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OVICIDAL AND LARVICIDAL PROPERTIES OF *ADHATODA VASICA* (L.) EXTRACTS AGAINST GASTROINTESTINAL NEMATODES OF SHEEP *IN VITRO*

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ABSTRACT

The main objective of this study was to evaluate the anthelmintic activity of *Adhatoda vasica* (Acanthaceae) *in vitro* against the gastrointestinal nematodes of sheep. The aqueous and ethanolic extracts of *Adhatoda vasica* aerial parts were evaluated by egg hatching and larval development assays. The aqueous and ethanolic extracts at 25-50 mg/ml concentrations exhibited ovicidal and larvicidal ($p<0.05$) activity against gastrointestinal nematodes. The plant extracts showed dose-dependent inhibition ($P<0.05$). The ethanolic extract at the concentration of 50.0 mg/ml was more effective in inhibiting egg hatching and larval development of gastrointestinal nematodes. The effective dose (ED_{50}) of aqueous and ethanolic extracts were determined graphically from linear regression equation with probit scale, y = 5. The results of this study suggested that *Adhatoda vasica* extracts may be useful in the control of gastrointestinal nematodes of sheep.

Keywords: *Adhatoda vasica*, egg hatch assay, gastrointestinal nematodes, larval development assay, sheep.

INTRODUCTION

Helminths control in domestic animals has been achieved almost exclusively by use of pharmaceutically derived anthelmintics. The misuse and/or widespread use of poor quality synthetic or semi-synthetic anthelmintics can develop multiple anthelmintic resistance that may lead to failure of control of worm parasites in farm animals. These constraints indicate that entire reliance on pharmaceutically derived anthelmintics may cause difficulties in management of gastrointestinal parasitic infections in livestock, necessitating alternative methods for helminths control (Pessoa *et al*., 2002; Githiori, 2004; Bizimenyera *et al*., 2006).

Recently, there has been an increasing interest in ethnomedical and ethno-veterinary practices across the world, especially the use of medicinal plants in various ailments. Use of indigenous plant preparations as livestock dewormers is gaining ground as one of the alternative and sustainable methods readily adapted to rural farming communities (Alawa *et al*., 2003; Bizimenyera *et al*., 2006). The present study was, therefore, carried out to evaluate the anthelmintic activity of *Adhatoda vasica* against gastrointestinal nematodes of sheep *in vitro*.

MATERIALS AND METHODS

Collection and preparation of plants

Plant materials of *A. vasica* were harvested from campus of Sindh Agriculture University (SAU), Tandojam, Pakistan. The plant materials were identified and authenticated by botanists in the Departments of Botany and Horticulture, Sindh Agriculture University, Tandojam, Pakistan. The collected plant materials were dried in shade at ambient temperature, ground and milled to powder form by electrical blender. The powdered materials were stored in dark tightly closed glass bottles until used.

Aqueous extract

The crude aqueous extract of *A. vasica* was prepared according the techniques described by Onyeyili *et al*. (2001) and Iqbal *et al*. (2006). Briefly, 100g of the powdered plant material was mixed with 500 ml of distilled water in 1L flask and boiled for 1.5 hours. Following cooling to 40°C, the residues were filtered using Whatman No.1 filter paper. The extracts then were evaporated to dryness by freeze dryer (ALPH 4-1, Martin Christ, Germany) and stored at 4°C until used.

Ethanolic extract

The powdered material of *A. vasica* was exhaustively extracted with 90% ethanol in a Soxhlet`s apparatus. Solvent was removed at temperature below 50°C in an oven. The residue (extract) of respective plant material was stored at 4°C until used.

