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# *IN VITRO* EVALUATION OF THE NEMATICIDAL EFFECT OF SOME MEDICINAL PLANTS ON *HAEMONCHUS CONTORTUS* (NEMATODA)

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## ABSTRACT

The aim of present study is to investigate the nematicidal efficacies of five medicinal plants, *Acacia nilotica, Azadirachta indica, Dalbergia sissoo, Ocimum sanctum and Ficus religiosa*. Aqueous extracts of these plants were investigated *in vitro* conditions for their anthelmintic activity against adults of *Haemonchus contotus*. Aqueous extract of each plant was used at five concentrations (10, 20, 30, 40 and 50 %). The results showed that all the extracts has a dose related effect on the motility of worms and the mortality of the worms increases as the concentration of the extract increases. The overall findings of the study revealed that aqueous extracts prepared from the medicinal plants studied have potential nematicidal properties and *in vivo* studies are suggested to make use of these plants as effective drugs against Haemonchosis in small ruminants as an alternative to chemotherapy.

**Keywords:** Nematoda, anthelmintic, nematicidal, medicinal plants, *Haemonchus contortus*

## INTRODUCTION

Helminthic parasitic diseases remained a major constrain to livestock productivity in different parts of the globe. *Haemonchus contortus* is a blood sucking trichostrongylid nematode of small ruminants, causing anorexia, anaemia, reduced growth and decreased wool production and high mortality (Waller and Thamsborg, 2004). This pathogenic nematode is major constraint to production of small ruminants. The strategies for control of this highly pathogenic nematode become ineffective due to emergence of drug resistance to all the broad and narrow spectrum classes of anthelmintics (Guo *et al,* 2016). In most cases, resistance seems clearly to be associated with heavy reliance on chemical control, applied frequently and sometimes haphazardly. This is most important problem acting against sustainable parasite control.

Parasite resistance enhances cost of treatment, reduces the efficacy of production, depletes the stock of effective control tools and increases the risk of environment contamination, as frequency of use and dose increases with declining effectiveness of anthelminitcs. As resistance to newer anthelminitics is emerging, there is a need in India for sustainable integrated parasite management which is multidisciplinary in nature and is underresourced because of its long-term nature (Sanyal, 1996). Also keeping in view the global economic conditions at present there is a dire need for the new anthelmintics as declared by Kornis (1996) in the 41th Annual Meeting of Veterinary Parasitologistis in USA. This calls for the new area of research in evolving drugs which are nonpollutant, safer and cost effective.

The growing awareness of environmental ecofriendly approaches and the global economic conditions have directed research for bio-safe, ecofriendly and economically viable approaches. The anthelmintic activities of various plant species have been tested by various workers in different part of the world.

Widiarso *et al* (2018) observed significant changes in morphology and morphometry of *Haemonchus contortus*. when treated with *Gigantochloa apus* crude aqueous extract. The reduced morphometric measurements were seen notably in body width, body length, width of cervical papillae, length of vulvar region in female and copulatory bursal parts (gubernaculum and spicules) in male worms. The anthelmintic properties of *Annona muricata* were evaluated by Ferreira et al (2013). They tested the aqueous leaf extract of *Annona muricata* under in vitro conditions on eggs, infective larval stages and adults of trichostrongylid nematode *Haemonchus contortus.* Jabeen *et al* (2015) studied the *in vitro* anthelmintic efficacy of fruit peel of *Punica granatum*, leaves and roots of *Berberis lyceum* against *Haemonchus contortu*s. They found that both these plant extracts show potent anthelmintic activity at concentration higher than 50 mg/mL. The methanolic root extracts exhibit more potent activity than the leaf extract at the same concentrations.

Kumar *et al* (2010) tested the *in vitro* anthelmintic activity of *Amaranthus spinosus, A. viridis* and *A. caudatus* by using earthworms (*Pheretima posthuma*) as experimental model animals. Their study using methanolic extracts of these three plants of Amaranthacae family showed a dose dependent mortality. Anthelmintic activities of *Anogeissus leiocarpus* and *Daniella oliveri* against *Haemonchus contortus* were investigated by Adama *et al* (2009). The effect of both these plant extracts was dose dependent on egg hatch and first stage larvae ($L_1$) but not in case of adult worms. *D. oliveri* stem bark extract is more ovicidal and larvicidal than *A. leiocarpus* leaves.

