs on the market has made helminthes control a daunting task for resource poor small-holder sheep producers in Zimbabwe (Hammond et al, 1997). This has prompted them to opt for the use of Ethno- Veterinary Medicine (EVM) to control nematode infestations in their flocks (Fielding, 1997). *A. Sativum* is one of such Ethno Veterinary Medicine whose well documented anti-parasitic properties prompted this research to investigate its anthelmintic ability. The objective of this research was to investigate the effectiveness of drenching Blackhead Persian ewes with an aqueous extract of *A. Sativum* to reduce nematode burdens.

## LITERATURE REVIEW

### Nematode Parasites

Nematodes are multicellular, cylindrically shaped bodies belonging to the helminth class. The helminth class includes the phyla Nemanthelminths (nematodes) and Platyhelminths (tapeworms and liver flukes). Nematodes, also commonly known as roundworms, make a large assemblage of relatively simple structures with a wide spread distribution (Smyth, 1994). The general morphological and anatomical characteristics include:

- Bilaterally symmetrical and striated bodies
- Rounded anteriorly and tapering posteriorly
- Transparent cuticle (proteinaceous) may be marked by striations.
- Well developed reproductive system with females' having ovaries, oviducts and uterus vulva, and males having testis and all the accessory glands.
- Reproduction can either be by gametes or pathogenesis
- Highly developed than flatworms with a complete digestive system and a primitive digestive system. They have a primitive fluid filled body cavity that lacks the complete lining found in higher animals (Smyth, 1994)

### Epizootic Cycle

A typical nematode cycle involves the stages in and out of the host. The cycle generally involves four phases which are contamination, free living, infective, and parasitic stages. Eggs are shed in the host animal's feces by adult parasites. This marks the contamination phase (Levine, 1963). The eggs hatch in a day or two, and develop through three larval stages which constitute the free living phase.

The first stage (L1) and the second stage (L2) feed on the bacteria and other organic material in the fecal pat. These two stages are not protected, and are vulnerable to adverse microclimatic conditions in the fecal pat (Levine, 1963). The third stage (L3) larvae retain the L2 cuticle and this ensheathed larval form is relatively resistant to adverse microclimate conditions in the fecal pat and climatic conditions after leaving the pat onto the herbage ( Padgham, 2000). The parasitic phase begins with ingestion of the L3. Exsheathment of L3 occurs in the rumen and the larvae migrate to the abomasum mucosa where they molt to the fourth stage (L4). The L4 emerges back into the lumen where they molt to the adult stage. Adults mate and eggs are passed out in the feces, thus completing the life cycle

(Padgham, 2000; Levine, 1963)

### **Ethno Veterinary Medicine**

Ethno veterinary medicine (EVM) was defined by Eskyer and Ploeger (2000) as the scientific term for traditional animal health care, which encompasses the knowledge, skill, practices, and beliefs about animal health care found among members of the community. Ethno veterinary medicine makes use of bioactive forages in animal disease control. Bioactive forages are plants that contain secondary plant substances (SPSs) and metabolites considered to be beneficial to the animal health. In the context a certain group of SPSs, the condensed tannins have been investigated for their antihelminthic effects. There are two explanations for their mode of action:

#### **Indirect mode of action**

When tannin-rich forages are consumed, the then released condensed tannins build complexes with proteins and protects them from ruminal degradation. The complexes dissociate in the abomasums and release protein ready for absorbing the intake of tanniferous forages that may balance protein loss, thereby increasing resilience (Rahmann, 2006)

#### **Direct Mode of Action**

Condensed tannins directly react with the proteins on the surface of the parasites and disturb the normal physiological functions of the nematode like mobility, food absorption, or reproduction (Heckerdorn, 2005).

Some Ethno Veterinary Medicines used locally for gastrointestinal parasite control include *Adansonia digitata* (baobab), *Uacapa kirkiana* (muzhanje), *Annonium senegalensis* (muroro), *Ozora veticulata* (mugaragunguwo), *Aloe vera* (gavakava), and *Allium Sativum* (garlic).

### **A. SATIVUM**

#### **Botany**

*A. Sativum* is a frost hardy bulbous perennial crop belonging to the Alliaceae family, together with *A. Cepa* (onion). It is an erect herb of 20-100 cm in height with narrow flat leaves and bears white flowers and bulbils.

