

## RESEARCH ARTICLE

## Nematicidal properties of neem leaf extract and neem oil against *Oesophagostomum columbianum* and *Trichuris ovis*

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### ABSTRACT

The present study was conducted to evaluate the *in vitro* effect of neem (*Azadirachta indica*) leaf extract and neem seed oil on gastrointestinal nematodes of sheep and goat. Two nematodes chosen for the present study were *Oesophagostomum columbianum* and *Trichuris ovis*. It was found that neem leaf extract and neem seed oil have the potential to control these gastrointestinal nematodes. The nematodes of both these species showed decreased motility when placed in different concentrations of the neem leaf extract and neem seed oil. The death of the nematodes took place under the effect of varying concentrations of the extract and oil treatments. The histological structure of the treated worms was severely altered. The effect was seen mainly on the body wall and microvillar brush border of intestinal epithelium.

**Keywords:** Neem leaf extract, Neem seed oil, gastrointestinal nematodes, anthelmintic, *Oesophagostomum columbianum*, *Trichuris ovis*.

### INTRODUCTION

Control of gastrointestinal nematodes of sheep and goat is mainly anthelmintic dependent. The hap-hazardous use and total reliance on anthelmintics have led to the emergence of the anthelmintic resistant strains of parasites. The problem has threatened the anthelmintic based parasite control programme and has necessitated further research on alternative methods of worm control [1]. In the 42<sup>nd</sup> Annual Meeting of Veterinary Parasitologists held at Reno (Navada), Sangster [2] stated that the problem of anthelmintic resistance is emerging in different parts of the globe. He also stated that in some sheep and goat producing areas of the world the resistance has rendered all the registered anthelmintic drugs ineffective. Sanyal [3] stated that keeping in view the problem of anthelmintic resistance, there is a global need for sustainable integrated parasite management. The rising cost of broad spectrum anthelmintics and concern about the development of the resistance in the parasites and their toxicity to the host are posing serious threat to the continued use of synthetic drugs.

In the present study, two gastrointestinal nematode parasites of sheep and goat were chosen. The effect of neem leaf extract and neem oil was tested against *Oesophagostomum columbianum* and *Trichuris ovis*. In India, the importance of neem in veterinary medicine dates back to Mahabharata as

reported by Indologist Vartak and presently neem products have internationally received a significant attention [4]. The neem tree is a very popular in most tropical and subtropical regions of the world. It is a hardy tree that grows vigorously in desert areas and in a harsh climate. Neem leaves are imparipinnately compound, alternate, exstipulate and 20 to 38 cms long and crowded at the ends of the branch. Each leaf has a swollen base and bears 8 or 9 opposite or alternate leaflets on a slender petiole. The seed of neem is ovoid or spherical, pointed above and has a thin testa. Under natural conditions the seed generally falls to the ground during rainy season.

The neem constituents belonging to chemically diverse classes have been divided into two major sections viz. isoprenoids and others. The isoprenoids include diterpenoids, triterpenoids and azadirachtins. The non-isoprenoid constituents include glycerides, polysaccharides, sulphurous compounds including flavonoids and their glucosides, amino acids, aliphatic compounds etc. [4]. The present research work on the *in vitro* effect of neem leaf extract throws a light on efficacy, manifested as to extent of damage on the various organ-systems of the nematode parasites leading to disruption in their fundamental activities which may result in their death.

## MATERIALS AND METHODS

### Collection of parasites

The infected intestines of sheep and goat were collected from the local abattoirs of Batala. For collecting *Oesophagostomum columbianum* and *Trichuris ovis* the intestine was cut open. The nematodes of both the species were placed in separate containers and washed several times with 0.85 % sodium chloride solution.

### Preparation of Neem leaf extract

An aqueous leaf extract of neem was prepared with the method given by Vyas and Mistry [5]. Mature green leaves of neem (*Azadirachta indica*) were collected, dried in shade. These dried leaves were crushed to powder with the help of an electric grinder. The powdered leaves were soaked in water in the ratio of 1:5 for overnight. The aqueous extract was strained through a fine muslin cloth. This was taken as stock solution (S). The experimental solution concentrations were prepared from the stock solution as 10%, 20%, 30%, 40% and 50%.

### Preparation of Neem seed oil

The kernels were collected from the ground under the neem tree, depulped and washed with water. These were dried and crushed using a crucible and blender. The oil was obtained by pressing the crushed kernels.