Ahmed *et al* (2012) tested the anthelmintic activity of crude extracts of 25 medicinal plants against *Haemonchus contortus.* In the second part of their experimentation they selected five plants out of twenty five plants chosen for preliminary experimentation. The five plants showing high efficacies were *Aloe ferox, Warburgia salutaris, Allium sativum, Ananas comosus and Lespedeza cuneata.* The aqueous, ethanolic and dichloromethane extracts of these plants were screened at 2.5, 5, 10 and 20 percent concentrations. More than 70 percent mortality of worms was seen in ethanolic extracts of *Lespedeza cuneata.* Eugale *et al* (2006) tested the potential anthelmintic effects of crude hydro-alcoholic extracts and aqueous extracts of seeds of *Acacia nilotica, Ekebergia capensis, Croton macrostachyus* and *Terminalia schimperiana.* Eugale *et al* (2007) investigated the *in vivo* and *in vitro* nematicidal activities of *Hedera helix* extracts. They tested the effect of aqueous and hydro-alcoholic extracts of *Hedera helix* on eggs and adults of *H. contortus.* The anthelmintic activity of crude extracts of *Carissa spinarum* and *Azadirachta indica* (leaves) and *Acacia torttilis* (stem bark) was tested against *H. contortus* under *in vitro* experimentation. A significant adult mortality rate (93.9 and 96.8 percent) was observed with the extracts of *Carissa spinarum* and *Azadirachta indica. Phytolacca dodecandra* and *Acacia torttilis* extracts showed adult mortality rates of 68.1 and 53.03 percent. Urban et al (2008) evaluated the *in vitro* anthelmintic effects of sixteen czeck medicinal plants on infective larvae of *Trichostrongylus colubriformis* and embryonated eggs of *Ascaris suum*.

Singh (2015) investigated the *in vitro* effect of neem (*Azadirachta indica*) leaf extract on the various organ-systems of the trichostrongylid nematode *H. contortus.* The various parameters related to motility, mortality and histomorphology was evaluated efter exposure to treatment with

varying concentrations of the aqueous extract. The maximum mortality rate was seen in 50 percent concentration after a time period of 6 to 12 hours. The anthelmintic activity of *Acacia nilotica* against *H. contortus* was also tested by Singh (2013). The motility of the worms was strongly inhibited after 5 to 10 hours of treatment in varying concentrations of the aqueous leaf and bark extracts.The anthelmintic activity of eight medicinal plants was tested against adult and larvae ($L_3$) of *Haemonchus contortus by* Akhtar *et al* (2015). The plants selected were neem, tamak, Korolla, halud, chatim, sharna lata and lazzabati, The results showed that neem and korolla showed maximum efficacy against adults and larval stages of *H. contortus*. The study also concluded that methanolic extracts proved better adulticidal and larvicidal than aqueous extracts. It was also suggested that further toxicological and pharmacological assessment is required to determine the recommended doses.

Mohammad *et al* (2013) tested the nematicidal properties of four medicinal plants *viz., Carissa spinarum, Phytolacca duodecandra, Acacia tortilis* and *Azadirachta indica.* Zenebe *et al* (2017) evaluated the nematicidal activity of crude extracts of various parts of *Cissus Quadrangularis* and leaves of *Schinus molle*. The *in vitro* adulticidal and egg hatching inhibitory effects of the crude methanolic extracts of *Schinus molle* and *Cissus Quadrangularis* were investigated. Mali and Mehta (2008), Qadir *et al* (2010), Rajeshwari (2014), Kuma *et al* (2015) reviewed the literature on anthelmintic properties of different plants. They briefly reviewed the research work carried out by different researchers to evaluate the plant species which are capable of reducing the helminthic infections

The present study was aimed to investigate the the nematicidal efficacies of aqueous extracts of five medicinal plants *viz., Acacia nilotica, Azadirachta indica, Dalbergia sissoo, Ocimum sanctum* and *Ficus religiosa* against *Haemonchus contortus.*

## MATERIAL AND METHODS

**(a) Collection of Plant leaves, seeds and Bark:**

Fresh leaves of *Acacia nilotica, Azadirachta indica, Dalbergia sissoo, Ocimum sanctum and Ficus religiosa* were collected from different parts of Gurdaspur and Barnala Districts of Punjab. The plant Samples were collected in the months of march- April and September- October.

