COMPARATIVE EFFICACY OF DOMESTIC GARLIC (*ALLIUM SATIVUM*) AND NEEM (*AZADIRACHTA INDICA*) AGAINST *HAEMONCHUS CONTORTUS* IN SMALL RUMINANTS

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Abstract. Livestock plays an important role in the economy of Pakistan. Small ruminants like sheep and goats, are highly susceptible to internal parasites, particularly gastrointestinal nematodes (GINs) including *Haemonchus contortus*. The present study was conducted in Pakistan to test the larvicidal and ovicidal activities with different concentrations of extracts of bulbs of domestic garlic (*Allium sativum*) and leaves of neem tree (*Azadirachta indica*) against *H. contortus*. In case of larval mortality, the highest mortality was recorded as 87% at 100% extract of neem leaves, while 100% extract of garlic bulb showed 67% mortality. The highest egg hatching inhibition (94%) was noted when neem leaves extract was used at 100% concentration, while in case of garlic extract, 100% concentration showed 80% inhibition of the eggs hatching. It was concluded that all the extracts of *A. indica* showed higher mortality and eggs hatching inhibition of *H. contortus* than *A. sativum*.
Keywords: *parasites, medicinal plants, eggs hatch assay, larval mortality assay*

Introduction

Agriculture always played a major role in the economy of Pakistan. In National Gross Domestic Product (GDP), agriculture contributes 24% in which share of livestock is 12%. According to the Ministry of National Food Security & Research the estimated population of domestic sheep (*Ovis aries*) was 30.5 million while domestic goats (*Capra aegagrus hircus*) were 74.1 million during 2017-18. It is also estimated that about 40000 tons of meat from sheep and 915000 tons from goats were obtained during the year 2017-18 (Economic Survey of Pakistan 2017-2018). But this important sector of agriculture is highly susceptible to gastrointestinal nematodes (GINs). Among GINs, *Haemonchus contortus*, a blood-sucking nematode that belongs to the family *Trichostrongylidae* and class *Secernentea*, causing a serious disease in small ruminants called Haemonchosis (Khattak et al., 2018).

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The clinical symptoms of this disease include hemorrhagic anemia, hypo-protein anemia, and parasitic gastroenteritis in animals especially in small ruminants like sheep and goats (Mola, 2018). *Haemonchus contortus* infestation causes major health problems including reduction in animal productivity in terms of meat, milk and wool. The major impacts of *H. contortus* in small ruminants are associated with the blood sucking activity that results in extensive blood loss (Bowman et al., 2009). Each parasite sucks 0.05 ml of blood per day. As a result, there is a decrease in total erythrocytes count, hemoglobin, packed cell volume, body weight, milk production and wool growth (Hepworth, 2006; Urquhart et al., 1996; Rasool et al., 1995). It is estimated that a sheep can lose 250 ml blood/day if infected with 5000 *H. contortus* worms. Life cycle of this parasite takes around 21 days to complete. Its life cycle is direct i.e. no secondary host is involved in cycle completion. Every adult female parasite lays 5000-10,000 eggs per day which are then passed out in the feces (Emery et al., 2016) as shown in *Figure 1*.

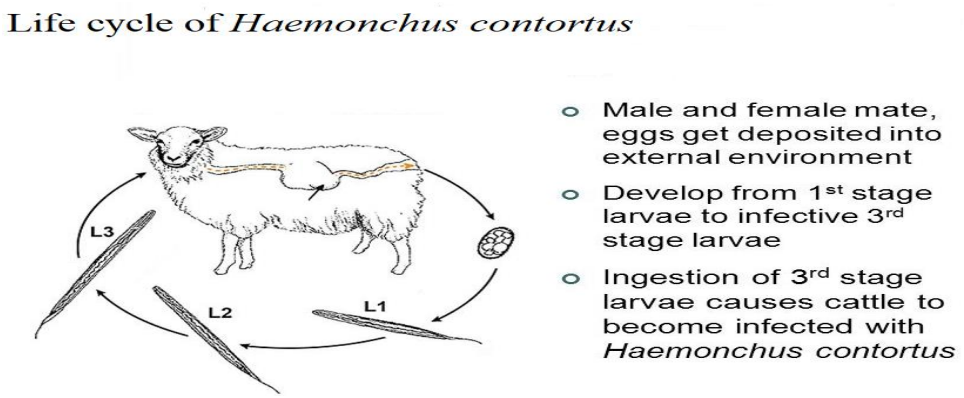

Figure 1. *Life cycle of Haemonchus contortus. (Reproduced from SlidePlayer.com)*