The edible underground stem (bulb) is made up of smaller bulb lets (cloves) which vary from 6-15 in number (Singh et al, 2004).

#### **History**

*A. Sativum* is thought to have originated in the high plains of West and Central Asia and has been used medicinally for some 5000 years ago. A multipurpose herb used for the treatment of antibiotic diseases which has also shown immunopotentiating power.

#### **Propagation**

*A. Sativum* can be grown all year round in mild climates. In the cold climates, cloves can be planted in the ground about 6 weeks before the soil freezes, and harvested in the late spring. Sandy soils or silt loam with high organic matter and high temperatures are optimum for the growth of the plant and bulb development.

#### **Chemical Properties**

The physiological activity of dietary *A. Sativum* is attributed to a number of organosulphate compounds found in the bulb. One of the compounds is unique amino acid alliin found in the intact bulb. When the bulb is cut or crushed, the C-S lyase enzymatic system called allinase converts alliin to allicin (diallyl thiosulphinate), which is responsible for the characteristic flavour of fresh *A. Sativum* and its anti-microbial properties (Velissek et al, 1996). Allicin is responsible for *A. Sativum*'s anti-microbial properties and the characteristic flavour of fresh *A. Sativum*.

#### **Medicinal Properties**

*A. Sativum* is an effective remedy for a variety of ailments such as heart problems, worms and tumors (Josling, 2000). Microorganisms are much more sensitive to the active constituents of *A. Sativum* than are higher organisms (Josling, 2000). Kirubakaran et al (1994) demonstrated the effect of crude extract of *A. Sativum* on a growth medium of *E. Coli*. At 4% final concentration a reduction of 100% in *E. Coli* population was achieved. Singh et al, (2004) identifies some of *A. Sativum*'s medicinal properties including its use as an antibiotic, for

pain relief, an anticoagulant, a remedy for internal worms and ring worms. In Israel it is administered as a boiled pulp to donkeys and horses in order to reduce worm burdens. A *Sativum* is known to stimulate T- lymphocyte and macrophage action, and support natural killer cells. Strong activity of these key cells promotes healthy immune system function, and strengthens the body's defense mechanism.

## Ethno Veterinary Applications of

### *A. Sativum*

In poultry farming, the addition of 2-5% *A. Sativum* (chips of extract) to the feed is used for the prevention of mycosis in the animals. A *Sativum* was also effective against *Candida albicans* and *Aspogillus fumigatus*. In veterinary practice, *A. Sativum* extract is used for treatment of infected wounds, in calves, and for promotion of wound healing (Josling, 2000). In India, *A. Sativum* preparation is used for scabies in swine (Josling, 2000). Orr (1998) investigated the use of *A. Sativum* based herbal formula as an antihelminthic in dairy goat production. The herbal group had a 0% infection rate with *Strongyloides*, whilst the chemical dewormer group had 29%. In a survey by Nemaunga (2006) on the use of ethno veterinary medicine in small-holder cattle production in Mutasa district, 4% of the farmers were using *A. Sativum* as a dewormer. The *A. Sativum* were ground and mixed with the feed to be given to animals.

### Residual Effects of *A. Sativum*

Like most tanniferous plants, *A. Sativum* is also toxic and can have adverse effects on animal health if consumed in large amounts or in its raw form. Some of the deleterious effects of *A. Sativum* are listed below:

- An acrid smell that makes it unpleasant to work with.
- It is toxic to cats and dogs.
- When consumed excessively, it can leave a distinct odor on the skin and breath, cause heartburn, upset the stomach, and trigger allergic reactions.
- A *Sativum* can also thin the blood,

as it causes platelet agglutination. This effect is most severe in horses.