**Table 1: List of five selected plants along with the parts used against *Haemonchus contortus***

| Botanical name of the Plant | Common name | Plant Part used |
|---|---|---|
| *Acacia nilotica* | Kikkar | Leaves |
| *Azadirachta indica* | Neem | Leaves |
| *Dalbergia sissoo* | Shisham | Leaves |
| *Ocimum sanctum* | Tulsi | Leaves |
| *Ficus religiosa* | Pipal | Leaves |

**(b) Preparation of Crude Aqueous Extracts:**

The leaves were washed, dried in shade and chopped into smaller pieces. Then these were grinded into fine powder. The powder was soaked in water in the ratio of 1:5 for overnight and next morning the extract was strained thrugh a piece of cloth. This was taken as Stock Solution. The experimental concentrations were prepared from stock solution. (Vyas and Mistry, 1996).

(c) **Collection of Parasites:**

Adult male and female worms of *Haemonchus contortus* were collected from the abomasums of infected sheep and goat from local abattoirs of batala, Barnala and Patiala. Immediately after slaughters, the stomach were collected. Abomasum were separated from the stomach and transported to the laboratory. The male and female worms were collected manually. The

parasites were washed in petri dishes containing phosphate buffered saline (PBS).

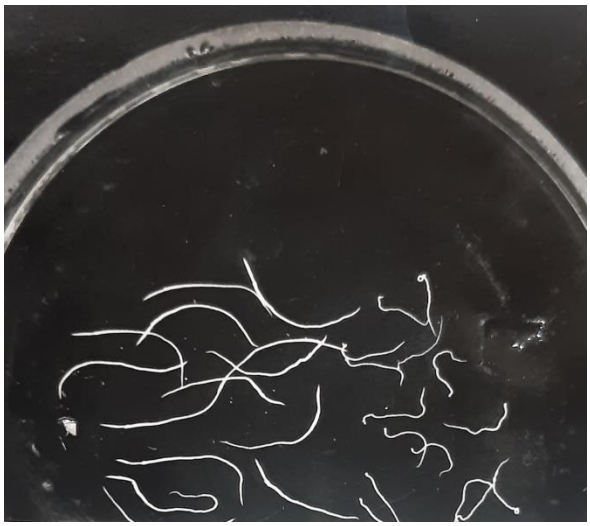

**Fig. 1 Male and female worms of *Haemonchus contortus***

(d) ***In vitro*** **testing of crude aqueous leaf extracts:**

Eight actively moving worms were placed in petri dishes containing 10, 20, 30, 40 and 50 percent of aqueous extract in phosphate buffered saline (PBS). Phosphate buffered saline alone used as control. Albendazole diluted PBS was used as positive control. The number of motile worms was confirmed by needleprick and placing the worms in warm saline for 10 seconds. A mortality index was calculated as number of dead nematodes by the total number of nematodes per petri dish. The percent mortality (M) of worms was calculated for each concentration of the extract by using the formula

Mortality Percent (M) = Number of worms died/ Number of worms in the petri dish X100

## RESULTS AND DISCUSSION

Results showing the nematicidal efficacy of aqueous extracts of *Acacia nilotica*, *Azadirachta indica*, *Dalbergia sissoo*, *Ocimum sanctum* and *Ficus religiosa* against *Haemonchus contortus* are summarized in table no. 2.

**Table 2: Efficacy of aqueous extract of six selected plants at 50 percent concentration against *Haemonchus contortus.***

| Name of Plant/Drug | Concentration (%) | Mean Time of Paralysis (in hours) | Mean Time of Death (in hours) |
|---|---|---|---|
| *Acacia nilotica* | 10 | 8 | 16 |
| | 20 | 8 | 16 |
| | 30 | 6 | 12 |
| | 40 | 6 | 12 |
| | 50 | 4 | 08 |
| *Azadirachta indica* | 10 | 8 | 14 |
| | 20 | 8 | 14 |
| | 30 | 6 | 10 |
| | 40 | 6 | 10 |
| | 50 | 4 | 06 |
| *Dalbergia sissoo* | 10 | 8 | 18 |
| | 20 | 8 | 18 |
| | 30 | 6 | 16 |
| | 40 | 6 | 16 |
| | 50 | 4 | 10 |
| *Ocimum sanctum* | 10 | 8 | 18 |
| | 20 | 8 | 18 |
| | 30 | 6 | 16 |
| | 40 | 6 | 16 |
| | 50 | 4 | 10 |
| *Ficus religiosa* | 10 | 10 | 20 |
| | 20 | 10 | 20 |
| | 30 | 8 | 16 |
| | 40 | 8 | 16 |
| | 50 | 6 | 14 |