The frequent and un-judicial use of anthelmintic drugs has led to a growing problem of parasitic resistance against the conventional therapies. *Haemonchus contortus* is showing resistance to the most commonly used broad spectrum anthelmintics belonging to the classes; Benzimidazoles, imidazothiazoles and Macro cyclic lactones (Kaplan, 2004; Jackson and Coop, 2000; Sangster, 1999; Miller et al., 1987).

Garlic (*Allium sativum*) has been reported to be a parasiticide, amoebicide, larvicide and immune-stimulant (Duke, 2002). Essential oil of these plants can also be used as alternative or supplement to the current anthelmintics therapies (Anthony et al., 2004). Neem plant, (*Azadirachta indica)* is known for its medicinal properties and has been used against gastrointestinal nematodes and related problems in many parts of the world (Biswas et al., 2002). Therefore, the present study was designed with the objective to evaluate the anthelmintic properties of the crude extracts of Garlic and Neem plants against *H. contortus* in small ruminants.

Materials and methods

Sampling area

Adult *H. contortus* were collected from the infected abomasums of small ruminants from slaughter houses in district Kohat of Khyber Pakhtunkhwa, Pakistan as shown in

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Figure 2. The climate in Kohat is referred to as a local steppe climate. There is little rainfall throughout the year. This location is 33°34'47.1"N/71°26'29.3"E, elevation is 395 m/1296 feet and barometric pressure is 97KPa. The temperature here averages 22.8 °C. The average annual rainfall is 529 mm.

Figure 2. *Sampling area of district Kohat. (Reproduced from Google Maps)*

Collection and isolation of H. contortus

After collection of the adult parasites, female *H. contortus* worms were separated and placed in the Petri dish containing Phosphate Buffer Saline (PBS). Petri dish containing worms were kept in incubator at 37 °C. The identification of *H. contortus* was carried out on the basis of morphological features using standard keys (Urquhart et al., 1996) as shown in *Figure 3*.

Cultivation of infective larvae

After separation of female parasites from male parasites, eggs were recovered by grinding the female with pistol and mortar by adding 5 ml PBS. Eggs were incubated at room temperature for 72 h for the development of the infective larvae (L3). After cultivation, the infective larvae (L3) were maintained in the laboratory at 25-30 °C in sterile conditions.

Collection of plant materials

Fresh and healthy plant parts of Garlic (bulbs) and Neem (leaves) (disease free) were collected from various parts of the Khyber Pakhtunkhwa, Pakistan. After collection, the plants were washed with distilled water and dried with clean clothes and then kept in the laboratory for further processing.

Preparation of plant extracts

After drying, the plant materials were cut into small pieces followed by grinding into fine powder. The powdered material was mixed with methanol in conical flask and filtered through Whattman filter paper No. 1. The filtrate was then allowed to evaporate and make them concentrated.

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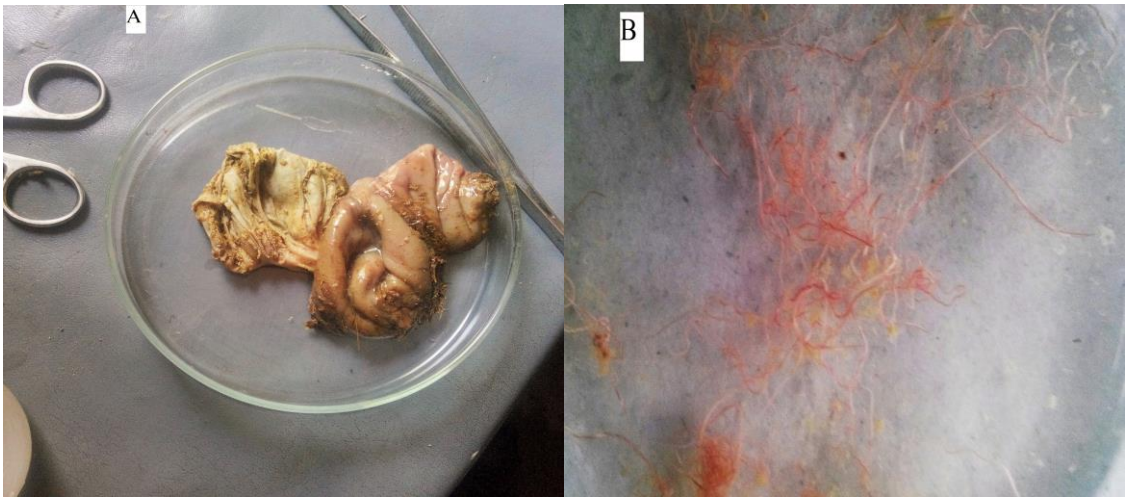

