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In vitro anthelmintic efficacy of some indigenous medicinal plants against gastrointestinal nematodes of cattle

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Abstract

The prevalence of natural gastrointestinal nematodes was observed in cattle during the period from June, 2004 to May, 2005 in Sadar upazila of Mymensingh district. The prevalence of gastrointestinal nematodes was 84.1% (rainy seasons-97%, summer-85.5% and winter seasons-69.8%). The prevalence of strongyles (*Haemonchus* sp., *Trichostrongylus* sp., *Oesophagostomum* sp. and *Mecistocirrus* sp.), *Bunostomum* sp., *Strongyloides* sp., *Trichuris* sp. and *Capillaria* sp. were 63.9%, 26.3%, 21.5%, 17.3% and 24.5%, respectively. Water extracts of 20 indigenous plants(neem, tobacco plant, barbados lilac, betel leaf, pineapple, jute, turmeric, garlic, devil's tree, papaya, lime tree, dodder, white teak, conessi tree, bitter gourd, sweet basil, white verticillia, pomegranate, sage, chaste tree) showed potential *in vitro* activities against adult parasites. Out of these, 20 plant extracts, 10 plants (neem, tobacco, barbados lilac, betel leaf, pineapple, jute, turmeric, garlic, dodder and bitter gourd) showed 100% efficacy against adult worms, 4 plants (devil's tree, papaya, white verticillia and chaste tree) showed 90-98% and others (lime tree, white teak, conessi tree, sweet basil, pomegranate and sage) showed below 90%.

Keywords: Anthelmintic, Gastrointestinal nematodes, *In vitro*, Prevalence, Medicinal plants

Introduction

Parasitism is an important limiting factor that responsible for deteriorating the health and productivity of livestock. The agro-ecological and geo-climatic conditions of Bangladesh are highly favorable for the growth and multiplication of parasites. As a result about 50% apparently healthy cattle populations are affected with different species of parasites (Garrels, 1975). Infections by gastrointestinal helminth parasites of livestock are among the most common, which are considered as economically important diseases of grazing livestock (Perry *et al.*, 2002). They are characterized by lower outputs of animal products (meat, milk, hides and skins), manure and traction, which all have impact of the livelihood of small holder farmers (Perry and Randolph, 1999). The production performances of these cattle are low, because of wide spread occurrence of pathogenic parasites. Parasitic diseases are considered important causing enormous economic losses through morbidity and mortality in livestock. Among the parasitic diseases, gastrointestinal nematodes such as *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp., *Oesophagostomum* spp., *Trichuris* spp. and *Strongyloides* spp. are most common (Qadir, 1981; Rahman and Mondal, 1983). Imported synthetic anthelmintics are considered the only effective way of controlling parasitic infection. However, as these are expensive and unavailable, livestock producers are not interested to use these anthelmintics. Furthermore, some serious disadvantages of using those anthelmintics, notably the development of resistance to helminth parasites (Waller and Prichard, 1985) against various anthelmintic compounds and classes, as well as their residues and toxicity problems (Kaemmerer and Butenkotter, 1973) poses hazards to livestock development and public health. For these reasons, interest in the screening of medicinal plants for their anthelmintic activity has remained of great scientific interest despite extensive use of synthetic chemicals in clinical practices (Akhtar *et al.*, 2000). Until today very little works are performed in our country to investigate the anthelmintic properties of indigenous medicinal plants in cattle. Considering all of these constraints, this work was undertaken with following objectives: i) screening of medicinal plants having *in vitro* anthelmintic activity, ii) prevalence of gastrointestinal nematodes in cattle of Sadar Upazila of Mymensingh district.

Materials and Methods

Collection of fecal samples

Fecal samples were collected from 1200 randomly selected cattle of Sadar upazila of Mymensingh district (100 month) were examined at various seasons (rainy seasons, summer and winter). Fecal samples were collected from the rectum of cattle by hand and kept in polythene bags and examined

by Floatation method (Rahman *et al*., 1996). The simple test tube flotation method is a qualitative test for the detection of nematode and cestode eggs and coccidia oocysts in the faeces. It is based on the separating of eggs from faecal material and concentrating them by means of a flotation fluid with an appropriate specific gravity.

Collection of indigenous medicinal plants

Indigenous medicinal plants (Table-3) from different location of Bangladesh were collected and the plants material were dried in shade and then dried off in the hot air oven at 55-60^{0}C to gain constant weight.