Anthelmintic activity tests

The recommendations by the World Association for the Advancement of Veterinary Parasitology (WAAVP) for the detection of anthelmintic resistance in nematodes of veterinary importance (Hubert and Kerbouet, 1992; Coles *et al*., 1992; Taylor *et al*., 2002) were slightly modified for the eggs preparation, egg hatch and larval development assays, as described below:

Recovery and preparation of eggs

Twelve sheep of local breeds were treated by single dose of commercial anthelmintic Levamizole (Nilverm 1.5 % w/v; ICI Pakistan Ltd.) at a dose of 7.5 mg/ml body weight and kept indoor at experimental farm, SAU, Tandojam. The absence of gastrointestinal parasites in faeces of treated sheep were microscopically verified. All animals were then infected experimentally with different L_3 infective larvae of gastrointestinal nematodes to serve as source of fresh mono-species nematode eggs for subsequent *in vitro* tests, namely, egg hatch and larval development assays.

To induce experimental infection, adult nematode females were collected from the abomasal and intestinal contents of sheep at necropsy from slaughter houses. The adult worms were crushed to liberate eggs that were cultured to obtain the L_3 infective larvae. Two animals were infected orally with one of the following L_3 infective larvae species: *Haemonchus contortus*, *Trichostrongylus spp*, *Ostertagia circumcincta*, *Strongyloides papillosus*, *Oesphagostomum Columbianum* and *Chabertia ovina*. Eggs were collected after four weeks of infection and the eggs per gram (EPG) were daily determined. The concentrations of eggs were estimated by counting the number of eggs in aliquots of 50 µl by McMaster slide technique, as described by Urquhart *et al*. (1996). The egg suspensions were used on the same day.

Egg hatch test/assay

The stock solution of the crude extract of *A. vasica* initially was prepared by dissolving the crude extract in dimethylsulfoxide (DMSO) to improve it solubility in water. Aliquot of stock solution (50 mg/ml) was taken for preparation of final concentrations of 3.12, 6.3, 12.5, 25.0 and 50.0 mg/ml. In the assays, approximately 100 eggs in 200 µl of egg suspension were pipetted into each well of 96-well microtitre plate. In test wells, 200 µl of *A. vasica* extract in concentrations of 3.12, 6.3, 12.5, 25.0, and 50.0 mg/ml was added. Levamisole at concentrations of 0.5, 0.5×10^{-1}, 0.5×10^{-2}, 0.5×10^{-3}, 0.5×10^{-4} mg/ml was used as a positive control, while distilled water was utilized as negative control. Three replicates for each concentration of *A. vasica* extract and control were performed. The plates were incubated under humidified condition at ambient temperature (27°C) for 48 hours. A drop of Lugol`s iodine solution was added to each well to stop further hatching, and all the unhatched eggs and L_1 larvae in each well were counted under an inverted microscope.

Larval development test/assay

Eggs were obtained and estimated as described in egg hatching assay. One hundred eggs in 170 µl of egg suspension were put into each well of 96-well microtitre plate. A 20 µl of nutritive media (comprising of 1g yeast in 90 ml of normal saline and 10 ml Earle's balanced salt) was added into each well. The plates were then incubated under humidified condition at ambient temperature for 48 hours. Then 200 µl of *A. vasica* extract at same concentrations as mentioned above and Levamizole control concentration were added to respective plates. There were three replicates for each extract concentration and control. The plates were further incubated for 5 days (total of 7 days), further development was stopped by addition of one drop of Lugol`s iodine solution. All L_1 and L_3 larvae in each well were counted under an inverted microscope.

Statistical analysis

The data from egg hatch assay/test and larval development assay/test were transformed by $\log_{10}$ and submitted to one-way analysis of variance. The means were compared by the Dunncan test with 5% significant level using the SPSS 15.0 programme. The effective dose (ED_{50}) values were calculated graphically from linear regression with probit scale y = 5. The ED_{50} values were back transformed and presented in milligram plant extract per milliliter.