Figure 3. A *Abomasum of infected sheep.* ***B*** *Adult worms harvested from infected abomasum*

Preparation of methanol extracts

10 gm of the Neem and Garlic powdered materials first weighted and then dissolved in 100 ml methanol solution in separate beakers in order to make 10% solution and mixed properly by magnetic stirrer for 15 min. The solutions were kept on rotary shaker for 24 h and the mixtures were then passed through Whattman filter paper No.1 to prepare filtrate. The filtrate was taken into cork labeled bottle in sterilized condition. Different concentrations of filtrate were made i.e. 50%, 25%, 12.5% and 6.25% by adding 2.5 ml PBS and 2.5 ml filtrate in separate petri dishes by serial dilution method. A 100% (5 ml) pure extract solution was taken as a mother solution having no PBS, where PBS (5 ml) was used as a control group.

Effects of plant extract on Haemonchus contortus

Mortality of *H. contortus* was checked after 6 h treatment to the various concentrations of Garlic (*Allium sativum*) and Neem (*Azadirachta indica*) extracts.

Statistical analysis

Student *t* test was used to statistically analyze the data and the significance level was kept as $P < 0.05$.

Results

Mean percentage mortality of L3 stage Larvae after 6 h post-exposure of various percentages of methanol neem extracts

The selected plants of different concentration of methanol extract, Neem (100%) was significantly effective against L3 stage larvae of *Haemonchus contortus.* The efficacy of methanol extracts of plants at different concentration presented in *Figure 4*. At 100% concentration, 87% mortality was observed. 53%, 43%, 37% and 23% mortality have showed by 50%, 25%, 12.5%, 6.25% concentrations, respectively. In case of L3, Neem showed highest mortality as compared to Garlic extract. In positive control there was 100% mortality as shown in *Figure 4*.

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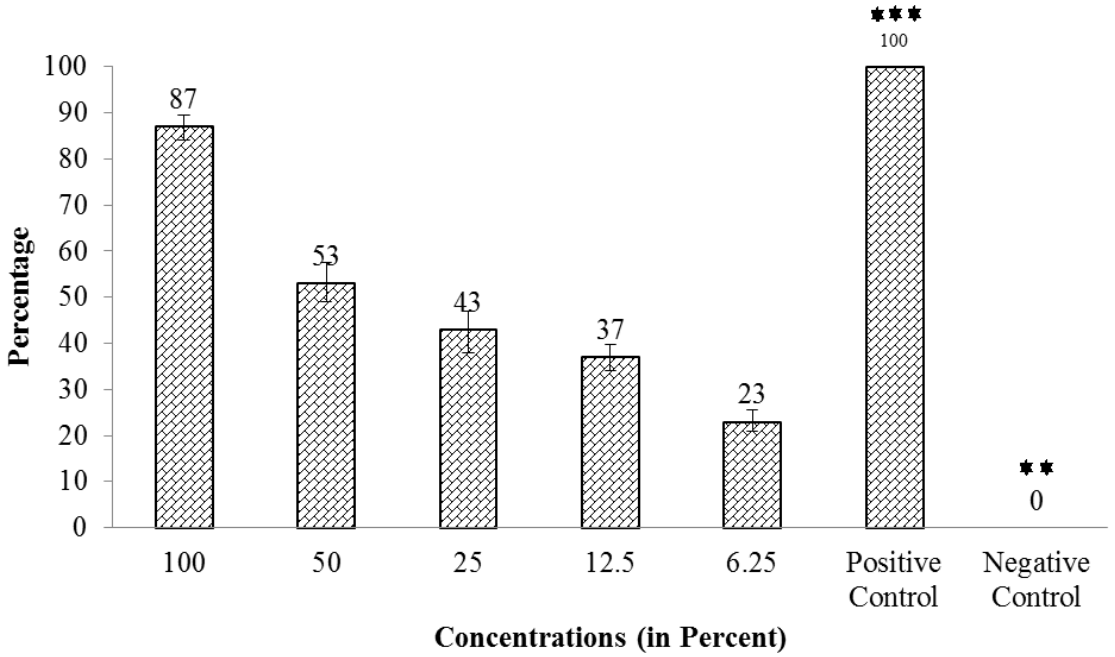