Preparation of plants powder

Powders were prepared by pulverizing the dried indigenous medicinal plants with the help of electric grinder. A 25-mesh diameter sieve was used to obtain fine dust and preserved them into airtight plastic container, till their use for extract preparation. Previously prepared plants powders were used for preparation of plants extract. Ten grams of each powder were taken in a 500 ml beaker and separately mixed with 100 ml of distilled water. Then the mixtures were stirred for 30 min by a magnetic stirrer (6000 rpm) and left as such for next 24 hrs. The extracts were then filtered through a fine cloth and again through filter paper (Whatman No. 1). The filtered material was taken into round bottom flask and then concentrated by evaporation of water from filtrate in a water bath at 50^{0}C till it reached the final volume of 10 ml. After the evaporation of water from filtrate, the condensed extracts were preserved in tightly corked-labelled bottle and stored in a refrigerator until used for screening of anthelmintic activity.

Preparation of stock solution

Stock solutions of plant extracts were prepared by diluting the condensed extracts with water. Different concentrations of each category of plant extracts were prepared by dissolving them in the water prior to anthelmintic screening.

Collection and maintenance of adult gastrointestinal nematodes in the laboratory

Collection of parasites from abomasum were done by following standard procedure as described by Taylor (1934) and Bell (1957). Viscera of cattle were collected from local market .The abomasums were opened through its lesser curvature with the help of scissors. The contents were emptied in glass jars containing normal saline. The abomasi were thoroughly washed and cleaned off ingesta and put in a different jar containing normal saline and left for an hour or two to release the attachments of parasites from the wall of the abomasum. The mucosal surface of the abomasum were rubbed carefully between the fingers to remove any remaining worms adhering to the abomasal wall. Finally the abomasal mucosa was examined with the help of a magnifying glass for any remaining parasites still adhering to the mucous lining of abomasum. The contents were washed several times with water and continued till the worms were free from debrises. The final wash was made with the normal saline. The sediments were examined in large petri-dishes over a black background. The parasites were collected with the help of curved needle and kept in normal saline. The parasites were cleared off debris by brushing with camel hairbrush or shaking in normal saline and identified according to the keys described by Rahman *et al.,* (1996) and Yamaguti (1958). They were kept in petri-dishes containing normal saline incubated at 37^{0}C.

***In vitro* screening of plant extracts for anthelmintic activity**

Screening of water extracts of plant at various concentration viz. 25 mg/ml, 50 mg/ml and 100 mg/ml were performed in the petri-dishes containing adult live stomach worms of cattle collected from slaughter house in Phosphate buffer saline (PBS). PBS (100ml) containing 50 adult worms (both male and female) were pipetted in 3 petri-dishes at ratio 0.1 ml, 0.05 ml and 0.025 ml water extracts were then added, respectively. The drug-parasite petri-dishes were incubated for three hours at room temperature and the efficacy was observed by counting the dead parasites and expressed in percentages (%).

Results and Discussion

The prevalence of gastrointestinal nematodes was highest in the month of June and July. The prevalence of gastrointestinal nematodes in cattle are shown in the Table 1 and Table 2. Highest prevalence of gastrointestinal nematodes were found in rainy seasons (July-October) followed by summer (March-June) and winter seasons (November-February). Prevalence of strongyles (*Haemonchus* sp., *Trichostrongylus* sp., *Oesophagostomum* sp. and *Mecistocirrus* sp.) infection was highest followed by *Bunostomum* sp., *Strongyloides* sp., *Trichuris* sp. and *Capillaria* sp. Waruiru *et al.* (2001) observed the epidemiology of gastrointestinal nematodes of dairy cattle in Central Kenya. The total worm burden in the animals was highest during the rainy season (March-June and October-December) and lowest during the dry seasons (July-September and January-February). This result is in conformity with earlier reports made by Keyyu *et al.* (2006), Jager *et al.* (2005), Soca *et al.* (2003) and Yildirim *et al.* (2000).

Table 1. Prevalence of gastrointestinal nematodes in cattle in Kotoally thana of Mymensingh district during different seasons

Name of Parasites	Prevalence percentage			
	Whole year	Rainy seasons	Summer	Winter seasons
Gastrointestinal nematodes	84.08%	97%	85.5%	69.75%
Strongyles (*Haemonchus* sp., *Trichostrongylus* sp., *Oesophagostomum* sp. and *Mecistocirrus* sp.)	63.92%	71%	66.25%	52%
Bunostomum sp.	26.33%	29%	27.25 %	22.75%
Strongyloides sp.	21.5%	24.25%	23.25%	17%
Trichuris sp.	17.25%	19.5%	18%	14.25%
Capillaria sp.	24.5%	27%	26%	20%

Table 2. Prevalence of Gastrointestinal nematodes in cattle in Kotoally thana of Mymensingh district on monthly basis