RESULTS

The results of anthelmintic activity evaluation of *A. vasica* extracts on the eggs and larvae of gastrointestinal nematodes in sheep by egg hatch and larval development tests/assays *in vitro* are shown in Tables 1-4. The aqueous and ethanolic extracts of *A. vasica* exhibited ovicidal and larvicidal activity ($p<0.05$) against the eggs and larvae of gastrointestinal nematodes. The extract concentrations of 25 and 50 mg/ml showed higher ($p<0.05$) inhibition rate compared to distilled water (negative control). The Levamizole (positive control) at the concentration of 0.5 mg/ml showed the highest ovicidal and larvicidal ($P<0.05$) inhibition rate, ranging from 90.0 to 99.0 per cent. All extract concentrations of the plant showed dose-dependent inhibition ($p<0.05$). The ethanolic extract was slightly more effective compared to aqueous extract on eggs and larvae of gastrointestinal nematodes. The highest inhibition rate of ethanolic extract was recorded at concentration of 50 mg/ml i.e. 88.0, 89.0, 85.8, 86.0, 86.0 and 84.0 per cent on eggs hatching and 82.7, 79.3, 84.3, 85.0, 81.0 and 82.7 per cent on larval development of *H. contortus*, *O. circumcincta*, *Trichostrongylus spp*, *S. papillosus*, *Oe. columbianum* and *Chabertia ovina* species, respectively.

The ED_{50} values of aqueous and ethanolic extracts of *A. vasica* on egg hatching and larval development of gastrointestinal nematodes of sheep are given in Table 5. The highest ED_{50} values of *A. vasica* extracts were recorded against the eggs of *Chabertia ovina* (18.2 and 18.2 mg/ml) and the lowest values were recorded against the eggs of *O. circumcincta* (12.59 and 11.48

mg/ml for ethanolic and aqueous extracts, respectively). Similarly, the ED_{50} values of *A. vasica* extracts against larvae of gastrointestinal nematodes were also determined. The higher ED_{50} values were recorded against larvae of *Oe. columbianum* (19. 50 and 18. 62 mg/ml) and the lower against the *H. contortus* larvae (15. 14 and 12. 88 mg/ml for aqueous and ethanolic extracts, respectively).

Table 1: Mean inhibition percentage (± SD) of different concentrations of *Adhatoda vasica* aqueous extract on eggs hatching of gastrointestinal nematodes of sheep

Concentration(mg/ml)	*H. contortus*	*O. circumcincta*	*Trichostrongylus Spp.*	*S. papillosus*	*Oe. columbianum*	*Chabertia ovina*
50.00	81.0 ± 6.6ab	86.0 ± 5.3b	81.3 ± 7.1b	81.0 ± 6.6a	83.0 ± 2.0b	81.0 ± 1.0a
25.00	74.3 ± 4.0b	79.3 ± 4.1c	79.3 ± 2.1b	76.3 ± 3.2a	70.3 ± 4.5c	63.3 ± 2.9b
12.50	40.7 ± 1.2c	60.7 ± 8.0d	40.0 ± 5.0d	41.3 ± 3.2c	40.0 ±5.0d	30.0 ± 5.0c
6.30	21.7 ± 2.1d	18.3 ± 3.8e	16.3 ± 1.5f	11.7 ± 1.5f	20.3 ±1.5e	18.7 ± 3.2d
3.12	13.3 ± 3.8e	9.7 ± 0.6f	11.0 ± 1.7f	11.0 ± 3.6f	10.3 ±1.5f	10.7 ± 2.1e
Levamizole	98.0 ± 1.0a	98.1 ± 1.0a	99.0 ± 1.0a	96.0 ± 1.0a	98.0 ±1.0a	97.0 ±1.0a
Distilled water	10.1 ± 1.0f	10.1 ± 1.0f	10.1 ± 0.9f	11.1 ± 06f	10.1 ± 1.0f	10.7 ± 0.9e

*Means with same letter within same column are not significantly different at P<0.05.