### To study *in vitro* effect of Neem leaf extract and Neem oil

The motile, fresh and active worms of either sex were extracted from intestine of sheep (*Ovis aries*). These were divided into 5 groups and placed in various concentrations of neem leaf extract plus Tyrode's solution. The extract and the Tyrode's solution were taken in the ratio of 1:1 at each dose. Simultaneously, one group of worms in Tyrode's solution alone served as control. The motility and mortality of all experimental groups and control was noted at regular intervals.

The mass motility of worms was graded as 0 (nil); ± (feeble); + (poor); ++ (moderate); +++ (good) and ++++ (vigorous). The death of the individual parasite was noted by the response of the worm to needle prick and also by placing them in warm saline at 42-45°C.

After death, the worms of all the groups were fixed in alcoholic Bouin's fixative and processed for sectioning. The external observations on motility and mortality were combined with internal histomorphology of organ-systems of all the groups. The results of all these groups were compared with the histomorphology of untreated worms to draw the final inferences.

## RESULTS AND DISCUSSION

### Motility and mortality of *Oesophagostomum columbianum*

The treated nematodes of all the groups of *Oesophagostomum* spp. showed a decrease in motility after different time intervals in varying concentrations of the extract. In higher concentrations of neem leaf extract i.e. 50 % and 40 %, the motility of nematode worms become feeble after 6 hours and 8 hours respectively. After an interval of 8 and 10 hours the worms placed in 50 % and 40 % concentrations showed no motility. After carefully observing the worms under microscope and placing them in saline solution the worms were found to be dead. In 50 % concentration of neem leaf extract 100 % mortality of *Oesophagostomum columbianum* occurred after 8 hours. (Table no. 1).

The various groups of nematodes placed in neem seed oil died earlier as compared to those placed in some concentrations of neem leaf extract. In 50 % concentration of neem seed oil the nematode worms showed feeble motility after 4 hours of treatment. They become sluggish and even after repeated pin pricks they showed little motility. The complete mortality of nematodes placed in 50 % and 40 % concentrations was after 6 hours and 8 hours respectively. The lower

concentrations of neem seed oil were also very effective. Both 10 % and 20 % concentrations of neem seed oil caused the death of nematode worms after 12 hours. The worms placed in these concentrations showed moderate motility after 8 hours. The observations on the effect of the various concentrations of neem leaf extract and neem seed oil on *Oesophagostomum columbianum* are given in table number 1 and 2.

**Table 1: Observations on motility and mortality of control and Neem leaf extract treated *Oesophagostomum columbianum***

S. No	Concentration of the Neem leaf Extract	Number of worms	Observations on motility after the interval of						Total mortality worms/hrs
			4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	14 hrs	
1	10 %	10	+++	+++	+++	++	±	0	10/14
2	20 %	10	+++	+++	++	+	0	0	10/12
3	30 %	10	+++	+++	++	±	0	0	10/12
4	40 %	10	+++	++	±	0	0	0	10/8
5	50 %	10	++	±	0	0	0	0	10/6
6	Control	10	++++	++++	++++	++++	++++	++++	-

**Table 2: Observations on motility and mortality of control and Neem seed oil treated *Oesophagostomum columbianum***

S. No	Concentration of the Neem leaf Extract	Number of worms	Observations on motility after the interval of						Total mortality worms/hrs
			4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	14 hrs	
1	10 %	10	+++	+++	++	±	0	0	10/12
2	20 %	10	+++	+++	++	±	0	0	10/12
3	30 %	10	+++	+++	++	0	0	0	10/10
4	40 %	10	+++	+	0	0	0	0	10/8
5	50 %	10	±	0	0	0	0	0	10/6
6	Control	10	++++	++++	++++	++++	++++	++++	-

**Table 3: Observations on motility and mortality of control and Neem leaf extract treated *Trichuris ovis***

S. No	Concentration of the Neem leaf Extract	Number of worms	Observations on motility after the interval of						Total mortality worms/hrs
			4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	14 hrs	
1	10 %	10	+++	+++	++	+	±	0	10/14
2	20 %	10	+++	+++	++	+	0	0	10/12
3	30 %	10	+++	+++	++	±	0	0	10/12
4	40 %	10	+++	++	±	0	0	0	10/10
5	50 %	10	++	±	0	0	0	0	10/8
6	Control	10	++++	++++	++++	++++	++++	++++	-