Month	Gastrointestinal nematodes	*Strongyles*	*Bunostomum*	*Strongyloides*	*Trichuris*	*Capillaria*
January	62%	46%	20%	14%	12%	18%
February	73%	55%	25%	16%	14%	20%
March	74%	57%	22%	18%	13%	22%
April	82%	64%	26%	24%	17%	24%
May	86%	68%	29%	26%	20%	28%
June	100%	76%	32%	25%	22%	30%
July	100%	72%	33%	28%	20%	28%
August	96%	75%	30%	22%	23%	30%
September	97%	70%	28%	25%	18%	26%
October	95%	67%	25%	22%	17%	24%
November	76%	58%	24%	21%	15%	23%
December	68%	49%	22%	17%	16%	19%

The *in vitro* efficacy of different concentrations of water extracts of 20 plants against adult gastrointestinal nematodes are shown in the Table 3. Water extract of 20 indigenous plants showed potential *in vitro* activities against adult parasites. The efficacy of water extract of these plants at the concentrations of 25 mg/ml and 50 mg/ml was much lower than that of concentration of 100 mg/ml except tobacco plants. Tobacco plants (25 mg/ml and 50 mg/ml) showed 100% *in vitro* efficacy against gastrointestinal nematodes of cattle. Neem, barbados lilac, betel leaf, papaya, dodder, bitter gourd and white verticillia (50 mg/ml) showed 70-80% *in vitro* efficacy against gastrointestinal nematodes of cattle. Turmeric (50 mg/ml) was 88% effective *in vitro* against gastrointestinal nematodes of cattle. The plants (100mg/ml) had highly significant activity (90-100%) against adult gastrointestinal nematodes *in vitro* were : neem (leaves and bark), tobacco plant (leaves), barbados lilac (leaves and bark), betel leaf (leaves), pineapple (leaves), jute (leaves), turmeric (rhizome), garlic (bulbs), devil's tree (leaves), papaya (leaves), dodder (whole plant), bitter gourd (leaves and seeds), white verticillia (leaves) and chaste tree (leaves). Iqbal *et al.* (2006) observed *in vitro* anthelmintic activity of *Nicotiana tabacum* leaves against gastrointestinal nematodes of sheep. The *in vitro* inhibitory effect was evident from the paralysis and/or mortality of worms noted at 6-hours post-exposure. Raje and Jangde (2003) found *in vitro* anthelmintic activity of decoction of *Nicotiana tabacum* against *Haemonchus contortus* of goats.

Table 3. *In vitro* anthelmintic efficacy of different concentrations of water extracts of 20 indigenous medicinal plants against adult gastrointestinal nematodes of cattle

Sl. No.	Indigenous plants				Percent non-motile adult worms at different concentrations of water extracts of plant		
	Local name	Scientific name	English name	Part used	25 mg/ml	50 mg/ml	100 mg/ml
1.	Nim	*Azadirachta indica*	Neem	Leaves	22	74	100*
	Nim	*Azadirachta indica*	Neem	Bark	32	76	100*
2.	Tamak	*Nicotiana tabacum*	Tobacco plant	Leaves	100*	100*	100*
3.	Ghora Nim	*Melia azedarach*	Barbados lilac	Leaves	26	78	100*
	Ghora Nim	*Melia azedarach*	Barbados lilac	Bark	28	74	98*
4.	Paan	*Piper betle*	Betel leaf	Leaves	22	72	100*
5.	Anaras	*Ananas comosus*	Pineapple	Leaves	52	66	100*
	Anaras	*Ananas comosus*	Pineapple	Fruit	18	40	64
6.	Deshi pat	*Corchorus capsularis*	Jute	Leaves	24	58	100*
7.	Halud	*Curcuma longa*	Turmeric	Rhizome	42	88	100*
8	Rashun	*Allium sativum*	Garlic	Bulbs	50	80	100*
9.	Chhatim	*Alstonia scholaris*	Devil's tree	Leaves	36	62	94*
10.	Pepe	*Carica papaya*	Papaya	Leaves	46	78	90*
	Pepe	*Carica papaya*	Papaya	Seeds	14	28	68
11.	Lebu	*Citrus aurantifolia*	Lime Tree	Leaves	20	38	56
12.	Swarnalata	*Cuscuta reflexa*	Dodder	Whole plant	32	72	100*
13.	Gamari	*Gmelina arborea*	White Teak	Leaves	26	44	74
14.	Kurchi	*Holarrhena antidysenterica*	Conessi tree	Leaves	22	48	82
15.	Karalla	*Momordica charantia*	Bitter Gourd	Leaves	24	80	100*
	Karalla	*Momordica charantia*	Bitter Gourd	Seeds	20	60	98*
16.	Tulsi	*Ocimum basilicum*	Sweet basil	Leaves	16	56	84
17.	Dondakalos	*Leucas aspera*	White verticillia	Leaves	42	70	94*
18.	Dalim	*Punica granatum*	Pomegranate	Leaves	30	52	86
19.	Bhui-Tulsi	*Salvia plebeja*	Sage	Leaves	36	64	88
20.	Nishinda	*Vitex negundo*	Chaste Tree	Leaves	26	56	92*

*Considered strong wormicidal

It may be concluded that the medicinal plants and/or its extracts may be used against gastrointestinal nematodes of cattle in Bangladesh. The pharmacokinetics together with its toxic effects need further studies.