Table 2: Mean inhibition percentage (± SD) of different concentrations of *Adhatoda vasica* ethanolic extract on eggs hatching of gastrointestinal nematodes of sheep

Concentration(mg/ml)	*H. contortus*	*O. circumcincta*	*Trichostrongylus Spp.*	*S. papillosus*	*Oe. columbianum*	*Chabertia ovina*
50.00	88.0 ± 2.7ab	89.0 ± 1.0ab	85.8 ± 6.0ab	86.0 ± 3.6a	86.0 ± 3.6a	84.0 ± 1.7a
25.00	80.0 ± 1.0b	83.3 ± 7.3b	77.3 ± 2.5b	76.0 ± 3.6a	76.0 ± 3.6a	64.0 ± 1.0b
12.50	46.3 ± 3.5c	60.0 ± 5.0c	57.3 ± 3.2c	52.0 ± 6.1b	52.0 ± 6.1b	31.7 ± 0.6c
6.30	22.3 ± 0.5d	19.7 ± 8.1e	17.0 ± 2.0e	23.0 ± 3.6c	23.0 ± 3.6c	19.3 ± 1.2d
3.12	13.0 ± 2.7e	11.3 ± 5.5f	13.3 ± 1.5f	11.0 ± 3.6e	11.0 ± 3.6e	10.3 ± 0.6e
Levamizole	98.0 ± 1.0a	98.1 ± 1.0a	99.0 ± 1.0a	96.0 ± 1.0a	96.0 ± 1.0a	97.0 ± 1.0a
Distilled water	10.1 ±1.0f	10.1 ± 1.0f	10.1 ± 0.9f	11.1 ± 06e	11.1 ± 06e	10.7 ± 0.9e

*Means with same letter within same column are not significantly different at P<0.05.

Table 3: Mean inhibition percentage (± SD) of different concentrations of *Adhatoda vasica* aqueous extract on larval development of gastrointestinal nematodes of sheep

Concentration(mg/ml)	*H. contortus*	*O. circumcincta*	*Trichostrongylus Spp.*	*S. papillosus*	*Oe. columbianum*	*Chabertia ovina*
50.00	80.7 ± 1.2b	79.7 ± 0.6b	76.7 ± 2.9b	82.0 ± 1.0b	79.3 ± 4.0b	80.0 ± 5.0b
25.00	74.7 ±1.5bc	68.0 ± 2.7c	70.0 ± 1.0c	60.7 ± 0.6c	59.0 ± 1.0c	61.3 ± 0.6c
12.50	47.7 ± 2.1d	31.0 ± 1.0d	30.7 ± 1.5d	30.7 ± 1.2d	28.0 ± 1.0d	30.3 ± 1.5d
6.30	15.3 ± 1.5f	21.0 ± 1.0e	20.3 ± 0.6e	20.7 ± 1.2e	19.7 ± 1.5e	20.0 ± 1.0e
3.12	11.3 ± 1.5f	11.7 ± 0.6f	12.0 ± 0.0e	11.7 ± 1.5f	9.3 ± 1.2f	9.7 ± 0.6f
Levamizole	90.3 ± 8.9a	96.0 ± 1.0a	96.0 ± 1.0a	96.0 ± 1.0a	94.3 ± 1.2a	95.3 ± 2.5a
Distilled water	11.1 ± 0.6f	10.9 ± 0.8f	10.5 ± 05f	11.3 ± 0.6f	10.1 ± 0.5f	10.1 ± 0.4f

*Means with same letter within same column are not significantly different at P<0.05.

Table 4: Mean inhibition percentage (± SD) of different concentrations of *Adhatoda vasica* ethanolic extract on larval development of gastrointestinal nematodes of sheep