The 100 percent mortality rate of adult *Haemonchus contortus* worms was seen after an exposure of 14 hours in 10 and 20 percnt concentrations of crude aqueous extracts of leaves of *Azadirachta indica.* However, the paralysis of worms started after 8 hours of treatment. The 30 and 40 percent concentrations of the same extract caused decreased motility of worms after 6 hours and 100 percent mortality after 10 hours of treatment. The crude aqueous leaf extract of *Azadirachta indica* of the 50 percent concentration category caused paralysis after

4 hours and 100 percent mortality after 6 hours. The maximum mortality rate of adult *Haemonchus contortus* worms was seen after an exposure of 8 hours in 50 percent concentrations of crude aqueous extracts of leaves of *Acacia nilotica*. However, the motility of worms started decreasing after 4 hours of treatment. The 30 and 40 percent concentrations of this extract caused paralysis of worms after 6 hours and maximum mortality after 12 hours of treatment. The crude aqueous leaf extract of *Acacia nilotica* (50 percent concentration) caused paralysis after 4 hours and total mortality occured within 8 hours. The complete mortality rate of adult *Haemonchus contortus* worms was observed after a treatment of 10 hours in 50 perccnt concentration of crude aqueous extracts of leaves of *Dalbergia sissoo* and *Ocimum sanctum.* However, the paralysis of worms started after 4 hours of treatment. The low concentrations of the same extract (10 and 20 percent) caused decreased motility of worms after 8 hours and significant mortality after 18 hours of treatment. The crude aqueous leaf extracts of *Dalbergia sissoo* and *Ocimum sanctum* of the 30 and 40 percent concentrations led to worm paralysis after 6 hours and 100 percent mortality after 16 hours. A significant mortality rate of adult *H. contortus* was seen after an exposure of 20 hours in lower concentrations of crude aqueous extracts of leaves of *Ficus religiosa*. The paralysis of nematodes started after 10 hours of treatment. The medium concentrations of the same extract caused decreased motility of worms after 8 hours and total mortality after 16 hours of treatment. The crude aqueous leaf extract of *Ficus religiosa* (50 percent concentration) resulted in paralysis after 6 hours and death of all the worms within 14 hours. Albendazole which served as a positive control show a dose dependent decrease in motility and effective mortality rate. However, the worms of control group were alive even after the end of the experimentation.

Prviously, Singh (2015) also found decreased motility, enhanced mortality and histomorphological changes in the ultrastructure of *H. contortus* following treatment with various concentrations of the extract of *Azadirachta indica.* The changes in motility, mortality and histology after treatment with different concentrations of the crude aqueous extract of *Acacia nilotica* were observed by Singh (2013). Kumar *et al* (2010) also found dose- dependent effect of three plants of Amaranthaceae family. Adama *et al* (2009) found a correlation between the dose of the extract of *Anogeissus leiocarpus* and *Daniella oliveri* and the mortality of first stage larvae ($L_1$) of *Haemonchus contortus.* The effect of both these plant extracts was dose dependent on egg hatch and first stage larvae ($L_1$) but not in case of adult worms. *D. oliveri* stem bark extract is more ovicidal and larvicidal than *A. leiocarpus* leaves.

The present investigation revealed that all the plants selected for *in vitro* experimentation showed nematicidal activity against adult *Haemonchus contortus*. It also supports the traditional use of these medicinal plants as nematicides. The *in vitro* studies provide preliminary report of the potential nematicidal activities of different plant extracts. Actually, the *in vivo* experimentation conditions always remain different due some factors like variable absorption of phyto-constituents from the absorptive surface of the parasite, metabolic biotransformation processes, probable interactions with other food materials, Therefore, the results of *in vitro* studies should be confirmed by *in vivo* studies. The results should be ascertained by qualitative phytochemical screening along with *in vivo* evaluation. The overall findings of the study revealed that aqueous extracts prepared from the medicinal plants studied have potential nematicidal properties and *in vivo* studies are suggested to make use of these plants as effective drugs.