Figure 4. *Mean percent mortality of L3 stage larvae after 6 h post-exposure of various concentrations of methanol neem extracts*

Mean percentage mortality of L3 larvae after 6 h post-exposure against various percentages of garlic methanol extracts

It is evident from the data that Garlic diluted filtrate showed anthelmintics activity against L3 larvae but highest mortality has showed by 100% concentration which was noted as 67%. Moreover, 50%, 33%, 23% and 3% mortalities were shown by 50%, 25%, 12.5% and 6.25% solution, respectively. Whereas, in control group no mortality was noted as shown in *Figure 5*.

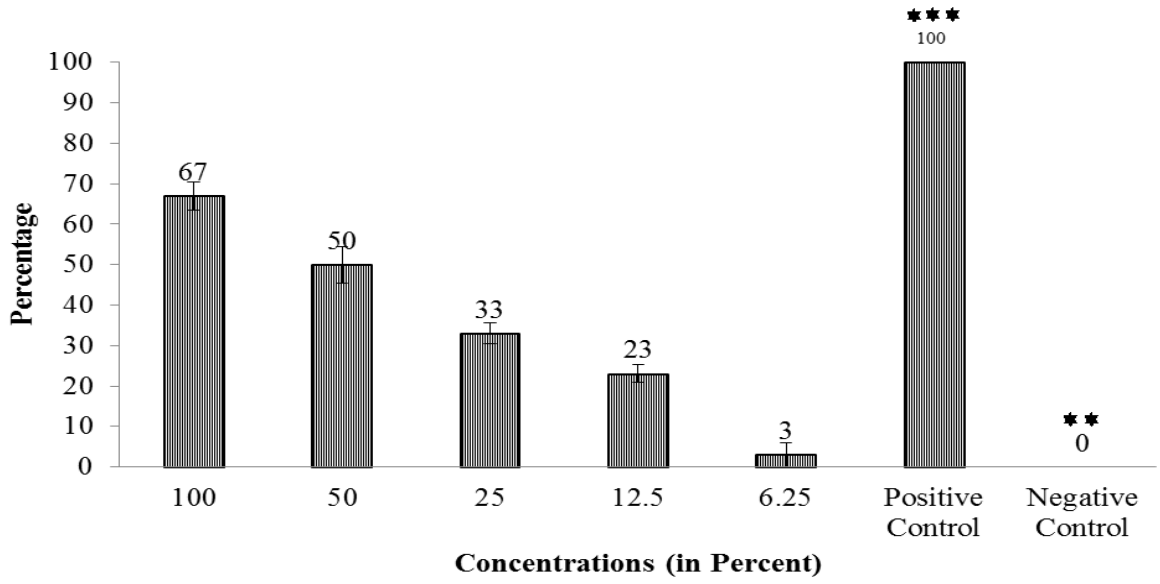

Figure 5. *Mean % mortality of larvae after treating 6 h with various concentration of methanol-based garlic extracts*

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Percent inhibition of eggs hatching of H. contortus against Neem extract

At 100% Neem extract showed highest egg hatch inhibition which was 94% (*Table 1*). Second highest egg hatch inhibition was recorded for 50% methanol extract of Neem (72%), followed by 25% (54%) and 12.5% extract (33%). The lowest inhibitions were observed for 6.25% and 3.125% that were 20% and 8%, respectively.