Concentration(mg/ml)	*H. contortus*	*O. circumcincta*	*Trichostrongylus Spp.*	*S. papillosus*	*Oe. columbianum*	*Chabertia ovina*
50.00	82.7 ± 2.5a	79.3 ± 4.1ab	84.3 ± 1.5b	85.0 ± 4.5b	81.0 ± 1.7a	82.7 ± 2.5b
25.00	74.0 ± 3.6a	71.0 ± 1.0b	70.0 ± 0.0c	60.3 ± 0.6c	59.7 ± 1.5b	63.3 ± 1.5c
12.50	47.7 ± 5.5b	27.3 ± 5.5c	30.7 ± 0.2d	30.0 ± 1.0d	29.0 ± 1.0c	29.3 ± 2.1d
6.30	26.7 ± 7.6c	22.0 ± 1.0de	20.3 ± 0.7e	20.7 ± 1.5e	20.0 ± 1.0d	19.7 ± 0.6e
3.12	14.7 ± 2.5e	11.3 ± 3.21f	13.7 ± 1.0e	12.0 ± 1.0f	9.7 ± 2.5e	10.0 ± 1.0f
Levamizole	90.3 ± 8.9a	96.0 ± 1.0a	96.0 ± 1.0a	96.0 ± 1.0a	94.3 ±1.2a	95.3 ± 2.5a
Distilled water	11.1 ± 0.6f	10.9 ± 0.8f	10.5 ± 05f	11.3 ± 0.6f	10.1 ± 0.5e	10.1 ± 0.4f

*Means with same letter within same column are not significantly different at P<0.05.

Table 5: The ED $_{50}$ (mg /ml) of aqueous and ethanol extracts of *Adhatoda vasica* on egg hatching and larval development of gastrointestinal nematodes in sheep

Nematode species	Egg hatching		Larval development	
	Aqueous	Ethanol	Aqueous	Ethanol
H. contortus	14.79	12.59	15.14	12.88
O. circumcincta	12.59	11.48	16.98	16.98
Trichostrongylus Spp.	14.79	12.59	17.38	15.85
S. papillosus	15.85	14.79	17.78	16.98
Oe. columbianum	15.49	14.79	19.50	18.62
Chabertia ovina	18.20	18.20	18.62	17.78

DISCUSSION

The main advantages of using *in vitro* assays to screen the anti-parasitic properties of the plants and plant extracts include low costs and rapid turnover which allow the screening of plants at large scale. In addition, these tests measured the effect of anthelmintic activity directly on the processes of hatching, development and motility of parasites without interfering the internal physiological functions of the host (Molan *et al*., 2003; Assis *et al*., 2003; Githiori *et al*., 2006). Several studies have been carried out in different parts of the world to evaluate the anthelmintic activity of medicinal plants against different nematode species of farm animal *in vitro* (Alawa *et al*., 2003; Asiss *et al*., 2003; Diehl *et al*., 2004; Githiori, 2004; Maciel *et al*., 2006; Bizimenyera *et al*., 2006).

In the present study, the frequencies of egg hatching and larval development (85 to 91%) observed in the absence of the *A. vasica* extracts showed that distilled water did not interfere with natural development of eggs and larvae. The results revealed that both ethanolic and aqueous extracts exhibited ovicidal and larvicidal activity on egg hatching and larval development of gastro Intestinal nematodes and the highest effectiveness ($P<0.05$) was at the concentration of 50 mg/ml. Assis *et al*. (2003) and Maciel *et al*. (2006) evaluated the anthelmintic activity of *Spigelia anthelmia* and *Melia azedarach* extracts on *H. contortus in vitro* by egg hatch and larval development tests and recorded similar results. Lateef (2002) and Lateef *et al*. (2003) conducted analogy studies on anthelmintic evaluation of *A. vasica* against gastrointestinal nematodes of sheep *in vitro* and *in vivo* through adult motility and faecal egg count reduction test (FECRT), respectively and reported that methanolic and aqueous extracts of *A. vasica* exhibited anthelmintic activity on adult worms *in vitro* and *in vivo* (37.4 per cent efficacy). The results of above workers are in agreement with findings of this study. The phytochemical analysis has revealed that the major constituents of *A. vasica* are vasicine, vascinone and saponins (Brain and Thapa, 1983; Akhter, 1988; Chauhan *et al*., 1999; Shaifali-Srivastava *et al*., 2001). The anthelmintic activity of this plant could be attributed to these compounds jointly or independently. The mechanism of action of active compounds of *A. vasica on* eggs and larvae of gastrointestinal nematodes is not yet clear but possibly these compounds interfere both with egg embryonation and direct effect on the larvae. The results of this study also showed that the ethanolic extracts were slightly more effective compared to the aqueous extracts on egg hatching and larval development of gastrointestinal nematodes of sheep. Cowan (1999) and Alawa *et al*. (2003) have stated that the isolation of botanical compounds from plant material largely depends on the solvent and method of extraction. Furthermore, they found that the organic solvent extracted more bioactive compounds from plants compared to water extract.

