Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article

In vitro anthelmintic activity of *Trachyspermum ammi* seeds

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ABSTRACT - Development of anthelmintic resistance against commonly used anthelmintics in gastrointestinal nematodes of small ruminants has awakened interest in the study of medicinal plants as alternative sources of anthelmintics. The present study was carried out to evaluate the ovicidal efficacy of *Trachyspermum ammi* seeds, used locally as anthelmintic for worm control in sheep. Egg hatch test (EHT) was conducted on *Haemonchus contortus* ova to investigate the *in vitro* ovicidal effect of crude aqueous (CAE) and methnolic extracts (CME). Lethal concentration 50 (LC_{50}) values of CAE and CME of *T. ammi* seeds were 0.1698 and 0.1828 mg/ml, respectively. This present study suggests further large scale pharmacological and toxicological studies for its safer use in veterinary medicine.

KEY WORDS - *Trachyspermum ammi*; Anthelmintic; *Haemonchus contortus*; Egg hatch test

INTRODUCTION

Gastrointestinal nematodes have been found to be of great economic importance in domesticated livestock throughout the world (1) because of their adverse effects on productivity. Livestock producers have generally derived substantial benefits from the use of anthelmintics in controlling livestock parasitosis. In developing countries, like Pakistan, small scale farmers have a limited access to the commercially available anthelmintics and veterinary services either due to their non-availability or high costs. Most of the farmers, therefore, have to rely on the ethnoveterinary medicine as in some other parts of the world (2).

Plants constitute major part of traditional veterinary practices and have been found to be a rich source of botanical anthelmintics in animals for centuries (3-5). *Trachyspermum ammi* (L.) *Sprague ex Turril* (Umbelliferae) locally named as Ajwain in Pakistan, is commonly used in ethnoveterinary medicine (6) in different digestive disorders (7) and as an anthelmintic (8) as 'massaulas' (physic drench/balls) and water decoction. The seeds of *T. ammi* have several therapeutic effects including diuretic, anti-emetic, analgesic, anti-asthma, and anti-dyspnea effects (9). They also have a therapeutic effect on some cutaneous, neural, and urinary tract disorders. *T. ammi* is, therefore, used in household remedies. A watery extract of this plant is widely used to relieve grippe in children. In diarrhea, either roasted seeds are taken or a watery extract is made from them and given as a draught (10). The use of *T. ammi* seeds as an anthelmintic lacks scientific evidence. The present study was, therefore, carried out to evaluate the *in vitro* anthelmintic/ovicidal activity of *T. ammi* seed against *Haemonchus contortus* eggs.

MATERIALS AND METHODS

Plant material

Trachyspermum ammi seeds were procured from local market (Faisalabad, Punjab), identified and authenticated by a botanist from the preserved samples in the Herbarium, Department of Botany, University of Agriculture, Faisalabad–Pakistan. The voucher specimen (No. 13/2002; *Trachyspermum ammi* seeds) is stored in the Ethnoveterinary Research and Development Centre (EVRDC), Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan. Dried seeds were ground to a powder in an electric mill and stored in cellophane bags at 4°C until use.

Preparation of the aqueous extract

Crude aqueous extract (CAE) of the powdered *T. ammi* seeds were prepared as described previously by Fenado et al., (11). Briefly, 100 g of the powdered seeds were mixed with 500mL of distilled water in a 1 L flask and boiled for 1.5 h. Following cooling to 40°C, the 'brew' was filtered using Whatman No.1 filter paper. The filtrate was then concentrated in a vacuum rotary evaporator (yield (%): 17.6 w/w) and the extract stored at 4°C until required.

Preparation of the methanolic extract:

Powdered *T. ammi* seeds were exhaustively extracted with methanol in a Soxhlet's apparatus (12). The crude methanolic extract (CME) was evaporated to dryness and stored at 4°C until used (yield (%): 11.3 w/w).

In vitro ovicidal activity

Egg recovery:

Adult female *Haemonchus contortus* were collected after giving the longitudinal incision along the greater curvature of abomasums of naturally infected sheep. The worms present in ingesta or attached to the surface of guts were picked manually using forceps and placed in a bottle containing cool (4°C) PBS (pH 7.2) and later were triturated in pestle and mortar. The suspension was filtered through sieves of different sizes based on the nematode species into a bowl. Filtrate was centrifuged in Clayton Lane tubes for 2 min at about 300 x *g* and supernatant was discarded. Tubes were agitated to loosen the sediment and then saturated sodium chloride solution was added until a meniscus formed above the tube. A cover slip was placed and sample re-centrifuged for 2 min at about 130 x *g*. Coverslip was plucked off carefully from tubes and eggs were washed off into a conical glass centrifuge tube. Tube was filled with water and centrifuged for 2 min at about 300 x *g*. Supernatant was decanted and eggs were re-suspended in water. The eggs were then washed thrice in distilled water and adjusted to a 500 eggs per milliliter using the McMaster technique (13).