Table 1. *Percent inhibition of eggs hatching of H. contortus against neem extract*

Concentration (%)	No. of egg inhibition in 1st trial	No. of egg inhibition in 2nd trial	No. of egg inhibition in 3rd trial	Mean of egg inhibition
100	94	96	92	94
50	75	70	72	72
25	58	53	50	54
12.5	35	32	33	33
6.25	20	18	21	20
3.125	8	9	7	8
Control	0	0	0	0

P = 0.9992 > 0.05, non-significant

Percent inhibition of eggs hatching of H. contortus against garlic extract

The 100% Garlic extract showed highest egg hatching inhibition i.e. 80% as shown in *Table 2*. At 50%, 25%, 12.5%, 6.25% and 3.125% egg hatching inhibition was recorded as 65%, 49%, 31%, 15% and 5%. The results infer that concentration is directly proportional to egg hatching inhibition**.**

Table 2. *Percent inhibition of eggs hatching of H. contortus against garlic extract*

Concentration (%)	No. of eggs after 3 days in 1st trial	No. of egg inhibition in 2nd trial	No. of egg inhibition in 3rd trial	Mean of egg inhibition
100	80	82	78	80
50	65	68	62	65
25	50	47	51	49
12.5	30	31	32	31
6.25	15	13	17	15
3.125	5	5	6	5
Control	0	0	0	0

P = 01.0000 > 0.05, Non-significant

Discussion

To control *Haemonchus contortus*, common anthelmintics are applied but not enough to erase this parasite completely as it is reported to be resistant to all three broad spectrum families of anthelmintics viz. benzimidazole, lmidazothiazole and ivermectin and against drugs with narrow spectrum of activity such as salicylanilides anthelmintics (Kaplan and Vidyashankar, 2012; Eckert, 2008). *H. contortus* is one of the most prevalent nematode parasites of small ruminants i.e. sheep and goats (Coles et al., 2005). Keeping in view the importance of this blood sucker, there is need of alternative

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methods other than common anthelmintics to prevent sheep and goats from this highly dangerous nematode parasite.

The present study was undertaken to check the mean percentage of egg hatching inhibition. Extract filtrate obtained from *Allium sativum* at different concentration showed mean of inhibited eggs of *H. contortus* in the range from 5% to 80% which is much similar when compared with work of (Hammad et al., 2012) who reported that there was a dose and time dependent antinematicidal activity of crude aqueous methanol extract (CAME) of *N. tabacum* leaves with estimated LC_{50} values of 0.566 in EHA. Another study conducted in Pakistan by (Sindhu et al., 2009) showed a similar result that in egg hatching test, based on the LC_{50} values, most effective plants (LC50 in ppm) in their order of activity were; *N. tabacum* (0.10), *S. lappa* (0.73), *A. indica* (1.73), *C. arvensis* (2.51), *T. chebula* (5.55) and *A. vasica* (15.74). The allicin may have an important role in penetration of nematode egg surface, resulting in death of juveniles inside hard and protective barriers. The Azadirachtin of *Azadirachta indica* may have an important role in penetration of nematode egg surface, resulting in death of juveniles inside hard and protective barriers.

The third stage larvae (L3) of *H. contortus* in the present study were treated with different concentrations of the selected plants. In case of larvae, the mortality was found to be 87% at 100% methanol *Azadirachta indica*. Whereas, in case of *Allium sativum* the highest mean percent mortality was 67% at 100% extract. The methanolic diluted filtrate extract of *Azadirachta indica* showed approximately same result to the published paper of Rahman (2002). Rahman (2002) recorded the highest efficacy of neem leaves in alcoholic extract whereas aqueous extract has the lower efficacy (92%) than alcoholic extract against gastrointestinal nematodiasis in goat. Sujon et al. (2008) also recorded that the efficacy of methanol extract of neem was 80% and 100% at 5% and 10% concentration, respectively. Niddhi et al. (2007), Rahman et al. (2011) and Radhakrishnan et al. (2007) also showed the efficacy of neem against Haemonchus contortus adult and L3 stage larvae.

Conclusion and recommendations

Medicinal plants traditionally used against *Haemonchus contortus* control showed therapeutic activity. The selected medicinal plants contain active agents especially Allicin in garlic and Azadirachtin in Neem. These plant extracts at absolute (100%) form have the ability to lower the *H. contortus* infestation in small ruminants i.e. sheep and goats. *Azadirachta indica* contain potent anthelmintics compounds and therefore this plant is strongly recommended for further research studies. Mode and mechanism of action of anthelmintics plants also need to be studied in detail.

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