The effective dose (ED_{50}) is defined as the concentration of drug or extract producing 50% inhibition on egg hatching or larval development (Varady *et al*., 2006). Amarante *et al*. (1996) stated that resistant species of gastrointestinal nematodes to a particular anthelmintic drug showed higher ED_{50} values than susceptible species. This study revealed that eggs of *O. circumcincta* and larvae of *H. contortus* were more susceptible to ovicidal and larvicidal effects of *A. vasica* extracts.

The *in vitro* ovicidal and lavicidal action of *A. vasica* extracts on eggs and larvae of gastrointestinal nematodes may not imply that the extracts would have similar action on the adult worm parasites. The relevance of *in vitro* studies to *in vivo* efficacy, in regard to anthelmintic activity, is greatly influenced by the differences in the physiology and bioavailability of plant preparations within host (Githiori *et al*., 2005).

Conclusions

A. vasica appears to possess some anthelmintic activity that may support the usage of this plant by local farmers to cure their animals in ethnoveterinary medicine. However, further research is required to study toxicity, mechanism of action, identification of phytochemichals and effectiveness of this plant *in vivo* before it can be recommended for use in animals.

REFERENCES

Akhter, M. S., 1988. Anthelmintic evaluation of indigenous medicinal plants for veterinary usage.

Final Research Report, Dept. Physiol. Pharmacol., Univ. Agri., Faisalabad, Pakistan.

Alawa, C. B. I., A. M. Adamu, J. O. Gefu, O. J. Ajanusic, P. A. Abdu, N. P. Chiezey, J. N. Alawa and D. D. Brwmam, 2003. *In vitro* screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. Vet. Parasitol., 133: 73-81.

Amarante, A. F. T., W. E. Pomroy, W. A. G. Charleston, D. M. Leathwick and M. T. T. Torneros, 1996. Evaluation of a larval development assay for the detection of anthelmintic resistance in *Ostertagia circumcincta*. Int. J. Parasitol., 27(3): 305-311.

Assis, L. M., C. M. L. Bevilaqua, M. Morais, L. S. Vieira, C. T. C. Costa and J. A. L. Souza, 2003. Ovicidal and larvicidal activity *in vitro* of *Spibelia anthelmia* Linn. extracts on *Haemonchus contortus*. Vet. Parasitol., 117: 43-49.

Bizimenyera, E. S., J. B. Githiori, J. N. Eloff and G. E. Swan, 2006. *In vitro* activity of *Peltophorum africanum* Sond (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode *Trichostrongylus colubriformis*. Vet. Parasitol., 142: 336-343

Brain, K. R. and B. B. Thapa, 1983. High performance liquid chromatographic vasicine and vasicinnone in *Adhatoda vasica*. J. Chromatogr., 258: 183-188.

Chauhan, S. K., G. P. Kimothi, B. P. Singh and Agrawal, 1999. Development of HPLC method for vasicine and vasicinone in *Adhatoda vasica*. Indian J. Nat. Prod., 15: 21-24

Coles, G. C., C. Bauer, F. H. M. Borgsteede, S. Geerts, T. R. Klei, M. A. Tylor and P. J. Waller, 1992. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol., 44: 35-44.