- Other side effects of taking *A. Sativum* supplements include loss of appetite, muscle aches, and dizziness.
- Raw *A. Sativum* juice if taken undiluted can cause superficial "burns" in the mouth and gastrointestinal tract.
- Raw *A. Sativum* juice can increase the susceptibility of an animal under treatment to frothy bloat if administered in its undiluted form or if left standing for long after preparation (Josling, 2000)

## EXPERIMENTAL PROCEDURE

### Study Site

The study was carried out at Terevania farm located 20 km north west of Harare, the capital city of Zimbabwe. This area is characterized by an annual rainfall of 750-1,000 mm in the summer season and an annual temperature range of 22-26°C, and rich red clay soils. Operations on the farm include small-scale sheep and goat production and commercial cropping. The sheep flock numbers up to 70 in total, and there is no defined deworming programme. The production system is semi-intensive, with grazing rotation between fenced paddocks. Experimental animals were Blackhead Persian ewes ranging from 3-4 years in age in their second parity, and weighing an average weight of 35 kg. Five different trials were run concurrently during the trial period from January to March 2008. The flock composed of 45 breeding ewes, 10 rams, and 15 lambs.

### Animal Selection and Identification

At the start of the trial, fecal sampling was done on all the ewes showing heavy infestation of worms (25 ewes). For the initial phase, animal identification was made possible through the use of numbered tags. In order to identify ewes within the medium to high (greater than 1,000 eggs per gram), naturally acquired parasite burdens, purposive sampling was used. The 15 selected animals were then randomly aggregated into five groups, three of which were assigned

**Table 1: Identification Procedure**

Trial group	Rope colour	Quarter tied
A	Red	RF
B	Black	LF
C	Red	RH
D	Black	LH
E	Red + Black	FQ

Key: RF-Right fore quarter; RH-Right hind quarter; LF-Left fore quarter; LH- Left hind quarter; FQ- Both forequarter.

code names, that is, A-E. Identification was made possible through the use of colour coded string pieces of rope tied onto hind or fore quarters, and distinct phenotypic characteristics as shown below. The most prevalent nematodes were *Trichostrongylus* (low EPG < 100) and *Strongyles* (high EPG values > 1000). The two nematodes were therefore used for the analysis in the trial. Distinct phenotypic characteristics such as size and shape of ears, and spots on the coats were noted and used to assist in identification.

Animals were assigned code names according to their respective groups: A1, A2, and A3.

### Experimental Design

The treatments were completely randomized among the experimental units (ewes). There were four treatments plus a control, with five replicates in each treatment. After determining the extent of naturally acquired parasite burdens, animal in groups A-D were subjected to a weekly drenching program using different dilutions of raw garlic juice (RGJ) that is 20%, 40%, 60%, and 80% respectively. Animals in group E were dewormed using Valbazen® as per the manufacturer's recommendations. Faecal samples were collected from experimental ewes every 2 weeks to determine their egg per gram (EPG) counts. Faecal analysis was done at the Veterinary laboratories.

### Extraction Method

Fresh *A. Sativum* was obtained from the local market and prepared as detailed below. A

200 g of dried *A. Sativum* cloves was soaked overnight in an equal volume of distilled water to allow easy removal of cuticles and extraction of RGJ.

The cloves, still immersed in their soaking water, were pounded until they were completely mushy. (kelli, please make this one paragraph) The crushed material was left to stand for 2 hours.

### RGJ was filtered out using poly cotton cloth

The filtered collosion was used as the standard for the treatments. Care was taken to ensure that the RGJ was used as soon as possible as the active compound alliicin prone to rapid decomposition.

### Raw Garlic Dosage Formulation and Administration

Animals in groups 1 – 4 received different dosages of RGJ, A, B, C, and D, diluted to make a final volume of 10 ml as tabulated in Table 2.

RGJ was stabilized using vegetable oil at a ratio of 1:10 to avoid acidification of mixture and imminent frothy bloat. Animals were removed from pasture about 12 hours before drenching to prevent susceptibility to bloat. Animals were individually handled and drenched using a 10 ml dosing syringe. Once an animal had been treated, paint was used to mark its ear or forehead to avoid repetition of treatment. Great care was also taken to monitor the drenching process as the RGJ has a tendency of “burning” if administered incorrectly or in its raw undiluted form.