Test Procedure

Egg hatch test was conducted by the method described by Coles et al., (14). Eggs suspension of (0.2 ml; 100 eggs) was distributed in a 24-flat-bottomed microtitre plate and mixed with the same volume of different concentrations (0.15 to 5 mg mL^{-1}) of plant extract i.e., CAE and CME. The control plates contained the diluent water and the egg solution. The eggs were incubated in this mixture at 27°C. After 48 h, a drop of Lugol's iodine solution was added to stop the eggs from hatching. All the eggs and first-stage larvae (L1) in each plate were counted. There were three replicates for each treatment and control.

Statistical analysis:

Probit transformation was performed to transform a typical sigmoid dose-response curve to linear function (15). The extract concentration required to prevent 50% i.e., lethal concentration 50 (LC_{50}) of eggs from hatching was calculated from this linear regression (for y = 0 on the probit scale).

Results and discussion

Both aqueous and methanolic extracts of *T. ammi* demonstrated inhibitory effects on hatching of eggs. The LC_{50} was determined graphically from the regression equation as shown in Figs 1 and 2.

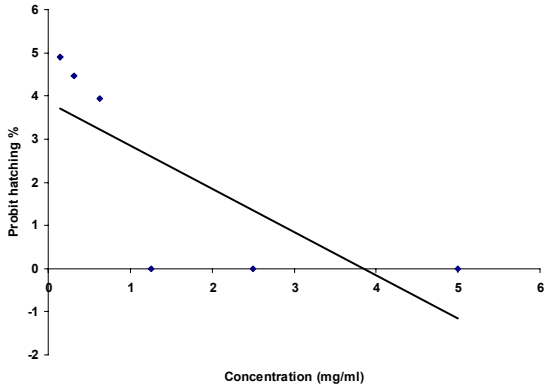

Fig. 1 Linear relationships between egg hatching % on the probit scale of trichostrongylid nematodes and T. ammi seeds aqueous extract concentrations (mg/ml)

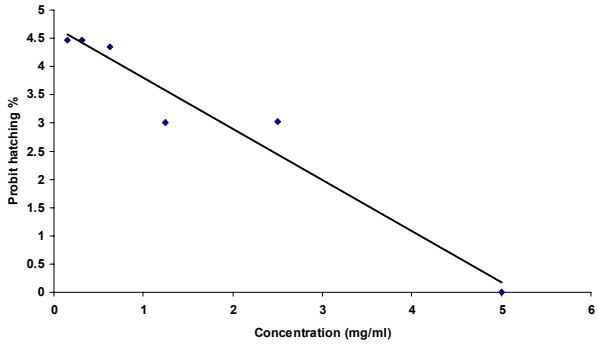

Fig. 2 Linear relationships between egg hatching % on the probit scale of trichostrongylid nematodes and T. ammi seeds methanolic extract concentrations (mg/ml)

The calculated LC_{50} values of aqueous and methanolic extracts of *T. ammi* were 0.1698 and 0.1828 mg/ml, respectively. The regression values and correlation of regression of the aqueous extract were y = -1.0008x + 3.8562 and R^2 = 0.575, respectively. Those of the methanolic extract were y = -0.9065x + 4.7076 and R^2 = 0.950. Aqueous extract was more effective against egg hatch compared to the methanolic extract (Figs 1 and 2).

In vitro tests using free living stages of parasitic nematodes offer a means of evaluating the

anthelmintic activity of new plant compounds as reported earlier (16,17). These *in vitro* tests measure the effects of anthelmintics directly on physiological processes such as hatch, development and motility of the parasites (18).

Higher levels of activity observed in the CAE suggest that the anthelmintic component of *T. ammi* is a relatively polar compound. *T. ammi* seeds have been reported to contain a variety of compounds. The trend of response was less linear in case of CAE as compared to CME which may be due to complex nature of compounds present in CAE and may not in CME. Thymol, a major component of *T. ammi* has been used as an antimicrobial (19) and antifungal (20). The following pharmacological effects of thymol, singly or in synergy, can be associated with the ovicidal activity of *T. ammi* seeds reported in the present study: (a) interference with the energy metabolism by potentiating ATPase activity (21) and thus loss of energy reserves required for embryonation of eggs; and (b) incorporation of thymol to membranes at the packing densities of natural membranes increasing the surface curvature and polarity (22).

CONCLUSION

T. ammi appears to possess some anthelmintic properties that may support the use of this plant by local farmers in traditional animal health care. Further, *in vitro* and *in vivo* experiments on *T. ammi* seeds or possibly isolated bioactive compounds that will incorporate toxicology/residues studies are required before it can be recommended for safe use.

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