Cowan, M. M., 1999. Plant products as antibacterial agents. Clin. Microbiol. Rev., 12(4): 564-582.

Diehl, M. S., K. K. Atindehou, H. Tere and B. Betschart, 2004. Prospect for anthelmintic plants in the Ivory Coast using ethno-botanical criteria. J. Ethno- pharmacol., 95: 277- 284.

Githiori, J. B., 2004. Evaluation of anthelmintic properties of ethnoveterinary plant preparations used as livestock dewormers by pastoralists and small holder farmers in Kenya. PhD Thesis, Swedish Univ. Agri. Sci., Uppsala, Sweden.

Githiori, J. B., J. Hoglund and P. J. Waller, 2005. Ethnoveterinary plant preparation as dewormers: practices, popular beliefs, pitfalls and prospects for future. Anim. Hlth. Res. Rev., 6: 91-103.

Githiori, J. B., S. Athansiadou and S. M. Thamsborg, 2006. Use of plants novel approaches for control of gastro Intestinal helminths in livestock with emphasis on small ruminants. Vet. Parasitol., 139: 308-320.

Hubert, J. and D. Kerbouef, 1992. A microlarval development assay for the detection of anthelmintic resistance in sheep nematodes. Vet. Rec., 130: 442-446.

Iqbal, Z., M. Lateef, A. Jabbar, M. N. Ghayur and A. H. Gilani, 2006. *In vitro* and *in vivo* anthelmintic activity of *Nicotiana tabacum* L. leaves against gastrointestinal nematodes of sheep. Phytotherapy Res., 20: 46-48.

Lateef, M., 2002. Trichostrongyloid nematodes of sheep: Epidemiological aspects and evaluation of anthelmintic activity of indigenous plants. PhD Thesis, Univ. Agri., Faisalabad, Pakistan.

Lateef, M., Z. Iqbal, M. N. Khan, M. S. Akhter and A. Jabber, 2003. Anthel mintic activity of *Adhatoda vasica* roots. Int. J. Agri. Biol., 5(1): 86-90.

Maciel, M. V., S. M. Morais, C. M. l. Bevilaqua, A. L. F Camurca-Vasconcelo, C. T. C. Costa and C. M. S Castro, 2006. Ovicidal and larvicidal effects of *Melia azedarach* extracts on *Haemonchus* contortus. Vet. Parasitol., 140: 98-104.

Molan, A. L., L. P. Meagher, P. A. Spencer and S. Sivakuman, 2003. Effect of flavan-3-ols on *in vitro* egg, larval development and viability of infective larvae of *Trichostrongylus colubriformis*. Int. J. Parasitol., 33: 1691-1698.

Onyeyili, P. A., C. O. Nwosu, J. D. Amin and J. I. Jibike, 2001. Anthelmintic activity of crude aqueous extract of *Nauclea latifolia* stem bark against ovine nematodes. Fitoterapia, 72: 12-21.

Pessoa, L. M., S. M Morais, C. M. L. Bevilaqua and J. H. S. Luciano, 2002. Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus contortus*. Vet. Parasitol., 109: 59-63.

Shaifali-Srivastava, R. K. Verma, M. M. Gupta, S. C. Singh, S. Kumar, S. Srivastava and S. Kumar, 2001. HPLC determination of vassicine and vasicinone in *Adhatoda vasica* with photo diode array detection. J. Liquid Chromatography Related Tech., 24: 153-159.

Taylor, M. A., K. R. Hunt and K. L. Goodyear, 2002. Anthelmintic resistance detection methods. Vet. Parasitol., 103:183-194.

Varady, M., D. Cernanska and J. Corba, 2006. Use of two *in vitro* methods for the detection of anthelmintic resistant nematode parasites on Slovak sheep farms. Vet. Parasitol., 135: 325-331.

Urquhart, G. M., J. Armour, J. L. Dunncan and F. W. Jennings, 1996. Veterinary Parasitology. 1st Ed., ELBS, Longman, London, UK.