**Table 2: Dosage Formulation**

Trial group	Volume of RGJ/ml	Volume of water/ml	Final Concentration of RGJ (%)
A	8.0	2.0	80
B	6.0	4.0	60
C	4.0	6.0	40
D	2.0	8.0	20

**Table 3. Response of nematodes to different dilution levels**

<i>A. Sativum</i> (%)	<i>Strongyles</i> (% reduction)	<i>Trichostrongyles</i> (% reduction)
20	23.37± 12.73	12.46 ± 5.62
40	19.92± 6.87	43.00 ± 18.49
60	35.08 ± 17.33	29.36 ± 9.70
80	42.30 ± 13.40	36.16 ± 11.01
Valbazen	46.30 ± 13.40	49.34 ±19.36

## Techniques

### Faecal Sampling

Faecal pellets were collected directly from the animal's rectum using a gloved hand. Care was taken to avoid cross infection by disinfecting the glove after each withdrawal. Collecting tubes were clearly labeled with individual animal data and sampling date. Faecal samples were taken to the veterinary laboratories for faecal analysis.

### Faecal Egg Counting

The faecal samples were analysed using the modified McMaster technique at the veterinary laboratories. The following procedure was followed:

- Measured faecal samples were crushed and then mixed with a substance of relatively high specific gravity that is, Fecasol.
- This results in worm eggs floating to the top of the liquid where they are collected on a glass or plastic slides.
- To determine egg per gram counts (EPG), the McMaster technique, which makes use of weighed faecal sample, was immersed in a floatation solution with a known dilution and

a specialized counting ( graduated slide).

- After the slide chambers were filled with faecal suspension in the floatation solution, the eggs were counted under a grid that defines a known volume of suspension that is usually the area between two grids. The results were averaged and multiplied by a dilution factor. Because the number of grams of faeces and their dilution is known, the results are an estimate of the number of eggs in a specific amount of faecal part per gram that is EPG.

### Statistical Analysis

Differences between groups for faecal egg counts (FECs) and egg per gram (EPG) counts were determined using Statistical Package for Social Scientists (SPSS) Version 14. Differences between groups for percent FEC reductions were analysed using one way ANOVA. All differences were considered at  $P < 0.05$ .

## RESULTS

### Faecal Egg Count

The results indicated that *A. Sativum* can significantly reduce the EPGs in Tricho-

strongyles and Strongyles after treatment ( $P < 0.05$ ). The FECs generally declined in all the experimental groups. There are however no overall significant difference between the treatment groups and the control (Table 3) for both species of parasites  $P < 0.05$ .

#### Effect of *A. Sativum* on Strongyles

The strongyles showed a gradual response to treatment. Although the percentage reduction in EPG counts generally increased with increasing concentration of *A. Sativum*, the variations were not significantly different ( $P < 0.05$ ). The highest overall FEC percentage reduction was recorded for the 80% concentration level of RGJ (97.33 %). There was however discrepancies between sampling results for group B as a result of sudden influx of parasitic larvae on the veld favored by heavy rainfall received during this period.

#### Effects of *A. Sativum* on the Trichostrongyles

This species showed rapid response to treatment with *A. Sativum*. The percent reduction in EPG counts generally increased with increasing concentration of *A. Sativum*.

However, the variations were not significantly different ( $P < 0.05$ ). The highest overall FEC percent reduction was 100%, recorded for group B. The large variations in initial mean FECs were a source of error, as some groups like B with lower levels of infestation achieved a 100% reduction earlier than others. Overall, there were no significant differences between the parasite's responses to the treatments (B – E).

#### DISCUSSION

The results of the study indicated that *A. Sativum* has the ability to control nematode parasites, Strongyles, and Trichostrongyles in particular. This concurs with Orr (1999), who conducted a study with dairy goats using *A. Sativum* based herbal formula. It was discovered that the herbal group had a 0 % infection rate for strongyles and 33 % protostrongyloides (lungworms), whilst the chemical had 29.5% and 50% infection rate for the respective nematodes. Anonymous (1993) further investigated *A. Sativum*'s antihelmentic properties and concluded that the herb is effective against lungworm, Ascaris, Nectobar, and Enterobius.