**Table 4: Observations on motility and mortality of control and Neem seed oil treated *Trichuris ovis***

S. No	Concentration of the Neem leaf Extract	Number of worms	Observations on motility after the interval of						Total mortality worms/hrs
			4 hrs	6 hrs	8 hrs	10 hrs	12 hrs	14 hrs	
1	10 %	10	+++	+++	++	+	0	0	10/12
2	20 %	10	+++	+++	++	±	0	0	10/12
3	30 %	10	+++	++	+	0	0	0	10/10
4	40 %	10	++	+	±	0	0	0	10/10
5	50 %	10	±	0	0	0	0	0	10/6
6	Control	10	++++	++++	++++	++++	++++	++++	-

**Key to Observations:**The mass motility of worms was graded as 0 (nil); ± (feeble); + (poor); ++ (moderate); +++ (good) and ++++ (vigorous).

In higher concentrations of neem leaf extract i.e. 40 % and 50 %, *Trichuris ovis* died after 10 hours and 8 hours respectively. In 50 % concentration of neem leaf extract, the motility of nematode worms became moderate after 4 hours of treatment. They showed feeble motility after 6 hours. The death of worms took place after 8 hours of neem leaf extract treatment. (Table no. 3).

The effect of neem seed oil on *Trichuris ovis* was quicker as compared to neem leaf extract. The nematode worms placed in higher concentrations i.e., 40 % and 50 % showed decreased motility after 4 hours of treatment. In 50 percent concentration of neem seed oil, the death of nematode worms took place after 4 hours. The lower concentrations of the neem seed oil showed decreased motility after 8 hours. A moderate motility was observed in worms placed in 10 % and 20 % concentrations of neem seed oil after 8 hours. Their motility became feeble after 10 hours of treatment. The death of the worms placed in 10 %, 20 % and 30 % concentrations were observed after 12 hours, 12 hours and 10 hours respectively. The observations on the effect of the various concentrations of neem leaf extract and neem seed oil on *Trichuris Ovis* are given in table number 3 and 4.

Normal control worms of both the species of nematodes studied remained active with whole body movements.

#### Alterations in histology

In case of *Oesophagostomum columbianum* the main effect of neem leaf extract and neem seed oil was on the body wall and intestinal microvillar brush border. The body wall of the neem treated worms showed marked alterations. The cuticle layer of these nematode worms showed peeling and etching. The musculature was also damaged. The brush border of intestinal epithelium was erupted. The erosion of intestinal epithelium indicated that it may have affected the absorption of nutrients and ultimately leading to the death of the worms. However, no marked change was observed in the reproductive tracts of male and female worms.

The effect of neem leaf extract and neem seed oil on the histological structure of *Trichuris ovis* was also on the digestive system. The microvillar brush border and intestinal epithelium was badly damaged. No effect was seen on the reproductive organs except the disappearance of bounding membrane of gonads.

The efficacy of different anthelmintic drugs was tested on various nematode species by a number of workers. The micro-morphological changes induced by anthelmintic piperazine were tested by Abdulazizov [6].

He assessed the *in vivo* effect of piperazine on *Ascaris lumbricoides*. Sujon *et al* [7] performed a detailed study to find out the anthelmintic properties of ten indigenous medicinal plants against gastrointestinal nematodes of goat. Badar *et al* [8] assessed the anthelmintic activity of *Acacia nilotica* bark and leaf extract against *Haemonchus contortus*. Lalchandama *et al* [9] studied the anthelmintic activity of *Acacia oxyphylla* stem bark against *Ascaridia galli*. Devasting structural alterations on the fine topography of *A. galli* were noticed. Severe shrinkage of the cuticle, loosening and collapsing of the lips, extensive irregular wrinkles all over the body surface and appearance of small blebs or swellings on cuticle were noticed.

The brush border of intestinal epithelium of *Oesophagostomum columbianum* was erupted. The erosion of intestinal brush border indicated that it may have affected the absorption of nutrients and ultimately leading to the death of worms. The effect of neem leaf extract and neem seed oil on the histological structure of *Trichuris ovis* was also on the digestive system. The microvillar brush border and intestinal epithelium was badly damaged.

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