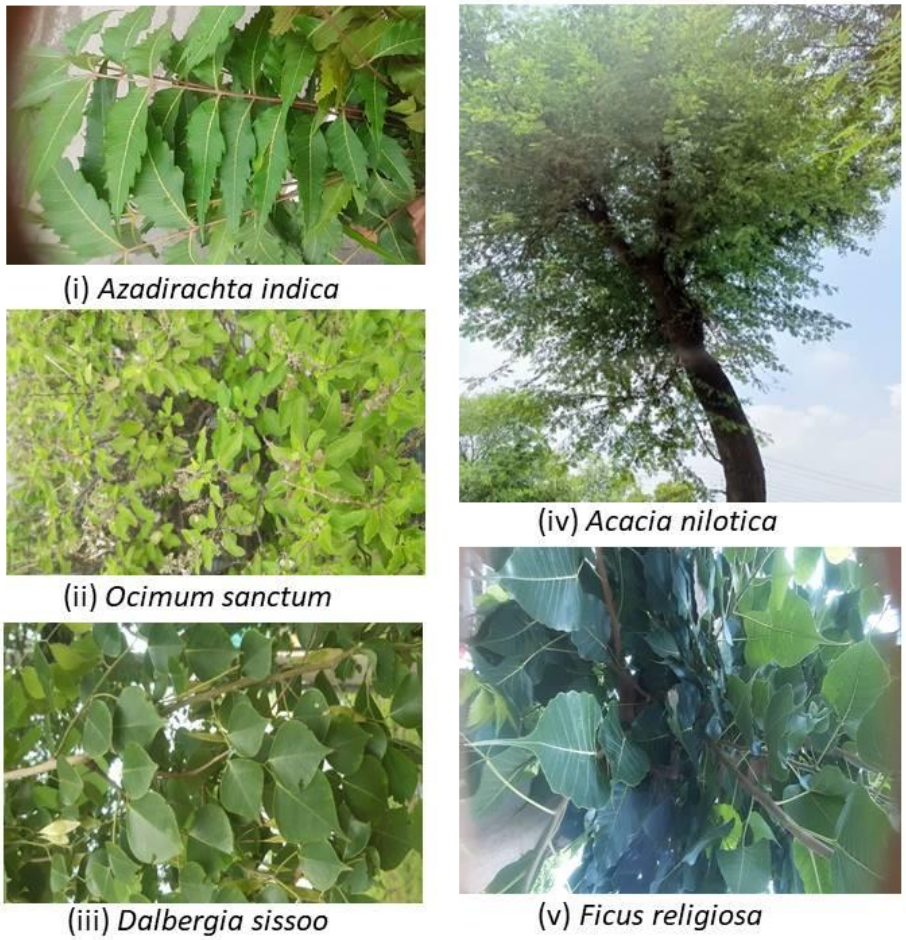


**Fig. 2: Plants used to test nematicidal properties against *Haemonchus contortus.***

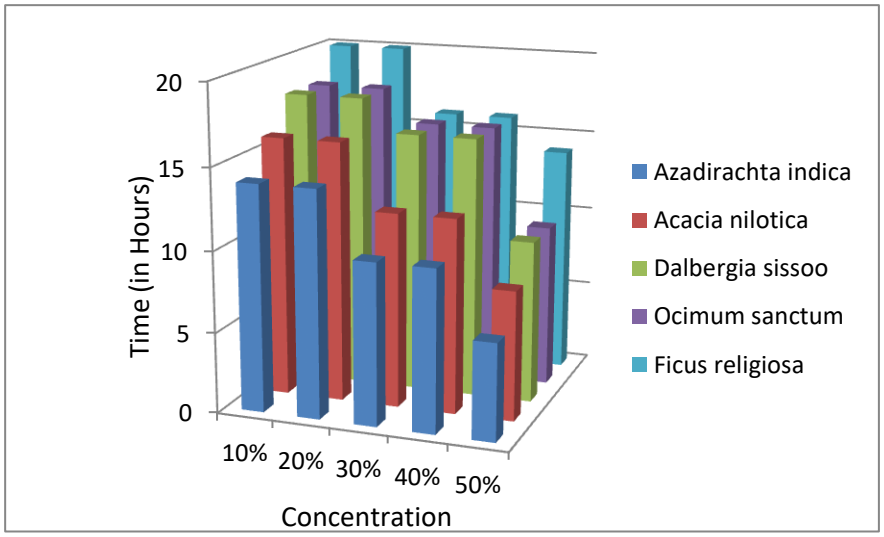


**Fig. 3: Plot showing time (in hours) for complete mortality of adult *H. contortus* treated with various concentrations of different crude aqueous extracts of different medicinal plants**

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