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References

Akhtar, M.S., Iqbal, Z., Khan, M.N. and Lateef, M. 2000. Anthelminitic activity of medicinal plants with particular reference to their use in animals in the Indo-Pakistan subcontinent. Small Rum. Res., 38: 99-107.

Bell, R.R. 1957. Survey of gastrointestinal parasites of cattle in North California. *Am. J. Vet. Res*., VII: 294.

Garrels, G. 1975. Gastro-intestinal parasitic infestation of cattle in some village of Dhaka and Tangail district in Bangladesh. *Bang. Vet. J*., 9(1-4): 9-10.

Iqbal, Z., Lateef, M., Jabbar, A., Ghayur, M.N. and Gilani, A.H. 2006. *In vitro* and *in vivo* anthelmintic activity of *Nicotiana tabacum* L. leaves against gastrointestinal nematodes of sheep. Phytother. Res., 20(1): 46-48.

Jager, M., Gauly, M., Bauer, C., Failing, K., Erhardt, G. and Zahner, H. 2005. Endoparasites in calves of beef cattle herds: management systems dependent and genetic influences. Vet. Parasitol., 131(3-4): 173-91.

Kaemmerer, K. and Butendotter, S. 1973. The problem of residues in meat of edible domestic animals after application or intake of organophosphate esters. Residure Review, 46:1.

Keyyu, J.D., Kassuku, A.A., Msalilwa, L.P., Monrad, J. and Kyvsgaard, N.C. 2006. Cross-sectional prevalence of helminth infections in cattle on traditional, small-scale and large-scale dairy farms in Iringa district, Tanzania. *Vet.* Res. Com., 30(1): 45-55.

Perry, B.D. and Randolph, T.F. 1999. Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. Vet. Parasitol., 84: 145-168.

Perry, B.D., Randolph, T.F., McDermott, J.J., Sones, K.R. and Thornton, P.K. 2002. *Investing in animal health research to alleviate poverty*. International Livestock Research Institute, Nairobi, Kenya, 148 pp.

Qadir, A.N.M.A. 1981. An observation on the seasonal influence on the gastro-intestinal nematode infection in goats under farm conditions. Bang. Vet. J*.,* 15(3-4): 11-15.

Rahman, M.H. Ahmed, S. and Mondal, M.M.H. 1996. Introduction to helminth parasites of animals and birds in Bangladesh. 1st edn., Sheba Printing Press. Bangladesh.

Rahman, M.H. and Mondal, M.M.H. 1983. Helminth parasites of cattle (*Bos tauras*) in Bangladesh. Ind. J. Parasitol., 7: 173-174.

Raje, A.A. and Jangde, C.R. 2003. *In vitro* anthelmintic activity of decoction of Nicotiana tabacum against Haemonchus contortus of goats. Ind. Vet. J., 80(4): 364-365.

Soca, M., Simon, L., Soca, M. and Garcia, E. 2003. Gastrointestinal nematodes of young cattle in commercial silvopastoral systems. I. "El Cangre" Livestock Enterprise. Pastos y Forrajes, 26(1): 47-52.

Taylor, E.L. 1934. A method of estimating the number of worms present in the 4th stomach and small intestine of sheep and cattle for the definite diagnosis of the parasitic gastritis. *Vet. Res.,* 14: 474.

Waller, P.J. and Prichard, R.K. 1985. Drug resistance in nematodes. In: Campbell, W.C., Rew, R.S. (Eds), Chemotherapy of Parasitic Infections. Phenum, New York, USA, 339-362pp.

Waruiru, R.M., Thamsborg, S.M., Nansen, P., Kyvsgaard, N.C., Bogh, H.O., Munyua, W.K. and Gathuma, J.M. 2001. The epidemiology of gastrointestinal nematodes of dairy cattle in Central Kenya. Trop. Anim. Healt. Produc., 33(3): 173-187.

Yamaguti, S. 1958. Systema Helminthum. Interscience Publishers, Inc. New York, USA, 3: 410-451.

Yildirim, A., Kozan, E., Kara, M. and Oge, H. 2000. The prevalence of helminth infections in cattle raised in barns in Kayseri province. Ankara Universitesi Veteriner Fakultesi Dergisi, 47(3): 333-337.