Differentiation in faecal egg counts

**Table 4:** Effects of *A. Sativum* on Strongyles EPGs

A. Sativum (%)	Initial EPG	Final EPG	% Reduction
20	1700.1	229.3	95.60
40	1338.3	500	62.30
60	1443.3	60	96.80
80	1843.1	49	97.33
Valbazen	2726.7	66.7	97.70

**Table 5.** Effects of *A. Sativum* on Trichostrongylus EPGs

A. Sativum (%)	Initial EPG	Final EPG	% Reduction
20	88.7	43.7	50.7
40	100	0	100
60	150	25	93.3
80	58	4.5	97.3
Valbazen	77	0	100

indicated that infection levels were substantially reduced from the start of the trial period to end. *A. Sativum*, like many other tanniferous plants, increases the supply and the digestible protein by forming non-biodegradable complexes with protein in the rumen, improving the host's immunity and resilience to nematode infestation. The high tannin content in *A. Sativum* may have direct antihelminthic effects on resident worm populations, disrupting the normal physiological functions like mobility, food absorption, and reproduction. The later mode of action agrees with Duval (2004), who asserted that *A. Sativum* does not prevent the production of eggs, but prevents the eggs of certain parasites from developing into larvae. This reduction in larvae on herbage will subsequently reduce the build up of nematode burdens in hosts. In the current study, however, *A. Sativum* appears to have direct antihelminthic effect on resident worms in the animals. The results indicate that there was a substantial die off, which allowed infectivity differential to be observed in the FCEs percent reductions. Individual faecal samples were used to verify the effect of *A. Sativum* on the nematode population. *A. Sativum* has the ability to reduce EFCs of treated animals. Allen et al (1998) also evaluated faecal cultures from lambs treated with *A. Sativum* tincture and concluded that the herb had parasite effect on nematode and reduced the EPG counts.

It was also discovered that aqueous *A. Sativum* extract can still exert its antibiotic properties, even at dilution rate of 1:1000. The percent reduction in faecal egg counts increased with increasing level of inclusion of the herb. There is need therefore to isolate the active compound in *A. Sativum* and determine the drug response curves of the parasite in question. Several options exist for administering *A. Sativum*. Even though freshly minced *A. Sativum* proved to be more efficient than other *A. Sativum* extracts in a study by Pena et al (1988) to control endoparasites in carp, it is not necessarily the most practical method on a day to day basis due to its low palatability.

Grieve (1981) suggested the use of *A. Sativum* milk made by boiling bulbs mashed in milk as a dewormer. Some researchers have, however, recommended not oiling *A. Sativum* as it reduces its effectiveness against parasites. The most practical method of administering *A. Sativum* juice is by oral drenching as this ensures intake and its effectiveness.

There is little documentation on the recommended dosages of *A. Sativum* intake. The rates seem to vary with physical form, in which the herb is being administered. Rahmann (2006) suggested a dosage of 5 ml of *A. Sativum* juice per animal per day. Orr (1999) suggested that two bulbs or whole plants be given daily. The high tannin content of *A. Sativum* (45-55g of CT/kg/ DM) makes it able for use in small concentration less than 20%. *A. Sativum* was effective against Strongyles and Trichostrongyles, reputed to be the most resistant strains because of their thick proteinaceous cuticles and mode of reproduction.

The slow response to treatment shown by Strongyles and longer time taken to level off infestations after influxes in FCEs indicate that *A. Sativum* should be administered as a homeopathic prophylactic treatment. This is because *A. Sativum* does not seem to have a long-term effect, and thus requires continuous administration. Its effectiveness was reduced if there were sudden influxes in the FECs. This assertion is supported by Duval (2004), who reported that plants with high contents of parasiticides may have a short-term effective curative use and may therefore be ideally used in a preventive fashion.

In the nineteenth century in Persia, Vi- enne recommended the use of *A. Sativum* as an additive rather than as a dewormer alone. *A. Sativum* can be incorporated into certain commercial homeopathic and allopathic dewormers and with other plant substances. The advantage of these mixes is that they will have a wider spectrum of action. *A. Sativum* can also be cultivated into pastures where they can be grazed by the animals.

However this application is limited by its palatability, ease of establishment, and its ability to withstand trampling.

## CONCLUSION AND RECOMMENDATIONS

It can be concluded from this study that *A. Sativum* can be used as a natural antihelmentic to reduce pasture infectivity and thus lower nematode burdens in the grazing sheep. *A. Sativum* has antihelmentic properties that can control nematodes in sheep if used at a dosage of 80 % concentration level of RGJ.

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